Characterization of Multi Drug Resistant (MDR) *Escherichia coli* Isolated from Chicken using Molecular and Bioinformatics Tools

By

Muhammad Rafique

Department of Microbiology

Faculty of Biological Sciences

Quaid-I-Azam University Islamabad,

Pakistan, 2023

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Thesis

Submitted in the Partial Fulfillment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

IN

MICROBIOLOGY

By

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Certificate of Approval

This is to certify that the research work presented in this thesis, entitled titled **"Characterization of Multi Drug Resistant (MDR) *E-coli* Isolated from Chicken using Molecular and Bioinformatics Tools"** was conducted by **Mr. Mohammad Rafique** under the supervision of **Prof. Dr. Naeem Ali and** Co-supervision of **Dr. Muhammad Athar Abbas.** No part of this thesis has been submitted anywhere else for any other degree. This thesis is submitted to the Department of Microbiology, Quaid-i-Azam University, Islamabad in partial fulfillment of the requirements for the degree of Doctor of Philosophy in field of **Microbiology**.

Student Name: **Mr. Mohammad Rafique** Signature:_____

Examination Committee:

a) External Examiner 1: Signature:_____

Dr. Shahzad Hussain
House No. 1082, Street No. 7
Oversease Block-6, Bahria Town
Phase-8, Rawalpindi

b) External Examiner 2: Signature:_____

Prof. Dr. Asma Gul
Department of Biotechnology and Bioinformatics
Islamic International University, Islamabad

Supervisor Name: **Prof. Dr. Naeem Ali** Signature:_____

Co-Supervisor Name: **Dr. Muhammad Athar Abbas** Signature:_____
NRLPD, ASI, NARC, Islamabad

Name of HOD: **Prof. Dr. Naeem Ali** Signature:_____

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Acknowledgements

Praise to ALMIGHTY ALLAH, whose blessings enabled me to achieve my goals. Tremendous praise for the Holy Prophet Hazrat Muhammad (Peace Be upon Him), who is forever a torch of guidance for the knowledge seekers and humanity as a whole.

I have great reverence and admiration for my research supervisor, Dr. Naeem Ali, Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan and co supervisor Dr. Muhammad Athar Abbas, (NARC) for their scholastic guidance, continuous encouragement, sincere criticism and moral support throughout the study. Their guidance helped me in all the time of research and writing of this thesis, with their patience and immense knowledge.

I would also like to thank the Chairperson of Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan, for his help and guidance throughout my Ph.D.

I do not find enough words to express my heartfelt gratitude for Prof. Dr. Gautam Dantas Washington University St. Louis, USA. He guided me during my research at Washington University during IRSIP. This experience would not have been as valuable without the guidance, support and inspiration provided by him. I am impressed by his scientific thinking and politeness. I am also very thankful to Robert and Alaric who helped me in data analysis by designing graphs and other bioinformatic tools to conclude my result. I would also like to thank Higher Education Commission, Pakistan, for providing me grant under the Project "International Research Support Initiative Program (IRSIP)" and Indigenous Scholarships.

I am extremely grateful to the entire faculty at the Department of Microbiology, Quaid-i-Azam University, Islamabad. I feel thankful to the non-teaching staff, Shabbir Sahib, Shahzad bahi, Sharjeel, Department of Microbiology, QAU, Islamabad, for their kind assistance. I extend my great depth of loving thanks to all my friends and lab mates

(seniors and juniors) especially Robert, Aura, Alaric, Athar Abbas, Abdul Rahim Muhammad Rafiq, Samin, Wasim Sajjad, Abdul Haleem, Abdul Haq, Ameer Afzal and many more. I cannot say enough thanks to Tariq Ahmed for their help and support. A non-payable debt to my loving Abu, Ami, brothers and sisters for bearing all the ups and downs of my research, motivating me for higher studies, sharing my burden and making sure that I sailed through smoothly. Completion of this work would not have been possible without the unconditional support and encouragement of my loving family members. Finally, I express my gratitude and apology to all those who provided me the opportunity to achieve my endeavors, but I missed to mention them personally.

Muhammad Rafique

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List of Abbreviations

ADH	Arginine Dihydrolase
AFLP	Amplified Fragment length Polymorphism
AIEC	Adherent Invasive *E. coli*
Ak	Amikacin
AMP	Amoxicillin
AMR	Antimicrobial resistance
APEC	Avian Pathogenic *E. coli*
API	Analytical profile index
ARG-ANNOT	Antibiotic resistance gene annotation
ARGs	Antibiotic-resistant gene
AST	Antibiotic sensitivity
GBD	Global burden of diseases
BLAST	Basic local alignment search tools
BR	Broad range
C30	Chloramphenicol
CARD	Comprehensive antibiotic resistance database
CDC	Centre for diseases control and prevention
CGE	Centre of Genomic Epidemiology
CIP	Ciprofloxacin
CH	fumC fimH typing
CLSI	Clinical laboratory & standard Institute
CN	Gentamicin
CN30	Gentamycin
CNF1	Cytotoxic necrotizing factor 1

CPS	Capsule Polysaccharide
CT10	Colistin Sulphate
DEC	Diarrheagenic *E. coli*
DNA	Deoxyribonucleic acid
DO30	Doxycycline
E. coli	*Escherichia coli*
E30	Erythromycin
EAEC	Enteroaggregative *E. coli*
EcMLST	*E. coli* Multilocus Sequence Typing
EFT30	Ceftiofur
EHEC	Enterohaemorrhagic *E. coli*
EMB	Eosin methylene Blue
ENR5	Enrofloxacin,
ENA	European nucleotide archive
EARSS	European Antimicrobial Resistance Surveillance System
ESBL	Extended spectrum beta lactamase
ESF	Exponential survival function
ETP10	Ertapenem
EPEC	Enteropathogenic *E. coli*
EUCAST	European Committee on antimicrobial susceptibility testing
ExPEC	Extraintestinal pathogenic *E. coli*
F300	Nitrofurantoin
FAST	Fast All
FAO	Food and agriculture organization
GD	Gautam Dantas
GDP	Gross domestic product
H5N1	Hemagglutinin type 5and Neuraminidase type 1

HPAI	Highly pathogenic avian influenza
IND	Indole
Kg	Kilogram
LAMP	Loop-mediated isothermal amplification
LDC	lysine decarboxylase
LPA	Low pathogenicity virus
MALDI-TOF	Matric-assisted laser desorption/ionization, Time of flight
mcr-1	Mobilized colistin resistance
MBLs	Metallo-β-lactamases
MDR	Multidrug resistance
MEM10	Meropenem
MHA	Muller Hinton agar
MIC	Minimum Inhibitory Concentration
MLEE	Multilocus Enzyme Electrophoresis
MLST	Multilocus Sequence Typing
MRSA	Methicillin-resistant Staphylococcus
MY15	Lincomycin
N30	Neomycin
NA	Neuraminidase
NAE	No antibiotics ever
NARMS	National Antimicrobial Resistance Monitoring
NARC	National agriculture research centre
ND	Newcastle Disease
NDM-1	New Delhi metallo-beta-lactamase
NGS	Next Generation Sequencing
NOR	Norfloxacin
NOR10	Norfloxacin

NTS	Nontyphoidal *Salmonellas*
NP	Nanoparticle
NRLPD	National reference lab for poultry diseases
NMEC	New-born Meningitis Associated *E. coli*
ODC	Ornithine decarboxylase
OIE	Office International des Epizooties
ORT	Ortnithobacterium rihinotracheale
ORF	Open reading frame
OT30	Oxytetracycline
P10	Penicillin
PAI	Pathogenicity Islands
PBS	Penicillin-binding protein
PCR	Polymerase chain reaction
PDR	Pan drug resistance
REP-PCR	Repetitive element sequenced base PCR
PFGE	Pulsed field gel electrophoresis
RAPD	Random amplified polymorphic DNA
QS	Quorum sensing
RFLP	Restriction fragment length polymorphism
ROS	Reactive oxygen species
RGI	Resistance gene identifier
RPA-PCR	Recombinase polymerase amplification PCR
RT-PCR	Real-time polymerase chain reaction
S25	Streptomycin
SH	Spectinomycin
SNP	Single nucleotide polymorphisms
SXT25	Sulfamethoxazole-trimethoprim

STEC	Shiga toxin producing *E. coli*
STEAEC	Shiga-Toxin Producing Enteroaggregative *E. coli*
TB	Tuberculosis
TD	Tag-mention
TDA	Tryptophan deaminase
TE	Transposable element (TE)
UB30	Flumequine
UPEC	Uropathogenic *E. coli*
URE	Urease
UTI	Urinary tract infection
VIM	Verona Integron-encoded
VP	Voges-Proskauer test
WGS	Whole-genome sequencing
WHO	World health organization
XDR	Extensively drug-resistant

Abstract

Global propagation of multidrug-resistant (MDR) *Escherichia coli* in food chains is a threat not only to animal health but also to human health. Some advanced countries have devised policies for antimicrobial use not only for humans but also for animal health treatment purposes. The poultry industry is an important component of Pakistan's gross domestic product. However, the Pakistani poultry industry faces several environmental conditions that threaten continued economic output, livestock health, and human health. One of these emerging threats is MDR *E. coli*. Until 2016 there was no policy in Pakistan regarding antimicrobial use or contaminant of antimicrobial resistance. Besides, public awareness regarding the presence of MDR pathogens in the food chain is scant.

In this study total of 1,219 liver samples were collected from the National Reference Laboratory for Poultry Diseases in Pakistan via federal and provincial sentinel surveillance laboratories under a national surveillance program from 2015 to 2017. In which 511, liver samples were taken from culled layer and broiler chickens having poor birth growth and reduced appetite but not otherwise symptomatic for colibacillosis. Of which 265 isolations were identified as *E. coli* which were tested initially for antimicrobial resistance (AMR) against 21 priority antibiotics used in poultry in Pakistan. Out of 265 isolates, 105 were further checked against the extended spectrum of 31 antibiotics and analyzed through Next Generation Sequencing.

The pattern of antibiotics resistance explains that commercial isolated *E. coli* has shown a higher percentage of resistance to penicillin, amoxicillin, lincomycin, oxytetracycline, doxycycline, erythromycin, sulfa-methazole-trimethoprim, spectinomycin, and nitrofurantoin as compared to the backyard poultry (domestic chicken), isolated *E. coli*. Moreover, the isolates from both commercial and backyard poultry have shown similar resistance patterns to two major groups of antibiotics, aminoglycosides (neomycin, gentamycin, streptomycin) and fluoroquinolones (flumequine, enrofloxacin, and norfloxacin, ciprofloxacin). However, some very important antibiotics like ceftiofur (thirdgeneration cephalosporin), colistin, and carbapenem were the least resistant drugs in both commercial and backyard poultry. The presence of colistin-resistant bacteria in chickens indicates a danger to public health in Pakistan. An Illumina whole-genome sequencing was performed on 92 *E. coli* isolates. Our analysis indicates that the isolates were predominantly from B1 and A clade. They harbour a diverse number of antibiotic resistance and virulence genes with no connection between phylogeny and antibiotic gene presence. However, some connection was found between phylogeny virulence gene, and SNP (single nucleotide polymorphism). Importantly, most of our isolates can be

classified as multidrug-resistant with one displaying extended drug resistance. These results provide the highest resolution analysis from poultry-associated *E. coli* isolates in an area with a high endemic burden of antibiotic resistance. The isolated *mcr-1* bearing strains *E. coli* 79 was used to transform colistin-resistant gene to the donor *E. coli* J53, via conjugation. *E. coli* 79 isolate was used as donors. While *E. coli* J53 (resistant to sodium-azide) was used as the recipient strain. Plasmid (*mcr1_Inc12*) was confirmed in our several strains by NGS data using Plasmid-finder.

Prominently, the findings of the study depict that out of 92 strains 82 were found to be MDR. One strain was reported as extensively drug-resistant (XDR) and 13 were *mcr-1* positive *E. coli*. In Multiple locus Sequence Typing (MLST) data analysis, nine strains of serotype ST 117 *E. coli* (09/92) reported were from phylogroup B2. More interestingly two of our isolated strains EC-83 and EC-35-B have *blaCTX-M-15* genes. However, in our study 12 of our isolates carried *blaCTX-M-15* is the second most common after *blaT-EM-1B* in the Extended-spectrum β-lactamase (ESBL) family. Furthermore, 08 sequence types (ST117, ST1011, ST2847, ST533, ST1324, ST2973, ST155, ST4516) have *mcr-1* genes. One of the very interesting results in the study was the EC-60 strain, of ST131 serotype is found in many infections worldwide. The strain ST131 is *blaCTX-M-1* gene having *IncFIB* (AP001918), *IncFIC* (F11), and *Incl* 1 plasmid replicons were reported in this strain. Overall, it is the first study from Pakistan that provide detailed information of antibiotic resistance pattern and genetic attributes of avian MDR *E. coli*. However, the limitation of the study was that with short Illumina reads, we were unable to unequivocally implicate ARGs or virulence genes present on mobilizable plasmids. Additionally, we do not have access to a chicken model of infection to demonstrate links between comparative pathogenicity of the strains and their virulence gene mosaic.

1.0 Introduction

The poultry industry has shown significant growth worldwide over the last several years. The growth of the poultry industry is faster than other livestock industries (Yegani & Korver, 2008; Windhorst, 2006). The poultry industry is playing a significant role to lower the magnitude of food shortage by providing high-quality animal proteins all over the world. Chicken meat is one-third of the total meat produced and consumed globally (Windhorst, 2006, Burlingame, 2004). The poultry industry is exposed to various stressful conditions including diseases and challenging environmental conditions. Consequently, extensive veterinary medicines including antibiotics are employed for the protection and cure of poultry diseases (Trafalska & Grzybowska, 2004, Fair & Tor, 2014). To investigate the mechanisms of drug resistance used by these multidrug-resistant bacteria, molecular and bioinformatics approaches are broadly in use (Hert *et al*., 2008). The above sector is vulnerable to high economic loss because of the outbreak of MDR bacteria. This can be regulated by identifying the exact mechanism of resistance in bacteria (Alexandratos & Bruinsma, 2012). Mobile plasmid genes can easily spread through a bacterial population and affect normal flora. Therefore, genomic profiling is increasing our understating and spreading of the wide range of these plasmids (Lesser, 2018).

The United States of America is a prominent chicken meat producer worldwide. It accounts to produce 19.3 million tons of chicken meat alone (Lesser, 2018). However, Brazil was the leading chicken meat exporter with 3.6 million tons in 2018. Chicken meat is not only an important source of nutrition but is also an economical source of animal protein for low-income countries (Lesser, 2018). The poultry industry is an important component of Pakistan's gross domestic product (Hussain *et al*., 2015a). In Pakistan, poultry is the most dynamic sector of livestock providing 1.5 million jobs all over the country. Its current investment has crossed Rs 700 billion. Also, poultry meat is 26.8% of the total meat consumed in the country. In the year 2016-17 the commercial layers, breeders, broiler stock, and rural poultry were developed by 7%,5.0%,10%, and 1.5% respectively and it contributes to the growth of GDP by 1.3% (Wing& Finance, 2008; Tahir *et al*., 2018).

In humans, 70% of enteric infections are caused by foodborne pathogens. Therefore, it is the 5th largest cause of death worldwide (Martens & Demain, 2017b). Every year more than 1500 million cases of diarrhea are reported of which 2.2 million die due to these infectious pathogens (Martens & Demain, 2017b).

Several bacteria, fungi, and viruses are involved in these infections. But most of them are bacteria specifically *E. coli* strains are involved in these diseases (Martens & Demain, 2017b; Singh & Barrett, 2006; Davies, 2007). Similarly, the Pakistan poultry industry has been facing several problems that affect economic production, human and livestock health (Hussain *et al*., 2015a). One of these emerging threats is avian pathogenic *Escherichia coli* (APEC) (Manges, 2016b). This animal pathogen is particularly relevant to human health, as *E. coli* can cause a diverse array of infections. It is often antibiotic-resistant through horizontally acquired antibiotic resistance genes (ARGs) (Croxen & Finlay, 2010). Given poultry susceptibility to APEC, several studies have been performed to analyze the pathogenicity of APEC and to identify virulence genes *ibeA*, *iss*, *sitA*, and *iroN* (Mellata *et al*., 2003; Sarowska *et al*., 2019).

Antibiotic resistance to all antibiotics classes is rapidly increasing. The seriousness of the issue (Antibiotic resistance) can be assessed from the fact that it has been given equal importance as given to terrorism and global warming (Brown, 2015, Rice, 2008). The report of "Monitoring Antimicrobial Resistance Trends" (SMART) in 2007-08 shown that in Latin America and Asian Pacific countries 30 percent of *Klebsiella* and 40 percent of *E. coli* species were ESBL positive extracted from patients with abdominal infections (Guembe *et al*., 2008). Millions of people die from bacterial infections worldwide every year. Two million persons in the United States per year are reported to be affected by MDR (multidrug-resistant bacteria) bacterial infections (Prestinaci *et al*., 2015b). Out of the total number, twenty-three thousand people succumb to these MDR infections. Similarly, twenty-five thousand people lose their lives in Europe to bacteria resistant to antibiotics (Martens & Demain, 2017b; Butler & Buss, 2006; Prestinaci *et al*., 2015b; Fischbach & Walsh, 2009). Along with, In-built resistance microbes also get antibiotic resistance genes from their environment. Hence, several mechanisms are being adopted by bacteria such as efficient efflux machinery, a mutation in genome or protein, and inactivation by enzymes to overcome the effects of antibiotics (Ajaiyeoba *et al*., 1992; Fernandez & Hancock, 2012).

To investigate these MDR bacteria, molecular analysis is done at large by reference as well as research laboratories. Some of the techniques like hybridization and polymerase chain reaction (PCR) are in practice for a long. However, a technique like complete genome sequencing is a new and advanced method of studying AMR (Antimicrobial-resistant) bacteria (Anjum, 2015; Anjum *et al*., 2017).

There are several applications of NGS and bioinformatics tools that have changed the dynamics of the research and diagnostic field. In the WGS outcome, for example, Resfineder and Card can predict

the possible resistance gene. This is relatively more time-saving and cost-effective than traditional PCR to test many antibiotic resistance genes (Jia *et al*., 2016; Joensen *et al*., 2015). The computational/bioinformatics tools have improved the analysis and storage of large WGS data. This data and information can be saved and accessed using both online software (i.e., Ridom, SeqSphere, BioNumerics) and a personal computer. Thus, NGS technology and bioinformatics have gained great popularity by reducing the response time to emerging disease outbreaks. The socio-economic benefits were enhanced by improving public health and reducing health care costs as well as reducing mortality rate due to illness (Lelieveld *et al*., 2016; Anjum *et al*., 2016; Sharma *et al*., 2016; Bradley *et al*., 2015).

In this study, an attempt is made to classify the MDR *E. coli* from the liver of chicken that had poor birth growth and reduced appetite but not otherwise symptomatic for colibacillosis were collected from Pakistan poultry. Further, a more comprehensive surveillance analysis is needed to get a clear picture of AMR prevalence in our country. Besides, large genomic data of pathogenic and non-pathogenic *E. coli* from humans and animals is needed to evaluate and draw a comprehensive scenario of MDR *E. coli* burden in the country. Certainly, comparative analysis of different MDR species needs to be done in the future.

1.1 *Escherichia coli*

E. coli was first described in 1885 by Theodore Von Escherichia. It was named *E. coli* in 1919 by Castellani and Chalmers (Collier & Sussman; 1998). The bulk of non-pathogenic and commensal species are *E. coli* strains. While others are distinguished by virulence factors and cause intestinal and extraintestinal diseases (Kaper, 2005). *E. coli* are a common enteric disease pathogen and causing from mild to severe digestive diseases in humans. They are harbored within food animals. *E. coli* outbreaks are often related to contaminated meat associated with food animals including beef, cattle, and poultry (Perepelo *et al*., 2007; Callaway *et al*., 2009). In April 2011 the *E. coli* outbreak in Germany was due to contaminated beef by Shiga toxin producing *E. coli* (STEC) O104:H4 strain (Borgatta *et al*., 2012). Broadly, *E. coli* can be categorized into, infectious and non-infectious *E. coli*. While the pathogenic group is further divided into subgroups including, Extraintestinal Pathogenic *E. coli*, Diarrheagenic *E. coli*, Uropathogenic *E. coli*, Avian Pathogenic, Endometrium pathogenic *E. coli*, Enteropathogenic *E. coli*, Enterotoxigenic *E. coli*, Enterohaemorrhagic *E. coli*, Enteroaggregative *E. coli*, Newborn Meningitis Associated *E. coli*, there are some other like Adherent Invasive *E. coli* and Shiga-Toxin Producing Enteroaggregative *E. coli* (Kunert *et al*., 2015).

1.2 Diseases Caused by *E. coli* in Humans

The term acute gastroenteritis also used for diarrheal diseases is characterized by loose stool, vomiting, fever, and abdominal cramps. Which can lead to acute and chronic intestinal complications (Callaway *et al*., 2009). The major bacterial entero-pathogens are NTS (nontyphoidal *Salmonella*), *Shigella*, *Yersinia Campylobacter*, and *E. coli* (Kotloff *et al*., 2017). The BGD (Global burden of diseases) reported in 2015, that worldwide diarrheal diseases have caused 1.3 million fatalities and 2.3 billion illnesses (GBD; 2015). Moreover, 40% of the children less than the age of five died due to diarrheal diseases. While 90% of these cases were reported from Africa and developing countries of South Asia (GBD, 2015, Kotloff, 2013). However, Kotloff reported five groups of *E. coli* are major infects to humans and are involved in diarrheal diseases, ETEC (Enterotoxigenic *E. coli*), Enteropathogenic, STEC (Shiga toxin-producing) is also known as Enterohemorrhagic, Enteroaggregative, and Enteroinvasive (Kotloff *et al*., 2017). Similarly, Ingle reported 185 typical *E. coli* (EPEC) were isolated causing diarrhea from five years old children from four Sub-Saharan Africa countries (Gambia, Mali, Kenya, Mozambique) and three South Asian countries included Pakistan, India, and Bangladesh. Interestingly, 65% of isolates were also immune to antibiotics in three or more drug classes (Ingle *et al*., 2018).

E. coli also causes extra-intestinal infection in humans. UTI or urinary tract infection has been linked to Extraintestinal Pathogenic *E. coli* (EXPEC) is the leading nonintentional infection in humans. In the USA, solely 7 million people need medical visits out of which 1 million reach out for emergency room visits. While 10000 people stay hospitalized for about a year (Manges *et al*., 2016). ExPEC does not cause gut infection, unlike enteric *E. coli*, but persists in the human gut until a chance to cause urinary tract infection e.g., infections acquired through sexual contact or the use of a catheter in a hospital are two examples. Environmental *E. coli* which resembles ExPEC (Genetic/Phenotypic) via virulence characteristic, had been gathered from sewage, household animals, wild animals and waterways, suggesting a variety of other human ExPEC non-human reservoirs (Platell *et al*., 2011; Ewers et., 2010; Dolejska *et al*., 2011; Dhanji, *et al*., 2011).

There are several lineages of *E. coli* that cause extra-intestinal infections in humans (Manges *et al*., 2008). For instance, *E. coli* (O25:H4-ST131) is worldwide considered as 60 percent of all *E. coli* infections are caused by this disseminated strain. *E. coli* O25:H4-ST131 is responsible of 70-78 percent infections are caused by ExPEC, an ESBL-producing fluoroquinolone-resistant pathogen

(Lazarus *et al*., 2014). While other ExPEC includes serotypes ST10, ST12, ST38, ST69, ST73, ST95, ST117, ST127, ST131and ST405. Serotypes can vary by geography (Blanco *et al*., 2011). However, the number of ST types causing UTI were isolated from poultry in British Columbia in a study conducted in 2012-2013 of 114 isolates of *E. coli* sequence type were ST69, ST10, ST95, ST131, ST117 (Manges *et al*., 2016).

1.3 Phylogeny of *E. coli*

The first approach used for epidemiological and genetic studies was Multilocus Enzyme Electrophoresis (MLEE) based on protein (enzyme) and gel electrophoresis instead of DNA (Saghrouni *et al*., 2013). Therefore, the banding mobility of different amino acids reveals the differences in amino acid sequence. However, the low-resolution power due to silent mutation in DNA (didn't change amino acid) decreases to count the micro-evolutionary changes (Saghrouni *et al*., 2013). While multi-locus sequence typing (MLST) is carried out on a theory close to that of MLEE. Where the genetic difference in strains is inferred by comparing electrophoretic mobility of housekeeping enzymes. However, less locus is targeted in MLEE, so the resolution is low. Hence, for high resolution in MLEE, more locus is needed (20 or more) which is more time-consuming and labor-intensive. Therefore, comparatively, MLST is targeting few loci (targeting an internal fragment of seven housekeeping genes) of 400-500 bp (Maiden *et al*., 1998). Both forward and reverse primers were used to sequence each DNA fragment to identify accurate mutations in the seven-housekeeping gene. However, strains have four common alleles that are placed in the same clonal group (Jolley *et al*., 2001, Urwin *et al*., 2003). In MLST the seven genes are *adk, aroE, fumC, gdh, pdhC, pgm*, and *abcZ* (Mayer *et al*., 2002).

The absence and presence of virulence determinants in *E. coli* is the main deference between virulent and non-virulent strains. In 1980 it was reported that the virulence caseates or virulence block located on chromosomes on UTI *E. coli* (UPEC) are different from other *E. coli* strains. A similar finding has been observed in intestinal pathogenic *E. coli* and pathogenic strains of bacteria (Hacker and Kaper, 2000). In 1990 these virulence blocks were called pathogenicity islands (PAI) (Hacker and Kaper, 2000). These PAIs have genes encoding one or more features of virulence (Figure 1).

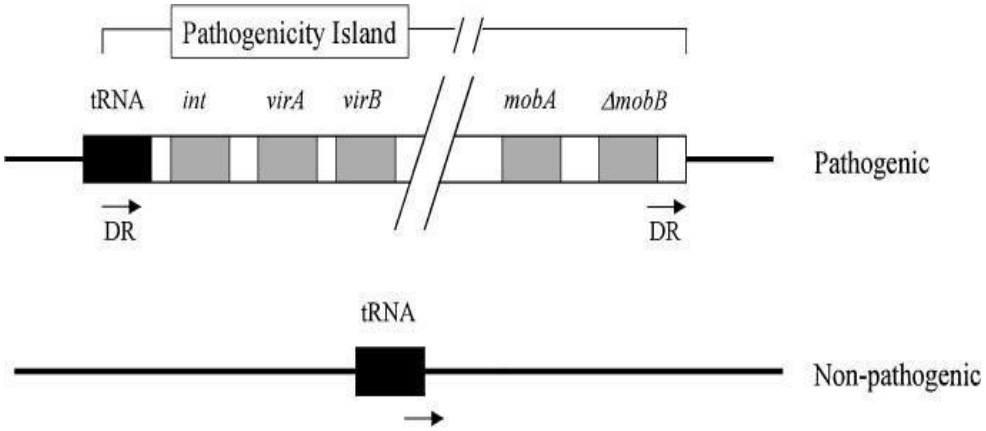


Figure 1 Bacterial pathogenicity island model. Regions of the core genome are represented by a thin bold line, while sequences unique to pathogenicity islands are highlighted. The box represents genes. Direct repeats are indicated by the arrows at the end of soft pathogenicity island. Direct repeats (DR), integrase gene (*int*), virulence-associated gene (*vir*), mobility gene (the mob), and pseudo-mobility gene (1mob). Integrases, transposons, and other enzymes are encoded by mob genes that are elaborate in the prokaryotic genome mobility (Hacker and Kaper, 2000).

1.4 Photo Genetic Grouping of *E. coli* Using Different MLST Techniques

Phylogenetic analysis enlisted *E. coli* in four primary classes (A, B1, B2, and D). It's been seen that commensal strain belongs to group A and B1 whereas the extra-intestinal pathogenic strains and other *E. coli* pathogenic strains fall within the B2 and D categories. (Chakraborty *et al*.,2015, Picard *et al*., 1999). The commensal strain (A and B1) possesses less virulence gene on PAI while phylogroup B2 and D strain possess more pathogenicity islands and virulence factors like adherence, high surface hydrophobicity, factor for biofilm production, siderophore production, and toxin "Hemolysin and CNF1" (Smith *et al*., 2007). A study was conducted by Picard in Paris (France) using a mice model to check the lethality of commensal strains belonging to classes A and B1 while pathogenic strains of B2 and D. The study concluded that strains of carboxylesterase B type (B2 and D) were more pathogenic by producing alpha hemolysin and mannose-resistant hemagglutinin hence, killed the mice, whereas the strains belonged to A and B1 were devoid of these factors (Picard *et al*., 1999). In many studies, Phylogenetic analysis has shown seven *E. coli* groups (A, B1, B2, C, D, E) by using the MLST Pasteur Scheme for seven genes *dinB, icdA, pabB, palB, putP, trpA, and uidA* (Jaureguy *et al*., 2008; Moissenet *et al*.,2010; Clermont *et al*.,2013). The comprehensive MLST data substantially enhanced our knowledge of the substructure of *E. coli*. The new phylogroup E which was previously unassigned strains (Including O157:H7), now it is well-recognized (Tenaillon *et al*., 2010). The wide spread MLST data sets and, to some degree, the

growing body of genome data have enhanced our understanding of the *E. coli* substructure. A phylogroup F has also been reported and it has been found to consist of strains forming a sister group to phylogroup B2 (Jaureguy *et al*., 2008; Clermont *et al*., 2011a).

A phylogenetic method was introduced in 2000 by Clermont *et al* to classify *E. coli* in four groups A, B1, B2, and D via the identification of three genes *chuA, yjaA*, and *TspE4.C22*, using Triplex PCR assay (Clermont *et al*., 2011a). The *E. coli* strains will be classified by the existence and lack of the three genes in one of these four phylogenetic classes. Being a cheap and simple method, the triplex PCR method has been extensively used for assigning the isolates of *E. coli* into four groups. It has also been evident that these strains in different groups have their unique genotypic and phenotypic, characteristics based on their habitat, environment, and pathogenicity (Tenaillon *et al*., 2010; Alm *et al*., 2011). However, *E. coli* MLST data from various hosts and environments succeeded to classify 80-85% of *E. coli* strains into four Clermont described phylogroups. Whereas a small number of strains were incorrectly assigned by using this method (Clermont *et al*., 2013). Consequently, in 2013 Clermont and fellows added another gene (*arpA*) in the already three marker genes as a modification of the triplex PCR method. This modified method was known as the Quadruplex PCR method and was used to arrange the *E. coli* strains into eight phylogroups A, B1, B2, C, D, E, F, and clade I, instead of four phylogroups (Clermont *et al*., 2013).

1.5 Serotyping Methods

Microorganism genotyping has played an important role in determining the pathogenic organisms' worldwide evolution by reviewing the genetic relationship through conducting epidemiological research, to determine their point source (Sharma-Kuinkel *et al*., 2014). In nucleotide profiling, restriction fragment length polymorphism (RFLP) has the method employed. In RFLP, molecular scissors are employed for DNA digestion. As result different lengths, fragments have been generated and used for the analysis of gel electrophoresis (Araujo & Sampaio-Maia, 2018, Smith, Hitchcock, Evans, Lacey, & Adams, 1989). Variations in length are due to deletion or alteration of the DNA sequence in the restriction site. Insertions and deletions between the recognition site of DNA are also held responsible for the variations (Smith et al., 1989). This approach is simple, cost-effective, and quick. Which demonstrates the discriminatory impact of local epidemiology. The only drawback of this method is the lack of reproducibility due to which it is not recommended and used for genotyping (Alanio *et al*., 2017; Araujo, 2014; Saghrouni *et al*., 2013).

1.6 Genotypic Typing Methods

In the 1980s, the pulsed-field gel electrophoresis (PFGE) revolutionized the eukaryotic genome analysis (Saghrouni *et al*., 2013). However, like RPLF, the PFGE also lacks reproducibility due to which it is not recommended for genotyping as well. In this process, a matrix of agarose gel separates large chromosomal DNA molecules. The gel periodically changes direction when an electric field is applied (Saghrouni *et al*., 2013). Conversely, in outbreak investigations, PFGE is the most used method for bacterial strain typing and is considered gold stranded for strain typing around the world (Dingle *et al*., 2015). The chromosomal DNA in PFGE is extracted by lysing bacterial cells embedded in an agarose plug to prevent mechanical sharing of DNA extraction. Then DNA fragments are formed with a restriction enzyme (SmaI). At last, the fragments of 80-800 kb are separated by alternating electric fields within the different pairs of electrodes. However, DNAs reorient to the anode through gel pore at different times that is inversely proportional to the size of fragments of DNA. However, it produces a quality resolution of mega DNA fragments on agarose gel (Reed *et al*., 2007). Finally, the agarose gel image generated PFGE pattern was studied through a system developed by Tenover *et al* using BioNumerics Software. It works as virtual barcodes (PFGE patterns) to identify the types of strains and to find the proximity of association among the different isolates (Tenover *et al*., 1995). Tenover *et al*, reported that PFGE is a laborious method and expensive instrumentation is required for its running. Therefore, it is confined to public health laboratories like CDC. Comparing PFGE bands is a challenging task but still, it is an important epidemiologic tool for disease outbreak investigation (Tenover *et al*., 1995). Later, Polymerase chain reaction-based methods were 78 for genotyping. Random amplified polymorphic DNA (RAPD) is a process based on PCR that amplifies large DNA sequences randomly by using many small primers about 10 bp in low stringency conditions. After loading on the acrylamide gel, different bands are obtained that vary amongst different isolates. This is a simple, easy to operate, and cost-effective method. Moreover, prior knowledge of DNA sequences is also not required in this method (Saghrouni *et al*., 2013). Nevertheless, along with poor reproducibility in the similar research lab, RAPD unable to identify unintended mixtures, is not a digitization move, and is stored in a database for further comparisons (Alanio *et al*., 2017). Base on the random process with unknown DNA sequences, the use of the RPAD method is limited for taxonomic and ploidy analysis (Guillamon & Barrio, 2017; Hryncewicz-Gwozdz *et al*., 2011). RAPD is still complementary to other approaches, such as the initial evaluation of the degree of polymorphism (Guillamon & Barrio, 2017).

As another multi-locus PCR technique, amplified fragment length polymorphism (AFLP) is used. It also doesn't require sequence knowledge (Saghrouni *et al*., 2013). Unlike previous methods, AFLP cut the DNA using two molecular scissors by using two PCR steps with unmarked primer in the first step/reaction and labeled primer in the 2nd reaction. Moreover, high stringency condition is maintained. This is more reproducible than RAPD (Saghrouni *et al*., 2013; Vos *et al*., 1995). However, an extra number of variables, the requirement of experienced technicians, and laborious methods are the disadvantages of this technique. Due to which it's not widely used for genotyping. However, AFLP was used for fungal genotyping like *Aspergillus Candida*, Fusarium, and Alternaria (Duarte-Escalante *et al*., 2013; Farahyar *et al*., 2013; Singh *et al*., 2015). Similarly, REP-PCR (repetitive sequence-based PCR) uses primers that target noncoding regions in the fungal genome and produce genomic fingerprints (Redkar *et al*., 1996). The amplified target region by rep-PCR is analyzed through electrophoresis. The main benefit of this approach is the ability to build a database using fingerprint patterns. Due to its' convenient nature, this technique is used in several genotyping studies (Pounder, Hansen, f& Woods, 2006; Sacheli *et al*., 2016). Due to low discriminatory problems, this method is more commonly used in combination with other techniques such as microsatellite for genotyping studies (Araujo & Sampaio-Maia, 2018).

1.7 Phenotypic Typing Method

Phenotypic typing includes color, colony morphology, odor, and other microrheological characteristics of microorganisms. All these phenotypic methods require strict experimental conditions. As these characteristics are more susceptible to environmental conditions. Serotyping has been the primary phenotypic approach used since the early days of microbiology (Van Belkum *et al*., 2007). In this typing different food-borne pathogenic bacteria are differentiated based on surface variable antigenic structures. These variables can be described by a series of immunological tests in which bacterial cells are combined to produce agglutination with antisera. The word serotypes or serovars is used for subtypes derived from these serological tests. The serotyping started in 1930, has been greatly useful because of host specificity, virulence factors, and pathogenicity as an associate with specific serotypes. As a result, the scheme makes easy for the health workers, scientist, and general public to identify and a prerequisite for surveillance and monitoring scheme (Uelze *et al*., 2020). Moreover, 2600 *Salmonella* and 190 *E. coli* serotypes were found (Grimont *et al*., 2007; Fratamico *et al*., 2016). While 54 serotypes of *Shigella spp* are also identified (Muthuirulandi *et al*., 2017). However, the o antigen and H antigen is the

flagella surface protein. Whereas only the O antigen is of concern for *Shigella* serotyping and capsule polysaccharide (CPS) is for *C. jejuni* serotyping (Pike *et al*., 2013). This is a comprehensive system for typing *E. coli* and *Salmonella* isolates in which most typing sera react with surface antigen. This system of serotyping is still used in food-related and health care laboratories. In this procedure, several defined sets of monoclonal and polyclonal antibodies are available (Frasch *et al*., 1994; Van Belkum *et al*., 2007). The method of serotyping can be reproducible with wide applicability. In which standardization of preparation and testing conditions is crucial. By combining serotyping with SDS-PAGE, which results in 'western' (Immuno) blotting, the problem of discrimination can often be improved (Tenover *et al*., 1994). However, some serotyping schemes used for *E. coli*, (from many years the O serogroup had been used to distinguish pathogenic from commensal *E. coli*) in which the O antigen is used composed of repeated subunits present on the surface of *E. coli*. Similarly, the flagellar antigen H is used in this serology scheme (Wolf *et al*. 1997) and M protein typing of *Streptococcus pyogenes* (Stanley *et al*., 1993) are replaced by their genotypic equivalents. In which the variation is found based on genes encoding the antigens (Nowakowska *et al*., 2006). Similarly, the discovery of the amplified cluster of O-antigen genes (molecular serotyping) appears to be a successful alternative to classical serotyping of *Shigella* and *E. coli spp* (Coimbra *et al*., 2000).

1.8 MLST Schemes

EnteroBase is a database that performs genomic assemblies from sequencing. It reads either submitted directly by the user or through the public domain (ENA short read archives) (Alikhan *et al*., 2018). EnteroBase is a web-based platform and contains legacy MLST genotypes as well as ribosomal gene (rMLST), core genome MLST (cgMLST), and whole-genome MLST (wgMLST) (Alikhan *et al*., 2018). This database also presents graphical visualization of all the above genotypes including SNPs (single nucleotide polymorphisms). Therefore, genomic genotyping with the help of a web database like EnteroBase can be employed to sort out all types of diversity within *E. coli*, *Salmonella*, *Shigella*, and other bacteria (Alikhan *et al*., 2018). EnteroBase offers a one-stop solution, instead of using many methods to deal with microbial diversity from genus to all way down to epidemiological tracing. Just like it provides comparable studies among different genera which it supports (*Escherichia*, *Yersinia*, and *Clostridium*) (http://enterobase.warwick.ac.uk). While within genera EnteroBase platform allows users to check relativity in the newly sequenced

genome within the framework of currently sequenced global diversity with more than 100,000 *Salmonella* genomes and 50,000 *Escherichia* genomes (Alikhan *et al*., 2018).

1.9 Three MLST Schemes

The three MLST Schemes websites are hosted by Warwick Medical School UK, Michigan State University USA, and Pasteur Institute France. Although, the three MLST schemes for *E. coli* have different combinations of genes (shown in table) all are housekeeping genes in which only one gene *icd* is common. The EcMLST created by Thomas Whittam was for EPEC enteropathogenic *E. coli* (Reid *et al*., 2000). While the two most common MLST schemes used in genotyping analysis were created by Mark Acthman also called the Acthman Warwick scheme (Wirth *et al*., 2006). Pasteur scheme developed by Sylvain Brisse and Erick Denamur which is hosted by the Pasteur Institute (Paris, France). However, in 2012 Kaas *et al* reported that nucleotide diversity was highest in the Pasteur MLST gene followed by the Michigan scheme genes and lowest for Warwick scheme MLST. However, Sahl *et al*, observed that the best Phylogeny can be obtained by using the Achtman Warwick scheme (Sahl *et al*., 2012). Besides these MLST schemes, other schemes were also developed using fever genes “Two locus approach" instead of using seven genes Achtman and Pasteur schemes. In this two genes or locus *fumC/fimH* are targeted (this process is also known as CH typing). It gives the excellent result of MLST based profiles (Weissman *et al*., 2012). Clermont *et al* reported that CH typing (Two locus scheme) shown excellent discriminative power among the data of different *E. coli* strain and gives more haplotypes than the Warwick seven-locus approach (Clermont & Denamur, 2015). Moreover, *E. coli* ST131 subtyping was consistent with the Pasteur scheme (Tchesnokova *et al*., 2013).

1.9.1 ECOR Collection

In 1983 Ochman and Selander assembled 72 reference *E. coli* strains from their isolated collection of 2600 *E. coli* isolated from the different environments (Ochman & Selander, 1984). This collection of reference strains is called the ECOR collection. These strains are representative of genetic diversity using the pioneer MLEE (Multilocus enzyme electrophoresis) serotyping method (Clermont & Denamur, 2015).

1.10 *E. coli* Genome

Escherichia coli is a prominent model organism in the laboratory and a widely studied bacterium. The bacteria are involved in bacterial conjugation, finding phage genetics,

recombination, and genetic regulations, and chromosomal replications (Hobman *et al*., 2007). Especially, the K-12 strains of *E. coli* were isolated in Palo Alto in 1922 from diphtheria patient feces. Since food animals and humans carry a large number of *E. coli* which makes possible antagonistic or commensal interactions with their hosts (Hobman *et al*., 2007, Bachmann *et al*., 1996). Therefore, both genetic and population determinants that shifted the commensal strains to pathogenic strains are necessary to determine. However, population genetics research based on MLEE and different gene markers recognized four major groups of *E. coli* "A, B1, D, and B2" (Gordon *et al*., 2008). The six sequenced *E. coli* contains an average genome size of 4.7- 5.2 Mb. In which 4627- 5129 protein-encoding genes. Moreover, the G+C content is 50.8% (Touchon *et al*., 2009).

1.10.1 Pan-genome and Core genome in *E. coli*

Bacterial sequencing has shown a broad variety of genomes of related strains in respect of size and gene content. In the study, sequencing three strains of *E. coli* have only 39% common set of genes (Welch *et al*., 2002). Therefore, to describe bacterial species using their genomes, the pan-genome approach has been used. The total complement of genetic factor in all the sequencing strains of species and genera, or other large groups is called pan-genome (Gordienko *et al*., 2013). The pangenome has been divided into three parts 1: The universal genome also called core genome consists of genes that are similar for all gather strains 2: strain-specific genes are found in a genome known as OR Fans, and 3: the periphery genome or the genetic factor in all the subgroups of strains (Lapierre *et al*., 2009, Rouli *et al*., 2015). Moreover, the pan-genome of *E. coli* is open such as not saturated like the closed pan-genome e.g., Bacillus anthracis pan-genome that remains unchanged after accounting for the first four genomes (Gordienko *et al*., 2013).

Therefore *S. enterica* and *E. coli* are close relatives. It has been assumed by the protein clock model that both these species share a common ancestor about 100 million years back (Gordienko *et al*., 2013). However, MLST data suggests that distinct *Shigella spp, S. boydii, S. dysenteriae, S. flexneri, and S. sonnei* are virulent *E. coli* that instigated independently 35,000 to 270,000 years ago (Pupo *et al*., 2000). Overall, Shigella spp. share phenotypic characteristics, such as being nonmotile, obligate pathogens, and having comparable metabolisms, which have been used as the foundation for first classifying them as a distinct genus (Gordienko *et al*., 2013).

The investigation of the first E. coli strain has altered knowledge of the evolution of gene repertoires in bacteria. analysis of the first *E. coli*. Variation in size of the genomes within the species was

observed as more than 1 Mb or even the gene repertoires of same size genomes vary (Welch *et al*., 2002, Willenbrock., 2007). Therefore, it was planned to examine the evolution of the gene repertoire in light of the extraordinary availability of 20 fully sequenced genomes of the same species. The initial step was to identify the *E. coli* core and pan-genomes, "the genes found in every genome" and the whole collection of non-orthologous genes across all genomes. The 4721 genes made up the average E. coli genome, in which 1976 genes made up the core genome, and 17 838 genes made up the pan-genome. There is only a 42% chance that a ubiquitous gene will be discovered by randomly selecting one gene from an *E. coli* genome. However, only one-fourth of the pan-genome found in an *E. coli* strain may be detected using complete sequencing. This suggests that no one strain can be regarded as particularly typical of the species, despite the fact that several essential processes may be examined well using a model strain. (Touchon *et al*., 2009). Although further *E. coli* genome samples are unlikely to dramatically alter estimates of the core genome, the pan-genome is still far from having been fully revealed. Core and pan-genome size estimates may be impacted by annotation and sequencing artifacts, such as falsely annotating insignificant genes or pseudogenes. We believe that by utilizing a consistent set of annotations, we have reduced such issues. However, we discovered that 40 genes in E. coli K-12 W3110 were classified as essential (Baba *et al*., 2006).

The sequencing of genomes was completed after an average of 12 times of coverage. The 6 newly sequenced *E. coli* chromosomes range in size from 4.7 Mb to 5.2 Mb, or 4627 to 5129 protein-coding genes which is somewhat more than the average value across the 20 genomes that we studied. With 4.6 Mb and 4500 protein-coding genes, the chromosome of *E. fergusonii* is substantially smaller. The G+C content is fairly comparable and near to the E. coli K-12 MG1655 value (50.8%) among the 6 strains. *E. fergusonii* has a lower 49.9% G+C concentration. Similar quantities of coding genes and stable RNA genes are present on these chromosomes. By contrast, the number of pseudogenes varies more widely, from 22 in *E. fergusonii* to 95 in strain ED1a (Touchon *et al*., 2009).

1.11 Hypothesis of the study

Multidrug resistant *E. coli can* be a real threat to the health of animals and Humans. Such as high morbidity and motility can be expected through these different MDR *E. coli* strains in poultry. Finally, these *E. coli* strains have plasmids having many ARGs gene that could accelerate the spread of antibiotic resistant genes to the pathogenic and non-pathogenic strains through food chain via horizontal gene transfer. Therefore, the detail investigation of these MDR *E. coli* could be helpful to combat this problem in Pakistan poultry.

1.11 Aim and Objectives

The present study was designed to find the MDR profile of *E. coli* strains in the local scenario of Pakistan from commercial and backyard poultry. Typically, it will focus on identifying different strains of *E. coli* commonly found in chicken liver samples of culled layer and broiler chickens having poor birth growth and reduced appetite. Moreover, the transmission potential of the AMR gene of *E. coli* would also be studied.

The main objectives of the study are.

- Samples will be isolated from the chicken liver of culled layer and broiler chickens having poor birth growth and reduced appetite and characterization of *E. coli* will be done through biochemical and molecular tools.
- Antibiotic susceptibility of different isolates of *E. coli*
- Determination of MDR and virulence genes
- Computational profiling identification and type of multidrug resistance genes in Avian *E. coli*.
- Phylogenetic classification through Roary and FastTree. Evaluation of transmission potential of MDR genes in *E. coli* through conjugation assay

2.0 Literature Review

2.1 Poultry Industry, International and Local Market

There has always been a constant increase in demand for the ever-growing human population to provide food with high-quality meat. The response to these demands is evident in massive industrialization observed in the livestock sector, particularly in the last two decades. Poultry production has been raised globally, and specifically in Asian countries, due to the usage of very cheap and economical feed (Harder *et al.*, 2016, Wu *et al.*, 2014, Leibler *et al.*, 2009). This growth of the livestock sector will be continuously increasing. In 2050, the human population is estimated to exceed 9.6 billion, where more than seventy percent of the world population will be living in cities. However, the income will increase by only 2 % per annum. Therefore, with such a huge vast population burden, the demand for food will increase up to 70 % by the end of 2050. The highest contributor will be poultry meat 121%, beef 66 %, and pork 43 % (Alexandratos & Bruinsma, 2012). The poultry production is mainly divided into broilers, layers, and the backyard (Gerber *et al.*, 2013). Up to 81 million tons of eggs and 120 million tons of chicken meat are produced globally. The statistical data shows that 96 % of meat comes from broilers, only 6 % from layers, and 8 % from backyard poultry. (Alston *et al.*, 2009, FAO, 2016, Carvalho, 2017).

In a country like Pakistan, the demand and supply gap of animal protein was highly reduced by the poultry industry. In Pakistan, the industry was launched back in 1960; it showed tremendous growth within the few years of its implantation. In Pakistan, poultry is the largest industry after the textile industry. It contributes more than 26.8 % of the meat produced in the country and 1.3 % of the country's total GDP. While more than 1500 thousand people are employed in this industry (Hussain *et al*., 2015a). The poultry industry has increased its share in meat production and consumption in the country. In 2010, poultry meat consumption was increased up to 26 % of the total meat pool in the country, and beef was reduced to 55 %. Similarly, mutton was reduced to 20 %, which was 2-25 % of chicken meat, beef 61 %, and mutton 37 % in the year 1971 (Hussain *et al*., 2015a).

2.2 Poultry Industry Challenges

The poultry industry is a crucial economic sector in many nations. However, the industry is facing several problems, including the rearing of facilities. Besides, diseases and worsening of environmental conditions have led to high financial problems, affecting the poultry industry immensely (Trafalska & Grzybowska, 2004). A large number of veterinary medicines are being used for the cure of

poultry diseases. However, the substantial use of antibiotics has been questioned, which has given extensive credentials to the evolution of MDR bacteria. Therefore, the use of antibiotics both for therapeutic and growth promoters in poultry has left severe concerns for humans and animals (Trafalska & Grzybowska, 2004).

2.3 Poultry Diseases

Like other animals, chicken also obtains their food through the intestinal tract and has the protective mechanism to protect the body from various pathogens. However, sometimes, these protective measures are bypassed by the pathogens like viruses, bacteria, and other infectious and non-infectious organisms (Naeem *et al.*, 2003). As a result, they cause many diseases in these animals**.** Some of the prevalent poultry diseases are Colibacillosis, infectious coryza, Enteritis, Owl pox, Avian influenza, Infectious bronchitis, Salmonellosis, Coccidiosis, Hydropericardium syndrome, and Newcastle (ND) diseases (Abbas *et al*., 2015, Javed *et al.*, 1994, Naeem *et al.*, 2003).

These diseases have adverse economic effects on the poultry industry. For instance, China lost 4.5 billion dollars due to the bird flu virus H5N1 attack in 2004 and 2005 (Nishiguchi *et al.*, 2007). Moreover, transportation and unhygienic utensils are also playing a key role in spreading these poultry diseases. Transport of living birds from one market to another market has also been the fundamental cause of many avian diseases, affecting the poultry industry all around the world. In this regard, the role of association of human movement cannot be ignored in spreading avian diseases between different flocks (Wang *et al.*, 2013, Paul *et al.*, 2011, Nishiguchi *et al.*, 2007).

2.4 Infectious Diseases and Potential Pathogens

Bacterial infections are affecting the bird's health and their yield. Nevertheless, antibiotics and vaccination have overcome the effect of bacterial infections. However, the appearance of MDR bacteria in developed nations has reopened a new chapter, including most of the previously known pathogens with MDR tag. There are many species of bacteria causing diseases in the poultry industry. Some have a very drastic history in terms of their effect on economy and mortality, such as *Salmonella enterica* and *Salmonella gallinarurm,* the causing agent of Fowl typhoid in animals (Wu *et al.*, 2018). Only in Asia, the antibiotic consumption in poultry has increased up to 12 9 % and more than 50 % of antibiotics are used as growth promoters in the poultry industry which corresponds to the use of antibiotics per kg of animal produced of ~148 mg kg^{-1} in chicken production. Therefore, resistance is not only observed in clinical *E. coli* strains but is highly common in avian *E. coli* (Chen *et al.*, 2015, Wu *et al.*, 2018, Van Boeckel *et al.*, 2015b)**.**

Three types of infections are prevalent. They are parasitic, viral, and bacterial.

2.4.1 Parasitic Infections

Many types of parasites and protozoans colonize the chicken gut. Where protozoans are the most common and cause various diseases. Coccidiosis is a familiar parasitic infection in commercial poultry. Its entire life cycle passes in the chicken gut without any other intermediate host. Nevertheless, many other parasites enter via the intermediate host and cause diseases in poultry. Such as nematodes, cestodes, and flukes (Yegani & Korver, 2008). Histomonas meleagridids are one of the prevalent parasitic disorders in birds; the disease is also called a blackhead. This agent is spread through eggs among the birds. Although, it badly affects the turkeys, and the mortality rate reaches 10-20 % accompanied by high morbidity. In many cases, the outbreak of these parasites was unnoticed (Yegani & Korver, 2008, Williams, 2005, McDougald, 1998).

2.4.2 Viral Infections

Viral infections are the real threat to the poultry industry in which several viral strains are involved, such as rotavirus, coronavirus, enterovirus, adenovirus, astrovirus, and reovirus. The deadliest virus is influenza. Type A influenza is responsible for infection in birds. There are 15 different types of combinations based on haemagglutinin (H1±H15) and neuraminidase (NA) that affect birds regularly. Influenza type A virus is divided into two main groups based on the severity of diseases in chickens (Bagust, 2013).

The most virulent type is the HPAI (Highly pathogenic avian influenza) virus, and the less virulent type is LPA (low pathogenicity virus). Both viruses are related to respiratory diseases. The types H5 and H7 are the most virulent type whose mortality sometimes reaches 100 %. From 1959 till 1995, 12 out of 17 HPAI attacked chickens in the USA and spread to other neighboring states. In 1994-1995 the victim of this viral attack was Mexico and Pakistan. (Alexander, 2000). The most common diseases are Newcastle disease, bursal disease, influenza, and bronchitis caused by RNA viruses, while Marek's disease is caused by DNA virus. These are some of the most challenging viral diseases worldwide, which need to be controlled and treated by the poultry industries (Alexander, 2000, Bagust, 2013).

2.4.3 Bacterial Infections

Pathogenic bacteria strongly affect chicken health and production. Therefore, it is also an essential factor of foodborne infections with *Salmonella* and *Campylobacter* species (Meade *et al.*, 2009). *E.*

coli was first reported by Theodore Von Escherichia in 1885, which he named Bacterium *coli* commune. However, later in 1919, it was renamed *Escherichia coli*. Although, a number of these strains are non-pathogenic and popular as commensals bacteria. However, they are also well associated with animal's food chain and human infections. Therefore, leading outbreaks of *E. coli* are mostly related to food in which up to 75 % are related to beef meat only. Additionally, several pathogenic strains have also been reported from chicken products (Minodier, 2011, Hussain *et al*., 2017, Kunert Filho *et al.*, 2015, Callaway *et al.*, 2009). Avian Pathogenic *E. coli* (APEC) is the causing agent of prominent poultry diseases, including pericarditis, airsacculitis, salpingitis, polyserositis, peritonitis, coli septicemia, diarrhea, synovitis, osteomyelitis, and swollen head syndrome. *E. coli* bacillosis is the common term used for all *E. coli* infections. Subsequently, these infections are the reason for enormous economic loss in the poultry industry in terms of severe morbidity, mortality, and retard growth in flocks along with carcass contamination (Nagy *et al.*, 2001, Paixao *et al*., 2016, Ewers *et al.*, 2004, GalMor & Finlay, 2006). The appearance of MDR bacteria and excessive use of antibiotics have reopened a new chapter of antibiotic-resistant bacteria, including all previously known pathogens such as *Clostridium perfringens* and APEC (Schuijffel *et al.*, 2006).

The US broiler business has converted to No Antibiotics Ever (NAE) for the majority of its output due to market demand, regulatory restrictions, and scientific concerns. Antimicrobial growth promoters (AGPs), which were previously used to protect broilers in integrator houses from bacterial infections including Avian Pathogenic *Escherichia coli* (APEC) and *Clostridium perfringens*, are now banned under the no antibiotics ever program. (Fancher et al., 2020). Avian pathogenic *E. coli* causes colibacillosis in broilers, a systemic illness marked by a triad of diseases including pericarditis, perihepatitis, and airsacculitis, all of which lead to septicemia. Subclinical colibacillosis, on the other hand, infects approximately 30% of broiler flocks in the United States. (Dziva & Stevens; 2008)**.** *C. perfringens* is a gram-positive bacterium that causes gangrenous dermatitis and necrotic enteritis in broilers that cost the broiler industry much money. High litter humidity, poor litter consistency, and viral exposure exacerbate gangrenous dermatitis, which is characterized by skin lesions and subcutaneous infection. Haemorrhagic enteritis, high morbidity, and mortality are all symptoms of necrotizing enteritis, which causes annual billion-dollar losses (Skinner et al., 2010, Timbermont et al**.,** 2011). In immunocompromised birds, necrotizing enteritis may occur as a primary infection or secondary infection (Fadly & Nair et al., 2008). Immune suppression induced by viral infections and intestinal erosion caused by coccidia both enhance the risk of NE (McReynolds

et al., 2004). Greater vulnerability to bacterial infections, reduced growth performance, and increased mortality and economic losses are all new problems for broiler production in the NAE system (Fancher et al., 2020**).** The Food and Drug Administration of USA concluded in 2009 that antibiotics could no longer be used for growth promotion. Long term subtherapeutic antibiotic use in food-producing animals has been linked to bacterial resistance growth and spread **(**Asai et al., 2007**)**, which can be spread between humans and animals through direct interaction or the environment **(**Ritter et al., 2019**).** Top retail consumers in the broiler industry announced in 2014 and 2015 that they would only serve antibiotic-free chicken. As of 2019, NAE programs cover more than half of all birds produced in the US (Fanche et al., 2020). The elimination of subtherapeutic AGPs has resulted in poor flock efficiency, reduced daily gain, higher risk of gastrointestinal problems, limited water intake, and significant mortality due to NAE (Ritter et al., 2019, Tabler & Wells,2017). NAE broiler chickens have a 25–50 % higher monthly mortality rate than traditional broiler chickens (Mulder & Zomer, 2017). The mortality rate in NAE broilers is about 4.2 %, compared to 2.9 %in traditional broilers **(**Gaucher et al., 2015). As a result, the increased prevalence of multiple bacterial diseases, including NE and colibacillosis, is related to higher mortality in NAE. the NAE program has a detrimental effect on feed conversion ratio, body weight growth, and GIT health in poultry, resulting in reduced bird performance and net output. (Gaucher et al., 2015, Fanche et al., 2020)

Some pathogens have a drastic history in terms of economic loss and mortality, such as *S. enterica and Salmonella Gallinarurm, the* causing agent of Fowl typhoid in animals. ORT (*Ornithobacterium rhinotracheale*) is another very important gram-negative bacterium. It causes respiratory diseases in poultry, including pneumonia and airsacculitis. While *Pasteurella multocida* causes fowl-cholera, is non-motile gram-negative bacteria (Wigley, 2013, Schuijffel *et al.*, 2005a, Schuijffel *et al.*, 2005b, Schuijffel *et al.*, 2006).

2.5 Molecular Resistance; Growing Trends

Undoubtedly, antibiotics have saved countless lives throughout history and are regarded as the most revolutionary drug in medical history (Yoneyama & Katsumata, 2006, Cohen, 2000). The actinomycete bacteria produce about 75% of the antibiotics. Surprisingly, Streptococcus of the Actinomyces group alone has provided 75% of global antibiotic synthesis (Hamaki *et al.*, 2005, Martens & Demain, 2017b, Ikeda *et al.*, 2003). The antibiotics used in agriculture have a similar makeup to those used in medicine, or nearly so (McEwen & Fedorka-Cray, 2002).

