Multidrug Resistant Genome Analysis of *E.coli* isolated from Avian Species

BY

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AUTHOR'S DECLARATION

I would like to declare that the data presented in this thesis "**Multidrug Resistant Genome Analysis of *E.coli* isolated from Avian Species**" is generated myself from original research work under the supervisionof **Dr. Muhammad Athar Abbas** at Department of Animal Genomics and Biotechnology (AGB), PARC Institute of Advanced Studies in Agriculture (PIASA), National Agriculture Research Centre (NARC), Quaid-I-Azam University Islamabad, Pakistan. The results and material used in this thesisneverpresentedanywhereelse earlier.

Syeda Laraib Fatima Bukhari

Dated:

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Iamgratefulto**ALLAHAlmighty**and**HOLYPROPHETHAZRATMUHAMMAD**ﷺ**. ALLAH** Almighty the most merciful and beneficentand **Prophet**ﷺis the torch bearer of knowledge for humanity. The wholefacilities,abilities,opportunities,strengthandpowersareblessedby**ALLAH**almightyforthe levelofstudyand research to suchavibrant environment.

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Syeda Laraib Fatima Bukhari

Dedication to

HOLYPROPHET

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TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION

MDR	Multi Drug Resistance
AMR	Antimicrobial Resistance
GDP	Gross Domestic Product
PW	Buffer Peptone Water
MA	MacConkey Agar
EMB	Eosin Methylene Blue
ONPG	Clinical and Laboratory Standards Institute
MHA	Muller Hinton Agar
NRLPD	National Reference Laboratory for Poultry Disease
PCR	Polymerase Chain Reaction
TBE	Tris Borate EDTA Buffer
NA	Nutrient Agar
AST	Antibiotic Susceptibility Test
CLSI	Clinical and Laboratory Standards Institute
TSI	Triple Sugar Iron
WHONET	World Health Organization software
µg	Microgram
bla	Gene encoding β-lactamase
aac(6′)- Ib	Aminoglycoside 6′-N-acetyl transferase type Ib
µl	Microliter
FAO	Food and Agriculture Organization

ABSTRACT

Escherichia coli are commensal adherent of the gastrointestinal tract of both human and animals. Although most *E. coli* strains are harmless, however, some strains are pathogenic for both human and animals as well. Pathogenic *E. coli* are usually categorized as intestinal pathogenic or extra intestinal pathogenic *E. coli*. The pathogenic group is further divided into further sub-pathotypes, e.g., enterohemorrhagic*E. coli* (EHEC), enterotoxigenic*E. coli* (ETEC), entero-aggregative *E. coli* (EAEC) and enteropathogenic*E. coli* (EPEC). Multidrug resistant (MDR) bacteria, usually described as those impervious to three or more antibiotic classes, especially are of great concern because MDR *E. coli* tends to harbor numerous resistance genes and shifts its resistance determinants to other strains, species or genera. The current study was designed to evaluate AMR in *E. coli* isolated from healthy chicken. A total 785 samples wereobtained from which 621 *E. coli* were isolated. About 221 (35%) isolates were subjected to Antimicrobial Susceptibility Tests using CLSI protocols. Phenotypically 100% resistant to Penicillin was observed in tested *E. coli* isolates. More than 50% resistance in tested *E. coli* isolates was observed against 18 (58%) out of 31 antibiotics analyzed by AST and these include Ampicillin, Ampicillin-Sulbactam, Azithromycin, Chloramphenicol, Ciprofloxacin, Clindamycin, Doxycycline, Enrofloxacin, Erythromycin, Florfenicol, Linezolid, Nalidixic acid, Penicillin, Quinupritin-Dalfopristin, Streptomycin, Co-trimoxazole, Tetracycline, and Teicoplanin. Highest sensitivity was observed against Pepracilline/Tazobactam, Amikacin and Meropenem antibiotics showing sensitivity in 88%, 80% and 79% respectively. Based on the phenotypic AMR pattern, 24 isolates were subjected to genotypic characterization against 20 AMR genes of 4 antibiotic classes which include B-lactams, Aminoglycosides, Tetracyclines and Polypeptides (Colistin). Among Beta-lactamase *blaTEM* was found in 22 (92%) isolates, while *blaCTX*-M was found in 4 (17%) isolates. The beta-lactamase producing *blaOXA* and *bla*FOX, were found in 2 (8%) isolates while *blaNDM*-1, *blaSHV*-1, weredetected in 1 (4%) isolate. Among Tetracycline tetA showed highest prevalence with 92% and detected in 22 isolates while tetM was detected in 2 (8%) isolates. The prevalence of aac(6')-Ib was found to be 46% as it was detected in 11 isolates while of aac(3)-II, aph(3)-II was 8% each with detection in 2 isolates. For Colistin resistance producing genes, mcr 1 to mcr 9 were tested and only mcr-9, mcr-5 and mcr-2 were detected

with mcr-9 was found in 12 (50%), mcr-5 detected in 6 (25%) and mcr-2 was detected in 3 (13%) isolates, however, mcr-1 reported previously was not detected in any of the isolates tested. On the basis of these results, it could be concluded that the chickens sold in retail sectors of Pakistan are harboring significant population of multi-drug resistant *E. coli*. The presence of MDR *E. coli* may be resulted by the persistent exposure of birds to multiple antibiotics during the rearing period. To relieve this issue, the public health authority ought to control non-judicial utilization of antibiotics at poultry production and stringent measure need to be adopted for future control of AMR issue in the country as well as globally. Further a surveillance network for detection of AMR in healthy poultry and other food animals need to be established on sustainable basis. While data collection and analysis needs to be carried out at larger scale for exact depiction of the AMR situation in the country. The control measures may be devised according to the situation of AMR in the country.

Key words:*E. coli*, antimicrobial resistance, isolation and identification, phenotypic antimicrobial resistance profile, genotypic antimicrobial resistance profile.

1. INTRODUCTION

The poultry industry has gained significance all over the world. According to Food and Agriculture Organization of United Nations (FAO) 103.5 million tons of annual global chicken meat production has been estimated which contributed more than 30% to global meat production (Pawar et al., 2016). In food, poultry meat and eggs are the most proficient protein sources (Sebho, 2016). A poultry endeavor produces meat in about one and half months in most of the world, and eggs in 24 weeks. Antibiotics are being used regularly in commercial poultry production as growth promoters, however, these are also utilized as preventive as well as curative agent against various infectious diseases. Furthermore, due to the use of high concentrations of antibiotic agents, poultry meat may exhibit high saturation of antibiotic deposits, which may lead to selection of multidrug resistant pathogens through promotion of AMR genes (Donoghue, 2003).

Escherichia coli (*E. coli*) is a Gram-negative, facultative anaerobic bacterium of the Enterobacteriaceae family. *E. coli* is a commensal individual from the gastrointestinal lining of people and other warm-blooded animals. It has been widely used to screen AMR in healthy food animals (Nhung, Chansiripornchai and Carrique-Mas, 2017). Moreover, some *E. coli* strains facilitated by poultry are thought to be a possible origin of AMR genes transmitted to people and thus may be the cause of increased AMR transmission to human (Overdevest *et al.*, 2011). *Escherichia coli* is one of the most-deliberately studied microorganism worldwide because of *E. coli* its behavior of continuously changing characteristics (Vila *et al.*, 2016)

Escherichia coli (*E. coli*), is a diverse bacterial variety reported with intestinal commensal lines to intestinal pathogenic, and afterwards extra-intestinal pathogenic lines causing urinary lining contamination, sepsis and meningitis (Levings *et al.*, 2005). *E. coli* is a main source of the environmental as well as nosocomially derived disease (Liang *et al.*, 2018). *E. coli*, just as Enterococcus spp., has for some time been utilized as a marker of fecal infection to evaluate the microbial nature of surface waters (Gomi *et al.*, 2017).

From the clinical point of view *E. coli* is divided into two categories commensal strains and pathogenic strains of *E. coli*. Commensal strains harmlessly colonize the intestine of sound mammals, however, sometimes triggering extra intestinal sickness within the sight of a huge inoculum or immune-compromised situation of the host. The pathogenic group is further divided into two other subgroups: diarrhea genic *E. coli* (DEC) and extra intestinal pathogenic *E. coli* termed as ExPEC (Huma *et al*., 2021). ExPEC are ordered into six subgroups: mammary pathogenic *E. coli* (MPEC), sepsis/newborn meningitis associated *E. coli* (NMEC), uropathogenic *E. coli* (UPEC), avian pathogenic *E. coli* (APEC), sepsis-associated pathogenic *E. coli* (SePEC) and endometrial pathogenic *E. coli* (EnPEC) (Latif *et al*., 2019). The DEC strains are additionally ordered into different pathotypes relying upon their harmful attributes. There are six sub-grouping of DEC, including Shiga Toxin-forming *E. coli* (STEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC), Enterotoxigenic *E. coli* (ETEC), and Diffusely Adherent *E. coli* (DAEC). Pathogenesis of DEC pathotypes relies on the presence and articulation of destructive genes (Gomes *et al*., 2016). A typical sensation of STEC pathogenesis brings about gastroenteritis and Hemolytic Uremic Syndrome eventually cause kidney malfunctioning in newborn mammals (Bielaszewska *et al*., 2007). Universally, EPEC attacks little youngsters by their attachment and subsequently destroying gastrointestinal cells surfaces due to the different enzymes and toxins. EPEC are additionally ordered into two subgroups: typical EPEC (tEPEC) and atypical EPEC (aEPEC). This subgrouping is based on the occurrence of bond and cluster determining pili (bfp) genes. The tEPEC are all perceived in developing nations while aEPEC are distributed around the globe (Ochoa *et al*., 2008). Pathogenesis of ETEC relies on the presence of heat stable (ST) and heat labile (LT) toxins. The grip proteins of EIEC help them to adhere with gastrointestinal cells which usually produce bacillary diarrhea in people, explicitly more normal in agricultural nations (Gomes *et al*., 2016; Lan *et al*., 2004).The EAEC through its impressive harming impacts on digestive epithelium is the main cause of watery diarrhea (Jensen *et al*., 2014).

E. coli is broadly acknowledged as an indicator of fecal contamination. It is effectively graspable and its essence in food, water and different sources is demonstrative of fecal contamination (Antony *et al*., 2016). The concentration of fecal

coliforms or *E. coli* is generally acknowledged microbiological quality boundary, deciding the sterile nature of food products implied for domestic and commercial purposes. (Manual, B. A. 2011). Significant types of *E. coli* are observed and reported in the lower gut of mammals and avians, sometimes reported as causative agent for gastroenteritis (Vet, 2008).Notwithstanding most *E. coli* strains are inoffensive, a few strains are pathogenic and cause increased danger of waterborne infections (Ishii and Sadowsky, 2008).

ExPEC have been isolated from humans, food animals, retail meat items and there is a critical assortment of incidental proof that a subset of ExPEC are zoonotic microorganisms (Vincent *et al.*, 2010; Nordstrom, Liu and Price, 2013; Manges, 2016).The harmfulness ability of such strains is controlled by combinations of different particular accessory traits, called as virulence factors, (Vila *et al.*, 2016)APECare essential causative agents of cellulitis, septicemia, and air sacculitis in poultry (History, 2014).

Commensal *E. coli* are known to be essential for the ordinary flora of the gastrointestinal track of human and animals without making any damage to their host (Delmani *et al.*, 2017; Sarowska *et al.*, 2019). A few *E. coli* strains have been utilized as marker in different investigations on AMR (Szmolka and Nagy, 2013; Delmani *et al.*, 2017). Though commensal *E. coli* are necessary microflora of the host organisms, however, the microscopic organisms can obtain drug resistant genes and work as a store for the spread of multidrug resistance (MDR) across the numerous host species through the food chain contamination. The hereditary structure of *E. coli* strains is generally enraged by a few components including the host and climate empowering the microbes to gain different AMR mechanisms (Tenaillon *et al.*, 2010; Szmolka and Nagy, 2013; AWORH *et al.*, 2020). Drug resistance in *E. coli* is reliably most elevated for antimicrobial agents that have been used the longest time in human and veterinary medication, like ampicillin. Nonetheless, in the recent twenty years sudden rise and spread of MDR microbes has been observed, counting strains impervious to more current antimicrobials, for example, fluoroquinolones and extended range of cephalosporin.

Certain *E. coli* strains, assigned as "avian pathogenic *E. coli*" (APEC) are the main cause of colibacillosis, one of the main cause of mortality in poultry around the world (Lutful Kabir, 2010). For quite a while APEC strains were viewed as just ingenious microbes and identified as members of various serogroups including O1, O2, O8, O78 and a few other serogroups as well (La Ragione and Woodward, 2002). It has been shown that sickness due to *E. coli* strains encode numerous presumed harmful genes and altogether vary from commensals. However, some strains carrying the ColV plasmid-related genes, which are viewed as indicators of poultry-modified pathogenic strains (Johnson *et al.*, 2008; Collingwood *et al.*, 2014; Stromberg *et al.*, 2017). Notwithstanding, few strains detached from infected chicken are detected with harm related genes that emphasis particular character of certain sorts of *E. coli* infection (Collingwood *et al.*, 2014). Abrasions related with colibacillosis in poultry basically comprise of airsacculitis, peritonitis, polyserositis and septicemia. Colibacillosis is typically viewed as an optional sickness, following contamination with respiratory microbes and ends with horrible ecological conditions(Vandekerchove *et al.*, 2010). APEC-like strains (conveying APEC associated characteristics) can be discovered additionally in the gut of healthy chicken (Kemmett *et al.*, 2013). It has been proposed that the APEC population makes out of definite sub pathotypes related with various stress conditions (Maturana, 2011), like the human extra-intestinal pathogenic *E. coli* (ExPEC).(Papouskova *et al.*, 2020). disease related to *E. coli*, represented around50% of layer rush mortalities during year 2012(Olsen *et al.*, 2012). Omphalitis as well as yolk sac contaminations, with or without septicemia are also observed. (Mokady, Gophna and Ron, 2005; Vandekerchove *et al.*, 2010). In various parts of the world, multi drug resistance strains of *E. coli* are omnipresent in both humans and other mammals, commensal or Pathogenic both most likely have a significant amount of resistant genes (Österblad *et al.*, 2000)

Antimicrobial resistance (AMR)is the capacity of microorganisms to proliferate within the sight of a medication that would typically cause death or decline in the growth of microorganisms(World Health Organization (WHO), 2014). AMR muddles the treatment of infections and is related with expanded morbidity and mortality. Antimicrobial obstruction isn't new, yet the quantity of resistant entities, the geographic areas influenced by drug opposition, and the extensiveness of obstruction

in organisms are unparallel and mounting (Levy, 2003).Diseases and sickness causing agents that were thought to be constrained by anti-microbials are habitual in new classes and have become impervious and more offensive to these treatments. Drug-resistant strains were found where antimicrobial agents were frequently used.(Levy, 2018). Resistance from numerous medications was first observed among enteric microscopic organisms specifically, *E. coli, Shigella* and *Salmonella*in the last part of the 1950s to mid-1960s. Such strains presented serious medical issues and can cause death of the infected person, especially in non-industrial nations(Levy and Bonnie, 2004).

Expanding obstruction of microbes to anti-microbial agents represents a significant issue for both human and veterinary medication, alluded as a worldwide threat. Microbial resistance from the impact of anti-microbials, comes from various causes joined with one another, making the scenario considerably riskier. The main elements adding to the advancement of bacterial obstructions is the selection pressure of antibiotics, recombination events prompt a trade of the hereditary material, and the horizontal distribution of genetically indistinguishable strains of a specific species (Sedláková *et al.*, 2014). This additionally happens in the living population specially humans, an expected source of resistant microorganisms might be taken up by animals or their items entering the human diet, for instance poultry. These antibiotics might be shown particularly in those animals that are regularly given water or feed containing antimicrobial specialties. This classification incorporates poultry. There are different ways of transmission of resistant microorganisms among mammals. The antibiotic resistant genes replicas are transmitted by means of the evolved way of life and direct or indirect contacts of individuals intently working with animals, for example, ranchers, farmers or veterinarians. Significant roles are likewise played by the climate and the water contaminated with excreta which are potential reservoirs of resistance genes(Press, 2015). The transmission of multi resistant strains from food animals to people is supposed to be basically connected with Gram-negative microbes delivering wide range beta-lactamases (Bardoň *et al.*, 2018).

The review of bacterial isolates that have contagious resistance from carbapenems and colistin holds the significant general wellbeing danger and they are considered as the last line of antibacterial agents for MDR Gram-negative bacterial

diseases (Liu *et al.*, 2016). AMR in humans is influenced by bacterial and naturally existing factors, including presentation of antimicrobials for clinical medication, ecological waste and pollution, food animals and creature husbandry (Armoni, Barbón and Petkou, 2002; Singer and Williams-Nguyen, 2014; Holmes *et al.*, 2016). The upgrading of AMR is essentially because of particular stress on microorganisms – due to the introduction to antimicrobials. There are a few components by which microorganisms adjust themselves and get impervious to antimicrobials; these integrate the formation of compounds, change of target sites, modification of metabolic pathways, change of external wall penetrability and efflux (Blair *et al.*, 2015). Hereditary variation is fundamental for microbial development and may emerge by an assortment of hereditary material including point changes, modifications of huge portions of DNA from one area of a plasmid or chromosome to another, or procurement of unfamiliar DNA from different microscopic organisms by horizontal exchange of versatile hereditary components. A slight change that presents AMR in a bacterium in a particular specie under selection pressure can empower endurance of that specific antibiotic gene form which remaining microbes are killed(Walsh, 2000). The resistant microbes can keep on reproducing, turning into the prevailing variation (Walsh, 2000).There is a perplexing interchange between people, animals and the climate comparable to the turn of events and spread of AMR (Holmes et al., 2016: Armoni, Barbón and Petkou, 2002). There have been numerous examinations investigating the relationship between antimicrobial use in animals and obstruction in people, including immediate and backhanded courses of transmission (Awasthi *et al.*, 2019)

Antimicrobial opposition (AMR) in zoonotic microbes adds to this danger (S. B. Levy & Bonnie, 2004; Varga *et al.,* 2019). It has become a fact that contaminations with antimicrobial resistant microscopic organisms are more hard to treat, and bring about higher bleakness and mortality(Caniça *et al.*, 2019). Improper antimicrobial use has been demonstrated to be one of the primary causes for the improvement of poultry production that has led to AMR in commensal and pathogenic microorganisms of poultry. Presentation to a specific antibiotic may make the microbes create resistance from different antibiotics. If resistant genes are situated on mobile hereditary components they might get transmitted horizontally and vertically. Moreover, these procured resistant genes may have the ability to continue even after the antibiotic

determination pressure closes and stress conditions for bacteria are reduced (Levy and Bonnie, 2004; Varga *et al.*, 2019)

Around 65 years prior, from when antibacterial agents turned out to be broadly accessible, they have been acclaimed as marvel drugs, skilled to annihilate illness triggering microorganisms. In any case, with each passing decade, microscopic organisms that used to resist single, however nowadays can oppose various antibiotic agents and have ability to cause more persistent and problematic disease to be controlled. (History, 2014) The danger of transfer of obstruction genes additionally rises due the high growth of resistant microscopic organisms(Davies, J., 1994). Normal micro flora especially *E. coli* takes approximately about 5 to 7 days for most of the drugs to get resistance. Review of studies shows that following 1 day of treatment cotrimoxazole, opposition levels significantly expands, yet ampicillin, doxycycline and cotrimoxazole obstruction levels showed significant increment after treatment for 2–7 days(Raum *et al.*, 2008).Quinolone resistance was also observed in intestinal *E. coli* derived from retail shops(Johnson *et al.*, 2005). Furthermore, Petersen et al.(2006) exhibited the more likely vertical transmission of fluoroquinolone resistance in *E. coli*(Petersen *et al.*, 2006).

Information on the degree of the carriage of drug resistant genes is probably going to be belittled on the grounds that many investigations confine phenotypic screening to antibiotic agents that are of clinical relevance for contamination control.(McKinnon, Roy Chowdhury and Djordjevic, 2018).

Drug resistant *E. coli* of healthy animal source may accommodate the human digestive system through a process called as zoonosis. Zoonosis can be characterized as illness or contamination normally contagious from animals to people and the other way around. (Maudoux et al., 2006). Verocytotoxigenic *Escherichia coli* is well known for an occurrence of food born zoonoses. Moreover, in evaluating the zoonotic danger of poultry items, it is crucial to consider that ExPEC confines are genotypically heterogeneous. They share genomic likenesses with commensal *E. coli* Nonpathogenic *E. coli* genotypic investigation alone is consequently not enough to authoritatively separate the different pathotypes, though ongoing proof shows that the assessment of phylotypes and commensal E. *coli* genotypes assures the separation of

ExPEC from commensal *E. coli*. Albeit sharing a bunch of genes that are normal to all *E. coli*, i.e., the *E. coli* central genome, these pathotypes vary according to the presence or non-existence of different accessory traits, which are nonessential for vegetative development yet decide the strains and its clinical behavior (Touchon et al., 2009)

Pathogenic *E. coli* comprises a significant danger to general physical condition, and the rise of expanded range Beta lactamase (ESBL) - producing *E. coli* with high destructive potential is alarming. Similar genome holds significant potential in understanding the genomics of such microbes and in this way recognizing and organizing microbes in different classification is applicable in the improvement of mediation procedures (Awasthi *et al.*, 2019)

Aims and Objectives:

Main objectives of current investigation are:

- To isolate and identify *E. coli* in apparently healthy chicken from poultry retail shops in selected areas of Pakistan.
- To evaluate phenotypic and genotypic MDR profiling and its comparative analysis in selected *E. coli* isolates.

2. MATERIALS AND METHODS

2.1 Sample collection

Through considerations were given to the stage of collection, control, and storage of the samples, involving biosafety gauges that are set up to forestall defilement of the climate or disclosure of animals and people to possibly irresistible infectious materials according to the OIE guidelines. Sterile strategies were applied for sample collection and samples are kept in refrigeration and sterile containers to evade contamination.

Caecum from freshly slaughtered broilers were taken in such a way that each retail shop was represented as a single sampling unit of chickens brought together up from one shed, and having encountered a similar antimicrobial disclosure. Samples were arbitrarily or efficiently taken from apparently healthy chicken. It was imperative to ensure that the caecum was intact. Pooling of samples from the same shop was also done and that sample was considered as one sample.The samples were kept in sampling boxes at refrigeration consistently supplied with ice packs from the place of collection until arrived at NRLPD within 24 hours after collection. The caeca contents if not pooled at the place of sample collection, were checked for the integrity of samples. The caeca contents were pooled in a sterile tube and mixed well using sterile swabs and kept at refrigeration (4^{o}C) until further evaluation or analysis.

2.2 Isolation and Identification of E. coli

Isolation of E. coli

Isolation of *E. coli* was carried out using the standard protocols (FAO, 2019), flow chart of brief protocols is given in Figure 1. Briefly pre-enrichment of the samples was done in pre-sterilized Buffered Peptone Water (Oxoid CM0009) prepared according to the manufacturer's instruction (Appendix-I). For pre purpose, caeca contents were inoculated in ratio of 1:9 in BPW and incubated aerobically at 37°C ± 1°C for 18 h to 22 h.