Unfortunately, resistant bacteria are also brought on by the widespread usage of this wonder medication. So, according to medical professionals, "this overuse of antibiotics will bring us back to the pre-antibiotic era." (Liu & Pop, 2009, Ventola, 2015b, Spellberg & Gilbert, 2014, Piddock, 2012).

Antibiotics are generated in large quantities around the world each year and are used to treat humans, animals, and agriculture-related diseases. These antibiotics eliminate the susceptible microbes, leaving the resistant ones. The question of how bacteria become resistant emerges in this situation? (Mellon *et al.*, 2001, Levy & Marshall, 2004b, Levy, 2005). The antibiotic-resistant gene can be resistant against one or a whole family of antimicrobials. Nevertheless, multiple genes can be found resistant against a single antimicrobial in the same organism. Similarly, the resistance approach in bacteria is also varied. Bacteria adopt a stepwise mechanism of mutation in chromosomes to show resistance against antibiotics in the absence of plasmids or transposons (Wang *et al.*, 2001, Levy & Marshall, 2004b, Schneiders *et al.*, 2003).

2.6 The Role of New Antibiotics in Controlling Diseases

In discovering new antibiotics, one of the main obstacles is that many bacteria cannot be cultured in the classical laboratory. But thanks to the recent advancement in cultivation techniques, which made the growth of uncultured bacteria possible. The microfluidic bioreactor is one of them in which 600000 pure actinobacteria can be grown microfluidic environment per hour (Zang *et al*., 2013). This was the step taken by the Nsicons company in 2006 to grow uncultured and unclassified fungi, actinomycetes, or other hard to isolate strains to produce new metabolites. Therefore, the effort was fruitful in the discovery of a new protein synthesis inhibitor orthoformimycin novel compounds (Monciardini *et al*., 2014). However, the combination of different antibiotics is also gaining importance to overcome antibiotic resistance. Several examples influence folate metabolisms, such as the combination of trimethoprim and sulfamethoxazole. Similarly, the combination of amoxicillin with clavulanic acid is an inhibitor of the β-lactamase drug resistance enzyme. They are broad-spectrum antibiotics that are active in suppressing the appearance of antibiotic resistance (Taylor *et al*., 2012). Another, compound aspergillomarasmine A, was successfully trailed in a mouse model against *NDM-1 Klebsiella pneumoniae* to reverse the carbapenem resistance (King *et al* 2014).

For HIV, TB, and bacterial infections, multicomponent compound therapy is widely recognized. In addition, throughout the resistance phase, β-lactam antibiotics and lactamase

inhibitors are viable alternatives. In the near term, new β-lactamase inhibitors like avibactam and tazobactam have been incorporated into a few versions of earlier antibiotic groups or combinations, providing some optimism. Eravacycline5, a next-generation tetracycline, and plazomicin6, a next-generation aminoglycoside with efficacy against Gram-negative pathogens, are two drugs that have entered Phase III trials (King *et al* 2014).

2.7 Intrinsic Resistance

Bacteria may develop resistance to antibiotics and metabolites through horizontal gene sharing, which involves changes in chromosomal genes. The intrinsic resistance of a bacterial species to an antibiotic is the capacity to withstand the antibiotic's effect due to inherent structural or functional characteristics (Chuanchuen *et al*., 2003). Antibiotic resistance is complicated, and it is mediated by several mechanisms. The whole family of β-lactam antibiotics is often linked with resistance to other antibacterial classes in carbapenem resistance. The result is widespread drug resistance, including PDR (Pan drug resistance). The development of carbapenemases or β-lactamase is a unique marker for MDR and extensive drug resistance (XDR) isolates. It is defined by the capacity of carbapenemase-encoding genes to propagate within and between species through non-chromosomal DNA (plasmid) transmission. (Livermore *et al*., 2012, Wang *et al*., 2013). In China, the first report of a plasmid-mediated *mcr-1* gene responsible for the horizontal transfer of resistance to colistin has been published. It was declared when the first colistin-resistant *E. coli* and *Klebsiella pneumoniae* isolates were recovered between the years 2011 and 2014 (Liu *et al*., 2016). Several significant reasons have tilted the balance towards the formation and uncontrollable spread of antimicrobial resistance over the past several decades, including the fast growth of the population and the intervention of new technologies.

In addition to this, many other factors (international traveling, migration, trade, and globalization) also played their role in the spreading of resistance (Stenhem *et al*., 2010). Several DNA (gene) coding areas responsible for intrinsic antibiotic resistance of different kinds, including β-lactams, fluoroquinolones, and aminoglycosides, have been discovered using recent technology. High-throughput screening of mutant libraries of high-density genomes was used to accomplish this. By targeted insertion or random transposable element (TE) mutagenesis, they have been generated in a variety of bacterial species, including *E. coli, Staphylococcus aureus*, and *Pseudomonas aeruginosa.* (Blake *et al*., 2013). In addition to inherent resistance, bacteria may acquire or evolve resistance to antibiotics from the environment. This may be done via a variety

of methods that fall into three categories: first, those that reduce antibiotic intracellular concentrations by exploiting the bacteria's efflux machinery or the antibiotic's low penetration ability. Second, the change or modification of the antibiotic target by genetic mutation or protein modification (enzyme); and third, those that inactivate the antibiotic by enzyme breakdown (hydrolysis) or modification. Each of the processes has been researched during the last several years (Fernández *et al*., 2013 Ajaiyeoba *et al*., 1992).

2.7.1 Acquired Antibiotic Resistance

In both community and nosocomial infections, horizontal gene transfer has played a major role in the development and spread of beta-lactam antibiotic resistance among enteric bacteria. In this scenario, the development of antibiotic-resistant bacteria occurred at the same time that new antibiotic families were introduced. These classes tend to compromise the most successful ones, such as *Pseudomonas aeruginosa*, resistant to beta-lactams and aminoglycosides, which are significant problems for cystic fibrosis patients (Davies *et al*., 2010).

The most popular horizontal gene transfer mechanism is antibiotic resistance via plasmid (Norman *et al*., 2009). The conjugative transmission of the AMR gene is several times higher in nature than that in laboratory conditions (Sorensen *et al*., 2005). However, in some species like *streptococci, meningococci,* the virulence and AMR gene are promiscuous, and the exchange occurs through transformation (Enright *et al*, 2005, Springman *et al*., 2009). While *Acinetobacter spp is* competent in taking naked DNA from the environment (Barbe *et al*., 2004). In all of the above, the role of plasmids, phages, integrons, and other genetic mechanisms is well established in the laboratory in the evolution of antibiotic-resistant bacteria. However, other mechanisms like bacterial cell-cell fusion also play a role in the mixed microbial communities in biofilm (Gillings *et al*., 2009). Experiments show that conjugative transmission frequencies in nature are likely to be several orders of magnitude higher than those seen in laboratories (Sorensen *et al*., 2005). Hall and stokes first discovered integrons in 1987 (Strahilevitz *et al*., 2009). They play a significant role in the transmission and expression of genetic resistance to antibiotics (Gillings *et al*., 2008).

Metagenomic study of bacterial samples from agriculture, hospitals, wastewater have several integrons carrying antibiotic resistance genes, showing the importance of integrons in the evolution of antibiotic resistance (D'Costa *et al*., 2006, Walsh *et al*., 2006). However, the genes already exist in the bacterial genome that generates phenotypic resistance is called intrinsic resistance. Many extrinsic and intrinsic gene activities in bacteria that lead to antibiotic resistance in clinical situations

have been discovered thanks to the availability of genome sequencing in this century. Gene amplification, for example, is a typical genetic pathway of resistance to sulfonamides and trimethoprim (Brochet *et al*., 2006). Therefore, bacteria carrying integrons have the potential to integrate mobile genetic elements (gene cassettes), which correspond to a promoter less open reading frame (ORF) linked with attc a recombination site in the genome (Escudero *et al*. 2015). However, in all integrons class 1, integrons are the well spread and clinically important ones. Integron often found on the plasmid and other mobile genetic elements like transposon class 1 integrons were reported having ampicillin (*Ampr*) trimethoprim (*Tmpr*) and streptomycin-spectinomycin (*Strr -Spcr*), resistance genes (Fluit & Schmitz 2004). Similarly, class 1 integron genes (*intl1*) were found in entering bacterial isolates having *Tmpr, Ampr*, and *Strr* resistant genes. The integrase genes among these strains were very similar (Antelo *et al*., 2018).

2.8 Antibiotic Resistance

Intestinal bacteria such as *E. coli, Shigella, and Salmonella* were the first to develop multiple drug antibiotic resistance in the 1950s and 1960s. Due to this many clinical issues originated throughout the globe, especially in underdeveloped nations (Levy; 2001). Typically, antibiotic-resistant genes are directed towards a particular antibiotic class or kind. It is possible for an organism to collect many genes with the same drug resistance characteristic, resistance mechanisms, like antibiotics themselves, are varied. Successive chromosomal modifications cause bacteria to transition from low-level to high-level resistance in the absence of plasmids and transposons, which typically mediate high-level resistance (Ren *et al*., 2012). This mechanism was central to the development of penicillin and tetracycline become resistant in *Neisseria gonorrhoeae*. Transposons containing highly resistant genes to these medications were subsequently acquired by the organism. Other Enterobacteriaceae, including *E. coli* have becoming more resistant to fluoroquinolones.

Enzyme (topoisomerases) mutations and increased production of membrane proteins that transport medicines out of cells cause this (Schneiders, *et al*., 2003, Wang *et al*., 2001). Though, other ubiquitous bacteria, including *E. coli*, *S. enterica,* and *Klebsiella pneumoniae* are the reason for several human and animal diseases. However, during the past five decades, a strong link has been discovered between the usage of antibiotics to treat these illnesses and the rise of antibiotic resistance (Davies & Davies, 2010). This is particularly evident with the antibiotics in the β-lactam class (containing the beta-lactam ring) and their related inactivating enzymes, β-lactamase. Now, several groups and classes of antibiotics have been described, comprising thousands of β-lactamases-

related resistances. These include, most recently recognized, gene groups and their mutant radiation (Davies & Davies, 2010).

2.9 Prevalence of Extended-Spectrum β-Lactamase Gene in *Enterobacteriaceae*

The most important mechanism of resistance among gram-negative bacteria to the β-lactam family of antibiotics, which involves the production of β-lactamase, is extended spectrum β-lactamases (ESBL) (Hirakata *et al*., 2010). By horizontal gene transfer, ESBLs are usually acquired or spread and confer resistance to the third generation of antibiotics, oxyimino-cephalosporins.

Moreover, some of them are mutant derivatives of proven β-lactamase plasmid-mediated derivatives. TEM/SHV or mobilized from the previous work shows their fast emergence and development under the constant pressure of antimicrobial usage, in addition to the pace of discovery and the capacity to identify these enzymes. Except for the unique genotype *CTX-M-2* from South America, most of the ESBL identified in the 1990s are *TEM/SHV* forms. Five majors *CTX-M* genotype families recognize the acquisition of the β-lactamases genes from various species of Klebsiella. Surveillance data identified elevated levels of *K*. *Pneumonia* strains generating ESBL. The production ranged up to 10 % in Japan and Australia, about 30 % for *K*. *Pneumonia* in Singapore and China. And for *E. coli* ESBL, the percentage was 11 % and 25 % respectively for the two countries (Hirakata *et al*., 2010).

Strains studied from China found *CTX-M-14* carbapenemases as the dominant genotype, which was found in the Far East region and recorded globally (Chanawong *et al*., 2002, Hawkey; 2008). The geographical regions are correlated with genotypes of (*CTX-M*). The type (*CTX-M15*) is the common genotype recorded from India, particularly from the subcontinent. It also has reported ST 131, a very competitive uropathogenic *E. coli* (Ensor *et al*., 2006, Nicolas-Chanoine *et al*., 2008). Moreover, an outbreak of both *E. coli* and *K. Pneumonia* generating *CTX-M-15* from the southern part of China has been identified. Where the genotype had been exceedingly rare before, it suggests the potential for extensive dominant form spread and change (*CTX-M-14*) and (*CTX-M-3*) ESBLs. (Liu *et al*., 2009). Significant changes in ESBL prevalence and types have been recorded in Europe since the end of the 20th century, producing the *CTX-M* genotype strains becoming dominant, mainly the *CTX-M-15* genotype. (Livermore *et al*., 2007). Although in some other parts of the world the isolates producing *CTX-M* remains in the stage of sporadic, most of Asia, Europe, and South America is the stage of endemic prevalence (Lahlaoui *et al*., 2014). According to the most recent data from SMART (Global

Study for Monitoring Antimicrobial Resistance Trends), 40 percent of *E. coli* and 30 percent of *Klebsiella* species identified from individuals in the Asia-Pacific and Latin America region who had abdominal illnesses were ESBL positive (de Microbiología; 2008).

Metallo-lactamases (MBLs) having antibacterial activity against carbapenems have recently emerged, such as the enzyme families VIM and IMP, has put the therapeutic efficacy of this class of antibiotics in jeopardy. Higher synthesis of either AmpC or ESBL, as well as reduced production of porin protein or increased bacterial efflux efficiency, may all lead to carbapenem resistance. (Walsh; 2008, Mena *et al*., 2006). Six of the 33 European nations that participated in According to data from the European Antimicrobial Resistance Surveillance System (EARSS), carbapenem resistance rates of about 0.25 percent in the isolates *of P. aeruginosa* in 2007, with Greece reporting the highest prevalence at 51 percent. Greece likewise has a high incidence of *Klebsiella* resistance (Souli & Giamarellou, 2008). In the third-generation group, pneumoniae was 46 percent for carbapenems, 58 percent for fluoroquinolones, and 63 percent for cephalosporins. Furthermore, it has also been confirmed that the VIM-2 in *Pseudomonas aeruginosa* is the utmost leading MBL and confers the real clinical problem (Souli & Giamarellou, 2008, Walsh, 2008). *VIM-2* type Metallo-β-lactamases have been recorded from over 36 countries across five continents. It was also reported that all antibiotic-containing β-lactam, excluding aztreonam, can be hydrolyzed by the MBL enzyme. VIM-1 represents the other major VIM MBLs that are phylogenetically associated. Furthermore, the related genotypes have become increasingly prevalent in the Enterobacteriaceae family, especially in Mediterranean nations. In 70 % of Brazilian *Pseudomonas aeruginosa* isolates, the gene encoding another mobile carbapenemase enzyme (blaSPM-1) was found. (Walsh, 2008). India has also expanded its usage of carbapenem antibiotics, putting strong selection pressure on the growth of carbapenemase-producing bacteria, including those that generate ESBLs. Japan, on the other hand, was the first nation to report IMP carbapenemase in 1991 (Herbert, *et al*., 2007). Fluoroquinolones bind to the enzymes DNA gyrase and topoisomerase-IV, resulting in bacterial DNA conformation changes during DNA replication and RNA synthesis. Resistance to fluoroquinolone antibiotics has been studied through gradual changes in the coding regions of the gyrase subunits, i.e., *gyrA* and *gyrB* and DNA topoisomerase IV *parC* (Lavilla *et al*., 2008). It has also been discovered that the widely widespread plasmid-encoded aminoglycoside-modifying enzyme (AAC-61- Ib-cr) piperazinyl-modified fluoroquinolone antibiotics ciprofloxacin and norfloxacin. The *qnr* genes also allow a non-chromosomal target protection mechanism. (Cattoir *et al*., 2009).

2.10 *E. coli* Epidemiology

A group of bacteria within the *Enterobacteriaceae* family has a diverse phylogenetic structure of *Escherichia coli* (Clermont *et al*., 2013). Many of them are the animals' normal flora, including humans (Nicolas *et al*., 2014). Nevertheless, certain polygenetic groups (B2 and D) in humans who have UTIs are suffering from extraintestinal infections caused by *E. coli.* including meningitis. (European Centre for Disease Prevention and Control, 2014). The strain of *E. coli*, O25b-ST131, causes urinary tract infections which are highly virulent and resistant strains spreading around the globe (Clermont *et al*., 2009).

There is little concern that antibiotic resistance in bacteria has risen only through the widespread use of antibiotics by humans (Davies and Davies, 2010; Hawkey and Jones, 2009). Bacterial resistance may occur by gene mutation or by sharing the antibiotic resistance gene with a plasmid and other mobile genetic components (integrons). Therefore, many antibiotics failed to treat bacterial infections, and only a few are susceptible to these antibiotic-resistant bacteria. In the recent era, antibiotic resistance is a severe health threat worldwide (Davies *et al*., 2011, Leonard *et al*., 2018). If the current trend continues, these antibiotic-resistant pathogens are expected to cause 10 million deaths per year by 2050 (Leonard *et al*., 2018). One important group in the antibiotic-resistant pathogens is ESBL producing Enterobacteriaceae, a special concern in human medicine, which is a critical priority by World Health Organization to develop new antibiotics against them (Tacconelli *et al*., 2017). In the presence of a wide variety of βlactam antibiotics, including cephalosporins and penicillin cephalosporins, bacteria producing ESBL enzymes may survive (Nordmann *et al*., 2012). Plasmid having ESBL gene, such as *bla*$_{CTX-M}$, can easily spread and often get resistant to multiple antibiotics e.g aminoglycosides, tetracycline, and fluoroquinolones (Johnson *et al*., 2010; Nordmann *et al*., 2012). There are various other ESBL genes, such as *bla*$_{TEM}$ and *bla*$_{SHV}$, but in 80 % of ESBL strains, *bla*$_{CTX-M}$ is the most common genotype in the world, particularly *bla*$_{CTX-M-15}$ (Amos *et al*., 2014). However, the rapid spread of ESBL *bla*$_{CTX-M}$ genotype is alarming to both animal and human health and its resistance to many frontline antibiotics, including third-generation cephalosporin (Collignon *et al*., 2009). Carbapenem is recommended against these bacteria, since 2010, the infections caused by the carbapenems producing strains have risen (European Centre for Disease Prevention and Control, 2016).

According to the "Enteric bacteria foodborne diseases burden epidemiology group" of WHO, there are 31 food diseases that have affected 600 million people and 420,000 casualties worldwide

(WHO, 2015). The highest mortality was reported from Africa (WHO, 2015). Most of these diseases were transmitted to humans by eating contaminated food containing associated microorganisms and chemicals (Kirk *et al*., 2015). However, 79 % of the foodborne diseases are caused by a microorganism, in which the most dominant organism is *Salmonella spp*, *E. coli*, *Campylobacter*, and Norovirus (Getie *et al*., 2019). Therefore, to overcome these bacterial diseases, many antibiotics are used in humans, animals, and agriculture. As a result, antibiotic resistance emerges in developing countries, and the enteric pathogens get resistant to ampicillin, tetracycline, streptomycin sulphadiazine, and chloramphenicol (Okeke *et al*., 2005 Getie *et al*., 2019). Moreover, in enteric pathogens fluoroquinolones is predominately resistant to *E. coli* (Robicsek *et al*., 2006), *Citrobacter freundii* (Cattoir *et al*., 2007) *Salmonella t*yphi (Baker *et al*., 2013), *Shigella flexneri* (Hata *et al*., 2005), *Salmonella enterica* (Yanagi *et al*., 2009). Resistance in these species can be caused by the point mutation of the *qyrA* and parC gene regions deciding the quinolone. Second, plasmid-mediated resistance of *qnr* alleles or *qepA* and *qxABB* quinolone efflux genes (Redgrave *et al*., 2014; Blair *et al*., 2015). *Salmonella* itself is responsible for around 3 billion human and animal infections (Crump *et al*., 2004; Coburn *et al*., 2007).

2.10.1 *E. coli* Infections in Humans

E. coli ST131 has been among high risk in *E. coli* strains. In the USA, 50 % of ESBL producing infections in adults, and 10-20 % of all clinical infections are caused by *E. coli* ST131 (Johnson *et al*., 2016, Banerjee *et al*., 2013). Similarly, the strain ST131-*H30* was also responsible for 44 % of ESBL-producing extraintestinal infections in children and 5.3 % of all other extraintestinal *E. coli* infections in the USA (Miles-Jay *et al*., 2017). However, in other parts of the world, the pandemic lineages of *E. coli* strains circulate in Nigeria, Guinea, South Africa, and Tanzania (Chattaway *et al*, 2016). The other pathogenic *E. coli* causing major outbreaks is EHEC, and its serotype 0157:H7 is the reason for the outbreaks in Australia, Europe and America (Allos *et al*., 2004; Angulo, 2007). In America, the biggest ETEC epidemic occurred in the state of Illinois in 1998, when about 3,300 individuals were thought to have gotten sick after eating meals prepared by infected workers (Beatty *et al*., 2006). EHEC's epidemiology has been a core issue and deemed significant to researchers, particularly in regard to the detection of EHEC serotype O157:H7, though other non-O157 strains have also been the source of several major outbreaks in certain regions, including North America, Australia, and Europe (Allos *et al*., 2004; Angulo, 2007). In South Asia and sub-Saharan Africa, the children are affected from mild to severe diarrhea due to *E. coli* and shigella

(Kotloff *et al*., 2013). Furthermore, diarrhea was the third greatest cause of mortality among five-year-old children in Sub-Saharan Africa, behind malaria and pneumonia, accounting for 12 percent of the projected 3.6 million fatalities in 2013 (Liu *et al*., 2015).

Antimicrobial resistance has been found in *E. coli* from a variety of environments, people in hospitals, and animals. In which many are MDR resistant to three categories of antibiotics. However, prime importance to those resistant to fluoroquinolones, producing ESBL and carbapenemases (Magiorakos *et al*., 2014; WHO., 2014). Moreover, several *E. coli* pathogens were reported from South Asia and Africa producing ESBL and resistant to gentamycin and ciprofloxacin (Lim *et al*., 2016; Mathai *et al*., 2008; Avasthi *et al*., 2011).

The outbreak of gastroenteritis, hemorrhagic colitis in Germany in 2011, was due to *E. coli* 0104:H4 (Buchholz *et al*. 2011; Frank *et al*. 2011). The ST69 *E. coli* was also reported in many parts of the world, causing UTI and BSI. These EXPEC lineages are pandemic, including ST73, ST95, ST69, ST131, and ST393 (Riley, 2014). This small group is responsible for third and a half of all the EXPEC related extraintestinal infections in the world (Riley, 2014; Salipante *et al*., 2015; Yamaji *et al*. 2018b). Poultry meat has been linked with the number of these pandemic EXPEC genotypes including ST10, ST69, ST95, ST117, and ST131 (Hussain *et al*. 2017; Manges 2016; Yamaji *et al*. 2018a). A study of retail meat in northern California showed that ST strains from chicken and turkey meat were identified in 21% of *E. coli* isolates from probable UTI patients. (Yamaji *et al*., 2018a). While, from a total of 1188 clinical and 2452 meat *E. coli*, 76 distinct STs were found in a study conducted in Arizona USA. All of twenty-seven, two were from poultry meat (Liu *et al*. 2018). Furthermore, many ESBL producing *E. coli* strains, including ST10, ST69, and ST131 in the Netherlands, were isolated from both chicken and human samples (Leverstein-van Hall *et al*., 2011). During this period, some ciprofloxacin resistant strains of ST10 were also found in human clinical samples and chicken from Italy (Leverstein-van Hall *et al*., 2011). Similarly, *E. coli* ST10 was the most common *E. coli*, isolated from chicken in the period 0f 2005 -2007 in Canada (Bergeron *et al*. 2012). While 34 % of the STEC *E. coli* types were reported from vegetables contaminated with *E. coli* strains (Adnan *et al*., 2017).

Globally, *CTX-M* type, particularly *CTX-M* 15 of ESBL producing *E. coli* is important and frequently reported from the chicken, environment, wildlife, and humans (Silva et al., 2016; Kim *et al*., 2015). Similarly, in Pakistan *CTX-M* are the most common ESBL genotype *E. coli* reported clinical isolates (Abrar *et al*., 2018; Abbas *et al*., 2019) and poultry Ur Rahman *et al*., 2018). While, these

genotypes are predominant in Asia, spreading by integrons and other mobile genetic elements (Ali *et al*., 2016).

2.11 Causes and Mechanism by Transferring Resistant Gene between Humans and Animals

The One Health approach, which links animal health to human health and their respective environments, provides a framework for understanding disease origin and transmission (Rupnik *et al*., 20008; Knetsch *et al*., 2018). A study conducted by Knetsch identified 247 strains of *C. difficile* RT078 from people and animals in 22 countries throughout Europe, North America, Asia, and Australia in research. (https://microreact.org/project/rJs-SYgMe). The high genomic similarity between human and animal isolates has proved that the above strains are frequently spread between humans and animals (Knetsch *et al*., 2018). This spread of *C. difficile* RT078 bacteria between humans and animals was also documented (Bakker *et al*., 2010; Keessen *et al*., 2011). Similarly, the MRSA strains isolated from humans' patients were identical to animals belonging to ST254, ST8, and ST22 genetic linage shared between animals and humans (Wieler *et al*., 2011). Furthermore, the transmission of veterinary-associated MRSA strain ST38 from horses to humans was reported (van Duijkeren *et al*., 2011). In the UK, the MRSA ST22 was transferred from companion animals to humans (Harrison *et al*., 2014). The first *E. coli* in which *CTX-M* ESBL enzyme was found, cefotaxime-resistant *E. coli* isolated from dog feces in Japan in 1986 (Matsumoto *et al*., 1988). After, that the ESBLs were disseminated in humans worldwide. Then, the ESBL positive uropathogenic *E. coli* was isolated from companion animals in Spain in 1998 (Teshager *et al*., 2000). Then *CTX-M* enzyme was found rapidly in bacteria related to human infections, and in animals, both commensals and clinical *E. coli* isolates have been producing β-lactamases *CTX-M* (Ewers *et al*., 2011; Pomba, 2017).

Recently, the MDR *E. coli* ST131 has been reported as a worldwide pandemic in humans (Wieler *et al*., 2011; Dierikx *et al*., 2012). The first fluoroquinolone-resistant *E. coli* ST131 clone had been reported from animals in a study conducted in Portugal. In that study, 41 isolates from the dog and 20 isolates from a cat were screened for ESBLs in the year 2004-2006 (Pomba *et al*., 2009). Moreover, a large number of human clinical isolated ST131 samples are similar to animals *E. coli* ST131 isolates based on ARG, virulence genotype, plasmid, and PFGE profile (Bogaerts *et al*., 2015 Ewers *et al*., 2011).

E. coli such as ST156, ST405, ST410, and ST648 have been found in both animals and humans (Wieler *et al*., 2011; Dierikx *et al*., 2012). The high prevalence of similar clones in foods, humans,

and non-humans' species like dogs, cats, horses, and poultry may suggest their transmission via animal and food (Platell *et al*., 2011). Therefore, the transmission is a contributory factor in the dissension of *E. coli*. Many carbapenems resistance strains were also reported in animals. NDM-1 and OXA-48 producing *E. coli* were isolated from companion animals in the USA and Europe (Stolle *et al*., 2013; Shaheen *et al*., 2013).

2.12 Extensive use of Antibiotics

Antimicrobial resistance has increased by many folds in the last several years across the globe to healthcare settings. Mortality attributed to antibiotic resistance is one of them coupled with infectious diseases burden (De Kraker *et al*., 2011, Köck *et al.*, 2010). Though the antibiotic resistance is not a new terminology and the resistant gene is present all around in our environment (Rolain *et al*., 2012). Globally, the use of antimicrobial in animal food production will increase by 67 % by 2030, particularly in countries like Brazil, China, India, South Africa, and Russia. In these countries, the large scale of animal farming is highly demanded due to the rise of income and meat consumption demand (Brower *et al.*, 2017; Van Boeckel *et al.*, 2014; Van Boeckel *et al*., 2015b).

About 80 %, of the antibiotics produced in the USA, are used as growth promotors and to control infections in the livestock. The sole purpose of antimicrobials use in livestock is to improve animal health, large yield, and quality products. These antibiotics are transferred from animals to humans when they consume animal meat in their food. This was reported 35 years back when antibiotic-resistant microbes were observed both in animals and the farmers' gut (Ventola, 2015a, Spellberg & Gilbert, 2014, Gross, 2013, Bartlett *et al.*, 2013).

The Union of Concerned Scientists, a national nonprofit organization established more than 50 years ago by scientists and students at the Massachusetts Institute of Technology (https://www.ucsusa.org/about) advocacy group recommends less use of antibiotics in agriculture. According to their report, 24.6 million pounds were consumed in animals (poultry, cattle, and swine) and only 3.0 million pounds are consumed for human medicines (Landers *et al.*, 2012a). Twelve major antibiotic classes are frequently used in animals. These are arsenicals, polypeptides, glycolipids, tetracycline, elfamycin, macrolides, lincosamides, polyether's, β-Lactams, quinoxaline, streptogramins, and sulfonamides are used in different phases of the poultry, cattle, and swine industry (*Sarmah et al.,* 2006; Barton, 2000).

2.13 Mechanism of Antibiotics Resistance

Antibiotics have disturbed the normal biochemical process of the targeted cell of pathogens. Most of these are bacteriostatic (inhibit the growth/division of cell) and bactericidal (kill bacterial cell) (Lewis, 2012). These abilities of metabolites produced by some bacteria and fungi were used to kill the human pathogen with very few side effects on the human cells (Lewis, 2012). It was first studied by Selman Waksman, a member of the discovery team of streptomycin. The spore-forming bacteria like actinomycetes more reported these metabolites. Waksman's strategy led to the foundation of many antibiotics' discoveries (Lewis, 2012; Lewis *et al.*, 2010). Waksman's strategy/approach was more systematic, and novel as compared to the accidental discovery of penicillin. He and his co-workers screened the growth inhibition zone of isolated soil microbes on agar plates providing different culture conditions. After that, they tested their inhabitation against specific bacteria. However, this was very painstaking work, screening thousands of microbes, but this approach of Waksman was very successful in yielding 20 antimicrobials, including the most essential streptomycin at that time (Santesmases *et al*., 2018; Waksman, 1945).

The platform also enforced success metrics that would be used in future drug development initiatives. The most common method for determining a compound's MIC (minimal inhibitory concentration) is to evaluate cell growth inhibition in vitro on a rich medium. Similarly, in the mid-1960s, the Waksman technique failed to generate novel and effective antibiotic scaffolds. (Cho *et al.*, 2014). Because these specialized metabolites evolved as a result of microbial evolution in a particular environment and were not created to be used as medicines. The majority of them had significant pharmacological or toxicological flaws. This situation led to the entry of medicinal chemistry in the field of antibiotics. In this period, many synthetic versions of natural antibiotics were produced. The golden era of antibiotics was more focused on the broad-spectrum use of antibiotics with a lower dose in which single antibiotics was effective against many pathogens and an effort to avoid the antibiotics resistance against the old antibiotics (Cho *et al.*, 2014; Brown, 2015; Dwyer *et al.*, 2015).

Novel medication combinations radically altered medical practice and ushered in the "miracles" of modern medicine that we now take for granted. Most of the antibiotics target the cell wall and interrupt the DNA and ribosome machinery; however, few of them can have multiple targets in the pathogenic cell. Such as the β-lactam class of antibiotics mainly targets the cell wall of bacteria, but it can do in multiple ways by modifying the enzyme called PBS (Penicillin-binding

proteins) (Navarro-Martinez *et al.*, 2005). Collectively, these enzymes are involved in the modeling and synthesis of bacterial growth and cell division. So, β-lactam targets the penicillin binding protein PBPs. It also reduces the chances of quick resistance of genes against these antibiotics. Evidence is mounting that β-lactam antibiotics disrupt the bacterial cell-wall manufacturing machinery in a way that is much more complex than simple inhibition (Yam *et al.*, 1998; Navarro-Martinez *et al.*, 2005; Brown, 2015). Secondary metabolites are categorized in a variety of ways, but the most common is based on their chemical structures, mechanism of action, and spectrum of activity. They may also be categorized by how they are administered, such as injectable, oral, or topical. The efficacy, toxicity, allergic potential, and side effects of all antibiotics in the same class will usually follow a similar pattern. Antibiotics are classified into chemical or molecular structure groups such as β-lactams, Macrolides, Tetracyclines, Quinolones, Aminoglycosides, Sulphonamides, Glycopeptides, and Oxazolidinones. (Adzitey *et al.*, 2015; van Hoek *et al.*, 2011; Roberts, 2002; Queenan & Bush, 2007)**.**

In the 1950s and 1960s, resistance to multiple antibiotics was initially discovered in gut bacteria such as *E. coli, Shigella, and Salmonella*. Due to which many clinical problems erupted all around the world, including causalities in developing countries (Levy, 2001; Sykes & Richmond, 1970). Indeed, the excessive usage of antibiotics has increased antibiotic resistance in the number of bacterial species. Especially, the situation is more pathetic in developing countries where antibiotics are readily available without a prescription. Bacteria may also develop antibiotic resistance via chromosomal gene mutations and horizontal gene exchange (Chuanchuen *et al*., 2003; Charlesworth, 2010; Smith & Coast, 2013; Bhullar *et al*., 2012). Moreover, it falls into three main groups. First, those that minimize the intracellular concentrations of the antibiotic by the efficient efflux machinery of bacteria or poor penetration capability of the antibiotics. Second, genetic mutagenesis or protein alteration of the antibiotic target. Third, those that degrade (hydrolyze) or modify antibiotics to render them inactive. Each of the processes listed above has been researched for many years (Fernandez & Hancock, 2012; Ajaiyeoba *et al*., 1992; Baquero, 2001). Approximately 30 different antibiotics were mixed in the food and water of animals. Recently, a study was conducted, which has revealed that more than 50 % of the antibiotics are used as a growth promoter for animals. In some developing countries, antibiotics are available without prescriptions which also add their part in the resistance (Singh & Barrett, 2006; Davies, 2007). The demand for antibiotics has increased in veterinary medicine in the international market. It was 8650 million

dollars in 1992 and had jumped to 20000 million dollars in 2010. It had almost reached 42.9 billion dollars at the end of 2018 (Hao *et al.*, 2014).

2.14 Resistance Controlling Strategies and Techniques/Alternatives

Antibiotic resistance can be addressed more effectively by taking cooperative efforts at the individual level, community-based, and national and international. Therefore, all strategies should be based on optimizing antibiotic usage, reducing the un-intended interaction between pathogenic microorganisms and antibiotics, and controlling the spread of resistant strains and sensible use of antibiotics (Tanwar *et al.*, 2014). To achieve this goal, an alliance was formed between FAO, WHO, and OIE with the principle of One health approach. Additionally, they issued a complete global action plan on AMR in 2015 (Hunter *et al.*, 2017). Meanwhile, FAO also issued AMR policy in 2016 to support the proper execution plan of WHO regarding the Global action plan in the food and agriculture sectors. Consequently, to aware people of AMU and AMR, the benefit of balanced use of antibiotics, minimizing the spread of infectious diseases, and giving proper dimension to research, resources for the spread of antibiotic resistance (Hunter *et al.*, 2017).

However, the alternative compounds used as antibiotics should be acceptable to the gastrointestinal tract, must be non-toxic, have high target accuracy, and be environmentally friendly. It has also enhanced feed efficiency and animal growth and more importantly, is free from resistance (Cheng *et al.*, 2014a; Bourlioux, 2013). Vaccination is considered the best alternative to antibiotics. It is the most effective method to control and eradicate infectious diseases. For instance, through vaccination, smallpox was eradicated. It can also restrain AMR bacteria, which will greatly impact animal and human health. Brucellosis is a very lethal and common disease around the world. The disease is caused by *Brucella*, which is intracellular gram-negative bacteria (Leylabadlo *et al*., 2015; Haque *et al*., 2011). Therefore, live attenuated and inactivated, and killed bacterial strains of *Brucella* are used to control brucellosis. RB-51 (*Brucella abortus*) is a typical example of vaccines used in these animals (Founou *et al.*, 2016; Woolhouse *et al.*, 2015). However, the safety assurance of recombinant vaccine must ascertain both for humans and animals before field trial (Poulet *et al.*, 2007). Moreover, phytocompounds were also used as an excellent alternative to antibiotics. For a long in human civilization, plants were used as traditional medicine, both for animals and human beings. The farmers of developing nations were more confident approaching the traditional and local experts for their animal's disorders. The given traditional remedies are mostly plant-based which were organic, non-toxic, cheap, and readily available. Such as, *Tagetes minuta* and some other

plants were used as a whole or its leaves and roots extract to cure tick and worm infection in animals. Interestingly, like antibiotics, these compounds have broad-spectrum activity, which can be used as the best alternative to antibiotics (Sharma *et al.*, 2017c; Panda & Dhal, 2014).

Probiotics, prebiotics have availed of great importance in the production of safe food and have increased gut microbiota especially after banning antibiotics in food (Baffoni *et al.*, 2012; Pineiro *et al.*, 2008). Therefore, with AMR, the role of probiotics was increased in both the medical and animal sectors. Additionally, probiotics are the best food supplement, consists of beneficial gut microbes, and improve the immune system (Tellez *et al.*, 2015; Isolauri *et al.*, 2002). Such as, bacteriocins are the ribosomal synthesized protein that restrains and kill the related species of bacteria (Yang *et al.*, 2014). Bacteriocin is produced both by gram-positive and gram-negative bacteria. Similarly, actcin, sacacin, lactobin, and nisin were well-studied bacteriocin produced by gram-positive bacteria. While colicin, microcin is produced by gram negative bacteria, particularly by *E. coli* (Karpinski & Szkaradkiewicz, 2013). Prebiotics are the food ingredients that selectively metabolize gut microbes. The number of gut microbes increases and enhances the immune system, which also acts as an antimicrobial to pathogens and gives other beneficial effects to the host. Some common prebiotics is oligosaccharides, polysaccharides, polyols, protein hydrolysates (Uyeno *et al.*, 2015; Isolauri *et al*., 2002).

2.14.1 Phage Therapy

As an alternative to antibiotics, "Phage Therapy" is the potential of bacteriophage to inhibit bacterial growth. And this lysis of bacterial cells has greatly inspired the researcher to use it as an antibacterial agent and will be used in both the medicinal and veterinary sectors. Though, it targets a very narrow group of bacterial species (Buttimer *et al.*, 2017; Lobocka *et al.*, 2004). Phage therapy could be promising alternatives to antibiotics (Chanishvili *et al*., 2019). The use of phage therapy against bacterial infections has gain attention because of its emergence. Historically, Frederick Twort was the first who identified the phage lysis zone related to phage infection in 1915. But Felix d'Herelle identified this phenomenon and used the term bacteriophage for bacterial viruses. He also gave an idea of using phage therapeutically and used phage first time against bacterial dysentery in des Enfants-Malades hospital Paris in 1919 successfully (Chanishvili *et al*., 2012). However, along with D' Herelle, other entrepreneurs in Brazil and the USA also attempted to commercialize phage production against *E. coli*, *streptococcus, staphylococcus,* and other multidrug-resistant bacteria (Sulakvelidze *et al*., 2001).

Using an animal model such as mice with sepsis caused by *pseudomonas aeruginosa*, the oral treatment decreased mortality by 66.7 percent compared to 0% in the control group (Watanabe *et al*., 2007). Single phage strain administration in mice model affected vancomycin-resistant *E. faecium* (Biswas *et al*., 2002), imipenem resistant *P. aeruginosa* (Wang *et al., 2006*). ESBL producing *E. coli* (Wang *et al*., 2006) was enough to give 100 % recovery from bacteremia. Human phage treatment studies had begun at several institutions, including the Eliva Institute of Bacteriophage, Immunology, and Experimental Therapy in Wroclaw, Poland. *Streptococcus spp, P.aeruginosa, Proteus spp, Enterococcus spp, S.aureus, E. coli, S.dysenteriae,* and *Salmonella spp* have all been treated with the phage at the Eliava Institute in clinical and preclinical trials (Kutateladze *et al*., 2008). Similarly, during the 1974 typhoid outbreak, 18577 children were enrolled in a preventive intervention study using typhoid phage. It decreases five times typhoid incidence as compared to placebo (Lin *et al*., 2017; Kutateladze *et al*., 2008). However, two major phage proteins lysin and holin, work together to lysis bacterial cells (Roach *et al*., 2015). These proteins are infective against eukaryotic cells and fast-acting, potent against the bacterial cell. As lysin was successfully employed in mice affected with bacteremia by MDR *A. baumannii* (Lood *et al*., 2015), MRSA (Schmelcher *et al*., 2015), *Streptococcus pneumoniae* (Witzenrath *et al*., 2009). During the lysis of the bacterial host, the majority of phage species use two main protein groups. One is holin, a transmembrane protein, and the other is endolysin, a peptidoglycan cell wall hydrolase (lysin). The bacterial cell lyses is triggered by these two proteins working together (Roach *et al*., 2015). Therefore, bacteriophages can be used as an alternative to antibiotics. However, pharmaceutical companies were reluctant to invest. Because phage therapy has some shortcomings such as chances of resistant or unknown gene transfer, lack of clinical trials, and safety issues. As a result, this is still passing through the research phase (Born *et al.*, 2015; Buttimer *et al*., 2017). Immunostimulants can be used to reduce the burden of antibiotics. These substances provoke the defense mechanism of the organism and enhance the host immune system against pathogens. They directly affect the immune system's innate responses by the activation of phagocytes, complement system, neutrophils, and increased lysozyme activity. Recently, the demand for immuno-stimulants is increasing, which is an alternative to antibiotics. Some common immuno-stimulants are proteins (arginine, leucine), vitamins: A, E, C, and many plant polysaccharides, hormones, cytokines, interferons, interleukin, and immunoglobin. With more research, immuno-stimulants will be a good alternative to antibiotics soon (Sharma *et al*., 2017c; Cheng *et al.*, 2014b; Song *et al.*, 2014; Masihi, 2000).

Bacteria communicate with themselves and the environment through quorum sensing, which plays a crucial role in pathogenesis. However, this communication can be disturbed by the degradation of the autoinducer, which inhibits the synthesis of auto induce. It will help to reduce bacterial pathogenicity. Such as homocysteine, which can disturb the QS signals (Pique *et al.*, 2015). However, different enzymes have been employed in the animal feed, enhancing the digestion capability and reducing drug abuse as a growth promotor in the animals. The enzyme also enhances the immune system, which directly reduces pathogens. Glycanases and phytases were the most commonly used enzyme in animal feed (Ravindran & Son, 2011; Bedford & Partridge, 2010; Selle & Ravindran, 2007).

2.14.2 Nanotechnology

In 1959, Richard Feynman introduced nanotechnology, and the term of nanotechnology was coined by Norio Taniguchi in 1974. This technology mainly consists of the fabrication and characterization of a small range of molecules (<100 nm). Nanotechnology is used to create, develop, and treat materials that are embedded with nanodevices or nanoparticles (Sharma *et al*., 2017; Momin and Joshi, 2015). Some nanoparticles have shown antimicrobial activity, such as copper that has inhibited the growth of many pathogens, including *S. aureus* and *L. monocytogenes,* and *E. coli* on a polymer composite after four hours of exposure (Cioffi *et al*., 2005). Copper nanoparticles reported many toxic effects like lipid peroxidation, reactive oxygen species (ROS), protein oxidation, and DNA degradation which might be the reason for its antibacterial activity (Sheikh *et al*., 2011; Chatterjee *et al*., 2014). Similarly, zinc nanoparticles also possess antifungal and antibacterial activity (Vermeiren *et al*., 2002).

Over the last several years, nanotechnology is well employed in food and animal science. Subsequently, it is also used to reduce AMR pathogens (Sharma *et al*., 2017c, Raguvaran *et al.*, 2015, Sharma *et al.*, 2017b). Therefore, NP (Nanoparticle) is a convenient vehicle for the transfer of antimicrobial agents to the target area. Many NPs would be the best alternative agents against bacteria and fungi. Such as the control of bovine mastitis by NP (Gomes & Henriques, 2016; Cardozo *et al.*, 2014; Berni *et al.*, 2013). The above techniques are very beneficial to control the spread of antibiotic resistance. But with appropriate use of antibiotics, antimicrobial stewardship programs, education, hygiene, and disinfection, the development of novel antibiotics is also very important in controlling AMR problems (Lee *et al.*, 2013; Dellit*et al.*, 2007).

2.15 Molecular Techniques to Investigate Antibiotic Resistance

Molecular description after the primary phenotypic result of antibiotic sensitivity testing is now the critical aspect of investigations of bacterial infections both in humans and animals. However, the phenotypic results are very much time taking or inconclusive than the molecular method. Therefore, molecular methods are the most appropriate method to study the desired biological problem at the gene level and find any mutation. The above characterization is often used as a reliable method to support or help epidemiological investigations. It is beneficial after an outbreak when phenotypic data does not provide enough information to make a strategy to hinder the diseases caused by MDR bacteria. In brief, AMR analysis through molecular biology is an essential pillar of AMR surveillance both on the regional and global stages. Therefore, the following are the important methods concerning AMR study such as NGS (Next-generation sequencing) and Real-time PCR (Stoesser *et al.*, 2013, Anjum, 2015, Fang *et al.*, 2008). A genome-based analysis is used broadly, both by reference and scientific laboratories. Thermocycler and hybridization are popular techniques. While methods like WGS and MALDITOF-MS (protein base method) are the new and advanced techniques in these laboratories. The main methods of genotypic methods for detecting AMR are (Stoesser *et al*., 2013; Anjum, 2015).

2.15.1 PCR (Polymerase Chain Reaction)

PCR is the most well-known technique in molecular biology, developed by Kary Mullis in1980s. PCR brought a new revolution in molecular biology. This discovery enabled fast and exponential amplification of target genes using PCR primer (forward and Reverse), DNA polymerase (known amplifying enzyme), and the presence of deoxyribonucleotides. The basic steps for every PCR are common. Like, denaturation of genomic DNA, annealing, and the extension of the target DNA (Arya *et al.*, 2005; Yuce *et al.*, 2014; Saiki *et al.*, 1988; Anjum *etal*., 2017).

In microbiology laboratories, these techniques are routinely used for the confirmation and detection of desired genes. It requires a targeted DNA sequence for designing primers. Then the amplified gene/sequence result is visualized by loading on the gel stained with specific loading dyes. This all procedure, from PCR amplification to visualization, takes 4 to 5 hours. With time, more advancements and development in PCR are noted (Anjum *et al*., 2017). These include Q-PCR, LAMP (loop-mediated isothermal amplification), and RPA-PCR (Recombinase polymerase amplification). However, Q-PCR is different from conventional PCR as amplification of targeted genes is

monitored from the start of the cycle because of shining dyes in the reaction rather than at the end of the reaction like in conventional PCR. That's why it is called Quantitative PCR.

Consequently, there is no need for agarose gel electrophoresis; hence it has saved considerable time and is much human and environment friendly because of no use of ethidium bromide (a carcinogen) (Anjum *et al*., 2013). LAMP and RPA, Isothermal PCR techniques are different from both conventional and Q-PCR, in which thermocycler run through constant temperature. In LAMP, 6 multiple primers are used at constant 65°C temperature. While for RPA-PCR temperature is around 40°C (Anjum *et al.*, 2013; Glais & Jacquot, 2015; Arya *et al*., 2005).

2.15.2 Multiplex PCR

In Multiplex PCR, multiple DNA sequences are amplified concurrently using standard and real time PCR and can be performed by using both conventional and Q-PCR. Today the use of multiplex PCR is more in studying AMR genes. This technique has become a more user-friendly and appropriate technique as compared to conventional and RT-PCR. In this process, the number of antibiotic-resistant genes can be detected using multiple primers (Dallenne *et al.*, 2010; Anjum *et al*., 2017; Solanki *et al.*, 2014). Using agarose gel electrophoresis or different dyes for real-time PCR, the amplified genes of various sizes may be seen. The above type of PCRs is convenient in identifying genes responsible for the same class of phenotype such as betalactamases genes resistant to the antibiotic cephalosporin group (Poirel *et al.*, 2011; Dallenne *et al*., 2010; Anjum *et al*., 2017).

2.15.3 Uses of Different Types of PCR

Both lamp & RPA PCR uses specific temperatures to amplify the targeted genome sequence (Zanoli & Spoto, 2013). These are based on isothermal amplification techniques using enzymes in vitro, which start DNA replication and do not need accurate temperature control or the conventional PCR temperature of 50 to 95°C (Gill & Ghaemi, 2013). These isothermal amplifications included LAMP (loop-mediated isothermal amplification), RPA (recombinase polymerase 71 amplification), MDA (Multiple displacement amplification), NASBA (nucleic acid sequence-based amplification), and HDA (helicase-dependent amplification) (Gill & Ghaemi, 2013; Yan *et al*., 2014).

RPA is a novel isothermal amplification technique that makes use of a recombinant enzyme that creates a nucleoprotein complex with oligonucleotide primers and allows the primers to be inserted into complementary DNA (Piepenburg *et al*., 2006). The primer amplifies the targeted region similar to conventional PCR. However, exo-probe allows the RPA PCR to monitor real time

detection (TwistDx, 2013). Similarly, LAMP is another important isothermal-based assay. The key advantage of the LAMP PCR over others is the use of a rapid and simple protocol that helps in rapid diagnosis. In the LAMP assay, only a single enzyme is used (DNA polymerase). Moreover, a single temperature is needed for amplification, and without using electrophoretic techniques, the amplification is diagnosed. While in LAMP, four primers are used in targeting six regions of DNA (Lee *et al*., 2017).

2.15.4 DNA Microarray Technology

DNA microarray is another very advanced genomic tool. Therefore, in the last few years, it is considered the most important technique in evaluating genomic diversity and confirming AMR genes in bacteria. In this assay, the glass slide is marked with specifically targeted probes based on genes (Barl *et al.*, 2008; Carter *et al.*, 2008). However, with the advancement in technology, several organisms were sequenced. Ultimately, the number of accessory genes was increased in DNA probes present on the microarray glass slide, which is not present in the reference strain. However, it is part of the pan-genome (the entire gene set of all the strains of a species). So now, using comparative genomic hybridizations, tested isolates and reference strain DNA/gene can be fluorescently tagged and hybridized to a DNA probe on a microarray slide (Yu *et al.*, 2004; Anjum *et al*., 2017). By analyzing the hybridization results, the desired gene in the test strain can be detected through the reference genome. This method finds the genomic diversity among many tested organisms for which complete genome sequencing is not found. (Barl *et al*., 2008; Call *et al.*, 2003). The microarray (ALERE technologies) was developed to study AMR and virulence genes on the plasmid, transposons of both commensal and pathogenic *E. coli*. However, later, this technique was extended to study the AMR gene in food and clinical isolation of organisms, particularly *Salmonella* and *E. coli* (Anjum *et al.*, 2007).

2.15.5 MALDI-TOF in Species Identification

Matrix-Assisted Laser Desorption Ionization-time of flight mass spectrometry (MALDI-TOF MS) is a new technique that has recently been opted by many research and clinical laboratories. Now it has been used to study biomolecules such as carbohydrates, DNA, and proteins. It first ionizes the biomolecules and changes them into the gaseous phase, so the time of flight is measured via these gas molecules. Therefore, the logic behind this is to measure the m/z (mass/charge) ratio of the gas molecules and is then used for molecular signature (Murray, 2012). This technique is performed on living organisms, including whole single organisms or complex biological samples such as

blood and urine. Moreover, each range (spectrum) generated by the machine is compared with commercial databases is helpful for species identification (Panda *et al.*, 2014).

Moreover, along with the detection of proteins and enzymes the MALDI-TOF MS is used for other biological molecules (Hrabak *et al.*, 2014; Panda *et al.*, 2014). MALDI-TOF MS has a protein base method. However, the antimicrobial biomolecules or their products degraded by enzymatic activity are detected by MALDI-TOF (Hrabák *et al*., 2013). Therefore, the specific enzyme-drug combination is targeted in this approach and specifically relevant subgroups of important drugs. Due to that, most studies identify the compatibility of this approach with carbapenem because of the carbapenemase enzyme (Burckhardt & Zimmermann, 2011). However, MALDI-TOF is not strongly recommended in the identification of resistance due to the various drawbacks. This method for detecting carbapenem medicines (imipenem and meropenem) and their enzyme-degraded derivatives in the clinical laboratory relies on a preincubation phase to provide time for degradation. Second, compared to species identification, these molecules must be analyzed across a narrower range (0 to 700 m/z). (Chong *et al*., 2015). Furthermore, many antimicrobial agents, such as vancomycin-resistant enterococci and MRSA, are not inactivated by enzymes. All of these flaws make this strategy unworkable (Anjum *et al*., 2018).

2.16 Sequencing Methods: Analysis to Detect AMR

Like polymerase chain reaction and DNA microarrays, NGS can also screen various genes and all kinds of biological mutations conferring antimicrobial resistance (Zankari *et al.*, 2012; Gupta *et al.*, 2014). The WGS has the advantage over other molecular methods to cover many different targets simultaneously. WGS technique for analysis has its pros and cons, like PCR and DNA microarray. However, unlike microarrays, the sequencing technology has made the addition of new target sequences rapidly to analysis database and do a quick analysis of already sequenced isolates in silico reanalysis (Hasman *et al.*, 2015; Anjum *et al*., 2017).

Current advanced sequencing and computational methods have generated large data that is easily analyzed compared to the old Sanger method. Currently, Illumina and Ion's torrent machines were the best in generating high throughput of bacterial genomes. This sequencing is also called '3rd Generation' or 'Next Generation Sequencing. Given the output of the short read, about 100400 base pairs depending upon the machine. Therefore, the sequenced gene part represents less of the gene representing antibiotic resistance. Secondly, 2nd generation sequencing has a higher error rate than

the old Sanger sequencing method (Kwong *et al.*, 2015; Piccinini *et al.*, 2012; Pilla *et al.*, 2013).To reduce the above problem, many 100-400 bp reads are generated for every genome.

Furthermore, it is mapped with referenced assembly or uses these reads to make large contigs through the de novo method (Padmanabhan *et al.*, 2013; Edwards & Holt, 2013). The NGS (Next-generation sequencing) quality and quantity of short reads for the correct downstream analysis was improved. WGS is a sensitive technique so it must be ensured that no contamination of the foreign DNA is present to give false-positive results. Unfortunately, a small amount of DNA from intraspecies contamination can't be detected. However, good library skills and positive and negative control are advantageous to avoid or minimize contamination during DNA extraction and sequence library preparation (Gargis *et al.*, 2012). Whole-genome sequencing has made significant contributions to the area of infectious diseases, particularly in terms of bettering our knowledge of transmission dynamics and epidemic analyses. Further, with this technology, we can screen the acquired antibiotic resistance gene and chromosomal mutations to predict the AST (Antibiotic Resistance Gene) results. Lastly, whole-genome sequencing is essential for guiding antibiotic treatment decisions (Tamma *et al.*, 2019, Cao *et al.*, 2016).

2.16.1 New Advanced Sequencing Methods

There are three axes in the presently available sequencing platform. Oxford Nanopore and Pacific Biosciences sequencers make advantage of single molecule detection on a per-reaction, well, or sensor basis. Ion Torrent, Roche 454, and Illumina platforms all detect colony amplified DNA in the same way. While axis two is based on Illumina's optical detection for base calls during sequencing optical detection to make sequencing base calls, as performed by Illumina, Roche 454 "detection of light via pyrosequencing" platforms, Pacific Biosciences "detection of fluorescently modified nucleotides" (Levy *et al*., 2016). Furthermore, as performed both by Oxford Nanopore measurement of the translocation of DNA through a nanopore sensor and Ion Torrent detection of the release of H+ during a polymerization reaction via a solid-state sensor. However, the most common axis is the third axis called sequencing by synthesis using polymerase or ligation process to do so. In which, the reaction product is measured, or direct DNA molecules are measured. Ion Torrent, Illumina, Pacific Biosciences (Quail et al., 2012) performed this type of sequencing. The third axis involves using a polymerase or ligation procedure to initiate a sequencing-by-synthesis reaction. The reaction's products are then analyzed to provide sequencing data or directly measure DNA molecules. A polymerase reaction is used in Illumina, Ion Torrent, Pacific Biosciences, and Roche 454

sequencing-by-synthesis processes. The Polonator platform and the old Applied Biosystems SOLiD platform, on the other hand, utilize ligation-mediated synthesis. The Oxford Nanopore platform measures DNA sequences in real-time. Depending on the chemistry and detection techniques employed, each publicly accessible stage both resembles and differs from the others. Because of these similarities and variances, the platforms have a range of capabilities and specifications, resulting in distinct strengths and disadvantages. Because of the variations between the platforms, especially their limits, numerous comparisons to assess their performance under comparable circumstances have been made. (Quail *et al*., 2012). It's also become more economical to combine several platforms in a single experiment to make use of each platform's capabilities. (Ku CS *et al*., 2013). The above all sequencing platforms are compared on the number of reads produced and the length of those read produced in a given instrument. While other factors considered are sample preparation cost, cost per base/per run, run time of the instrument, etc. (Burghel *et al*., 2015; Szalay *et al*., 2015). The Illumina and Ion Torrent platforms have dramatically reduced the cost per sequenced base and boosted data output in short-read sequencing methods. While there has been an increase in the duration of reads (35-350 bases per reading). Illumina compensates for short read lengths by allowing paired-end sequencing, which involves sequencing both ends of the same DNA molecule to the full read length. (Leinonen *et al*., 2011).

The Illumina platform has more suitable in NGS and exome sequencing and applications that count reads, such RNA sequencing (RNA-seq) (Illumina; 2014). However, in term of output, the two highest Illumina's machines are The HiSeq 4000 and HiSeq X for genome sequencing may produce more than 6 billion paired-end reads despite having a 150-nt read limit.approximately 12 billion overall readings per instrument run for general purposes (Li *et al*., 2014; Lou *et al*.,2013). Oxford Nanopore and Pacific Biosciences are the two powerful technologies that produce long reads in the case of a long read. They produce read lengths each reading in the tens of thousands of bases. In a zero-mode waveguide, a sequencing-by-synthesis technique is used by Pacific Biosciences to detect sequences optically. while, Oxford Nanopore uses nanopore for detection (Levene *et al*., 2003). Moreover, nanopore sequencing was first commercialized by Oxford Nanopore, which they did in the Minion sequencer. It was the most feasible sequencer machine that was handheld and operates only through the USB port. It was the most cost-effective sequencer released at that time of $ 1,000 (Reuter *et al*., 2015). Secondly, up to 100 Mb of data of the long-read length of six KB has generated by the MinION platform is per 16-hour run (Ashton *et al*., 2015). A hybrid assembly is created using data

from nanopores and Illumina technology (Goodwin *et al*., 2015; Risse *et al*., 2015). Library preparation techniques and analysis algorithms for long read sequencing data are rapidly evolving, including scaffolding approaches for assembling draught genomes and tools for analyzing and visualizing MinION data. (Warren *et al*., 2015 126, 15), including error corrections improvement and read accuracy (Jain *et al*., 2015 55; Szalay *et al*., 2015).

Second-generation sequencing has progressed significantly in the past decade in terms of detecting genetic variations in any species. However, for complicated structural variants identification, such as copy number variations, these short reads of 100-500 bp are insufficient. (Magi *et al*., 2018). However, third-generation sequencing had mostly resolved this issue in the last few years by producing long reads with different strategies. In which Oxford Nanopore technology including MinION, GridION X5, and PromethION analyze the DNA through the nanoscopic pore (Korlach *et al*., 2010; Jain, *et al*., 2016). However, GridION X5 is cost-effective in Oxford Nanopore technologies. GridION X5 is a small benchtop sequencer that can use five MinION Flow Cells at a time, which drastically increases the MinION experiment up to 100 GB of DNA sequence data output of 48 hrs of sequencing run (Bolognini *et al*., 2019).

2.17 Bioinformatics approaches to study WGS (Whole Genome Sequencing) Data

Getting the relevant information from the DNA related to AMR from WGS data is easy now. It requires appropriate bioinformatics tools to analyze WGS. Further, target databases of WGS are working on the same principle as the primer does in PCR or in DNA microarray to detect the specific target in the genome (Ellington *et al.*, 2017; Padmanabhan *et al*., 2013). In the computational approach BLAST and mapping of raw reads are the two essential bioinformatics tools to detect the desired gene from the sequenced data (Gargis *et al*., 2012; Kwong *et al*., 2015).

Additionally, advancement in computational link with biology has increased the role of bioinformatics tools for analyzing and detecting genetic determinants for the antibiotic-resistant gene in WGS data. These bioinformatics tools can be found online or downloaded and used on a PC (Personal computer). However, it needs Linux or Unix operating window. For example, Resfinder, CARD "Comprehensive Antibiotic Resistance Database" ARG-ANNOT "Antibiotic Resistance Gene-Annotation" Prokka and plasmid finder (Martens & Demain, 2017a).

Most of the bioinformatics tools identify genome sequences that reduce the AMR Susceptibility pattern, including other mutations. However, to study the correlation between the

genome and phenotypic expression is only known through the antibiotic resistance digital library and previous research papers. The only drawback of such a library is no new genes and mutations are identified. Because they are based on preloaded data, fit in the database (Clausen *et al*., 2016; Arya *et al*., 2005). Regular monitoring of the database and updating is needed with the discovery of new genes. Although all international and local surveillance of AMRs is strongly dependent on target databases, they offer a unique resolution of gene variants which is not an easy job to obtain by phenotypic approach or through other molecular methods. However, NGS methods have reanalyzed the previous data and updated the library by finding new genes and mutations. Similarly, the expected existing data for colistin was reanalyzed when it was first reported in China (Liu *et al.*, 2016b, Arya *et al*., 2005). Now the *mcr-1* gene is recovered from GenBank is added in ResFinder software, used to screen colistin-resistant genes (Thomsen *et al*., 2016).

Overall, the screening of the AMR gene through the reference mapping technique is more sensitive compared to BLAST-based analysis. Because the de nova doesn't assemble all the sequences of a gene. And it may be because the ratio of the resistant gene contigs is meager in the DNA for complete assembly or more resistant genes with similar DNA sequences. Resultantly, the assembler split this fragment and ultimately splitting the resistant gene (Clausen *et al*., 2016; Martens & Demain, 2017a).

2.17.1 Newly Advanced Bioinformatics Database use for AMR

With more advancement in computational link with biology, the use of the different computational databases was increased to screen AMR genes from sequencing data. These bioinformatics databases are accessed online or easily downloaded through the internet and can be used on one's personal computer (McArthur & Tsang, 2017).

2.17.2 ResFinder

A BLAST-based alignment database uses the FASTA format to screen AMR sequences from the WGS data. After the BLAST, the 30 bp overlap of the top hit is shown as a result. Due to which the same gene position can be located in a different position. Results are based on minimum percentage length and minimum percentage identity of the alignment sequences. Therefore, those genes will be reported which meet these parameters (Larsen *et al.*, 2012; Zankari *et al*., 2012). So, the above two selection parameters are strongly dependent on the quality of the desire sequence and the purpose of its use. Both preassembled data and raw data from different sequencing sources are uploaded. The target sequences of WGS data are assembled by Velvet assembler then analyzed by ResFinder.

ResFinder is also a web service on the Center of Genomic Epidemiology (CGE) bacterial analysis platform to study AMR genes from WGS data (Thomsen *et al.*, 2016). Resfinder is a well-suited technique to study AMR surveillance. Moreover, it only deals with those genes which are acquired or present on the plasmid and do not deal with chromosomal mutation, multidrug transporter, and other intrinsic resistance in bacteria (Thomsen *et al*., 2016; Zankari *et al*., 2012).

2.17.3 CARD

CARD is another antibiotic resistance algorithm. It has two options of analysis as blast and RGI (resistance gene identifier). The first approach performs BLAST searches on smaller DNA sequences supplied by the user to the CARD reference sequences. The RGI analyzes the data by two methods. The first method of resistant gene detection is based on BLAST sequenced similarity called the protein homolog model. The second method is called "protein variant models" which detect mutations in the target genome and confer AMR (McArthur *et al*., 2013). As the RGI currently works on protein sequences, when any contigs of WGS are submitted to the tool. After predication or identification of the open reading frame, it will then analyze the predicted protein. However, it can also analyze the bulk of the genome at a time (McArthur & Wright, 2015, McArthur *et al.*, 2013).

2.17.4 ARG-ANNOT

ARG-ANNOT is another antibiotic resistance gene database. It works by using the combination of BioEdit and the local BLAST algorithm. The software can be easily downloaded on a PC and can analyze the data without internet access. The ARG-ANNOT provides three databases. Firstly, the FASTA format and the header will have the information. Secondly, plasmid bearing antibiotic resistance genes with protein or DNA nucleotide sequences. The third is the mutation identifying database. Now, with this tool, we cannot detect mutations automatically. However, the target gene sequence will match with the reference sequence, and the mutation can be found manually by the user (Gupta *et al*., 2014, Anjum *et al*., 2017).

Following are some other genomic and phenotypic searches that can be found on the website of "Center for genomic epidemiology"

LRE-finderhttps://cge.cbs.dtu.dk/services/LRE-finder/

PathogenFinderhttps://cge.cbs.dtu.dk/services/PathogenFinder/

VirulenceFinderhttps://cge.cbs.dtu.dk/services/VirulenceFinder/

MLSThttps://cge.cbs.dtu.dk/services/MLST/

PlasmidFinderhttps://cge.cbs.dtu.dk/services/PlasmidFinder/
SpeciesFinderhttps://cge.cbs.dtu.dk/services/SpeciesFinder/

2.17.5 Kmer Resistance

Kmer resistance is another mapping tool available both as command-line and web server version. The methodology of the Kmer tool is to count the number of K-mer occur between raw data and reference database and perform mapping against the ResFinder gene database. To find probable AMR impurities and reduced false-positive results by adding a novel quality validation estimation of data. The quality of identified resistant gene is measured by exponential survival function (Clausen *et al*., 2016; Anjum *et al*., 2017). Bioinformatics applications have been significantly important for the analysis and interpretation of next-generation sequencing data. More advanced computational tools can now analyze and interpret the NGS data in a more standard fashion and provide workable results (Lelieveld *et al*., 2016; Thomsen *et al*., 2016).

Based on the amount of overlapping (co-occurring) k-mers, the KmerFinder determines the bacterial species (6-mers). In the reference database, there is a k-mer overlap between the query genome and the reference genome. (Hasman *et al*., 2014). If the first 16-mer starts at position N and finishes at position "N + 15," all of the data was divided into overlapping 16-mers. The next 16-mer starts at position N + 1 and finishes at position N + 16, and so on. Only 16-mers with the prefix ATGAC were retained to decrease the size of the final 16-mer database. Finally, the query genome is projected to the genome's species. It is also compared to the common 16-mers, regardless of the 16-mers' position. (Hasman *et al*., 2014)

Detection of genetic determinants of antibiotic resistance requires analytical tools and databases, which will be very useful to determine these genotypic-phenotypic correlations. Therefore, the combination of concerned bioinformatics and biological competencies is based on the mutual collaboration of these two systems. Lastly, the need for proper validation of software and target databases will be completely documented and calibrated (Anjum *et al*., 2017).

3.0 Methodology

3.1 Sample collection

In this study all the chicken liver samples were collected from the National Reference Laboratory for Poultry Diseases in Pakistan via federal and provincial sentinel surveillance laboratories under a national surveillance program from 2015 to 2017. The samples were collected from four provinces Punjab, Sindh, Khyber-Pakhtunkhwa, Baluchistan, and the capital Islamabad. In this study chicken liver from culled layer and broiler chickens that had poor birth growth and reduced appetite but not otherwise symptomatic for colibacillosis were aseptically removed using fully sterilized gloves and then placed in a sterilized container. However, the liver was easy in collection and transportation as compared to other parts like blood as well as more invasive and competitive bacteria to reach the liver. Moreover, each liver was separately packed in a sterilized plastic bag and then ship in an icebox to the National Reference Lab for Poultry Diseases NRLPD. As National lab of poultry diseases (NRLPD) has a well-established surveillance system in coordination with provincial livestock departments in Pakistan. The samples were then received by the well-trained person in the NRLPD reception. Moreover, before transfer to the bacteriology lab, a proper record was maintained of each received sample with a given lab ID.

3.2 Microbiological Analysis of Poultry Isolated *E. coli* 3.2.1 Isolation

The liver received in the bacteriology lab was sterile from the surface by a hot iron strip. After sterilization from the surface, a loopful of the liver inside was inoculated onto the autoclaved nutrient broth and incubated at 37°C for 24 hours. Thereafter, the loopfuls from the broth were streaked on EMB (Eosin methylene blue) and MA (MacConkey Agar) (Quinn *et al.*, 2002). Agar plates were incubated for 24 hours at 37°C. Subsequently, on the next day, as lactose-fermenting *E. coli* had flat, dark colonies with a green metallic sheen on EMB, and dry, flat, pink colonies with a surrounding darker pink area were observed on MA. The suspected colony was picked up and streaked on blood agar for morphological and biochemical identification (Amer *et al.*, 2018, Quinn *et al.*, 2002). However, the strains were further confirmed by using API 20E kits (Biome Rieux, Durham, NC).

3.3 Gram Staining and Colony Morphology

Gram-negative bacteria (*E. coli*) were studied based on size (large, moderate, small), colony shape (round, irregular), colony color (green-metallic sheen, pink), and colony margins and elevation

(concave, convex, raised). The only single isolated colony of such characteristics was further examined by gram staining. Few drops of saline water were placed on the sterile glass. A part of the isolated colony was mixed with saline drop with a sterile wire loop and could air dry and heat fixed. Crystal violet was applied on the dried smear sustain for 1 min. After washing with distilled water, the smear was covered with mordent (iodine) solution for 1 min. Then, the smear was washed with a decolorizer (ethanol) for few secs, after washing with water. Before the smear was covered with Safranin (counterstain) for 30 secs, it was washed with water. After 30 secs, the slide was washed, air-dried, and examined under the microscope (100X), applying immersion oil (Quinn *et al.*, 2002).

3.4 Biochemical Confirmation

E. coli were further confirmed by using the following biochemical tests

3.4.1 Catalase Test

This test is used to differentiate *E. coli* (catalase-positive) from non-catalase (*Streptococcus*) species. The test was performed to detect the presence of catalase enzyme in the organism by using hydrogen peroxide (H_2O_2). A drop of 3% hydrogen peroxide was placed on a clean, sterile glass slide. A colony was picked with the help of a sterile toothpick and mixed with an H_2O_2 drop. Bubble production indicates a positive result. Catalase enzyme converts H_2O_2 into O_2 and H_2O. Therefore, no bubble formation was indicative of a catalase-negative organism (Dezfulian *et al.*, 2010, Bertrand *et al.*, 2002).

3.5 Motility Test by Hanging Drop Method

This test is performed to check if bacteria are motile employing flagella. As non-motile bacteria do not possess flagella. While *E. coli* are a motile organism.

Procedure

- A single drop of water was placed on the center of the coverslip
- A single colony of bacteria was mixed with water on the coverslip
- Coverslip was placed on the central dispersion side of the glass slide. The coverslip was stick with the slide, when the slide has inverted the drop of bacteria had suspended with the Eppendorf wall.
- In the end, the motility was examined under X400 of the microscope.

3.6 Analytical Profile Index (API20E)

API 20E is series of biochemical tests for the identification and differentiation of the members of the Enterobacteriaceae family. It consists of plastic strips having twenty mini test chambers and has a chemically defined composition for each test. The list of tests included in API20E are, ONPG, ADH, LDC, ODC, CIT, H2S, URE, TDA, IND, VP, GEL, GLU, MAN, INO, SOR, RHA, SAC, MEL, AMY, ARA, and OX test are included in the API20E (Robinson *et al.*, 1995; Miller, 1991).

Protocol

- A single and isolated colony was picked from a pure culture mixed with sterile distilled water or in normal saline.
- API20E strip compartments were filled up with bacterial culture according to API20E guidelines.
- Two drops of sterile wood oil were added to ADH, LDC, ODC, H2S, and URE compartments after the bacterial suspension.
- The stray was sprayed with sterile water to keep the environment moist for bacterial growth.
- API tray was labeled with identification number and date.
- The tray was having a bacterial culture and was incubated at 37°C for 18-24 hrs. Reagents adding on next day

Some of the test results were read directly based on the change in color after 24 hrs.

While in some we added reagents before reading. Following reagents were added in specific compartments.

1. TDA compartments added one drop of Ferric Chloride
2. IND compartments added one drop of Kovascs reagent
3. VP compartments added one drop of VP1 reagent and one drop of VP2 reagent

(Waited for 10 min to note its result).

However, the API-20E kits were incubated at 37°C for 24 hrs. Quality control of the kits was performed by inoculating kits with quality-control microorganism "*E. coli*". In addition, autoclaved distal water was processed alongside every suspected *E. coli* as a negative control. Further, the online API web.bioMérieux.com analytical was used to identify both the presumptive Avian *E. coli* and confidence associated with the identification (Robinson *et al.*, 1995, Miller, 1991).

3.7 MALDI-TOF used for Sample Identification

MALDI-TOF (matrix-assisted laser desorption/ionization-time of flight mass spectrometry) was used for the *E. coli* identification before further molecular analysis. It is a new technique used for bacterial and fungi identification in many research laboratories (Wieser *et al.*, 2012, Eigner *et al.*, 2009). The isolated strains were streaked on blood agar and incubated at 37°C for 24 hrs, before MALDI-TOF.

The protocol followed for MALDI-TOF

- The pure isolated colony was mixed with 1 ul of matrix solution on the target plate (steel plate) to dry
- The matrix solution was crystalized with sample
- The loaded targeted plate was inserted into the machine and then it was transferred to the measuring chamber
- With the insertion of the loaded plate, the air was introduced to create a high vacuum before sample analysis
- Then the samples were exposed to laser pulses
- The laser pulses vaporized the loaded samples with solution matrix, lead the ionization of proteins (ribosomal)
- An electromagnetic field of 20 kV accelerated the ions before they entered the flight test tube
- The time of flight (TOF) of the ions particles or analytes were measured reaching the detector end
- Both masses of protein and degree of ionization was based to measure the time of flight (TOF)
- Based on the time of flight (TOF) information, a spectrum was generated by an automatic machine which was unique for every species.

3.8 Antibiotic Susceptibility Profiling

Antibiotic susceptibility test of the isolated *E. coli* was conducted by using the Kirby-Bauer disk diffusion method (Boyen *et al.*, 2010). The test was performed according to the guideline of the clinical and Laboratory standard Institute (CLSI, 2017). Muller Hinton agar was prepared following the manufacturer's instructions (Oxiod, UK). Control strains used in this study were *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 (Jones *et al.*, 2005). A panel of Twenty-one (21) antibiotics was tested in Pakistan, included amoxicillin (25 µg), penicillin (10 µg), gentamycin (30

µg), neomycin (30 µg), spectinomycin(100 µg), streptomycin (25 µg), ciprofloxacin (5 µg), flumequine (30 µg), enrofloxacin (5 µg), norfloxacin (10 µg), sulfamethoxazole-trimethoprim, (25 µg), erythromycin (30 µg), chloramphenicol (30 µg), ceftiofur (30 µg), doxycycline (30 µg),oxytetracycline (30 µg), meropenem(10 µg), ertapenem (10 µg), colistin Sulphate (25 µg), nitrofurantoin (300 µg), lincomycin (15 µg) were selected in first phase of this study.

The *E. coli* strains that had been preserved in 50% glycerol and PBS solution were sent in dry ice to Gautam Lab Washington University St. Louis United States of America. However, some of the strains had lost their viability and the revive was re-tested against the thirty-one (31) antibiotics for extended spectrum in GD lab USA. The antibiotics panel were included ampicillin (10 µg), cefazolin (30 µg), cefotetan (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefepime (30 µg) meropenem (10 µg), imipenem (10 µg), piperacillin-tazobactam (100/10 µg), ceftolozane-tazobactam (30/10 µg), ceftazidime-avibactam (30/20 µg), ampicillin-sulbactam (20 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), gentamicin (10 µg), amikacin (30 µg) trimethoprim-sulfa (25 µg), fosfomycin (200 µg), colistin (10 µg), aztreonam (30 µg), doxycycline (30 µg), minocycline (30 µg), tigecycline (15 µg), nitrofurantoin (300 µg), chloramphenicol (30 µg), norfloxacin (10 µg), streptomycin (10 µg), lincomycin (2 µg), ceftiofur (30 µg), oxytetracycline (30 µg), spectinomycin (100 µg) and delafloxacin (5 µg).

The interpretation of the zone of clearance was used to create a heatmap with hierarchical clustering for each isolate in the heatmap (R studio). ComASP^{TM} (Liofilchem) was used exactly in concordance with the manufacturer's instructions to quantify colistin resistance in isolates with identified in silico resistance determinants.

Table 3.0 Antibiotics and zone of diameters

		Zone Diameters in mm		
Antibiotics	**Disc code**	**Resistant**	**Intermediate**	**Susceptible**
Amikacin	AK	≤14	15-16	≥17
Amoxycillin	AMC	≤13	14-17	≥18
Ampicillin	AMP	≤13	14-16	≥17
Aztreonam	ATM	≤17	18-20	≤21
Cefazolin	KZ	≤19	20-22	≤23
Cefepime	FEP	≤14	15-17	≥18
Cefixime	CFM	≤15	16-18	≥19
Ceftazidime	CAZ	≤17	18-20	≥21
Ceftriaxone	CRO	≤19	20-22	≥23
Chloramphenicol	C	≤12	13-17	≥18
Ciprofloxacin	CIP	≤20	21-30	≤31
Colistin	CT	≤10	----	≥11
Doxycycline	DO	≤10	11 -13	≥14
Ertapenem	ETP	≤18	19 -21	≥22
Erythromycin	E	≤13	14-22	≥23
Gentamicin	CN	≤12	13-14	≥15
Imipenem	IPM	≤13	14-15	≥16
Levofloxacin	LEV	≤13	14-16	≥17
Meropenem	MEM	≤19	20-22	≥23
Minocycline	MH	≤12	13-15	≥16
Nitrofurantoin	F	≤14	15-16	≥17
Norfloxacin	NOR	≤12	13-16	≥17
Penicillin	P	≤26	27-46	≥47
Piperacillin - Tazobactam	TZP	≤17	18-20	≥21
Spectinomycin	SH	≤14	15-17	≥18

Streptomycin	S	≤11	12-14	≥15
Sulfisoxazole	SFX	≤12	13-16	≥17
Tetracycline	TE	≤11	12-14	≥15
Tigecycline	TGC	≤15	16-23	≥24
Trimethoprim	W	≤10	11-15	≥16
Trimethoprim-Sulfamethoxazole	SXT	≤10	11-15	≥16

3.8.1 Protocol used in AST (Antibiotic Susceptibility Testing)

The following steps were followed in performing the Kirby Bauer disc diffusion method

- Muller Hinton agar plates were prepared under the manufacturer's instructions and incubated at 37°C for 18-24 hours to see any contamination 5 ml of sterile normal saline was taken in a sterilized 5 ml glass tube
- 2-5 isolated colonies were picked with help of a sterile cotton swab, inoculated in the tube containing normal saline, and mixed well.
- The turbidity of the tubes was adjusted and made equal to 0.5 McFarland standard solution.
- Then a new sterile swab was dipped and pushed against the wall of the tubes to remove the extra solution.
- The dipped swab was used to make lawn on Muller Hinton agar plates. This all process was completed within 15 minutes.
- Followed by antibiotic discs were placed with the help of sterilized forceps
- On Muller Hinton agar plates the antibiotic discs were placed 24 mm apart from each other
- Then MHA Plates were incubated at 37°C for 24 hours.
- After the above incubation time, zones were measured with the help of a scale, and readings were recorded.

3.9 Molecular Characterization

Biochemical and morphological identification was followed by more advanced and authentic

molecular identification techniques. However, DNA extraction was the initial step for most molecular identification and characterization techniques. The methods followed were previously described by (Potter *et al.*, 2018b).

3.9.1 Culture Preparation

Poultry isolates were identified as *E. coli* by MALDI-TOF, and biochemical techniques were refreshed on blood agar plates by streaking and were incubated at 37°C for 18-24 hours in aerobic conditions.

3.9.2 DNA Extraction

Genomic DNA extraction was done by using a bacteremia DNA isolation Kit (Qiagen, Germantown, MD).
CB1: Suspending Buffer CB2: DNA digestion solution CB3: DNA Digestion powder CB4: DNA capture Buffer CB5: DNA elution Buffer

Protocol

- Sterile wooden swaps were used to pick isolated colonies from blood agar plates and were transferred to a 2 ml collection tube. And were then centrifuged at 13,000 x g for 2 minutes. The supernatant of each tube was decanted.
- Added 450 µL of prewarmed (55°C) of CB1 solution to the pellet and resuspended by pipetting. Lysates were transferred to the 2 mL microbead tubes. Vortexed for 10 seconds and then placed in a 70°C heat block for 15 minutes.
- Microbead tubes were again vortexed for 10 minutes (secure to vortexer with tape). After vortex, microbead tubes were centrifuged at 10,000 x g for 1 minute and the supernatants were transferred to new 2 mL collection tubes.
- Added 100 µL of CB2 solution in each tube, were mixed by vortex and then incubated at room temperature for 5 minutes followed by centrifugation at 10,000 x g for 1 minute. The supernatant of every tube was transferred to new collection tubes.
- Added 1 mL of CB3 solution into the supernatant was vortexed to mix and centrifuged briefly to collect any liquid from the top of the lid.

Loaded 600 µL of lysate onto each spin filter and centrifuged at 10,000 x g for 1 minute to bind. Flow-through was discarded and placed the Spin Filters back into the same 2 mL collection tubes. That step was repeated twice more until all the lysates were loaded onto the spin filters.

- Spin Filter was transferred to a new 2 mL collection tubes and washed by adding 500 µL CB4 solution to each column. Centrifuged at 10,000 x g for 1 minute. Discarded the flow-through and placed the columns back in the same collection tubes.
- The washing step was repeated with another 500 uL Solution CB4 and spin at 10,000 x g for 1 minute. Again, discarded the flow-through and placed the columns back in the same collection tubes.
    - To dry out the columns, the 2 ml tubes were centrifuged at 13,000 x g for 2 minutes.
- Spin Filters of each tube were transferred to a new collection tube and eluted by adding F50 uL of CB5 solution directly to the center of the columns. Incubated at room temperature for up to 5 minutes to maximize the elution efficiency. Centrifuged at 10,000 x g for 1 minute. Caped the 2 mL collection tubes containing the genomic DNA and Stored at -20°C in the freezer.

3.9.3 Preparation of Sequencing Libraries using the NextEra DNA Library Prep Kit

In preparation of the NextEra library, 0.5ng of genomic DNA was used as input for constructing NextEra Illumina sequencing libraries (Illumina, San Diego, CA) protocol followed in the previous study (Baym *et al.*, 2015a, Adey & Shendure, 2012).

3.10.1 Reagents

- Ethanol (80%)
- Nuclease free water
- Resuspension buffer (10 mM Tris-Cl, mM EDTA, 0.05% Tween 20, pH8.0)
- Agencourt Magnetic beads

3.9.3.1 Preparation

The following settings were set on a thermocycler (Bio-Rad)

- Preheated block at 55°C for 15 min
- Incubated samples at 55°C for min
- 4°C for forever

Note: The above parameter setting was used for a thermocycler. As the blocking temperature reached 55°C, the sample plate was placed in a thermocycler and pressed the skip button followed by a pressed (ok) button to incubate the samples for 15 min. After 15 min, the run was canceled, and the plate was taken out and placed on the ice.

- The magnetic bead was brought out onto the working bench, as 30 min earlier before used.
- Tag-mentation DNA buffer (TD) was Thaw on ice.
- TD Enzyme was taken out from -20 just before use, was kept on an ice bag and the leftover was immediately returned to the freezer.

3.9.3 Procedure

3.9.3.1 DNA sample preparation

For library preparation, the DNA was diluted up to the final concentration of 0.5 ng/ µL in nuclease-free water.

3.9.4.2 Precautions

- Nuclease-free water was used as a diluent.
- We had diluted DNA just before use to avoid any degradation
- As the possible smaller volume of pipetting was avoided

3.9.4.3 Tag Mentation

- Both TD and TDE1 were thaw on ice.
- Arrayed diluted genomic DNA,0.5 ng/µl in a 96- well plate
- Preheated Bio-Rad thermocycler to 55°C
- Tag mentation master mix was prepared

Table 3.1 Reagents quantified for PCR reaction

Component	µl per rxn	µl in the mix (100X)
TD Buffer	1,25	125
TDE1 Enzyme	0.125	12.5
Nuclease Free Water	0.125	12.5
Genomic DNA	1	

- Distributed 1.5 µL TMM into 96-well plate by using the electronic repeat pipette. The first dispense was wasted to avoid air bubble
- Transferred 1 µL of genomic DNA to all wells containing TMM solution by using a multichannel pipette.
  - Sealed 96-well plate and spin to bring all solution down
  - Incubated plate in Bio-Rad thermocycler for 15 min at 55°C (using above instruction)
  - During this period index plate was thawed at room temperature. Once thawed, the plate

was spinning to bring all solutions down.

• Index Plate was cooled down on the ice before proceeding to adapter addition.

Note. In tag mentation steps, the 96-well plate was placed on ice before and after incubation at 55°C

3.9.4.4 Adapter Addition

- Indexing primers aliquoted were mixed at 5 µM and in 10µl aliquots.
- Added 11.2 µl KAPA HiFi PCR master mix into each well, by using 100 µl electronic pipette
- Then Distributed 8.8 µl of primer in each well-having DNA, mixed well by using a multichannel pipette up and down 10x
- Followed by spinning the sealed plate After spinning the plate was placed in a thermocycler and the following program was run on the machine

1. 72°C for 3 min
2. 98°C for 5 min
3. 63°C for 30 secs
4. 72°C for 30 sec
5. Step 3-

5 repeated 13x (total 14 cycles)

6. 72°C for 5 min

7. 7. 4°C forever

3.9.4.5 PCR Clean Up

a) Reagent preparation

- AMPure XP beads were brought on the working bench, resuspended thoroughly. In parallel, the resuspension buffer from the NextEra Kit was thawed.
- However, we can also use 10mM Tris-Cl + 1mM EDTA+ 0.05% Tween-20 (pH 8.0) instead of resuspension buffer as in (Baym *et al*., 2015).
- Fresh 80% of ethanol was prepared
- The plate was centrifuged as a post PCR process. b) Binding
- Added 22.5 µL of beads to each well and mixed thoroughly by pipetting.
- Incubated the plate for 5 minutes
- After incubation, the plate was placed on a magnetic stand for 2 minutes to separate beads
- The supernatant was removed and discarded from the plate, but the beads were not disturbed

c) Washing

- Washed 2x (two times) the plate with 200 µl of 80% ethanol with a gap of 30 seconds, as beads were not disturbed in washing steps
- Followed by the complete removal of ethanol the plate could air dry for 15 minutes at room temperature

d) Recovery

- The plate was removed from the magnetic stand. Added 60 µL of resuspension buffer and pipette up and down 10-15x to mixed (avoided bubble formation at this step)
- The plate was incubated at room temperature for 5 minutes
- The plate was again placed on a magnetic stand, allowed the liquid to be clarified for 2 minutes
- Transferred 57 µL to new 96 well plate

Note: At this point, the plates were sealed and stored at -20°C till sequencing.

DNA quantification using Qubit Fluorometer

- PCR tubes (0.5 ml) were labeled for standards and samples.

1. The assay required

a) BR dsDNA kit was selected for our DNA quantification b) DNA samples and 2 standards

- Qubit working solution was made by diluting the Qubit dsDNA BR reagent 1200 in Qubit ds DNA BR buffer. And for this clean plastic tube was selected

  • Loaded 190 µl of Qubit working solution into each standards tube
- Followed by added 10 µl Qubit standards to the appropriate tubes (standard 1 & standard 2 were mixed by vortexing for 3-4 seconds)

  • Qubit working solution of 198 µl was added into individual assay tubes
- To gain the final volume of 200 µl, 2 µl samples were added into appropriate assay tubes

  • All tubes were incubated at room temperature for 2-3 minutes
- After selecting the Quant-IT dsDNA BR on the home screen of the Qubit 2.0 fluorometer, the GO button was pushed

  • Then on the Standard screen, "new calibration option was chosen"
- Standard #1 was inserted in the machine, the lid was closed, and pressed the go option (each read will take 3 seconds)
- Standard #1 was removed, inserted standard # 2 and pressed the repeat option to read the samples, standard # 2 was removed, and inserted the next sample "selected
  The sample option" and the "Go" option was pressed, the process was repeated until all samples were done (Mandal *et al.*, 2016).

3.10 Illumina Whole Genome Sequencing

Frozen stocks of *E. coli* isolates were plated onto blood agar using four-quadrant streaking and ~10colonies from the fourth quadrant were used as input for the Bacteraemia genomic DNA extraction kit (Qiagen, Germantown, MD, USA). 0.5 ng of genomic DNA per isolate was used to create sequencing libraries with the NextEra Kit (Illumina, San Diego, CA, USA) (Baym *et al.*, 2015b). The libraries were pooled together at equimolar concentrations and sequenced on a NextSeq 500 to obtain 25X-183X coverage of each genome with 2 x 150 bp reads. The reads were demultiplexed by barcode and Illumina adaptors and contaminating sequences were removed with Trimmomatic v.38 (Bolger *et al.*, 2014) and Deconseq v.4.3 (Schmieder & Edwards, 2011), respectively. The processed reads were used to construct de-novo assemblies of each genome with SPAdes v3.13.0 (Bankevich *et al.*, 2012). The assembly metrics of the *scaffolds.fasta* files were assessed with QUAST v4.5 (Gurevich *et al.*, 2013) and open reading frames identified with Prokka v1.12 (Seemann, 2014). 92 genomes with acceptable coverage were chosen for downstream genomic and phenotypic analysis.

3.11 Antibiotic Resistance Gene Identification Visualization

The coding region of the assembler sequences was identified by using prokka version 1.12. (Seemann; 2014). Resfinder was used to study the resistance genes in the annotated assembly. Subsequently, version 4.0 was loaded on the local cluster. Aminoglycoside, Colistin, Fosfomycin, Glycopeptide, Nitroimidazole, Oxazolidinone, Quinolone, Rifampicin, Tetracycline, Beta-lactam, fusidic acid, Macrolide, Phenicol, Sulphonamide and Trimethoprim (Clausen *et al*., 2016).

3.12 In Silico Analysis

To obtain phylogroup information for each *E. coli* genome, we used 11 *E. coli* genome publicly, available (Figure 2.0). The publicly available genomes were gathered from known *E. coli* phylogroups and identified open reading frames using Prokka (Hutton *et al.*, 2018, Schreiber *et al.*, 2017). The *.gff* files from Prokka for the phylogroup reference strains and the genomes sequenced in this study were used as input for Roary v3.12.0 to construct a core-genome alignment of the F core-genes (Core genome of 2,755 was used because that is the number of genes roary identified in the core genome) with PRANK v1.0 (Page *et al.*, 2015, Loytynoja, 2014).

The core-genome alignment file was converted into an approximate maximum likelihood tree with FastTree v2.1.10 and the resulting Newick file was uploaded to iToL (https://itol.embl.de/) (Price *et al.*, 2010, Letunic & Bork, 2007). In parallel, we identified *in silico* antibiotic resistance determinants for acquired antimicrobial genes using ResFinder v4.0 and for *E. coli* single nucleotide polymorphisms with PointFinder v4.0 (Kleinheinz *et al.*, 2014, Zankari *et al.*, 2017). Additionally, we identified known virulence genes with VirulenceFinder v1.5 (Kleinheinz *et al*., 2014). Hypergeometric tests were used to determine the significant enrichment of isolate groups within phylogenetic clades or dendrogram clusters, with Bonferroni correction for multiple hypothesis testing.

3.13 Conjugation Assay

The reference Recipient strain of *E. coli* J53 strains is sodium azide resistant strains**.** It is *Escherichia coli* K-12 is modified to j53, and finally transferred to J53 by spontaneous mutations. The J53 is a 4.5 Mb long genome, and 50.8 mol% GC content. It contains 4,484 genes (Yi *et al*., 2012). The *E. coli* J53 is resistant to azide because of mutation in the *secA* gene (Oliver *et al*., 1990). We did a conjugation experiment to determine the *mcr1* positive isolates (EC_79) and could confirm the transfer of the *mcr1* gene to wild-type *E. coli* J53 as previously described (Potter

et al., 2018a). The presence of the *mcr1* gene on the plasmid was already confirmed by Illumina Whole-genome sequencing.

3.13.1 Protocol for Conjugation Assay

- Tryptic soya broth was prepared according to the manufacturer's instructions (Sigma Aldrich, St Louis, MO, USA).
- Both donor (*E. coli* 79) and recipient (*E. coli*) strains were separately suspended in TS broth
- The strains were diluted to 0.005 OD600.

After dilution, 100 µl of donor strain (*E. coli* 79) was suspended in the 100 µl of recipient strains (11 ratios) and diluted up to 5000 µl with TS broth

Now the Co-cultures were incubated at 37°C for 24 hours

On the next day 50 µl of co-cultures were suspended on agar plats (MacConkey agar) contained sodium azide and colistin (5mg/ml)

- The liquid co-culture was spread with glass beads on agar plates and was incubated at 37°C for 18-24 hours
  - However, individual trans-conjugants colonies were suspended in TS broth supplemented with colistin (5mg/ml) and were incubated at 37°C on a shaker of 220 rpm (Potter *et al*., 2018a).

3.14 PCR Optimization for Molecular Detection of *mcr-1* Gene

For molecular confirmation of conjugation assay, PCR conditions were optimized to amplify the *mcr1* gene in both E-79 and J53 strains. The gene was also amplified in E-07, E-25, E-50, and E98. For this purpose, a known pair of primer of 200 bp of the Forward 5'-AAATCAGCCAAACCTATCCC -3' and reverse primer 5'- CGTATCATAGACCGT GCCAT -3'. (Table 3.1), previously described (Zhang *et al.*, 2017).

Table 3.1: Scheme for a total volume of PCR reaction for *mcr1* gene amplification

PCR Reaction components	Stock concentration	Final concentrations	Volume per Reaction
PCR Master Mix	2X	1X	12.5 µl
Forward Primers Each	100 pmol/µl	2 pmol/µl	0.5 µl x 9 = 4.5 µl
Reverse Primers Each	100 pmol/µl	2 pmol/µl	0.5 µl x 9 = 4.5 µl
Nuclease Free Water	-	-	1.5 µl
Template DNA	-	-	2 µl
Total PCR volume			25 µl

Mplification of the desired amplicons bands, the PCR program was set as the first denaturation of DNA for (5 minutes at 95°C). Then, 32 cycles each consisting (30 seconds at 95°C) to carry out denaturation. Primer annealing was done at 55°C and extension at 72°C. Followed by extension for 10 minutes at 72°C and 4ºC forever. The optimization temperature was recorded at 55ºC for the *mcr1* gene.

3.14.1 Gel Electrophoresis

Agarose gels are prepared using a w/v percentage solution. Therefore, 1 % agarose was dissolved in 100ml of TAE buffers (40 mM Tris-acetate, 1 mM EDTA). The mixture of agarose/buffer was then melted in the microwave. After completely dissolved the agarose in the buffer, at a concentration of 0.5 µg/ml, ethidium bromide was added. The solution was cooled in a 65°C water bath. The gel tray was taped and pour the molten gel was into the gel tray. Moreover, wells were created by comb. At last, the comb has removed the gel was placed in the gel box (Lee *et al*., 2012).

PCR product was analyzed by gel electrophoresis. Then 2-5µl of the reaction product was combined with the loading dye for this. For this 2-5µl of the reaction product and 2-5µl of loading dye (Thermo scientific) was loaded into each well. In the first well, a DNA marker (Biolabs 100bp ladder) was also loaded. The gel was then run for 50 minutes of 90V in a 1X TBE buffer BIO-RAD gel electrophoresis tank. After the defined time, the gel was observed under an ultraviolet trans-illuminator to visualize bands. Photograph of PCR bands was saved in the BIO-RAD gel documentation system (Bintvihok *et al.*, 2016).

4.0 Results

4.1 Sample collection

In the total of five hundred and eleven (n=511) bacterial samples, two hundred and sixty-five (n=265) of *E. coli* were collected through the surveillance system of NRLPD. However, out of which twenty-five (n=25) were collected from Baluchistan, one hundred and twelve from Islamabad (n=112), Twenty-eight from Khyber-Pakhtunkhwa (n=28), Eighty-eight from Punjab (n=88), and Twelve of the *E. coli* from Sindh (n=12). Furthermore, out of 265 *E. coli* isolates, 225 were isolated from commercial and 40 isolates from backyard poultry (Table 1).

4.2 Gram staining and biochemical analysis

On selective media, dark convex and small size colonies with a green metallic sheen appeared on EMB (eosin methylene blue) (Figure 4.1a). While flat, pink colonies with a surrounding darker pink area were seen on MA (MacConkey Agar) (Figure 4.1b). Further, the isolated samples were observed under the microscope after differential staining (Gram staining). In addition, another biochemical analysis was done through the catalase test, where a rapid bubble was observed after the addition of few drops of hydrogen peroxide on the inoculum. The identification codes (ID) of API20E (Analytical profile index) and motility tests using the hanging drop method also given further biochemical identification (Appendix1).

a b

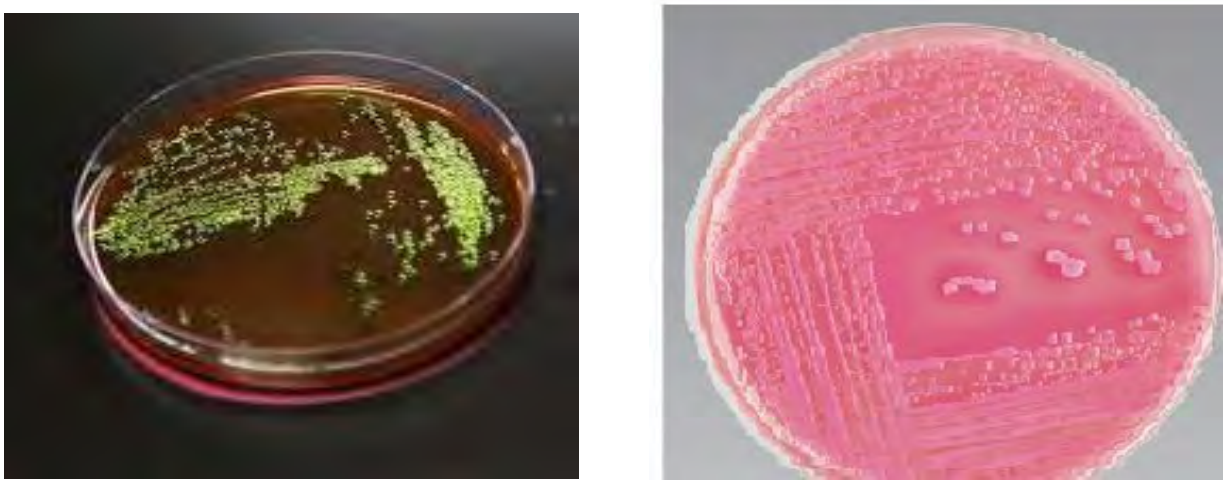


Figure-1 Colonies of *E. coli* growth on EMB (a) and MA (b).

S. No	Bacterial ID	Area/regions	Isolation	Type of samples	Isolates sequenced
1	15N-0006	Punjab	*E. coli*	Liver	Nil
2	15N-0061	KPK	*E. coli*	Liver	Nil
3	15N-0091	Punjab	*E. coli*	Liver	Nil
4	15N-0107	Islamabad	*E. coli*	Liver	Nil
5	15N-0107	Islamabad	*E. coli*	Liver	Nil
6	15N-0113	Islamabad	*E. coli*	Liver	Nil
7	15N-0229-3	Punjab	*E. coli*	Liver	Nil
8	15N-0230-1	Punjab	*E. coli*	Liver	Nil
9	15N-0230-2	Punjab	*E. coli*	Liver	Nil
10	15N-0264	Islamabad	*E. coli*	Liver	Nil
11	15N-0365-A	Islamabad	*E. coli*	Liver	Nil
12	15N-0365-B	Islamabad	*E. coli*	Liver	Nil
13	15N-0648	Sindh	*E. coli*	Liver	Nil
14	15N-0687-1	Islamabad	*E. coli*	Liver	Nil
15	15N-0744	Islamabad	*E. coli*	Liver	Nil
16	15N-0755	Islamabad	*E. coli*	Liver	Nil
17	15N-0758	Punjab	*E. coli*	Liver	Nil
18	15N-0763	Islamabad	*E. coli*	Liver	Nil
19	15N-0764	Islamabad	*E. coli*	Liver	Nil
20	15N-0765	Islamabad	*E. coli*	Liver	Nil
21	15N-0884	Punjab	*E. coli*	Liver	Nil
22	15N-0969	Sindh	*E. coli*	Liver	Nil
23	15N-1051	Islamabad	*E. coli*	Liver	Nil
24	15N-1097	Punjab	*E. coli*	Liver	Nil
25	15N-1098	Punjab	*E. coli*	Liver	Nil
26	15N-1100	Punjab	*E. coli*	Liver	Nil

27	15N-1132	Islamabad	*E. coli*	Liver	Nil
28	15N-1133	Islamabad	*E. coli*	Liver	Nil
29	15N-1134	Islamabad	*E. coli*	Liver	Nil
30	15N-1135	Islamabad	*E. coli*	Liver	Nil
31	15N-1136	Punjab	*E. coli*	Liver	Nil
32	15N-1137	Punjab	*E. coli*	Liver	Nil
33	15N-1138	Punjab	*E. coli*	Liver	Nil
34	15N-1156	Punjab	*E. coli*	Liver	Nil
35	15N-1162	Islamabad	*E. coli*	Liver	Nil
36	15N-1163	Punjab	*E. coli*	Liver	Nil
37	15N-1189	Islamabad	*E. coli*	Liver	Nil
38	15N-1441	Punjab	*E. coli*	Liver	Nil
39	15N-1444	Islamabad	*E. coli*	Liver	Nil
40	15N-1446-1	KPK	*E. coli*	Liver	Nil
41	15N-1446-2	KPK	*E. coli*	Liver	Nil
42	15N-1448	Punjab	*E. coli*	Liver	Nil
43	15N-1499	Punjab	*E. coli*	Liver	Nil
44	15N-1558	Punjab	*E. coli*	Liver	Nil
45	15N-1559	Islamabad	*E. coli*	Liver	Nil
46	15N-1656	Islamabad	*E. coli*	Liver	Nil
47	15N-1657	Punjab	*E. coli*	Liver	Nil
48	15N-1659	Islamabad	*E. coli*	Liver	Nil
49	15N-1661	Islamabad	*E. coli*	Liver	Nil
50	15N-1664	Quetta	*E. coli*	Liver	Nil
51	15N-1665	Quetta	*E. coli*	Liver	Nil
52	15N-1674	Quetta	*E. coli*	Liver	Nil
53	15N-1675	Quetta	*E. coli*	Liver	Nil
54	15N-1677	Punjab	*E. coli*	Liver	Nil
55	15N-1678	Punjab	*E. coli*	Liver	Nil
56	15N-1687	Quetta	*E. coli*	Liver	Nil
57	15N-1693	Quetta	*E. coli*	Liver	Nil
58	15N-1791	Sindh	*E. coli*	Liver	Ec-54
59	15N-1808	Peshawar	*E. coli*	Liver	Ec-101
60	15N-1818	Peshawar	*E. coli*	Liver	Ec-102
61	15N-1819	Islamabad	*E. coli*	Liver	Nil

62	15N-1832	Islamabad	*E. coli*	Liver	Nil
63	15N-1852	Punjab	*E. coli*	Liver	Nil
64	15N-1872	Quetta	*E. coli*	Liver	Nil
65	15N-1874	Islamabad	*E. coli*	Liver	Nil
66	15N-1877	Punjab	*E. coli*	Liver	Nil
67	15N-1904-1	Punjab	*E. coli*	Liver	Nil
68	15N-1904-2	Punjab	*E. coli*	Spleen	Nil
69	15N-1905	Islamabad	*E. coli*	Spleen	Nil
70	15N-1911	Islamabad	*E. coli*	Liver	Nil
71	15N-1923	Punjab	*E. coli*	Liver	Nil
72	15N-1924	Punjab	*E. coli*	Liver	Ec-100
73	15N-1942-1	KPK	*E. coli*	Liver	Nil
74	15N-1945	Islamabad	*E. coli*	Liver	Nil
75	15N-1946	Islamabad	*E. coli*	Liver	Nil
76	15N-1948	Islamabad	*E. coli*	Liver	Nil
77	15N-1950	Sindh	*E. coli*	Liver	Nil
78	15N-1951	Islamabad	*E. coli*	Liver	Nil
79	15N-1952	Islamabad	*E. coli*	Liver	Nil
80	15N-1953	Islamabad	*E. coli*	Liver	Nil
81	15N-1954	Islamabad	*E. coli*	Liver	Nil
82	15N-1956	Islamabad	*E. coli*	Liver	Nil
83	15N-1958	Islamabad	*E. coli*	Liver	Nil
84	15N-1960	Islamabad	*E. coli*	Liver	Nil
85	15N-1961	Islamabad	*E. coli*	Liver	Nil
86	15N-1965	Islamabad	*E. coli*	Liver	Nil
87	15N-1967-3	Islamabad	*E. coli*	Liver	Nil
88	15N-1985	KPK	*E. coli*	Liver	Nil
89	16N-0057	Punjab	*E. coli*	Liver	Nil
90	16N-0058	Punjab	*E. coli*	Liver	Nil
91	16N-0079	Islamabad	*E. coli*	Liver	Nil
92	16N-0105	Punjab	*E. coli*	Liver	Ec-21
93	16N-0188	Islamabad	*E. coli*	Liver	Ec-88
94	16N-0189	Punjab	*E. coli*	Liver	Ec-52
95	16N-0195	Punjab	*E. coli*	Liver	Ec-43
96	16N-0229	Punjab	*E. coli*	Liver	Ec-28
97	16N-0230	Peshawar	*E. coli*	Liver	Nil
98	16N-0231	Islamabad	*E. coli*	Liver	Ec-56
99	16N-0238	Islamabad	*E. coli*	Liver	Nil
100	16N-0243	Islamabad	*E. coli*	Liver	Nil
101	16N-0259	Islamabad	*E. coli*	Liver	Nil
102	16N-0263	Islamabad	*E. coli*	Liver	Ec-57

103	16N-0266	Islamabad	*E. coli*	Liver	Nil
104	16N-0295	Islamabad	*E. coli*	Liver	Ec-19
105	16N-0331	Islamabad	*E. coli*	Liver	Ec-44
106	16N-0403	Punjab	*E. coli*	Liver	Ec-22
107	16N-0412	Punjab	*E. coli*	Liver	Nil
108	16N-0469	Islamabad	*E. coli*	Liver	Nil
109	16N-0471	KPK	*E. coli*	Liver	Nil
110	16N-0491	Islamabad	*E. coli*	Liver	Nil
111	16N-0512-1	Islamabad	*E. coli*	Liver	Nil
112	16N-0549	Islamabad	*E. coli*	Liver	Nil
113	16N-0562	Punjab	*E. coli*	Liver	Nil
114	16N-0569	Islamabad	*E. coli*	Liver	Nil
115	16N-0572	Punjab	*E. coli*	Liver	Ec-55
116	16N-0583	Islamabad	*E. coli*	Liver	Nil
117	16N-0584	Punjab	*E. coli*	Liver	Nil
118	16N-0650	Islamabad	*E. coli*	Liver	Ec-108
119	16N-0650	Islamabad	*E. coli*	Liver	Nil
120	16N-0677	Islamabad	*E. coli*	Liver	Nil
121	16N-0679	Islamabad	*E. coli*	Liver	Nil
122	16N-0683	Islamabad	*E. coli*	Liver	Ec-60
123	16N-0685	Punjab	*E. coli*	Liver	Ec-32
124	16N-0695	Islamabad	*E. coli*	Liver	Ec-9
125	16N-0696	Punjab	*E. coli*	Liver	Ec-93
126	16N-0697	Islamabad	*E. coli*	Liver	Ec-1
127	16N-0698	Punjab	*E. coli*	Liver	Ec-46
128	16N-0703	Quetta	*E. coli*	Liver	Nil
129	16N-0705	Quetta	*E. coli*	Liver	Ec-45
130	16N-0706	Quetta	*E. coli*	Liver	Ec-23
131	16N-0707	Quetta	*E. coli*	Liver	Nil
132	16N-0709-A	Quetta	*E. coli*	Liver	Ec-33 A
133	16N-0709-B	Quetta	*E. coli*	Liver	Ec-33 B
134	16N-0711	Quetta	*E. coli*	Liver	Nil
135	16N-0712	Quetta	*E. coli*	Liver	Nil
136	16N-0713-B	Peshawar	*E. coli*	Liver	Ec-49
137	16N-0713-A	Quetta	*E. coli*	Liver	Ec-48 A
138	16N-0714	Quetta	*E. coli*	Liver	Ec-47
139	16N-0715	Quetta	*E. coli*	Liver	Nil
140	16N-0717	Quetta	*E. coli*	Liver	Ec-53
141	16N-0801	Islamabad	*E. coli*	Liver	Nil
142	16N-0813	Quetta	*E. coli*	Liver	Ec-24
143	16N-0814	Quetta	*E. coli*	Liver	Ec-25

144	16N-0815	Quetta	*E. coli*	Liver	Ec-59
145	16N-0819	Quetta	*E. coli*	Liver	Ec-61
146	16N-0820	Quetta	*E. coli*	Liver	Ec-27
147	16N-0821	Quetta	*E. coli*	Liver	Ec-26
148	16N-0832	KPK	*E. coli*	Liver	Nil
149	16N-0833-1	Islamabad	*E. coli*	Liver	Nil
150	16N-0833-2	Islamabad	*E. coli*	Liver	Nil
151	16N-0834	Islamabad	*E. coli*	Liver	Nil
152	16N-0835	Islamabad	*E. coli*	Liver	Nil
153	16N-0836	Islamabad	*E. coli*	Liver	Ec-62
154	16N-0837	Islamabad	*E. coli*	Liver	Nil
155	16N-0840-1	Islamabad	*E. coli*	Liver	Nil
156	16N-0840-2	Islamabad	*E. coli*	Liver	Ec-30
157	16N-0843	Punjab	*E. coli*	Liver	Ec-50
158	16N-0844-1	Punjab	*E. coli*	Liver	Nil
159	16N-0844-2	Punjab	*E. coli*	Liver	Ec-51
160	16N-0847-2	Punjab	*E. coli*	Liver	Nil
161	16N-0847-3	Punjab	*E. coli*	Liver	Ec-63
162	16N-0847-4	Punjab	*E. coli*	Liver	Ec-92
163	16N-0848	Punjab	*E. coli*	Liver	Ec-29
164	16N-0900	KPK	*E. coli*	Liver	Ec-96
165	16N-0901	KPK	*E. coli*	Liver	Ec-99
166	16N-0902	KPK	*E. coli*	Liver	Ec-72
167	16N-0903	KPK	*E. coli*	Liver	Nil
168	16N-0904	Punjab	*E. coli*	Liver	Ec-79
169	16N-0905-1	Punjab	*E. coli*	Liver	Ec-75
170	16N-0907	Islamabad	*E. coli*	Liver	Nil
171	16N-0909	Islamabad	*E. coli*	Liver	Nil
172	16N-0911-1	Punjab	*E. coli*	Liver	Nil
173	16N-0911-2	Islamabad	*E. coli*	Liver	Nil
174	16N-0915	Punjab	*E. coli*	Liver	Ec-64
175	16N-0917	Islamabad	*E. coli*	Liver	Nil
176	16N-0918	Islamabad	*E. coli*	Liver	Ec-84
177	16N-0921	Islamabad	*E. coli*	Liver	Ec-39
178	16N-0922	Islamabad	*E. coli*	Liver	Nil
179	16N-0924	KPK	*E. coli*	Liver	Ec-104
180	16N-0937-1	Punjab	*E. coli*	Liver	Ec-97
181	16N-0937-2	Punjab	*E. coli*	Liver	Nil
182	16N-0956-1	Islamabad	*E. coli*	Liver	Nil
183	16N-0956-2	Islamabad	*E. coli*	Liver	Nil
184	16N-0968	Punjab	*E. coli*	Liver	Ec-66

185	16N-0972	Islamabad	*E. coli*	Liver	Ec-87
186	16N-0973	Islamabad	*E. coli*	Liver	Ec-76
187	16N-0995-A	Islamabad	*E. coli*	Liver	Ec-94
188	16N-0995-B	Islamabad	*E. coli*	Liver	Ec-95
189	16N-0997	Punjab	*E. coli*	Liver	Ec-65
190	16N-0999	Islamabad	*E. coli*	Liver	Ec-90
191	16N-1003	Islamabad	*E. coli*	Liver	Ec-67
192	16N-1007	Islamabad	*E. coli*	Liver	Ec-10
193	16N-1012	KPK	*E. coli*	Liver	Nil
194	16N-1026	KPK	*E. coli*	Liver	Ec-77
195	16N-1038	KPK	*E. coli*	Liver	Ec-3
196	16N-1039	KPK	*E. coli*	Liver	Ec-71
197	16N-1043	Islamabad	*E. coli*	Liver	Nil
198	16N-1052	KPK	*E. coli*	Liver	Ec-89
199	16N-1062	Islamabad	*E. coli*	Liver	Ec-78
200	16N-1067	Islamabad	*E. coli*	Liver	Ec-73
201	16N-1068	KPK	*E. coli*	Liver	Nil
202	16N-1070	Islamabad	*E. coli*	Liver	Ec-70
203	16N-1073-1	Punjab	*E. coli*	Liver	Ec-86
204	16N-1073-2	Punjab	*E. coli*	Liver	Nil
205	16N-1074	KPK	*E. coli*	Liver	Ec-82
206	16N-1076	Islamabad	*E. coli*	Liver	Nil
207	16N-1078	Punjab	*E. coli*	Liver	Ec-98
208	16N-1081	Punjab	*E. coli*	Liver	Ec-83
209	16N-1082	Punjab	*E. coli*	Liver	Ec-85
210	16N-1083	Punjab	*E. coli*	Liver	Ec-80
211	16N-1086	Sindh	*E. coli*	Liver	Ec-69
212	16N-1099	Islamabad	*E. coli*	Liver	Ec-88
213	16N-1124-1	Sindh	*E. coli*	Liver	Ec-91
214	16N-1124-2	Sindh	*E. coli*	Liver	Ec-74
215	16N-1130-1	Punjab	*E. coli*	Liver	Ec-38
216	16N-1130-2	Punjab	*E. coli*	Liver	Ec-8
217	16N-1130-3	Punjab	*E. coli*	Liver	Ec-15
218	16N-1131-1	Islamabad	*E. coli*	Liver	Ec-31
219	16N-1131-2	Islamabad	*E. coli*	Liver	Ec-17
220	16N-1138-1	Punjab	*E. coli*	Liver	Ec-4
221	16N-1138-2	Punjab	*E. coli*	Liver	Ec-14
222	16N-1138-3 A	Punjab	*E. coli*	Liver	Ec-35 A
223	16N-1138-3 B	Punjab	*E. coli*	Liver	Ec-35 B
224	16N-1138-4	Punjab	*E. coli*	Liver	Ec-16
225	16N-1138-5	KPK	*E. coli*	Liver	Ec-5

226	16N-1139-A	Sindh	*E. coli*	Liver	Ec-34 A
227	16N-1139-B	Sindh	*E. coli*	Liver	Ec-34 1
228	16N-1140-1	Sindh	*E. coli*	Liver	Ec-2
229	16N-1140-2	Sindh	*E. coli*	Liver	Ec-20
230	17N-0002	Punjab	*E. coli*	Liver	Ec-36
231	17N-0049	KPK	*E. coli*	Liver	Ec-42
232	17N-0056	Punjab	*E. coli*	Liver	Ec-6
233	17N-0057	Punjab	*E. coli*	Liver	Ec-13
234	17N-0065	Punjab	*E. coli*	Liver	Ec-38
235	17N-0069	Islamabad	*E. coli*	Liver	Nil
236	17N-0070	Punjab	*E. coli*	Liver	Ec-7
237	17N-0071-1	Punjab	*E. coli*	Liver	Nil
238	17N-0071-2	Punjab	*E. coli*	Liver	Nil
239	17N-0076	Punjab	*E. coli*	Liver	Ec-18
240	17N-0077	Punjab	*E. coli*	Liver	Nil
241	17N-0078	Punjab	*E. coli*	Liver	Nil
242	17N-0081	Punjab	*E. coli*	Liver	Ec-12
243	17N-0083	Islamabad	*E. coli*	Liver	Nil
244	17N-0084	Islamabad	*E. coli*	Liver	Nil
245	17N-0085	Punjab	*E. coli*	Liver	Nil
246	17N-0105	Islamabad	*E. coli*	Liver	Ec-103
247	17N-0110	Sindh	*E. coli*	Liver	Ec-41
248	17N-0117	Punjab	*E. coli*	Liver	Ec-40
249	17N-0121	Islamabad	*E. coli*	Liver	Nil
250	17N-0177	Islamabad	*E. coli*	Liver	Nil
251	17N-0189	Islamabad	*E. coli*	Liver	Nil
252	17N-0190	Islamabad	*E. coli*	Liver	Nil
253	17N-0201	Punjab	*E. coli*	Liver	Nil
254	17N-0224	Punjab	*E. coli*	Liver	Ec-11
255	17N-0230	Punjab	*E. coli*	Liver	Nil
256	17N-0257	Islamabad	*E. coli*	Liver	Nil
257	17N-0258	Islamabad	*E. coli*	Liver	Nil
258	17N-0259-1	Islamabad	*E. coli*	Liver	Ec-37
259	17N-0272	Punjab	*E. coli*	Liver	Nil
260	17N-0291	Islamabad	*E. coli*	Liver	Nil
261	17N-0294-2	Punjab	*E. coli*	Liver	Nil
262	17N-0294-3	Punjab	*E. coli*	Liver	Nil
263	17N-0312	KPK	*E. coli*	Liver	Nil
264	17N-0313	Islamabad	*E. coli*	Liver	Nil
265	17N-0330	Islamabad	*E. coli*	Liver	Nil

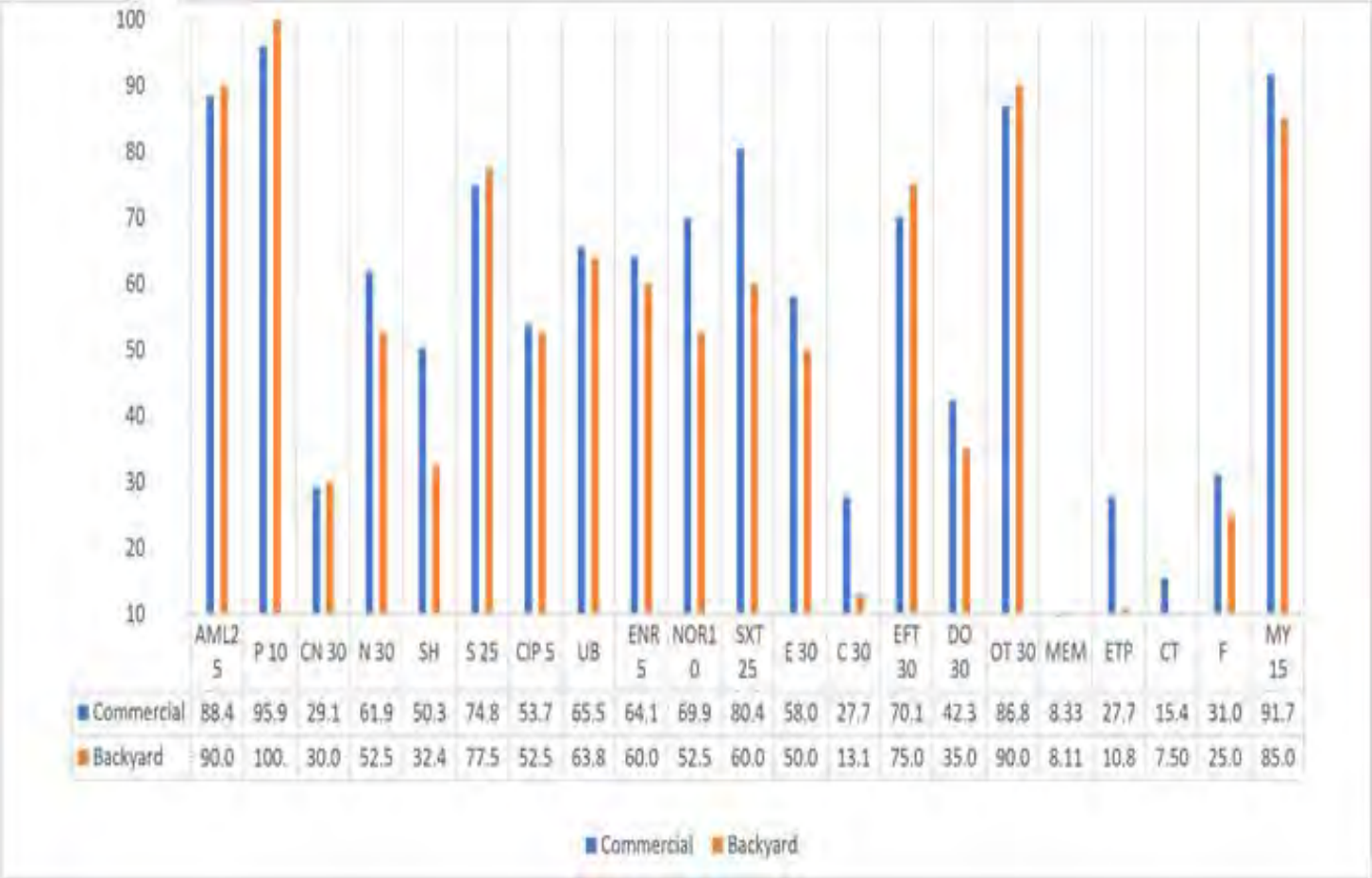
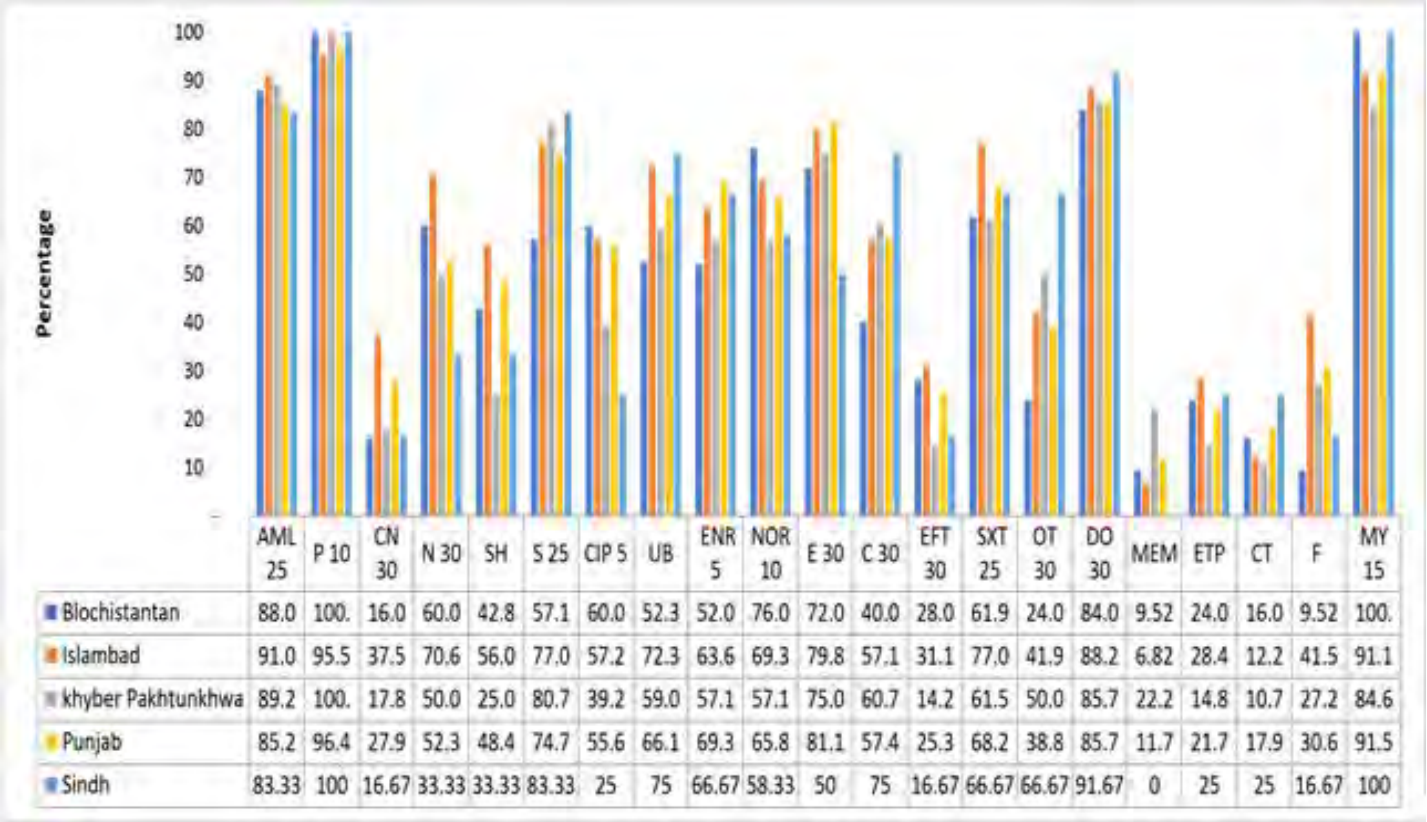


Figure 4.2 Antibiotic-resistant patterns of *E. coli* isolates from commercial (blue) and backyard (red) poultry, each antibiotic is written in percentage on the x-axis. Amp25 amoxicillin, p10 penicillin, cn30 gentamycin, n30 neomycin, spectinomycin, s25 streptomycin, cip5 ciprofloxacin, ub30 flumequine, enr5 enrofloxacin, nor10 norfloxacin, sxt25 sulfamethoxazoletrimethoprim, e30 erythromycin, c30 chloramphenicol, eft30 ceftiofur, do30 doxycycline, ot30 oxytetracycline, mem10 meropenem, etp10 ertapenem, ct10 colistin sulfate, f300 nitrofurantoin, my15 lincomycin.