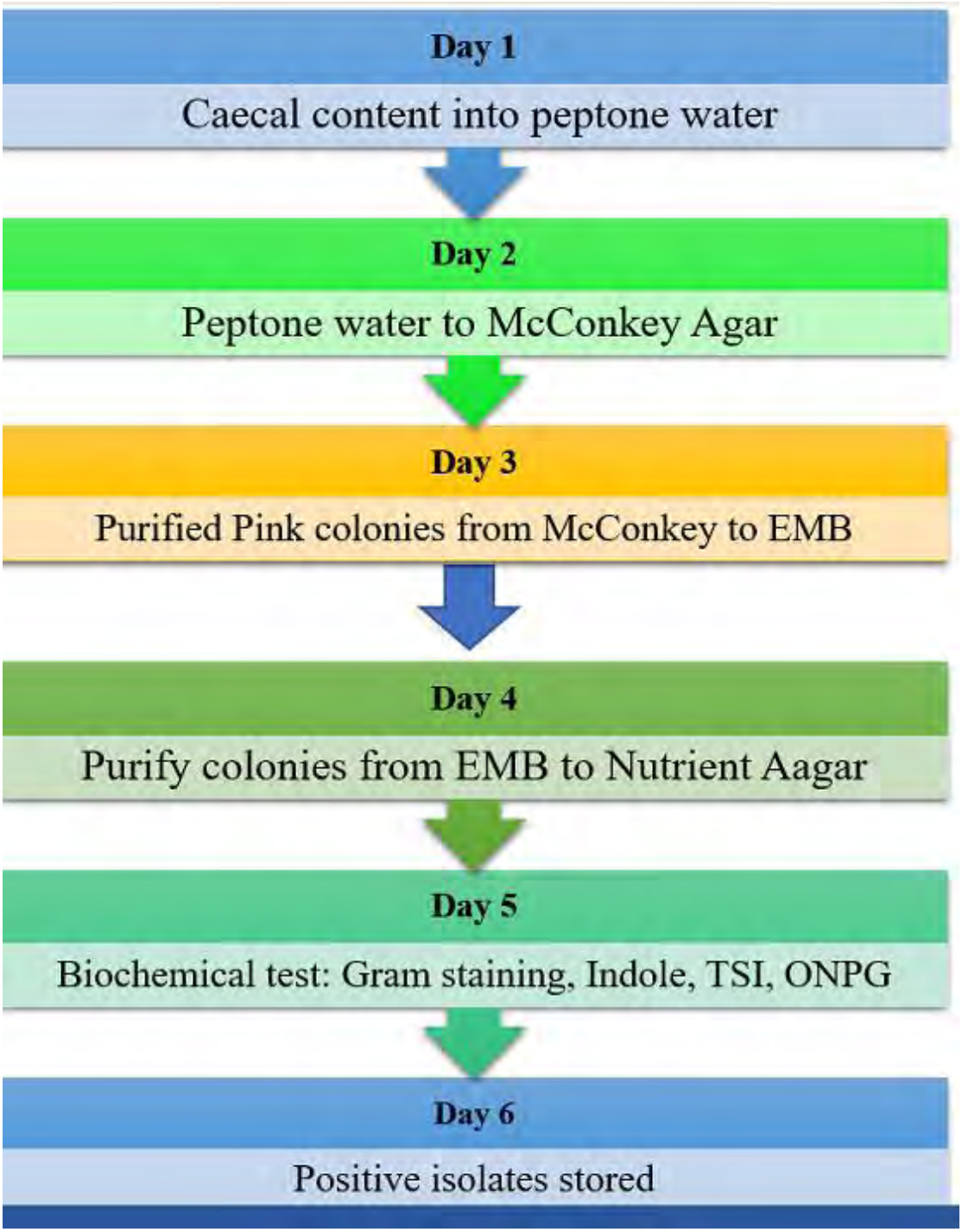

Figure 1: Flow chart for *E. coli* identification according to FAO protocols from Caeca Samples

One loop full of the overnight culture of BPW was applied by quadrate streaking method onto a sterile MacConkey (OXOID Cat # CM007) agar plate, prepared according to the manufacturer's instructions (Appendix II). After overnight incubation 2-3 pink colonies suspected to be *E. coli* were selected and further streaked using quadrate streaking technique on to sterile Eosin Methylene Blue (EMB) agar (OXOID Cat # CM0069) prepared according to the manufacturer's instructions (Appendix-III) and incubated at 37ºC overnight. The cultured colonies which gave green metallic sheen color on EMB after overnight incubation, were selected for confirmation using biochemical tests. The corresponding colonies (2-3) were again restreaked on to MA for confirmation purpose and a single pink colony was picked up and streaked on to sterile Nutrient Agar (NA) plates (Oxoid Cat# CM0003B) prepared according to manufacturer's protocols(Appendix-IV) and incubated at 37ºC overnight. The pure cultures obtained were stored at refrigeration until further use.

Identification of E. coli using Gram staining and Biochemical tests

The pure cultures obtained at NA were subjected to different biochemical tests for identification and confirmation of *E. coli*. For this purpose single suspected colony from either NA or MA was streaked on to sterile NA plates and incubated at 37ºC for 18-24 hours using quadrate streak method. Overnight pure cultures obtained this way were subjected to gram staining, indole, Triple sugar iron and ONPG tests

Gram staining

Gram staining was done according to previously describe methods (Beveridge & Beveridge, 2009) using Gram's Staining Solution set (Liofilchem, Italy Cat # 80293). For this purpose, a suspension of single microbial colony was made on a glass slide and parched by delicate warming to obtain a heat fixed smear. Multiple drops of crystal violet were added to the heat fixed smear and permitted to stand on the slide for 30 seconds, followed by rinsing with tap water to get excessive stain cleared from the slide. Gram's iodine solution was flooded on the smear for 30 seconds and washing to remove excessive stains from the slide was done by rinsing under the tap water gently. This was followed by decolorizing agent only for a few seconds and rinsed it with tap water. As a final step of Gram staining Safranin

solution was added on the slide for 60 seconds. The slide was rinsed with tap water and allowed to dry after removing excess of water with a blotting paper by gentle tapping. The stained smear was visualized using cedar wood oil under the oil immersion lens of microscope making sure that no air bubbles in oil were present at the slide.

Indole Test

The minimum requirement for *E. coli* confirmation was to test for indole production for verification of the species. Tryptone water (Oxoid Cat# CM 0087) was prepared according to manufacturer's instructions (Appendix-V) boiled and dispensed 10 ml in each of 25ml tube and autoclaved at 121ºC and 15psi for 15 minutes, sterility of the tryptone water containing tubes was checked by incubating aerobically at 37°C ± 1°C for 18-22 hours. The sterile media confirmed after sterility check was stored at refrigeration until use. Refrigerated bottles were brought to normal room temperature before using. Freshly overnight incubated pure culture was used for inoculation and 4-5 colonies were inoculated in 10 ml of sterile tryptone water and then incubated aerobically at 37°C ± 1°C for 18-22h (Macwilliams, 2016). After the accomplishment of incubation 3-4 drops of kovacs reagent (Oxoid Cat # MB0209A) was added. Formation of pink ring on the top of incubated media was considered as positive for *E. coli* presence.

Triple Sugar Iron Agar Test

Triple sugar iron (TSI) agar (Oxoid Cat # CM 0277) was prepared according to the manufacturer's instructions (Appendix-VI). Autoclaved media (15ml) was dispensed in 25ml test tube each and allowed it to solidify by placing those tubes slightly inclined on the surface by the pivoting them on stands to give it butt and slant. After solidification sterility was checked for each tube to ensure no contamination by overnight aerobic incubation at 37°C. The sterile media tubes were stored at 4ºC until use. The refrigerated sterile media was brought to room temperature before use. Freshly overnight incubate pure culture on non-selective medium was used for inoculation on to TSI slants. The slant of TSI was inoculated by straight wire loop with a suspected colony using stabbing method. Inoculated slants of TSI were

incubated at 37ºC for 18-22 hours aerobically. After incubation media with culture growth showing yellow slants and yellow butt was considered positive for presence of *E. coli*.

Orthonitrophenyl-β-D-galact opyranoside test

The orthonitrophenyl-β-D-galact opyranoside (ONPG) test was additionally used to verify apparently positive *E. coli* isolates. ONPG base (BIOLab® Cat# EONB 20500) was prepared according to the manufacturer's instructions (Appendix-VII). The prepared 2 ml ONPG media was dispensed per 5ml test tube and subjected for sterility test by overnight incubation. The sterile media was stored at refrigeration until use. The refrigerated media was brought to normal room temperature before use and 5-10 colonies were inoculated as described earlier (Boadi *et al.,* n.d.; Frampton & Restaino, 1993). Inoculated ONPG was incubated aerobically for 18-24 hours at 37°C ± 1°C. After incubation the inoculated ONPG that turned its color to yellow was considered positive for presence of *E. coli*.

2.3 Antimicrobial Susceptibility Testing (AST)

The antibiotic susceptibility test (AST) was performed according to Clinical and Laboratory Standards Institute (CLSI) as previous described(P. Weinstein, 2020).

Mueller-Hinton agar (OXOID Cat # CM0337) was prepared according to the manufacturer's guidelines (Appendix-VIII). The autoclaved medium was brought to 50°C and 20ml of medium was poured to each of 90mm pre-sterilized plastic petri plates. The medium was allowed to cool down to solidify and then sterility was checked by overnight incubation at 37°C ± 1°C. The sterile plates with no contamination were stored at refrigeration (4-8°C) after marking the date of preparation until use (Lalitha, 2007). The inoculum was prepared by making direct suspension of segregated pure culture colonies from non-selective medium in presterilized distilled water in accordance with the 0.5 McFarland turbidity standards. The inoculums thus prepared were used within 10-15 minutes after preparation. For inoculation on to the sterile MH agar plates the inoculum was spread by the help of sterile swabs to make a lawn of the inoculums on to the petri plates (Lalitha, 2007). A panel of 31 antibiotics from different classes were selected for application and tested

against the pure cultures of *E. coli* recovered from the healthy chicken. The list of antibiotics, their corresponding potency and cat No. are given in table No. 1. The inoculated plates after application of antibiotic discs were then incubated at 35ºC ± 1°C for 18-24 hours. The corresponding diameter of zone of inhibition where produced were recorded with AST rular and recorded for further analysis.

Following 18 hours at 35°C, each plate was inspected. Zones of inhibition were measured utilizing vernier calipers or AST roller. For the purpose of measuring AST the Petri plates were held against a dark, nonreflecting background and enlightened with light. Interpretations were made according to CLSI 2020 using WHONET software that were given in table-1. For the purpose of internal quality assurance, standard ATC strains (ATCC 25922 *E. coli*, 25923 *S. aureus* and 27953 *P. aeruginosa*) were also used for AST analysis.

2.4 Genotypic Characterization of Selected Isolates against AMR Producing Genes

The 221 isolates which were subjected to antimicrobial susceptibility test were analyzed used CLSI through WHONET. Highly resistant and highly sensitive isolates were selected for genotypic characterization on the basis of date of sample collection and area/region of sampling. From each city/region at least 3 isolates were selected for genotypic characterization. A total 24 isolates were subjected for AMR genotypic characterization.

DNA extraction:

Colonies from freshly cultured Nutrient Agar were dissolved in 5ml distilled water and subjected to two methods of DNA extraction.

Boiling method for whole DNA

For extraction of DNA, 200µLsample suspension was taken in a 1.5ml micro tube and boiled it at 100°C using water bath or heat block for 10 minutes followed by immediately placing the samples in ice box (-20ºC) for 5 minutes.

Table1: Interpretation Matrix against different antibiotics with Disc Diffusion Method for antibiotic sensitivity according to CLSI

Antibiotics + potency per disc	Classes of antibiotics	Resistant	Intermediate	Sensitive
Penicillin (P-10) Cat #CT0043B	Penicillin	≤28		≥29
Ampicillin (AMP-10) Cat #CT0003B		≤13	14-16	≥17
Amoxicillin Clavulanate/ Augmentin (Aug-30) Cat #CT00223B	Beta-lactamase inhibitor	≤13	14-17	≥18
Ampicillin-Sulbactam (SAM-20) Cat #CT0012B		≤11	12-14	≥15
Pipercilline-tazobactam (TZP-10) Cat #CT16288B		≤17	16-20	≥21
Cefazolin (KZ-30) Cat #CT0011B	Cephalosporin	≤19	20-22	≥23
Ceftiofur (EFT-30) Cat #CT1751B		≤17	18-20	≥21
Cefepime (FEP-30) Cat #CT0774B		≤18	19-24	≥25
Cefotaxime (CTX-30) Cat #CT0166B		≤22	23-25	≥26
Ceftazidime (CAZ-30) Cat #CT0412B		≤17	18-20	≥21
Chloramphenicol (C-30) Cat #CT0013B	Phenicol	≤12	13-17	≥18
Florfenicol (FFC-30)Cat #CT1754B		≤14	15-18	≥19
Ciprofloxacin (CIP-5) Cat #CT0623B	Quinolones	≤21	22-25	≥26
Enrofloxacin (ENR-5) Cat #CT0639B		≤16	17-22	≥23
nalidixic acid (NA 30) Cat #CT0031B		≤13	14-18	≥19
Colistin (CT-10/CS-10) Cat #CT0065B	Polypeptides cyclic	≤10		≥11
Ertapenem (ETP-10) Cat #CT0043B	Carbapenems	≤18	19-21	≥22
Meropenem (MEM-10 /MRP-10) Cat #CT0774B		≤19	20-22	≥23

Antibiotics + potency per disc	**Classes of antibiotics**	**Resistant**	**Intermediate**	**Sensitive**
Imipenem (IMI-10/ 1PM-10) Cat #CT0455B		≤19	20-22	≥23
Erythromycin (E-15) Cat #CT0019B	Macrolide	≤13	14-22	≥23
Azithromycin (AZM-15) Cat #CT0906B		≤12		≥13
Linezolid (LNZ-30) Cat #CT1649B	Oxazolidinone	≤20		≥21
sulfamethoxazole/ trimethoprim (SXT-25) Cat #CT0052B	Sulfonamide	≤10	11-15	≥16
Teicoplanin (TEC-30) Cat #CT00647B	Glycopeptide antibiotic	≤10	11-13	≥14
Streptomycin (S-10) Cat #CT0046B	Aminoglycoside	≤11	11-14	≥15
Amikacin (AK-30) Cat #CT01073B		≤14	15-16	≥17
Quinopristin/ Dalforistin (QDA-15) Cat #CT1644B		≤15	16-18	≥19
Minocycline (MH-30) Cat #CT0030B	Streptogramin	≤12	13-15	≥16
Gentamicin (CN-10) Cat #CT0072B	Tetracyclines	≤12	13-15	≥16
doxycycline (DO-30) Cat #CT0018B		≤10	11-13	≥14
Clindamycin (DA- 20) Cat #CT0064B		≤14	15-20	≥21
Tetracycline (TE-30) Cat #CT0053B		≤11	11-14	≥15

These micro centrifuge tubes are then centrifuged for 10 minutes at 14000 rpm at 8ºC. Supernatant derived after centrifugation was separated into another sterile micro centrifuge tube and palette was discarded. The supernatant containing the extracted DNA was stored at -20ºC until use.

Plasmid DNA extraction Kit method

Plasmid extraction was performed using (Favorogen Cat #FAPDE 001) according to the manufacturer's instructions (LUO & A, 2017)Briefly a few overnight cultured pure colonies of test samples were mixed in 3mL of sterile distilled water and vortexed to make a uniform suspension, then 1mL of this suspension was transferred to a micro centrifuge tube. The suspension was then centrifuged at 11,000 x g for 1 minute at refrigeration to pellet the cells and disposed the supernatant off., the cell pellet was resuspended in 200µL of FAPDI Buffer by pipetting gently. The 200µL of FAPD2 Buffer was added and mixed gently by inclining the tubes multiple times. The mixture was then incubated at 25ºC for 5 minutes to lyse the cells. It was kept in consideration. Then 300µL of FAPD3 Buffer was added and again the mixture was homogenized by tilting gently for multiple times promptly. After that the tubes were centrifuged at 18,000 x g, for 5 min at 8ºC to settle the lysate down. FAPD Column were adjusted in a collection tubes, then transferred the supernatant to the FAPD Column at the center of the membrane and centrifuged at 11,000 x g for 30 seconds at 8ºC. Disposed of the course through and placed the column again to the Collection Tube. Then 400 µL of WP Buffer was added to the FAPD Column and centrifuged at 11,000 x g for 30 seconds at 8ºC. Then the flow through was discarded and columns were placed back to the Collection Tube again. After that 700µL of Wash Buffer was added to the FAPD Column and centrifuged at 11,000 x g for 30 seconds at 8ºC. Disposed of the flow through and placed the section back to the Collection Tube. It was centrifuged again at 18,000 x g for an extra 3 minutes at 8ºC to dry the FAPD Column membrane. The FAPD Columns were fixed over new sterile 1.5mL micro centrifuge tubes. Then 70 µL of Elution Buffer was added to the central membrane of the FAPD Column. The column was then held for 1 minute and centrifuged at 18,000 x g for at 8ºC for 1 minute to elute plasmid DNA and stored the DNA at -20ºC until use.

Detection of antimicrobial resistance genes using PCR

Polymerase chain reaction (PCR) was used to amplify the antimicrobial resistance producing genes of the selected *E. coli* isolates according to the protocols already published. PCR was done using Dream Taq Green PCR Master Mix (Thermo Scientific, US Cat# K1081). In this investigation AMR genes against 4 categories of antibiotics were detected using PCR named as Beta-lactams, Aminoglycoside, tetracyclineand polypeptides (colistin). The primers sequences for Beta-lactamase genes included *bla*-OXA48, *bla*NDM, *bla*OXA8, *bla*CTX-M *bla*SHV and *bla*TEM (table 2) were used according to the previously describes protocols., The Aminoglycoside resistant genes included aac3-II, aph3 and aac6-Ib (table 3), tetracycline genes included tetA and tetM genes (table 4) whereas all colistin resistance producing genes mcr1 to mcr-9 (table 5) were utilized from the previous studies (F. M. Aarestrup et al., 2000; Frank Møller Aarestrup et al., 2000; Agersö et al., 2002; Aminov et al., 2002; Borowiak et al., 2020; Cirit et al., 2019; Daoud et al., 2015; Evans et al., 2008; Hatrongjit et al., 2018; Lan et al., 2008; Lescat et al., 2018; Liu et al., 2020; Miranda et al., 2003; Pérez-Pérez & Hanson, 2002; Poirel et al., 2011; Qiu et al., 2019; Rebelo et al., 2018; Van et al., 2008).

Primers synthesized by Eurofins Genomics (Canada). The ABI Proflex thermal cycler (Applied Biosystems / Thermo, USA) was used to amplify the gene products. PCR conditions for *bla*-OXA8 and *bla*-NDM gene (table 6) was done according to the protocol (Poirel *et al.,* 2011). Thermal profile foramplification of *bla*-OXA48, *bla*-SHV and *bla*-TEM genes (table 7) were used according to the previous protocols described by Daoud and coworkers (Daoud *et al.,* 2015).. Thermal cycling conditions for *bla*-FOX (table 8) were used according to the protocols (Pérez-Pérez & Hanson, 2002). PCR conditions for mcr-7 to 9 gene (table 9) were performed according to the protocols described by Liu and others (J. Liu *et al.,* 2020). PCR amplification of mcr-6 gene (table 10)was performed according to the previous studies (Borowiak *et al.,* 2020), For amplification of mcr-1, mcr-2, mcr-3 and mcr-4 genes (table 11) thermal conditions were used according to protocols described by Lescat and others (Lescat *et al.,* 2018)While amplification of tetA, aac3-II and aac6-

Ib genes (table 12) was done using thermal profile as described in previous study (Qiu *et al.,* 2019). For aph gene amplification, PCR conditions (table 13) were implemented according to the protocol developed by Miro et al., (Miró *et al.,* 2013). For tet M gene thermal condition are given in table 14 according to the directions of (Agersö *et al.,* 2002)**.** Master mix volume of 25µL (table 15) was made for PCR reaction using DreamTaq Green PCR Master Mix (2X). Briefly for PCR reaction 1µL of each primer (25pmol/µL) with 5µL of sample (template DNA) was used. Total of 12.5µL of 2x PCR mater mix used and made the volume to 25µL using 5.5µL of nuclease free distilled water. The PCR was done according to the relevant thermal profile and the PCR amplicons were stored at -20ºC until further used or analyzed through gel electrophoresis. The corresponding PCR product was analyzed by using 1.5% agarose gel electrophoresis (Appendix-IX). The gel was loaded with 12µL of PCR amplicons and a reference GeneRuler 100 bp DNA Ladder (Thermo Fisher Scientific, USA Cat# 15628-050) . The gel was run in TBE Buffer (Appendix-X) at 100 volts for 30 minutes. The gel was visualized with Ethidium Bromide under UV light using Gel Documentation System (Vilber Lourmat Sté, France). The genotypic results of each isolate were recorded and analyzed for comparison with corresponding phenotypic characteristics of AMR.

Table 2: Primer sequences for PCR amplification of Beta-lactamase producing genes

AMR genes	Forward and Reverse Sequences of primers	Product Bps	References
blaSHV-1	R 5'- CTTTATCGGCCCTCACTCAA-3'. F 5'- AGGTGCTCATCATGGGAAAG-3	237bp	(Daoud *et al.,* 2015)
blaTEM-1	F5'-CGCCGCATACACTTTCTCAGAATGA-3' R 5'- ACGCTCACCGGCTCCAGTTTAT-3'	444bp	(Daoud *et al.,* 2015)
blaFOX	F5'-GGTTTGGCGATCTGGTTTTC-3' R 5'-CGGAATGGCTCATCACGATC-3'	190bp	(Pérez-Pérez & Hanson, 2002)
blaNDM-1	F 5'-AACCCACGATGTGGGTAGC-3' R 5'-TCGCGTTAAGCGGATGATGC -3'	621bp	(Poirel *et al.,* 2011)
blaCTX-M-8	F5'-GTTAAATAGTGTTCTTGGAG-3' R5'-CTAAGATATGGCTCTAACAA-3'	687bp	(Woodford *et al.,* 2006)
blaOxa	F 5'-CATCAAGTTCAACCCAACCG-3' R 5'-GCGTGGTTAAGGATGAACAC-3'	438bp	(Poirel *et al.,* 2011)

Table 3: Primer sequences for PCR amplification of Aminoglycoside resistance producing genes

AMR genes	Forward and Reverse Sequences of primers	Product Bps	References
aac(3)-II	F5'-CTCCGTCAGCGTTTCAGCTA-3' R 5'ACTGTGATGGGATACGCGTC-3'	237bp	(Qiu *et al.,* 2019)
aac(6')-Ib	F5'-CAAAGCGCGTAACCGGATTGG-3' R5'-AACATGGGGTATCAGGGAGATG-3'	346bp	(Pérez-Pérez & Hanson, 2002)
aph(3)-II	R 5'- CTTTATCGGCCCTCACTCAA-3'. F 5'- AGGTGCTCATCATGGGAAAG-3	680bp	(Miró *et al.,* 2013)

Table 4: Primer sequences for PCR amplification of Tetracycline resistance producing genes

AMR genes	Forward and Reverse Sequences of primers	Product Bps	References
tet A	F 5'-GCTACATCCTGCTTGCCTTC-3' R 5'-CATAGATCGCCGTGAAGAGG-3'	211bp	(Qiu *et al.,* 2019)
tetM	F5'-GTTAAATAGTGTTCTTGGAG-3' R 5'-CTAAGATATGGCTCTAACAA-3'.	657bp	(Agersö *et al.,* 2002)

Table 5: Primer sequences for PCR amplification of Colistin resistance producing genes

AMR genes	Forward and Reverse Sequences of primers	Product Bps	References
mcr-1	F 5'-TCGGCAAATTGCGCTTTTGGC-3'. R 5'-ATGCCAGTTTCTTTCGCGTG-3'	502bp	(Lescat *et al.*, 2018)
mcr-2	F5'-TCGGCAAATTGCGCTTTTGGC-3'. R 5'-ATGCCAGTTTCTTTCGCGTG-3'.	379bp	(Lescat *et al.*, 2018
mcr-3	F5'-GATGGCGGTCTATCCTGTAT-3'. R 5'-AAGGCTGACACCCCATGTCAT-3'.	296bp	(Lescat *et al.*, 2018
mcr-4	F5'-AGGACAACCTCGTCATAGCA-3'. R 5'-ACCAGTAAATCTGGTGGCGT-3'.	207bp	(Lescat *et al.*, 2018
mcr-5	F5'-TTGCAGACGCCCATGGAATA-3'. R 5'-GCCGCATGAGCTAGTATCGT-3'.	608bp	(Lescat *et al.*, 2018
mcr-6	F5'-GGACGCGACTCCCTAACTTC-3'. R 5'-ACAACCAGTACGAGAGCACG-3'.	252bp	(Borowiak *et al.*, 2020)
mcr-7	F5'-AGCTATGTCAATCCCGTGAT-3'. R 5'-ATTGGCTAGGTTGTCAATC-3'.	791bp	(J. Liu *et al.*, 2020)
mcr-8	F5'-GTCAGTTACGCCATGCTCAA-3'. R 5'-TTCTTGTCGCAGAACTGTGG-3'.	943bp	(J. Liu *et al.*, 2020)
mcr-9	F5'-GCCATAGCACCTCAACACCT-3'. R 5'-AAACTGAACCCGGTACAACG-3'.	635bp	(J. Liu *et al.*, 2020)

Table 6: PCR Thermal Profile for amplification of *blaOXA*-8 and *blaNDM*-1 genes

Description	Temperature	Time	Cycles
Initial denaturation	94°C	10m	1
Denaturation	94°C	30s	30
Annealing	52°C	40s	
Elongation	72°C	5min	
Final extension	72ºC	∞	1

s = seconds, m= minutes

Table 7:PCR Thermal Profile for amplification of *blaOXA*-48, *bla* SHV-1 and *blaTEM* genes

Description	Temperature	Time	Cycles
Initial denaturation	95°C	15m	1
Denaturation	94°C	30s	30
Annealing	62°C	90s	
Elongation	72°C	10min	
Final extension	72ºC	∞	1

s = seconds m= minutes

Table 8: PCR Thermal Profile for amplification of *blaFOX* gene

Description	Temperature	Time	Cycles
Initial denaturation	94°C	3m	1
Denaturation	94°C	30s	25
Annealing	64°C	30s	
Elongation	72°C	1min	
Final extension	72ºC	∞	1

s = seconds m= minutes

Table 9:PCR Thermal Profile for amplification of mcr-7, mcr-8 and mcr-9 genes

Description	Temperature	Time	Cycles
Initial denaturation	94°C	4m	1
Denaturation	94°C	5s	40
Annealing	59°C	15s	
Elongation	72°C	5min	
Final extension	72ºC	∞	1

s = seconds m= minutes

Table 10:PCR Thermal Profile for amplification of mcr-6 gene

Description	Temperature	Time	Cycles
Initial denaturation	95°C	5m	1
Denaturation	95°C	30s	25
Annealing	52°C	30s	
Elongation	72°C	5min	
Final extension	72ºC	∞	1

s = seconds m= minutes

Table 11: PCR Thermal Profile for amplification of mcr-1 to mcr-5 gene

Description	Temperature	Time	Cycles
Initial denaturation	94°C	4m	1
Denaturation	94°C	5s	30
Annealing	59°C	20s	
Elongation	72°C	5min	
Final extension	72ºC	∞	1

s = seconds m= minutes

Table 12:PCR Thermal Profile for amplification of tet A, aac(3)-II and aac(6')-Ib genes

Description	Temperature	Time	Cycles
Initial denaturation	94°C	5m	1
Denaturation	94°C	60s	30
Annealing	56°C	55s	
Elongation	68°C	90s	
Final Elongation	72°C	10m	
Final extension	72ºC	∞	1

s = seconds m= minutes

Table 13: PCR Thermal Profile for amplification of aph (3)-II gene

Description	Temperature	Time	Cycles
Initial denaturation	94°C	5m	1
Denaturation	94°C	30s	30
Annealing	55°C	30s	
Elongation	72°C	10m	
Final extension	72ºC	∞	1

s = seconds m= minutes

Table 14:PCR Thermal Profile for amplification of tet M gene

Description	Temperature	Time	Cycles
Initial denaturation	94°C	3m	1
Denaturation	94°C	1m	35
Annealing	45°C	1m	
Elongation	72°C	10m	
Final extension	72ºC	∞	1

s = seconds m= minutes

Table 15: Master Mix recipe for PCR

Chemicals	Volume
Dream Taq Green PCR Master Mix	12.5µl
Water, Nuclease free	5µl
Forward Primer	1 µl
Reverse Primer	1µl
Sample	7µl

3. RESULTS

3.1 Sample Collection for Isolation of E. coli

For the current study, sampling strategy was adopted from the guidelines of FAO and the sampling was done in selected cities of all the provinces including Punjab, Sindh, Khyber Pakhtunkhwa (KP) and Balochistan while Azad Jammu and Kashmir (AJK) and Gilgit Baltistan (GB) were also added in later stages for sample collection. The sampling was done from main selected cities by the provincial and/or regional staff from the public sector livestock / poultry institutes. And samples were sent to NRLPD, NARC for analysis through isolation and identification of *E. coli* and AST evaluation using standard protocols. In this regard a total 785 samples were collected during July2020 to February 2021 from different regions of Pakistan. Out of these 785 collected samples,the number of received samples from selected areas ofPunjab Province, Lahore was 151 along with 21 sample received from Rawalpindiwhile 144 samples were collected from Sindh (Karachi). The number of samples from KP (Peshawar) was 148 and from Balochistan province 135 samples were received from the city of Quetta. Further a total of 153 samples were collected from Islamabad capital territory by NRLPD and from AJK, Muzaffarabad city was the selected area from where 33 samples were received. While from Gilgit city of Gilgit-Baltistan, only 21 samples were collected and received for further analysis. The data of samples collected and analyzed is depicted in table 16.

3.2 Isolation and Identification of E. coli

Total 785 samples were received during the year of 2020 July to February 2021 from different regions of Pakistan and Azad Jammu and Kashmir (table 16). All those samples were subjected to different tests for isolation and identification of *E. coli* for AMR surveillance. For this purpose the samples were pre-enriched using BPW (Fig-2), while the overnight culture from BPW was inoculated on to MA (Fig-3), and red colonies from MA were streaked for confirmation on EMB agar (Fig-4). The isolates producing green metallic sheen were purified by culturing on MA Agar and then non selective NA medium was used to get purified culture. There were about 640 out of 785 samples were observed with suspected *E. coli* cultures, which were

then subjected to other biochemical tests along with gram staining for confirmation of the *E. coli*. For this purpose the pure culture of each suspected isolate was subjected to Gram's Staining (Fig-5), Indole test along with TSI (Fig-6) and ONPG test (Fig-7). After analysis through biochemical tests a total of 621 isolates were confirmed as *E. coli*(Table-16). Number of samples received from selected areas of Punjab Province was 151 (Lahore) from which 113(75%) and from Rawalpindi 16(76%) were found positive for the presence of *E. coli*. While out of 144 received samples from Sindh (Karachi),84 were positive for *E. coli*with 58% prevalence rate. The number of received samples from KP (Peshawar) was 148 and positive isolates were 45%(68) recovered from these samples while from Balochistan province sampling number was 135 and positive isolates were 96 with 71% frequency from the city of Quetta. Highest number of positive isolates 129 were isolated from Islamabad capital territory with sample size of 153 with isolation rate of 84%, while from AJK, Muzaffarabad city was the selected area from where 33 samples were collected from which 26 were positive for *E. coli* with arecovery rate of 78.21%. Whereas 42% samples were found positive for the presence of *E. coli* with a total number of 9 out of 21 samples received and tested from Gilgit city of Gilgit-Baltistan region(table 16).

3.3 AMR profiling of isolated E. coli

A total 621 *E. coli* were isolated from the total number of 785 samples collected from all regions of Pakistan, where 221 (35%) isolates were subjected to Antimicrobial Susceptibility Tess (Figure 8) using CLSI protocols against a panel of 31 antibiotics from different classes. More than 50% resistance in tested *E. coli* isolates was observed against 11 out of 31 antibiotics analyzed and these include Penicillin, Ampicillin, Doxycycline, Azithromycin, Erythromycin, Linezolid, Streptomycin, Tetracycline and Teicoplanin (Table 17).

Table 16: Region wise sampling and isolation data for *E. coli*

Region	No. of Samples collected	No. of *E. coli* isolated Total, (%age)	No. of AST Performed
Islamabad Capital Territory	153	129 (84%)	3
Muzaffarabad	33	26 (78%)	3
Rawalpindi	21	16 (76%)	3
Lahore	151	113 (75%)	3
Quetta	135	96 (71%)	3
Karachi	144	84 (58%)	3
Peshawar	148	68 (45%)	3
Gilgit	21	9 (42%)	3

Figure 2: Enrichment of samples for isolation of *E. coli* through inoculation into Peptone Water. At left turbid Peptone water is positive for samples whereas at right PW is negative control

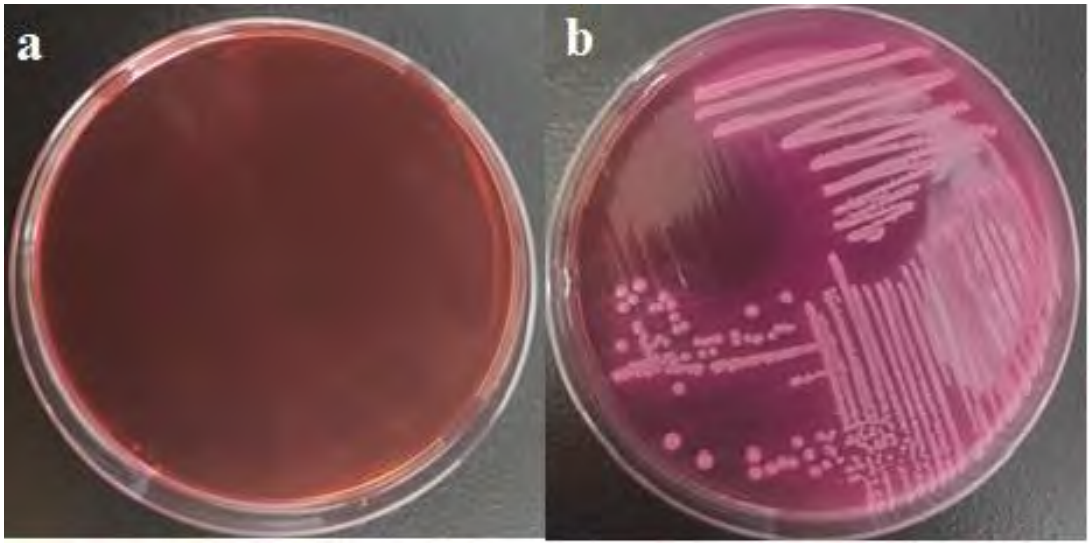

Figure 3: Isolation of *E. coli* on selective medium (MacConkey Agar)

a) MacConkey Agar plate with no growth (b) MacConkey plate with pink colonies of *E. coli*

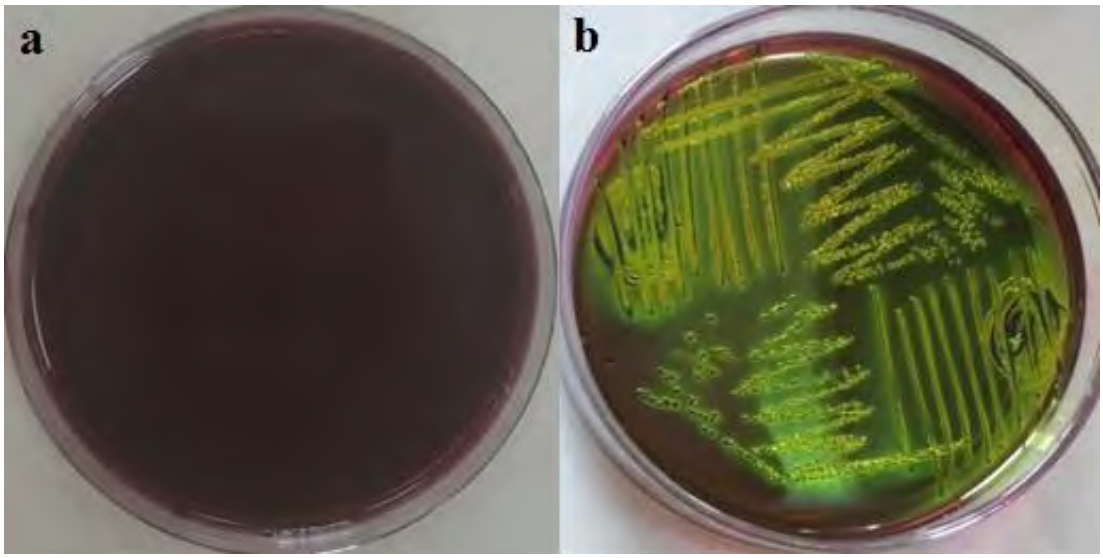

Figure 4: Identification of *E. coli* on selective medium (EMB Agar)

a) EMB Agar with no growth (b) Green metallic sheen colonies on EMB

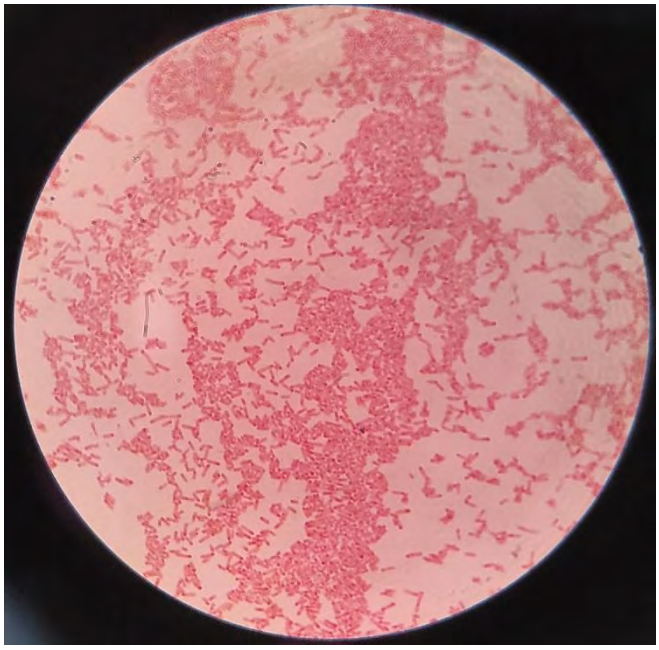

Figure 5: Microscopic view of *E. coli* after Gram staining

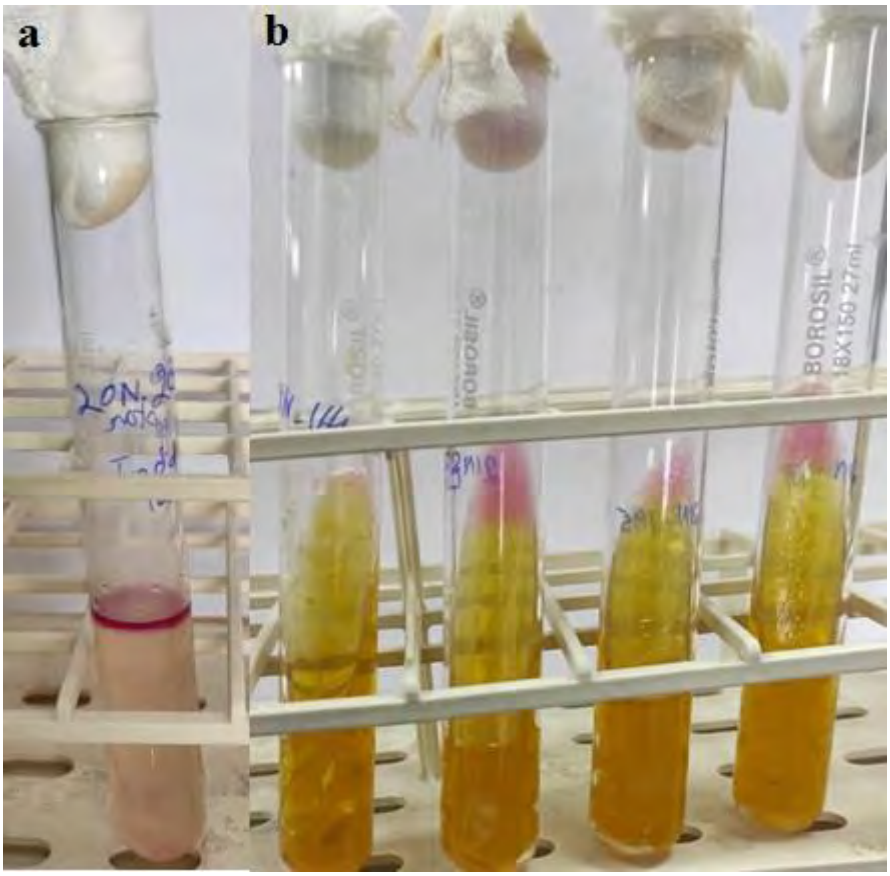

Figure 6: Identification of *E. coli* by using indole and TSI biochemical tests

(a) Positive Indole for *E. coli,* Pink ring at the rim; (b) Negative TSI for *E. coli,* yellow butt and yellow slant

Figure 7: Identification of *E. coli* using ONPG

ONPG positive for *E. coli* and negative control for ONPG

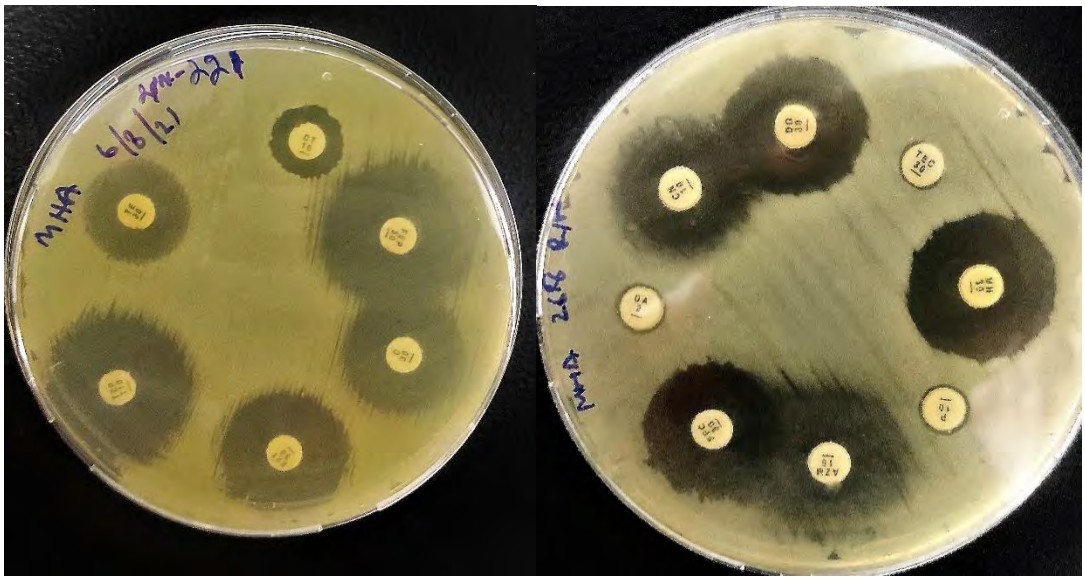

Figure 8: Antibiotic susceptibility test showing zone of inhibition against different antibiotics

Table 17: AMR profiling of selected *E. coli* isolates

Antibiotics	% S	% I	% R	Antibiotics	% S	% I	% R
Amikacin	80%	14%	6%	Erythromycin	5%	3%	92%
Amoxicillin Clavulanate/ Augmentin	39%	31%	30%	Florfenicol	30%	2%	68%
Ampicillin	15%	1%	84%	Gentamicin	57%	8%	35%
Ampicillin-Sulbactam	33%	15%	52%	Imipenem	59%	18%	23%
Azithromycin	47%	0%	53%	Linezolid	10%	6%	84%
Cefazolin	26%	30%	44%	Meropenem	79%	13%	8%
Cefepime	68%	16%	16%	Minocycline	46%	30%	24%
Cefotaxime	60%	21%	18%	Nalidixic Acid	4%	6%	89%
Ceftazidime	50%	26%	24%	Penicillin	0%	0%	100%
Chloramphenicol	30%	2%	68%	Pipercillin-Tazobactam	88%	8%	5%
Ciprofloxacin	10%	6%	84%	Quinopristin/Dalforistin	16%	4%	80%
Clindamycin	6%	6%	87%	Streptomycin	16%	1%	83%
Colistin	50%	0%	50%	Sulfamethoxazole-Trimethoprim	32%	5%	63%
Doxycycline	2%	0%	98%	Teicoplanin	11%	03%	86%
Enrofloxacin	11%	9%	80%	Tetracycline	10%	1%	89%

S= Sensitive; I= Intermediate; R= Resistance

The most resistant antibiotic observed was Penicillin with a result of 100% resistance followed by Doxycycline (98%), Erythromycin (92%) resistance, while Nalidixic acid and tetracycline were observed with a resistance frequency of 89% each. The other most resistant antibiotics observed were Clindamycin (87%), Teicolpanin (86%), Enrofloxacin (85%) and Linezolid along with Ampicillin with a resistance frequency of 84% each, while Streptomycin was observed with 83% resistance (Table 17, Fig-9). Other highly resistant antibiotic observed was Quinopristin/Delfopristin which was 80% resistant against the tested isolates (Fig-9).

A total of 9 out of 31 antibiotics tested were found with sensitivity in more than 50% of tested isolates (Table 17). Highest sensitivity was observed for Piperacillin/Tazobactam, Amikacin and Meropenem antibiotics with a sensitivity of 88%, 80% and 79% respectively against tested isolates. The other most sensitive antibiotics observed were Cefepime, Cefotaxime, Imipenem and Gentamycin with 68%, 60%, 59% and 57% frequency of sensitivity respectively (Table 17, Figure 10). On the other hand some of the antibiotics were observed with intermediate sensitivity range according to the CLSI interpretation matrix and these were Minocycline and Cefazolin with frequency of 30% each followed by Ceftazidime and Cefotaxime exhibiting sensitivity frequency of 26% and 21% respectively (Table 17, Figure 11). While 0% frequency in intermediate category of sensitivity was found against Penicillin and Doxycycline observed with 100% resistance along with Colistin and Azithromycin recorded with 50% & 53% resistance respectively (Fig-11). The complete AMR profile of all the isolates against tested antibiotics with all categories of sensitivity (Sensitive, Resistance, Intermediate) are depicted in Figure 12.

Among the total isolates tested for AST analysis, maximum resistance observed was 90% against the 31 tested antibiotics. While 60 (27%) isolates were observed for more than 50% resistance against the tested antibiotics. On the other hand only 76 (34%) isolates were observed with more than 50% sensitivity against the tested panel of antibiotics, while maximum sensitivity observed was 89% (Appendix 19-26).