	AML 25	P 10	CN 30	N 30	SH	S 25	CIP 5	UB	ENR 5	NOR 10	E 30	C 30	EFT 30	SXT 25	OT 30	DO 30	MEM	ETP	CT	F	MY 15
Blochistantan	88.0	100.	16.0	60.0	42.8	57.1	60.0	52.3	52.0	76.0	72.0	40.0	28.0	61.9	24.0	84.0	9.52	24.0	16.0	9.52	100.
Islambad	91.0	95.5	37.5	70.6	56.0	77.0	57.2	72.3	63.6	69.3	79.8	57.1	31.1	77.0	41.9	88.2	6.82	28.4	12.2	41.5	91.1
khyber Pakhtunkhwa	89.2	100.	17.8	50.0	25.0	80.7	39.2	59.0	57.1	57.1	75.0	60.7	14.2	61.5	50.0	85.7	22.2	14.8	10.7	27.2	84.6
Punjab	85.2	96.4	27.9	52.3	48.4	74.7	55.6	66.1	69.3	65.8	81.1	57.4	25.3	68.2	38.8	85.7	11.7	21.7	17.9	30.6	91.5
Sindh	83.33	100	16.67	33.33	33.33	83.33	25	75	66.67	58.33	50	75	16.67	66.67	66.67	91.67	0	25	25	16.67	100

Figure 4.3 Prevalence of antibiotic resistance data among *E coli* isolates from Baluchistan, Islamabad, Khyber Pakhtunkhwa, Punjab, and Sindh. Amp25 amoxicillin, p10 penicillin, cn30 gentamycin, n30 neomycin, sh spectinomycin, s25 streptomycin, cip5 ciprofloxacin, ub flumequine, enr5 enrofloxacin, nor10 norfloxacin, sxt25 sulfamethoxazole-trimethoprim, e30 erythromycin, c30 chloramphenicol, eft30 ceftiofur, do30 doxycycline, ot30 oxytetracycline, mem meropenem, etp ertapenem, colistin sulfate, f300nitrofurantoin, my15 lincomycin.

4.3 Data Availability

All genomes sequenced in this study have been uploaded to the NCBI WGS database associated with Bio Project PRJNA522294.

4.4 *E. coli* Revealed from Glycerol were Sequenced through WGS

E. coli samples frozen in 50% glycerol and PBS solution were transferred in fully sterile dry ice packing to Gautam lab Washington University St. Louis America. In which 92 of the *E. coli* strains were revived from glycerol stock after plating on blood agar. *E. coli* that was used for whole-genome sequencing and phenotypic analysis, are isolated 41.3% (38/92) from Punjab and 26% (24/92) from Islamabad. While the remaining isolates were originated from Khyber Pakhtunkhwa, 14% (13/92), Baluchistan, 12% (11/92), and Sindh, 7% (7/92) respectively (Figure 4.4).

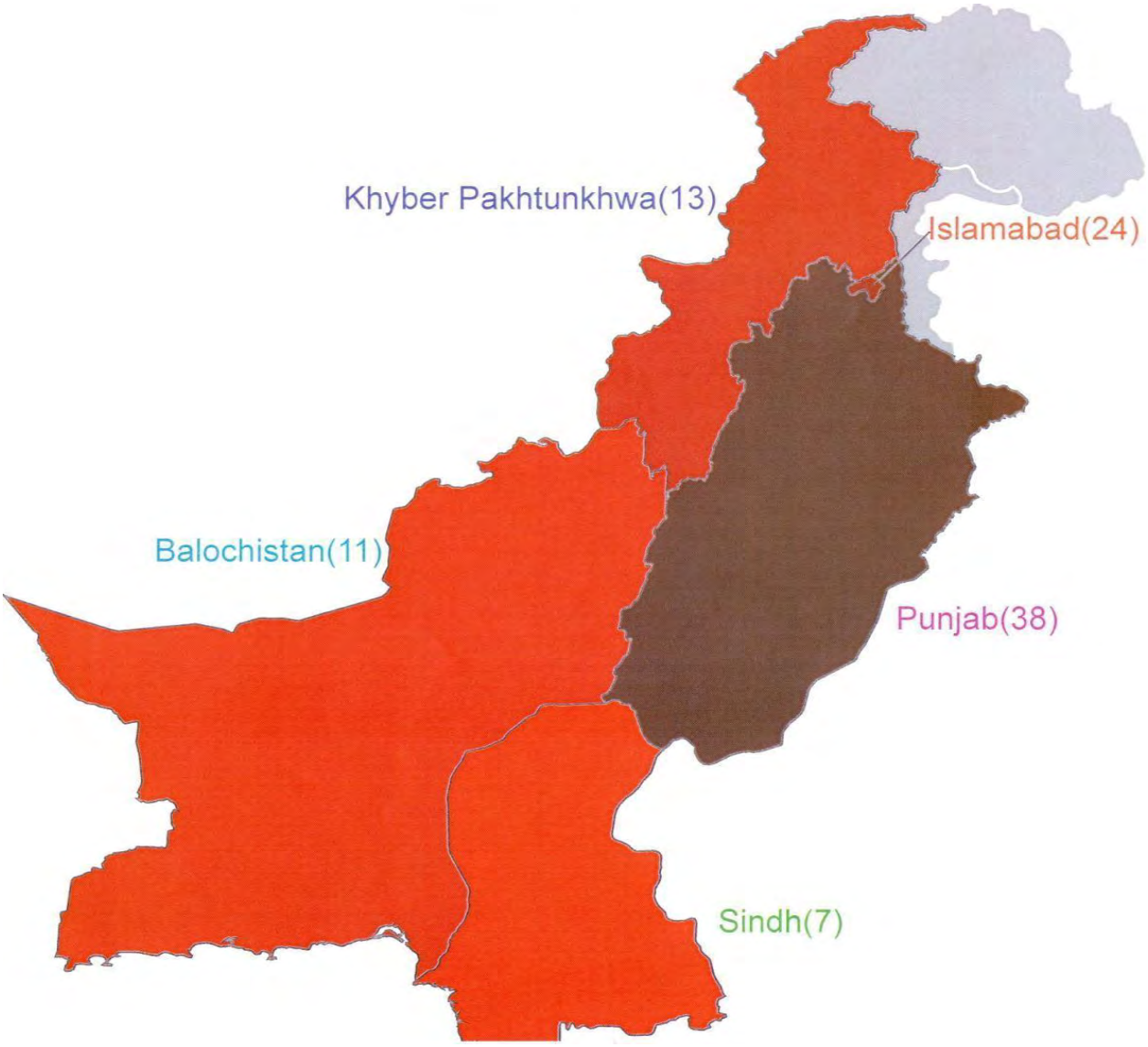


Figure 4.4: *E. coli* cohort originated from 5 regions in Pakistan Map depicting the five regions of Pakistan from where sampling of *E. coli* (from the chicken liver) was carried. The number of isolates showed adjacent to the provinces, and colors correspond to isolate from each region.

4.5 Antibiotic Profiling of the Isolated *E. coli*

To assess the effect of ARG burden on phenotypic antibiotic resistance, we performed antimicrobial susceptibility testing using the Kirby-Bauer Disk Diffusion method. Moreover, the Clinical Laboratory and Standards Institute (CLSI) interpretative criteria from the M100 (Edition 29) and VET01 (Edition 5) for Enterobacteriaceae on a variety of antibiotics relevant for human and veterinary use (Magiorakos et al., 2012). Using the definition of multidrug-resistant (MDR) as non-susceptibility to at least one agent in three or more antimicrobial classes, and extensively drug-resistant (XDR) as susceptibility to at least one agent in only one or two classes assayed, we found that 82/92 are MDR but only 1/92 are XDR (Figure 4.5) (Magiorakos et al.,2012). Consistent with the presence of *mdf* (A) in all the genomes, all isolates were resistant to the lincosamide antibiotic lincomycin.

We identified 3 *tet* ARGs (76 isolates), and the quinolone resistance gene *qnrS1* (13 isolates). PointFinder identified amino acid changes in *GyrA* (D87G 4/92 isolates, D87N 44/92 isolates, D87Y 3/92 isolates, and S83L 53/92 isolates), *ParC* (E84A 1/92isolates, S80I 51/92 isolates, S80R 3/92 isolates), *ParE* (I464F 2/92isolates), and *PmrB* (V161G 1/92 isolates). Hierarchal clustering on the phenotypic resistant data (using 1 for resistant, 0 for intermediate, and -1 for susceptible) with a cluster cut off just below the second node of the dendrogram resulted in seven clusters ($k = 7$) (Figure 4.5). While there was almost universal resistance to streptomycin, ampicillin, lincomycin, and oxytetracycline across all clusters, the first cluster (from left to right) was characterized by susceptibility to quinolones and was enriched for B2 isolates ($p < 0.01$); this result reflects the B2 clustering observed when the isolates were clustered on ARG (antibiotic resistance gene) presence, and the ARGs detected in these isolates reflect the observed phenotypic resistance profiles (Figure 4.5). Isolates in the third cluster were almost entirely resistant to the quinolones tested and were enriched for B1 isolates ($p < 0.01$); all isolates in the fourth cluster were additionally resistant to chloramphenicol and trimethoprim-sulfamethoxazole and were enriched for A isolates ($p < 0.05$); finally, the sixth cluster was characterized by an additional resistance to some Blactams and was dominated by B1 isolates, though this phylogroup was not found to be significantly enriched in this phenotypic cluster.

Isolates 79 and 98 were found to be resistant to colistin; both contained *mcr-1*. The one isolates

With an SNP in *pmrB* (Isolate 55) was not found to be phenotypically resistant to colistin. Meropenem, imipenem, cefotetan, piperacillin-tazobactam, tigecycline, fosfomycin, and amikacin also had 100% efficacy against the cohort (Figure 4.5).

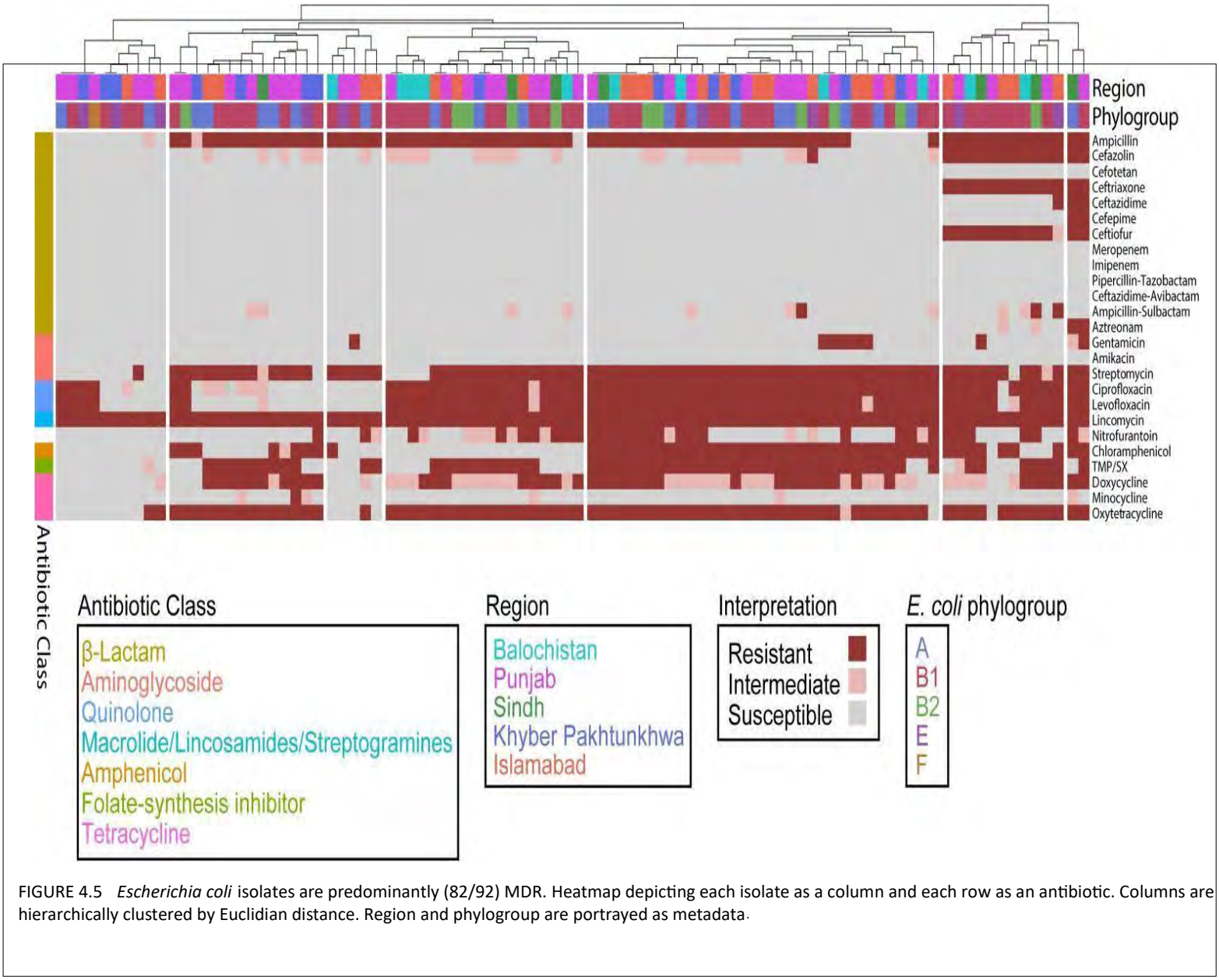


FIGURE 4.5 *Escherichia coli* isolates are predominantly (82/92) MDR. Heatmap depicting each isolate as a column and each row as an antibiotic. Columns are hierarchically clustered by Euclidian distance. Region and phylogroup are portrayed as metadata.

Table 4.2: Phenotypic and Genotypic resistance (Gene) in sequenced *E. coli* strains

***E. coli* strains**	**Antibiotics (Phenotypic resistance)**	**Resistant Gene(genotypic)**	***E. coli* strains**	**Antibiotics (Phenotypic resistance)**	**Resistant (Genotypic)**
EC-2	Trimethoprim resistance	*dfr$_{A17}$*	EC-4	Sulphonamide resistance	*sul3*
EC-2	Aminoglycoside resistance	*aph$_{(6)-Id}$*	EC-4	Aminoglycoside resistance	*aph(3')-Ia*
EC-2	Aminoglycoside resistance	*aad$_{A5}$*	EC-4	Tetracycline resistance	*tet(A)*
EC-2	Sulphonamide resistance	*sul1*	EC-4	Beta-lactam resistance	*bla$_{CTX-M-15}$*
EC-2	Tetracycline resistance	*tet(B)*	EC-4	Aminoglycoside resistance	*aadA1*
EC-2	Beta-lactam resistance	*bla$_{TEM-1B}$*	EC-4	Beta-lactam resistance	*bla$_{TEM-1B}$*
EC-2	Aminoglycoside resistance	*aph(3'')-Ib*	EC-4	Aminoglycoside resistance	*aadA2*
EC-2	Phenicol resistance	*catA1*	EC-4	Phenicol resistance	*cmlA1*
EC-2	Sulphonamide resistance	*sul2*	EC-4	Aminoglycoside resistance	*strA*
EC-5	Tetracycline resistance	*tet(A)*	EC-4	Aminoglycoside resistance	*aph(6)-Id*
EC-5	Beta-lactam resistance	*bla$_{CTX-M-15}$*	EC-6	Quinolone resistance	*QnrS1*
EC-5	Phenicol resistance	*floR*	EC-6	Phenicol resistance	*cmlA1*
EC-5	Quinolone resistance	*QnrS1*	EC-6	Phenicol resistance	*floR*
EC-7	Aminoglycoside resistance	*aadA1*	EC-6	Sulphonamide resistance	*sul3*
EC-7	Aminoglycoside resistance	*aph(6)-Id*	EC-6	Aminoglycoside resistance	*aph(3')-Ia*
EC-7	Colistin resistance	*mcr-1*	EC-6	Aminoglycoside resistance	*aadA1*

EC-7	Trimethoprim resistance	*dfrA1*	EC-6	Aminoglycoside resistance	*aadA2*
EC-7	Tetracycline resistance	*tet(A)*	EC-6	Aminoglycoside resistance	*aph(6)-Id*
EC-7	Sulphonamide resistance	*sul1*	EC-6	Tetracycline resistance	*tet(A)*
EC-7	Sulphonamide	*sul2*	EC-6	Tetracycline	*tet(M)*
EC-7	Quinolone resistance	*QnrS1*	EC-6	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-7	Beta-lactam resistance	*bla*$_{CTX-M-15}$	EC-6	Sulphonamide resistance	*sul2*
EC-8	Aminoglycoside resistance	*aph(6)-Id*	EC-6	Trimethoprim resistance	*dfrA15*
EC-8	Aminoglycoside resistance	*aph(3'')-Ib*	EC-9	Phenicol resistance	*catA1*
EC-8	Beta-lactam resistance	*bla*$_{CTX-M-1}$	EC-9	Tetracycline resistance	*tet(A)*
EC-8	Tetracycline resistance	*tet(A)*	EC-9	Sulphonamide resistance	*sul1*
EC-8	Sulphonamide resistance	*sul2*	EC-9	Trimethoprim resistance	*dfrA12*
EC-8	Phenicol resistance	*floR*	EC-9	Sulphonamide resistance	*sul2*
EC-10	Aminoglycoside resistance	*aph(3')-Ia*	EC-9	Aminoglycoside resistance	*aph(3'')-Ib*
EC-10	Trimethoprim resistance	*dfrA12*	EC-9	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-10	Aminoglycoside resistance	*aph(6)-Id*	EC-9	Aminoglycoside resistance	*aph(6)-Id*
EC-10	Aminoglycoside resistance	*aadA2*	EC-9	Aminoglycoside resistance	*aadA2*
EC-10	Aminoglycoside resistance	*aph(3'')-Ib*	EC-12	Beta-lactam resistance	*bla*$_{CTX-M-15}$
EC-10	Colistin resistance	*mcr-1*	EC-12	Colistin resistance	*mcr-1*
EC-10	Tetracycline resistance	*tet(A)*	EC-13	Tetracycline resistance	*tet(A)*

EC-10	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-13	Sulphonamide resistance	*sul2*
EC-10	Sulphonamide resistance	*sul2*	EC-13	Aminoglycoside resistance	*aph(3'')-Ib*
EC-10	Sulphonamide resistance	*sul1*	EC-13	Aminoglycoside resistance	*aph(3')-Ia*
EC-10	Sulphonamide resistance	*sul2*	EC-13	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-11	Aminoglycoside resistance	*aph(6)-Id*	EC-13	Aminoglycoside resistance	*aph(6)-Id*
EC-11	Quinolone resistance	*QnrS1*	EC-16	Lincosamide resistance	*lnu(F)*
EC-11	Trimethoprim	*dfrA14*	EC-16	Phenicol	*cmlA1*
EC-11	Tetracycline resistance	*tet(A)*	EC-16	Beta-lactam resistance	*bla*$_{CTX-M-15}$
EC-11	Aminoglycoside resistance	*aph(3')-Ia*	EC-16	Tetracycline resistance	*tet(A)*
EC-11	Aminoglycoside resistance	*aac(3)-IIa*	EC-16	Phenicol resistance	*floR*
EC-11	Beta-lactam resistance	*bla*$_{CTX-M-15}$	EC-16	Sulphonamide resistance	*sul3*
EC-11	Aminoglycoside resistance	*aph(3'')-Ib*	EC-16	Aminoglycoside resistance	*aph(4)-Ia*
EC-11	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-16	Aminoglycoside resistance	*aph(3')-Ia*
EC-11	Phenicol resistance	*floR*	EC-16	Aminoglycoside resistance	*aadA2*
EC-17	Aminoglycoside resistance	*aph(3'')-Ib*	EC-16	Aminoglycoside resistance	*aadA1*
EC-17	Tetracycline resistance	*tet(B)*	EC-16	Aminoglycoside resistance	*aph(6)-Id*
EC-17	Aminoglycoside resistance	*aph(6)-Id*	EC-20	Aminoglycoside resistance	*aph(3'')-Ib*
EC-17	Aminoglycoside resistance	*aadA5*	EC-20	Aminoglycoside resistance	*aph(6)-Id*
EC-17	Macrolide resistance	*mph(A)*	EC-20	Aminoglycoside resistance	*aadA5*
EC-17	Aminoglycoside resistance	*aac(3)-IId*	EC-20	Tetracycline resistance	*tet(B)*
EC-17	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-20	Beta-lactam resistance	*bla*$_{TEM-1B}$

EC-17	Phenicol resistance	*catA1*	EC-20	Phenicol resistance	*catA1*
EC-17	Sulphonamide resistance	*sul2*	EC-20	Sulphonamide resistance	*sul2*
EC-17	Sulphonamide resistance	*sul1*	EC-20	Sulphonamide resistance	*sul1*
EC-17	Trimethoprim resistance	*dfrA17*	EC-20	Trimethoprim resistance	*dfrA17*
EC-21	Aminoglycoside resistance	*aph(3'')-Ib*	EC-23	Aminoglycoside resistance	*aph(3'')-Ib*
EC-21	Aminoglycoside resistance	*aph(3')-Ia*	EC-23	Aminoglycoside resistance	*aph(6)-Id*
EC-21	Aminoglycoside resistance	*aadA1*	EC-23	Tetracycline resistance	*tet(A)*
EC-21	Aminoglycoside	*aadA2*	EC-23	Beta-lactam	*bla*$_{TEM-1B}$
EC-21	Aminoglycoside resistance	*aph(6)-Id*	EC-23	Sulphonamide resistance	*sul2*
EC-21	Tetracycline resistance	*tet(A)*	EC-23	Trimethoprim resistance	*dfrA14*
EC-21	Phenicol resistance	*floR*	EC-24	Aminoglycoside resistance	*aph(6)-Id*
EC-21	Phenicol resistance	*cmlA1*	EC-24	Aminoglycoside resistance	*aph(3'')-Ib*
EC-21	Sulphonamide resistance	*sul3*	EC-24	Tetracycline resistance	*tet(A)*
EC-21	Sulphonamide resistance	*sul2*	EC-24	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-21	Trimethoprim resistance	*dfrA17*	EC-24	Sulphonamide resistance	*sul2*
EC-22	Aminoglycoside resistance	*aph(3'')-Ib*	EC-24	Trimethoprim resistance	*dfrA14*
EC-22	Aminoglycoside resistance	*aph(6)-Id*	EC-25	Aminoglycoside resistance	*aph(3'')-Ib*
EC-22	Aminoglycoside resistance	*aadA1*	EC-25	Aminoglycoside resistance	*aph(6)-Id*
EC-22	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-25	Aminoglycoside resistance	*aadA1*
EC-22	Macrolide resistance	*mph(B)*	EC-25	Aminoglycoside resistance	*aadA2*
EC-22	Phenicol resistance	*floR*	EC-25	Colistin resistance	*mcr-1*

EC-22	Sulphonamide resistance	*sul2*	EC-25	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-22	Sulphonamide resistance	*sul1*	EC-25	Phenicol resistance	*floR*
EC-22	Trimethoprim resistance	*dfrA1*	EC-25	Phenicol resistance	*cmlA1*
EC-26	Aminoglycoside resistance	*aph(3'')-Ib*	EC-25	Sulphonamide resistance	*sul2*
EC-26	Aminoglycoside resistance	*aph(6)-Id*	EC-25	Sulphonamide resistance	*sul3*
EC-26	Tetracycline resistance	*tet(A)*	EC-25	Trimethoprim resistance	*dfrA12*
EC-26	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-28	Tetracycline resistance	*tet(A)*
EC-26	Sulphonamide resistance	*sul2*	EC-28	Trimethoprim resistance	*dfrA5*
EC-26	Trimethoprim	*dfrA14*	EC-29	Aminoglycoside	*aph(6)-Id*
EC-27	Aminoglycoside resistance	*strA*	EC-29	Aminoglycoside resistance	*strA*
EC-27	Aminoglycoside resistance	*aph(6)-Id*	EC-29	Aminoglycoside resistance	*aadA1*
EC-27	Aminoglycoside resistance	*aph(3')-Ia*	EC-29	Aminoglycoside resistance	*aadA2*
EC-27	Aminoglycoside resistance	*aadA1*	EC-29	Tetracycline resistance	*tet(M)*
EC-27	Quinolone resistance	*QnrS1*	EC-29	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-27	Tetracycline resistance	*tet(A)*	EC-29	Phenicol resistance	*cmlA1*
EC-27	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-29	Sulphonamide resistance	*sul3*
EC-27	Phenicol resistance	*floR*	EC-30	Aminoglycoside resistance	*strA*
EC-27	Trimethoprim resistance	*dfrA1*	EC-30	Aminoglycoside resistance	*aadA2*
EC-31	Aminoglycoside resistance	*aadA5*	EC-30	Tetracycline resistance	*tet(A)*
EC-31	Aminoglycoside resistance	*aph(3'')-Ib*	EC-30	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-31	Aminoglycoside resistance	*aac(3)-IId*	EC-30	Phenicol resistance	*floR*

EC-31	Aminoglycoside resistance	*aph(6)-Id*	EC-30	Sulphonamide resistance	*sul1*
EC-31	Tetracycline resistance	*tet(B)*	EC-30	Trimethoprim resistance	*dfrA12*
EC-31	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-32	Tetracycline resistance	*tet(A)*
EC-31	Macrolide resistance	*mph(A)*	EC-32	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-31	Phenicol resistance	*catA1*	EC-37	Aminoglycoside resistance	*aph(3')-Ia*
EC-31	Sulphonamide resistance	*sul2*	EC-37	Aminoglycoside resistance	*aadA2*
EC-31	Sulphonamide resistance	*sul1*	EC-37	Aminoglycoside resistance	*aph(6)-Id*
EC-31	Trimethoprim resistance	*dfrA17*	EC-37	Tetracycline resistance	*tet(A)*
EC-38	Colistin resistance	*mcr-1*	EC-37	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-38	Phenicol	*floR*	EC-37	Phenicol	*floR*
EC-39	Aminoglycoside resistance	*aph(3')-Ia*	EC-37	Sulphonamide resistance	*sul2*
EC-39	Aminoglycoside resistance	*aph(6)-Id*	EC-37	Sulphonamide resistance	*sul1*
EC-39	Aminoglycoside resistance	*aph(3'')-Ib*	EC-37	Trimethoprim resistance	*dfrA12*
EC-39	Tetracycline resistance	*tet(B)*	EC-42	Aminoglycoside resistance	*aadA5*
EC-39	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-42	Aminoglycoside resistance	*aph(3'')-Ib*
EC-39	Sulphonamide resistance	*sul2*	EC-42	Aminoglycoside resistance	*aph(6)-Id*
EC-39	Trimethoprim resistance	*dfrA17*	EC-42	Tetracycline resistance	*tet(B)*
EC-43	Aminoglycoside resistance	*aph(6)-Id*	EC-42	Tetracycline resistance	*tet(A)*
EC-43	Aminoglycoside resistance	*aph(3'')-Ib*	EC-42	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-43	Tetracycline resistance	*tet(A)*	EC-46	Aminoglycoside resistance	*aph(6)-Id*
EC-43	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-46	Aminoglycoside resistance	*aph(3'')-Ib*

EC-43	Phenicol resistance	*floR*	EC-46	Quinolone resistance	*QnrS1*
EC-43	Trimethoprim resistance	*dfrA14*	EC-46	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-44	Aminoglycoside resistance	*aph(3'')-Ib*	EC-46	Sulphonamide resistance	*sul2*
EC-44	Aminoglycoside resistance	*aadA2*	EC-47	Aminoglycoside resistance	*aph(6)-Id*
EC-44	Aminoglycoside resistance	*aph(6)-Id*	EC-47	Aminoglycoside resistance	*aph(3'')-Ib*
EC-44	Aminoglycoside resistance	*aac(3)-IId*	EC-47	Tetracycline resistance	*tet(A)*
EC-44	Aminoglycoside resistance	*aadA1*	EC-47	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-44	Aminoglycoside resistance	*aadA5*	EC-47	Sulphonamide resistance	*sul2*
EC-44	Colistin resistance	*mcr-1*	EC-47	Trimethoprim resistance	*dfrA14*
EC-44	Tetracycline resistance	*tet(A)*	EC-48	Quinolone resistance	*QnrS1*
EC-44	Beta-lactam	*bla*$_{CTX-M-15}$	EC-48	Tetracycline	*tet(A)*
EC-44	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-49	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-44	Macrolide resistance	*mph(A)*	EC-49	Trimethoprim resistance	*dfrA14*
EC-44	Phenicol resistance	*cmlA1*	EC-49	Aminoglycoside resistance	*aph(3'')-Ib*
EC-44	Sulphonamide resistance	*sul1*	EC-49	Aminoglycoside resistance	*aph(6)-Id*
EC-44	Sulphonamide resistance	*sul3*	EC-49	Tetracycline resistance	*tet(A)*
EC-44	Trimethoprim resistance	*dfrA17*	EC-49	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-50	Aminoglycoside resistance	*strA*	EC-49	Sulphonamide resistance	*sul2*
EC-50	Aminoglycoside resistance	*aph(4)-Ia*	EC-49	Trimethoprim resistance	*dfrA14*
EC-50	Aminoglycoside resistance	*aac(3)-IVa*	EC-51	Aminoglycoside resistance	*aph(3'')-Ib*
EC-50	Aminoglycoside resistance	*aadA2*	EC-51	Aminoglycoside resistance	*aph(6)-Id*

EC-50	Aminoglycoside resistance	*aph(6)-Id*	EC-51	Aminoglycoside resistance	*aadA2*
EC-50	Aminoglycoside resistance	*aadA1*	EC-51	Aminoglycoside resistance	*aadA1*
EC-50	Colistin resistance	*mcr-1*	EC-51	Colistin resistance	*mcr-1*
EC-50	Tetracycline resistance	*tet(A)*	EC-51	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-50	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-51	Phenicol resistance	*cmlA1*
EC-50	Phenicol resistance	*cmlA1*	EC-51	Phenicol resistance	*floR*
EC-50	Sulphonamide resistance	*sul3*	EC-51	Sulphonamide resistance	*sul2*
EC-50	Trimethoprim resistance	*dfrA1*	EC-51	Sulphonamide resistance	*sul3*
EC-52	Aminoglycoside resistance	*aadA1*	EC-51	Trimethoprim resistance	*dfrA12*
EC-52	Aminoglycoside resistance	*aadA2*	EC-54	Aminoglycoside resistance	*aph(3')-Ia*
EC-52	Tetracycline resistance	*tet(A)*	EC-54	Aminoglycoside resistance	*aph(3'')-Ib*
EC-52	Beta-lactam	*bla*$_{TEM-1B}$	EC-54	Aminoglycoside	*aph(6)-Id*
EC-52	Macrolide resistance	*mph(A)*	EC-54	Tetracycline resistance	*tet(B)*
EC-52	Phenicol resistance	*cmlA1*	EC-54	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-52	Sulphonamide resistance	*sul3*	EC-54	Sulphonamide resistance	*sul2*
EC-52	Sulphonamide resistance	*sul1*	EC-54	Trimethoprim resistance	*dfrA17*
EC-52	Trimethoprim resistance	*dfrA12*	EC-56	Aminoglycoside resistance	*aph(3'')-Ib*
EC-55	Aminoglycoside resistance	*strA*	EC-56	Aminoglycoside resistance	*aph(6)-Id*
EC-55	Aminoglycoside resistance	*aadA2*	EC-56	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-55	Aminoglycoside resistance	*aacA4*	EC-56	Sulphonamide resistance	*sul2*
EC-55	Aminoglycoside resistance	*aph(6)-Id*	EC-56	Trimethoprim resistance	*dfrA14*

EC-55	Aminoglycoside resistance	*aph(3')-Ia*	EC-57	Aminoglycoside resistance	*aph(6)-Id*
EC-55	Aaminoglycoside resistance	*aac(6')Ib-cr*	EC-57	Aminoglycoside resistance	*aph(3'')-Ib*
EC-55	Tetracycline resistance	*tet(B)*	EC-57	Tetracycline resistance	*tet(A)*
EC-55	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-57	Beta-lactam resistance	*bla*$_{TEM-1A}$
EC-55	Beta-lactam resistance	*bla*$_{CMY-2}$	EC-57	Phenicol resistance	*floR*
EC-55	Phenicol resistance	*catA1*	EC-57	Sulphonamide resistance	*sul2*
EC-55	Phenicol resistance	*catB3*	EC-57	Trimethoprim resistance	*dfrA14*
EC-55	Sulphonamide resistance	*sul1*	EC-58	Aminoglycoside resistance	*aadA1*
EC-55	Sulphonamide resistance	*sul1*	EC-58	Aminoglycoside resistance	*aadA2*
EC-55	Trimethoprim resistance	*dfrA1*	EC-58	Tetracycline resistance	*tet(A)*
EC-55	Trimethoprim resistance	*dfrA10*	EC-58	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-61	Aminoglycoside	*aph(6)-Id*	EC-58	Macrolide	*mph(A)*
EC-61	Aminoglycoside resistance	*aph(3'')-Ib*	EC-58	Phenicol resistance	*cmlA1*
EC-61	Tetracycline resistance	*tet(A)*	EC-58	Sulphonamide resistance	*sul3*
EC-61	Sulphonamide resistance	*sul2*	EC-58	Sulphonamide resistance	*sul1*
EC-62	Aminoglycoside resistance	*aph(6)-Id*	EC-58	Trimethoprim resistance	*dfrA12*
EC-62	Aminoglycoside resistance	*aph(3'')-Ib*	EC-59	Aminoglycoside resistance	*aadA2*
EC-62	Colistin resistance	*mcr-1*	EC-59	Aminoglycoside resistance	*aph(6)-Id*
EC-62	Tetracycline resistance	*tet(A)*	EC-59	Tetracycline resistance	*tet(A)*
EC-62	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-59	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-62	Phenicol resistance	*floR*	EC-59	Sulphonamide resistance	*sul2*

EC-62	Trimethoprim resistance	*dfrA14*	EC-59	Sulphonamide resistance	*sul1*
EC-63	Tetracycline resistance	*tet(A)*	EC-59	Trimethoprim resistance	*dfrA12*
EC-63	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-64	Aminoglycoside resistance	*aph(3')-Ia*
EC-66	Aminoglycoside resistance	*aph(3')-Ia*	EC-64	Aminoglycoside resistance	*aph(6)-Id*
EC-66	Aminoglycoside resistance	*aph(6)-Id*	EC-64	Quinolone resistance	*QnrS1*
EC-66	Aminoglycoside resistance	*strA*	EC-64	Tetracycline resistance	*tet(A)*
EC-66	Tetracycline resistance	*tet(A)*	EC-64	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-66	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-64	Beta-lactam resistance	*bla*$_{CTX-M-15}$
EC-67	Aminoglycoside resistance	*aph(3')-Ia*	EC-64	Phenicol resistance	*floR*
EC-67	Aminoglycoside resistance	*aadA2*	EC-64	Sulphonamide resistance	*sul2*
EC-67	Aminoglycoside resistance	*aph(6)-Id*	EC-65	Aminoglycoside resistance	*aadA2*
EC-67	Aminoglycoside resistance	*aph(3'')-Ib*	EC-65	Aminoglycoside resistance	*aph(3')-Ia*
EC-67	Colistin	*mcr-1*	EC-65	Aminoglycoside	*aph(6)-Id*
EC-67	Tetracycline resistance	*tet(A)*	EC-65	Aminoglycoside resistance	*strA*
EC-67	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-65	Tetracycline resistance	*tet(A)*
EC-67	Sulphonamide resistance	*sul1*	EC-65	Beta-lactam resistance	*bla*$_{CTX-M-15}$
EC-67	Sulphonamide resistance	*sul2*	EC-65	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-68	Aminoglycoside resistance	*aph(3'')-Ib*	EC-65	Sulphonamide resistance	*sul1*
EC-68	Aminoglycoside resistance	*aph(6)-Id*	EC-65	Sulphonamide resistance	*sul2*
EC-68	Colistin resistance	*mcr-1*	EC-65	Trimethoprim resistance	*dfrA12*
EC-68	Tetracycline resistance	*tet(A)*	EC-73	Aminoglycoside resistance	*strA*

EC-71	Aminoglycoside resistance	*aph(3'')-Ib*	EC-73	Aminoglycoside resistance	*aph(6)-Id*
EC-71	Aminoglycoside resistance	*aadA1*	EC-73	Aminoglycoside resistance	*aph(3')-Ia*
EC-71	Tetracycline resistance	*tet(A)*	EC-73	Tetracycline resistance	*tet(A)*
EC-71	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-73	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-71	Sulphonamide resistance	*sul2*	EC-73	Phenicol resistance	*floR*
EC-71	Trimethoprim resistance	*dfrA1*	EC-73	Sulphonamide resistance	*sul2*
EC-72	Aminoglycoside resistance	*aph(3')-Ia*	EC-74	Aminoglycoside resistance	*aph(6)-Id*
EC-72	Aminoglycoside resistance	*aph(6)-Id*	EC-74	Aminoglycoside resistance	*aadA1*
EC-72	Aminoglycoside resistance	*aph(3'')-Ib*	EC-74	Aminoglycoside resistance	*aadA2*
EC-72	Tetracycline resistance	*tet(B)*	EC-74	Aminoglycoside resistance	*aadA5*
EC-72	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-74	Aminoglycoside resistance	*aph(3'')-Ib*
EC-72	Macrolide resistance	*mph(A)*	EC-74	Tetracycline resistance	*tet(A)*
EC-72	Sulphonamide resistance	*sul2*	EC-74	Phenicol resistance	*cmlA1*
EC-72	Trimethoprim	*dfrA14*	EC-74	Phenicol	*floR*
EC-75	Aminoglycoside resistance	*strA*	EC-74	Sulphonamide resistance	*sul2*
EC-75	Aminoglycoside resistance	*aph(6)-Id*	EC-74	Sulphonamide resistance	*sul3*
EC-75	Aminoglycoside resistance	*aadA2*	EC-74	Trimethoprim resistance	*dfrA17*
EC-75	Aminoglycoside resistance	*aadA1*	EC-79	Aminoglycoside resistance	*aph(3'')-Ib*
EC-75	Quinolone resistance	*QnrS1*	EC-79	Aminoglycoside resistance	*aph(6)-Id*
EC-75	Tetracycline resistance	*tet(A)*	EC-79	Aminoglycoside resistance	*aadA1*
EC-75	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-79	Aminoglycoside resistance	*aadA2*

EC-75	Macrolide resistance	*mef(B)*	EC-79	Colistin resistance	*mcr-1*
EC-75	Phenicol resistance	*cmlA1*	EC-79	Tetracycline resistance	*tet(A)*
EC-75	Sulphonamide resistance	*sul3*	EC-79	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-75	Trimethoprim resistance	*dfrA12*	EC-79	Phenicol resistance	*floR*
EC-76	Aminoglycoside resistance	*aph(3'')-Ib*	EC-79	Phenicol resistance	*cmlA1*
EC-76	Aminoglycoside resistance	*aph(6)-Id*	EC-79	Sulphonamide resistance	*sul2*
EC-76	Aminoglycoside resistance	*aadA1*	EC-79	Sulphonamide resistance	*sul3*
EC-76	Tetracycline resistance	*tet(A)*	EC-79	Trimethoprim resistance	*dfrA12*
EC-76	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-81	Tetracycline resistance	*tet(A)*
EC-76	Macrolide resistance	*mph(B)*	EC-81	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-76	Sulphonamide resistance	*sul2*	EC-82	Aminoglycoside resistance	*aac(3)-IId*
EC-76	Sulphonamide resistance	*sul1*	EC-82	Aminoglycoside resistance	*aadA1*
EC-76	Trimethoprim resistance	*dfrA1*	EC-82	Aminoglycoside resistance	*aac(3)-VIa*
EC-84	Aminoglycoside resistance	*aph(6)-Id*	EC-82	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-84	Aminoglycoside	*aph(3'')-Ib*	EC-82	Sulphonamide	*sul1*
EC-84	Tetracycline resistance	*tet(A)*	EC-83	Aminoglycoside resistance	*aadA2*
EC-84	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-83	Aminoglycoside resistance	*aph(6)-Id*
EC-84	Phenicol resistance	*floR*	EC-83	Aminoglycoside resistance	*strA*
EC-84	Sulphonamide resistance	*sul2*	EC-83	Aminoglycoside resistance	*aadA1*
EC-84	Trimethoprim resistance	*dfrA14*	EC-83	Quinolone resistance	*QnrS1*

EC-85	Tetracycline resistance	*tet(A)*	EC-83	Tetracycline resistance	*tet(A)*
EC-88	Aminoglycoside resistance	*aadA2*	EC-83	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-88	Tetracycline resistance	*tet(A)*	EC-83	Phenicol resistance	*cmlA1*
EC-88	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-83	Sulphonamide resistance	*sul3*
EC-88	Beta-lactam resistance	*bla*$_{CTX-M-15}$	EC-83	Trimethoprim resistance	*dfrA15*
EC-88	Macrolide resistance	*mph(A)*	EC-83	Trimethoprim resistance	*dfrA1*
EC-88	Sulphonamide resistance	*sul1*	EC-89	Aminoglycoside resistance	*aph(6)-Id*
EC-88	Trimethoprim resistance	*dfrA12*	EC-89	Aminoglycoside resistance	*strA*
EC-91	Aminoglycoside resistance	*aph(3'')-Ib*	EC-90	Aminoglycoside resistance	*aadA2*
EC-91	Aminoglycoside resistance	*aph(6)-Id*	EC-90	Aminoglycoside resistance	*aph(3')-Ia*
EC-91	Tetracycline resistance	*tet(A)*	EC-90	Tetracycline resistance	*tet(A)*
EC-91	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-90	Beta-lactam resistance	*bla*$_{CTX-M-15}$
EC-91	Sulphonamide resistance	*sul2*	EC-90	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-92	Aminoglycoside resistance	*aph(3')-Ia*	EC-90	Sulphonamide resistance	*sul2*
EC-92	Aminoglycoside resistance	*aph(6)-Id*	EC-90	Sulphonamide resistance	*sul1*
EC-92	Aminoglycoside resistance	*strA*	EC-90	Trimethoprim resistance	*dfrA12*
EC-92	Tetracycline	*tet(A)*	EC-95	Aminoglycoside	*aph(3')-Ia*
EC-92	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-95	Aminoglycoside resistance	*aph(6)-Id*
EC-97	Aminoglycoside resistance	*aph(3'')-Ib*	EC-95	Aminoglycoside resistance	*strA*
EC-97	Aminoglycoside resistance	*aph(6)-Id*	EC-95	Tetracycline resistance	*tet(A)*
EC-97	Tetracycline resistance	*tet(A)*	EC-95	Beta-lactam resistance	*bla*$_{TEM-1B}$

EC-97	Phenicol resistance	*floR*	EC-96	Aminoglycoside resistance	*aadA1*
EC-97	Sulphonamide resistance	*sul2*	EC-96	Aminoglycoside resistance	*aph(3')-Ia*
EC-98	Aminoglycoside resistance	*aph(6)-Id*	EC-96	Tetracycline resistance	*tet(A)*
EC-98	Aminoglycoside resistance	*aph(3'')-Ib*	EC-96	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-98	Colistin resistance	*mcr-1*	EC-96	Sulphonamide resistance	*sul1*
EC-98	Tetracycline resistance	*tet(A)*	EC-96	Sulphonamide resistance	*sul2*
EC-98	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-96	Trimethoprim resistance	*dfrA15*
EC-98	Sulphonamide resistance	*sul2*	EC-100	Aminoglycoside resistance	*aph(6)-Id*
EC-98	Trimethoprim resistance	*dfrA14*	EC-100	Aminoglycoside resistance	*aadA2*
EC-102	Aminoglycoside resistance	*aac(3)-VIa*	EC-100	Aminoglycoside resistance	*aph(3'')-Ib*
EC-102	Aminoglycoside resistance	*aadA2*	EC-100	Tetracycline resistance	*tet(A)*
EC-102	Aminoglycoside resistance	*aadA1*	EC-100	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-102	Tetracycline resistance	*tet(A)*	EC-100	Phenicol resistance	*floR*
EC-102	Phenicol resistance	*catA1*	EC-100	Sulphonamide resistance	*sul2*
EC-102	Phenicol resistance	*catA2*	EC-100	Sulphonamide resistance	*sul1*
EC-102	Sulphonamide resistance	*sul2*	EC-100	Trimethoprim resistance	*dfrA12*
EC-102	Sulphonamide resistance	*sul1*	EC-101	Aminoglycoside resistance	*aac(3)-VIa*

EC-102	Trimethoprim	*dfrA12*	EC-101	Aminoglycoside	*aadA2*
EC-103	Aminoglycoside resistance	*aph(3'')-Ib*	EC-101	Aminoglycoside resistance	*aadA1*
EC-103	Aminoglycoside resistance	*aph(3')-Ia*	EC-101	Tetracycline resistance	*tet(A)*
EC-103	Aminoglycoside resistance	*aadA2*	EC-101	Phenicol resistance	*catA1*
EC-103	Aminoglycoside resistance	*aph(6)-Id*	EC-101	Phenicol resistance	*catA2*
EC-103	Aminoglycoside resistance	*aadA1*	EC-101	Sulphonamide resistance	*sul2*
EC-103	Tetracycline resistance	*tet(A)*	EC-101	Sulphonamide resistance	*sul1*
EC-103	Phenicol resistance	*floR*	EC-101	Trimethoprim resistance	*dfrA12*
EC-103	Phenicol resistance	*cmlA1*	EC-33B	Quinolone resistance	*QnrS1*
EC-103	Sulphonamide resistance	*sul2*	EC-33B	Tetracycline resistance	*tet(A)*
EC-103	Trimethoprim resistance	*dfrA17*	EC-33B	Beta-lactam resistance	*bla*$_{TEM-1B}$
EC-104	Aminoglycoside resistance	*aadA1*	EC-33B	Trimethoprim resistance	*dfrA14*
EC-104	Aminoglycoside resistance	*aph(6)-Id*	EC-34A	Aminoglycoside resistance	*aph(3'')-Ib*
EC-104	Aminoglycoside resistance	*aph(3')-Ia*	EC-34A	Aminoglycoside resistance	*aph(6)-Ic*
EC-104	Aminoglycoside resistance	*aph(3'')-Ib*	EC-34A	Aminoglycoside resistance	*aph(3')-IIa*
EC-104	Aminoglycoside resistance	*aac(3)-VIa*	EC-34A	Aminoglycoside resistance	*aph(6)-Id*
EC-104	Tetracycline resistance	*tet(B)*	EC-34A	Aminoglycoside resistance	*aadA1*
EC-104	Tetracycline resistance	*tet(A)*	EC-34A	Aminoglycoside resistance	*aadA5*
EC-104	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-34A	Aminoglycoside resistance	*aadA2*
EC-104	Macrolide resistance	*mph(B)*	EC-34A	Tetracycline resistance	*tet(A)*
EC-104	Sulphonamide resistance	*sul2*	EC-34A	Beta-lactam resistance	*bla*$_{CARB-2}$
EC-104	Sulphonamide resistance	*sul1*	EC-34A	Phenicol resistance	*catA1*

EC-104	Trimethoprim	*dfrA1*	EC-34A	Phenicol	*cmlA1*
EC-104	Trimethoprim	*dfrA*	EC-34A	Phenicol	*floR*
EC-34 B	Aminoglycoside resistance	*aph(3'')-Ib*	EC-34A	Sulphonamide resistance	*sul1*
EC-34 B	Aminoglycoside	*aph(6)-Id*	EC-34A	Sulphonamide	*sul2*
EC-34 B	Aminoglycoside	*aadA1*	EC-34A	Sulphonamide	*sul3*
EC-34 B	Aminoglycoside	*aadA2*	EC-34A	Trimethoprim	*dfrA16*
EC-34 B	Tetracycline	*tet(A)*	EC-34A	Trimethoprim	*dfrA17*
EC-34 B	Beta-lactam resistance	*bla*$_{TEM-1B}$	35 A	Aminoglycoside resistance	*aph(3')-Ia*
EC-34 B	Beta-lactam	*bla*$_{CARB-2}$	35 A	Aminoglycoside	*aph(6)-Id*
EC-34 B	Phenicol resistance	*cmlA1*	35 A	Aminoglycoside resistance	*strA*
EC-34 B	Sulphonamide resistance	*sul3*	35 A	Aminoglycoside resistance	*aadA1*
EC-34 B	Trimethoprim resistance	*dfrA16*	35 A	Aminoglycoside resistance	*aadA2*
35 B	Aminoglycoside resistance	*aph(3')-Ia*	35 A	Tetracycline resistance	*tet(A)*
35 B	Aminoglycoside resistance	*aph(6)-Id*	35 A	Beta-lactam resistance	*bla*$_{TEM-1B}$
35 B	Aminoglycoside resistance	*strA*	35 A	Beta-lactam resistance	*bla*$_{CTX-M-15}$
35 B	Aminoglycoside resistance	*aadA1*	35 A	Phenicol resistance	*cmlA1*
35 B	Aminoglycoside resistance	*aadA2*	35 A	Sulphonamide resistance	*sul3*
35 B	Tetracycline resistance	*tet(A)*	EC-36B	Aminoglycoside resistance	*aadA1*
35 B	Beta-lactam resistance	*bla*$_{TEM-1B}$	EC-36B	Aminoglycoside resistance	*aph(6)-Id*
35 B	Beta-lactam resistance	*bla*$_{CTX-M-15}$	EC-36B	Aminoglycoside resistance	*aadA2*
35 B	Phenicol resistance	*cmlA1*	EC-36B	Quinolone resistance	*QnrS1*
35 B	Sulphonamide resistance	*sul3*	EC-36B	Tetracycline resistance	*tet(A)*
EC-36B	Beta-lactam	*bla*$_{TEM-1B}$	EC-36B	Macrolide	*mef(B)*
EC-36B	Phenicol resistance	*cmlA1*	EC-36B	Macrolide resistance	*mef(B)*

4.6 Phylogenomic Analysis of Isolated Strains of *E. coli*

Phylogenetic reconstruction of the similarity between the *E. coli* within our cohort and with 11 known phylogroup strains or control strains as mentioned (Figure 4.6), but there is not a clear association between phylogroup and geographic region, although we noted isolates from Baluchistan fell exclusively in the B1 Clade (Figure 4.6 a). However, 53.2% (49/92) of the cohort are in the B1 clade. The rest of the isolates are in clade A (22/92), B2 (11/92), E (9/92), and F (1/92). The most abundant sequence types were ST155 (16/92) and ST117 (9/92) belong to clade B2 (Figure 4.6a). The ST131 type (EC-60) is isolated from Punjab and having the $bla_{CTX\text{-}M1}$ gene. We applied Plasmid-Finder using the *Enterobacteriaceae* database on our *E. coli* cohort to identify known plasmid replicons (Figure 4.6D). 90/92 isolates had plasmid replicons identified, with a maximum of 7 in EC_10, EC_44, and EC_67, and a median of 4. 26 different replicons were identified, among which members of the IncF plasmid replicon family were the most prevalent (12/26). IncFIB (AP001918) and IncFII were the most prevalent among the isolates with 65/92 and 41/92 identified within the cohort, respectively (Figure 4.6D).

To identify a genotypic basis for phenotypic antibiotic resistance, following Illumina Wholegenome sequencing, we applied ResFinder to identify acquired ARGs and PointFinder to locate relevant SNP resistance determinants. Consistent with previous reports on genomic analysis of *E. coli* isolates, we have identified a mosaic of antibiotic-resistant determinants and virulence genes within our cohort with no clear association between ARG composition, phylogroup, and region source, other than the identification of all isolates from Balochistan as members of the B1 phylogroup ($p < 0.01$, hypergeometric test) (Figure 4.6a). In total, we identified 49 unique ARGs (Figure 4.6b) and 5 previously validated antibiotic resistanceconferring SNPs (Zankari *et al*., 2017).