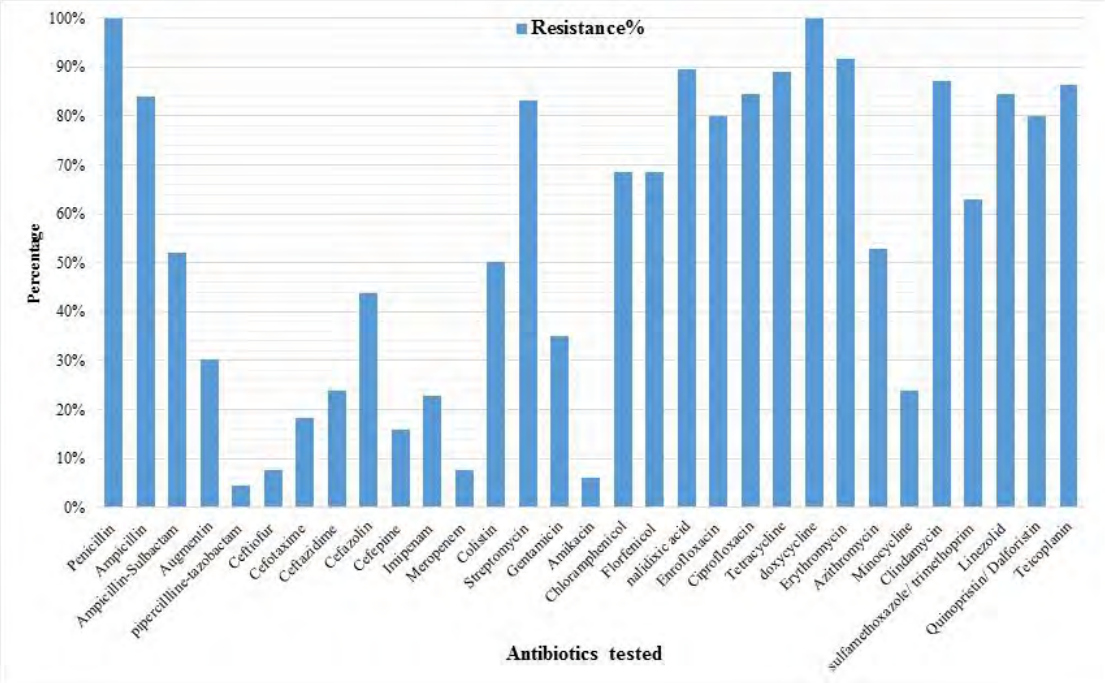

Figure 9: Antimicrobial resistance profile of *E. coli* isolates analyzed against selected panel of antibiotics

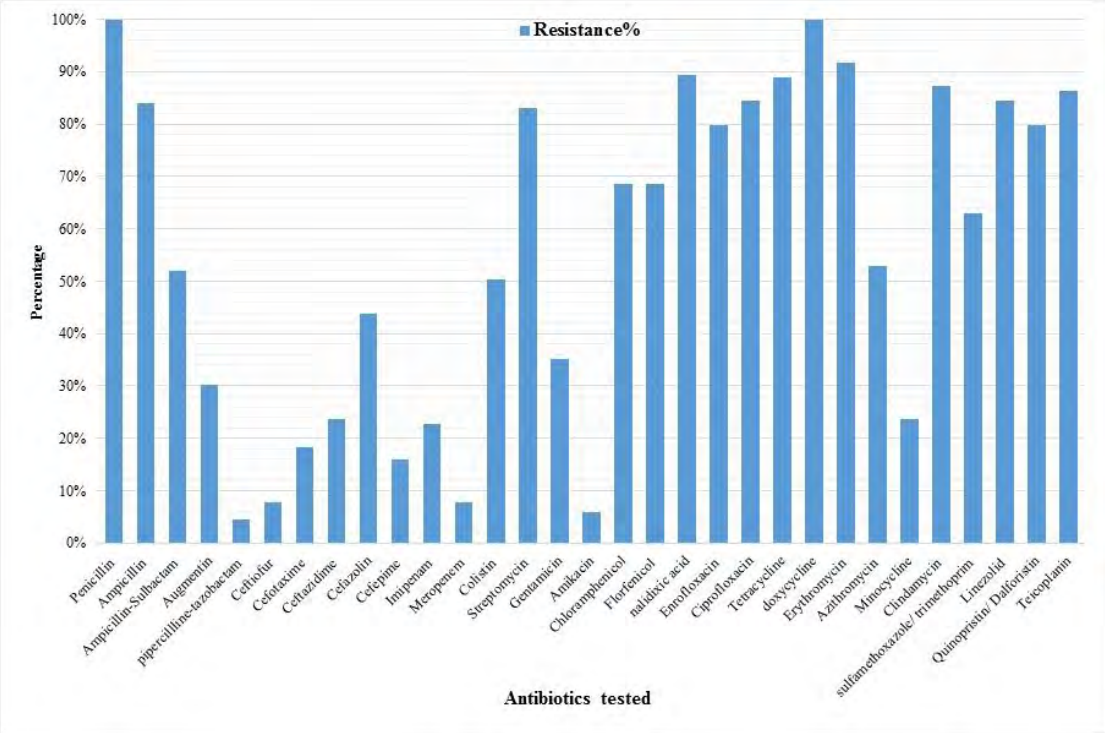

Figure 10: AMR profiling of *E. coli* isolates showing sensitivity against selected antibiotic panel

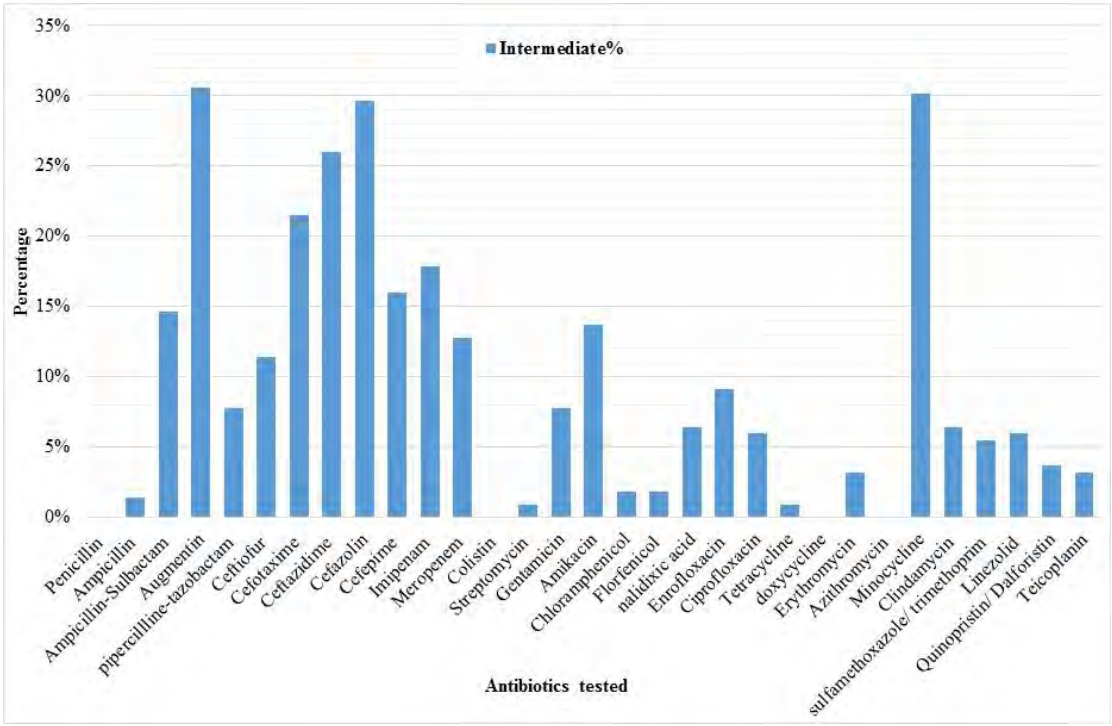

Figure 11: AMR profiling of *E. coli* isolates showing Intermediate category of sensitivity

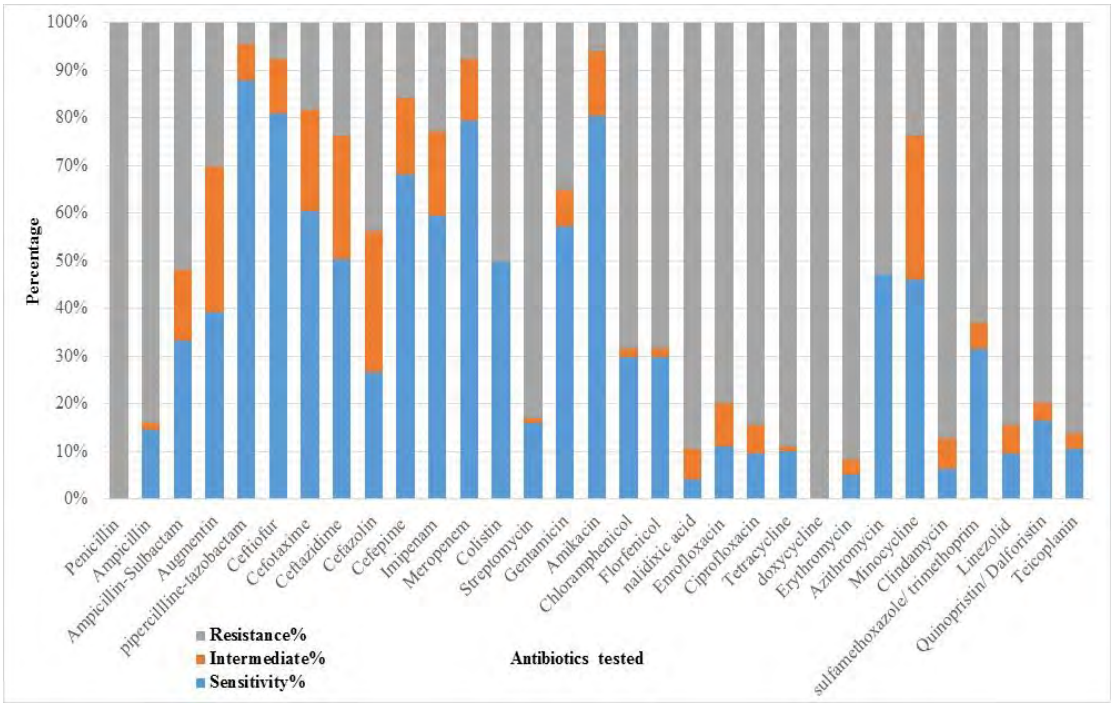

Figure 12: AMR profilingof selected*E. coli* isolated across the country

Area wise AMR profiling of E. coli isolated

Out of 129 positive isolates from Islamabad region, a total of 62 isolates were subjected to antimicrobial susceptibility test. The most resistant antibiotics observed were Penicillin with a result of 100% resistance each followed by Erythromycin, Linezolid, and Clindamycin with 97% resistance, while Tetracycline was observed with a resistance frequency of 95% each. The other most resistant antibiotics observed were Nalidixic Acid (94%) and Enrofloxacin (94%) while Doxycycline was observed with 92% resistance, whereas Ciprofloxacin along with Chloramphenicol were observed with 90% resistance (Appendix-XI). Other highly resistant antibiotic observed was Streptomycin with 89% resistance frequency and Quinupristin/dalfopristin along with Ampicillin which was found 85% resistant against the tested isolates (Appendix-XI).

A total of 10 out of 31 antibiotics tested were found with sensitivity more than 50% of isolates (Appendix-XI). Highest sensitivity was observed for Ceftiofur, Piperacillin/tazobactam and Meropenem antibiotics with frequency of 94%, 90% and 82% respectively against the tested isolates. The other most sensitive antibiotics observed were Amikacin and Cefotaxime with 81% frequency of sensitivity each. The remaining most sensitive antibiotics observed were Cefepime and Colistin with a 77% and 58% whereas Gentamycin along with Imipenem with 56% each frequency of sensitivity respectively. On the other hand, some of the antibiotics were observed with intermediate sensitivity range according to the CLSI interpretation matrix and these were Cefazolin and Minocycline with a frequency of 32% and 26% followed by Ampicillin-Sulbactam with a frequency of 25% sensitivity falling in intermediate category (Figure 13). The complete AMR profile of all the isolates recovered from Islamabad region against tested antibiotics with all categories of sensitivity (Sensitive, Resistance, Intermediate) are depicted in Figure 13.

Among the total isolates tested for AST analysis, maximum resistance observed was 78% against the 31 tested antibiotics. While 11 (17.7%) isolates were observed for more than 50% resistance against the tested antibiotics. On the other hand only 28 (45%) isolates were observed with more than 50% sensitivity against the

tested panel of antibiotics. Whereas maximum sensitivity observed was 89%(Appendix-XIX).

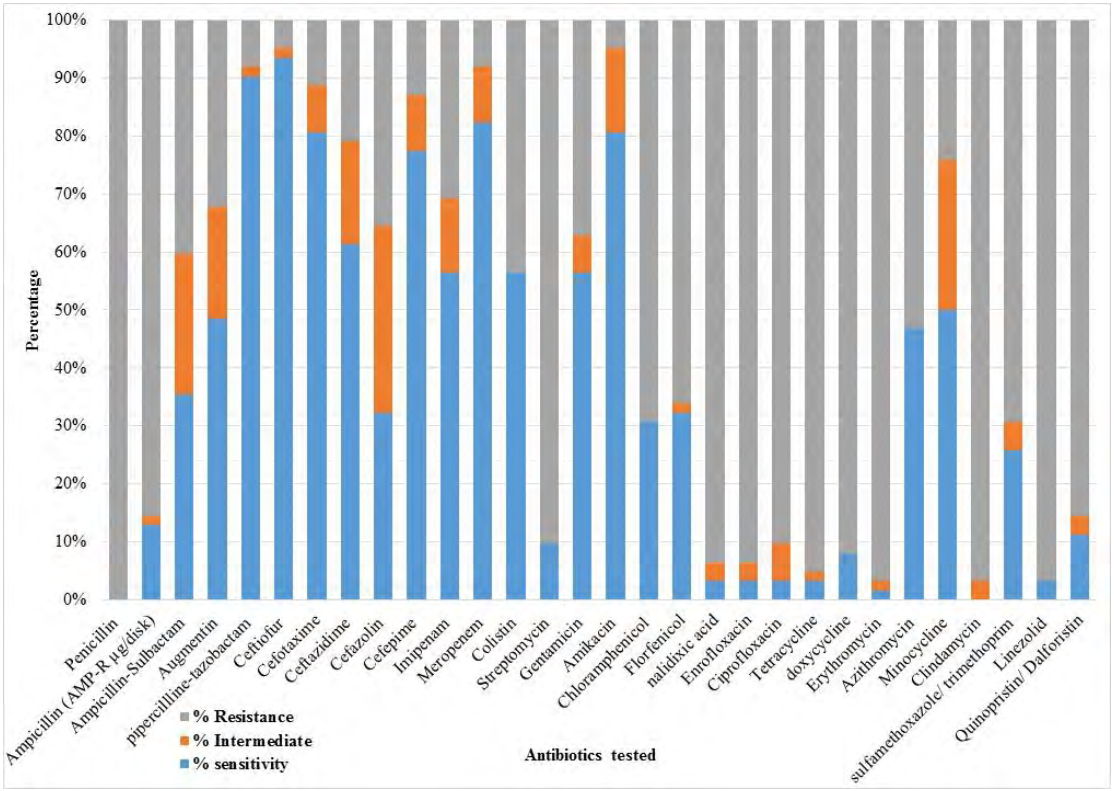

Figure 13: AMR profiling of selected *E. coli* isolated from Islamabad

A total of 18 (85%) out of 21 isolated *E. coli (*from Gilgit) were analyzed for AST, whereas more than 50% resistance was observed against 18 out of 31 antibiotics analyzed and these include Penicillin, Doxycycline, Erythromycin, Tetracycline, Teicoplanin, Quinupristin/dalfopristin, Clindamycin, Ciprofloxacin, Streptomycin, Nalidixic acid and Enrofloxacin. The most resistant antibiotics observed were Penicillin, Tetracycline, Streptomycin, Nalidixic acid, Quinupristin/Dalfopristin, Ciprofloxacin and Enrofloxacin with a result of 100% resistance followed by Erythromycin, Linezolid, Teicoplanin, Chloramphenicol,Doxycycline and Ampicillin with a resistance frequency of 94% each. The other most resistant antibiotics observed were Clindamycin, Sulfamethoxazole/trimethprim and Florfenicol with resistance frequency of 89% each (Fig-14).

A total of 7 out of 31 antibiotics tested were found with sensitivity more than 50% of isolates (Appendix-XII). Highest sensitivity was observed for Meropenem, Ceftiofur, and Amikacin antibiotics with frequencies of sensitivity recorded 89%, 83% and 78% respectively against tested isolates. The other most sensitive antibiotic observed wasPiperacillin/tazobactam, with a 72% frequency of sensitivity (Figure 14). Furthermore, some of the antibiotics were observed with intermediate sensitivity range according to the CLSI interpretation matrix and these were Augmentin61%and Cefazoline 50% followed by Ceftazidimewith an intermediate frequency of 39% (Appendix-XII, Figure 14). The complete AMR results of all the isolates against tested antibiotics with all categories of sensitivity (Sensitive, Resistance and Intermediate) are depicted in Figure 14.

Among the total isolates tested for AST analysis, maximum resistance observed was 76% against all the tested antibiotics. While 5 (27%) isolates were observed for more than 50% resistance. On the other hand, only 2 (11%) isolates were observed with more than 50% sensitivity against the tested panel of antibiotics, here the maximum sensitivity observed was 89% among the tested isolates of *E. coli* (Appendix-XX).

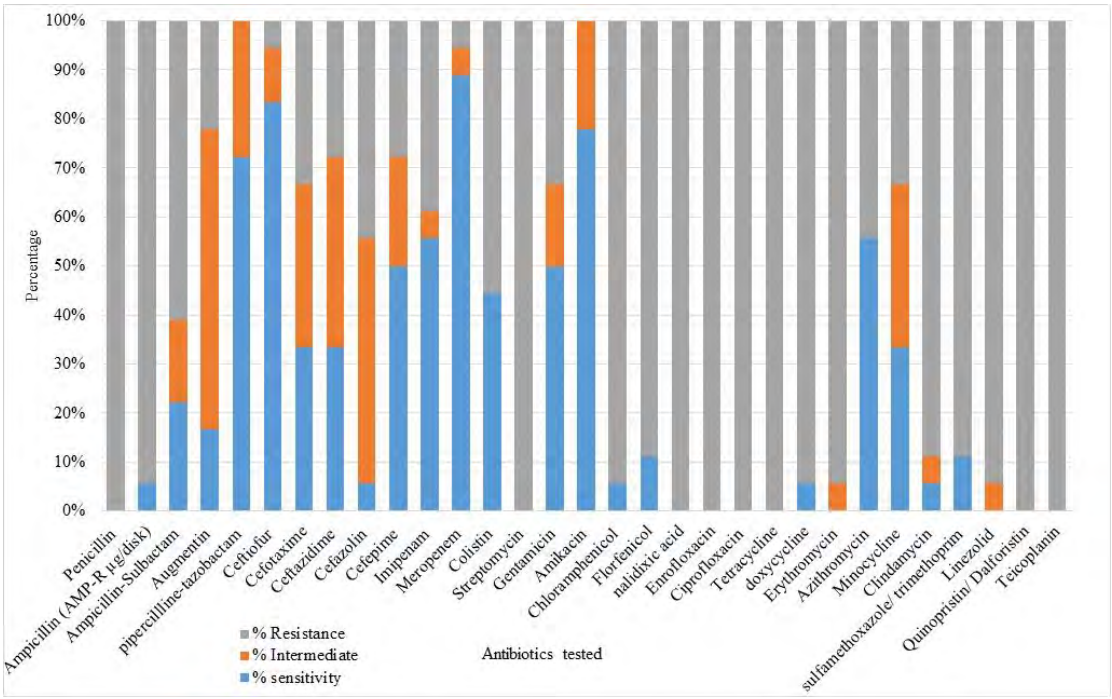

Figure 14: AMR profiling of selected *E. coli* isolated from Gilgit

The selected isolates from Karachi for AST analysis were 31 (37%) out of 84 total isolated *E. coli*, where 100 % resistant was observed against Penicillin and Doxycycline among the tested *E. coli* isolates, followed by Nalidixic acid (87%), Erythromycin (77%) resistance, while tetracycline and Ciprofloxacin were observed with a resistance frequency of 74% each. More than 50% resistance in tested *E. coli* isolates was observed against 17 (54%) out of 31 antibiotics analyzed by AST and these include Penicillin, Doxycycline, Erythromycin, Tetracycline, Teicoplanin, Quinupristin/Dalfopristin, Clindamycin, Ciprofloxacin, Streptomycin, Nalidixic acid and Enrofloxacin (Appendix-XIII, Figure 15).

A total of 11 out of 31 antibiotics tested were found with sensitivity more than 50% against the tested isolates (Appendix-XIII). Highest sensitivity was observed for Piperacillin-tazobactam and Amikacin antibiotics with a value of 90%. The other most sensitive antibiotics observed were Meropenem, Imipenem and Gentamycin with a 87%, 84% and 74% frequency of sensitivity respectively (Figure 15). On the other hand some of the antibiotics were observed with intermediate sensitivity range according to the CLSI interpretation matrix and these were Minocycline with a value of 32% followed by Augmentin, Ceftazidime and Enrofloxacin a frequency of 26%, 23% and 22% respectively (Appendix–XIII). The complete AMR results of all the isolates against tested antibiotics with all categories of sensitivity (Sensitive, Resistance, and Intermediate) are depicted in Appendix-XXI.

Among the total isolates tested for AST analysis, maximum resistance observed was 67% against the 31 tested antibiotics. While 11 (35%) isolates were observed for more than 50% resistance against the tested antibiotics. On the other hand only 17 (54%) isolates were observed with more than 50% sensitivity against the tested panel of antibiotics. While maximum sensitivity observed was 87 % (Appendix-XXI).

From Quetta city of Balochistan province, a total of 135 samples were received and 96 were found positive for presence of *E. coli*, where 29 (30%) isolates were subjected to AST analysis. The isolates tested for AST were found 100% resistant against Penicillin, and Erythromycin (Appendix-XIV, Figure 16).

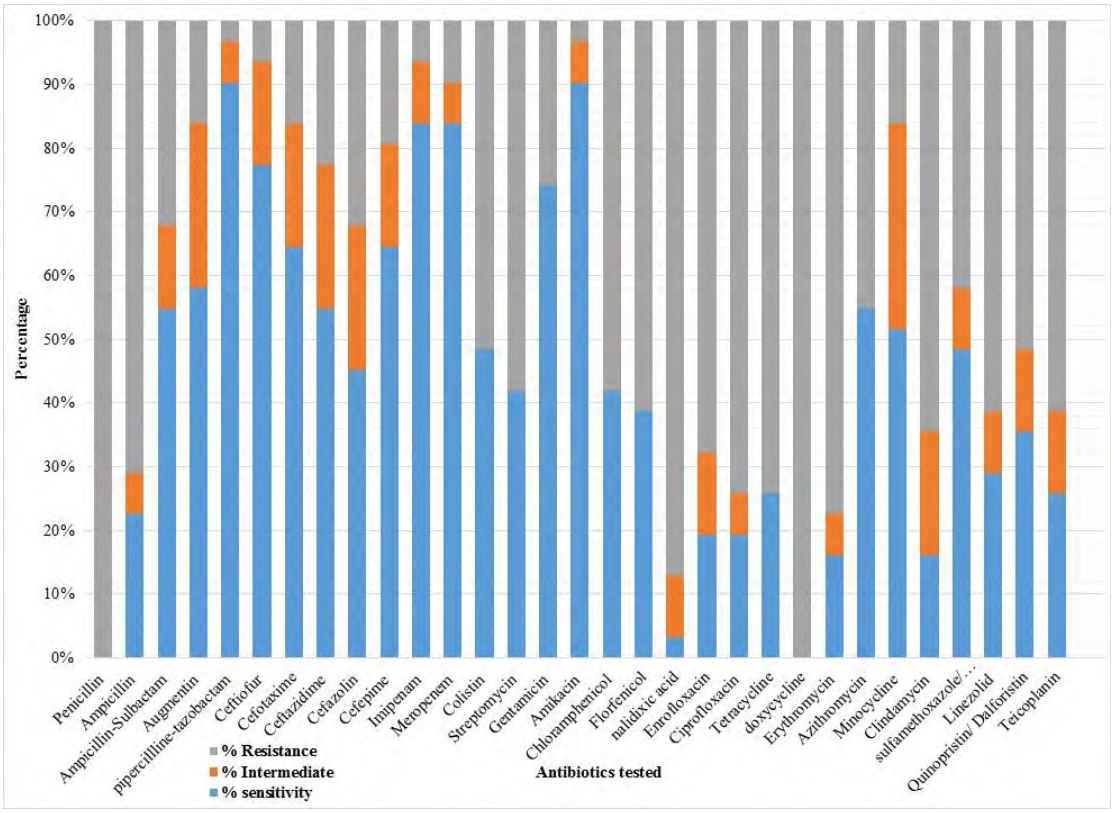

Figure 15: AMR profiling of selected *E. coli* isolated from Karachi

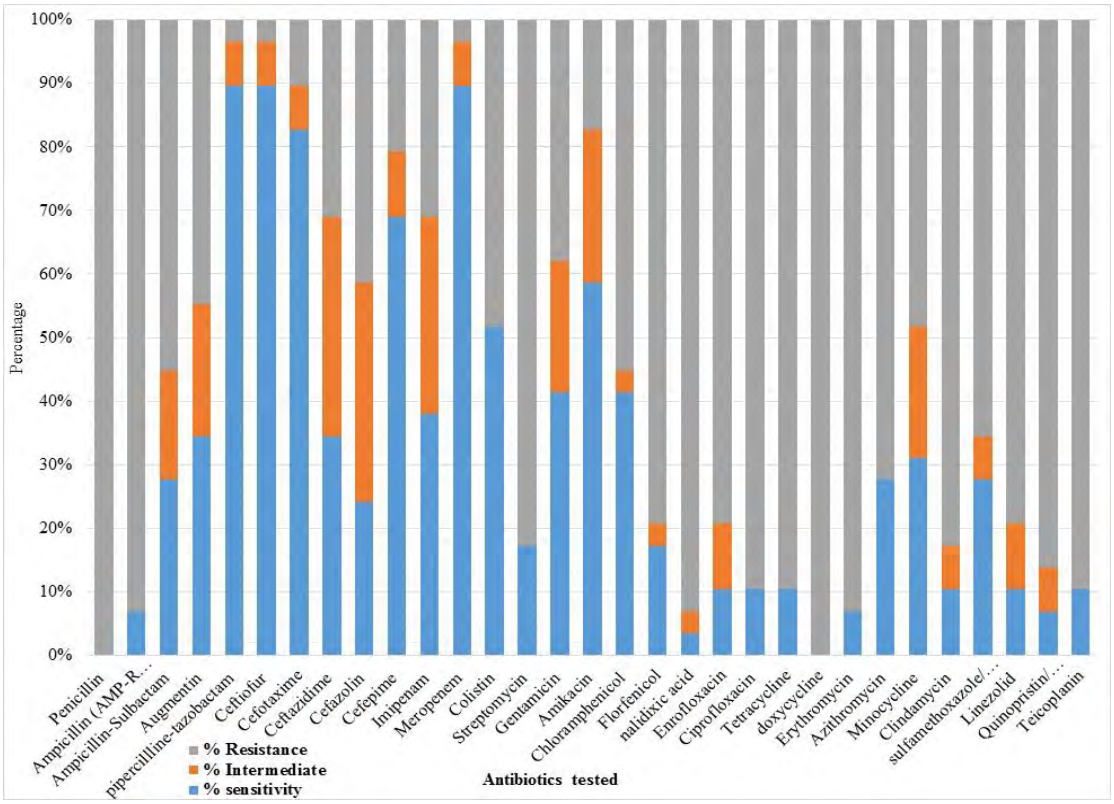

Figure 16: AMR profiling of selected *E. coli* isolated from Quetta

The other most resistant antibiotics observed were Ampicillin, Nalidixic acid and Erythromycin with resistance frequency of 93% each. Among all the antibiotics tested 19 antibiotics were found to be resistance in more than 50% of the total *E. coli* isolates tested (Appendix XIV, Figure 16). On the other hand, 7 out of 31 antibiotics tested were found sensitive in more than 50% of isolates (Appendix-XIV). Highest sensitivity was observed for piperacillin/tazobactam and Meropenem antibiotics with a sensitivity of 90% against tested isolates. The other most sensitive antibiotic observed was Colistin,Ceftazidime and Cefotaxime with 83% frequency of sensitivity respectively (Figure 16). Whereas some of the antibiotics were observed with intermediate sensitivity range and these included Ceftazidime along with Cefazoline 35% each and Imipenem with a frequency of31% (Appendix-XIV). The complete AMR results of all the isolates against tested antibiotics with all categories of sensitivity (Sensitive, Resistance, and Intermediate) are depicted in Appendix-XXII.

Among the total isolates for Quetta tested for AST analysis, maximum resistance observed was 78% against the 31 tested antibiotics. While 19 (65.5%) isolates were observed for more than 50% resistance against the tested antibiotics. On the other hand, only 7 (24%) isolates were observed with more than 50% sensitivity against the tested panel of antibiotics. While maximum sensitivity observed among isolates was 67% (Appendix-XXII).

A total of 113(75%) isolates were recovered out of 151 samples collected from Lahore, whereas 25 (22%) isolates were subjected to AST analysis. The tested isolates for AST were found highly resistant to Penicillin and Doxycycline with 100% frequency followed by Erythromycin and Ampicillin (88%) resistance for each, while Clindamycin and Teicoplanin were observed with a resistance frequency of 84% each (Fig-17). More than 50% resistance in tested *E. coli* isolates was observed against 19 (61%) out of 31 antibiotics analyzed and these include Penicillin, Ampicillin, Doxycycline, Azithromycin, Erythromycin, Linezolid, Streptomycin, Tetracycline, Teicoplanin, Ciprofloxacin, Quinopristin/Dalfopristin, Nalidixic acid, Colistin, Florfenicol. Enrofloxacin, Cefazolin and Sulfamethoxazole/ trimethoprim.

A total of 5 (16%) out of 31 antibiotics tested were found with sensitivity more than 50% (Table 17) which were Pipercillline-tazobactam, Amikacin, Imipenam, Gentamicin and Ceftiofur. Highest sensitivity was observed forPiperacillin/tazobactam, Amikacin antibiotics with a sensitivity of 76% each against tested isolates. The other most sensitive antibiotics observed were Imipenem, Gentamycin and Meropenem with a 64%, 60% and 56% frequency of sensitivity respectively (Figure 17). On the other hand, some of the antibiotics were observed with intermediate sensitivity range and these were Cefotaxime and Cefepime with a frequency of 40% and 36% respectively (Appendix-XV, Figure 17). The complete AMR results of all the isolates from Lahore against tested antibiotics with all categories of sensitivity (Sensitive, Resistance, and Intermediate) are depicted in Appendix-XXII.

Among the total isolates (obtained from Lahore) tested for AST analysis, maximum resistance observed was 84% against the 31 tested antibiotics. While 19 isolates were observed for more than 50% resistance against the tested antibiotics. On the other hand, only 4 isolates were observed with more than 50% sensitivity against the tested panel of antibiotics. While maximum sensitivity observed was 71% among all the tested *E. coli* isolates (Appendix-XV).

Among selected isolates for AST analysis from Peshawar, more than 50% resistance was observed against 19(61%) out of 31 antibiotics analyzed and these include Penicillin, Erythromycin, Ciprofloxacin, Clindamycin, Nalidixic acid, Quinopristin/ Dalforistin, Tetracycline, Linezolid, Streptomycin, Teicoplanin, Ampicillin, Azithromycin, Enrofloxacin, Chloramphenicol,Doxycycline, Florfenicol, Colistin and Sulfamethoxazole/ trimethoprim. The most resistant antibiotic observed was Penicilllin with 100% resistance followed by Erythromycin (88%), Ciprofloxacin and Clindamycin with a frequency of 85% each, Nalidixic acid (81%), while Quinopristin/ Dalforistin and Tetracycline shared a same resistance frequency of 77%. Whereas Streptomycin, Teicoplanin and Linezolid with a resistance frequency 73% recorded for each (Fig-18).Among these isolates 16(59%) isolates were found resistant against more than 50% antibiotics tested while maximum resistance was observed 71% in these isolates. However, the minimum resistance observed was 16% in the selected isolates for AST analysis (Appendix XVI).

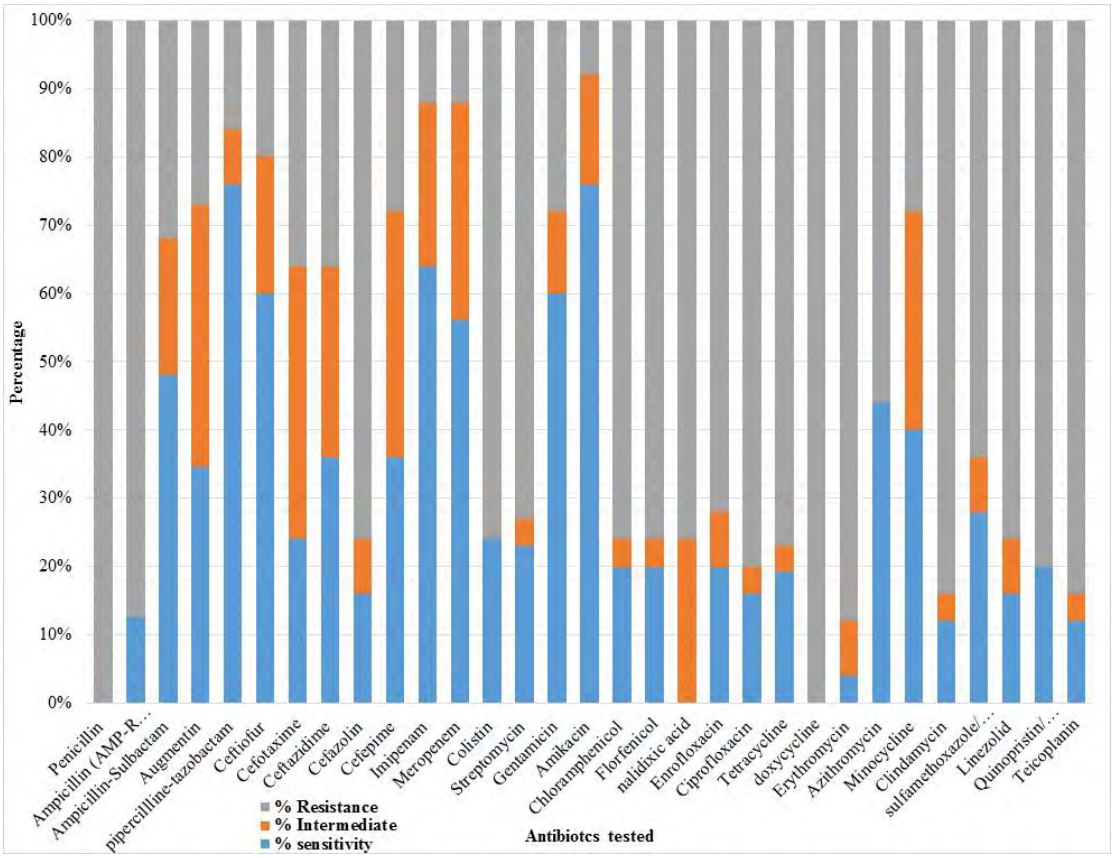

Figure 17: AMR profiling of selected *E. coli* isolated fromLahore

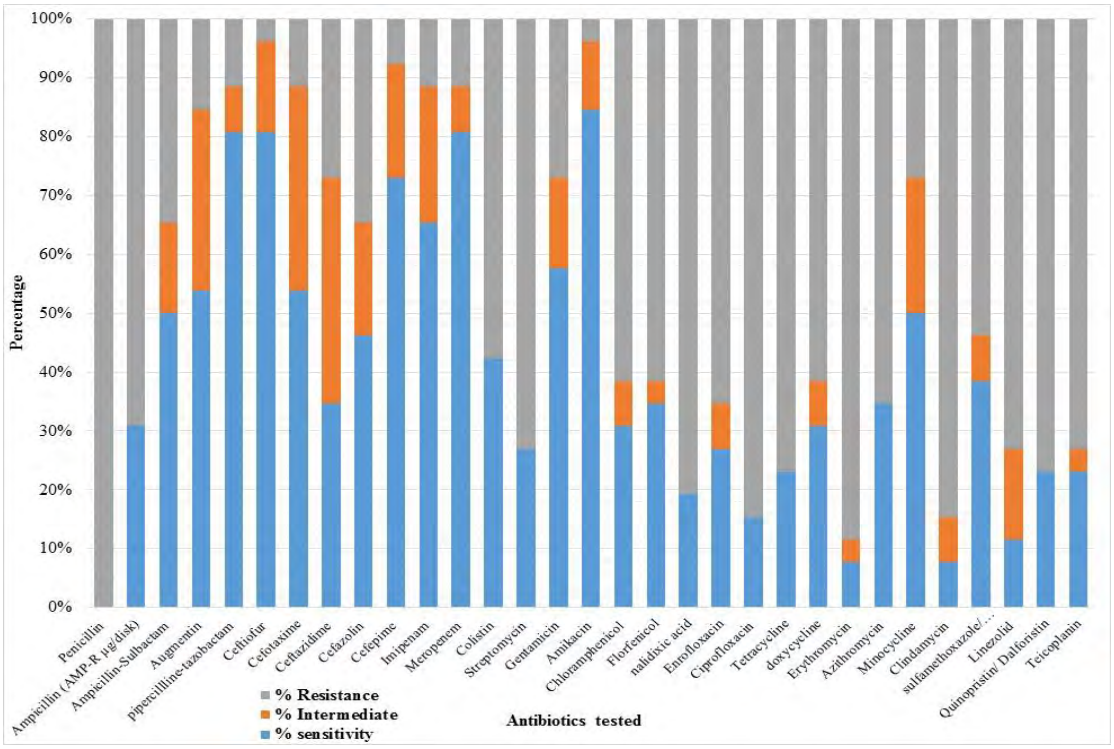

Figure 18: AMR profiling of selected *E. coli* isolated fromPeshawar

In comparison to resistance,12 (38%) out of 31 antibiotics tested were found sensitive in more than 50% of tested isolates (Appendix-XIII). Highest sensitivity was observed for Amikacin (85%),followed by Meropenem,Pipercillline-tazobactam and Ceftiofur with a sensitivity frequency of 81% each. The other most sensitive antibiotics observed were Cefepime and Imipenemwith 73% and 65% frequency of sensitivity respectively (Figure 18). The complete AMR results of all the isolates against tested antibiotics with all categories of sensitivity (Sensitive, Resistance, and Intermediate) are depicted in Appendix-XXV and Figure 18.

Among the total isolates tested for AST analysis, maximum resistance observed was 71% against all the tested antibiotics. While 19 (73%) isolates were observed for more than 50% resistance against the tested antibiotics, in contrast 12(46%) isolates were observed with more than 50% sensitivity against the tested panel of antibiotics, while maximum sensitivity observed was 77% (Appendix-XXV).

Among selected isolates for AST analysis from Muzaffarabad, more than 50% resistance was observed against 21 (67.7%) out of 31 antibiotics analyzed and these include Doxycycline, Quinopristin/Dalforistin, Sulfamethoxazole/ trimethoprim, Teicoplanin, Penicillin, Erythromycin, Tetracycline,Linezolid, Florfenicol, Colistin,Nalidixic acid, Ampicillin-Sulbactam, Clindamycin, Ciprofloxacin, Chloramphenicol, Ampicillin, Streptomycin, Enrofloxacin, Azithromycin and Minocycline. The most resistant antibiotics observed were Doxycycline, Quinopristin/Dalforistin, Sulfamethoxazole/ trimethoprim, Teicoplanin and Penicillin with 100% resistance followed by Erythromycin, Tetracycline and Linezolidantibiotics with resistance frequency of 95% recorded for each (Fig-19). Among these isolates 11 (52%) isolates were found resistant against more than 50% antibiotics tested while maximum resistance was observed 89% in these isolates. However, the minimum resistance observed was 11% in the selected isolates for AST analysis (Appendix XVI).

In comparison to resistance, 2 out of 31 antibiotics tested were found sensitive in more than 50% of tested isolates (Appendix-XVI). Highest sensitivity was observed for Piperacillin/tazobactam antibiotics with a value of 76% respectively. The

other most sensitive antibiotics observed were Cefepime and Gentamycin with 64% and 61% frequency of sensitivity respectively (Figure 19).

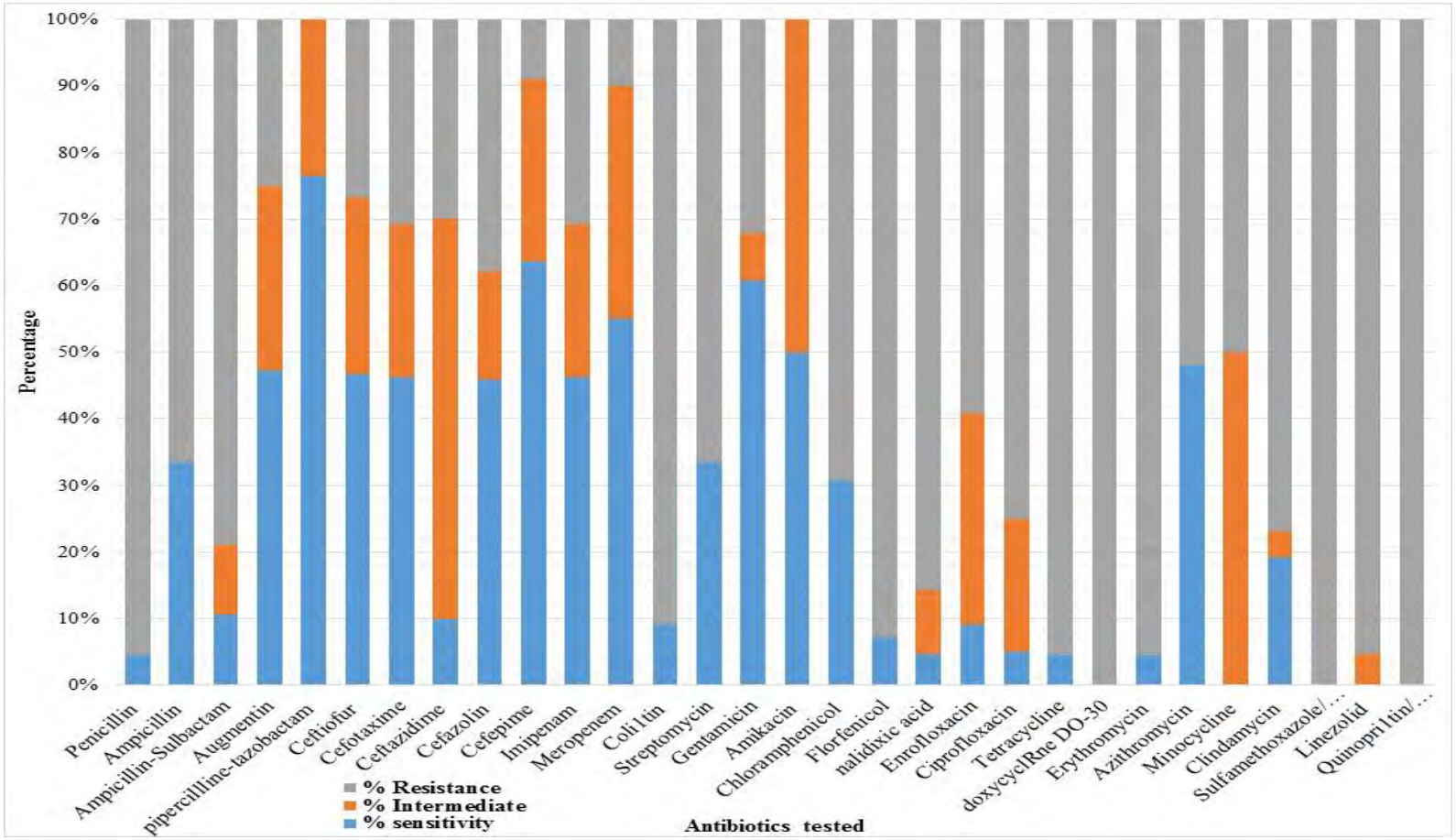

Figure 19: AMR profiling of selected *E. coli* isolated from Muzaffarabad

The complete AMR results of all the isolates against tested antibiotics with all categories of sensitivity (Sensitive, Resistance, and Intermediate) are depicted in Appendix-XXV and Figure 19.

Among the total isolates tested for AST analysis, maximum resistance observed was 89% against all the tested antibiotics. While 11(52%) isolates were observed for more than 50% resistance against the tested antibiotics, in contrast 2 isolates were observed with more than 50% sensitivity against the tested panel of antibiotics, while maximum sensitivity observed was 78% (Appendix-XXV).

A total of 16 isolates were recovered out of 21samples collected from Rawalpindi, where 10(47%) isolates were subjected to AST analysis. The tested isolates for AST were found highly resistant to Penicillin,Streptomycin, Enrofloxacin, Ciprofloxacin, Tetracycline, Doxycycline, Erythromycin, Minocycline, Sulfamethoxazole/ trimethoprim and Teicoplanin with 100% frequency followed by AmpicillinandAzithromycin were observed with a resistance frequency of 90% and 80% respectively (Fig-19). More than 50% resistance in tested *E. coli* isolates was observed against 15 out of 31 antibiotics analyzed and these include Doxycycline, Sulfamethoxazole/ trimethoprim, Teicoplanin, Penicillin, Erythromycin, Tetracycline,Linezolid, Clindamycin, Ciprofloxacin, Chloramphenicol, Ampicillin, Streptomycin, Enrofloxacin, Azithromycin and Minocycline(Appendix XVII). Further, among the total isolates (obtained from Rawalpindi) tested for AST analysis, maximum resistance observed was 56% against the 31 tested antibiotics. While 15 isolates were observed for more than 50% resistance against the tested antibiotics (Appendix XXVI).

A total of 5 (16%) out of 31 antibiotics tested were found with sensitivity more than 50% (Fig-20) which wereImipenem, Meropenem, pipercillline-tazobactam, Cefepime, Colistin, Ceftazidime, Ceftiofur, Amikacin, Clindamycin, Linezolid, Quinopristin/ Dalforistin, Ampicillin-Sulbactam and Cefotaxime. Highest sensitivity was observed for Imipenem and Meropenemantibiotics with a sensitivity of 100% against tested isolates, followed by Pipercillline-tazobactam and Cefepime with 90% frequency each. The other most sensitive antibiotics observed were Colistin, and Ceftazidime with 80% frequency of sensitivity each (Fig-20).

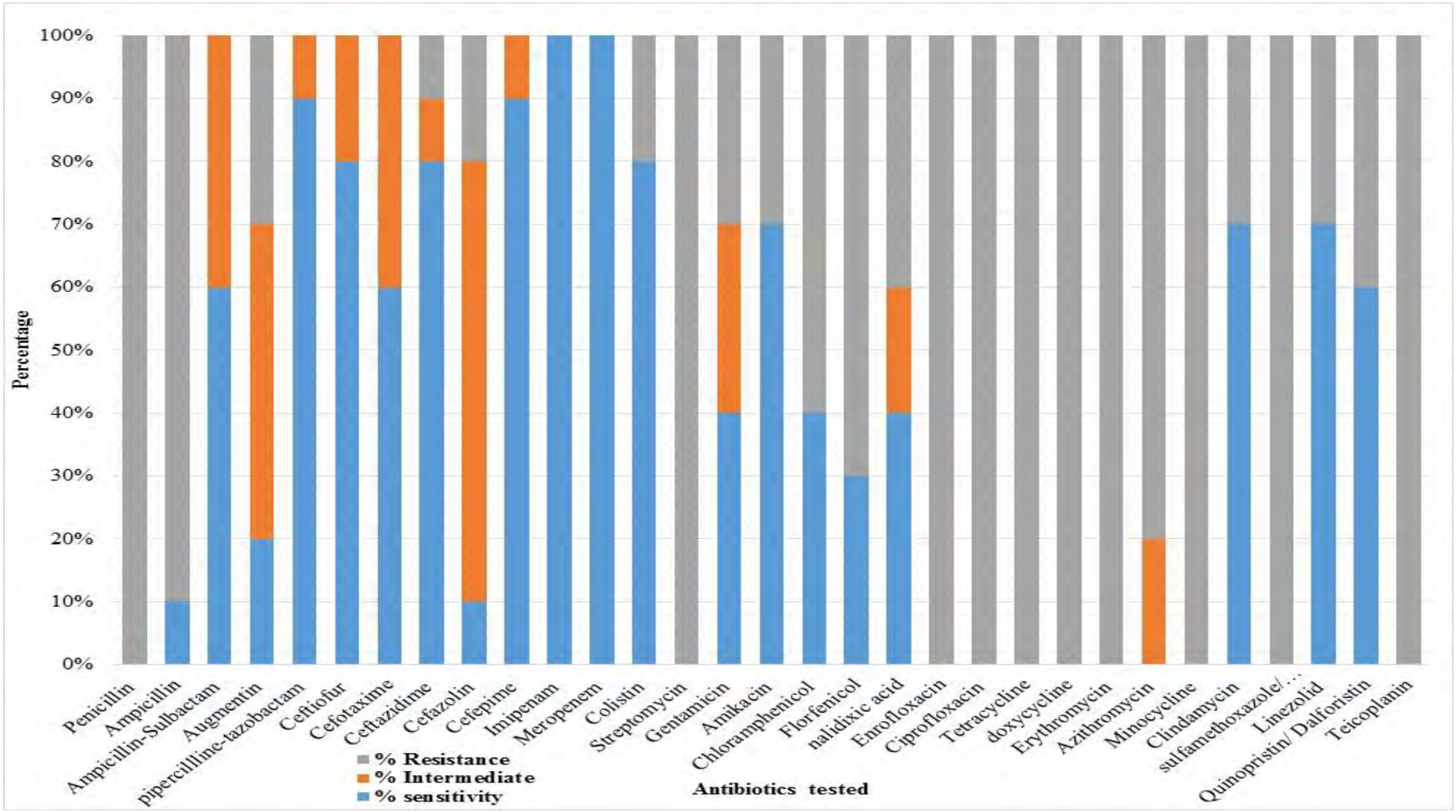

Figure 20: AMR profiling of selected *E. coli* isolated from Rawalpindi

On the other hand, some of the antibiotics were observed with intermediate sensitivity range and these were Cefazoline and Augmentin with a frequency of 70% and 50% respectively (Appendix-XVII, Figure 20). The complete AMR results of all the isolates from Lahore against tested antibiotics with all categories of sensitivity (Sensitive, Resistance, and Intermediate) are depicted in Appendix-XXVI. Furthermore, only 5 (30%) isolates were observed with more than 50% sensitivity against the tested panel of antibiotics. While maximum sensitivity observed was 58% among all the tested *E. coli* isolates (Appendix-XV).

3.4 Genotypic characterization of avian E. coli isolated from healthy chicken

For genotypic characterization 20 AMR genes were selected for 24 isolates, covering each city equally. Maximum number of resistant genes among selected isolates are 9 detected through PCR. Least number of detected AMR gene was for the sensitive isolate with 0 gene detected through PCR. Among Beta-lactamase genes *blaOXA*-48 and *bla*FOX are detected in only 2 isolates which shows 8% prevalence. While *bla*NDM-1 and *bla*SHV-1 are detected only in single isolate whereas *bla*TEM-1 was maximum detected in 22 isolates with 92% prevalence among the resistant isolates. Among all selected isolates *blaCTX*-M-8 was detected in only 4 isolates (table 18). For the class of Polypeptides, the resistance producing genes against Colistin were analyzed. The genes belong to family of mcr (1-9) genes.

There was no detection of mcr-1, mcr-3, mcr-4, mcr-6, mcr-7 and mcr-8 genes was recorded (table 19), however, mcr-2 was detected in 3 (12.5%) isolates, while mcr-5 was detected in 6 (25%) isolates and mcr-9 was detected in 12 (50%) isolates (Table19).For Tetracycline class only two gene were analyzed that were tetA and tetM. Among them tetA showed 92% prevalence with 22 positive reactions of PCR however tetM was present in only 2 (8%) isolates (Table 20). Among the resistance producing genes against Aminoglycosides, aac(3)-II and aph(3)-II are detected only in 2 (8%) isolates however from the same class aac(6')-Ib was detected in 11 (45.6%) isolates (table 21). The amplification of different genes in the selected *E.* coli isolates are shown in figures 21 to 23 visualized through gel documentation system.