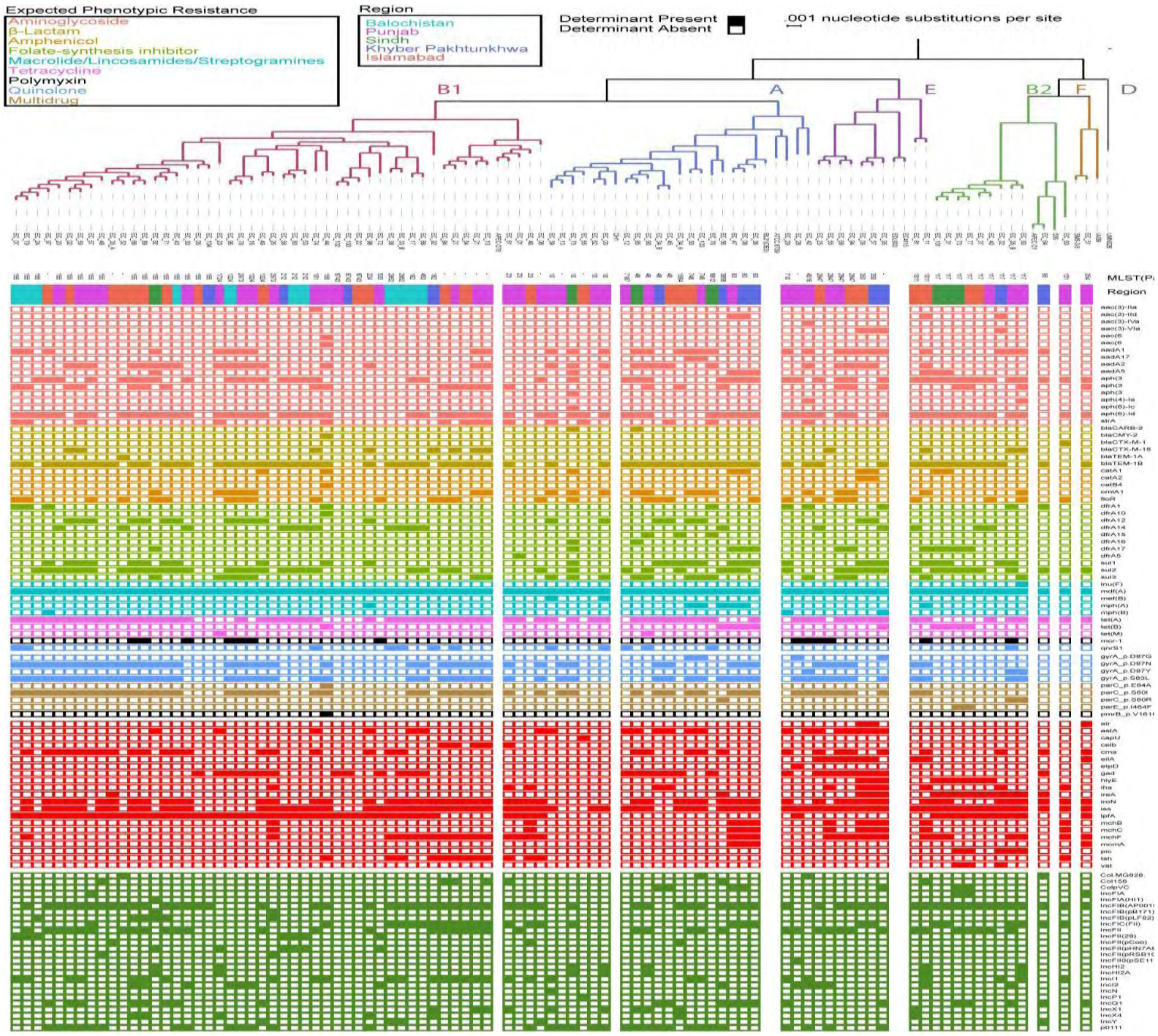


FIGURE 4.6 *Escherichia coli* genomes are predominantly in B1 and A phylogroup with a mosaic of antibiotic resistance determinants and virulence genes (A) Population structure of the *E. coli* cohort depicting the phylogroup, MLST, and region obtained. Presence absence of antibiotic resistance determinants (B) virulence genes (C), and plasmid replicons (D) were identified using ResFinder, PointFinder, VirulenceFinder, and PlasmidFinder on the cohort.

4.7 Comparative phylogenetic grouping *in E. coli* based upon virulence factor, ARG or SNP (Single Nucleotide Polymorphism)

The median number of ARGs per isolate was 7 and the median prevalence for each ARG was 8. Therefore, 17/49 of the ARGs are predicted to have activity against aminoglycosides, 11/49 against folate-synthesis inhibitors, 6/49 against β-lactamases, 5 against amphenicols, and 5 against lincosamides (Figure 4.7a). In addition, we identified 3 *tet* ARGs, the colistin resistance gene *mcr-1*, and the quinolone resistance gene *qnrS1*. The SNPs identified by PointFinder are D87G, D87N, D87Y, and S83L in *gyrA* as well as E84A, S80I, S80R, in *parC*, I464F in *parE*, and V161G in *pmrB*. (Figure 4.7c). The aminoglycoside ARGs include representatives of the *aac*, *aadA*, and *aph* families. No carbapenem resistance genes were identified within this *E. coli* cohort. The most prevalent β-lactamase was *blaTEM-1B* found in (66/92) isolates (Figure 4.7a)

The only non-class A β-lactamase present was *bla*$_{\text{CMY-2}}$, which was found in a single isolate. The most conserved gene, *mdf(A)*, was found in 92/92 of the *E. coli* isolates, indicating that it is a core gene within this cohort. 7/92 of the isolates had *mdf(A)* as the sole ARG. Despite this conservation, the other lincosamide ARGs*, Inu(F)* (1/92), *mef(B)* (2/92), *mph(A)* (7/92), and *mph(B) (*3/92) were at or below the median ARG prevalence. Alarmingly, 13/92 of the *E. coli* isolates contained the colistin ARG *mcr-1*. The prevalence of *mcr-1* was much higher than SNPs against colistin as only 1/92 isolates contained the V161G mutation in *pmrB*. When hierarchically clustered on ARG presence (k = 6), no segregation of isolates by region or phylogroup is observed, except for enrichment of B2 isolates ($p < 0.01$) in a cluster characterized by a high ARG load that includes *blaTEM-1B*, *tet(A)*, and *mdf(A)*, as do most of the isolates, but also *aadA2*, *aadA1*, *sul3*, and *cmlA1* (Figure 4.7a).

When hierarchically clustered on antibiotic resistance-conferring SNPs, the isolates are Segregated into two major clades, one with a preponderance of SNPs in *gyrA* and *parC*, and a clade of isolates most of which carry no SNPs (Figure 4.7c). B2 isolates are enriched in the latter clade ($p < 0.001$). We applied Virulence Finder on the sequenced *E. coli* cohort to annotate genes putatively involved in poultry infections (Figure 4.7b). 21 virulence genes were identified and the median number of virulence genes per isolate was 4. 2/92 of the isolates had no known virulence genes identified. Consistent with their previously identified roles in APEC virulence, the serum survival gene *iss* was found in 78.2% (72/92) of the isolates, the iron acquisition gene *iroN* was in 60.9%

(56/92) of the cohort, and the long polar fimbriae gene *lpfA* was in 59.8% (55/92) of the cohort. When hierarchically clustered on virulence gene presence (k = 5), we again observed no segregation of the isolates by geographic region (Figure 4.8b). However, the B2 and E isolates were each enriched in their clusters ($p < 1E\text{-}9$ and $p < 0.01$, respectively) characterized by different virulence gene profiles. While the larger B1 phylogroup was more distributed across the clusters, there was one cluster that was exclusively comprised of B1 isolates ($p < 0.01$). In conclusion, these data indicated that the phylogroups segregate better by virulence gene presence than antibiotic resistance gene presence; the lack of segregation by geographic region suggests that APEC strains are readily transmitted across Pakistan (Figure 4.7a).

The first cluster (from left to right) was characterized by susceptibility to b-lactams. There was widespread resistance to quinolones and the aminoglycoside streptomycin across all clusters, while the two rightmost clusters were characterized by additional resistance to the blactams cefazolin, ceftriaxone, and ceftiofur. There was no significant association between any cluster and any region or phylogroup, unlike the associations detected between clusters formed on genetic features and phylogroup (Figure 4.7).

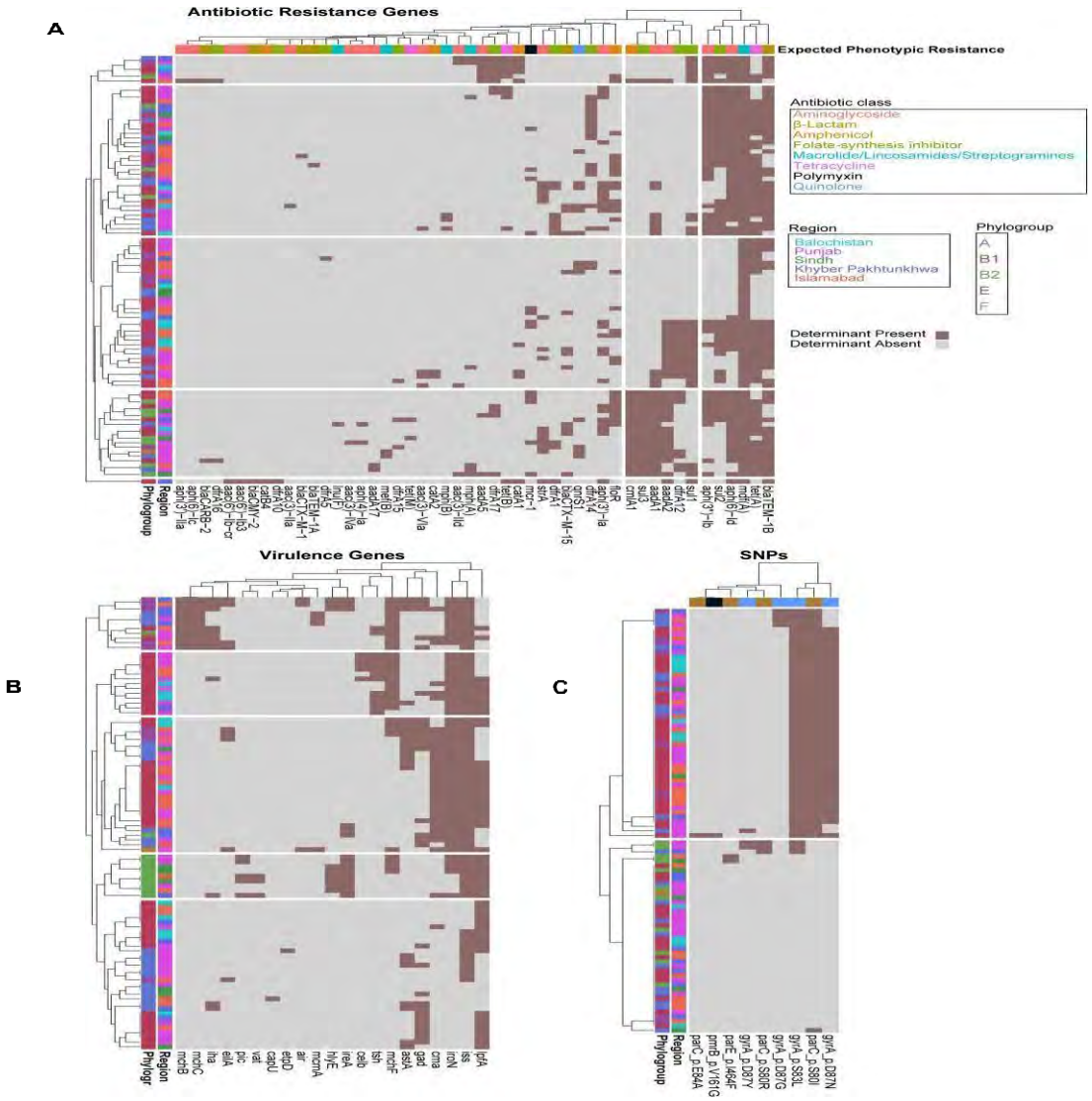


FIGURE 4.7 *Escherichia coli* phylogroups segregate better by virulence gene presence than ARG or SNP presence. Heatmaps depicting isolates as rows and (A), virulence genes (B), or SNPs (C) as columns. Rows and columns are hierarchically clustered by Euclidian distance. Region, phylogroup, or expected phen resistance conferred by ARGs are portrayed as metadata, as indicated.

4.8 *mcr-1* Gene was Confirmed in some of the Isolates

Given problems using disk diffusion testing for colistin resistance, we performed a broth minimum inhibitory concentration (MIC) assay using the ComASPTM colistin test on all mcr-1 positive isolate, the pmrB SNP isolate 55, quality control strain *E. coli* ATCC 25922, and the mcr-1 positive *E. coli* AR Bank #0350 from the CDC and FDA Antibiotic Resistance Isolate Bank (Table 1). All mcr-1 positive isolates had MIC values of 4 or 8 mg/mL but the pmrB SNP isolate 55 had an MIC of 0.25 mg/mL. This cohort demonstrated 100% in vitro susceptibility to meropenem, imipenem, cefotetan, piperacillin-tazobactam, and amikacin. We found a strong association between phenotypic resistance within the 3rd generation cephalosporin ceftriaxone and ceftiofur (R2 = 0.9004) and quinolones ciprofloxacin and norfloxacin (R2 = 0.8897) (Supplementary Figure S1). One isolate (EC_44) was discordant for the 3rd generation cephalosporins and tested as resistant to ceftriaxone but intermediate to the veterinary antibiotic ceftiofur and contained bla_{CMY-2} and bla_{TEM-1B}. While there are not CLSI interpretative criteria for norfloxacin, one isolate (EC_72) without any identified quinolone resistance determinants tested as ciprofloxacin susceptible but had a comparatively low disk diffusion radius to norfloxacin.

The strain EC_79 was selected for conjugation assay has confirmed the *mcr-1* gene. Thus, with EC_79 strains 12 other *E. coli* strains including EC_07, EC_10, EC_12, EC_25, EC_38, EC_44, EC_50, EC_51, EC_55, EC_62, EC_67, EC_68, EC_98, had also mcr-1 gene, confirmed through Resfinder, with 100% identity. The following strains Ec-10(not shown), Ec-12(ST7187), Ec-15(NC), Ec-18(?), Ec-25(ST2847), Ec-38(ST2852), Ec-41(NC), Ec44(ST156), Ec-50(ST746), Ec-51(ST354), Ec-62(ST10), Ec-67(ST155), Ec-68(ST2847), Ec79(ST155), Ec-07(ST155), Ec-98(ST224) have *mcr-1* gene (Table 4.2).

Table 4.3: Summary of the prevalence of *mcr-1* gene in *E. coli* strains

Bacterial ID	Resistance gene	Identity	Query/ HSP	Contigs	Position in contigs	Phenotype	Accession no
EC_10	*mcr-1*	100	1626/1626	NODE_47_length_20584_cov_65.554 445	6686..8311	Colistin	KP347127
EC_12	*mcr-1*	100	1626/1626	NODE_44_length_26809_cov_23.727 480	8977..10602	Colistin	KP347127
EC_15	*mcr-1*	100	1626/1415	NODE_35_length_10453_cov_6.0105 05	1..1415	Colistin	KY685071
EC_18	*mcr-1*	100	1626/1626	NODE_135_length_12667_cov_11.27 3709	2003..3628	Colistin	KP347127
EC_25	*mcr-1*	100	1626/1626	NODE_27_length_59130_cov_154.79 5116	9040..10665	Colistin	KP347127
EC_38	*mcr-1*	100	1626/1626	NODE_37_length_26368_cov_30.652 885	8978..10603	Colistin	KP347127
EC_41	*mcr-1*	100	1626/1626	NODE_81_length_12677_cov_20.841 032	9040..10665	Colistin	KP347127
EC_44	*mcr-1*	100	1626/1626	NODE_50_length_27614_cov_45.9	9862..1148733072	Colistin	KP347127

EC_50	*mcr-1*	100	1626/1626	NODE_39_length_26809_cov_37.896790	16208..17833	Colistin	KP347127
EC_51	mcr-1	100	1626/1626	NODE_44_length_26683_cov_43.849733	8977..10602	Colistin	KP347127
EC_62	mcr-1	100	1626/1626	NODE_44_length_26756_cov_40.218486	9040..10665	Colistin	KP347127
EC_67	*mcr-1*	100	1626/1626	NODE_43_length_20584_cov_54.644268	6686..8311	Colistin	KP347127
EC_68	*mcr-1*	100	1626/1626	NODE_78_length_13789_cov_35.888273	10118..11743	Colistin	KP347127
EC_79	*mcr-1*	100	1626/1626	NODE_45_length_27097_cov_41.510400	16433..18058	Colistin	KP347127
EC_07	*mcr-1*	100	1626/1626	NODE_58_length_12574_cov_96.291430	1911..3536	Colistin	KP347127
EC_91	*mcr-1*	100	1626/1626	NODE_79_length_13141_cov_16.244718	1983..3608	Colistin	KP347127
EC_98	*mcr-1*	100	1626/1626	NODE_27_length_61122_cov_32.390417	48530..50155	Colistin	KP347127

4.9 Screening of pmcr_IncI2 mcr-1 Bearing Plasmid in *E. coli*

The presence of *mcr-1* harboring plasmids was important for conjugation assay. As the *mcr-1* gene present on a plasmid can be transferred to the recipient strain. All our strains having *mcr-1* gene caring pmcr1_ IncI2 plasmid was confirmed by using plasmid-finder on the NGS sequences (Table 4.3). The data of IncI2 plasmid showed that 100 of identity. Moreover, all the strains having *mcr-1* gene/IncI2 have similar accession numbers KP347127. Thus, the determination of the IncI2 plasmid has justified the transformation of the *mcr-1* gene through conjugation assay. Which was previously confirmed by (Zhang, 2017).

Table 4.4: Summary data of *pmcr1_Inc12* plasmid isolated in *mcr-1* bearing *E. coli*

Bacterial ID	Plasmid	Identity	Query/ HSP	Contigs	Position in contigs	Accession no
25	IncI2	100	1626/1626	61122_cov_32.390417	29520..29835	KP347127
50	IncI2	100	1626/1626	46464_cov_97.621273	29456..29771	KP347127
79	IncI2	100	1626/1626	32122_cov_39.750819	29468..29783	KP347127
7	IncI2	100	1626/1626	32110_cov_36.449224	29456..29771	KP347127
98	IncI2	100	1626/1626	59130_cov_154.795116	29360..29675	KP347127

Table 4-5: Phenotypic resistance of *mcr-1* isolates

Isolates	Putative determinant	resistant	Colistin MIC (µg/mL)
EC_07	*mcr-1*	8	
EC_10	*mcr-1*		4
EC_12	*mcr-1*		4
EC_25	*mcr-1*		4
EC_38	*mcr-1*		4
EC_44	*mcr-1*		8
EC_50	*mcr-1*		4
EC_51	*mcr-1*		4
EC_55	*pmrB mutation*		0.25
EC_62	*mcr-1*		4
EC_67	*mcr-1*		4
EC_68	*mcr-1*		4
EC_79	*mcr-1*		4
EC_98	*mcr-1*		8
E. coli ATCC 25922	none		0.5
E. coli AR Bank #0350	mcr-1		4

4.10 Conjugation Assay

The confirmed mcr-1 gene was transferred from strain EC_79 to *E. coli* J53, via conjugation assay. EC_79 isolate was used as donors, and *E. coli* J53 (resistant to sodium-azide) was used as the recipient strain respectively (Fig 4.10). Therefore, transfer of the mcr-1 bearing plasmid from EC_79 to *E. coli* J53 was successful as observed on the mixed growth plate (having sodium azide and colistin), because the strain lacking the mcr-1 gene was unable to grow on colistin mixed media. In contrast, no growth was observed on the J53 control plate (Fig 4.10). Subsequently, plasmid mcr1_Inc12 was confirmed in several of our strains by NGS data using Plasmid-finder (Table 4.3). Furthermore, the transfer of the mcr-1 gene via IncI2 plasmid was confirmed through PCR amplification in the J53 recipient (Fig 4.11). Similarly, the result also showed the mcr-1 gene likely located on a plasmid, not was chromosomally encoded in strain EC_79 and was confirmed by PCR amplification (Figure 4.11).

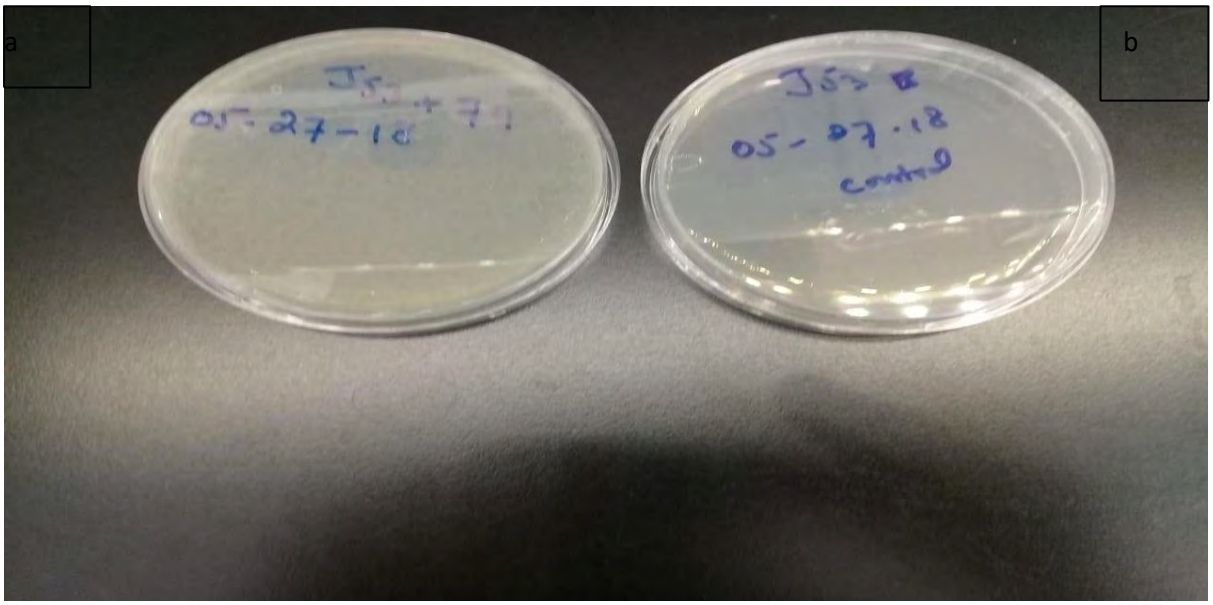


Figure 4.8 :(a) Mixed growth of j53 (recipient) and EC_79 (Donor) *E. coli*. (b) While on the Other plate no growth was observed on J53 as control.

4.11 Statistical Analysis

Statistical analysis for the prevalence of antimicrobial resistance between commercial and backyard poultry and among different regions was carried out by using two-way Anvvoa. The test was implemented in graph pad prism software online (version 7.00). *P-values* ≤ 0.0001 among the commercial and backyard poultry resistance pattern, which is considered statistically significant (Appendix-3). Furthermore, the antibiotic resistance pattern isolates of *E. coli* from provinces (Baluchistan, Punjab, Sindh, Khyber Pakhtunkhwa, Sindh) and capital Islamabad were also the very significant difference of antibiotic resistance of having P -values ≤ 0.0001 (Appendix-4).

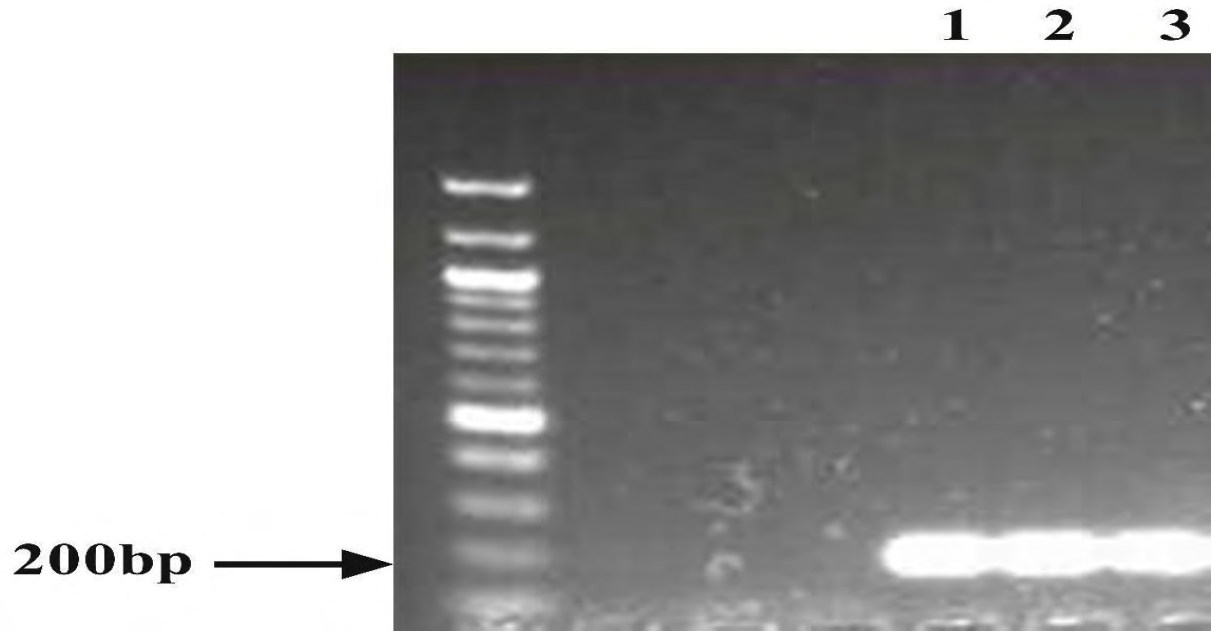


Figure 4.9: Gel Electrophoresis of *mcr-1* gene amplification (1) *mcr-1* positive control strain (2) EC_79 donor strain and (3) the J53 recipient strain of *mcr-1* gene of 200bp

The Supplementary material for this thesis can be found online at:
https://www.frontiersin.org/articles/10.3389/fmicb.2019.03052/full#sup plementary-materia

5.0 Discussion

Escherichia coli is living as a commensal inhabitant in vertebrates and is frequently reported as a microbial contaminant in meat products. The NARMS (National Antimicrobial Resistance Monitoring System) has continually monitored antibiotic resistance in foodborne *E. coli*. NARMS, on the other hand, is a United States government initiative that began in 1996 and collaborated with the US Department of Agriculture (USDA), the Food and Drug Administration (FDA), and the Centers for Disease Control and Prevention (CDC). The NARMS, collect and provide data of antimicrobial susceptibility of the enteric organism isolated from both human and animal populations (Ginevan, 2002). Resistance was increased in both clinical and foodborne *E. coli*. (Davis *et al.*, 2018b). Nevertheless, the highest antibiotics resistance was observed in livestock isolates than it did in clinical human isolates (Davis *et al.*, 2018b, Tadesse *et al.*, 2012). Therefore, the overuse of antibiotics in food-producing animals may have a significant threat and global health issue, which can be the source of producing MDR bacteria either capable of causing human diseases or transforming resistant genes to other human pathogens, according to the following studies (Alali *et al.*, 2010, Lazarus *et al.*, 2014, Miranda *et al.*, 2008a, Miranda *et al.*, 2008b). The above phenomena were supported by the isolation of gene encoding transferable colistin resistance gene *mcr-1* in humans and animals were first reported. However, colistin has been not approved for humans in china while used in animals as a therapeutic and growth promoter since 1980. Thus, it is expected that *the mcr-1* gene first occurred in animals then spread to humans (Liu *et al*., 2016). In the same way, in Switzerland, 16 transmissible plasmids were unanimously determined, including eight assuredly having *bla*$_{CTX\text{-}M\text{-}1}$-encoding essential genes from nine MDR *E. coli* in food animals and likely humans (Wang *et al*., 2014). Furthermore, in Switzerland, 3rd generation cephalosporin-resistant *E. coli* was identified from food animals (Wang *et al*., 2013).

The possible hypothesis is that various food and poultry products are reliable sources of *E. coli* that cause extraintestinal pathogenic *E. coli* active infections (ExPEC) supported by multiple lines of evidence 1: Genetic relationship between human EXPEC and Avian *E. coli.* 2: Many experimental studies scientifically show the potential of APEC's disease in a mammalian animal model, similarly the potential of the disease of EXPEC in avian model isolated from typical humans. 3: The close genetic relationship of *E. coli* isolated from human, poultry, retail

chicken meat based on molecular epidemiological data. 4: Furthermore, the observations in the past had shown that specific strains of *E. coli* in a specific community had caused a large number of infections in a short period, like cryptic outbreaks (Manges *et al*.,2016; Lazarus *et al*., 2015). *E. coli* (O25b: H4-ST131) and variant human EXPEC strains have been isolated from chicken farms in Spain (Cortés *et al*., 2010) and from other animals in Europe that causes infections in subtle humans (Ewers *et al*., 2010). Moreover, *E. coli* strains (O45:K1:H7-B2-ST95 DST371/ST 2676) have been recovered from patients with colibacillosis and poultry from France and Spain, in particular, which more than 85 % of clusters similarity were detected (Mora *et al*., 2013). From the previous hypothesis, this comparative study was typically conducted to determine the apparent prevalence of MDR *E. coli* in Pakistan poultry. From 2015 to 2017, a total of 1,219 liver samples from culled layer and broiler chickens with limited birth growth and reduced appetite but no other colibacillosis symptoms were collected from Pakistan's National Reference Laboratory for Poultry Diseases via federal and provincial sentinel surveillance laboratories as part of a national surveillance program. Most of the isolates, according to our findings, belong to the B1 or A clades. (Figure 4.5). With the more rigorous criterion of sequencing isolates obtained from active chicken illnesses and chickens with failure to develop, a more significant number of genetic signatures linked to phylogroup may be uniquely discovered. These results are comparable to those reported in the literature for *E. coli* from bovine mastitis in Ireland, mainly producing B1 and A clade genomes (Keane, 2016). A comparison of avian associated *E. coli* isolates from several leading European countries discovered that there was variation within each country, but that the A1 and B2 phylogroups were the most common, indicating that on a continental scale, geographic location may affect *E. coli* fascinating background (Cordoni *et al*., 2016). B2 and A emerged as the main phylogroups in the broader study of European avian associated *E. coli* sequesters (Mora *et al*., 2013). Because our research did not include a control chicken gut arm, the B1 and A clade isolates we discovered were identified as commensals in a higher percentage of the chickens. Except for the discovery that all Balochistan isolates were B1, we discovered that the phylogroups are equally distributed throughout Pakistan. This could be due to the small sample size from that region (11 isolates). The important aspect is that we found several phylogroups. Similarly sized isolate sets from Sindh and Khyber Pakhtunkhwa, and that the Sulaiman and Brahui ranges physically divide most of Balochistan from the rest of Pakistan (southern offshoots of the Hindu Kush Himalayan Region) shows the possibility of finding phylogeneticseparation of chicken associated *E. coli* in a larger collection of isolates throughout Pakistan's huge area.

When hierarchically grouped by ARG, virulence gene, or SNP presence, as well as phenotypic resistance, the B2 isolates often separate, indicating the longer branch lengths of these isolates in the phylogeny in (Figure 4.6A) compared to the majority of the prospective cohort. The phylogroups have been separated more effectively by the presence of virulence genes than ARGs, perhaps due to ARGs' greater mobility. The most common plasmid replicons in our sample were IncFIB plasmid replicons. In a study of *E. coli* in the United States, IncFIB plasmids were identified in substantially higher numbers in *E. coli* suspected of being avian pathogens or from retail chicken than in human and avian commensals (Johnson *et al*., 2016). The sensitivity pattern of specific antibiotics has been tested in Pakistan and the USA, respectively, a model similar for meropenem, ciprofloxacin, gentamycin, trimethoprim, doxycycline, nitrofurantoin, chloramphenicol, norfloxacin, streptomycin, lincomycin, ceftiofur, and oxytetracycline (Supplementary Tables S4, S5). While only the two antibiotics, colistin and spectinomycin, had revealed some differences (Supplementary Tables S4, S5). These core differences may be due to the miss interpretation of the resistant and intermediate zone or the possible use of complex MacFarland solution in Pakistani sensitivity protocol (Supplementary Tables S5).

MLST data in this study had shown that 11/16 of ST155 serotypes were positive for *bla*$_{\text{TEM-1b.}}$ similar finding reported in (Wang *et al*., 2014). While two of the ST155 serotypes (EC-59 and EC02) were positive for *bla*$_{\text{CTX-M-15,}}$ and the rest of ST155 have no beta-lactamase resistant genes (Figure 4.6). Therefore, these findings are endorsed with the study conducted in France on foodproducing animals, in which *bla*$_{\text{CTX-M-1}}$, *bla*$_{\text{CTX-M-15}}$ genes of *E. coli* strains were isolated from swine, poultry, cattle (Meunier *et al*., 2006), that these genes have well spread in both animals and humans. Meanwhile, *the E. coli* ST155 serotype was previously reported in the zoonotic transmission of ESBL genes to humans (Salim *et al*., 2019). However, all our ST155 strains belonged to B1 phylogroups (Figure 4.6). Formerly, the same ST155 type of *E. coli* strains was isolated from chicken of B1 phylogroup, from Switzerland (Wang *et al*., 2014). In recent years the study conducted in Ghana on poultry meat also reported that *bla*$_{\text{CTX-M-15}}$ was predominant in ST155 serotypes (Eibach *et al*., 2018). These findings support the phenomena of transmission of AMR genes between poultry and human reservoirs. One of the fascinating findings in this study was the EC-60 strain of phylogroup ST131. The ST131 strain of *E. coli* is frequently reported and related to human infections, including community acquired UTI infections and bacteremia worldwide (Chen *et al*., 2016).

The EC-60 ST131 has *bla*$_{\text{CTX-M-1}}$ gene along with IncFIB (AP001918), IncFIC (F11), and Incl 1 plasmid replicons (Figure 4.6). *E. coli* ST131 has been universally reported from humans, animals, the environment, and food (Rogers *et al*., 2011; Jamborova *et al*., 2018). Therefore, a study on isolation and characterization of sewage *E. coli* in Pakistan has also reported *E. coli* ST131 strains

(Zahra *et al*., 2018). Similarly, Ali and co-workers found 68 ST131 isolates out of 148 from clinical samples processed in Pakistan (Ali *et al*.,2016), which shows that ST131 is common in Pakistan clinical samples.

In ESBL, *bla*$_{CTX\text{-}M\text{-}1}$ is more commonly reported in animals isolated samples (Madec *et al*., 2015). Our finding agreed with the study conducted on the broiler in Switzerland that *the bla*$_{CTX\text{-}M\text{-}1}$ gene has disseminated on *IncI1* plasmid (Zurfluh *et al*., 2014). The presence of ST131 *E. coli with* ESBL in our poultry samples support the evidence of zoonotic transmission between poultry and humans. It is another line of evidence that *E. coli* could disseminate among animals, the environment, and humans (Figure 4.6). MALST data has shown another important serotype ST 117, in the samples. All the nine ST 117 (09/92) strains were reported in this study to belong to phylogroups B2 (Figure 4.6). Two EC-83 and EC-35-B interestingly have *bla*$_{CTX\text{-}M\text{-}15}$ genes while the rest have *bla*$_{TEM\text{-}1b}$ genes (Figure 4.6). However, the same serotype ST117 *E. coli* has been isolated from human stool and chicken meat simultaneously in the Netherlands (Overdevest *et al*., 2011). Similarly, in Italy, the ST117 strains were reported from human and avian *E. coli* samples (Giufrè, *et al*., 2012). In Sweden, together with other *E. coli* (ST69, ST10) serotypes, ST117 had fifty percent of the ESBL *E. coli* recovered from domestic chicken meat (Egervärn *et al*., 2014). However, a study in the United Kingdom documented *bla*$_{CTX\text{-}M\text{-}1}$ is the common ESBL gene in chicken isolated *E. coli* (Day *et al*., 2019). Similarly, a study in Germany reported *bla*$_{CTX\text{-}M\text{-}1}$ in ST117 isolates from chicken samples (Freitag *et al*., 2017). This is opposed with our investigation the diverse linage in the different geographical regions. However, this sequence type ST117 has been reported in causing diseases both in animals (Mora *et al*., 2012) and human sepsis and urinary tract diseases (Manges and Johnson, 2012). Conclusively, the serotype ST117 with *bla*$_{CTX\text{-}M\text{-}15}$ has been found in human, and poultry samples support the hypothesis of the zoonotic potential of this avian *E. coli*.

One of our considerable strains Ec-74 (ST101), also has the *bla*$_{CTX\text{-}M\text{-}15}$ gene. The sequence type (ST101) has been isolated from humans and swine with *bla*$_{CTX\text{-}M}$ genes, in which 95.75% of their isolates have ESBL *bla*$_{CTX\text{-}M}$ group; the study was conducted in northern Thailand in 2019 on healthy humans and swine. (Seenama *et al*., 2019). Therefore, it agrees with our finding that ST101 has similar ESBL genes and lineage of human *E. coli*.

Comparatively, antibiotic resistance from our isolates and isolates from human *E. coli* particularly, in ESBL positive isolates, has different results. However, in the current study, 12 of our isolates carried *bla*$_{CTX\text{-}M\text{-}15}$ is the second most common after *bla*$_{T\text{-}EM\text{-}1B}$. (Figure 4.6). Similarly, Rubab and co-workers also reported the same *bla*$_{CTX\text{-}M\text{-}15}$ genes in 34 isolates from sewage isolated *E. coli* in which one isolate carried *bla*$_{CTX\text{-}M\text{-}27}$ from Pakistan (Zahra *et al*., 2018). However, a study

conducted on ESBL derived *E. coli* of humans and animals in the UK, *bla*$_{CTX-M-15}$ has only reported in human samples about 77% but not in animals (Day *et al*., 2019), it is revealed that Pakistani linage of avian *E. coli* is more relevant to human pathogenic *E. coli* reported from the rest of the world. And the high prevalence of this gene in Pakistani *E. coli* strains, as compared to other *bla*$_{CTX-M}$ groups, particularly *bla*$_{CTX-M-1}$, has been reported in only one of our isolate.

However, a study was conducted between year 2012-2014 in Pakistan, where 148 uropathogenic *E. coli* were isolated from the Pakistan Institute of Medical Sciences (PIMS), Islamabad (Ali *et al*., 2016), in which most of the ESBL positive strains have *the bla*$_{CTX-M-15}$ gene. As a result, all these findings have shown that *the bla*$_{CTX-M-15}$ gene is more common in the Pakistani gene pool whether it was isolated from poultry, environment, and human samples. However, based on the previous study, the phylogenic linage was dominated by the B2 group (Ali *et al*., 2016), while most of our strains were from B1, which shown that different linage exists in humans and animals.

Colistin is considered a last resort drug to treat gram-negative bacterial infections caused by MDR bacteria (Olaitan *et al*., 2014). In veterinary, colistin is administered in food to control bacterial infections in poultry (Kempf *et al*., 2013). Furthermore, the transmission of *the mcr-1* gene exhibited a threat to human health from animals (Yao *et al*., 2016). In Pakistan, antibiotics usage for food animals (poultry, cattle) is enormously dominant for food safety (Mohsin *et al.,* 2017). Because Pakistan is in the top 10 countries, where intense farming practices (high use of antibiotics) in the animal industry (poultry, cattle) have been administrated as a growth promoter and disease control. However, woefully, there is no proper check and balance by the government of Pakistan on the use of antibiotics (Mohsin *et al.,* 2017; ur Rahman *et al.,* 2018b). That's why it is tough to find out the exact amount of antibiotics for growth promoters and disease control used in food-producing animals in Pakistan (Mohsin *et al.,* 2017).

In the present study, we isolated 13 *E. coli* strains with different sequence types (ST117, ST1011, ST2847, ST533, ST1324, ST2973, ST155, ST4516) have *mcr-1* genes (Figure4.6). Three of the *E. coli* were ST155, one strain was ST117, and the rest were 1=1011, 2=2847, 1= no group, 1=533, 2=1324,1=2973, 1=4516 serotypes (Figure 4.6). To the best of our knowledge, this is the first study that we had reported a large number of *mcr-1 E. coli* strains from poultry in Pakistan. However, small-scale studies have previously been conducted in Pakistan poultry and reported the *mcr-1* positive *E. coli* from chicken (Azam *et al.,* 2017; Lv *et al.,* 2018; Mohsin *et al*., 2019). Our findings also suggested that *the mcr-1* gene is well-spread in Pakistan poultry. Additionally, a similar study was conducted in China in which 40 *mcr-1* positive *E. coli* isolates have been reported

from different sequence types. All the above *mcr-1* samples were reported from 13 provinces of China in the year 2010-2015 (Yang *et al*., 2017), belonging to different sequence types.

The discovery of the mobilizable colistin ARG, *mcr-1*, in 14 percent (13/92) of the isolates, all of which showed phenotypic resistance to colistin, was of considerable concern given our study of the ARG content within the cohort. Initially discovered in *E. coli* from Chinese swine farms, it has subsequently been found in a wide range of bacteria, including a cohort of avian *E. coli* isolates from Egypt and China, albeit at a considerably lower frequency of 1% (12/1220 isolates). (Liu*et al*., 2016; Lima *et al*., 2017). Moreover, *mcr-1* was identified in 4% (58/1136) of the genomes of *E. coli* from chickens in 13 Chinese provinces, according to a thorough study (Yang *et al*., 2017), while in a study of 100 *E. coli* chicken isolates from Faisalabad, Pakistan, the frequency of *mcr-1* was reported to be 8% (Lv *et al*., 2018).

An *E. coli* strain from a Tunisian chicken farm was discovered to have both bla_{CMY-2} and *mcr-1* and antibiotic resistance to many additional classes (Maamar *et al*., 2018). Our cohort only included one bla_{CMY-2} isolate, which did not contain *mcr-1* but did have the *pmrB V161G* mutation, which has previously been demonstrated to confer colistin resistance but did not in our research. (Delannoy *et al*., 2017). It was the most frequent virulence gene found in us cohort, similar to earlier studies of *E. coli* isolates from poultry (Keane, 2016). *E. coli* growth in serum was substantially disrupted when an isolate was deleted. (Huja *et al*., 2015). Finally, adopting the criteria of MDR as non-susceptibility to at least one drug in three or more antimicrobial classes, we discovered that 82/92 isolates are MDR. (Magiorakos *et al*., 2012). This finding is comparable to that of a study of avian *E. coli* isolates from Nepal, which found that 94 percent (47/50) of the cohort was MDR (Subedi *et al*., 2018); and another study from Hebei, China, which found that 100% (87/87) of the cohort was MDR (Li *et al*., 2015).

One study of avian-associated *E. coli* from Pakistan's Punjab area showed nearly universal resistance to ampicillin (98.6%), while we found just 79.3% (73/92) (Azam *et al*., 2019). One study in Egypt showed 100% resistance to ampicillin in 116 avian-associated *E. coli* isolates (116/116) (Awad *et al*., 2016). According to a research of retail poultry goods from the United States, *E. coli* from turkey products showed a greater incidence of ampicillin resistance (62%) than *E. coli* from chicken products (20 percent) (Davis *et al*., 2018).

Fortunately, no phenotypic resistance was found in many therapeutically important antibiotics (Meropenem, imipenem, cefotetan, piperacillin-tazobactam, and amikacin). This finding is in line with a recent study of *E. coli* isolates from the ecosystem in Japan, which the authors characterized as having a virulence gene profile comparable to isolates linked with avian illnesses and being

100 percent sensitive to carbapenems and aminoglycosides (Hayashi *et al*., 2019). We were unable to clearly identify ARGs or virulence genes present on mobilizable plasmids using short Illumina reads, which limited our research. Moreover, we could not discuss the similarities and differences between the liver-borne isolates and commensal sites due to the lack of sequencing of isolates obtained from the intestinal contents of indicated hens.

Furthermore, we do not have access to a chicken infection model to show connections between the strains' comparative pathogenicity and their virulence gene mosaic. Because clinical breakpoints are limited, some isolates with genetic markers of resistance may test phenotypically susceptible. There was no apparent connection between phenotypic resistance and the genotypic existence of a resistant determinant since we examined several drugs of the same class. We were unable to provide MIC values since we utilized disc diffusion instead of quantitative broth microdilution to evaluate antimicrobial susceptibility (except in instances of *mcr-1* positive isolates).

In conclusion, we gathered a group of chicken-associated *E. coli* isolates from Pakistan's various regions. The bulk of the isolates in this cohort are from the B1 and A clades, and they contain a variety of ARGs and virulence genes, which may make human infection treatment more difficult.

1.1. Future Prospects

In Pakistan and worldwide food security and food safety is highly important. Livestock have number of challenges. Considering the one health concept certain pathogenic microbes in humans, animals and environment are common and exposed to antibiotics. Therefore, current study basis to point out certain indicator organism of Enterobacterace. We must deal with them in terms of knowing their pathogenicity, MDR, virulence genes and their potential of transforming these factors to another organism. Such organisms with such common ability of pathogenicity and antibiotic resistance should be figure out under the umbrella of one health concept and deal with specific drugs so the diseases burden is reduced.

Specific future goals are including:

- Further analysis of the mcr-1 positive strains through detailed draft genome sequence is require due to one health concept as it is reported from animal, humans and environment simultaneously.
- Zoonosis and molecular epidemiology of MDR *E. coli* on basis of human and animal intersection.
- Surveillance of integron sequence in MDR *E. coli* can provide useful information regarding the evolutionary changes of genes cassettes
- Characterization based on pathogenesis in *E. coli* and other related species is required

- Comparison with related species and other bacteria and white prevalence as indicator organism
- Sequencing of the conjugated plasmid through nanopore sequencing is needed for molecular evidence of conjugation assay.
- Large scale National surveillance and epidemiological analysis of AMR would present clear picture of AMR prevalence in the country

6.0 References

Aarestrup, F. M., F. Bager and J. S. ANDERSEN, 2000: Association between the use of avilamycin for growth promotion and the occurrence of resistance among Enterococcus faecium from broilers: epidemiological study and changes over time. *Microbial Drug Resistance,* **6,** 7175.

Abbas, G., I. Khan, and M. Mohsin. 2019. High rates of CTX-M group-1 extended-spectrum blactamases producing Escherichia coli from pets and their owners in Faisalabad, Pakistan. Infect. Drug. Resist. 12:571–578.

Abbas, G., S. H. Khan, M. Hassan, S. Mahmood, S. Naz and S. S. Gilani, 2015: Incidence of poultry diseases in different seasons in Khushab district, Pakistan. *Journal of Advanced Veterinary and Animal Research,* **2,** 141-145.

Abdelgader, S. A., D. Shi, M. Chen, L. Zhang, H. M. A. Hejair, U. Muhammad, H. Yao and W. Zhang, 2018: Antibiotics Resistance Genes Screening and Comparative Genomics Analysis of Commensal Escherichia coli Isolated from Poultry Farms between China and Sudan. *BioMed research international***,** 5327450.

Abo-Amer, A. E., M. Y. Shobrak and A. D. Altalhi, 2018: Isolation and antimicrobial resistance of Escherichia coli isolated from farm chickens in Taif, Saudi Arabia. *Journal of global antimicrobial resistance,* **15,** 65-68.

Abrar, S., S. Hussain, R.A. Khan, N. Ul Ain, H. Haider, and S. Riaz. 2018. Prevalence of extended-spectrum-blactamase-producing Enterobacteriaceae: first systematic meta-analysis report from Pakistan. Antimicrob. Resist. Infect. Control 7:1–11

Adey, A. and J. Shendure, 2012: Ultra-low-input, tagmentation-based whole-genome bisulfite sequencing. *Genome research,* **22,** 1139-1143.

Adnan, M., Khan, H., Kashif, J., Ahmad, S., Gohar, A., Ali, A., Khan, M.A., Shah, S.S.A., Hassan, M.F. and Irshad, M., 2017. Clonal expansion of sulfonamide.

Adzitey, F., S. Nafisah and A. Haruna, 2015: Antibiotic Susceptibility of Escherichia coli Isolated from some Drinking Water Sources in Tamale Metropolis of Ghana.

Ahmed, A. M., T. Shimamoto and T. Shimamoto, 2013: Molecular characterization of multidrug-resistant avian pathogenic Escherichia coli isolated from septicemic broilers. *International Journal of Medical Microbiology,* **303,** 475-483.

Ajaiyeoba, I. A., H. U. Pindiga and E. E. Akang, 1992: Tumours of the eye and orbit in Ibadan. *East Afr Med J,* **69,** 487-489.

Ajaiyeoba, I. A., Pindiga, H. U., & Akang, E. E. (1992). Tumours of the eye and orbit in Ibadan. *East African medical journal*, *69*(9), 487-489.

Akhtar, F., I. Hussain, A. Khan and S. Rahman, 2010: Prevalence and antibiogram studies of Salmonella enteritidis isolated from human and poultry sources. *Pakistan Veterinary Journal,* **30,** 25-28.

Alali, W. Q., S. Thakur, R. D. Berghaus, M. P. Martin and W. A. Gebreyes, 2010: Prevalence and distribution of Salmonella in organic and conventional broiler poultry farms. *Foodborne Pathogens and Disease,* **7,** 1363-1371.

Alexander, D. J., 2000: A review of avian influenza in different bird species. *Veterinary microbiology,* **74,** 3-13.

Alexandratos, N. and J. Bruinsma, 2012: World agriculture towards 2030/2050: the 2012 revision. ESA Working paper FAO, Rome.

Ali T, Zhang L, Shahid M, et al., 2016. ESBL-producing Escherichia coli from cows suffering mastitis in China contain clinical class 1 integrons with CTX-M linked to ISCR1. Front Microbiol 7:1931

Ali, I., Rafaque, Z., Dasti, J. I., Graham, S. E., Salzman, E., & Foxman, B. (2016, December). Uropathogenic *E. coli* from Pakistan have high prevalence of multidrug resistance, ESBL, and O25b-ST131. In *Open Forum Infectious Diseases* (Vol. 3, No. suppl_1, p. 2008). Oxford University Press.

Alikhan, N. F., Zhou, Z., Sergeant, M. J., & Achtman, M. 2018. A genomic overview of the population structure of Salmonella. *PLoS genetics*, *14*(4).

Allos, B. M., Moore, M. R., Griffin, P. M., & Tauxe, R. V. (2004). Surveillance for sporadic foodborne disease in the 21st century: the FoodNet perspective.

Alston, J. M., J. M. Beddow and P. G. Pardey, 2009: Agricultural research, productivity, and food prices in the long run. *Science,* **325,** 1209-1210.

Amer, M. M., H. M. Mekky, A. M. Amer and H. S. Fedawy, 2018: Antimicrobial resistance genes in pathogenic Escherichia coli isolated from diseased broiler chickens in Egypt and their relationship with the phenotypic resistance characteristics. *Vet World,* **11,** 1082-1088.

Amos, G. C., Hawkey, P. M., Gaze, W. H., & Wellington, E. M. (2014). Wastewater effluent contributes to the dissemination of CTX-M-15 in the natural environment. *Journal of Antimicrobial Chemotherapy*, *69*(7), 1785-1791.

Andersson, D. I. and D. Hughes, 2010: Antibiotic resistance and its cost: is it possible to reverse resistance? *Nature Reviews Microbiology,* **8,** 260.

Angulo, P., Hui, J. M., Marchesini, G., Bugianesi, E., George, J., Farrell, G. C., ... & Day, C. P. (2007). The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology*, *45*(4), 846-854.

Anjum, M. F., 2015: Screening methods for the detection of antimicrobial resistance genes present in bacterial isolates and the microbiota. *Future Microbiol,* **10,** 317-320.

Anjum, M. F., E. Zankari and H. Hasman, 2017: Molecular Methods for Detection of Antimicrobial Resistance. *Microbiol Spectr,* **5**.

Anjum, M. F., F. Lemma, D. J. Cork, D. Meunier, N. Murphy, S. E. North, N. Woodford, J.

Anjum, M. F., M. Mafura, P. Slickers, K. Ballmer, P. Kuhnert, M. J. Woodward and R. Ehricht, 2007: Pathotyping Escherichia coli by using miniaturized DNA microarrays. Applied and environmental microbiology*,* **73,** 5692-5697.

Anjum, M. F., N. A. Duggett, M. AbuOun, L. Randall, J. Nunez-Garcia, R. J. Ellis, J. Rogers, R. Horton, C. Brena, S. Williamson, F. Martelli, R. Davies and C. Teale, 2016: Colistin resistance in Salmonella and Escherichia coli isolates from a pig farm in Great Britain. *The Journal of antimicrobial chemotherapy,* **71,** 2306-2313.

Antelo, V., Guerout, A. M., Mazel, D., Romero, V., Sotelo-Silveira, J., & Batista, S. (2018). Bacteria from Fildes Peninsula carry class 1 integrons and antibiotic resistance genes in conjugative plasmids. *Antarctic Science*, *30*(1), 22-28.

ANTIMICROBIAL RESISTANCE FROM SMALL SCALE POULTRY SLAUGHTERHOUSES IN PAKISTAN. APPLIED ECOLOGY AND ENVIRONMENTAL RESEARCH, 17, 3049-3063. application in foods and pharmaceuticals. *Frontiers in microbiology,* **5,** 241.

Araujo, R., & Sampaio-Maia, B. 2018. Fungal Genomes and Genotyping. In *Advances in applied microbiology* (Vol. 102, pp. 37-81). Academic Press.

Arya, M., I. S. Shergill, M. Williamson, L. Gommersall, N. Arya and H. R. Patel, 2005: Basic principles of real-time quantitative PCR. *Expert Rev Mol Diagn,* **5,** 209-219.

Asai, T., H. Esaki, A. Kojima, K. Ishihara, Y. Tamura and T. Takahashi, 2006: Antimicrobial resistance in Salmonella isolates from apparently healthy food-producing animal from 2000 to 2003: the first stage of Japanese Veterinary Antimicrobial Resistance Monitoring (JVARM). *Journal of Veterinary Medical Science,* **68,** 881-884.

Asai, T., K. Masani, C. Sato, M. Hiki, M. Usui, K. Baba, M. Ozawa, K. Harada, H. Aoki and T. Sawada, 2011: Phylogenetic groups and cephalosporin resistance genes of Escherichia coli from diseased food-producing animals in Japan. *Acta Veterinaria Scandinavica,* **53,** 52.

Ashton PM, Nair S, Dallman T, Rubino S, Rabsch W, et al. 2015. MinION nanopore sequencing identifies the position and structure of a bacterial antibiotic resistance island. Nat. Biotechnol. 33:296–300.

Avasthi, T. S., Kumar, N., Baddam, R., Hussain, A., Nandanwar, N., Jadhav, S., & Ahmed, N. (2011). Genome of multidrug-resistant uropathogenic Escherichia coli strain NA114 from India.

Awad, A., N. Arafat and M. Elhadidy, 2016: Genetic elements associated with antimicrobial resistance among avian pathogenic Escherichia coli. *Annals of clinical microbiology and antimicrobials,* **15,** 59.

Azam, M., M. Mohsin, R. Sajjad Ur and M. K. Saleemi, 2019: Virulence-associated genes and antimicrobial resistance among avian pathogenic Escherichia coli from colibacillosis affected broilers in Pakistan. *Tropical animal health and production*.

Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., & Baba, M. (2006). KA 542

Bachmann, B. J. (1996). Derivations and genotypes of some mutant derivatives of Escherichia coli K-12. *Escherichia coli and Salmonella: cellular and molecular biology, 2nd ed. ASM Press, Washington, DC*, 2460-2488.

Baffoni, L., F. Gaggia, D. Di Gioia, C. Santini, L. Mogna and B. Biavati, 2012: A Bifidobacterium-based synbiotic product to reduce the transmission of C. jejuni along the poultry food chain. *Int J Food Microbiol,* **157,** 156-161.

Bagust, T. J., 2013: Poultry health and disease control in developing countries. *the role of poultry in human nutrition***,** 96.

Bailey, J. K., J. L. Pinyon, S. Anantham and R. M. Hall, 2011: Distribution of the blaTEM gene and blaTEM-containing transposons in commensal Escherichia coli. *The Journal of antimicrobial chemotherapy,* **66,** 745-751.

Baker, S., Duy, P. T., Nga, T. V. T., Dung, T. T. N., Phat, V. V., Chau, T. T., ... & Boni, M. F. (2013). Fitness benefits in fluoroquinolone-resistant Salmonella Typhi in the absence of antimicrobial pressure. *Elife*, *2*, e01229.

Bakker D, Corver J, Harmanus C, Goorhuis A, Keessen EC, Fawley WN, Wilcox 331 MH, Kuijper EJ. 2010. Relatedness of human and animal Clostridium difficile PCR 332 ribotype 078 isolates determined on the basis of multilocus variable -number tandem - 333 repeat analysis and tetracycline resistance. J Clin Microbiol 48:3744 -3749.

Banerjee, R., Robicsek, A., Kuskowski, M. A., Porter, S., Johnston, B. D., Sokurenko, E., ... & Johnson, J. R. (2013). Molecular epidemiology of Escherichia coli sequence type 131 and its H30 and H30-Rx subclones among extended-spectrum-β-lactamase-positive and-negative E. coli clinical isolates from the Chicago region, 2007 to 2010. *Antimicrobial agents and chemotherapy*, *57*(12), 6385-6388.

Bankevich, A., S. Nurk, D. Antipov, A. A. Gurevich, M. Dvorkin, A. S. Kulikov, V. M. Lesin, S. Baquero, F., 2001: Low-level antibacterial resistance: a gateway to clinical resistance. *Drug Resistance Updates,* **4,** 93-105.

Barbe, V., D. Vallenet, N. Fonknechten, A. Kreimeyer, S. Oztas, L. Labarre, Barbee, L. A., O. O. Soge, K. K. Holmes and M. R. Golden, 2014: In vitro synergy testing of novel antimicrobial combination therapies against Neisseria gonorrhoeae. *The Journal of antimicrobial chemotherapy,* **69,** 1572-1578.

Barl, T., U. Dobrindt, X. Yu, D. J. Katcoff, D. Sompolinsky, S. Bonacorsi, J. Hacker and T. T. Bachmann, 2008: Genotyping DNA chip for the simultaneous assessment of antibiotic resistance and pathogenic potential of extraintestinal pathogenic Escherichia coli. *Int J Antimicrob Agents,* **32,** 272-277.

Bartlett, J. G., D. N. Gilbert and B. Spellberg, 2013: Seven ways to preserve the miracle of antibiotics. *Clin Infect Dis,* **56,** 1445-1450.

Barton, M. D., 2000: Antibiotic use in animal feed and its impact on human healt. *Nutrition research reviews,* **13,** 279-299.

Bassetti, M. and E. Righi, 2014: Eravacycline for the treatment of intra-abdominal infections.

Baym, M., S. Kryazhimskiy, T. D. Lieberman, H. Chung, M. M. Desai and R. Kishony, 2015a: Inexpensive multiplexed library preparation for megabase-sized genomes. *PLoS One,* **10,** e0128036.

Baym, M., S. Kryazhimskiy, T. D. Lieberman, H. Chung, M. M. Desai and R. Kishony, 2015b: Inexpensive multiplexed library preparation for megabase-sized genomes. *PloS one,* **10,** e0128036-e0128036.

Beatty, M. E., Adcock, P. M., Smith, S. W., Quinlan, K., Kamimoto, L. A., Rowe, S. Y., ... & Mintz, E. D. (2006). Epidemic diarrhea due to enterotoxigenic Escherichia coli. *Clinical infectious diseases*, *42*(3), 329-334.

Bedford, M. R. and G. G. Partridge, 2010: *Enzymes in farm animal nutrition*. CABI.

Benameur, Q., H. Tali-Maamar, F. Assaous, B. Guettou, N. TAHRAT, N. Aggoune, K. Rahal and M.-H. Ben-Mahdi, 2018: Isolation of Escherichia coli carrying the blaCTX-M-1 and qnrS1 genes from reproductive organs of broiler breeders and internal contents of hatching eggs. *Journal of Veterinary Medical Science***,** 18-0283.

Bergeron CR, Prussing C, Boerlin P, Daignault D, Dutil L, et al. 2012. Chicken as reservoir for extraintestinal pathogenic Escherichia coli in humans, Canada. Emerg. Infect. Dis. 18:415–21

Berni, E., P. Marcato, G. Nakazato, R. Kobayashi, F. Vacchi, G. Umbuzeiro and N. Durán, Violacein/poly (ϵ-caprolactone)/chitosan nanoparticles against bovine mastistis: Antibacterial and ecotoxicity evaluation. in Proceedings of the Journal of Physics: Conference Series, 2013, p. 012030.

Bertrand, X., Y. Huguenin and D. Talon, 2002: First report of a catalase-negative methicillin resistant Staphylococcus aureus. Diagnostic microbiology and infectious disease, **43,** 245-246.

Bhargava, H. and P. A. Leonard, 1996: Triclosan: applications and safety. *American journal of infection control,* **24,** 209-218.

Bhullar, K., N. Waglechner, A. Pawlowski, K. Koteva, E. D. Banks, M. D. Johnston, H. A. Barton and G. D. Wright, 2012: Antibiotic resistance is prevalent in an isolated cave microbiome. *PloS one,* **7,** e34953.

Bilecen, K., G. Yaman, U. Ciftci and Y. R. Laleli, 2015: Performances and Reliability of Bruker Microflex LT and VITEK MS MALDI-TOF Mass Spectrometry Systems for the Identification of Clinical Microorganisms. *BioMed research international***,** 516410-516410.

Bintvihok, A., S. Treebonmuang, K. Srisakwattana, W. Nuanchun, K. Patthanachai and S. Usawang, 2016: A Rapid and Sensitive Detection of Aflatoxin-producing Fungus Using an Optimized Polymerase Chain Reaction (PCR). *Toxicol Res,* **32,** 81-87.

Biswas, B., Adhya, S., Washart, P., Paul, B., Trostel, A. N., Powell, B., & Merril, C. R. 2002. Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin resistant Enterococcus faecium. *Infection and immunity*, *70*(1), 204-210.

Blair, J. M., M. A. Webber, A. J. Baylay, D. O. Ogbolu and L. J. Piddock, 2015: Molecular mechanisms of antibiotic resistance. *Nature reviews microbiology,* **13,** 42.

Blair, J. M., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. (2015). Molecular mechanisms of antibiotic resistance. *Nature reviews microbiology*, *13*(1), 42-51.

Blake, K. L. and A. J. O'Neill, 2013: Transposon library screening for identification of genetic loci participating in intrinsic susceptibility and acquired resistance to anti- staphylococcal agents.

Blake, K. L., & O'Neill, A. J. 2013. Transposon library screening for identification of genetic loci participating in intrinsic susceptibility and acquired resistance to anti-staphylococcal agents. *Journal of Antimicrobial Chemotherapy*, *68*(1), 12-16.

Bogaerts, P., Huang, T. D., Bouchahrouf, W., Bauraing, C., Berhin, C., El Garch, F, & ComPath Study Group. (2015). Characterization of ESBL-and AmpC-producing Enterobacteriaceae from diseased companion animals in Europe. *Microbial Drug*

Bolger, A. M., M. Lohse and B. Usadel, 2014: Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics,* **30,** 2114-2120.

Bolognini, D., Bartalucci, N., Mingrino, A., Vannucchi, A. M., & Magi, A. (2019). NanoR: A user-friendly R package to analyze and compare nanopore sequencing data. *PloS one*, *14*(5).

Borah, M., R. Islam, M. Sarma, J. Mahanta and N. Kalita, 2017: Prevalence and seasonal variation of certain microbial diseases in Kamrup and Kamrup (Metro) Districts of Assam. *International Journal of Chemical Studies,* **5,** 724-726.

Borgatta, B., Kmet-Lunaček, N., & Rello, J. (2012). E. coli O104: H4 outbreak and haemolytic–uraemic syndrome. *Medicina Intensiva (English Edition)*, *36*(8), 576-583.

Born, Y., L. Bosshard, B. Duffy, M. J. Loessner and L. Fieseler, 2015: Protection of Erwinia amylovora bacteriophage Y2 from UV-induced damage by natural compounds. *Bacteriophage,* **5,** e1074330.

Bourlioux, P., 2013: Which alternatives are at our disposal in the anti-infectious therapeutics face to multi-drug resistant bacteria? *Annales pharmaceutiques francaises,* **71,** 150-158.

Boyen, F., F. Vangroenweghe, P. Butaye, E. De Graef, F. Castryck, P. Heylen, M. Vanrobaeys and F. Haesebrouck, 2010: Disk prediffusion is a reliable method for testing colistin susceptibility in porcine E. coli strains. *Veterinary microbiology,* **144,** 359-362.

Bradley, P., N. C. Gordon, T. M. Walker, L. Dunn, S. Heys, B. Huang, S. Earle, L. J. Pankhurst, Braykov, N. P., J. N. Eisenberg, M. Grossman, L. Zhang, K. Vasco, W. Cevallos, D. Munoz, A. Acevedo, K. A. Moser, C. F. Marrs, B. Foxman, J. Trostle, G. Trueba and K. Levy, 2016: Antibiotic Resistance in Animal and Environmental Samples Associated with Small-Scale Poultry Farming in Northwestern Ecuador. *mSphere,* **1**.

Brochet, M., E. Couve´, M. Zouine, C. Poyart, and P. Glaser. 2008. A naturally occurring gene amplification leading to sulfonamide and trimethoprim resistance in *Streptococcus agalactiae*. J. Bacteriol. **190:**672–680.

Brogan, D. M. and E. Mossialos, 2016: A critical analysis of the review on antimicrobial resistance report and the infectious disease financing facility. *Globalization and health,* **12,** 8.

Brower, C. H., S. Mandal, S. Hayer, M. Sran, A. Zehra, S. J. Patel, R. Kaur, L. Chatterjee, S. Mishra, B. R. Das, P. Singh, R. Singh, J. P. S. Gill and R. Laxminarayan, 2017: The Prevalence

of Extended-Spectrum Beta-Lactamase-Producing Multidrug-Resistant Escherichia Coli in Poultry Chickens and Variation According to Farming Practices in Punjab, India. *Environ Health Perspect,* **125,** 077015.

Brown, D., 2015: Antibiotic resistance breakers: can repurposed drugs fill the antibiotic discovery void? *Nat Rev Drug Discov,* **14,** 821-832.

Buchholz U, Bernard H, Werber D, Bohmer MM, Remschmidt, et al. 2011. German outbreak of Escherichia coli O104:H4 associated with sprouts. N. Engl. J. Med. 365:1763–70

Burckhardt I, Zimmermann S. 2011. Using matrix-assisted laser desorption ionization-time of flight mass spectrometry to detect carbapenem resistance within 1 to 2.5 hours. J Clin Microbiol 49:3321–3324 http://dx.doi.org/10.1128/JCM.00287-11.

Burghel GJ, Hurst CD, Watson CM, Chambers PA, Dickinson H, et al. 2015. Towards a nextgeneration sequencing diagnostic service for tumour genotyping: a comparison of panels and platforms. Biomed. Res. Int. 2015:478017.

Burlingame, B., 2004: Fostering quality data in food composition databases: visions for the future. *Journal of Food Composition and Analysis,* **17,** 251-258.

Butler, M. S. and A. D. Buss, 2006: Natural products--the future scaffolds for novel antibiotics? *Biochem Pharmacol,* **71,** 919-929.

Buttimer, C., O. McAuliffe, R. P. Ross, C. Hill, J. O'Mahony and A. Coffey, 2017: Bacteriophages and Bacterial Plant Diseases. *Frontiers in microbiology,* **8,** 34.

Caffrey, N., O. Nekouei, S. Gow, A. Agunos and S. Checkley, 2017: Risk factors associated with the A2C resistance pattern among E. coli isolates from broiler flocks in Canada. *Preventive veterinary medicine,* **148,** 115-120.

Cain, A. K. and R. M. Hall, 2012: Evolution of a multiple antibiotic resistance region in IncHI1 plasmids: reshaping resistance regions in situ. *The Journal of antimicrobial chemotherapy,* **67,** 2848-2853.

Cain, A. K., X. Liu, S. P. Djordjevic and R. M. Hall, 2010: Transposons related to Tn1696 in IncHI2 plasmids in multiply antibiotic resistant Salmonella enterica serovar Typhimurium from Australian animals. *Microb Drug Resist,* **16,** 197-202.

Call, D. R., M. K. Bakko, M. J. Krug and M. C. Roberts, 2003: Identifying antimicrobial resistance genes with DNA microarrays. *Antimicrobial agents and chemotherapy,* **47,** 32903295.

Callaway TR, Carr MA, Edrington TS, Anderson RC, Nisbet DJ. Diet, *Escherichia coli* O157:H7, and Cattle: A review After 10 Years. Current Issues in Molecular Biology. 2009; 11:67-80.

Callaway, T. R., M. Carr, T. Edrington, R. C. Anderson and D. J. Nisbet, 2009: Diet, Escherichia coli O157: H7, and cattle: a review after 10 years. *Current issues in molecular biology,* **11,** 67.

Cao, M. D., D. Ganesamoorthy, A. G. Elliott, H. Zhang, M. A. Cooper and L. J. Coin, 2016: Streaming algorithms for identification of pathogens and antibiotic resistance potential from realtime MinION (TM) sequencing. *GigaScience,* **5,** 32.

Cardozo, V. F., C. A. Lancheros, A. M. Narciso, E. C. Valereto, R. K. Kobayashi, A. B. Seabra and G. Nakazato, 2014: Evaluation of antibacterial activity of nitric oxide-releasing polymeric particles against Staphylococcus aureus and Escherichia coli from bovine mastitis. *International journal of pharmaceutics,* **473,** 20-29.

Carter, B., G. Wu, M. J. Woodward and M. F. Anjum, 2008: A process for analysis of microarray comparative genomics hybridisation studies for bacterial genomes. *BMC genomics,* **9,** 53.

Carvalho, F. P., 2017: Pesticides, environment, and food safety. *Food and Energy Security,* **6,** 48-60.

Cattoir, V., & Nordmann, P. (2009). Plasmid-mediated quinolone resistance in gram-negative bacterial species: an update. *Current medicinal chemistry*, *16*(8), 1028-1046.

Cattoir, V., Poirel, L., Rotimi, V., Soussy, C. J., & Nordmann, P. (2007). Multiplex PCR for detection of plasmid-mediated quinolone resistance qnr genes in ESBL-producing enterobacterial isolates. *Journal of antimicrobial chemotherapy*, *60*(2), 394-397.

Cerceo, E., S. B. Deitelzweig, B. M. Sherman and A. N. Amin, 2016: Multidrug-resistant gramnegative bacterial infections in the hospital setting: overview, implications for clinical practice, and emerging treatment options. *Microbial Drug Resistance,* **22,** 412-431.

Chakraborty, A., Saralaya, V., Adhikari, P., Shenoy, S., Baliga, S., & Hegde, A. 2015. Characterization of Escherichia coli phylogenetic groups associated with extraintestinal infections in South Indian population. *Annals of medical and health sciences research*, *5*(4), 241246.

Chanawong, A., M'Zali, F. H., Heritage, J., Xiong, J. H., & Hawkey, P. M. (2002). Three cefotaximases, CTX-M-9, CTX-M-13, and CTX-M-14, among Enterobacteriaceae in the People's Republic of China. *Antimicrobial agents and chemotherapy*, *46*(3), 630-637.

Chanishvili, N. (2012). Phage therapy—history from Twort and d'Herelle through Soviet experience to current approaches. In *Advances in virus research* (Vol. 83, pp. 3-40). Academic Press.

Chanishvili, N., & Aminov, R. (2019). Bacteriophage therapy: coping with the growing antibiotic resistance problem. *Microbiology Australia*.

Charlesworth, B., 2010: *Elements of evolutionary genetics*. Roberts Publishers.

Chattaway, M. A., Aboderin, A. O., Fashae, K., Okoro, C. K., Opintan, J. A., & Okeke, I. N. (2016). Fluoroquinolone-resistant enteric bacteria in sub-Saharan Africa: clones, implications and research needs. *Frontiers in microbiology*, *7*, 558.

Chatterjee, A. K., Chakraborty, R., & Basu, T. (2014). Mechanism of antibacterial activity of copper nanoparticles. *Nanotechnology*, *25*(13), 135101.

Chen, J., H. Li, J. Yang, R. Zhan, A. Chen and Y. Yan, 2015: Prevalence and characterization of Integrons in Multidrug Resistant Acinetobacter baumannii in Eastern China: A Multiple-Hospital Study. *Int J Environ Res Public Health,* **12,** 10093-10105.

Chen, P. A., Hung, C. H., Huang, P. C., Chen, J. R., Huang, I. F., Chen, W. L., ... & Cheng, M. Cheng, G., H. Hao, S. Xie, X. Wang, M. Dai, L. Huang and Z. Yuan, 2014a: Antibiotic alternatives: the substitution of antibiotics in animal husbandry? *Frontiers in microbiology,* **5,** 217.

Cheng, G., H. Hao, S. Xie, X. Wang, M. Dai, L. Huang and Z. Yuan, 2014b: Antibiotic alternatives: the substitution of antibiotics in animal husbandry? *Frontiers in microbiology,* **5,** 217.

Cho, H., T. Uehara and T. G. Bernhardt, 2014: Beta-lactam antibiotics induce a lethal malfunctioning of the bacterial cell wall synthesis machinery. *Cell,* **159,** 1300-1311.

Chong PM, McCorrister SJ, Unger MS, Boyd DA, Mulvey MR, Westmacott GR. 2015. MALDITOF MS detection of carbapenemase activity in clinical isolates of Enterobacteriaceae spp., Pseudomonas aeruginosa, and Acinetobacter baumannii compared against the Carba NP assay. J

Choudhary, U., B. Tiwary, A. Prasad and S. Ganguly, 2012: Study on incidence of infectious bursal disease in and around Ranchi. *Indian Journal of Animal Research,* **46**.

Chuanchuen, R., Karkhoff-Schweizer, R. R., & Schweizer, H. P. 2003. High-level triclosan resistance in Pseudomonas aeruginosa is solely a result of efflux. *American journal of infection control*, *31*(2), 124-127.

Cioffi, N., Torsi, L., Ditaranto, N., Tantillo, G., Ghibelli, L., Sabbatini, L., & Traversa, E. (2005). Copper nanoparticle/polymer composites with antifungal and bacteriostatic properties. *Chemistry of Materials*, *17*(21), 5255-5262.

Clausen, P. T., E. Zankari, F. M. Aarestrup and O. Lund, 2016: Benchmarking of methods for identification of antimicrobial resistance genes in bacterial whole genome data. *The Journal of antimicrobial chemotherapy,* **71,** 2484-2488.

Clermont, O., Christenson, J. K., Denamur, E., & Gordon, D. M. (2013). The C lermont E scherichia coli phylo‐typing method revisited: improvement of specificity and detection of new phylo‐ groups. *Environmental microbiology reports*, *5*(1), 58-65.

Clermont, O., Christenson, J. K., Denamur, E., & Gordon, D. M. (2013). The C lermont E scherichia coli phylo‐typing method revisited: improvement of specificity and detection of new phylo‐ groups. *Environmental microbiology reports*, *5*(1), 58-65.

Clermont, O., Dhanji, H., Upton, M., Gibreel, T., Fox, A., Boyd, D., ... & Frank, T. (2009). Rapid detection of the O25b-ST131 clone of Escherichia coli encompassing the CTX-M-15producing strains. *Journal of Antimicrobial Chemotherapy*, *64*(2), 274-277.

Clermont, O., Olier, M., Hoede, C., Diancourt, L., Brisse, S., Keroudean, M., et al. 2011a. Animal and human pathogenic Escherichia coli strains share common genetic backgrounds. Infect Genet Evol 11: 654–662

CLSI, 2018: Performance Standards for Antimicrobial Disk Susceptibility Tests.

Coburn, B., Grassl, G. A., & Finlay, B. B. (2007). Salmonella, the host and disease: a brief review. *Immunology and cell biology*, *85*(2), 112-118

Cohen, M. L., 2000: Changing patterns of infectious disease. *Nature,* **406,** 762-767.

Coimbra, R. S., Grimont, F., Lenormand, P., Burguière, P., Beutin, L., & Grimont, P. A. 2000. Identification of Escherichia coli O-serogroups by restrictionof the amplified O-antigen gene cluster (rfb-RFLP). *Research in microbiology*, *151*(8), 639-654.

Collier, L. B., & A Sussman, M. (1998). Topley & Wilson's Microbiology and microbial infections, Vol 3-Bacterial infections.

Collignon, P. C., Conly, J. M., Andremont, A., McEwen, S. A., Aidara-Kane, A., World Health Organization Advisory Group, Bogotá Meeting on Integrated Surveillance of Antimicrobial Resistance (WHO-AGISAR), ... & Dang Ninh, T. (2016). World Health Organization ranking of antimicrobials according to their importance in human medicine: a critical step for developing risk management strategies to control antimicrobial resistance from food animal production. *Clinical Infectious Diseases*, *63*(8), 1087-1093.

Collingwood, C., K. Kemmett, N. Williams and P. Wigley, 2014: Is the Concept of Avian Pathogenic Escherichia coli as a Single Pathotype Fundamentally Flawed? *Frontiers in veterinary science,* **1,** 5.

Cordoni, G., M. J. Woodward, H. Wu, M. Alanazi, T. Wallis and R. M. La Ragione, 2016: Comparative genomics of European avian pathogenic E. Coli (APEC). *BMC genomics,* **17,** 960.

Cortés, P., Blanc, V., Mora, A., Dahbi, G., Blanco, J. E., Blanco, M., ... & Bou, G. (2010). Isolation and characterization of potentially pathogenic antimicrobial-resistant Escherichia coli strains from chicken and pig farms in Spain. *Appl. Environ. Microbiol.*, *76*(9), 2799-2805

Croxen, M. A. and B. B. Finlay, 2010: Molecular mechanisms of *Escherichia coli* pathogenicity.

Crump, J. A., Luby, S. P., & Mintz, E. D. (2004). The global burden of typhoid fever. *Bulletin of the World Health Organization*, *82*, 346-353.

D'Costa, V. M., K. M. McGrann, D. W. Hughes, and G. D. Wright. 2006. Sampling the antibiotic resistome. Science **311:**374–377.

Da Silva, J. and C. Hollenbach, 2010: Fluorquinolonas x Resistência Bacteriana na Medicina Veterinária. *Arquivos do Instituto Biológico,* **77,** 363-369.

Dallenne, C., A. Da Costa, D. Decre, C. Favier and G. Arlet, 2010: Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. *The Journal of antimicrobial chemotherapy,* **65,** 490-495.

Dandachi, I., E. S. Sokhn, E. A. Dahdouh, E. Azar, B. El-Bazzal, J.-M. Rolain and Z. Daoud, 2018: Prevalence and characterization of multi-drug-resistant gram-negative bacilli isolated from Lebanese poultry: A nationwide study. *Frontiers in microbiology,* **9,** 550.

Datsenko, M. Tomita, BL Wanner, and H. Mori. 2006. Construction of 543 Escherichia coli K12 in-frame, single-gene knockout mutants: the Keio 544 collection. *Mol Syst Biol*, *2*, 0008.

Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiol. Mol. Biol. Rev.*, *74*(3), 417-433.

Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and molecular biology reviews*, *74*(3), 417-433.

Davies, J., 2007: Microbes have the last word. A drastic re-evaluation of antimicrobial treatment is needed to overcome the threat of antibiotic-resistant bacteria. *EMBO Rep,* **8,** 616-621.

Davies, S. C., Fowler, T., Watson, J., Livermore, D. M., & Walker, D. (2013). Annual Report of the Chief Medical Officer: infection and the rise of antimicrobial resistance. *The Lancet*, *381*(9878), 1606-1609.

Davis, G. S., K. Waits, L. Nordstrom, H. Grande, B. Weaver, K. Papp, J. Horwinski, B. Koch, B. A. Hungate and C. M. Liu, 2018a: Antibiotic-resistant *Escherichia coli* from retail poultry meat with different antibiotic use claims. *BMC microbiology,* **18,** 174.

Davis, G. S., K. Waits, L. Nordstrom, H. Grande, B. Weaver, K. Papp, J. Horwinski, B. Koch, B. A. Hungate, C. M. Liu and L. B. Price, 2018b: Antibiotic-resistant Escherichia coli from retail poultry meat with different antibiotic use claims. *BMC Microbiol,* **18,** 174.

Day, M. J., Hopkins, K. L., Wareham, D. W., Toleman, M. A., Elviss, N., Randall, L., ... & Ellington, M. J. (2019). Extended-spectrum β-lactamase-producing Escherichia coli in humanderived and foodchain-derived samples from England, Wales, and Scotland: an

epidemiological surveillance and typing study. *The Lancet Infectious Diseases*, *19*(12), 1325-1335.

Day, M. J., Hopkins, K. L., Wareham, D. W., Toleman, M. A., Elviss, N., Randall, L., ... & Ellington, M. J. (2019). Extended-spectrum β-lactamase-producing Escherichia coli in humanderived and foodchain-derived samples from England, Wales, and Scotland: an epidemiological surveillance and typing study. *The Lancet Infectious Diseases*, *19*(12), 1325-1335.

Day, M. J., Hopkins, K. L., Wareham, D. W., Toleman, M. A., Elviss, N., Randall, L., ... & Livermore, D. M. (2019). Extended-spectrum β-lactamase-producing Escherichia coli in humanderived and foodchain-derived samples from England, Wales, and Scotland: an epidemiological surveillance and typing study. *The Lancet Infectious Diseases*, *19*(12), 1325-1335.

de Kraker, M. E., P. G. Davey, H. Grundmann and B. s. group, 2011: Mortality and hospital stay associated with resistant Staphylococcus aureus and *Escherichia coli* bacteremia: estimating the burden of antibiotic resistance in Europe. *PLoS Med,* **8,** e1001104.

de Microbiología, S. (2008). Evolution of antimicrobial susceptibility patterns of aerobic and facultative gram-negative bacilli causing intra-abdominal infections: results from the SMART studies 2003-2007. *Rev Esp Quimioter*, *21*(3), 166-173.

Delannoy, S., L. Le Devendec, E. Jouy, P. Fach, D. Drider and I. Kempf, 2017: Characterization of Colistin-Resistant Escherichia coli Isolated from Diseased Pigs in France. *Frontiers in microbiology,* **8,** 2278.

Dellit, T. H., R. C. Owens, J. E. McGowan, D. N. Gerding, R. A. Weinstein, J. P. Burke, W. C. Huskins, D. L. Paterson, N. O. Fishman and C. F. Carpenter, 2007: Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America guidelines for developing an institutional program to enhance antimicrobial stewardship. *Clinical Infectious Diseases,* **44,** 159-177.

Dezfulian, A., M. Salehian, V. Amini, H. Dabiri, M. Azimirad, M. M. Aslani, M. Zali and I. Fazel, 2010: Catalase-negative Staphylococcus aureus isolated from a diabetic foot ulcer. *Iran J Microbiol,* **2,** 165-167.

Dhanji, H., Murphy, N. M., Akhigbe, C., Doumith, M., Hope, R., Livermore, D. M., & Woodford, N. 2011. Isolation of fluoroquinolone-resistant O25b: H4-ST131 Escherichia coli with CTX-M-14 extended-spectrum β-lactamase from UK river water. *Journal of antimicrobial chemotherapy*, *66*(3), 512-516.

Dierikx, C. M., J. A. van der Goot, H. E. Smith, A. Kant and D. J. Mevius, 2013: Presence of ESBL/AmpC-producing Escherichia coli in the broiler production pyramid: a descriptive study. *PloS one,* **8,** e79005.

Dierikx, C. M., van Duijkeren, E., Schoormans, A. H. W., van Essen-Zandbergen, A., Veldman, K., Kant, A., ... & Mevius, D. J. (2012). Occurrence and characteristics of extended-spectrum-βlactamase-and AmpC-producing clinical isolates derived from companion animals and horses. *Journal of Antimicrobial Chemotherapy*, *67*(6), 1368-1374

Dingle, T. C., & MacCannell, D. R. (2015). Molecular strain typing and characterization of toxigenic Clostridium difficile. In *Methods in Microbiology* (Vol. 42, pp. 329-357). Academic Press.

Doi, Y., J. I. Wachino and Y. Arakawa, 2016: Aminoglycoside Resistance: The Emergence of Acquired 16S Ribosomal RNA Methyltransferases. *Infectious disease clinics of North America,* **30,** 523-537.

Dolejska, M., Frolkova, P., Florek, M., Jamborova, I., Purgertova, M., Kutilova, I., ... & Literak, I. 2011. CTX-M-15-producing Escherichia coli clone B2-O25b-ST131 and Klebsiella spp. isolates in municipal wastewater treatment plant effluents. *Journal of antimicrobial chemotherapy*, *66*(12), 2784-2790.

Dozois, C. M., J. M. Fairbrother, J. Harel and M. Bosse, 1992: pap-and pil-related DNA sequences and other virulence determinants associated with Escherichia coli isolated from septicemic chickens and turkeys. *Infection and immunity,* **60,** 2648-2656.

Duarte-Escalante, E., Zúñiga, G., Frías-De-León, M. G., Canteros, C., Castañón-Olivares, L. R., & del Reyes-Montes, M. R. 2013. AFLP analysis reveals high genetic diversity but low population structure in Coccidioides posadasii isolates from Mexico and Argentina. *BMC infectious diseases*, *13*(1), 411

Durão, P., R. Balbontín and I. Gordo, 2018: Evolutionary mechanisms shaping the maintenance of antibiotic resistance. *Trends in microbiology,* **26,** 677-691.

Dutil, L., R. Irwin, R. Finley, L. K. Ng, B. Avery, P. Boerlin, A.-M. Bourgault, L. Cole, D. Daignault and A. Desruisseau, 2010: Ceftiofur resistance in Salmonella enterica serovar Heidelberg from chicken meat and humans, Canada. *Emerging infectious diseases,* **16,** 48.

Dwyer, D. J., J. J. Collins and G. C. Walker, 2015: Unraveling the physiological complexities of antibiotic lethality. *Annual review of pharmacology and toxicology,* **55,** 313-332.

Dziva, F. and M. P. Stevens, 2008: Colibacillosis in poultry: unravelling the molecular basis of virulence of avian pathogenic Escherichia coli in their natural hosts. *Avian Pathol,* **37,** 355-366.

Edwards, D. J. and K. E. Holt, 2013: Beginner's guide to comparative bacterial genome analysis using next-generation sequence data. *Microb Inform Exp,* **3,** 2.

Egervärn, M., Börjesson, S., Byfors, S., Finn, M., Kaipe, C., Englund, S., & Lindblad, M. (2014). Escherichia coli with extended-spectrum beta-lactamases or transferable AmpC betalactamases and Salmonella on meat imported into Sweden. *International journal of food microbiology*, *171*, 8-14.

Egervärn, M., Börjesson, S., Byfors, S., Finn, M., Kaipe, C., Englund, S., & Lindblad, M. (2014). Escherichia coli with extended-spectrum beta-lactamases or transferable AmpC betalactamases and Salmonella on meat imported into Sweden. *International journal of food microbiology*, *171*, 8-14.

Egervarn, M., S. Borjesson, S. Byfors, M. Finn, C. Kaipe, S. Englund and M. Lindblad, 2014: Escherichia coli with extended-spectrum beta-lactamases or transferable AmpC beta-lactamases and Salmonella on meat imported into Sweden. *International journal of food microbiology,* **171,** 8-14.

Eibach, D., D. Dekker, K. Gyau Boahen, C. Wiafe Akenten, N. Sarpong, C. Belmar Campos, L. Berneking, M. Aepfelbacher, R. Krumkamp, E. Owusu-Dabo and J. May, 2018: Extendedspectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in local and imported poultry meat in Ghana. *Veterinary microbiology,* **217,** 7-12.

Eibach, D., Dekker, D., Boahen, K. G., Akenten, C. W., Sarpong, N., Campos, C. B., ... & May, J. (2018). Extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in local and imported poultry meat in Ghana. *Veterinary microbiology*, *217*, 7-12.

Eigner, U., M. Holfelder, K. Oberdorfer, U. Betz-Wild, D. Bertsch and A.-M. Fahr, 2009: Performance of a matrix-assisted laser desorption ionization-time-of-flight mass spectrometry system for the identification of bacterial isolates in the clinical routine laboratory. *Clinical Laboratory Journal for Clinical Laboratories and Laboratories Related,* **55,** 289.

Ellington, M. J., O. Ekelund, F. M. Aarestrup, R. Canton, M. Doumith, C. Giske, H. Grundman, El-Shazly, D., S. Nasef, F. Mahmoud and D. Jonas, 2017: Expanded spectrum β–lactamase producing Escherichia coli isolated from chickens with colibacillosis in Egypt. *Poultry science,* **96,** 2375-2384.

EMBORG, H., 2004: Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark. *DANMAP 2004*.

Emerging avian pathogenic Escherichia coli strains belonging to clonal groups O111: H4-DST2085 and O111: H4-D-ST117 with high virulence-gene content and zoonotic potential. *Veterinary microbiology*, *156*(3-4), 347-352.

Emerging avian pathogenic Escherichia coli strains belonging to clonal groups O111: H4-DST2085 and O111: H4-D-ST117 with high virulence-gene content and zoonotic potential. *Veterinary microbiology*, *156*(3-4), 347-352.

Enright, M. C., D. A. Robinson, G. Randle, E. J. Feil, H. Grundmann, and B. G. Spratt. 2002. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc. Natl. Acad. Sci. U. S. A. 99**:**7687–7692.

Ensor, V. M., Shahid, M., Evans, J. T., & Hawkey, P. M. (2006). Occurrence, prevalence and genetic environment of CTX-M β-lactamases in Enterobacteriaceae from Indian hospitals. *Journal of Antimicrobial Chemotherapy*, *58*(6), 1260-1263. Escherichia coli (APEC) from broiler chickens in Chitwan, Nepal. *BMC veterinary research,* **14,** 113.

ESCUDERO, J.A., LOOT, C., NIVINA, A. & MAZEL, D. 2015. The integron: adaptation on demand. Microbiology Spectrum, 3, 10.1128/ microbiolspec. MDN A3-0019-2014.

European Centre for Disease Prevention and Control Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-NETet) Antimicrobial Resistance Surveillance in Europe, The European Centre for Disease Prevention.

Ewers C, Grobbel M, Stamm I, Kopp PA, Diehl I, Semmler T, et al (2010). Emergence of human pandemic O25:H4-ST131 CTX-M-15 extendedspectrum-β-lactamase-producing Escherichia coli among companion animals. J Antimicrob Chemother 2010; 65:651–60.

Ewers, C., Grobbel, M., Bethe, A., Wieler, L. H., & Guenther, S. (2011). Extended-spectrum beta-lactamases-producing gram-negative bacteria in companion animals: action is clearly warranted. *Berliner und Münchener Tierärztliche Wochenschrift*, *124*(3/4), 4-101.

Ewers, C., Grobbel, M., Bethe, A., Wieler, L. H., & Guenther, S. (2011). Extended-spectrum beta-lactamases-producing gram-negative bacteria in companion animals: action is clearly warranted. *Berliner und Münchener Tierärztliche Wochenschrift*, *124*(3/4), 4-101.

Ewers, C., Grobbel, M., Stamm, I., Kopp, P. A., Diehl, I., Semmler, T., ... & Guenther, S. 2010. Emergence of human pandemic O25: H4-ST131 CTX-M-15 extended-spectrum-β-lactamase producing *Escherichia coli* among companion animals. *Journal of antimicrobial chemotherapy*, *65*(4), 651-660.

Ewers, C., T. Janssen, S. Kiessling, H. C. Philipp and L. H. Wieler, 2004: Molecular epidemiology of avian pathogenic Escherichia coli (APEC) isolated from colisepticemia in poultry. *Veterinary microbiology,* **104,** 91-101. *Expert opinion on investigational drugs,* **23,** 1575-1584.

F. (2016). Characteristics of CTX-M extended-spectrum β-lactamase-producing Escherichia coli strains isolated from multiple rivers in Southern Taiwan. *Appl. Environ. Microbiol.*, *82*(6), 18891897.

Fair, R. J. and Y. Tor, 2014: Antibiotics and bacterial resistance in the 21st century. *Perspectives in medicinal chemistry,* **6,** PMC. S14459.

Falagas, M. E., D. E. Karageorgopoulos and P. Nordmann, 2011: Therapeutic options for infections with Enterobacteriaceae producing carbapenem-hydrolyzing enzymes. *Future microbiology,* **6,** 653-666.

Fang, H., F. Ataker, G. Hedin and K. Dornbusch, 2008: Molecular epidemiology of extendedspectrum β-lactamases among Escherichia coli isolates collected in a Swedish hospital and its associated health care facilities from 2001 to 2006. *Journal of clinical microbiology,* **46,** 707712.

FAO, I., 2016: WFP (2015), The State of Food Insecurity in the World 2015. Meeting the 2015 international hunger targets: taking stock of uneven progress. *Food and Agriculture Organization Publications, Rome*.

Farahyar, S., Zaini, F., Kordbacheh, P., Rezaie, S., Safara, M., Raoofian, R., & Heidari, M. 2013. Overexpression of aldo-keto-reductase in azole-resistant clinical isolates of Candida glabrata determined by cDNA-AFLP. *DARU Journal of Pharmaceutical Sciences*, *21*(1), 1.

Feng, Y., 2018: Transferability of MCR-1/2 polymyxin resistance: complex dissemination and genetic mechanism. *ACS infectious diseases,* **4,** 291-300.

Fernandes, R., P. Amador and C. Prudêncio, 2013: β-Lactams: chemical structure, mode of action and mechanisms of resistance. *Reviews in Medical Microbiology,* **24,** 7-17.

Fernandez, L. and R. E. Hancock, 2012: Adaptive and mutational resistance: role of porins and efflux pumps in drug resistance. *Clin Microbiol Rev,* **25,** 661-681.

Fernández, L., & Hancock, R. E. (2013). Adaptive and mutational resistance: role of porins and efflux pumps in drug resistance. *Clinical Microbiology Reviews*, *26*(1), 163

Fischbach, M. A. and C. T. Walsh, 2009: Antibiotics for emerging pathogens. *Science,* **325,** 1089-1093.

FLUIT, A.C. & SCHMITZ, F.-J. 2004. Resistance integrons and super integrons. Clinical Microbiology and Infection, 10, 10.1111/j.1198- 743X.2004.00858.x

Forsberg, K. J., A. Reyes, B. Wang, E. M. Selleck, M. O. Sommer and G. Dantas, 2012: The shared antibiotic resistome of soil bacteria and human pathogens. *science,* **337,** 1107-1111.

Founou, L. L., R. C. Founou and S. Y. Essack, 2016: Antibiotic Resistance in the Food Chain: A Developing Country-Perspective. *Frontiers in microbiology,* **7,** 1881.

Frank C, Werber D, Cramer JP, Askar M, Faber M, et al. 2011. Epidemic profile of Shiga-toxinproducing Escherichia coli O104:H4 outbreak in Germany. N. Engl. J. Med. 365:1771–80

Frasch, C. E. 1994. Serogroup and serotype classification of bacterial pathogens. *Methods in enzymology*, *235*, 159-174.

Fratamico, P. M., DebRoy, C., Liu, Y., Needleman, D. S., Baranzoni, G. M., & Feng, P. (2016). Advances in molecular serotyping and subtyping of Escherichia coli. *Frontiers in microbiology*, *7*, 644.

Freitag, C., Michael, G. B., Kadlec, K., Hassel, M., & Schwarz, S. (2017). Detection of plasmidborne extended-spectrum β-lactamase (ESBL) genes in Escherichia coli isolates from bovine mastitis. *Veterinary microbiology*, *200*, 151-156.

Freitag, C., Michael, G. B., Kadlec, K., Hassel, M., & Schwarz, S. (2017). Detection of plasmidborne extended-spectrum β-lactamase (ESBL) genes in Escherichia coli isolates from bovine mastitis. *Veterinary microbiology*, *200*, 151-156.

Funk, J. A., J. T. Lejeune, T. E. Wittum and P. J. Rajala-Schultz, 2006: The effect of subtherapeutic chlortetracycline on antimicrobial resistance in the fecal flora of swine. *Microbial drug resistance,* **12,** 210-218.

Fuoco, D., 2012: Classification framework and chemical biology of tetracycline-structure-based drugs. *Antibiotics,* **1,** 1.

G. Compton, S. Das, D. H. Farkas, M. J. Ferber, B. H. Funke, M. R. Furtado, L. M. GanovaRaeva, U. Geigenmuller, S. J. Gunselman, M. R. Hegde, P. L. Johnson, A. Kasarskis, S. Kulkarni, T. Lenk, C. S. Liu, M. Manion, T. A. Manolio, E. R. Mardis, J. D. Merker, M. S. Rajeevan, M. G. Reese, H. L. Rehm, B. B. Simen, J. M. Yeakley, J. M. Zook and I. M. Lubin, 2012: Assuring the quality of next-generation sequencing in clinical laboratory practice. *Nat Biotechnol,* **30,** 1033-1036.

Gal-Mor, O. and B. B. Finlay, 2006: Pathogenicity islands: a molecular toolbox for bacterial virulence. *Cellular microbiology,* **8,** 1707-1719.

Gardiner, B. J., A. J. Stewardson, I. J. Abbott and A. Y. Peleg, 2019: Nitrofurantoin and fosfomycin for resistant urinary tract infections: old drugs for emerging problems. *Australian prescriber,* **42,** 14.

Gargis, A. S., L. Kalman, M. W. Berry, D. P. Bick, D. P. Dimmock, T. Hambuch, F. Lu, E. Lyon, K. V. Voelkerding, B. A. Zehnbauer, R. Agarwala, S. F. Bennett, B. Chen, E. L. Chin, J. Garneau-Tsodikova, S. and K. J. Labby, 2016: Mechanisms of resistance to aminoglycoside antibiotics: overview and perspectives. *MedChemComm,* **7,** 11-27.

GBD 2015 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet 2016; 388:1545–602.

GBD 2015 Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet 2016;388: 1459–544.

Gerber, P. J., A. N. Hristov, B. Henderson, H. Makkar, J. Oh, C. Lee, R. Meinen, F. Montes, T. Ott, J. Firkins, A. Rotz, C. Dell, A. T. Adesogan, W. Z. Yang, J. M. Tricarico, E. Kebreab, G. Waghorn, J. Dijkstra and S. Oosting, 2013: Technical options for the mitigation of direct methane and nitrous oxide emissions from livestock: a review. *Animal: an international journal of animal bioscience,* **7** Suppl 2, 220-234.

Getie, M., Abebe, W., & Tessema, B. (2019). Prevalence of enteric bacteria and their antimicrobial susceptibility patterns among food handlers in Gondar town, Northwest Ethiopia. *Antimicrobial Resistance & Infection Control*, *8*(1), 111.

Giamarellou, H., 2016: Epidemiology of infections caused by polymyxin-resistant pathogens. *International journal of antimicrobial agents,* **48,** 614-621.

Gill, P., & Ghaemi, A. (2008). Nucleic acid isothermal amplification technologies—A review. 502 Nucleosides Nucleotides & Nucleic Acids, 27(3), 224–243

Gillings, M. R., M. P. Holley, and H. W. Stokes. 2009. Evidence for dynamic exchange of *qac* gene cassettes between class 1 integrons and other integrons in freshwater biofilms. FEMS Microbiol. Lett. 296**:**282–288

Gillings, M., Y. Boucher, M. Labbate, A. Holmes, S. S. Krishnan, M.Holley, and H. W. Stokes**.** 2008. The evolution of class 1 integrons and the rise of antibiotic resistance. J. Bacteriol. 190:5095–5100

Ginevan, M. E. (2002). Assessment of the National Antimicrobial Resistance Monitoring System (NARMS) and its value in critical decision-making. *International journal of infectious diseases*, *6*, S8-S15.

Giufrè, M., Graziani, C., Accogli, M., Luzzi, I., Busani, L., & Cerquetti, M. (2012). Escherichia coli of human and avian origin: detection of clonal groups associated with fluoroquinolone and multidrug resistance in Italy. *Journal of antimicrobial chemotherapy*, *67*(4), 860-867.

Glais, L. and E. Jacquot, 2015: Detection and Characterization of Viral Species/Subspecies Using Isothermal Recombinase Polymerase Amplification (RPA) Assays. *Methods in molecular biology (Clifton, N.J.),* 1302**,** 207-225.

Gomes, F. and M. Henriques, 2016: Control of Bovine Mastitis: Old and Recent Therapeutic Approaches. *Curr Microbiol,* **72,** 377-382.

Goodwin S, Gurtowski J, Ethe-Sayers S, Deshpande P, Schatz MC, McCombie WR. 2015. Oxford Nanopore sequencing, hybrid error correction, and de novo assembly of a eukaryotic genome. Genome Res. 25:1750–56

Gordienko, E. N., Kazanov, M. D., & Gelfand, M. S. (2013). Evolution of pan-genomes of *Escherichia coli,* Shigella spp., and Salmonella enterica. *Journal of bacteriology*, *195*(12), 27862792.

Gordon, D. M., Clermont, O., Tolley, H., & Denamur, E. (2008). Assigning Escherichia coli strains to phylogenetic groups: multi‐ locus sequence typing versus the PCR triplex method. *Environmental microbiology*, *10*(10), 2484-2496.

Gouvêa, R., F. dos Santos and M. de Aquino, 2015: Fluoroquinolones in industrial poultry production, bacterial resistance and food residues: a review. *Brazilian Journal of Poultry Science,* **17,** 1-10.

Grimont, P. A., & Weill, F. X. 2007. Antigenic formulae of the Salmonella serovars. *WHO collaborating centre for reference and research on Salmonella*, *9*,1-166.

Gross, M., 2013: Antibiotics in crisis. *Current biology: CB,* **23,** R1063-1065.

Grundmann, H., C. Glasner, B. Albiger, D. M. Aanensen, C. T. Tomlinson, A. T. Andrasević, R. Cantón, Y. Carmeli, A. W. Friedrich and C. G. Giske, 2017: Occurrence of carbapenemaseproducing Klebsiella pneumoniae and Escherichia coli in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE): a prospective, multinational study. *The Lancet Infectious Diseases,* **17,** 153-163.

Guembe, M., E. Cercenado, L. Alcala, M. Marin, R. Insa and E. Bouza, 2008: Evolution of

antimicrobial susceptibility patterns of aerobic and facultative gram-negative bacilli causing intra-abdominal infections: results from the SMART studies 2003-2007. *Rev Esp Quimioter,* **21,** 166-173.

Guillamón, J. M., & Barrio, E. (2017). Genetic polymorphism in wine yeasts: mechanisms and methods for its detection. *Frontiers in microbiology*, *8*, 806

Gupta, S. K., B. R. Padmanabhan, S. M. Diene, R. Lopez-Rojas, M. Kempf, L. Landraud and J. M. Rolain, 2014: ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. *Antimicrobial agents and chemotherapy,* **58,** 212-220.

Gurevich, A., V. Saveliev, N. Vyahhi and G. Tesler, 2013: QUAST: quality assessment tool for genome assemblies. *Bioinformatics (Oxford, England),* **29,** 1072-1075.

Gutiérrez, C., J. Zenis, P. Legarraga, J. Cabrera-Pardo, P. García, H. Bello-Toledo, A. OpazoCapurro and G. González-Rocha, 2019: Genetic analysis of the first mcr-1 positive *Escherichia coli* isolate collected from an outpatient in Chile. *The Brazilian journal of infectious diseases: an official publication of the Brazilian Society of Infectious Diseases*.

H. Hasman, M. T. G. Holden, K. L. Hopkins, J. Iredell, G. Kahlmeter, C. U. Koser, A. Hacker, J., and Kaper, J.B. 2000. Pathogenicity islands and the evolution of microbes. *Annu Rev Microbiol* **54**: 641– 679.

Haines and L. P. Randall, 2013: Isolation and detection of extended spectrum beta-lactamase (ESBL)-producing enterobacteriaceae from meat using chromogenic agars and isothermal loopmediated amplification (LAMP) assays. *J Food Sci,* **78,** M1892-1898.

Hamaki, T., M. Suzuki, R. Fudou, Y. Jojima, T. Kajiura, A. Tabuchi, K. Sen and H. Shibai, 2005: Isolation of novel bacteria and actinomycetes using soil-extract agar medium. *Journal of bioscience and bioengineering,* **99,** 485-492.

Hao, H., G. Cheng, Z. Iqbal, X. Ai, H. I. Hussain, L. Huang, M. Dai, Y. Wang, Z. Liu and Z. Yuan, 2014: Benefits and risks of antimicrobial use in food-producing animals. *Frontiers in microbiology,* **5,** 288.

Haque, N., Bari, M. S., Hossain, M. A., Muhammad, N., Ahmed, S., Rahman, A., ... & Islam, A. (2011). An overview of Brucellosis. *Mymensingh medical journal: MMJ*, *20*(4), 742-747.

Harder, T. C., S. Buda, H. Hengel, M. Beer and T. C. Mettenleiter, 2016: Poultry food products-

a source of avian influenza virus transmission to humans? *Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases,* **22,** 141-146.

Harrison, E. M., Weinert, L. A., Holden, M. T., Welch, J. J., Wilson, K., Morgan, F. J., & Paterson, G. K. (2014). A shared population of epidemic methicillin-resistant Staphylococcus aureus 15 circulates in humans and companion animals. *MBio*, *5*(3), e00985-13.

Hasan, B., R. Faruque, M. Drobni, J. Waldenström, A. Sadique, K. U. Ahmed, Z. Islam, M. H. Parvez, B. Olsen and M. Alam, 2011: High prevalence of antibiotic resistance in pathogenic Escherichia coli from large-and small-scale poultry farms in Bangladesh. *Avian diseases,* **55,** 689-692.

Hasman, H., A. M. Hammerum, F. Hansen, R. S. Hendriksen, B. Olesen, Y. Agersø, E. Zankari, P. Leekitcharoenphon, M. Stegger and R. S. Kaas, 2015: Detection of mcr-1 encoding plasmidmediated colistin-resistant Escherichia coli isolates from human bloodstream infection and imported chicken meat, Denmark 2015. *Eurosurveillance (Online Edition),* **20,** 1-5.

Hasman, H., Saputra, D., Sicheritz-Ponten, T., Lund, O., Svendsen, C. A., Frimodt-Møller, N., & Aarestrup, F. M. (2014). Rapid whole-genome sequencing for detection and characterization of microorganisms directly from clinical samples. *Journal of clinical microbiology*, *52*(1), 139-146.

Hata, M., Suzuki, M., Matsumoto, M., Takahashi, M., Sato, K., Ibe, S., & Sakae, K. (2005). Cloning of a novel gene for quinolone resistance from a transferable plasmid in Shigella flexneri 2b. *Antimicrobial agents and chemotherapy*, *49*(2), 801-803.

Hawkey, P. M. 2008. Prevalence and clonality of extended‐spectrum β‐lactamases in Asia. *Clinical Microbiology and Infection*, *14*, 159-165.

Hawkey, P. M., & Jones, A. M. (2009). The changing epidemiology of resistance. *Journal of Antimicrobial Chemotherapy*, *64*(suppl_1), i3-i10.

Hayashi, W., Tanaka, H., Taniguchi, Y., Iimura, M., Soga, E., Kubo, R., ... & Nagano, N. 2019. Acquisition of mcr-1 and cocarriage of virulence genes in avian pathogenic Escherichia coli isolates from municipal wastewater influents in Japan. *Applied and environmental microbiology*, *85*(22).

Herbert, S., Halvorsen, D. S., Leong, T., Franklin, C., Harrington, G., & Spelman, D. 2007. Large outbreak of infection and colonization with gram-negative pathogens carrying the

metallo β-lactamase gene bla IMP-4 at a 320-bed tertiary hospital in Australia. *Infection Control & Hospital Epidemiology*, *28*(1), 98-101. here is the storm! *Trends in molecular medicine*, *18*(5), 263-272.

Hert, D. G., C. P. Fredlake and A. E. Barron, 2008: Advantages and limitations of nextgeneration sequencing technologies: a comparison of electrophoresis and non-electrophoresis methods. *Electrophoresis,* **29,** 4618-4626.

Hirakata, Y., Matsuda, J., Miyazaki, Y., Kamihira, S., Kawakami, S., Miyazawa, Y., ... & Kohno, S. (2005). Regional variation in the prevalence of extended-spectrum β-lactamase–producing clinical isolates in the Asia-Pacific region (SENTRY 1998–2002). *Diagnostic microbiology and infectious disease*, *52*(4), 323-329.

Hobman, J. L., Penn, C. W., & Pallen, M. J. (2007). Laboratory strains of Escherichia coli: model citizens or deceitful delinquents growing old disgracefully. *Molecular microbiology*, *64*(4), 881-885.

Hrabak, J., E. Chudackova and C. C. Papagiannitsis, 2014: Detection of carbapenemases in Enterobacteriaceae: a challenge for diagnostic microbiological laboratories. *Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases,* **20,** 839-853.

Hryncewicz-Gwóźdź, A., Jagielski, T., Dobrowolska, A., Szepietowski, J. C., & Baran, E.

Hsu, S. C., T. H. Chiu, J. C. Pang, C. H. Hsuan-Yuan, G. N. Chang and H. Y. Tsen, 2006: Characterisation of antimicrobial resistance patterns and class 1 integrons among Escherichia coli and Salmonella enterica serovar Choleraesuis strains isolated from humans and swine in Taiwan. *Int J Antimicrob Agents,* **27,** 383-391. http://www.illumina.com/techniques/sequencing/ngslibrary-prep/library-prep-methods.htm

Huja, S., Y. Oren, E. Trost, E. Brzuszkiewicz, D. Biran, J. Blom, A. Goesmann, G. Gottschalk, J. Hacker, E. Z. Ron and U. Dobrindt, 2015: Genomic avenue to avian colisepticemia. *mBio,* **6**.

Hunter, M. C., R. G. Smith, M. E. Schipanski, L. W. Atwood and D. A. Mortensen, 2017: Agriculture in 2050: Recalibrating targets for sustainable intensification. *Bioscience,* **67,** 386391.

Hussain, H. I., Z. Iqbal, M. N. Seleem, D. Huang, A. Sattar, H. Hao and Z. Yuan, 2017: Virulence and transcriptome profile of multidrug-resistant Escherichia coli from chicken. *Sci Rep,* **7,** 8335.

Hussain, J., I. Rabbani, S. Aslam and H. A. Ahmad, 2015b: An overview of poultry industry in Pakistan. *Worlds Poult Sci J,* **71,** 689-700.

Hussain, J., I. Rabbani, S. Aslam and H. Ahmad, 2015a: An overview of poultry industry in Pakistan. *World's poultry science journal,* **71,** 689-700.

Hutton, T. A., G. K. Innes, J. Harel, P. Garneau, A. Cucchiara, D. M. Schifferli and S. C. Rankin, 2018: Phylogroup and virulence gene association with clinical characteristics of Escherichia coli urinary tract infections from dogs and cats. *Journal of veterinary diagnostic investigation: official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc,* **30,** 64-70.

Hwang, K.-P., Y.-F. Tang and Y.-H. Shen, 2009: Activity of ertapenem, ciprofloxacin, ceftriaxone, piperacillin-tazobactam, and ampicillin-sulbactam against 12 common clinical isolates of community-acquired bacteremia. *J Microbiol Immunol Infect,* **42,** 433-438.

I. Nikolenko, S. Pham, A. D. Prjibelski, A. V. Pyshkin, A. V. Sirotkin, N. Vyahhi, G. Tesler, M. A. Alekseyev and P. A. Pevzner, 2012: SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *Journal of computational biology: a journal of computational molecular cell biology,* **19,** 455-477.

Ikeda, H., J. Ishikawa, A. Hanamoto, M. Shinose, H. Kikuchi, T. Shiba, Y. Sakaki, M. Hattori and S. Omura, 2003: Complete genome sequence and comparative analysis of the industrial microorganism Streptomyces avermitilis. *Nat Biotechnol,* **21,** 526-531.

Illumina. 2014. Sequencing methods review: a review of publications featuring Illumina R

Ingle, D. J., Levine, M. M., Kotloff, K. L., Holt, K. E., & Robins-Browne, R. M. 2018. Dynamics of antimicrobial resistance in intestinal Escherichia coli from children in community settings in South Asia and sub-Saharan Africa. *Nature microbiology*, *3*(9), 1063-1073 Institute. *Medecine et maladies infectieuses*, *38*(8), 426-430.

Iseman, M. D., 1993: Treatment of multidrug-resistant tuberculosis. *The New England journal of medicine,* **329,** 784-791.

Isolauri, E., P. Kirjavainen and S. Salminen, 2002: Probiotics: a role in the treatment of intestinal infection and inflammation? *Gut,* **50,** iii54-iii59.

Ivarsson, M. E., J. C. Leroux and B. Castagner, 2015: Investigational new treatments for Clostridium difficile infection. *Drug discovery today,* **20,** 602-608.

Jain M, Fiddes IT, Miga KH, Olsen HE, Paten B, Akeson M. 2015. Improved data analysis for the MinION nanopore sequencer. Nat. Methods 12:351–56

Jain, M., Olsen, H. E., Paten, B., & Akeson, M. (2016). The Oxford Nanopore MinION: delivery of nanopore sequencing to the genomics community. *Genome biology*, *17*(1), 239.

Jamal, M., M. Shareef and S. Sajid, 2017: Lincomycin and tetracycline resistance in poultry. Review. *Matrix Sci. Pharma,* **1,** 33-38.

Jamborova I, Johnston BD, Papousek I, Kachlikova K, Micenkova L, Clabots C, Skalova A, Chudejova K, Dolejska M, Literak I, Johnson JR. 2018. Extensive genetic commonality among wildlife, wastewater, community, and nosocomial isolates of *Escherichia coli* sequence type 131 (*H*30R1 and *H*30Rx Subclones) that carry *bla*CTX-M-27 or *bla*CTX-M-15. Antimicrob Agents Chemother 62: e00519-18. https://doi.org/10.1128/AAC.00519-18.

Jaureguy, F., Landraud, L., Passet, V., Diancourt, L., Frapy, E., Guigon, G., & Picard, B. 2008. Phylogenetic and genomic diversity of human bacteremic Escherichia coli strains. *BMC genomics*, *9*(1), 560.

Javed, T., M. Khan and A. Khan, 1994: Epidemiological investigation and economic losses due to hydro-pericardium syndrome in layer and parent flocks in Lahore dist. *Proc. 8th Int. Cong. Anim. Hygiene*.

Jia, B., A. R. Raphenya, B. Alcock, N. Waglechner, P. Guo, K. K. Tsang, B. A. Lago, B. M. Dave, S. Pereira and A. N. Sharma, 2016: CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. *Nucleic acids research***,** gkw1004.

Jiang, H.-X., D.-H. Lü, Z.-L. Chen, X.-M. Wang, J.-R. Chen, Y.-H. Liu, X.-P. Liao, J.-H. Liu and Z.-L. Zeng, 2011: High prevalence and widespread distribution of multi-resistant Escherichia coli isolates in pigs and poultry in China. *The veterinary journal,* **187,** 99-103.

Jiménez-Belenguer, A., E. Doménech, A. Villagrá, A. Fenollar and M. A. Ferrús, 2016: Antimicrobial resistance of Escherichia coli isolated in newly hatched chickens and effect of amoxicillin treatment during their growth. *Avian Pathology,* **45,** 501-507.

Joensen, K. G., A. M. Tetzschner, A. Iguchi, F. M. Aarestrup and F. Scheutz, 2015: Rapid and Easy in Silico Serotyping of *Escherichia coli* Isolates by Use of Whole-Genome Sequencing Data. *Journal of clinical microbiology,* **53,** 2410-2426.

Johnson, J. R., Menard, M., Johnston, B., Kuskowski, M. A., Nichol, K., & Zhanel, G. G. (2009). Epidemic clonal groups of Escherichia coli as a cause of antimicrobial-resistant urinary tract infections in Canada, 2002 to 2004. *Antimicrobial agents and chemotherapy*, *53*(7), 27332739.

Johnson, J. R., Thuras, P., Johnston, B. D., Weissman, S. J., Limaye, A. P., Riddell, K., ... & Sokurenko, E. (2016). The Pandemic H 30 Subclone of Escherichia coli Sequence Type 131 Is Associated with Persistent Infections and Adverse Outcomes Independent from Its Multidrug Jolley KA, Feil EJ, Chan MS, Maiden MC. Sequence type analysis and recombinational tests (START). Bioinformatics 2001; 17:12301

Jones, R. N., T. R. Anderegg and J. M. Swenson, 2005: Quality control guidelines for testing gram-negative control strains with polymyxin B and colistin (polymyxin E) by standardized methods. *Journal of clinical microbiology,* **43,** 925-927.

Jørgensen, S. B., A. Søraas, L. S. Arnesen, T. Leegaard, A. Sundsfjord and P. A. Jenum, 2017: First environmental sample containing plasmid‐mediated colistin‐resistant ESBL‐producing Escherichia coli detected in Norway. *Apmis,* **125,** 822-825.

Joshi, P. R., R. Thummeepak, S. Paudel, M. Acharya, S. Pradhan, M. R. Banjara, U. Leungtongkam and S. Sitthisak, 2019: Molecular Characterization of Colistin-Resistant Escherichia coli Isolated from Chickens: First Report from Nepal. *Microbial drug resistance (Larchmont, N.Y.)*.

Kaas R. S., Friis C., Ussery D. W., Aarestrup F. M.. 2012; Estimating variation within the genes and inferring the phylogeny of 186 sequenced diverse *Escherichia coli* genomes. *BMC Genomics*13:577

Kamboh, A. A., M. Shoaib, S. H. Abro, M. A. Khan, K. K. Malhi and S. Yu, 2018: Antimicrobial Resistance in Enterobacteriaceae Isolated from Liver of Commercial Broilers and Backyard Chickens. *The Journal of Applied Poultry Research,* **27,** 627-634.

Kaper JB. Pathogenic *Escherichia coli*. International Journal of Medical Microbiology. 2005; 295:355-356.

Karpinski, T. M. and A. K. Szkaradkiewicz, 2013: Characteristic of bacteriocines and their application. *Pol J Microbiol,* **62,** 223-235.

Keane, O. M., 2016a: Genetic diversity, the virulence gene profile and antimicrobial resistance of clinical mastitis-associated Escherichia coli. *Research in microbiology,* **167,** 678-684.

Keane, O. M., 2016b: Genetic diversity, the virulence gene profile and antimicrobial resistance of clinical mastitis-associated Escherichia coli. *Research in microbiology,* **167,** 678-684.

Keessen EC, Gaastra W, Lipman LJ. 2011. Clostridium difficile infection in humans 338 and animals, differences and similarities. Vet Microbiol 153:205 -217.

Kempf I, Fleury MA, Drider D, Bruneau M, Sanders P, Chauvin C, Madec JY, Jouy E. 2013. What do we know about resistance to colistin in Enterobacteriaceae in avian and pig production in Europe? Int J Antimicrob Agents **42**:379-383.

Kempf I, Fleury MA, Drider D, Bruneau M, Sanders P, Chauvin C, Madec JY, Jouy E. 2013. What do we know about resistance to colistin in Enterobacteriaceae in avian and pig production in Europe? Int J Antimicrob Agents **42**:379-383.

Kempf, I., M. A. Fleury, D. Drider, M. Bruneau, P. Sanders, C. Chauvin, J.-Y. Madec and E. Jouy, 2013: What do we know about resistance to colistin in Enterobacteriaceae in avian and pig production in Europe? *International journal of antimicrobial agents,* **42,** 379-383.

Kieffer, N., L. Poirel, P. Nordmann, J.-Y. Madec and M. Haenni, 2014: Emergence of colistin resistance in Klebsiella pneumoniae from veterinary medicine. *Journal of Antimicrobial Chemotherapy,* **70,** 1265-1267.

Kim, S., H.W. Kang, and G.J. Woo. 2015. Prevalence of CTX-M-15 extended-spectrum betalactamase-producing Salmonella isolated from chicken in Korea. Foodborne Pathog. Dis. 12:661–663.