Table 18: Identification of antibiotic resistant gene against Beta-lactamase class

Sample ID	*bla*FOX	*bla*NDM-1	*bla*SHV-1	*bla*TEM-1	*bla*CTX-M-8	*bla*OXA-48
21N- 205	-ive	-ive	Positive	Positive	-ive	Positive
21N- 288	-ive	-ive	-ive	Positive	Positive	-ive
20N- 2307	-ive	Positive	-ive	Positive	Positive	Positive
20N-1159	positive	-ive	-ive	Positive	Positive	-ive
20N-1203	-ive	-ive	-ive	Positive	-ive	-ive
20N-1236	-ive	-ive	-ive	Positive	Positive	-ive
20N-1713	-ive	-ive	-ive	Positive	-ive	-ive
20N-1744	-ive	-ive	-ive	Positive	-ive	-ive
20N-2151	-ive	-ive	-ive	Positive	-ive	-ive
20N-1780	-ive	-ive	-ive	Positive	-ive	-ive
21N- 23	positive	-ive	-ive	Positive	-ive	-ive
21N-195	-ive	-ive	-ive	Positive	-ive	-ive
21N-234	-ive	-ive	-ive	-ive	-ive	-ive
21N-284	-ive	-ive	-ive	Positive	-ive	-ive
21N-177	-ive	-ive	-ive	Positive	-ive	-ive
21N-199	-ive	-ive	-ive	Positive	-ive	-ive
21N-339	-ive	-ive	-ive	Positive	-ive	-ive
20N-2240	-ive	-ive	-ive	Positive	-ive	-ive
20N-2030	-ive	-ive	-ive	Positive	-ive	-ive
20N-1952	-ive	-ive	-ive	Positive	-ive	-ive
21N-186	-ive	-ive	-ive	-ive	-ive	-ive
20N-2169	-ive	-ive	-ive	Positive	-ive	-ive
20N-2004	-ive	-ive	-ive	Positive	-ive	-ive
21N-12	-ive	-ive	-ive	Positive	-ive	-ive

Table 19: Identification of Colistin resistant genes.

Sample IDs	mcr-1	mcr-2	mcr-3	mcr-4	mcr-5	mcr-6	mcr-7	mcr-8	mcr-9
21N- 205	-ive	Positive	-ive	-ive	-ive	-ive	-ive	-ive	-ive
21N- 288	-ive	Positive	-ive	-ive	Positive	-ive	-ive	-ive	-ive
20N- 2307	-ive	-ive	-ive	-ive	Positive	-ive	-ive	-ive	-ive
20N-1159	-ive	Positive	-ive	-ive	-ive	-ive	-ive	-ive	-ive
20N-1203	-ive	-ive	-ive	-ive	-ive	-ive	-ive	-ive	-ive
20N-1236	-ive	-ive	-ive	-ive	Positive	-ive	-ive	-ive	positive
20N-1713	-ive	-ive	-ive	-ive	Positive	-ive	-ive	-ive	-ive
20N-1744	-ive	-ive	-ive	-ive	Positive	-ive	-ive	-ive	-ive
20N-2151	-ive	-ive	-ive	-ive	-ive	-ive	-ive	-ive	positive
20N-1780	-ive	-ive	-ive	-ive	-ive	-ive	-ive	-ive	positive
21N- 23	-ive	-ive	-ive	-ive	Positive	-ive	-ive	-ive	positive
21N-195	-ive	-ive	-ive	-ive	-ive	-ive	-ive	-ive	positive
21N-234	-ive	-ive	-ive	-ive	-ive	-ive	-ive	-ive	-ive
21N-284	-ive	-ive	-ive	-ive	-ive	-ive	-ive	-ive	-ive
21N-177	-ive	-ive	-ive	-ive	-ive	-ive	-ive	-ive	-ive
21N-199	-ive	-ive	-ive	-ive	-ive	-ive	-ive	-ive	positive
21N-339	-ive	-ive	-ive	-ive	-ive	-ive	-ive	-ive	positive
20N-2240	-ive	-ive	-ive	-ive	-ive	-ive	-ive	-ive	positive
20N-2030	-ive	-ive	-ive	-ive	-ive	-ive	-ive	-ive	-ive
20N-1952	-ive	-ive	-ive	-ive	-ive	-ive	-ive	-ive	-ive
21N-186	-ive	-ive	-ive	-ive	-ive	-ive	-ive	-ive	positive
20N-2169	-ive	-ive	-ive	-ive	-ive	-ive	-ive	-ive	positive
20N-2004	-ive	-ive	-ive	-ive	-ive	-ive	-ive	-ive	positive
21N-12	-ive	-ive	-ive	-ive	-ive	-ive	-ive	-ive	positive

Table 20: Identification of Tetracycline resistant genes.

Sample IDs	tetA	tetM	Sample IDs	tetA	tetM
21N- 205	Positive	-ive	**21N-234**	-ive	-ive
21N- 288	Positive	Positive	**21N-284**	Positive	-ive
20N- 2307	Positive	Positive	**21N-177**	Positive	-ive
20N-1159	Positive	-ive	**21N-199**	Positive	-ive
20N-1203	Positive	-ive	**21N-339**	Positive	-ive
20N-1236	Positive	-ive	**20N-2240**	Positive	-ive
20N-1713	Positive	-ive	**20N-2030**	-ive	-ive
20N-1744	Positive	-ive	**20N-1952**	Positive	-ive
20N-2151	Positive	-ive	**21N-186**	Positive	-ive
20N-1780	Positive	-ive	**20N-2169**	Positive	-ive
21N- 23	Positive	-ive	**20N-2004**	Positive	-ive
21N-195	Positive	-ive	**21N-12**	Positive	-ive

Table 21: Detection of Aminoglycosides resistant genes.

Sample IDs	aac(3)-II	aac(6')-Ib	aph(3)-II
21N- 205	Positive	Positive	Positive
21N- 288	-ive	Positive	-ive
20N- 2307	Positive	-ive	Positive
20N-1159	-ive	Positive	-ive
20N-1203	-ive	-ive	-ive
20N-1236	-ive	-ive	-ive
20N-1713	-ive	-ive	-ive
20N-1744	-ive	-ive	-ive
20N-2151	-ive	Positive	-ive
20N-1780	-ive	Positive	-ive
21N- 23	-ive	-ive	-ive
21N-195	-ive	Positive	-ive
21N-234	-ive	-ive	-ive
21N-284	-ive	Positive	-ive
21N-177	-ive	Positive	-ive
21N-199	-ive	Positive	-ive
21N-339	-ive	-ive	-ive
20N-2240	-ive	Positive	-ive
20N-2030	-ive	-ive	-ive
20N-1952	-ive	-ive	-ive
21N-186	-ive	Positive	-ive
20N-2169	-ive	-ive	-ive
20N-2004	-ive	-ive	-ive
21N-12	-ive	-ive	-ive

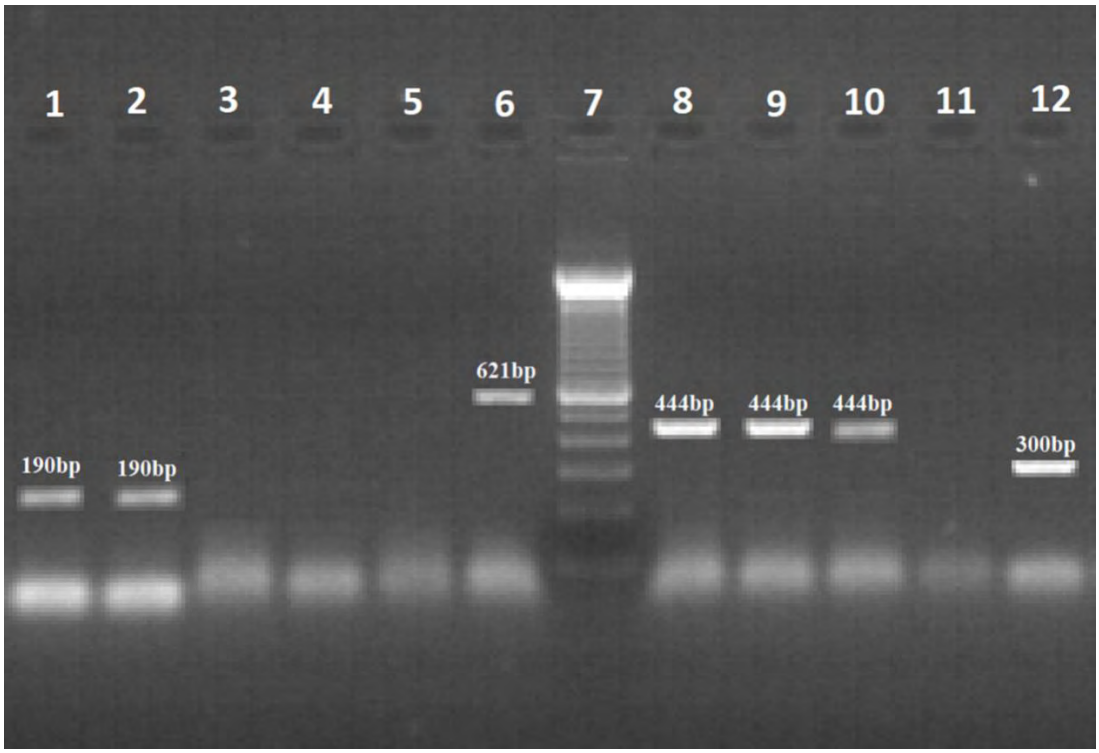

Figure 21: PCR results of amplified blaFOX, blaNDM, blaTEM genes

Lane 1: *bla*FOX gene positive for sample 23 with190bp

Lane 2: *bla*FOX gene positive sample 1159 with190bp

Lane 3: *bla*FOX gene negative for 2307 with 190bp

Lane 4: *bla*NDM gene negative sample 23 with 621bp

Lane 5: *bla*NDM gene negative sample 1159 with 621 bp

Lane 6: *bla*NDM gene positive sample 2307 with 621bp

Lane 7: Marker 100bp DNA step ladder

Lane 8: *bla*TEM gene positive sample 23 with 444bp

Lane 9: *bla*TEM gene positive sample 1159 with 444bp

Lane 10: *bla*TEM gene positive sample 2307 with 444bp

Lane 11: Negative control

Lane 12: Positive control of 300bp

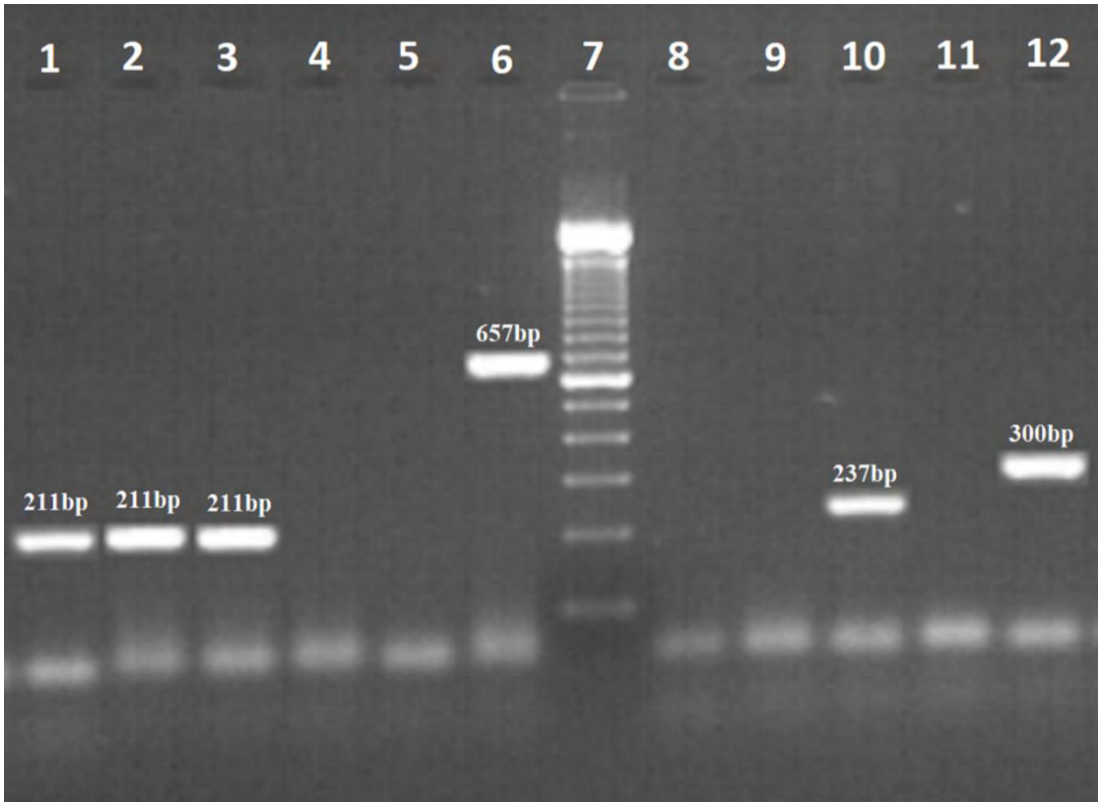

Figure 22: PCR results of amplified tetA, tetM, aac3-II genes

Lane 1: tet A gene positive for sample 23 with 211bp

Lane 2: tet A gene positive sample 1159 with 211bp

Lane 3: tet A gene positive for 2307 with 211bp

Lane 4: tet M gene negative sample 23 With 657bp

Lane 5: tet M gene negative sample 1159 with 657bp

Lane 6: tet M gene positive sample 2307 with 657bp

Lane 7: Marker100bp DNA step ladder

Lane 8: aac3 II gene negative sample 23 with 237bp

Lane 9: aac3 II gene negative sample 1159 with 237bp

Lane 10: aac3 II gene positive sample 2307 with 237bp

Lane 11: Negative control

Lane 12: Positive control of 300bp

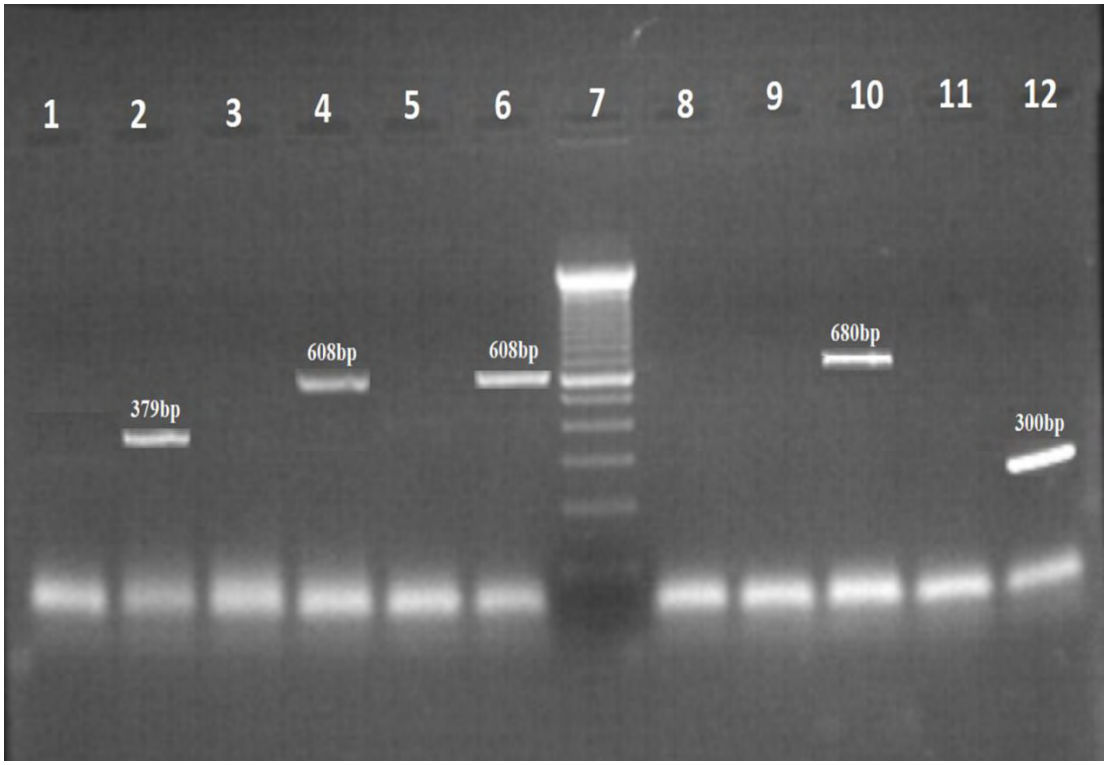

Figure 23: PCR results of amplified mcr-2, mce-5 and aph3-II genes

Lane 1: mcr-2 gene negative for sample 23 with 379bp

Lane 2: mcr-2 gene positive sample 1159 with 379bp

Lane 3: mcr-2 gene negative for 2307 with 379bp

Lane 4: mcr-5 gene positive sample 23 for 608bp

Lane 5: mcr-5 gene negative sample 1159 for 608bp

Lane 6: mcr-5 gene positive sample 2307 with 608bp

Lane 7: Marker100bp DNA step ladder

Lane 8: aph 3 II gene negative sample 23 with 680bp

Lane 9: aph 3 II gene negative sample 1159 with 680bp

Lane 10: aph 3 II gene positive sample 2307 with 680bp

Lane 11: Negative control

Lane 12: Positive control of 300bp

4. DISCUSSION

The poultry industry has gained significance all over the world due to its low cost production of quality meat around the world after commercialization in early 19th century. According to Food and Agriculture Organization of United Nations (FAO) 103.5 million tons of annual global chicken meat production has been estimated which contributed more than 30% to global meat production (Pawar et al., 2016). In food, poultry meat and eggs are the most proficient protein sources (Sebho, 2016). After being commercialized and heavy investments on research, poultry production gained a sudden boost in recent years. A commercial farm currently produces meat in about one and half months in most of the world, and eggs are being produced in 24 weeks after the placement of commercial poultry in the farms. Antibiotics are being used regularly in commercial poultry production as growth promoters, however, these are also utilized as preventive as well as curative agent against various infectious diseases. Furthermore, due to the use of high concentrations of antibiotic agents, poultry meat may exhibit high saturation of antibiotic deposits, which may lead to selection of multidrug resistant pathogens through promotion of AMR genes (Donoghue, 2003).

Escherichia coli (*E. coli*) is a Gram-negative, facultative anaerobic bacterium of the Enterobacteriaceae family. *E. coli* is a main source of the environmental as well as nosocomially derived disease (Liang *et al.*, 2018), and it has also been utilized as a marker of fecal infection to evaluate the microbial nature of surface waters (Gomi *et al.*, 2017).*E. coli* are commensal adherent of the gastrointestinal tract of both human and animals. It has been widely used to screen AMR in healthy food animals in recent years(Nhung, Chansiripornchai and Carrique-Mas, 2017). Moreover, some *E. coli* strains facilitated by poultry are thought to be a possible origin of AMR genes transmitted to human(Overdevest *et al.*, 2011). *Escherichia coli* is one of the mostdeliberately studied microorganism worldwide because of its behavior of continuously changing characteristics (Vila *et al.*, 2016). *E. coli* is a diverse bacterial variety reported with intestinal commensal lines to intestinal pathogenic, and afterwards extra-intestinal pathogenic lines causing urinary lining contamination, sepsis and meningitis (Levings *et al.*, 2005).Although most *E. coli* strains are harmless, however, some strains are pathogenic for both human and animals as well.

Multidrug resistant (MDR) bacteria, usually described as those impervious to three or more antibiotic classes, especially are of great concern because MDR *E. coli* tends to harbor numerous resistance genes and shifts its resistance determinants to other strains, species or genera. The AMR in microorganisms is thought to be the leading public health issue in next coming era, and it is assumed that if not contained properly, there would be more deaths in both humans as well as animals by AMR issue as compared to any other public health issue across the world. Therefore, more work is being carried out across the world to estimate the burden of AMR in both animals and humans through short experimentation and surveillance. The serious practices of anti-microbial in domesticated animals and industries for development and infection prevention, form which clinical exploitation has provoke significant antimicrobial contamination, dangerous to human and other organisms' wellbeing (Ma *et al.,* 2017; Qian *et al.,* 2017). The issue of expanding protection from antimicrobial agents has compromised the health around whole world. Development of beta-lactamase, which hydrolyses and inactivates beta-lactam antimicrobial agents, has been quite possibly the main opposition mechanism of various bacterial species, predominantly in the Enterobacteriaceae family (Kaftandzieva et al., 2011). Natural chicken fertilizers have been demonstrated to be an excellent repository of MDR bacteria and AMR genes(Cui *et al.,* 2016) that speeds up transformative patterns toward AMR, are a significant threat to human and other animals(Harbarth *et al.,* 2015; World Health Organization, 2015).The consumption of antibiotics in poultry production rises the selection pressure for antibiotic-impervious bacteria (Diarra & Malouin, 2014).

In Pakistan commercial poultry production is the second largest industry and effervescent segments of agriculture industry providing employment and income for about 1.5 million people (Anonymous, 2021). Recently Pakistan has developed it strategy for AMR surveillance in healthy food animals and the strategy was implemented as pilot phase in selected areas of Pakistan according to the guidelines developed by FAO. The current study was designed to evaluate AMR in *E. coli* isolated from healthy chicken in Pakistan. The samples were collected by NRLPD and provincial coordinating units according to the AMR surveillance strategy in food animals focusing on sampling of broiler chickens form live bird markets. For this purpose 785 caecal samples were collected from selected areas of the country during

July-2020 to February-2021. The samples were transported to NRLPD using serile and biosafe containment practices using specified sample transportation boxed with ice packs. The samples were analyzed for isolation and identification of of bacterial pathogens from which 621 *E. coli*isolates were identified thourgh biochemical testing. These comprise about 79% recovery of *E. coli* from caecal samples of apparently healthy poultry. Of these 621 isolates, 221 (35%) pure *E. coli* were subjected to AST analysis and found that most of the isolates were phenotypically 100% resistant to Penicillin and Doxycycline. Whereas more than 50% resistance in tested isolates was observed against 18 (58%) out of 31 antibiotics analyzed by AST and these include Ampicillin, Ampicillin-Sulbactam, Azithromycin, Chloramphenicol, Ciprofloxacin, Clindamycin, Doxycycline, Enrofloxacin, Erythromycin, Florfenicol, Linezolid, Nalidixic acid, Penicillin, Quinupritin-Dalfopristin, Streptomycin, Co-trimoxazole, Tetracycline, and Teicoplanin (Table 17). Highest sensitivity was observed against Pipracilline/Tazobactam, Amikacin and Meropenam antibiotics showing sensitivity in 88%, 80% and 79% respectively. Among these most resistance was observed in the *E.coli* isolated from Gilgit samples exhibiting 100% resistance against Penicillin, Streptomycin, Nalidixic Acid, Enrofloxacin, Ciprofloxacin, Tetracycline, Quinupristin/dalfopristin (Fig-14). While Penicillin and Doxycycline were found 100% resistant against the tested *E.coli* from most of the regions (Figures 13-20). The most sensitive isolates were observed with sensitivity frequency of 89% while most resistant isolates were observed with a frequency of 90% resistance (Appendix XIX-XXVI).

Based on the phenotypic AMR pattern, 24 isolates were subjected to genotypic characterization against 20 AMR genes of 4 antibiotic classes which include B-lactams, Aminoglycosides, Tetracyclins and Polypeptides (Colistin). Among Beta-lactamase *blaTEM* was found in 22 (92%) isolates, while *blaCTX*-M was found in 4 (17%) isolates. The beta-lactamase producing *blaOXA* and *bla*FOX, were found in 2 (8%) isolates while *blaNDM*-1, *blaSHV*-1, weredetected in 1 (4%) isolates (Table-18). For Colistin resistance producing genes, mcr 1 to mcr 9 were tested and only mcr-9, mcr-5 and mcr-2 were detected with mcr-9 was found in 12 (50%), mcr-5 detected in 6 (25%) and mcr-2 was detected in 3 (13%) isolates (Table 19), however, mcr-1 reported previously was not detected in any of the isolates tested.Among Tetracycline tetA showed highest prevalence with 92% and detected in 22 isolates while tetM was

detected in 2 (8%) isolates (Table 20). Among the prevalence of Aminoglycosides resistance producing genes, aac(6')-Ib was found to be 46% as it was detected in 11 isolates while of aac(3)-II, aph(3)-II was 8% each with detection in 2 isolates (Table 21).