King, A. M. *et al.* Aspergillomarasmine A overcomes metallo-β-lactamase antibiotic resistance. *Nature* 510**,** 503–506 (2014).

Kirk, M. D., Pires, S. M., Black, R. E., Caipo, M., Crump, J. A., Devleesschauwer, B., ... & Hall, A. J. (2015). World Health Organization estimates of the global and regional disease burden of 22 foodborne bacterial, protozoal, and viral diseases, 2010: a data synthesis. *PLoS medicine*, *12*(12).

Kleinheinz, K. A., K. G. Joensen and M. V. Larsen, 2014: Applying the ResFinder and

VirulenceFinder web-services for easy identification of acquired antibiotic resistance and E. coli virulence genes in bacteriophage and prophage nucleotide sequences. *Bacteriophage,* **4,** e27943.

Kmietowicz, Z., 2017: WHO downgrades oseltamivir on drugs list after reviewing evidence. *BMJ,* **357,** j2841.

Knetsch, C. W., Kumar, N., Forster, S. C., Connor, T. R., Browne, H. P., Harmanus, C., & Perry, M. (2018). Zoonotic transfer of Clostridium difficile harboring antimicrobial resistance between farm animals and humans. *Journal of clinical microbiology*, *56*(3), e01384-17

Köck, R., I. Daniels-Haardt, K. Becker, A. Mellmann, A. Friedrich, D. Mevius, S. Schwarz and A. Jurke, 2018: Carbapenem-resistant Enterobacteriaceae in wildlife, food-producing, and companion animals: a systematic review. *Clinical Microbiology and Infection,* **24,** 1241-1250.

Köck, R., K. Becker, B. Cookson, J. van Gemert-Pijnen, S. Harbarth, J. Kluytmans, M. Mielke, G. Peters, R. Skov and M. Struelens, 2010: Methicillin-resistant Staphylococcus aureus (MRSA): burden of disease and control challenges in Europe.

Koga, V. L., S. Scandorieiro, E. C. Vespero, A. Oba, B. G. de Brito, K. C. de Brito, G. Nakazato and R. K. Kobayashi, 2015: Comparison of Antibiotic Resistance and Virulence Factors among Escherichia coli Isolated from Conventional and Free-Range Poultry. *BioMed research international,* 2015**,** 618752.

Korlach, J., Bjornson, K. P., Chaudhuri, B. P., Cicero, R. L., Flusberg, B. A., Gray, J. J., ... & Turner, S. W. (2010). Real-time DNA sequencing from single polymerase molecules. In *Methods in enzymology* (Vol. 472, pp. 431-455). Academic Press.

Kotloff KL, Nataro JP, Blackwelder WC, et al. (2013) Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet; 382:209–22

Kotloff, K. L. 2017. The burden and etiology of diarrheal illness in developing countries. *Pediatric Clinics*, *64*(4), 799-814.

Kozak, G. K., D. L. Pearl, J. Parkman, R. J. Reid-Smith, A. Deckert and P. Boerlin, 2009: Distribution of sulfonamide resistance genes in Escherichia coli and Salmonella isolates from swine and chickens at abattoirs in Ontario and Quebec, Canada. *Appl. Environ. Microbiol.,* **75,** 5999-6001.

Krause, K. M., A. W. Serio, T. R. Kane and L. E. Connolly, 2016: Aminoglycosides: An Overview. *Cold Spring Harbor perspectives in medicine,* **6**.

Ku CS, Polychronakos C, Tan EK, Naidoo N, Pawitan Y, et al. 2013. A new paradigm emerges from the study of de novo mutations in the context of neurodevelopmental disease. Mol.

Kunert Filho, H. C., Brito, K. C. T., Cavalli, L. S., & Brito, B. G. 2015. Avian pathogenic Escherichia coli (APEC)-an update on the control. *The battle against microbial pathogens: basic science, technological advances and educational programs*, *2*, 598-618

Kunert Filho, H., K. Brito, L. Cavalli and B. Brito, 2015: Avian pathogenic Escherichia coli (APEC)-an update on the control. *The battle against microbial pathogens: basic science, technological advances and educational programs,* **2,** 598-618.

Kutateladze, Á., & Adamia, R. 2008. Phage therapy experience at the Eliava Kwong, J. C., N. McCallum, V. Sintchenko and B. P. Howden, 2015: Whole genome sequencing in clinical and public health microbiology. *Pathology,* **47,** 199-210.
L. Anson, M. de Cesare, P. Piazza, A. A. Votintseva, T. Golubchik, D. J. Wilson, D. H. Wyllie, R. Diel, S. Niemann, S. Feuerriegel, T. A. Kohl, N. Ismail, S. V. Omar, E. G. Smith, D. Buck, G. McVean, A. S. Walker, T. E. Peto, D. W. Crook and Z. Iqbal, 2015: Rapid antibiotic-resistance predictions from genome sequence data for Staphylococcus aureus and Mycobacterium tuberculosis. *Nature communications,* **6,** 10063.

L., Skrivankova V., Johnston B., Gileva A. *et al.* 2013; Predictive diagnostics for *Escherichia coli* infections based on the clonal association of antimicrobial resistance and clinical outcome. *J Clin Microbiol*51:2991–2999

Laarem, M., A. Barguigua, K. Nayme, A. Akila, K. Zerouali, N. El Mdaghri and M. Timinouni, 2017: Occurrence of plasmid-mediated quinolone resistance and virulence genes in avian Escherichia coli isolates from Algeria. *The Journal of Infection in Developing Countries,* **11,** 143-151.

Lahlaoui, H., Khalifa, A. B. H., & Moussa, M. B. (2014). Epidemiology of Enterobacteriaceae producing CTX-M type extended spectrum β-lactamase (ESBL). *Medecine et maladies infectieuses*, *44*(9), 400-404.

Landers, T. F., B. Cohen, T. E. Wittum and E. L. Larson, 2012a: A review of antibiotic use in food animals: perspective, policy, and potential. *Public Health Rep,* **127,** 4-22.

Landers, T. F., B. Cohen, T. E. Wittum and E. L. Larson, 2012b: A review of antibiotic use in food animals: perspective, policy, and potential. *Public health reports,* **127,** 4-22.

Landman, D., C. Georgescu, D. A. Martin and J. Quale, 2008: Polymyxins revisited. *Clinical microbiology reviews,* **21,** 449-465.

Lapierre, L., J. Cornejo, C. Borie, C. Toro and B. San Martín, 2008: Genetic characterization of antibiotic resistance genes linked to class 1 and class 2 integrons in commensal strains of Escherichia coli isolated from poultry and swine. *Microbial Drug Resistance,* **14,** 265-272.

Lapierre, P., & Gogarten, J. P. 2009. Estimating the size of the bacterial pan-genome. *Trends in genetics*, *25*(3), 107-110.

Larsen, M. V., S. Cosentino, S. Rasmussen, C. Friis, H. Hasman, R. L. Marvig, L. Jelsbak, T. Sicheritz-Ponten, D. W. Ussery, F. M. Aarestrup and O. Lund, 2012: Multilocus sequence typing of total-genome-sequenced bacteria. *Journal of clinical microbiology,* **50,** 1355-1361.

Lavilla, S., Gonzalez-Lopez, J. J., Sabate, M., Garcia-Fernandez, A., Larrosa, M. N., Bartolome, R. M., & Prats, G. (2008). Prevalence of qnr genes among extended-spectrum β-lactamaseproducing enterobacterial isolates in Barcelona, Spain. *Journal of antimicrobial chemotherapy*, *61*(2), 291-295.

Laxminarayan, R., T. Van Boeckel and A. Teillant, 2015: The economic costs of withdrawing antimicrobial growth promoters from the livestock sector.

Lazarus, B., D. L. Paterson, J. L. Mollinger and B. A. Rogers, 2014: Do human extraintestinal Escherichia coli infections resistant to expanded spectrum cephalosporins originate from foodproducing animals? A systematic review. *Clinical Infectious Diseases,* **60,** 439-452.

Lederberg, J., 2000: Infectious history. *Science,* **288,** 287-293.

Lee, C. R., I. H. Cho, B. C. Jeong and S. H. Lee, 2013: Strategies to minimize antibiotic resistance. *Int J Environ Res Public Health,* **10,** 4274-4305.

Lee, P. L. (2017). DNA amplification in the field: move over PCR, here comes LAMP. *Molecular ecology resources*, *17*(2), 138-141.

Lee, P. Y., Costumbrado, J., Hsu, C. Y., & Kim, Y. H. (2012). Agarose gel electrophoresis for the separation of DNA fragments. *JoVE (Journal of Visualized Experiments)*, (62), e3923

Leibler, J. H., J. Otte, D. Roland-Holst, D. U. Pfeiffer, R. Soares Magalhaes, J. Rushton, J. P. Graham and E. K. Silbergeld, 2009: Industrial food animal production and global health risks: exploring the ecosystems and economics of avian influenza. *EcoHealth,* **6,** 58-70.

Leinonen R, Sugawara H, Shumway M (Int. Nucleotide Seq. Database Consort.). 2011. The sequence read archive. Nucleic Acids Res. 39: D19–21

Lelieveld, S. H., J. A. Veltman and C. Gilissen, 2016: Novel bioinformatic developments for exome sequencing. *Hum Genet,* **135,** 603-614.

Leonard, A. F., Zhang, L., Balfour, A. J., Garside, R., Hawkey, P. M., Murray, A. K., ... & Gaze, W. H. (2018). Exposure to and colonisation by antibiotic-resistant E. coli in UK coastal water users: Environmental surveillance, exposure assessment, and epidemiological study (Beach Bum Survey). *Environment international*, *114*, 326-333.

Lesser, W. H., 2018: *Marketing livestock and meat*. CRC Press.

Letunic, I. and P. Bork, 2007: Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. *Bioinformatics (Oxford, England),* **23,** 127-128.

Levene MJ, Korlach J, Turner SW, FoquetM, Craighead HG, Webb WW. 2003. Zero-mode waveguides for single-molecule analysis at high concentrations. Science 299:682–86

Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J, Voets GM, van den Munckhof MP, et al. 2011. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains.Clin. Microbiol. Infect. 17:873–80

Leverstein-van Hall, M. A., C. M. Dierikx, J. Cohen Stuart, G. M. Voets, M. P. van den Munckhof, A. van Essen-Zandbergen, T. Platteel, A. C. Fluit, N. van de Sande-Bruinsma, J. Scharinga, M. J. Bonten and D. J. Mevius, 2011: Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. *Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases,* **17,** 873-880.

Levy, S. B. (2001). Antibiotic resistance: consequences of inaction. *Clinical Infectious Diseases*, *33*(Supplement_3), S124-S129.

Levy, S. B. and B. Marshall, 2004a: Antibacterial resistance worldwide: causes, challenges and responses. *Nat Med,* **10,** S122-129.

Levy, S. B. and B. Marshall, 2004b: Antibacterial resistance worldwide: causes, challenges and responses. *Nature medicine,* **10,** S122.

Levy, S. B., 2001: Antibiotic resistance: consequences of inaction. *Clinical Infectious Diseases,* **33,** S124-S129.

Levy, S. B., 2005: Antibiotic resistance—the problem intensifies. *Advanced drug delivery reviews,* **57,** 1446-1450.

Levy, S. E., & Myers, R. M. (2016). Advancements in next-generation sequencing. *Annual review of genomics and human genetics*, *17*, 95-115.

Lewis, K., 2012: Antibiotics: Recover the lost art of drug discovery. *Nature,* **485,** 439-440.

Lewis, K., S. Epstein, A. D'Onofrio and L. L. Ling, 2010: Uncultured microorganisms as a source of secondary metabolites. *J Antibiot (Tokyo),* **63,** 468-476.

Leylabadlo, H. E., Bialvaei, A. Z., & Samadi Kafil, H. (2015). Brucellosis in Iran: why not eradicated? *Clinical Infectious Diseases*, *61*(10), 1629-1630.

Li Q, Li Y, Song J, Xu H, Xu J, et al. 2014. High accuracy de novo assembly and SNP detection of chloroplast genomes using a SMRT circular consensus sequencing strategy. New Phytol. 204:1041–49

Li, S., M. Zhao, J. Liu, Y. Zhou and Z. Miao, 2016: Prevalence and Antibiotic Resistance Profiles of Extended-Spectrum β-Lactamase–Producing Escherichia coli Isolated from Healthy Broilers in Shandong Province, China. *Journal of food protection,* **79,** 1169-1173.

Li, Y., L. Chen, X. Wu and S. Huo, 2015a: Molecular characterization of multidrug-resistant avian pathogenic Escherichia coli isolated from septicemic broilers. *Poultry science,* **94,** 601611.

Li, Y., L. Chen, X. Wu and S. Huo, 2015b: Molecular characterization of multidrug-resistant avian pathogenic Escherichia coli isolated from septicemic broilers. *Poultry science,* **94,** 601611.

Lim, C., Takahashi, E., Hongsuwan, M., Wuthiekanun, V., Thamlikitkul, V., Hinjoy, S., ... & Limmathurotsakul, D. (2016). Epidemiology and burden of multidrug-resistant bacterial infection in a developing country. *Elife*, *5*, e18082.

Lima Barbieri, N., D. W. Nielsen, Y. Wannemuehler, T. Cavender, A. Hussein, S. G. Yan, L. K. Nolan and C. M. Logue, 2017: mcr-1 identified in Avian Pathogenic Escherichia coli (APEC). *PloS one,* **12,** e0172997.

Lin, D. M., Koskella, B., & Lin, H. C. (2017). Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. *World journal of gastrointestinal pharmacology and therapeutics*, *8*(3), 162.

Liu CM, Stegger M, Aziz M, Johnson TJ, Waits K, et al. 2018. Escherichia coli ST131-H22 as a foodborne uropathogen. mBio 9:e00470-18

Liu, A., L. Tran, E. Becket, K. Lee, L. Chinn, E. Park, K. Tran and J. H. Miller, 2010: Antibiotic sensitivity profiles determined with an Escherichia coli gene knockout collection: generating an antibiotic bar code. *Antimicrobial agents and chemotherapy,* **54,** 1393-1403.

Liu, B. and M. Pop, 2009: ARDB--Antibiotic Resistance Genes Database. *Nucleic acids research,* **37,** D443-447.

Liu, W. E., Chen, L., Li, H., Duan, H., Zhang, Y., Liang, X., & Hawkey, P. M. (2009). Novel CTX-M β-lactamase genotype distribution and spread into multiple species of Enterobacteriaceae in Changsha, Southern China. *Journal of antimicrobial chemotherapy*, *63*(5), 895-900.

Liu, Y. Y., Wang, Y., Walsh, T. R., Yi, L. X., Zhang, R., Spencer, J., & Shen, J. (2016). Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *The Lancet infectious diseases*, *16*(2), 161-168.

Liu, Y. Y., Y. Wang, T. R. Walsh, L. X. Yi, R. Zhang, J. Spencer, Y. Doi, G. Tian, B. Dong, X. Huang, L. F. Yu, D. Gu, H. Ren, X. Chen, L. Lv, D. He, H. Zhou, Z. Liang, J. H. Liu and J. Shen, 2016b: Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *The Lancet. Infectious diseases,* **16,** 161-168.

Liu, Y.-Y., Y. Wang, T. R. Walsh, L.-X. Yi, R. Zhang, J. Spencer, Y. Doi, G. Tian, B. Dong and X. Huang, 2016a: Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *The Lancet infectious diseases,* **16,** 161-168.

Livermore, D. M. (2012). Fourteen years in resistance. *International journal of antimicrobial agents*, *39*(4), 283-294.

Livermore, D. M., & Hawkey, P. M. (2005). CTX-M: changing the face of ESBLs in the UK.

Livermore, D. M., Canton, R., Gniadkowski, M., Nordmann, P., Rossolini, G. M., Arlet, G., ... & Woodford, N. (2007). CTX-M: changing the face of ESBLs in Europe. *Journal of Antimicrobial Chemotherapy*, *59*(2), 165-174.

Livestock-Associated Methicillin Resistant Staphylococcus aureus (LA-MRSA) Clonal Complex (CC) 398 Isolated from UK Animals belong to European Lineages. *Frontiers in microbiology,* **7,** 1741.

Lobocka, M. B., D. J. Rose, G. Plunkett, 3rd, M. Rusin, A. Samojedny, H. Lehnherr, M. B. Yarmolinsky and F. R. Blattner, 2004: Genome of bacteriophage P1. *Journal of bacteriology,* 186**,** 7032-7068.

Lood, R., Winer, B. Y., Pelzek, A. J., Diez-Martinez, R., Thandar, M., Euler, C. W., ... & Fischetti, V. A. (2015). Novel phage lysin capable of killing the multidrug-resistant gramnegative bacterium Acinetobacter baumannii in a mouse bacteremia model. *Antimicrobial agents and chemotherapy*, *59*(4), 1983-1991.

Lood, R., Winer, B. Y., Pelzek, A. J., Diez-Martinez, R., Thandar, M., Euler, C. W., ... & Fischetti, V. A. (2015). Novel phage lysin capable of killing the multidrug-resistant gramnegative bacterium Acinetobacter baumannii in a mouse bacteremia model. *Antimicrobial agents and chemotherapy*, *59*(4), 1983-1991.

Lou DI, Hussmann JA,McBee RM, Acevedo A, Andino R, et al. 2013. High-throughput DNA sequencing errors are reduced by orders of magnitude using circle sequencing. PNAS 110:19872–77

Loytynoja, A., 2014: Phylogeny-aware alignment with PRANK. *Methods in molecular biology (Clifton, N.J.),* **1079,** 155-170.

Lv, J., M. Mohsin, S. Lei, S. Srinivas, R. T. Wiqar, J. Lin and Y. Feng, 2018: Discovery of a mcr-1-bearing plasmid in commensal colistin-resistant Escherichia coli from healthy broilers in Faisalabad, Pakistan. *Virulence,* **9,** 994-999.

Maamar, E., C. A. Alonso, Z. Hamzaoui, N. Dakhli, M. S. Abbassi, S. Ferjani, M. Saidani, I. Boutiba-Ben Boubaker and C. Torres, 2018: Emergence of plasmid-mediated colistin-resistance in CMY-2-producing Escherichia coli of lineage ST2197 in a Tunisian poultry farm. *International journal of food microbiology,* **269,** 60-63.

MacGowan, D. Mevius, M. Mulvey, T. Naas, T. Peto, J. M. Rolain, O. Samuelsen and N. Woodford, 2017: The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases,* **23,** 2-22.

Madec, J. Y., Haenni, M., Métayer, V., Saras, E., & Nicolas-Chanoine, M. H. (2015). High prevalence of the animal-associated blaCTX-M-1 IncI1/ST3 plasmid in human Escherichia coli isolates. *Antimicrobial agents and chemotherapy*, *59*(9), 5860-5861.

Magiorakos, A. P., A. Srinivasan, R. B. Carey, Y. Carmeli, M. E. Falagas, C. G. Giske, S.

Harbarth, J. F. Hindler, G. Kahlmeter, B. Olsson-Liljequist, D. L. Paterson, L. B. Rice, J. Stelling, M. J. Struelens, A. Vatopoulos, J. T. Weber and D. L. Monnet, 2012: Multidrugresistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases,* **18,** 268-281.

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., & Paterson, D. L. (2012). Multidrug‐resistant, extensively drug‐resistant and pandrug‐resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical microbiology and infection*, *18*(3), 268-281.

Maiden MC, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, et al. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc Natl Acad Sci USA 1998; 95:31405

Mandal, R. K., T. Jiang, A. A. Al-Rubaye, D. D. Rhoads, R. F. Wideman, J. Zhao, I. Pevzner and Y. M. Kwon, 2016: An investigation into blood microbiota and its potential association with Bacterial Chondronecrosis with Osteomyelitis (BCO) in Broilers. *Sci Rep,* **6,** 25882.

Manges AR. 2016. Escherichia coli and urinary tract infections: the role of poultry-meat. Clin. Microbiol. Infect. 22:122–29

Manges, A. R. and J. R. Johnson, 2012: Food-borne origins of Escherichia coli causing extraintestinal infections. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America,* **55,** 712-719.

Manges, A. R., & Johnson, J. R. (2012). Food-borne origins of Escherichia coli causing extraintestinal infections. *Clinical infectious diseases*, *55*(5), 712-719.

Manges, A. R., 2016a: Escherichia coli and urinary tract infections: the role of poultry-meat. *Clinical Microbiology and Infection,* **22,** 122-129.

Manges, A. R., 2016b: Escherichia coli and urinary tract infections: the role of poultry-meat. Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases, **22,** 122-129.

Manges, A. R., Tabor, H., Tellis, P., Vincent, C., & Tellier, P. P. 2008. Endemic and epidemic lineages of Escherichia coli that cause urinary tract infections. *Emerging infectious diseases*, *14*(10), 1575.

Martens, E. and A. L. Demain, 2017a: The antibiotic resistance crisis, with a focus on the United States. *The Journal of antibiotics,* **70,** 520.

Martens, E. and A. L. Demain, 2017b: The antibiotic resistance crisis, with a focus on the United States. *J Antibiot (Tokyo),* **70,** 520-526.

Masihi, K. N., 2000: Immunomodulators in infectious diseases: panoply of possibilities. *International journal of immunopharmacology,* **22,** 1083-1091.

Matamoros, S., J. M. Van Hattem, M. S. Arcilla, N. Willemse, D. C. Melles, J. Penders, T. N. Vinh, N. T. Hoa, M. D. de Jong and C. Schultsz, 2017: Global phylogenetic analysis of Escherichia coli and plasmids carrying the mcr-1 gene indicates bacterial diversity but plasmid restriction. *Scientific reports,* **7,** 15364.

Mathai, E., Chandy, S., Thomas, K., Antoniswamy, B., Joseph, I., Mathai, M., & Holloway, K. (2008). Antimicrobial resistance surveillance among commensal Escherichia coli in rural and urban areas in Southern India. *Tropical medicine & international health*, *13*(1), 41-45.

Matsumoto, Y. O. S. H. I. M. I., Ikeda, F. U. M. I. A. K. I., Kamimura, T. O. S. H. I. A. K. I., Yokota, Y. O. S. H. I. K. O., & Mine, Y. A. S. U. H. I. R. O. (1988). Novel plasmid-mediated beta-lactamase from Escherichia coli that inactivates oxyimino-cephalosporins. *Antimicrobial agents and chemotherapy*, *32*(8), 1243-1246.

Mayer LW, Reeves MW, Al-Hamdan N, Sacchi CT, Taha MK, Ajello GW, et al. Outbreak of W135 meningococcal disease in 2000: not emergence of a new W135 strain but clonal expansion within the electophoretic type-37 complex. J Infect Dis 2002;185: 1596605

McArthur, A. G. and G. D. Wright, 2015: Bioinformatics of antimicrobial resistance in the age of molecular epidemiology. *Curr Opin Microbiol,* **27,** 45-50.

McArthur, A. G. and K. K. Tsang, 2017: Antimicrobial resistance surveillance in the genomic age. *Ann N Y Acad Sci,* 1388**,** 78-91.

McArthur, A. G., N. Waglechner, F. Nizam, A. Yan, M. A. Azad, A. J. Baylay, K. Bhullar, M. J. Canova, G. De Pascale, L. Ejim, L. Kalan, A. M. King, K. Koteva, M. Morar, M. R. Mulvey, J. McDougald, L. R., 1998: Intestinal protozoa important to poultry. *Poultry science,* **77,** 11561158.

McEwen, S. A. and P. J. Fedorka-Cray, 2002: Antimicrobial use and resistance in animals. *Clin Infect Dis,* 34 Suppl 3, S93-S106.

McEwen, S. A., J. F. Prescott and P. Boerlin, 2010: Antibiotics and poultry—A comment. *The*

Canadian Veterinary Journal, 51**,** 561.

McKenna, M., 2013: Antibiotic resistance: the last resort. *Nature News,* 499**,** 394.

Meade, K. G., F. Narciandi, S. Cahalane, C. Reiman, B. Allan and C. O'Farrelly, 2009: Comparative in vivo infection models yield insights on early host immune response to Campylobacter in chickens. *Immunogenetics,* **61,** 101-110.

Mellata, M., 2013: Human and avian extraintestinal pathogenic Escherichia coli: infections, zoonotic risks, and antibiotic resistance trends. *Foodborne Pathog Dis,* **10,** 916-932.

Mellata, M., M. Dho-Moulin, C. M. Dozois, R. Curtiss, 3rd, B. Lehoux and J. M. Fairbrother, 2003: Role of avian pathogenic Escherichia coli virulence factors in bacterial interaction with chicken heterophils and macrophages. *Infection and immunity,* **71,** 494-503.

Mellon, M., C. Benbrook and K. Benbrook, 2001: Hogging it: Estimates of antimicrobial abuse in livestock. Union of Concerned Scientists, Cambridge, MA. *Hogging it: Estimates of antimicrobial abuse in livestock. Union of Concerned Scientists, Cambridge, MA.***,** -.

Mena, A., Plasencia, V., García, L., Hidalgo, O., Ayestarán, J. I., Alberti, S., ... & Oliver, A. (2006). Characterization of a large outbreak by CTX-M-1-producing Klebsiella pneumoniae and mechanisms leading to in vivo carbapenem resistance development. *Journal of clinical microbiology*, *44*(8), 2831-2837.

Mendonça, N., R. Figueiredo, C. Mendes, R. Card, M. Anjum and G. da Silva, 2016: Microarray evaluation of antimicrobial resistance and virulence of Escherichia coli isolates from Portuguese poultry. *Antibiotics,* **5,** 4.

Meunier, D., Jouy, E., Lazizzera, C., Kobisch, M., & Madec, J. Y. (2006). CTX-M-1-and CTXM-15-type β-lactamases in clinical Escherichia coli isolates recovered from food-producing animals in France. *International journal of antimicrobial agents*, *28*(5), 402-407.

Microbiol Methods 111:21–23 http://dx.doi.org/10.1016/j.- mimet.2015.01.024

Miles-Jay, A., Weissman, S. J., Adler, A. L., Tchesnokova, V., Sokurenko, E. V., Baseman, J. G., & Zerr, D. M. 2017. Epidemiology of the multidrug-resistant ST131-H30 subclone among extraintestinal Escherichia coli collected from US children. *bioRxiv*, 122416.

Miller, J., 1991: Evaluating biochemical identification systems. *Journal of clinical microbiology,* **29,** 1559.

Minodier, P., 2011: Escherichia coli [esh″ə-rik′e-ə co′lī]. *Euro Surveill,* **16,** 19815.

Miranda, J., B. Vazquez, C. Fente, P. Calo-Mata, A. Cepeda and C. Franco, 2008b: Comparison of antimicrobial resistance in Escherichia coli, Staphylococcus aureus, and Listeria monocytogenes strains isolated from organic and conventional poultry meat. *Journal of food protection,* **71,** 2537-2542.

Miranda, J., M. Guarddon, B. Vázquez, C. Fente, J. Barros-Velazquez, A. Cepeda and C. Franco, 2008a: Antimicrobial resistance in Enterobacteriaceae strains isolated from organic chicken, conventional chicken and conventional turkey meat: A comparative survey. *Food control,* **19,** 412-416.

Mohsin, M., Azam, M., ur Rahman, S., Esposito, F., Sellera, F. P., Monte, D. F., ... & Lincopan,

Mohsin, M., Raza, S., Schaufler, K., Roschanski, N., Sarwar, F., Semmler, T., ... & Guenther, S. (2017). High prevalence of CTX-M-15-Type ESBL-producing E. coli from migratory avian species in Pakistan. *Frontiers in microbiology*, *8*, 2476.

Mohsin, M., S. Raza, K. Schaufler, N. Roschanski, F. Sarwar, T. Semmler, P. Schierack and S. Guenther, 2017b: High prevalence of CTX-M-15-Type ESBL-producing E. coli from migratory avian species in Pakistan. *Frontiers in microbiology,* **8,** 2476.

Mohsin, M., S. Raza, N. Roschanski, S. Guenther, A. Ali and P. Schierack, 2017a: Description of the first Escherichia coli clinical isolate harboring the colistin resistance gene mcr-1 from the Indian subcontinent. *Antimicrobial agents and chemotherapy,* **61,** e01945-01916.

Moissenet, D., Salauze, B., Clermont, O., Bingen, E., Arlet, G., Denamur, E., & Vu-Thien, H. 2010. Meningitis caused by Escherichia coli producing TEM-52 extended-spectrum β-lactamase within an extensive outbreak in a neonatal ward: epidemiological investigation and characterization of the strain. *Journal of clinical microbiology*, *48*(7), 2459-2463.

Momin, J. K., & Joshi, B. H. (2015). Nanotechnology in foods. In *Nanotechnologies in Food and Agriculture* (pp. 3-24). Springer, Cham.

Monciardini, P., Iorio, M., Maffioli, S., Sosio, M., and Donadio, S. (2014) Discovering new bioactive molecules from microbial sources. Microb Biotechnol 7: 209–220.

Mora A, Viso S, López C, Alonso MP, García-Garrote F, Dabhi G, et al. Poultry as reservoir for extraintestinal pathogenic Escherichia coli O45: K1:H7-B2-ST95 in humans. Vet Microbiol 2013; 167:506–12.

Mora, A., López, C., Herrera, A., Viso, S., Mamani, R., Dhabi, G., ... & Blanco, J. (2012).
Mora, A., S. Viso, C. Lopez, M. P. Alonso, F. Garcia-Garrote, G. Dabhi, R. Mamani, A. Herrera, J. Marzoa, M. Blanco, J. E. Blanco, M. Moulin-Schouleur, C. Schouler and J. Blanco, 2013: Poultry as reservoir for extraintestinal pathogenic Escherichia coli O45:K1:H7-B2-ST95 in humans. *Veterinary microbiology,* **167,** 506-512.

Murray, P. R., 2012: What is new in clinical microbiology-microbial identification by MALDITOF mass spectrometry: a paper from the 2011 William Beaumont Hospital Symposium on molecular pathology. *J Mol Diagn,* **14,** 419-423.

Muthuirulandi Sethuvel, D. P., Devanga Ragupathi, N. K., Anandan, S., & Veeraraghavan, B. 2017. Update on: Shigella new serogroups/serotypes and their antimicrobial resistance. *Letters in applied microbiology*, *64*(1), 8-18.

N. (2019). Genomic background of a colistin-resistant and highly virulent MCR-1-positive Escherichia coli ST6395 from a broiler chicken in Pakistan. *Pathogens and disease*, *77*(7), ftz064.

Naeem, K., M. Naurin, S. Rashid and S. Bano, 2003: Seroprevalence of avian influenza virus and its relationship with increased mortality and decreased egg production. *Avian pathology,* **32,** 283-287.

Nagy, G., U. Dobrindt, M. Kupfer, L. Emody, H. Karch and J. Hacker, 2001: Expression of hemin receptor molecule ChuA is influenced by RfaH in uropathogenic Escherichia coli strain 536. *Infection and immunity,* **69,** 1924-1928.

Narrod, C., J. Zinsstag and M. Tiongco, 2012: A one health framework for estimating the economic costs of zoonotic diseases on society. *EcoHealth,* **9,** 150-162. *Nature reviews. Microbiology,* **8,** 26-38.

Navarro-Martinez, M. D., E. Navarro-Peran, J. Cabezas-Herrera, J. Ruiz-Gomez, F. GarciaCanovas and J. N. Rodriguez-Lopez, 2005: Antifolate activity of epigallocatechin gallate against Stenotrophomonas maltophilia. *Antimicrobial agents and chemotherapy,* **49,** 2914-2920.

Nawarangpur district, Odisha, India. *World Journal of Pharmacy & Pharmaceutical Science,* **3,** 787-798.

Nhung, N. T., N. Chansiripornchai and J. J. Carrique-Mas, 2017: Antimicrobial resistance in bacterial poultry pathogens: a review. *Frontiers in veterinary science,* **4,** 126.

Nicolas-Chanoine, M. H., Bertrand, X., & Madec, J. Y. (2014). Escherichia coli ST131, an intriguing clonal group. *Clinical microbiology reviews*, *27*(3), 543-57

Nicolas-Chanoine, M. H., Blanco, J., Leflon-Guibout, V., Demarty, R., Alonso, M. P., Caniça, M. M., ... & Johnson, J. R. (2008). Intercontinental emergence of Escherichia coli clone O25: H4-ST131 producing CTX-M-15. *Journal of Antimicrobial Chemotherapy*, *61*(2), 273-281.

Niero, G., V. Bortolaia, M. Vanni, L. Intorre, L. Guardabassi and A. Piccirillo, 2018: High diversity of genes and plasmids encoding resistance to third generation cephalosporins and quinolones in clinical Escherichia coli from commercial poultry flocks in Italy. *Veterinary microbiology,* **216,** 93-98.

Nishiguchi, A., S. Kobayashi, T. Yamamoto, Y. Ouchi, T. Sugizaki and T. Tsutsui, 2007: Risk factors for the introduction of avian influenza virus into commercial layer chicken farms during the outbreaks caused by a low‐pathogenic H5N2 virus in Japan in 2005. *Zoonoses and public health,* **54,** 337-343.

Nordmann, P., Dortet, L., & Poirel, L. (2012). Carbapenem resistance in Enterobacteriaceae: Norman, A., L. H. Hansen, and S. J. Sorensen**,** 2009. Conjugative plasmids: vessels of the communal gene pool. Philos. Trans. R. Soc. Lond. B. Biol. Sci.

Nowakowska, D., Colón, I., Remington, J. S., Grigg, M., Golab, E., Wilczynski, J., & Sibley, L. D. 2006. Genotyping of Toxoplasma gondii by multiplex PCR and peptide-based serological testing of samples from infants in Poland diagnosed with congenital toxoplasmosis. *Journal of Clinical Microbiology*, *44*(4), 1382-1389.

O'Neil, J., 2014: Review on Antimicrobial Resistance. Antimicrobial Resistance: Tackling a Crisis for the Health and Wealth of Nations 2014.

Ochman H., Selander R. K. 1984; Standard reference strains of *Escherichia coli* from natural populations. *J Bacteriol*157:690–693

Ohsaki, Y., W. Hayashi, S. Saito, S. Osaka, Y. Taniguchi, S. Koide, K. Kawamura, Y. Nagano, Y. Arakawa and N. Nagano, 2017: First detection of Escherichia coli harboring mcr-1 gene from retail domestic chicken meat in Japan. *Japanese journal of infectious diseases***,** JJID. 2016.2572.

Okeke, I. N., Laxminarayan, R., Bhutta, Z. A., Duse, A. G., Jenkins, P., O'Brien, T. F., ... & Klugman, K. P. (2005). Antimicrobial resistance in developing countries. Part I: recent trends and current status. *The Lancet infectious diseases*, *5*(8), 481-493

Olaitan AO, Morand S, Rolain JM. 2014. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Front Microbiol **5**:1-18.

Oliver, D. B., Cabelli, R. J., Dolan, K. M., & Jarosik, G. P. (1990). Azide-resistant mutants of Escherichia coli alter the SecA protein, an azide-sensitive component of the protein export machinery. *Proceedings of the National Academy of Sciences*, *87*(21), 8227-8231

Overdevest, I., Willemsen, I., Rijnsburger, M., Eustace, A., Xu, L., Hawkey, P., ... & Huijsdens, X. (2011). Extended-spectrum β-lactamase genes of Escherichia coli in chicken meat and humans, The Netherlands. *Emerging infectious diseases*, *17*(7), 1216.

Overdevest, I., Willemsen, I., Rijnsburger, M., Eustace, A., Xu, L., Hawkey, P., ... & Huijsdens, Padmanabhan, R., A. K. Mishra, D. Raoult and P.-E. Fournier, 2013: Genomics and metagenomics in medical microbiology. *Journal of microbiological methods,* **95,** 415-424.

Page S and Gautier P, 2012. Use of antimicrobial agents in livestock. Revue Scientifique et Technique-OIE 31:145.

Page, A. J., C. A. Cummins, M. Hunt, V. K. Wong, S. Reuter, M. T. Holden, M. Fookes, D. Falush, J. A. Keane and J. Parkhill, 2015: Roary: rapid large-scale prokaryote pan genome analysis. *Bioinformatics (Oxford, England),* **31,** 3691-3693.

Paixao, A. C., A. C. Ferreira, M. Fontes, P. Themudo, T. Albuquerque, M. C. Soares, M. Fevereiro, L. Martins and M. I. Correa de Sa, 2016: Detection of virulence-associated genes in pathogenic and commensal avian Escherichia coli isolates. *Poult Sci,* 95**,** 1646-1652.

Panda, A., S. Kurapati, J. C. Samantaray, A. Srinivasan and S. Khalil, 2014: MALDI-TOF mass spectrometry proteomic based identification of clinical bacterial isolates. *The Indian journal of medical research,* 140**,** 770.

Panda, S. and N. Dhal, 2014: Plants used in ethno-veterinary medicine by native people of

Paudel, S., B. Stessl, C. Hess, A. Zloch and M. Hess, 2016: High genetic diversity among extraintestinal Escherichia coli isolates in pullets and layers revealed by a longitudinal study. *BMC veterinary research,* **12,** 221.

Paul, M., S. Wongnarkpet, P. Gasqui, C. Poolkhet, S. Thongratsakul, C. Ducrot and F. Roger, 2011: Risk factors for highly pathogenic avian influenza (HPAI) H5N1 infection in backyard chicken farms, Thailand. *Acta tropica,* 118**,** 209-216.

Perepelo AV, Han W, Senchenkova SN, Shevelev SD,Shashkov AS, Feng L,Liu Y, Knirel YA,

Perreten, V. and P. Boerlin, 2003: A new sulfonamide resistance gene (sul3) in Escherichia coli is widespread in the pig population of Switzerland. *Antimicrobial agents and chemotherapy,* **47,** 1169-1172.

Petrillo, M., A. Angers-Loustau and J. Kreysa, 2016: Possible genetic events producing colistin resistance gene mcr-1. *The Lancet infectious diseases,* **16,** 280.

Picard, B., Garcia, J. S., Gouriou, S., Duriez, P., Brahimi, N., Bingen, E., ... & Denamur, E. 1999. The link between phylogeny and virulence inEscherichia coli extraintestinal infection. *Infection and immunity*, *67*(2), 546-553.

Piccinini, R., R. Tassi, V. Daprà, R. Pilla, J. Fenner, B. Carter and M. F. Anjum, 2012: Study of Staphylococcus aureus collected at slaughter from dairy cows with chronic mastitis. *Journal of dairy research,* **79,** 249-255.

Piddock, L. J., 2012: The crisis of no new antibiotics--what is the way forward? *Lancet Infect Dis,* **12,** 249-253.

Piepenburg, O., Williams, C. H., Stemple, D. L., and Armes, N. A. (2006). DNA detection 558 using recombination proteins. PLoS Biology, 4(7), 1115–112

Pike, B. L., Guerry, P., & Poly, F. 2013. Global distribution of Campylobacter jejuni Penner serotypes: a systematic review. *PloS one*, *8*(6).

Pilla, R., V. Castiglioni, M. Gelain, E. Scanziani, V. Lorenzi, M. Anjum and R. Piccinini, 2013: Long-term study of MRSA ST1, t127 mastitis in a dairy cow. *Veterinary Record Case Reports,* **1,** e100510.

Pineiro, M., N.-G. Asp, G. Reid, S. Macfarlane, L. Morelli, O. Brunser and K. Tuohy, 2008: FAO Technical meeting on prebiotics. *Journal of clinical gastroenterology,* **42,** S156-S159.

Pique, N., D. Minana-Galbis, S. Merino and J. M. Tomas, 2015: Virulence Factors of Erwinia amylovora: A Review. *Int J Mol Sci,* **16,** 12836-12854.

Platell, J. L., Cobbold, R. N., Johnson, J. R., Heisig, A., Heisig, P., Clabots, C., ... & Trott, D. J. (2011). Commonality among fluoroquinolone-resistant sequence type ST131 extraintestinal Escherichia coli isolates from humans and companion animals in Australia. *Antimicrobial agents and chemotherapy*, *55*(8), 3782-3787.

Poirel, L., T. R. Walsh, V. Cuvillier and P. Nordmann, 2011: Multiplex PCR for detection of acquired carbapenemase genes. *Diagnostic microbiology and infectious disease,* **70,** 119-123.

Pomba, C., da Fonseca, J. D., Baptista, B. C., Correia, J. D., & Martínez-Martínez, L. (2009). Detection of the pandemic O25-ST131 human virulent Escherichia coli CTX-M-15-producing clone harboring the qnrB2 and aac (6′)-Ib-cr genes in a dog. *Antimicrobial agents and chemotherapy*, *53*(1), 327-328.

Pomba, C., Rantala, M., Greko, C., Baptiste, K. E., Catry, B., Van Duijkeren, E., ... & Sanders, P. (2017). Public health risk of antimicrobial resistance transfer from companion animals. *Journal of Antimicrobial Chemotherapy*, *72*(4), 957-968.

Potter, R. F., A. W. D'Souza, M. A. Wallace, A. Shupe, S. Patel, D. Gul, J. H. Kwon, W. Beatty, S. Andleeb, C. D. Burnham and G. Dantas, 2018a: Superficieibacter electus gen. nov., sp. nov., an Extended-Spectrum beta-Lactamase Possessing Member of the Enterobacteriaceae Family, Isolated From Intensive Care Unit Surfaces. *Frontiers in microbiology,* **9,** 1629.

Potter, R. F., W. Lainhart, J. Twentyman, M. A. Wallace, B. Wang, C. A. Burnham, D. A. Rosen and G. Dantas, 2018b: Population Structure, Antibiotic Resistance, and Uropathogenicity of Klebsiella variicola. *MBio,* **9**.

Poulet, H., J. Minke, M. C. Pardo, V. Juillard, B. Nordgren and J. C. Audonnet, 2007: Development and registration of recombinant veterinary vaccines. The example of the canarypox vector platform. *Vaccine,* **25,** 5606-5612.

Pounder, J. I., Hansen, D., & Woods, G. L. 2006. Identification of Histoplasma capsulatum, Blastomyces dermatitidis, and Coccidioides species by repetitive-sequence-based PCR. *Journal of clinical microbiology*, *44*(8), 2977-2982.

Prestinaci, F., P. Pezzotti and A. Pantosti, 2015a: Antimicrobial resistance: a global multifaceted phenomenon. *Pathogens and global health,* **109,** 309-318.

Prestinaci, F., P. Pezzotti and A. Pantosti, 2015b: Antimicrobial resistance: a global multifaceted phenomenon. *Pathogens and global health,* **109,** 309-318.

Price, M. N., P. S. Dehal and A. P. Arkin, 2010: FastTree 2--approximately maximum-likelihood trees for large alignments. *PLoS One,* **5,** e9490.*Proceedings of the National Academy of Sciences,* **112,** 5649-5654.

Proudfoot, F., H. Hulan, E. Jackson and C. Salisbury, 1990: Effect of lincomycin as a growth promoter for broiler chicks. *British poultry science,* **31,** 181-187. Psychiatry 18:141–53

Pupo, G. M., Lan, R., & Reeves, P. R. 2000. Multiple independent origins of Shigella clones of Escherichia coli and convergent evolution of many of their characteristics. *Proceedings of*

the National Academy of Sciences, *97*(19), 10567-10572.

Quail MA, Smith M, Coupland P, Otto TD, Harris SR, et al. 2012. A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. BMC Genom. 13:341

Queenan, A. M. and K. Bush, 2007: Carbapenemases: the versatile beta-lactamases. *Clin Microbiol Rev,* **20,** 440-458, table of contents.

Quesada, A., M. Ugarte-Ruiz, M. R. Iglesias, M. C. Porrero, R. Martínez, D. Florez-Cuadrado, M. J. Campos, M. García, S. Píriz and J. L. Sáez, 2016: Detection of plasmid mediated colistin resistance (MCR-1) in Escherichia coli and Salmonella enterica isolated from poultry and swine in Spain. *Research in veterinary science,* **105,** 134-135.

Quinn, P., M. Carter, B. Markey, W. Donnoly and F. Leonard, 2002: Veterinary microbiology and microbial diseases 166-1117 Osney Mead. Oxford first LTd, Registered at the United Kingdom.

R., Maiden M. C. *et al.* 2006; Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Mol Microbiol*60:1136–1151

Raguvaran, R., A. Manuja and B. K. Manuja, 2015: Zinc oxide nanoparticles: opportunities and challenges in veterinary sciences. *Immunome Research,* 11, 1.

Ramos, S., G. Igrejas, J. Rodrigues, J. L. Capelo-Martinez and P. Poeta, 2012: Genetic characterisation of antibiotic resistance and virulence factors in vanA-containing enterococci from cattle, sheep and pigs subsequent to the discontinuation of the use of avoparcin. *Vet J,* **193,** 301-303.

Ravindran, V. and J. H. Son, 2011: Feed enzyme technology: present status and future developments. *Recent Pat Food Nutr Agric,* **3,** 102-109.

Redgrave, L. S., Sutton, S. B., Webber, M. A., & Piddock, L. J. (2014). Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success. *Trends in microbiology*, *22*(8), 438-445.

Redkar, R. J., Dube, M. P., McCleskey, F. K., Rinaldi, M. G., & Del Vecchio, V. G. 1996. DNA fingerprinting of Candida rugosa via repetitive sequence-based PCR. *Journal of Clinical Microbiology*, *34*(7), 1677-1681.

Reed, K. D., Stemper, M. E., & Shukla, S. K. (2007). Pulsed-field gel electrophoresis of MRSA.

In *Methicillin-Resistant Staphylococcus aureus (MRSA) Protocols* (pp. 59-69). Humana Press.

Reid S. D., Herbelin C. J., Bumbaugh A. C., Selander R. K., Whittam T. S. 2000; Parallel evolution of virulence in pathogenic *Escherichia coli*. *Nature*406:64–67

REN, Y. N., ZHEN, P. P., LI, J., GUO, Y. F., WANG, L. H., REN, J. Y., ... & JIANG, H. X. Resistance and Associations with Compromised Hosts. *Clinical Infectious Diseases*, *62*(12), 1529-1536.*Resistance*,*21*(6),643-650.

Resistant*Enterobacteriaceae*Availablehttp://ecdc.europa.eu/en/publications/Publications/carba penem-resistant-enterobacteriaceae-risk-assessment-april-2016.pdf (2016).

Reuter JA, Spacek DV, Snyder MP. 2015. High-throughput sequencing technologies. Mol. Cell 58: 586–97

Rice, L. B., 2008: Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. The University of Chicago Press.

Riley LW. 2014. Pandemic lineages of extraintestinal pathogenic Escherichia coli. Clin. Microbiol. Infect. 20:380– 90

Risse J, Thomson M, Patrick S, Blakely G, Koutsovoulos G, et al. 2015. A single chromosome assembly of Bacteroides fragilis strain BE1 from Illumina and MinION nanopore sequencing data. GigaScience 4:60

Roach, D. R., & Donovan, D. M. (2015). Antimicrobial bacteriophage-derived proteins and therapeutic applications. *Bacteriophage*, *5*(3), e1062590.

Roberts, M. C., 2002: Resistance to tetracycline, macrolide-lincosamide-streptogramin, trimethoprim, and sulfonamide drug classes. *Molecular biotechnology,* **20,** 261-283.

Robicsek, A., Strahilevitz, J., Sahm, D. F., Jacoby, G. A., & Hooper, D. C. (2006). qnr prevalence in ceftazidime resistant Enterobacteriaceae isolates from the United States. *Antimicrobial agents and chemotherapy*, *50*(8), 2872-2874.

Robinson, A., Y. S. McCarter and J. Tetreault, 1995: Comparison of Crystal Enteric/Nonfermenter system, API 20E system, and Vitek Auto Microbic system for identification of gram-negative bacilli. *Journal of clinical microbiology,* **33,** 364-370.

Rogers BA, Sidjabat HE, Paterson DL**.** 2011. *Escherichia coli* O25b-ST131: a pandemic, multiresistant, community-associated strain. The Journal of antimicrobial chemotherapy 66:114.

Rolain, J. M., R. Canton and G. Cornaglia, 2012: Emergence of antibiotic resistance: need for a new paradigm. Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases, **18,** 615-616.

Rosengren, L. B., C. L. Waldner, R. J. Reid-Smith, S. L. Checkley, M. E. McFall and A. Rajić, 2008: Antimicrobial resistance of fecal Escherichia coli isolated from grow-finish pigs in 20 herds in Alberta and Saskatchewan. *Canadian Journal of Veterinary Research,* **72,** 160.

Rozwandowicz, M., M. S. M. Brouwer, J. Fischer, J. A. Wagenaar, B. Gonzalez-Zorn, B. Guerra, D. J. Mevius and J. Hordijk, 2018: Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. *The Journal of antimicrobial chemotherapy,* **73,** 1121-1137.

Rugumisa, B. T., D. R. Call, G. O. Mwanyika, R. I. Mrutu, C. M. Luanda, B. M. Lyimo, M. Subbiah and J. J. Buza, 2016: Prevalence of Antibiotic-Resistant Fecal Escherichia coli Isolates from Penned Broiler and Scavenging Local Chickens in Arusha, Tanzania. *J Food Prot,* **79,** 1424-1429.

Rupnik M, Widmer A, Zimmermann O, Eckert C, Barbut F. 2008. Clostridium 316 difficile toxinotype V, ribotype 078, in animals and humans. J Clin Microbiol 317 46:2146.

S. Cruveiller, C. Robert, S. Duprat, P. Wincker, L. N. Ornston, J. Weissenbach, P. Marlie`re, G. N. Cohen, and C. Me´digue. 2004. Unique features revealed by the genome sequence of *Acinetobacter* sp. ADP1, a versatile and naturally transformation competent bacterium. Nucleic Acids Res.**32:**5766–5779.

S. O'Brien, A. C. Pawlowski, L. J. Piddock, P. Spanogiannopoulos, A. D. Sutherland, I. Tang, P. L. Taylor, M. Thaker, W. Wang, M. Yan, T. Yu and G. D. Wright, 2013: The comprehensive antibiotic resistance database. *Antimicrobial agents and chemotherapy,* **57,** 3348-3357.

Sacheli, R., Adjetey, C., Darfouf, R., Harag, S., Huynen, P., Meex, C., & Hayette, M. P. 2016. A one-year survey of Microsporum audouinii infections in Belgium: epidemiological and genotypic characterization. *Clinical microbiology and infection*, *22*(3), 285-e9.

Saghrouni, F. B. A. J., Ben Abdeljelil, J., Boukadida, J., & Ben Said, M. 2013. Molecular methods for strain typing of C andida albicans: a review. *Journal of applied microbiology*, *114*(6), 1559-1574

Sahl J. W., Matalka M. N., Rasko D. A**.** 2012; Phylomark, a tool to identify conserved phylogenetic markers from whole-genome alignments. *Appl Environ Microbiol*78:4884–4892

Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis and

H. A. Erlich, 1988: Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science,* **239,** 487-491.

Salim, A., Babu, P., Mohan, K., Moorthy, M., Raj, D., Thirumeni, S. K., ... & Pal, S. (2019). Draft Genome Sequence of an Escherichia coli Sequence Type 155 Strain Isolated from Sewage in Kerala, India. *Microbiology resource announcements*, *8*(27).

Salipante SJ, Roach DJ, Kitzman JO, Snyder MW, Stackhouse B, et al. 2015. Large-scale genomic sequencing of extraintestinal pathogenic Escherichia coli strains. Genome Res. 25:119– 28

Santesmases, M. J. (2018). Modern Times: Screening Antibiotics and the Factory Line. In *The Circulation of Penicillin in Spain* (pp. 107-131). Palgrave Macmillan, Cham.

Sapunaric, F. M., M. Aldema-Ramos and L. M. McMurry, 2005: Tetracycline resistance: efflux, mutation, and other mechanisms. *Frontiers in Antimicrobial Resistance.* American Society of Microbiology.

Sarmah, A. K., M. T. Meyer and A. B. Boxall, 2006: A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere,* **65,** 725-759.

Sarowska, J., B. Futoma-Koloch, A. Jama-Kmiecik, M. Frej-Madrzak, M. Ksiazczyk, G. BuglaPloskonska and I. Choroszy-Krol, 2019: Virulence factors, prevalence and potential transmission of extraintestinal pathogenic Escherichia coli isolated from different sources: recent reports. *Gut pathogens,* **11,** 10.

Schatz, W. Timp and P. J. Simner, 2019: Applying Rapid Whole-Genome Sequencing To Predict Phenotypic Antimicrobial Susceptibility Testing Results among Carbapenem-Resistant Klebsiella pneumoniae Clinical Isolates. *Antimicrobial agents and chemotherapy,* **63**.

Schmelcher, M., Shen, Y., Nelson, D. C., Eugster, M. R., Eichenseher, F., Hanke, D. C., ... & Becker, S. C. (2015). Evolutionarily distinct bacteriophage endolysins featuring conserved peptidoglycan cleavage sites protect mice from MRSA infection. *Journal of Antimicrobial Chemotherapy*, *70*(5), 1453-1465.

Schmieder, R. and R. Edwards, 2011: Quality control and preprocessing of metagenomic datasets. *Bioinformatics (Oxford, England),* **27,** 863-864.

Schneiders, T., S. G. Amyes and S. B. Levy, 2003: Role of AcrR and ramA in fluoroquinolone resistance in clinical Klebsiella pneumoniae isolates from Singapore. *Antimicrobial agents and chemotherapy,* **47,** 2831-2837.

Schreiber, H. L. t., M. S. Conover, W. C. Chou, M. E. Hibbing, A. L. Manson, K. W. Dodson, T. J. Hannan, P. L. Roberts, A. E. Stapleton, T. M. Hooton, J. Livny, A. M. Earl and S. J. Hultgren, 2017: Bacterial virulence phenotypes of Escherichia coli and host susceptibility determine risk for urinary tract infections. *Science translational medicine,* **9**.

Schuijffel, D., P. Van Empel, A. Pennings, J. Van Putten and P. Nuijten, 2005a: Passive immunization of immune-suppressed animals: chicken antibodies protect against Ornithobacterium rhinotracheale infection. *Vaccine,* **23,** 3404-3411.

Schuijffel, D., P. Van Empel, A. Pennings, J. Van Putten and P. Nuijten, 2005b: Successful selection of cross-protective vaccine candidates for Ornithobacterium rhinotracheale infection. *Infection and immunity,* **73,** 6812-6821.

Schuijffel, D., P. Van Empel, R. Segers, J. Van Putten and P. Nuijten, 2006: Vaccine potential of recombinant Ornithobacterium rhinotracheale antigens. *Vaccine,* **24,** 1858-1867.

Schwarz, S. and E. Chaslus-Dancla, 2001: Use of antimicrobials in veterinary medicine and mechanisms of resistance. *Veterinary research,* **32,** 201-225.

Schweizer, H. P., 2001: Triclosan: a widely used biocide and its link to antibiotics. *FEMS microbiology letters,* **202,** 1-7.

Scott, M. G., H. Yan and R. E. Hancock, 1999: Biological properties of structurally related αhelical cationic antimicrobial peptides. *Infection and immunity,* **67,** 2005-2009.

Seemann, T., 2014: Prokka: rapid prokaryotic genome annotation. *Bioinformatics (Oxford, England),* **30,** 2068-2069.

Seenama, C., Thamlikitkul, V., & Ratthawongjirakul, P. (2019). Multilocus sequence typing and blaESBL characterization of extended-spectrum beta-lactamase-producing Escherichia coli isolated from healthy humans and swine in Northern Thailand. *Infection and drug resistance*, *12*, 2201.

Seiffert, S. N., A. Carattoli, S. Schwendener, A. Collaud, A. Endimiani and V. Perreten, 2017: Plasmids Carrying blaCMY -2/4 in Escherichia coli from Poultry, Poultry Meat, and Humans Belong to a Novel IncK Subgroup Designated IncK2. *Frontiers in microbiology,* **8,** 407.

Selle, P. H. and V. Ravindran, 2007: Microbial phytase in poultry nutrition. *Animal Feed Science and Technology,* **135,** 1-41.

Shad, A. A. and W. A. Shad, 2018: E. coli plasmid-mediated colistin resistance in Faisalabad, Pakistan: A glance. *Journal of Experimental Medicine & Biology,* **1**.

Shah, N. S., A. Wright, G. H. Bai, L. Barrera, F. Boulahbal, N. Martin-Casabona, F. Drobniewski, C. Gilpin, M. Havelkova, R. Lepe, R. Lumb, B. Metchock, F. Portaels, M. F. Rodrigues, S. Rusch-Gerdes, A. Van Deun, V. Vincent, K. Laserson, C. Wells and J. P. Cegielski, 2007: Worldwide emergence of extensively drug-resistant tuberculosis. *Emerg Infect Dis,* **13,** 380-387.

Shaheen, B. W., Nayak, R., & Boothe, D. M. (2013). Emergence of a New Delhi metallo-βlactamase (NDM-1)-encoding gene in clinical Escherichia coli isolates recovered from companion animals in the United States. *Antimicrobial agents and chemotherapy*, *57*(6), 29022903.

Sharma, C., Dhiman, R., Rokana, N., & Panwar, H. (2017). Nanotechnology: an untapped resource for food packaging. *Frontiers in microbiology*, *8*, 1735.

Sharma, C., N. Rokana, M. Chandra, B. P. Singh, R. D. Gulhane, J. P. S. Gill, P. Ray, A. K. Puniya and H. Panwar, 2017c: Antimicrobial Resistance: Its Surveillance, Impact, and Alternative Management Strategies in Dairy Animals. *Front Vet Sci,* **4,** 237.

Sharma, C., R. Dhiman, N. Rokana and H. Panwar, 2017a: Nanotechnology: An Untapped Resource for Food Packaging. *Frontiers in microbiology,* **8,** 1735.

Sharma, C., R. Dhiman, N. Rokana and H. Panwar, 2017b: Nanotechnology: an untapped resource for food packaging. *Frontiers in microbiology,* **8,** 1735.

Sharma, M., J. Nunez-Garcia, A. M. Kearns, M. Doumith, P. R. Butaye, M. A. Argudin, A. Lahuerta-Marin, B. Pichon, M. AbuOun, J. Rogers, R. J. Ellis, C. Teale and M. F. Anjum, 2016:

Sharma-Kuinkel, B. K., Rude, T. H., & Fowler, V. G. 2014. Pulse field gel electrophoresis. In *the Genetic Manipulation of Staphylococci* (pp. 117-130). Humana Press, New York, NY.

Sheikh, F. A., Kanjwal, M. A., Saran, S., Chung, W. J., & Kim, H. (2011). Polyurethane nanofibers containing copper nanoparticles as future materials. *Applied Surface Science*, *257*(7), 3020-3026.

Silva, K.C., M. Moreno, C. Cabrera, B. Spira, L. Cerdeira, N. Lincopan, and A.M. Moreno. 2016. First characterization of CTX-M-15-producing Escherichia coli strains beto sequence

type (ST) 410, ST224, and ST1284 from commercial swine in South America. Antimicrob. Agents Chemother. 60:2505–2508. Singapore. *Antimicrobial agents and chemotherapy*, *47*(9), 2831-2837.

Singh, D., Radhakrishnan, T., Kumar, V., Bagwan, N. B., Basu, M. S., Dobaria, J. R.,& Chanda, S. V. 2015. Molecular characterization of Aspergillus flavus isolates from peanut fields in India using AFLP. *Brazilian Journal of Microbiology*, *46*(3), 673-682.

Singh, S. B. and J. F. Barrett, 2006: Empirical antibacterial drug discovery--foundation in natural products. *Biochem Pharmacol,* **71,** 1006-1015.Smith, C. L. and K. R. Powell, 2000: Review of the sulfonamides and trimethoprim. *Pediatrics*

in review, **21,** 368-371.

Smith, J. L., Fratamico, P. M., & Gunther, N. W. 2007. Extraintestinal pathogenic Escherichia coli. *Foodborne pathogens and disease*, *4*(2), 134-163

Smith, R. A., Hitchcock, C. A., Evans, E. G. V., Lacey, C. J. N., & Adams, D. J. (1989). The identification of Candida albicans strains by restriction fragment length polymorphism analysis of DNA. *Journal of Medical and Veterinary Mycology*, *27*(6), 431-434.

Smith, R. and J. Coast, 2013: The true cost of antimicrobial resistance. *Bmj,* **346,** f1493.

Solanki, R., L. Vanjari, S. Subramanian, A. B, N. E and V. Lakshmi, 2014: Comparative Evaluation of Multiplex PCR and Routine Laboratory Phenotypic Methods for Detection of Carbapenemases among Gram Negative Bacilli. *J Clin Diagn Res,* **8,** Dc23-26.

Song, S. K., B. R. Beck, D. Kim, J. Park, J. Kim, H. D. Kim and E. Ringo, 2014: Prebiotics as immunostimulants in aquaculture: a review. *Fish & shellfish immunology,* **40,** 40-48.

Sorensen, S. J., M. Bailey, L. H. Hansen, N. Kroer, and S. Wuertz. 2005. Studying plasmid horizontal transfer in situ: a critical review. Nat. Rev.Microbiol. 3**:**700–710

Sotgiu, G., G. Ferrara, A. Matteelli, M. D. Richardson, R. Centis, S. Ruesch-Gerdes, O. Toungoussova, J. P. Zellweger, A. Spanevello, D. Cirillo, C. Lange and G. B. Migliori, 2009: Epidemiology and clinical management of XDR-TB: a systematic review by TBNET. *Eur Respir J,* **33,** 871-881.

Souli, M., Galani, I., & Giamarellou, H. (2008). Emergence of extensively drug-resistant and pandrug-resistant Gram-negative bacilli in Europe. *Eurosurveillance*, *13*(47), 19045.

Sousa, A., N. Frazão, R. S. Ramiro and I. Gordo, 2017: Evolution of commensal bacteria in the intestinal tract of mice. *Current opinion in microbiology,* **38,** 114-121.

Souza, L., D. Araújo, J. Astolphi, L. Dias, A. Ambiel, L. Santos, A. Carmo and P. Silva, Probiótico e antibiótico como promotores de crescimento para frangos de corte. in Proceedings of the Colloquium Agrariae, 2010, p. 33-39.

Spellberg, B. and D. N. Gilbert, 2014: The future of antibiotics and resistance: a tribute to a career of leadership by John Bartlett. *Clin Infect Dis,* 59 Suppl 2**,** S71-75.

Springman, A. C., D. W. Lacher, G. Wu, N. Milton, T. S. Whittam, H. D. Davies, and S. D. Manning. 2009. Selection, recombination, and virulence gene diversity among group B streptococcal genotypes. J. Bacteriol. 191**:**5419–5427.

Stanley, J., Linton, D., Desai, M., Efstratiou, A., & George, R. 1995. Molecular subtyping of prevalent M serotypes of Streptococcus pyogenes causing invasive disease. *Journal of clinical microbiology*, *33*(11), 2850-2855.

Stenhem, M., A. Ortqvist, H. Ringberg, L. Larsson, B. Olsson Liljequist, S. Haeggman, M. Kalin and K. Ekdahl, 2010: Imported methicillin-resistant Staphylococcus aureus, Sweden. *Emerg Infect Dis,* **16,** 189-196.

Stenhem, M., Örtqvist, Å., Ringberg, H., Larsson, L., Olsson-Liljequist, B., Hæggman, S., & Ekdahl, K. (2010). Imported methicillin-resistant Staphylococcus aureus, Sweden. *Emerging infectious diseases*, *16*(2), 189.

Stoesser, N., E. Batty, D. Eyre, M. Morgan, D. Wyllie, C. Del Ojo Elias, J. Johnson, A. Walker, T. Peto and D. Crook, 2013: Predicting antimicrobial susceptibilities for Escherichia coli and Klebsiella pneumoniae isolates using whole genomic sequence data. *Journal of Antimicrobial Chemotherapy,* **68,** 2234-2244.

Stolle, I., Prenger-Berninghoff, E., Stamm, I., Scheufen, S., Hassdenteufel, E., Guenther, S., ... & Ewers, C. (2013). Emergence of OXA-48 carbapenemase-producing Escherichia coli and Klebsiella pneumoniae in dogs. *Journal of Antimicrobial Chemotherapy*, *68*(12), 2802-2808.

Strahilevitz, J., G. A. Jacoby, D. C. Hooper, and A. Robicsek**.** 2009. Plasmidmediated quinolone resistance: a multifaceted threat. Clin. Microbiol. Rev.22**:**664–689.

Subedi, M., H. Luitel, B. Devkota, R. K. Bhattarai, S. Phuyal, P. Panthi, A. Shrestha and D. K. Chaudhary, 2018b: Antibiotic resistance pattern and virulence genes content in avian pathogenic

Subedi, M., R. K. Bhattarai, B. Devkota, S. Phuyal and H. Luitel, 2018a: Correction to: Antibiotic resistance pattern and virulence genes content in avian pathogenic Escherichia coli (APEC) from broiler chickens in Chitwan, Nepal. *BMC veterinary research,* **14,** 166.

Sulakvelidze, A., Alavidze, Z., & Morris, J. G. (2001). Bacteriophage therapy. *Antimicrobial agents and chemotherapy*, *45*(3), 649-659.

Sun, J., L. X. Fang, Z. Wu, H. Deng, R. S. Yang, X. P. Li, S. M. Li, X. P. Liao, Y. Feng and Y. H. Liu, 2017: Genetic Analysis of the IncX4 Plasmids: Implications for a Unique Pattern in the mcr-1 Acquisition. *Scientific reports,* **7,** 424.

Sunde, M. and M. Norström, 2006: The prevalence of, associations between and conjugal transfer of antibiotic resistance genes in Escherichia coli isolated from Norwegian meat and meat products. *Journal of Antimicrobial Chemotherapy,* **58,** 741-747.

Sykes, R. B. and M. H. Richmond, 1970: Intergeneric transfer of a beta-lactamase gene between Ps. aeruginosa and E. coli. *Nature,* **226,** 952-954.

Szalay T, Golovchenko JA. 2015. De novo sequencing and variant calling with nanopores using PoreSeq. Nat. Biotechnol. 33:1087–91

Tacconelli, E., Magrini, N., Kahlmeter, G., & Singh, N. (2017). Global priority list of antibioticresistant bacteria to guide research, discovery, and development of new antibiotics. *World Health Organization*, *27*.

Tadesse, D. A., S. Zhao, E. Tong, S. Ayers, A. Singh, M. J. Bartholomew and P. F. McDermott, 2012: Antimicrobial drug resistance in Escherichia coli from humans and food animals, United States, 1950-2002. *Emerg Infect Dis,* **18,** 741-749.

Tahir, M. F., M. A. Abbas, T. Ghafoor, S. Dil, M. A. Shahid, M. M. Bullo, Q. ul Ain, M. A. Ranjha, M. A. Khan and M. T. Naseem, 2018: Seroprevalence and risk factors of avian influenza H9 virus among poultry professionals in Rawalpindi, Pakistan. *Journal of infection and public health*.

Tamma, P. D., Y. Fan, Y. Bergman, G. Pertea, A. Q. Kazmi, S. Lewis, K. C. Carroll, M. C.

Tanwar, J., S. Das, Z. Fatima and S. Hameed, 2014: Multidrug resistance: an emerging crisis. *Interdiscip Perspect Infect Dis,* **2014,** 541340.

Tasho, R. P. and J. Y. Cho, 2016: Veterinary antibiotics in animal waste, its distribution in soil and uptake by plants: a review. *Science of the Total Environment,* **563,** 366-376.

Taylor, P. L., Rossi, L., De Pascale, G. & Wright, G. D. (2012). A forward chemical screen identifies antibiotic adjuvants in *Escherichia coli*. *ACS Chem. Biol.* **7,** 1547–1555.

Tchesnokova V., Billig M., Chattopadhyay S., Linardopoulou E., Aprikian P., Roberts P.

Team, E. E., 2013: The European Union summary report on trends and sources of zoonoses, 20449. Technology. Publ. No. 073-2014-001, Illumina, San Diego, CA.

Teillant and R. Laxminarayan, 2015a: Global trends in antimicrobial use in food animals.

Tellez, G., A. Laukova, J. D. Latorre, X. Hernandez-Velasco, B. M. Hargis and T. Callaway, 2015: Food-producing animals and their health in relation to human health. *Microb Ecol Health Dis,* **26,** 25876.

Tenaillon, O., Skurnik, D., Picard, B., and Denamur, E. 2010. The population genetics of commensal Escherichia coli. Nat Rev Microbiol 8: 207–217.

Tenover, F. C., Arbeit, R. D., Goering, R. V., Mickelsen, P. A., Murray, B. E., Persing, D. H., & Swaminathan, B. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsedfield gel electrophoresis: criteria for bacterial strain typing. *Journal of clinical microbiology*, *33*(9), 2233.

Teshager, T., Dom nguez, L., Moreno, M. A., Sa nz, Y., Torres, C., & Carde osa, S. (2000). Isolation of an SHV-12 β-lactamase-producing Escherichia coli strain from a dog with recurrent urinary tract infections. *Antimicrobial agents and chemotherapy*, *44*(12), 3483-3484.

The Journal of antimicrobial chemotherapy, **68,** 12-16.

Thomsen, M. C., J. Ahrenfeldt, J. L. Cisneros, V. Jurtz, M. V. Larsen, H. Hasman, F. M. Aarestrup and O. Lund, 2016: A Bacterial Analysis Platform: An Integrated System for Analysing Bacterial Whole Genome Sequencing Data for Clinical Diagnostics and Surveillance. *PLoS One,* **11,** e0157718.

Tijet, N., D. Faccone, M. Rapoport, C. Seah, F. Pasteran, P. Ceriana, E. Albornoz, A. Corso, A. Petroni and R. G. Melano, 2017: Molecular characteristics of mcr-1-carrying plasmids and new mcr-1 variant recovered from polyclonal clinical Escherichia coli from Argentina and Canada. *PLoS One,* **12,** e0180347.

Touchon, M., Hoede, C., Tenaillon, O., Barbe, V., Baeriswyl, S., Bidet, P., ... & Denamur, E. (2009). Organised genome dynamics in the Escherichia coli species results in highly diverse adaptive paths. *PLoS genet*, *5*(1), e1000344.

Trafalska, E. and K. Grzybowska, 2004: [Probiotics--an alternative for antibiotics?]. *Wiad Lek,* **57,** 491-498.

Trongjit, S., S. Angkittitrakul and R. Chuanchuen, 2016: Occurrence and molecular characteristics of antimicrobial resistance of Escherichia coli from broilers, pigs and meat products in Thailand and Cambodia provinces. *Microbiol Immunol,* **60,** 575-585.

Trung, N. V., H. N. Nhung, J. J. Carrique-Mas, H. H. Mai, H. T. Tuyen, J. Campbell, N. T. Nhung, P. Van Minh, J. A. Wagenaar, N. T. Mai, T. Q. Hieu, C. Schultsz and N. T. Hoa, 2016: Colonization of Enteroaggregative Escherichia coli and Shiga toxin-producing Escherichia coli in chickens and humans in southern Vietnam. *BMC microbiology,* **16,** 208.

Twist Dx, TwistAmp DNA amplification kits, Combined instruction manual (2013).

Uddin, M. N., M. M. Farooq, M. Waqas, N. U. Khan, W. A. Khan, I. Khan, N. Karim and M. Rizwan, 2018: 10. Antibiotic assays of Salmonella isolated from poultry chicken of various locations in districts Swat. *Pure and Applied Biology (PAB),* **7,** 78-84.

Uelze, L., Grützke, J., Borowiak, M., Hammerl, J. A., Juraschek, K., Deneke, C., & Malorny, B. 2020. Typing methods based on whole genome sequencing data. *One Health Outlook*, *2*(1), 1-19.

Ur Rahman, S. and M. Mohsin, 2019: The under reported issue of antibiotic-resistance in foodproducing animals in Pakistan. *Pak. Vet. J,* **1,** 1-16.

Ur Rahman, S., S. Ahmad, and I. Khan. 2018. Incidence of ESBL-producing-Escherichia coli in poultry farm environment and retail poultry meat. Pak. Vet. J. 39:116–120

Urwin R, Maiden MC. Multi-locus sequence typing: a tool for global epidemiology. Trends Microbiol 2003;11: 47987

Usui, M., S. Ozawa, H. Onozato, R. Kuge, Y. Obata, T. Uemae, P. T. Ngoc, A. Heriyanto, T. Chalemchaikit and K. Makita, 2014: Antimicrobial susceptibility of indicator bacteria isolated from chickens in Southeast Asian countries (Vietnam, Indonesia and Thailand). *Journal of Veterinary Medical Science***,** 13-0423.

Uyeno, Y., S. Shigemori and T. Shimosato, 2015: Effect of Probiotics/Prebiotics on Cattle Health and Productivity. *Microbes Environ,* **30,** 126-132.

Van Belkum, A., Tassios, P. T., Dijkshoorn, L., Haeggman, S., Cookson, B., Fry, N. K., & Brisse, S. 2007. Guidelines for the validation and application of typing methods for use in bacterial epidemiology. *Clinical Microbiology and Infection*, *13*, 1-46.

Van Boeckel, T. P., C. Brower, M. Gilbert, B. T. Grenfell, S. A. Levin, T. P. Robinson, A. Teillant and R. Laxminarayan, 2015b: Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences of the United States of America,* 112**,** 56495654.

Van Boeckel, T. P., S. Gandra, A. Ashok, Q. Caudron, B. T. Grenfell, S. A. Levin and R. Laxminarayan, 2014: Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. *Lancet Infect Dis,* 14**,** 742-750.

Van Cuong, N., N. T. Nhung, N. H. Nghia, N. T. M. Hoa, N. V. Trung, G. Thwaites and J. Carrique-Mas, 2016: Antimicrobial consumption in medicated feeds in Vietnamese pig and poultry production. *Ecohealth,* **13,** 490-498.

Van Duijkeren, E., ten Horn, L., Wagenaar, J. A., de Bruijn, M., Laarhoven, L., Verstappen, K., & Duim, B. (2011). Suspected horse-to-human transmission of MRSA ST398. *Emerging infectious diseases*, *17*(6), 1137.

Van Hoek, A. H., D. Mevius, B. Guerra, P. Mullany, A. P. Roberts and H. J. Aarts, 2011: Acquired antibiotic resistance genes: an overview. *Frontiers in microbiology,* **2,** 203.

Ventola, C. L., 2015a: The antibiotic resistance crisis: part 1: causes and threats. *P T,* **40,** 277283.

Ventola, C. L., 2015b: The antibiotic resistance crisis: part 1: causes and threats. *Pharmacy and Therapeutics,* **40,** 277.

Vermeiren, L., Devlieghere, F., & Debevere, J. (2002). Effectiveness of some recent antimicrobial packaging concepts. *Food Additives & Contaminants*, *19*(S1), 163-171.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Lee, T. V. D., Hornes, M., & Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic acids research*, *23*(21), 4407-4414.

Waksman, S. A. (1945). *Microbial antagonisms and antibiotic substances* (Vol. 59, No. 6, p. 482). LWW.

Walsh, T. R. (2008). Clinically significant carbapenemases: an update. *Current opinion in infectious diseases*, *21*(4), 367-371.

Walsh, T. R. 2006. Combinatorial genetic evolution of multi resistanc. Opin. Microbiol. **9:**476–482.

Wang L. Structure of the Opolysaccharide of *Escherichia coli* O150 containing 2-acetamido-4O-[(S)-1-carboxyethyl]-2-deoxy-D glucose. Carbohydrate Research. 2007; 342:648-652.

Wang, H., Dzink-Fox, J. L., Chen, M., & Levy, S. B. (2001). Genetic characterization of highly fluoroquinolone-resistant clinical Escherichia coli strains from China: role ofacrR mutations. *Antimicrobial agents and chemotherapy*, *45*(5), 1515-1521.

Wang, H., J. L. Dzink-Fox, M. Chen and S. B. Levy, 2001: Genetic characterization of highly fluoroquinolone-resistant clinical Escherichia coli strains from China: role of acrR mutations. *Antimicrobial agents and chemotherapy,* **45,** 1515-1521.

Wang, J., Hu, B., Xu, M., Yan, Q., Liu, S., Zhu, X., ... & Gong, J. (2006). Therapeutic effectiveness of bacteriophages in the rescue of mice with extended spectrum β-lactamaseproducing Escherichia coli bacteremia. *International journal of molecular medicine*, *17*(2), 347355.

Wang, J., Hu, B., Xu, M., Yan, Q., Liu, S., Zhu, X., ... & Li, Q. Q. (2006). Use of bacteriophage in the treatment of experimental animal bacteremia from imipenem-resistant Pseudomonas aeruginosa. *International journal of molecular medicine*, *17*(2), 309-317.

Wang, J., Stephan, R., Power, K., Yan, Q., Hächler, H., & Fanning, S. (2014). Nucleotide sequences of 16 transmissible plasmids identified in nine multidrug-resistant Escherichia coli isolates expressing an ESBL phenotype isolated from food-producing animals and healthy humans. *Journal of Antimicrobial Chemotherapy*, *69*(10), 2658-2668.

Wang, N., X. Yang, S. Jiao, J. Zhang, B. Ye and S. Gao, 2014: Sulfonamide-resistant bacteria and their resistance genes in soils fertilized with manures from Jiangsu Province, Southeastern China. *PloS one,* **9,** e112626.

Wang, Q., J. Sun, J. Li, Y. Ding, X. P. Li, J. Lin, B. Hassan and Y. Feng, 2017a: Expanding landscapes of the diversified mcr-1-bearing plasmid reservoirs. *Microbiome,* **5,** 70.

Wang, R., L. van Dorp, L. P. Shaw, P. Bradley, Q. Wang, X. Wang, L. Jin, Q. Zhang, Y. Liu and A. Rieux, 2018a: The global distribution and spread of the mobilized colistin resistance gene mcr-1. *Nature communications,* **9,** 1179.

Wang, X., Liu, W., Zou, D., Li, X., Wei, X., Shang, W.,& Yuan, J. (2013). High rate of New Delhi Metallo-β-Lactamase 1–producing bacterial infection in China. *Clinical infectious diseases*, *56*(1), 161-162.

Wang, X., Y. Wang, Y. Zhou, J. Li, W. Yin, S. Wang, S. Zhang, J. Shen, Z. Shen and Y. Wang, 2018b: Emergence of a novel mobile colistin resistance gene, mcr-8, in NDM-producing Klebsiella pneumoniae. *Emerging microbes & infections,* **7,** 122.

Wang, Y., R. Zhang, J. Li, Z. Wu, W. Yin, S. Schwarz, J. M. Tyrrell, Y. Zheng, S. Wang and Z. Shen, 2017b: Comprehensive resistome analysis reveals the prevalence of NDM and MCR-1 in Chinese poultry production. *Nature microbiology,* **2,** 16260.

Wang, Y., Z. Jiang, Z. Jin, H. Tan and B. Xu, 2013: Risk factors for infectious diseases in backyard poultry farms in the Poyang Lake area, China. *PLoS One,* **8,** e67366.

Warren RL, Yang C, Vandervalk BP, Behsaz B, Lagman A, et al. 2015. LINKS: scalable, alignment-free scaffolding of draft genomes with long reads. GigaScience 4:35

Waseem, H., J. Ali, A. Jamal and M. ALI, 2019: POTENTIAL DISSEMINATION OF

Watanabe, R., Matsumoto, T., Sano, G., Ishii, Y., Tateda, K., Sumiyama, Y., ... & Yamaguchi, K. (2007). Efficacy of bacteriophage therapy against gut-derived sepsis caused by Pseudomonas aeruginosa in mice. *Antimicrobial agents and chemotherapy*, *51*(2), 446-452.

Weissman S.J., Johnson J.R., Tchesnokova V., Billig M., Dykhuizen D., Riddell K., Rogers P., Qin X., Butler-Wu S. *et al.* 2012; High-resolution two-locus clonal typing of extraintestinal pathogenic *Escherichia coli*. *Appl Environ Microbiol*78:1353–1360

Weist, K., A. Muller, D. Monnet and O. Heuer, 2014: Surveillance of antimicrobial consumption in Europe. *European Centre for Disease Prevention and Control*.

Welch, R. A., Burland, V., Plunkett, G. I. I. I., Redford, P., Roesch, P., Rasko, D., ... & Stroud, D. 2002. Extensive mosaic structure revealed by the complete genome sequence of uropathogenic Escherichia coli. *Proceedings of the National Academy of Sciences*, *99*(26), 17020-17024

Welch, R. A., Burland, V., Plunkett, G. I. I. I., Redford, P., Roesch, P., Rasko, D., & Blattner, F. R. (2002). Extensive mosaic structure revealed by the complete genome sequence of uropathogenic Escherichia coli. *Proceedings of the National Academy of Sciences*, *99*(26), 17020-17024.

Wieler, L. H., Ewers, C., Guenther, S., Walther, B., & Lübke-Becker, A. (2011). Methicillinresistant staphylococci (MRS) and extended-spectrum beta-lactamases (ESBL)-producing Enterobacteriaceae in companion animals: nosocomial infections as one reason for the rising prevalence of these potential zoonotic pathogens in clinical samples. *International journal of medical microbiology*, *301*(8), 635-641.

Wieler, L. H., Ewers, C., Guenther, S., Walther, B., & Lübke-Becker, A. (2011). Methicillinresistant staphylococci (MRS) and extended-spectrum beta-lactamases (ESBL)-producing Enterobacteriaceae in companion animals: nosocomial infections as one reason for the rising prevalence of these potential zoonotic pathogens in clinical samples. *International journal of medical microbiology*, *301*(8), 635-641.

Wieser, A., L. Schneider, J. Jung and S. Schubert, 2012: MALDI-TOF MS in microbiological diagnostics-identification of microorganisms and beyond (mini review). *Appl Microbiol Biotechnol,* **93,** 965-974.

Wigley, P., 2013: Immunity to bacterial infection in the chicken. *Dev Comp Immunol,* **41,** 413417.

Willenbrock, H., Hallin, P. F., Wassenaar, T. M., & Ussery, D. W. (2007). Characterization of probiotic Escherichia coli isolates with a novel pan-genome microarray. *Genome biology*, *8*(12), 1-16.

Williams, R., 2005: Intercurrent coccidiosis and necrotic enteritis of chickens: rational, integrated disease management by maintenance of gut integrity. *Avian pathology,* **34,** 159-180.

Windhorst, H.-W., 2006: Changes in poultry production and trade worldwide. *World's Poultry Science Journal,* **62,** 585-602.

Wing, E. A. s. and D. Finance, 2008: Economic Sur. *Economic Survey,* **2015,** 16. Wirth T., Falush D., Lan R., Colles F., Mensa P., Wieler L.H., Karch H., Reeves P.

Witzenrath, M., Schmeck, B., Doehn, J. M., Tschernig, T., Zahlten, J., Loeffler, J. M., ... & Hippenstiel, S. (2009). Systemic use of the endolysin Cpl-1 rescues mice with fatal pneumococcal pneumonia. *Critical care medicine*, *37*(2), 642-649.

Wolf MK. Occurrence, distribution and associations of O and H serogroups, colonization factor antigens and toxins of enterotoxigenic *Escherichia coli*. *Clin Microbiol Rev* 1997; **10**: 569–584

Woolhouse, M., M. Ward, B. van Bunnik and J. Farrar, 2015: Antimicrobial resistance in humans, livestock and the wider environment. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences,* **370,** 20140083.

World Health Organization. WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007–2015?

Wu, C., Y. Wang, X. Shi, S. Wang, H. Ren, Z. Shen, Y. Wang, J. Lin and S. Wang, 2018: Rapid rise of the ESBL and mcr-1 genes in Escherichia coli of chicken origin in China, 2008-2014. *Emerg Microbes Infect,* **7,** 30.

Wu, G., J. Fanzo, D. D. Miller, P. Pingali, M. Post, J. L. Steiner and A. E. Thalacker-Mercer, 2014: Production and supply of high-quality food protein for human consumption: sustainability, challenges, and innovations. *Ann N Y Acad Sci,* **1321,** 1-19.

X. (2011). Extended-spectrum β-lactamase genes of Escherichia coli in chicken meat and humans, The Netherlands. *Emerging infectious diseases*, *17*(7), 1216.

Yam, T. S., J. M. Hamilton-Miller and S. Shah, 1998: The effect of a component of tea (Camellia sinensis) on methicillin resistance, PBP2' synthesis, and beta-lactamase production in Staphylococcus aureus. *The Journal of antimicrobial chemotherapy,* **42,** 211-216.

Yamaji R, Friedman CR, Rubin J, Suh J, Thys E, et al. 2018a. A population-based surveillance study of shared genotypes of Escherichia coli isolates from retail meat and suspected cases of urinary tract infections. mSphere 3(4): e00179-18

Yamaji R, Rubin J, Thys E, Friedman CR, Riley LW. 2018b. Persistent pandemic lineages of uropathogenic Escherichia coli in a college community from 1999 to 2017. J. Clin. Microbiol. 56(4): e01834-17

Yan, L., Zhou, J., Zheng, Y., Gamson, A. S., Roembke, B. T., Nakayama, S., & Sintim, H. O. 615 (2014). Isothermal amplified detection of DNA and RNA. Molecular BioSystem, 10(5), 616 970–1003.

Yanagi, D., de Vries, G. C., Rahardjo, D., Alimsardjono, L., Wasito, E. B., De, I., ... & Kawabata, M. (2009). Emergence of fluoroquinolone-resistant strains of Salmonella enterica in Surabaya, Indonesia. *Diagnostic microbiology and infectious disease*, *64*(4), 422-426

Yang, H., S. Chen, D. G. White, S. Zhao, P. McDermott, R. Walker and J. Meng, 2004: Characterization of multiple-antimicrobial-resistant Escherichia coli isolates from diseased chickens and swine in China. *Journal of clinical microbiology,* **42,** 3483-3489.

Yang, S. C., C. H. Lin, C. T. Sung and J. Y. Fang, 2014: Antibacterial activities of bacteriocins:
Yang, Y. Q., Li, Y. X., Song, T., Yang, Y. X., Jiang, W., Zhang, A. Y., ... & Wang, H. N. (2017). Colistin resistance gene mcr-1 and its variant in Escherichia coli isolates from chickens in China. *Antimicrobial agents and chemotherapy*, *61*(5).

Yang, Y. Q., Y. X. Li, T. Song, Y. X. Yang, W. Jiang, A. Y. Zhang, X. Y. Guo, B. H. Liu, Y. X. Wang, C. W. Lei, R. Xiang and H. N. Wang, 2017: Colistin Resistance Gene mcr-1 and Its Variant in Escherichia coli Isolates from Chickens in China. *Antimicrobial agents and chemotherapy,* **61**.

Yao X, Doi Y, Zeng L, Lv L, Liu JH. 2016. Carbapenem-resistant and colistin-resistant *Escherichia* 192 *coli* co-producing NDM-9 and MCR-1. Lancet Infect Dis **16**:288-289.

Yao X, Doi Y, Zeng L, Lv L, Liu JH. 2016. Carbapenem-resistant and colistin-resistant *Escherichia* 192 *coli* co-producing NDM-9 and MCR-1. Lancet Infect Dis **16**:288-289.

Yegani, M. and D. R. Korver, 2008: Factors affecting intestinal health in poultry. *Poult Sci,* **87,** 2052-2063.

Yi, H., Cho, Y. J., Yong, D., & Chun, J. (2012). Genome sequence of Escherichia coli J53, a reference strain for genetic studies.

Yoneyama, H. and R. Katsumata, 2006: Antibiotic resistance in bacteria and its future for novel antibiotic development. *Biosci Biotechnol Biochem,* **70,** 1060-1075.

Younis, G., A. Awad and N. Mohamed, 2017: Phenotypic and genotypic characterization of antimicrobial susceptibility of avian pathogenic Escherichia coli isolated from broiler chickens. *Vet World,* **10,** 1167-1172.

Yu, X., M. Susa, C. Knabbe, R. D. Schmid and T. T. Bachmann, 2004: Development and validation of a diagnostic DNA microarray to detect quinolone-resistant Escherichia coli among clinical isolates. *Journal of clinical microbiology,* **42,** 4083-4091.

Yuce, M., H. Kurt, V. R. Mokkapati and H. Budak, 2014: Employment of nanomaterials in polymerase chain reaction: insight into the impacts and putative operating mechanisms of nanoadditives in PCR. *RSC Advances,* **4,** 36800-36814.

Zahra, R., Javeed, S., Malala, B., Babenko, D., & Toleman, M. A. (2018). Analysis of Escherichia coli STs and resistance mechanisms in sewage from Islamabad, Pakistan indicates

a difference in E. coli carriage types between South Asia and Europe. *Journal of Antimicrobial Chemotherapy*, *73*(7), 1781-1785.

Zang, E., Brandes, S., Tovar, M., Martin, K., Mech, F., Horbert, P., et al. (2013) Real-time image processing for label-free enrichment of Actinobacteria cultivated in picolitre droplets. Lab Chip 13: 3707–3713.

Zankari, E., H. Hasman, S. Cosentino, M. Vestergaard, S. Rasmussen, O. Lund, F. M. Aarestrup and M. V. Larsen, 2012: Identification of acquired antimicrobial resistance genes. *The Journal of antimicrobial chemotherapy,* **67,** 2640-2644.

Zankari, E., R. Allesoe, K. G. Joensen, L. M. Cavaco, O. Lund and F. M. Aarestrup, 2017: PointFinder: a novel web tool for WGS-based detection of antimicrobial resistance associated with chromosomal point mutations in bacterial pathogens. *The Journal of antimicrobial chemotherapy,* **72,** 2764-2768.

Zanoli, L. M., & Spoto, G. (2013). Isothermal amplification methods for the detection of 620 nucleic acids in microfluidic devices. Biosensors, 3(1), 18–43

Zhang, H., M. Miao, J. Yan, M. Wang, Y. W. Tang, B. N. Kreiswirth, X. Zhang, L. Chen and H. Du, 2017: Expression characteristics of the plasmid-borne mcr-1 colistin resistance gene. *Oncotarget,* **8,** 107596-107602.

Zhao, S., J. J. Maurer, S. Hubert, J. F. De Villena, P. F. McDermott, J. Meng, S. Ayers, L. English and D. G. White, 2005: Antimicrobial susceptibility and molecular characterization of avian pathogenic Escherichia coli isolates. *Veterinary microbiology,* **107,** 215-224.

Zhao, S., K. Blickenstaff, S. Bodeis-Jones, S. Gaines, E. Tong and P. McDermott, 2012: Comparison of the prevalences and antimicrobial resistances of Escherichia coli isolates from different retail meats in the United States, 2002 to 2008. *Appl. Environ. Microbiol.,* **78,** 17011707.

Zhu, L., J. Lin, J. Ma, J. E. Cronan and H. Wang, 2010: Triclosan resistance of Pseudomonas aeruginosa PAO1 is due to FabV, a triclosan-resistant enoyl-acyl carrier protein reductase. *Antimicrobial agents and chemotherapy,* **54,** 689-698.

Zurfluh, K., Wang, J., Klumpp, J., Nüesch-Inderbinen, M., Fanning, S., & Stephan, R. (2014). Vertical transmission of highly similar blaCTX-M-1-harbouring IncI1 plasmids in Escherichia coli with different MLST types in the poultry production pyramid. *Frontiers in microbiology*, *5*, 519.

Zurfluh, K., Wang, J., Klumpp, J., Nüesch-Inderbinen, M., Fanning, S., & Stephan, R. (2014). Vertical transmission of highly similar blaCTX-M-1-harbouring IncI1 plasmids in Escherichia coli with different MLST types in the poultry production pyramid. *Frontiers in microbiology*, *5*, 519.

Zurfuh, K., L. Poirel, P. Nordmann, M. Nüesch-Inderbinen, H. Hächler and R. Stephan, 2016: Occurrence of the plasmid-borne mcr-1 colistin resistance gene in extended-spectrum-βlactamase-producing Enterobacteriaceae in river water and imported vegetable samples in Switzerland. *Antimicrobial agents and chemotherapy,* **60,** 2594-2595.

7.0 Appendix

A-1 Papers submitted / published links

1: https://www.frontiersin.org/articles/10.3389/fmicb.2019.03052/full

2:https://innspub.net/ijb/mcr-1-gene-transformationthrough-conjugation-assay-avian-pathogenic-e-coli-pakistan/

3:The Supplementary material for this thesis can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2019.03052/full#supplementary-material

A-2 Summary of all Sequenced Strains

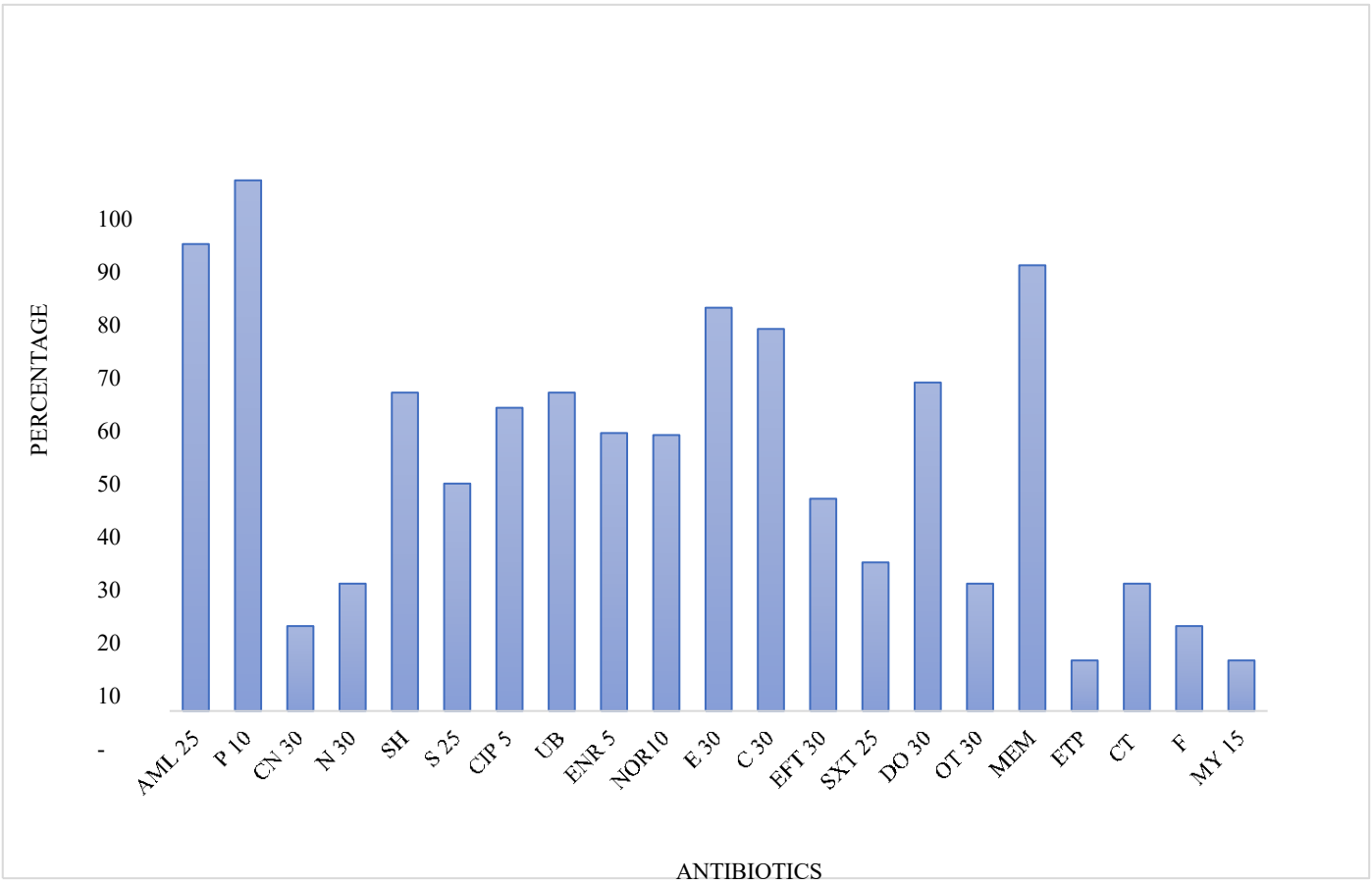
***E. coli* Strains**	**Serotype**	**Clade**	**Region**
EC-51	ST354	F	Punjab
EC_60	ST131	B2	Punjab
EC_83	ST117	B2	Punjab
EC_64	ST95	B2	KPK
EC_35**B**	ST117	B2	Punjab
EC_32	ST117	B2	KPK
EC_40	ST117	B2	Punjab
EC_37	ST117	B2	Islamabad
EC_77	ST117	B2	Islamabad
EC_73	ST117	B2	Sindh
EC_31	ST117	B2	Sindh
EC_101	ST117	B2	Sindh
EC_11	ST1011	E	Islamabad

EC_81	ST1011	E	Islamabad
EC_95	NIL	E	KPK
EC_57	ST350	E	KPK
EC_09	ST350	E	Islamabad
EC_94	ST2847	E	Islamabad
EC_68	ST2847	E	Punjab
EC_55	ST2847	E	Punjab
EC_26	ST2847	E	Islamabad
EC_42	ST4516	A	Punjab
EC_28	Nil	A	Punjab
EC_29	ST710	A	Punjab
EC_36	ST93	A	KPK
EC_08	ST93	A	KPK
EC_47	ST93	A	Punjab
EC_56	ST3856	A	KPK
EC_76	ST6812	A	Sindh
EC_103	ST746	A	Punjab
EC_50	ST746	A	Islamabad
EC_34A	ST1564	A	Islamabad
EC_45	ST46	A	Islamabad
EC_34B	ST48	A	KPK
EC_90	ST48	A	Punjab

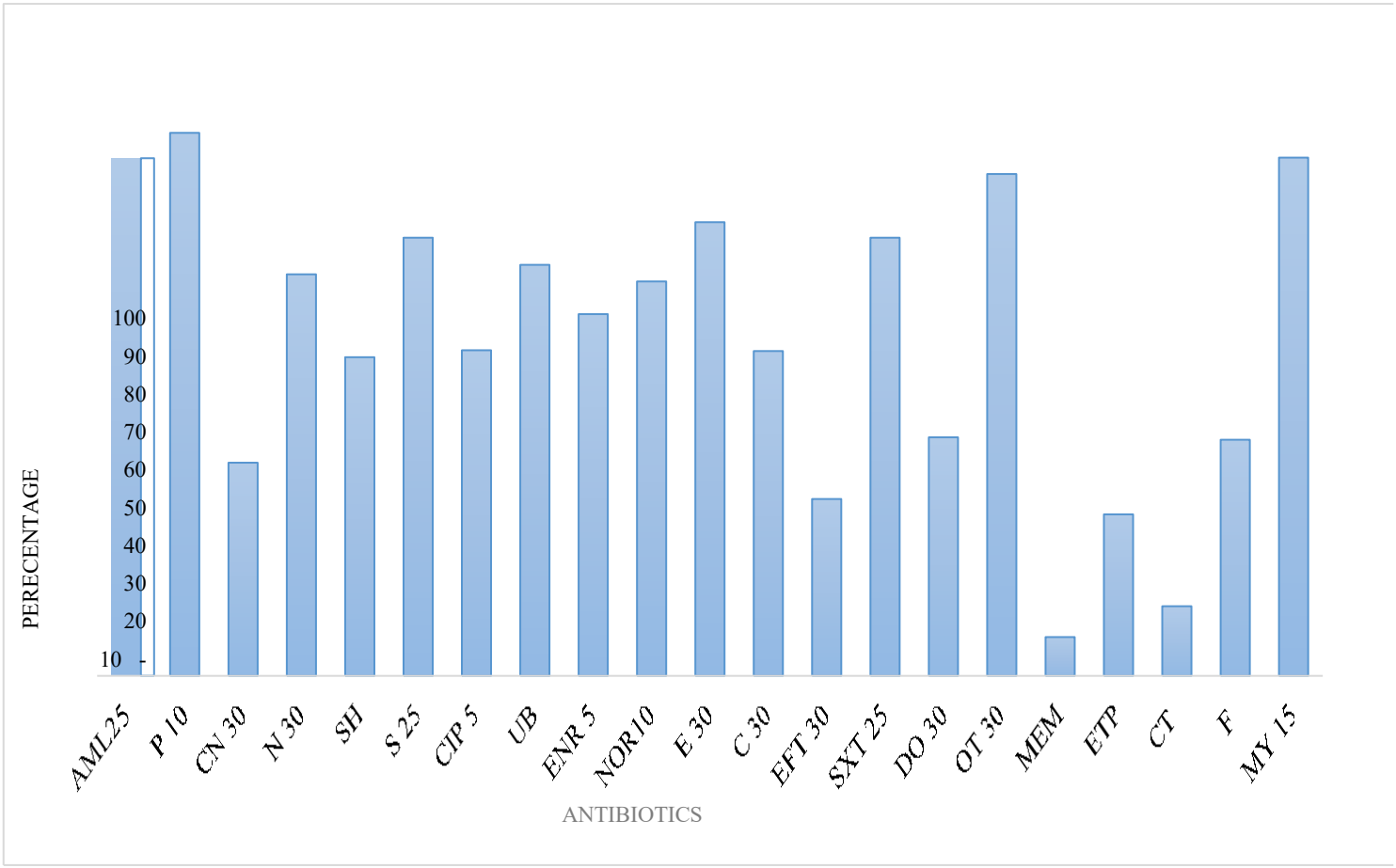
EC_85	ST48	A	Sindh
EC_12	ST7187	A	Punjab
EC_03	ST10	A	Punjab
EC_62	ST10	A	Punjab
EC_65	Nil	A	Islamabad
EC_75	ST10	A	Sindh
EC_13	Nil	A	Punjab
EC_39	ST10	A	Punjab
EC_06	Nil	B1	Punjab
EC_46	ST23	B1	Islamabad
EC_01	ST23	B1	Punjab
EC_61	ST23	B1	Punjab
EC_10	Nil	B1	Punjab
EC_21	Nil	B1	Punjab
EC_54	Nil	B1	Islamabad
EC_27	Nil	B1	Punjab
EC_84	Nil	B1	Punjab
EC_92	ST162	B1	KPK
EC_88	ST453	B1	Punjab
EC_17	ST453	B1	Punjab
EC_33-B	ST2852	B1	Balochistan
EC_38	ST2852	B1	Balochistan

EC_72	ST533	B1	Punjab
EC_98	ST224	B1	Islamabad
EC_22	ST76	B1	Islamabad
EC_100	ST6743	B1	KPK
EC_102	ST7643	B1	Punjab
EC_44	ST156	B1	Punjab
EC_74	ST101	B1	Punjab
EC_63	ST212	B1	Baluchistan
EC_80	ST212	B1	Baluchistan
EC_58	ST101	B1	KPK
EC_26	ST2973	B1	Punjab
EC_49	ST1326	B1	Islamabad
EC_16	ST1326	B1	Punjab
EC_78	ST2973	B1	Punjab
EC_96	ST1326	B1	Baluchistan
EC_23	ST1724	B1	Punjab
EC_104	ST155	B1	KPK
EC_05	ST155	B1	Islamabad
EC_30	ST155	B1	Punjab
EC_43	ST155	B1	Baluchistan
EC_71	ST155	B1	Islamabad
EC_82	ST155	B1	Sindh

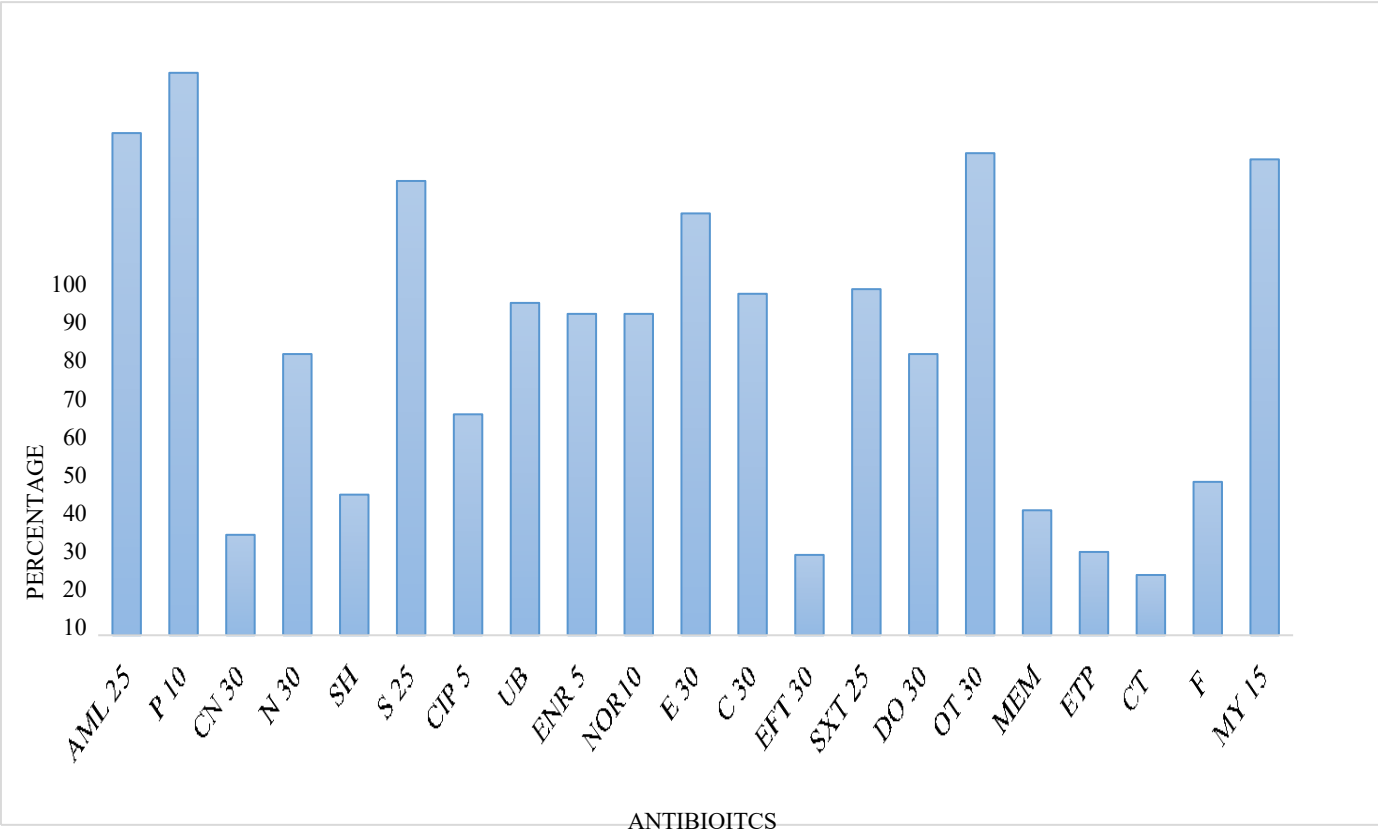
EC_89	ST155	B1	Islamabad
EC_66	ST155	B1	Islamabad
EC_52	Nil	B1	Islamabad
EC_35A	Nil	B1	Islamabad
EC_48	ST155	B1	Punjab
EC_67	ST155	B1	Punjab
EC_59	ST155	B1	Punjab
EC_02	ST155	B1	Islamabad
EC_20	ST155	B1	Punjab
EC_97	Nil	B1	Islamabad
EC_24	ST155	B1	Baluchistan
EC_79	ST155	B1	Baluchistan
EC_07	ST155	B1	Baluchistan



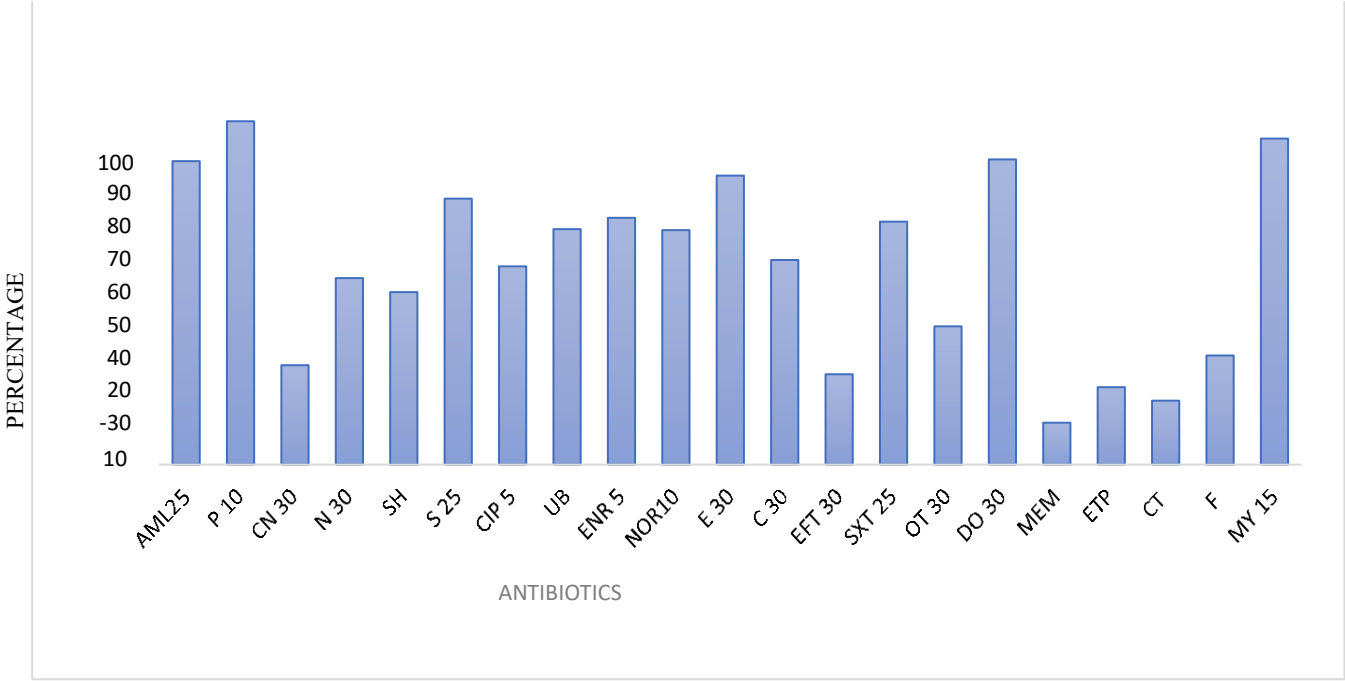
A-3 Prevalence of antibiotic resistant data among *E. coli* isolates from Baluchistan against different antibiotics.



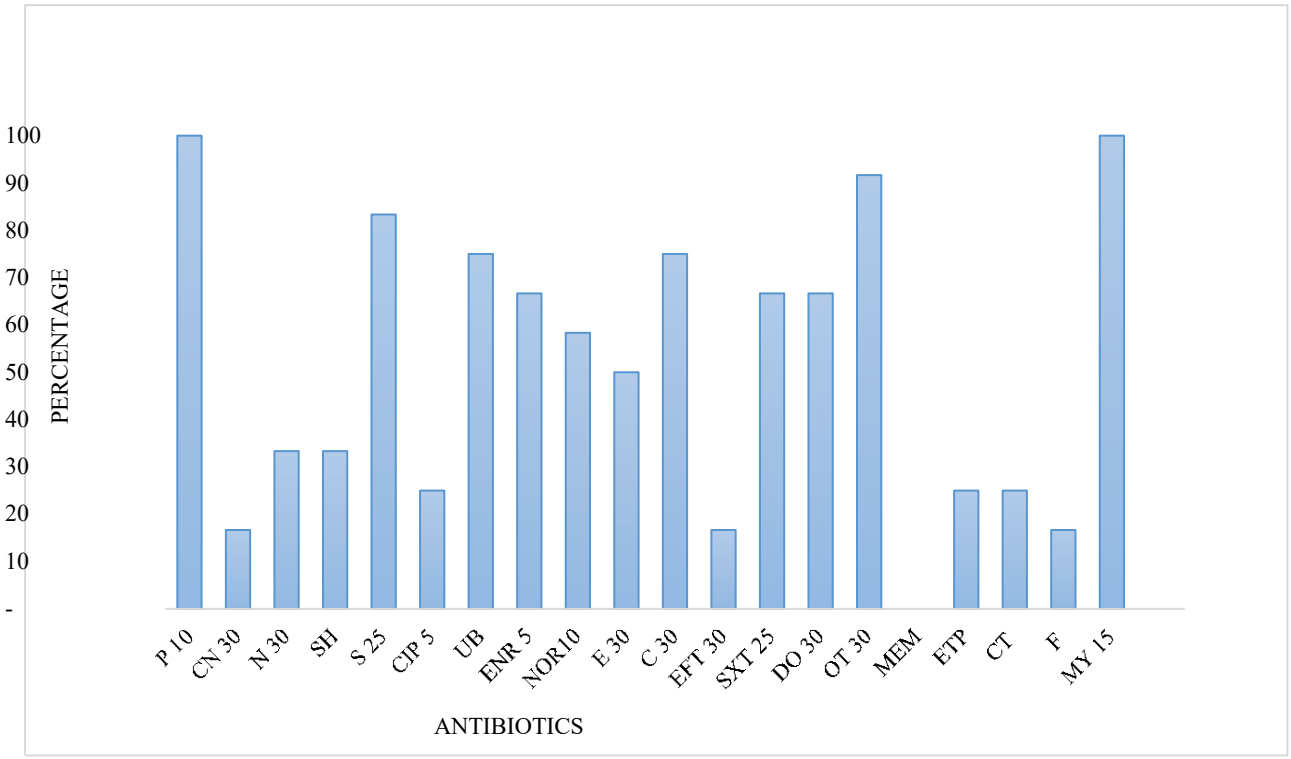
A-4 Prevalence of antibiotic resistant data among *E. coli* isolates from Islamabad against different antibiotics



A-5 Prevalence of antibiotic resistant data among *E. coli* isolates from Khyber-Pakhtunkhwa against different antibiotics.



A-6 Prevalence of antibiotic resistant data among *E. coli* isolates from Punjab against different antibiotics



A-7 Prevalence of antibiotic resistant data among *E. coli* isolates from Sindh against different antibiotics

A-8 Summary of backyard and commercial antibiotic resistance through GraphPad prism

Table Analyzed	Two-way ANOVA				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Row Factor	1.176	0.1420	ns	No	
Column Factor	88.76	<0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Row Factor	359.5	1	359.5	F (1, 20) = 2.337	P=0.1420
Column Factor	27123	20	1356	F (20, 20) = 8.816	P<0.0001
Residual	3077	20	153.8		
Number of missing values	0				

A-9 Summary of Antibiotic resistance among different regions through GraphPad prism

Table Analyzed	Two-way ANOVA				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Row Factor	1.259	0.0254	*	Yes	
Column Factor	90.17	<0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Row Factor	1001	4	250.2	F (4, 80) = 2.94	P=0.0254
Column Factor	71649	20	3582	F (20, 80) = 42.09	P<0.0001
Residual	6809	80	85.11		
Number of missing values	0				

A-10 Whole genome sequences of the isolated *E. coli* strains

Strain	Accession No	links
EC_01	SAMN10926008	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926008
EC_02	SAMN10926093	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926093
EC_03	SAMN10926045	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926045
EC_05	SAMN10926083	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926083
EC_06	SAMN10926098	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926098
EC_07	SAMN10926053	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926053
EC_08	SAMN10926026	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926026
EC_09	SAMN10926019	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926019
EC_10	SAMN10926043	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926043
EC_11	SAMN10926052	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926052
EC_12	SAMN10926054	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926054
EC_13	SAMN10926049	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926049
EC_16	SAMN10926084	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926084
EC_17	SAMN10926033	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926033
EC_20	SAMN10926094	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926094
EC_21	SAMN10926044	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926044
EC_22	SAMN10926067	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926067
EC_23	SAMN10926036	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926036

EC_24	SAMN10926068	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926068)
EC_25	SAMN10926069	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926069
EC_26	SAMN10926070	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926070
EC_27	SAMN10926071	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926071
EC_28	SAMN10926075	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926075
EC_29	SAMN10926029	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926029
EC_30	SAMN10926099	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926099
EC_31	SAMN10926027	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926027
EC_32	SAMN10926013	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926013
EC_33_B	SAMN10926040	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926040
EC_34_A	SAMN10926096	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926096
EC_34_B	SAMN10926097	https://www.ncbi.nlm.nih.gov/biosample/SAMN109260977
EC_35_A	SAMN10926086	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926086
EC_35_B	SAMN10926087	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926087
EC_36	SAMN10926035	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926035
EC_37	SAMN10926063	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926063
EC_38	SAMN10926056	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926056
EC_39	SAMN10926080	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926080
EC_40	SAMN10926051	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926051
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EC_50	SAMN10926065	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926065
EC_51	SAMN10926024	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926024
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EC_54	SAMN10926095	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926095
EC_55	SAMN10926023	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926023
EC_56	SAMN10926077	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926077
EC_57	SAMN10926018	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926018
EC_58	SAMN10926057	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926057
EC_59	SAMN10926072	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926072
EC_60	SAMN10926014	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926014
EC_61	SAMN10926073	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926073
EC_62	SAMN10926090	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926090

EC_63	SAMN10926030	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926030
EC_64	SAMN10926076	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926076
EC_65	SAMN10926092	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926062
EC_66	SAMN10926074	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926074
EC_67	SAMN10926017	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926017
EC_68	SAMN10926028	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926028
EC_72	SAMN10926047	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926047
EC_73	SAMN10926061	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926061
EC_74	SAMN10926022	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926022
EC_75	SAMN10926041	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926041
EC_76	SAMN10926088	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926088
EC_77	SAMN10926048	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926048
EC_78	SAMN10926015	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926015
EC_79	SAMN10926034	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926034
EC_80	SAMN10926055	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926055
EC_81	SAMN10926089	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926076
EC_82	SAMN10926079	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926079
EC_83	SAMN10926025	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926025
EC_84	SAMN10926078	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926078
EC_85	SAMN10926042	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926042

EC_88	SAMN10926011	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926011
EC_89	SAMN10926016	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926016
EC_90	SAMN10926012	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926012
EC_92	SAMN10926031	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926031
EC_94	SAMN10926020	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926020
EC_95	SAMN10926021	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926021
EC_96	SAMN10926089	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926032
EC_97	SAMN10926081	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926081
EC_98	SAMN10926091	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926091
EC_100	SAMN10926085	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926085
EC_101	SAMN10926009	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926009
EC_102	SAMN10926010	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926010
EC_103	SAMN10926060	https://www.ncbi.nlm.nih.gov/biosample/SAMN10926060
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ORIGINAL RESEARCH
published: 17 January 2020
doi: 10.3389/fmicb.2019.03052

Genomic Characterization of Antibiotic Resistant *Escherichia coli* Isolated From Domestic Chickens in Pakistan

OPEN ACCESS

Edited by:
Axel Cloeckaert,
Institut National de la Recherche Agronomique (INRA), France

Muhammad Rafique[1,2†], Robert F. Potter[2†], Aura Ferreiro[2,3], Meghan A. Wallace[4], Abdul Rahim[5], Akbar Ali Malik[5], Naila Siddique[5,6], Muhammad Athar Abbas[5,6], Alaric W. D'Souza[2], Carey-Ann D. Burnham[4,7,8*], Naeem Ali[1*] and Gautam Dantas[2,3,4,7*]

[1] Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan, [2] The Edison Family Center for Genome Sciences & Systems Biology, Washington University School of Medicine, St. Louis, MO, United States, [3] Department

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