Among the tested antibiotics Ampicillin was found with >80% resistant except in the *E. coli* isolated from Karachi and Peshawar. There was above 70% resistance observed against Streptomycin in all the regions except Quetta and Karachi which has the resistance value of above 47%. The Resistance against Chloramphenicol is above 55% across the country except Quetta which has a resistance frequency of 17% (Figure 13-20). Similar was observed for Florfenicol with 60% resistance for all areas of Pakistan except Quetta. The resistance against Nalidixic Acid for all area was found above 80% except for Quetta and Karachi. Above 70% resistance was observed against Ciprofloxacin, while resistance in Tetracycline was above 90% for all area except Lahore, Karachi and Peshawar for which resistance value is about 70%. The observed resistance against Doxycycline in different regions of Pakistan was above 90% except for Peshawar, Muzaffarabad and Karachi. Against Erythromycin the resistance value is above 85% for all selected area of Pakistan except Karachi. There was more than 70% resistance observed against Linezolid except Karachi and Quetta. Teicoplanin was observed with above 80% resistance in all areas of Pakistan except Peshawar and Karachi (Figure 13-20).

The resistance values for Islamabad and Rawalpindi are observed almost in similar range difference against all antibiotics. There is a possibility of geographical closeness between the two locations and retail shop holders get supplies from the nearest location to them. On review of chicken supply history, it is also observed that in Islamabad and Rawalpindi broiler supplies are mostly provided from similar area i.e., Abbottabad, Rawalpindi, Islamabad and Sargodha regions. Similar is the case for Quetta and Karachi, as there is frequent flock supplies between the two areas where Quetta is getting most of its marketable poultry supply from Hub City near to Karachi (Sindh) and from Multan and DG Khan areas of Punjab. In the area of Gilgit the commercial poultry production is not developed and there is no broiler farms avialble in all the region of Gilgit-Baltistan. Broiler supplies are provided in Gilgit from different locations which are Mansehra, Swat and other northern areas of province

KP. Almost all first-generation antibiotics were observed resistant in the tested *E.coli* strainsrecovered across the country perhaps there are similar animal husbandry practices may also be present. The similarities in resistance pattern between distinct areas are observed due to the resistance against first generation of all antibiotic classes used in this study. There might be a possibility that local farmers are frequently using similar antibiotics for the purpose of growth promotion of their flocks. The variations in resistance pattern among different area of Pakistan may be due to the application of different poultry medical practices in some area, as Quetta and Karachi usually showed less resistance values as compared to other area of Pakistan.

According to a study in Brazil resistance rates to penicillin and ampicillin were around 75% and 65%. While the same research depicted that in Spain the antimicrobial resistance rate against ampicillin was approximately 70%. While variation among the resiatnce against Cotrimoxazole was observed in Brazil (Roth *et al.,* 2016). In Pakistan Ampicillin is observed as 80% resistant and against Penicillin resistance rate is 100% under current study. Other studies presented resistance rates of 27% to 28% for the combination of sulfamethoxazole and trimethoprim (Pessanha and Filho, 2001; Korb *et al.*,2015). While in Czech Republic, 30% resistance was observed against Co-trimoxazole(Bardoň et al., 2018). In current studies, variations among the resistance pattern against co-trimoxazole was observedin all the areas except Peshawar and Karachi where its resistance rate observed was 54% and 42% respectively.In Spain antimicrobial resistance against ciprofloxacin in *E. coli* was reported to be 17% in 2001 and reported as 91% in 2016 (Roth *et al.,* 2016) whereas in 84% resistance was observed against Ciprofloxacin in current study. Increased resistance against Nalidixic Acid was reportedfrom 60% to 88% during 2014-16 in European countries, while resistance against tetracycline was reported as 70% in 2016. Whereas low Colistin resistance was observed during this period(Roth *et al.,* 2016), however current study depicted high resistance rate against Colistin (>50%)while resistance against Nalidixic acid is 89%. There may be some increasing patterns of drug resistance in the tested microbial organisms in Pakistan as well, as there was no comprehensive and coordinated work was previously done in healthy poultry. However, variable resistance patterns in different regions of Pakistan were also reported in short studies. The only coordinated research was carried out by Rafique and others during 2016-2018, but it was carried out in domestic chicken and

reported as 100% resistance against chloramphenicol, colistin while 93% against doxycycline and 92% against ampicillin. On the other hand gentamicin and ciprofloxacin were observed with 86% and 67% resistance respectively.(Gohar et al., 2015; Rafique et al., 2020; Yasin et al., 2019),which was almost similar in current study except for Gentamycin and Colistin with 34% and 50% resistance respectively. Further most of work around the world reported increasing patterns of AMR whether in developing or developed countries (Roth *et al.,* 2016; Pessanha and Filho, 2001; Korb *et al.,*2015)

The main objective of the current study was to evaluate AMR on genomic level, and for these 20 genes responsible to produce AMR in different bacterial species were identified and evaluated through PCR. The antibiotics covered with these genes were B-lactams (Penicillins, Cephalosporins and Carbapenems), Aminoglycosides, Polypeptides (Colistin) and Tetracyclines. The results depicted high prevalence of*bla*TEM and tetA (92% each) for B-lactams and Tetracyclines followed by mcr-9 (50%), aac(6′)- Ib (46%), mcr-5 (25%), *bla*CTX-M (17%) and mcr-2 (13%) whereas*bla*FOX, blaOXA, aac(3)-II, aph(3)-II, and tetM were detected in 8% isolates tested. Some of the frequencies recorded in the current study were in accordance to the previous studies carried out in the country however, some of the observation were found contrary to the previous observations.

Most of the *E. coli* carried tetA trailed by tetB according (DePaola & Roberts, 1995), where the two recognized tet genes belonged to a group of gene famous for *E. coli*. The greater part of the tet determinants were related with their assembled components, which clarifies their wide conveyance among bacteria (Delsol et al., 2003; Thaker et al., 2010). A portion of the genome in a study reported high prevalence oftetA or tetB genes. A large portion of the *E. coli* contained tetA determinant, and this was observed in concurrence with Miranda who reported that 44% of Gram negative microbes contains tetA while few isolates contained tetB(Miranda *et al.,* 2003). There were some other reports that 60% of the *E. coli* in bovines contains tetB gene(SN *et al.,* 2013) but none of the researcher reported so high prevalence of tetB in poultry at least. Whereas in another report it was presented that just 2% of Shigella spp. and *E. coli* sheltered from similar topographical areas harbored both tetA and tetB genes (Hartman *et al.,* 2003). Dolejska reported that none

of the *E. coli* in their study carried a mix of tetA and tetB(Dolejska *et al.,* 2007). Notwithstanding, tetM was found phenomenal in coliforms that were Gram-negative like *E. coli* (Chang *et al.,* 2008). tetM was distinguished in 13 of the 99 (13.1%) tetracycline resistant *E. coli*. That might demonstrated a potential exchange of tetM gene from other digestive system bacteria (Jurado-Rabadán *et al.,* 2014). According to Song et al,. no Tetracycline gene was detected in their investigation(Song *et al.,* 2020). Whereas in a previous study from PakistanTetM was the most prevalent and detected in 71% *E.coli* of avian origin through WGS (Rafique et al., 2020), however, in current study tetM was detected in only 8% isolates tested, whereas tetA was the most prevalent with 92% detections.

The revelation of plasmid-intervened colistin resistant gene mcr-1 in *E. coli* isolates from Chinese patients and other organism addressed a high threat of colistin resistance (Liu *et al.,* 2016). By and large, 94.7% of the mcr-1–positive isolates were found with beta-lactamase resistance genes (Song *et al.,* 2020). According to one study the mcr-4 prevalence was 72.8%, while 26.4% were positive for mcr-1,and 3.6% was the prevalence of mcr-5 gene (Flament-simon *et al.,* 2018). Whereas in current studies we detected mcr-9 with 50% prevalence in Pakistan while mcr-5 with 25% and mcr-2 with 13% detections, while no mcr-1 gene is detected in Pakistan. Whereas other colistin resistance genes of mcr family (mcr-3, mcr-4, mcr-6, mcr-7 and mcr-8),were also not detected under current study. However, mcr-1 gene was reported as the only gene available in genepool of bacterial isolates analyzed in Pakistan previously (Mohsin et al., 2019; Rafique et al., 2020)and also reported as transmissible to the other bacterial pathogens as well (Rafique et al., 2019). Where it was also reported that in commensal *Escherichia coli* derived from food-animals, the plasmid-origin mcr-1 gene was found adaptable to other bacterial species as well (Tong *et al.,* 2015). Further examination uncovered versatile defense mechanism against colistin in *E. coli*. After the underlying report from China, a few new variations of the mcr-1 genes (mrc1-mcr-9) were further identified (Aghapour *et al.,* 2019) therefore not much investigations were carried out for mcr family variants specially on mcr-9 in Pakistan.

The most important genes producing AMR in bacterial species are Beta lactamases against Penicillin, Cephalosporins and Carbapenems, where some of the

genes were also reported responsible for resistance in Aminoglycosides with some altered mechanisms. The Beta-lactamase *bla*TEM with highest prevalence rate of 93.5%, while *bla*CTX-M with 82.6% detection were reported earlier. Whereas *bla*SHV was at very low prevalence rate of 4% (Jena *et al.,* 2017), while in another investigation 58% prevalence for *bla*TEM was recorded, whereas *bla*CTX-M was found with 74% and *bla*SHV with 27% prevalence (Gundran *et al.,* 2019). Diabonga *et al.,* 2016 found 20.6% prevalence for *bla*TEM and 62% for *blaCTX*-M, while in case of *bla*SHV, low prevalence of about 5% was observed. According to another research β-lactamase genes *bla*TEM, *bla*SHVand *bla*FOX were detected by PCR and the prevalence was reported as 96.9%, 16.9%, and 27.7% respectively in *E. coli* isolates recovered from poultry (Osman *et al.,* 2018). Inf our findings *bla*TEM is detected in 92% *E.c oli* isolates, *bla*NDM along with *bla*SHVwere dected in 4%, whereas *bla*FOXalong with*bla*OXA-48 are present in 8% and *bla*CTX-M is detcted in 17 % *E. Coli* isolates tested.

Among the AMR genes responsible for resistance against Aminoglycosides, 3 genes including aph(3)-II, aac(3)-II, aac(6′)-Ib were tested through PCR, it was found that aac(6′)-Ib was present in most of isolates tested with 46% detections, whereas the other two genes were detected in 8% isolates only. However, genotypic resistance against Aminoglycosides wasreported as 23.3% by aac(6′)-II while aac(6′)-Ib was not linked with any resistance against Gentamicin(Hassan *et al.,* 2012). In another study 78.7% (59/75) bacterial community tested contained Aminoglycoside resistance gene aph-3(Song *et al.,* 2020).As indicated by another examination the presence of aac (6′)-Ib was observed as 11.3% (Pitout *et al.,* 2004). Xiao et al., reported thataac (3)-II was accounted for AMR among Chinese clinical isolates of *E. coli*. According to their investigations, the aac(3)-II was accounted for resistance in 162 isolates genotypically, whereas aph(3)-II gene was present in only 20 isolates. The prevailing enzyme, aac(3)-II was observed with the ability to exhibit resistance againstGentamicin, Tobramycin, Netilmicin and Dibekacin (Xiao and Hu, 2012).

In current studies, the identified genes for resistance genotypically were *bla*TEM,*bla*CTX-M, *bla*SHV, *bla*OXA, *bla*FOX, *bla*NDM for resisrance against β-lactams, while mcr-9, mcr-5 and mcr-2 detcted against Colistin, whereas tetM and tetA for Tetracyclines while aph(3)-II, aac(3)-II, aac(6′)-Ib were stected as

Aminoglycosides resistance producing genes. However, only mcr-1 gene was reported for colistin resistance from Pakistan previously (Mohsin et al., 2019; Rafique et al., 2020)whereas no *E. coli* isolate, tested under current study, was found harbouring mcr-1 gene. Further the previously report genes from Pakistan were mph against Aminoglycosides,tetAgenes for Tetracyclines whereas *bla*CTX-M were detected against β-lactams (Mohsin et al., 2019; Rafique et al., 2020)

On the basis of these results, it could be concluded that the chickens sold in retail sectors of Pakistan are harboring significant population of multi-drug resistant *E. coli*. The presence of MDR *E. coli* may be resulted by the persistent exposure of birds to multiple antibiotics during the rearing period. For containment of this issue, public health authorities ought to control non-judicial utilization of antibiotics at poultry production and stringent measure need to be adopted for future control of AMR issue in the country as well as globally. Further a surveillance network for detection of AMR in healthy poultry and other food animals need to be established on sustainable basis. While data collection and analysis need to be carried out at larger scale for exact depiction of the AMR situation in the country. The control measures may be devised according to the situation of AMR in the country in future by the relevant authorities.

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6. APPENDICES

Appendix-I: Peptone Water

Cat #	CM0009
Peptone	10.0g
Sodium chloride	5.0g

Appendix-II: MacConkey Agar

Cat #	CM0007
Peptone	20g
Lactose	10.0g
Bile salts	5.0g
Sodium chloride	5.0g
Neutral Red	0.075g
Agar	12.0g

Appendix-III: Eosin Methylene Blue

Cat #	**CM0069**
Peptone	10.0g
Lactose	10.0g
Di-Potassium Hydrogen Phosphate	2.0g
Eosin Y	0.4g
Methylene Blue	0.06g
Agar	15.0g

Appendix-IV: Nutrient Agar

Lab-Lemco' powder	1.0
Yeast extract	2.0
Peptone	5.0
Sodium chloride	5.0
Agar	15.0

Appendix-V: Tryptone water

Cat #	CM0087
tryptone	10.0g
Sodium chloride	5.0g

Appendix-VI: Triple Sugar Iron Test

Cat #	CM0277
Lab-Lemco Powder	3.0g
Yeast extract	3.0g
Peptone	20.0g
Lactose	10.0g
Sodium chloride	5.0g
Sucrose	10.0g
Glucose	1.0g
Ferric citrate	0.3g
Sodium thiosulphate	0.3g
Phenol red	0.024g
Agar	12.0g

Appendix-VII: ONPG Biolab

Cat #	EONB20500
o-Nitrophenyl-β-D-Galactopyranoside	0.30 g
Buffer	0.3g

ONPG base was prepared according to the manufacturer's instructions. ONPG supplement was added in autoclaved ONPG base. Supplement was dissolved in 5ml of distilled water and poured in the ONPG base of 500ml volume.

APPENDIX-VIII: Muller Hinton Agar

Cat #	CM0337
Beef dehydrated infusion	300.0g
Casein hydrolysate	17.5g
Starch	1.5g
Agar	17.0g

APPENDIX-XI: (1.5%) Agarose Gel for Electrophoresis

Agarose	0.8 gm
IX TBE Buffer	80 ml
Ethidium Bromide	8ul

0.8g of Agarose was dissolved in 80 ml of 1X TBE Buffer and 8ul of Ethidium bromide. The mixture was heated until it boiled and was poured in a mould to set with a gel comb.

APPENDIX-X: 5X- Tris Borate EDTA Buffer (TBE)

Stock solution of 5X/liter was prepared by adding the following:

TRIS Pure (Research Organics, Cat # 30950T)	54 gm
Boric Acid (Fisher Scientific, Cat # 10043-35-3)	27.5 gm
0.5M EDTA (pH 8.00) (MP Biomedicals, Cat# 195173)	20 ml

All above mentioned chemicals were dissolved in 980 ml of pure distilled water
Working solution was prepared as follows
1x TBE was prepared by dissolving 200 ml of 5X Stock solution in 800 ml of distilled water.

APPENDIX-XIDetailed frequencies in sensitivity, resistance and intermediate for samples of Islamabad according to CLSI, 2020 and WHONET

Antibiotics	% S	% I	% R	Antibiotics	% S	% I	% R
Augmentin	48%	19%	32%	Enrofloxacin	3%	3%	94%
Amikacin	81%	15%	5%	Erythromycin	2%	2%	97%
Ampicillin (AMP-R)	13%	2%	85%	Florfenicol	32%	2%	66%
Ampicillin-Sulbactam	35%	24%	40%	Gentamicin	56%	6%	37%
Azithromycin	47%	0%	53%	Imipenam	56%	13%	31%
Cefazolin	32%	32%	35%	Linezolid	3%	0%	97%
Cefepime	77%	10%	13%	Meropenem	82%	10%	8%
Cefotaxime	81%	8%	11%	Minocycline	50%	26%	24%
Ceftazidime	61%	18%	21%	nalidixic acid	3%	3%	94%
Ceftiofur	94%	2%	5%	Penicillin	0%	0%	100%
Chloramphenicol	31%	0%	69%	pipercillline-tazobactam	90%	2%	8%
Ciprofloxacin	3%	6%	90%	Quinopristin/ Dalforistin	11%	3%	85%
Clindamycin	0%	3%	97%	Streptomycin	10%	0%	90%
Colistin	56%	0%	44%	sulfamethoxazole/ trimethoprim	26%	5%	69%
doxycycline	8%	0%	92%	Tetracycline	3%	2%	95%

S= Sensitive; I= Intermediate; R= Resistance

APPENDIX-XII: Detailed AMR frequencies for the area of Karachi according to CLSI, 2020 and WHONET

Antibiotics	% S	% I	% R	Antibiotics	% S	% I	% R
Augmentin	17%	61%	22%	Erythromycin	0%	6%	94%
Amikacin	78%	22%	0%	Florfenicol	11%	0%	89%
Ampicillin	6%	0%	94%	Gentamicin	50%	17%	33%
Ampicillin-Sulbactam	22%	17%	61%	Imipenam	56%	6%	39%
Azithromycin	56%	0%	44%	Linezolid	0%	6%	94%
Cefazolin	6%	50%	44%	Meropenem	89%	6%	6%
Cefepime	50%	22%	28%	Minocycline	33%	33%	33%
Cefotaxime	33%	33%	33%	nalidixic acid	0%	0%	100%
Ceftazidime	33%	39%	28%	Penicillin	0%	0%	100%
Ceftiofur	83%	11%	6%	pipercillline-tazobactam	72%	28%	0%
Chloramphenicol	6%	0%	94%	Quinopristin/ Dalforistin	0%	0%	100%
Ciprofloxacin	0%	0%	100%	Streptomycin	0%	0%	100%
Clindamycin	6%	6%	89%	sulfamethoxazole/ trimethoprim	11%	0%	89%
Colistin	44%	0%	56%	Tetracycline	0%	0%	100%
doxycycline	6%	0%	94%	Enrofloxacin	0%	0%	100%

S= Sensitive; I= Intermediate; R= Resistance

APPENDIX-XIII Detailed Frequencies in sensitivity, resistance and intermediate for samples of Gilgit according to CLSI, 2020 and WHONET

Antibiotics	% S	% I	% R	Antibiotics	% S	% I	% R
Augmentin	58%	26%	16%	Erythromycin	16%	6%	77%
Amikacin	90%	6%	3%	Florfenicol	39%	0%	61%
Ampicillin (AMP-R µg/disk)	23%	6%	71%	Gentamicin	74%	0%	26%
Ampicillin-Sulbactam	55%	13%	32%	Imipenam	84%	10%	6%
Azithromycin	55%	0%	45%	Linezolid	29%	10%	61%
Cefazolin	45%	23%	32%	Meropenem	84%	6%	10%
Cefepime	65%	16%	19%	Minocycline	52%	32%	16%
Cefotaxime	65%	19%	16%	nalidixic acid	3%	10%	87%
Ceftazidime	55%	23%	23%	Penicillin	0%	0%	100%
Ceftiofur	77%	16%	6%	pipercillline-tazobactam	90%	6%	3%
Chloramphenicol	42%	0%	58%	Quinopristin/ Dalforistin	35%	13%	52%
Ciprofloxacin	19%	6%	74%	Streptomycin	42%	0%	58%
Clindamycin	16%	19%	65%	sulfamethoxazole/ trimethoprim	48%	10%	42%
Colistin	48%	0%	52%	Teicoplanin	26%	13%	61%
doxycycline	0%	0%	100%	Tetracycline	26%	0%	74%
Enrofloxacin	19%	13%	68%				

Sensitive; I= Intermediate; R= Resistance

APPENDIX-XIV: Detailed Analysis AMR frequencies for Quetta according to CLSI, 2020 and WHONET

Antibiotics	% S	% I	% R	Antibiotics	% S	% I	% R
Augmentin	34%	21%	45%	Erythromycin	7%	0%	93%
Amikacin	59%	24%	17%	Florfenicol	17%	3%	79%
Ampicillin (AMP-R µg/disk)	7%	0%	93%	Gentamicin	41%	21%	38%
Ampicillin-Sulbactam	28%	17%	55%	Imipenam	38%	31%	31%
Azithromycin	28%	0%	72%	Linezolid	10%	10%	79%
Cefazolin	24%	34%	41%	Meropenem	90%	7%	3%
Cefepime	69%	10%	21%	Minocycline	31%	21%	48%
Cefotaxime	83%	7%	10%	nalidixic acid	3%	3%	93%
Ceftazidime	34%	34%	31%	Penicillin	0%	0%	100%
Ceftiofur	90%	7%	3%	pipercillline-tazobactam	90%	7%	3%
Chloramphenicol	41%	3%	55%	Quinopristin/ Dalforistin	7%	7%	86%
Ciprofloxacin	10%	0%	90%	Streptomycin	17%	0%	83%
Clindamycin	10%	7%	83%	sulfamethoxazole/ trimethoprim	28%	7%	66%
Colistin	52%	0%	48%	Teicoplanin	10%	0%	90%
doxycycline	0%	0%	100%	Tetracycline	10%	0%	90%
Enrofloxacin	10%	10%	79%				

S= Sensitive; I= Intermediate; R= Resistance

APPENDIX-XV: Detailed AMR frequencies for Lahore isolates.

Antibiotics	% S	% I	% R	Antibiotics	% S	% I	% R
Augmentin	35%	38%	27%	Erythromycin	4%	8%	88%
Amikacin	76%	16%	8%	Florfenicol	20%	4%	76%
Ampicillin (AMP-R µg/disk)	13%	0%	88%	Gentamicin	60%	12%	28%
Ampicillin-Sulbactam	48%	20%	32%	Imipenam	64%	24%	12%
Azithromycin	44%	0%	56%	Linezolid	16%	8%	76%
Cefazolin	16%	8%	76%	Meropenem	56%	32%	12%
Cefepime	36%	36%	28%	Minocycline	40%	32%	28%
Cefotaxime	24%	40%	36%	nalidixic acid	0%	24%	76%
Ceftazidime	36%	28%	36%	Penicillin	0%	0%	100%
Ceftiofur	60%	20%	20%	pipercillline-tazobactam	76%	8%	16%
Chloramphenicol	20%	4%	76%	Quinopristin/ Dalforistin	20%	0%	80%
Ciprofloxacin	16%	4%	80%	Streptomycin	23%	4%	73%
Clindamycin	12%	4%	84%	sulfamethoxazole/ trimethoprim	28%	8%	64%
Colistin	24%	0%	76%	Teicoplanin	12%	4%	84%
doxycycline	0%	0%	100%	Tetracycline	19%	4%	77%
Enrofloxacin	20%	8%	72%				

S= Sensitive; I= Intermediate; R= Resistance

APPENDIX-XVI: Detailed AMR frequencies for isolates derived from Peshawar samples according to CLSI, 2020 and WHONET.

Antibiotics	% S	% I	% R	Antibiotics	% S	% I	% R
Augmentin	54%	31%	15%	Erythromycin	8%	4%	88%
Amikacin	85%	12%	4%	Florfenicol	35%	4%	62%
Ampicillin (AMP-R µg/disk)	31%	0%	69%	Gentamicin	58%	15%	27%
Ampicillin-Sulbactam	50%	15%	35%	Imipenam	65%	23%	12%
Azithromycin	35%	0%	65%	Linezolid	12%	15%	73%
Cefazolin	46%	19%	35%	Meropenem	81%	8%	12%
Cefepime	73%	19%	8%	Minocycline	50%	23%	27%
Cefotaxime	54%	35%	12%	nalidixic acid	19%	0%	81%
Ceftazidime	35%	38%	27%	Penicillin	0%	0%	100%
Ceftiofur	81%	15%	4%	pipercillline-tazobactam	81%	8%	12%
Chloramphenicol	31%	8%	62%	Quinopristin/ Dalforistin	23%	0%	77%
Ciprofloxacin	15%	0%	85%	Streptomycin	27%	0%	73%
Clindamycin	8%	8%	85%	sulfamethoxazole/ trimethoprim	38%	8%	54%
Colistin	42%	0%	58%	Teicoplanin	23%	4%	73%
doxycycline	31%	8%	62%	Tetracycline	23%	0%	77%
Enrofloxacin	27%	8%	65%				

S= Sensitive; I= Intermediate; R= Resistance

APPENDIX-XVII: Detailed AMR frequencies for isolates derived from Muzaffarabad, according to CLSI, 2020 and WHONET

Antibiotics	% S	% I	% R	Antibiotics	% S	% I	% R
Augmentin	47%	28%	25%	Erythromycin	5%	0%	95%
Amikacin	50%	50%	0%	Florfenicol	7%	0%	93%
Ampicillin (AMP-R µg/disk)	33%	0%	67%	Gentamicin	61%	7%	32%
Ampicillin-Sulbactam	11%	11%	79%	Imipenam	46%	23%	31%
Azithromycin	48%	0%	52%	Linezolid	0%	5%	95%
Cefazolin	46%	16%	38%	Meropenem	55%	35%	10%
Cefepime	64%	27%	9%	Minocycline	0%	50%	50%
Cefotaxime	46%	23%	31%	nalidixic acid	5%	10%	86%
Ceftazidime	10%	60%	30%	Penicillin	5%	0%	95%
Ceftiofur	47%	27%	27%	pipercillline-tazobactam	76%	24%	0%
Chloramphenicol	31%	0%	69%	Quinopristin/ Dalforistin	0%	0%	100%
Ciprofloxacin	5%	20%	75%	Streptomycin	33%	0%	67%
Clindamycin	19%	4%	77%	sulfamethoxazole/ trimethoprim	0%	0%	100%
Colistin	9%	0%	91%	Teicoplanin	0%	0%	100%
doxycycline	0%	0%	100%	Tetracycline	5%	0%	95%
Enrofloxacin	9%	32%	59%	Erythromycin	5%	0%	95%

S= Sensitive; I= Intermediate; R= Resistance

APPENDIX-XVIII:Detailed AMR frequencies in sensitivity, resistance and intermediate for the isolates derived from Rawalpindi

Antibiotics	% S	% I	% R	Antibiotics	% S	% I	% R
Augmentin	20%	50%	30%	Erythromycin	0%	0%	100%
Amikacin	70%	0%	30%	Florfenicol	30%	0%	70%
Ampicillin (AMP-R µg/disk)	10%	0%	90%	Gentamicin	40%	30%	30%
Ampicillin-Sulbactam	60%	40%	0%	Imipenam	100%	0%	0%
Azithromycin	0%	20%	80%	Linezolid	70%	0%	30%
Cefazolin	10%	70%	20%	Meropenem	100%	0%	0%
Cefepime	90%	10%	0%	Minocycline	0%	0%	100%
Cefotaxime	60%	40%	0%	nalidixic acid	40%	20%	40%
Ceftazidime	80%	10%	10%	Penicillin	0%	0%	100%
Ceftiofur	80%	20%	0%	pipercillline-tazobactam	90%	10%	0%
Chloramphenicol	40%	0%	60%	Quinopristin/ Dalforistin	60%	0%	40%
Ciprofloxacin	0%	0%	100%	Streptomycin	0%	0%	100%
Clindamycin	70%	0%	30%	sulfamethoxazole/ trimethoprim	0%	0%	100%
Colistin	80%	0%	20%	Teicoplanin	0%	0%	100%
doxycycline	0%	0%	100%	Tetracycline	0%	0%	100%
Enrofloxacin	0%	0%	100%				

S= Sensitive; I= Intermediate; R= Resistance

Appendix-XIX: Complete AST results of isolates derived from the samples derived from Islamabad.

ID number	CITY	Penicillin	Ampicillin (AMP-R µg/disk)	Ampicillin-Sulbactam	Augmentin	pipercilline-tazobactam	Ceftiofur	Cefotaxime	Ceftazidime	Cefazolin	Cefepime	Imipenam	Meropenem	Colistin	Streptomycin	Gentamicin	Amikacin	Chloramphenicol	Florfenicol	nalidixic acid	Enrofloxacin	Ciprofloxacin	Tetracycline	doxycycline	Erythromycin	Azithromycin	Minocycline	Clindamycin	Sulfamethoxazole/ trimethoprim	Linezolid	Quinopristin/ Dalfloristin	Teicoplanin
20N-1200	isb	R	R	S	R	S	S	S	I	R	I	R	S	S	S	S	S	R	R	I	R	S	R	R	R	S	I	R	R	R	R	R
20N-1963	isb	R	R	I	I	S	S	R	S	R	I	S	S	R	R	R	S	R	I	I	S	R	R	I	R	R	S	R	S	R	S	R
20N-1964	isb	R	R	S	I	S	S	I	S	I	S	S	S	R	R	S	S	R	R	R	R	R	R	R	R	R	S	R	S	R	S	R
20N-1965	isb	R	R	S	I	S	S	S	S	S	S	R	S	R	R	S	S	R	R	R	S	R	R	I	R	S	S	R	S	R	S	S
20N-1966	isb	R	R	S	S	S	S	S	S	S	S	R	S	R	R	S	S	R	R	R	S	R	R	R	R	S	S	R	S	R	S	R
20N-1967	isb	R	I	S	I	S	S	S	S	I	S	S	S	S	I	S	S	R	R	R	S	S	R	S	R	S	S	R	S	R	S	R
20N-1969	isb	R	R	I	I	S	S	S	S	R	S	S	S	R	R	S	S	R	R	R	R	R	I	S	R	S	I	R	R	R	R	R
20N-1970	isb	R	S	S	S	S	S	S	R	S	I	S	I	S	S	S	I	R	R	R	R	S	S	R	R	R	S	R	S	R	R	R
21N-23	isb	R	R	I	R	S	S	R	R	R	S	R	S	R	R	R	I	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R
21N-26	isb	R	R	I	I	S	I	S	S	R	S	S	S	S	S	R	S	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R
20N-2421	isb	R	R	I	S	S	S	I	S	R	S	S	I	R	R	S	S	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R
20N-2422	isb	R	R	I	R	S	S	S	S	R	S	R	I	R	S	S	S	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R
20N-2424	isb	R	R	I	S	S	S	S	S	R	S	S	S	R	R	S	S	R	R	I	S	R	R	R	R	S	I	R	R	R	R	R
20N-2425	isb	R	R	I	R	S	S	S	S	S	R	R	S	R	S	S	S	R	R	R	R	R	R	I	R	R	I	R	R	R	R	R
20N-2426	isb	R	R	S	S	S	S	S	S	R	R	R	S	R	S	S	S	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R
20N-2427	isb	R	R	S	R	S	S	S	S	S	S	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
20N-2428	isb	R	S	S	S	I	S	S	S	R	S	R	S	R	R	S	S	R	R	R	R	R	R	R	R	S	I	R	R	R	R	R

Appendix-XX: Complete AST results of isolates derived from the samples derived from Gilgit.

ID number	CITY	Penicillin	Ampicillin (AMP-R µg/disk)	Ampicillin-Sulbactam	Augmentin	pipercillline-	Ceftiofur	Cefotaxime	Ceftazidime	Cefazolin	Cefepime	Imipenam	Meropenem	Colistin	Streptomycin	Gentamicin	Amikacin	Chloramphenicol	Florfenicol	nalidixic acid	Enrofloxacin	Ciprofloxacin	Tetracycline	doxycycline	Erythromycin	Azithromycin	Minocycline	Clindamycin	Sulfamethoxazole/ trimethoprim	Linezolid	Quinopristin/ Dalforistin	Teicoplanin

Sample ID	Source	Penicillin	Ampicillin	Amoxicillin-clavulanic acid	Piperacillin-tazobactam	Cefuroxime	Ceftazidime	Cefotaxime	Ceftriaxone	Cefepime	Aztreonam	Imipenem	Meropenem	Ertapenem	Gentamicin	Amikacin	Tobramycin	Ciprofloxacin	Levofloxacin	Tetracycline	Doxycycline	Chloramphenicol	Azithromycin	Erythromycin	Clindamycin	Sulfamethoxazole/Trimethoprim	Linezolid	Daptomycin	Tigecycline	Tekoplanin
21N-190	GB	R	R	S	I	S	S	R	R	R	R	S	S	R	R	S	S	S	S	R	R	R	R	I	R	S	I	R	R	R	R	R
21N-191	GB	R	R	R	R	S	S	S	R	R	R	S	S	S	R	S	S	R	S	R	R	R	S	R	R	S	I	R	S	R	R	R
21N-192	GB	R	R	I	I	I	S	I	I	R	R	S	S	R	R	I	I	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R
21N-193	GB	R	R	S	I	S	S	S	I	R	S	S	S	R	R	S	S	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R
21N-194	GB	R	R	S	I	I	S	S	R	R	S	S	S	R	R	R	S	R	R	R	R	R	R	S	I	S	S	R	S	I	R	R
21N-196	GB	R	R	S	I	S	I	I	I	S	S	S	R	R	S	S	R	R	R	R	R	R	R	R	R	S	S	I	R	R	R	R
21N-197	GB	R	R	I	I	I	R	I	R	I	S	S	R	R	I	I	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R
21N-198	GB	R	R	S	S	S	R	I	S	S	R	R	R	R	S	S	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R
21N-195	GB	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
21N-199	GB	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
21N-200	GB	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
21N-338	GB	R	R	I	S	S	S	I	I	R	S	S	R	R	I	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R
21N-339	GB	R	S	S	S	S	S	S	S	R	R	S	R	R	S	R	R	R	R	R	I	R	R	R	I	R	R	R	R	R	R	I
21N-340	GB	R	I	S	I	S	S	I	I	I	S	R	S	S	R	S	I	R	R	R	R	R	I	R	R	S	S	R	R	R	R	R
21N-341	GB	R	I	S	I	S	S	R	I	I	R	R	S	R	R	S	S	R	R	R	R	R	I	I	R	R	R	R	R	R	R	R
2021-1747	Karachi	R	R	S	S	I	S	S	S	S	R	R	S	R	R	S	I	R	R	R	R	R	I	R	R	S	R	R	R	R	R	R
21N-344	GB	R	R	S	S	S	S	R	S	I	S	R	S	R	R	I	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R
2021-1748	Karachi	R	R	S	R	S	S	R	S	I	S	R	S	R	R	I	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R
21N-345	GB	R	R	S	R	S	S	S	S	S	R	S	S	R	S	S	R	S	S	R	R	R	R	R	R	S	R	R	R	R	R	R
2021-1749	Karachi	R	R	S	R	S	S	S	S	S	R	S	S	R	S	S	R	S	S	R	R	R	R	R	R	S	R	R	R	R	R	R

Appendix-XXI: AST results of isolates derived from Karachi sampling.

20N-1753	Karachi	R	R	S	I	S	S	S	S	I	S	S	S	S	R	R	S	R	R	R	R	R	R	R	R	R	R	R	S	S	R
20N-1908	Karachi	R	R	I	S	S	I	S	R	R	R	S	I	R	R	S	I	S	R	S	R	R	I	S	R	R	S	I	S	R	I	I
20N-1909	Karachi	R	R	S	S	S	I	R	S	R	R	S	S	R	S	S	S	S	S	R	R	R	R	S	R	R	S	S	S	S	S	S
20N-1948	Karachi	R	S	S	S	S	S	I	I	S	R	S	I	R	S	S	S	R	R	R	I	R	R	S	R	S	S	I	S	R	I	S
20N-1952	Karachi	R	S	S	I	R	R	R	R	S	R	I	S	R	S	R	S	I	R	R	S	R	R	R	I	R	R	R	S	I	S	R
20N-1960	Karachi	R	R	S	S	S	S	S	I	S	R	S	S	R	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S
20N-2045	Karachi	R	R	S	S	S	S	S	I	I	S	S	S	R	R	S	S	S	S	R	R	R	R	I	R	R	S	R	I	S	I	S
20N-2047	Karachi	R	S	S	S	S	S	S	I	S	S	R	S	R	S	S	S	S	S	I	S	S	S	S	R	R	S	I	S	S	R	S
20N-2050	Karachi	R	S	S	S	S	S	S	I	I	S	S	S	R	I	S	S	R	S	R	R	R	R	S	I	I	I	R	R	R	I	I
20N-2052	Karachi	R	S	S	S	S	S	S	I	I	S	S	S	R	I	S	S	R	S	I	S	S	I	S	I	S	S	I	S	S	I	I
20N-2054	Karachi	R	S	S	S	S	S	S	I	I	S	S	S	R	I	S	S	R	S	R	R	R	R	S	R	R	S	I	S	S	I	I
20N-2055	Karachi	R	R	S	S	S	S	S	I	I	S	S	S	R	I	S	S	R	S	R	R	R	R	S	R	R	S	I	S	S	I	I
20N-2193	Karachi	R	R	I	I	S	I	R	R	R	I	S	S	R	I	S	R	R	S	R	R	R	R	S	R	R	S	I	R	R	S	R
20N-2197	Karachi	R	R	I	I	S	I	I	I	R	S	S	S	S	R	I	S	S	R	I	R	S	R	R	R	R	S	R	R	R	R	R
20N-2304	Karachi	R	R	S	S	S	S	S	S	I	S	S	S	S	R	R	S	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R
20N-2307	Karachi	R	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S	R	R	R	R	R	R	R	R	S	R	R	R	S	R	R
20N-2311	Karachi	R	R	S	R	S	S	S	S	I	S	S	S	S	R	R	S	R	R	R	R	R	R	R	R	S	I	R	R	R	R	R
20N-1707	Quetta	R	R	S	R	S	S	S	S	I	S	S	S	S	R	R	S	R	R	R	R	R	R	R	R	S	I	R	R	R	R	R
20N-2312	Karachi	R	R	S	S	S	S	S	S	I	S	S	S	S	R	R	S	R	R	R	R	R	R	R	R	S	I	R	R	R	R	R
20N-1708	Quetta	R	R	S	S	S	S	S	S	I	S	S	S	S	R	R	S	R	R	R	R	R	R	R	R	S	I	R	R	R	R	R
20N-2313	Karachi	R	R	S	S	S	S	S	S	I	S	S	S	S	R	R	S	R	R	R	R	R	R	R	R	S	I	R	R	R	R	R
20N-1709	Quetta	R	R	S	S	S	S	S	S	I	S	S	S	S	R	R	S	R	R	R	R	R	R	R	R	S	I	R	R	R	R	R
20N-2314	Karachi	R	R	S	S	S	S	S	S	I	S	S	S	S	R	R	S	R	R	R	R	R	R	R	R	S	I	R	R	R	R	R
20N-1710	Quetta	R	R	S	S	S	S	S	S	I	S	S	S	S	R	R	S	R	R	R	R	R	R	R	R	S	I	R	R	R	R	R
20N-2316	Karachi	R	R	S	I	S	S	S	S	R	S	S	S	S	R	R	I	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R
20N-2440	Karachi	R	S	S	S	S	S	S	R	S	S	S	S	R	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S
21N-188	Karachi	R	S	S	S	S	S	S	S	S	S	I	S	S	R	S	S	S	S	R	I	I	R	R	R	S	S	R	R	S	R	R
21N-189	Karachi	R	S	S	S	R	R	S	S	S	R	I	S	R	S	S	S	R	R	R	S	S	R	R	S	S	R	S	R	I	R	R
21N-36	Karachi	R	R	I	I	S	S	I	S	R	I	I	S	R	R	S	S	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R
20N-1794	Karachi	R	S	S	S	S	R	S	S	S	R	S	S	R	S	R	S	S	R	R	I	R	S	R	S	S	I	R	S	R	I	R
21N-179	Karachi	R	R	I	S	S	S	I	S	R	S	S	S	S	I	S	I	R	R	R	R	R	R	I	S	S	S	R	R	S	S	S
21N-186	Karachi	R	R	I	I	I	I	R	I	R	I	S	S	S	R	S	S	R	R	R	R	R	R	R	I	S	I	R	R	I	S	R

Appendix-XXII: AST results for selected isolates derived from samples of Quetta

20N-1711	Quetta	R	R	S	R	S	S	S	S	S	R	I	S	S	R	R	S	R	R	R	I	R	R	R	R	S	S	R	R	R	R	R
20N-1712	Quetta	R	R	S	I	S	S	S	S	I	S	R	S	R	R	S	I	S	R	R	R	R	R	R	R	S	R	R	R	R	R	R
20N-1713	Quetta	R	R	I	R	R	S	R	R	R	S	R	S	R	R	R	I	S	R	I	R	R	R	R	R	R	I	R	R	R	R	R
20N-1714	Quetta	R	R	R	R	S	S	S	I	R	S	R	S	S	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
20N-1715	Quetta	R	R	S	R	S	S	S	S	I	S	R	S	S	R	I	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
20N-1716	Quetta	R	R	S	R	S	S	S	I	I	S	I	S	S	R	R	S	S	R	R	R	R	R	R	R	R	I	R	R	R	R	R
20N-1717	Quetta	R	R	S	R	S	S	S	I	S	S	S	S	R	R	I	I	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R
20N-1718	Quetta	R	S	S	S	R	S	I	R	S	I	R	S	S	R	I	S	S	R	S	S	S	R	S	R	R	S	R	R	I	R	R
20N-1719	Quetta	R	R	S	R	R	S	S	I	I	S	I	S	R	R	I	I	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R
20N-1720	Quetta	R	R	S	S	S	S	S	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R
20N-1721	Quetta	R	R	S	I	S	S	S	R	I	S	R	S	R	R	R	R	R	R	R	R	R	R	R	R	S	I	R	R	R	R	R
20N-1722	Quetta	R	R	S	S	S	S	S	R	I	S	R	S	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R
20N-1792	Quetta	R	R	R	S	S	S	S	S	S	S	S	S	R	S	S	S	R	R	R	S	R	R	R	R	S	S	R	R	I	I	S
20N-1794	Quetta	R	R	S	I	S	S	S	R	R	I	S	S	S	S	S	S	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R
20N-1798	Quetta	R	S	R	S	S	S	S	S	R	R	S	S	R	S	S	S	R	R	R	R	S	R	R	S	S	I	R	R	S	R	R
20N-1979	Quetta	R	S	S	S	S	S	S	I	S	I	S	S	R	S	S	S	S	S	R	R	R	S	I	R	R	S	S	S	S	S	R
20N-1991	Quetta	R	R	S	R	S	S	S	R	R	S	R	S	R	R	R	I	I	I	R	R	R	S	S	R	S	S	I	S	I	R	S
20N-1994	LHR	R	R	S	S	S	S	S	R	R	S	R	S	R	R	R	I	I	I	R	R	R	S	S	R	S	S	I	S	I	R	S
20N-2162	Quetta	R	R	I	I	S	S	S	S	R	S	S	S	R	R	R	I	I	I	R	R	R	S	S	R	S	S	I	S	I	R	S
20N-2163	LHR	R	R	I	I	S	S	S	S	R	S	S	S	R	R	R	I	I	I	R	R	R	S	S	R	S	S	I	S	I	R	S
20N-2166	LHR	R	R	S	I	S	S	S	S	R	S	I	S	R	R	R	I	I	I	R	R	R	S	S	R	S	S	I	S	I	R	S
20N-2167	LHR	R	R	S	I	S	S	S	S	R	S	I	S	R	R	R	I	I	I	R	R	R	S	S	R	S	S	I	S	I	R	S
20N-2168	Quetta	R	R	S	I	S	S	S	S	R	S	I	S	R	R	R	I	I	I	R	R	R	S	S	R	S	S	I	S	I	R	S
20N-2169	LHR	R	R	S	I	S	S	S	S	R	S	I	S	R	R	R	I	I	I	R	R	R	S	S	R	S	S	I	S	I	R	S
20N-2231	Quetta	R	R	S	I	S	S	S	S	R	S	S	S	R	R	S	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R
20N-2234	Quetta	R	R	I	I	S	S	S	S	R	S	I	I	S	R	S	S	S	R	R	R	R	R	R	R	S	R	S	R	R	R	R
20N-2415	Quetta	R	R	S	R	S	I	R	R	R	R	S	S	R	R	S	R	S	S	R	I	R	S	R	R	R	S	S	I	R	S	S
20N-2418	Quetta	R	R	S	S	S	S	I	S	R	S	S	S	S	R	S	R	R	R	R	R	R	R	I	R	R	I	R	R	R	R	R
21N-12	Quetta	R	R	S	S	S	S	S	S	I	S	S	S	S	R	S	S	R	R	R	S	S	R	R	R	R	I	R	R	R	R	R

Appendix-XXIII: Complete AST results of isolates derived from the samples of Lahore

ID Number	Source	Penicillin	Ampicillin (AMP-K 10µg/disk)	Amoxicillin	Augmentin	Piperacillin	Cefaclor	Cefotaxime	Ceftazidime	Cefazolin	Cefepime	Cefoxitin	Imipenem	Meropenem	Cotrimoxazole	Streptomycin	Gentamicin	Kanamycin	Chloramphenicol	Nitrofurantoin	Nalidixic acid	Enrofloxacin	Ciprofloxacin	Tetracycline	Doxycycline	Erythromycin	Azithromycin	Minocycline	Clindamycin	Sulfamethoxazole/Trimethoprim	Linezolid	Quinupristin/Dalfopristin	Teicoplanin
20N-2014	LHR	R	S	S	S	S	S	I	R	S	I	S	S	R	S	S	S	S	S	I	S	R	S	S	R	R	S	S	S	S	S	S	S
20N-2016	LHR	R	S	S	S	S	S	I	R	S	I	S	I	R	S	S	S	S	S	I	S	S	I	S	R	R	S	S	I	I	S	S	S
20N-2146	LHR	R	R	I	I	S	S	S	S	R	I	S	S	S	R	S	S	R	R	I	R	R	R	R	R	R	S	R	R	R	R	R	R
20N-2148	LHR	R	R	S	I	S	I	R	I	R	I	S	S	R	R	S	I	R	R	R	R	R	R	R	R	S	I	R	R	R	R	R	R
20N-2151	LHR	R	R	R	R	R	R	S	S	R	R	I	R	R	R	R	I	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R
20N-2216	LHR	R	R	R	R	S	S	I	I	R	I	I	R	S	R	S	S	R	R	R	R	R	R	I	R	R	S	R	R	R	R	R	R
20N-2217	LHR	R	R	I	R	S	S	S	S	R	S	S	S	R	I	I	I	R	R	R	R	R	R	I	R	S	S	R	R	R	R	R	R
20N-2228	LHR	R	R	S	R	S	R	R	S	R	R	S	S	R	R	I	S	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R
20N-2240	LHR	R	R	R	R	S	I	I	I	R	I	I	I	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
20N-2237	LHR	R	R	S	I	S	S	I	I	R	I	S	S	R	R	I	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
21N-205	LHR	R	R	R	I	R	R	R	R	R	R	R	I	R	R	I	S	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R
21N-211	LHR	R	S	S	S	S	S	S	R	S	S	S	I	R	R	S	S	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R
21N-213	LHR	R	R	R	S	I	I	R	I	R	I	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R
21N-253	LHR	R	R	I	I	S	S	R	R	R	R	S	S	S	R	S	S	R	R	R	R	R	R	R	R	S	I	R	R	R	R	R	R
21N-256	LHR	R	R	R	S	S	S	I	R	I	S	S	S	S	R	S	S	R	R	R	R	I	R	R	I	R	R	R	R	S	R	R	R
21N-257	LHR	R	R	I	R	S	S	R	R	R	I	R	I	R	R	R	S	R	R	R	R	R	R	R	R	S	I	R	R	R	R	R	R
21N-258	LHR	R	R	S	I	S	S	I	R	I	S	S	S	R	R	S	S	R	S	I	I	R	R	R	R	S	S	R	R	R	R	R	R
20N-2328	PES	R	S	S	S	S	S	S	R	S	S	S	S	R	R	S	S	R	S	I	R	R	S	R	R	S	S	R	R	R	R	R	R
21N-262	LHR	R	R	R	I	S	R	R	I	R	S	S	S	R	R	S	S	R	R	R	R	R	S	R	R	S	S	R	R	I	R	R	R
21N-269	LHR	R	R	R	I	S	R	R	I	R	S	S	S	R	R	S	S	R	R	R	R	R	S	R	R	S	S	R	R	I	R	R	R
20N-1486	PES	R	R	S	S	S	S	S	S	S	I	S	S	S	S	S	S	R	S	S	R	R	S	R	R	S	I	R	R	R	R	R	R
20N-1430	LHR	R	R	S	S	S	S	S	S	S	I	S	S	S	S	S	S	R	S	S	R	R	S	R	R	S	I	R	R	R	R	R	R
20N-1488	PES	R	R	I	I	S	S	S	S	I	S	S	S	S	R	S	S	R	R	R	R	R	S	R	R	S	S	R	R	R	R	R	R

Appendix-XXIV: Complete AST of isolates derived from samples of Peshawar

		Penicillin	Ampicillin (AMP-K 10µg/disk)	Amoxicillin	Augmentin	Piperacillin-tazobactam	Cefoxitin	Ceftriaxone	Ceftazidime	Cefazolin	Cefepime	Imipenem	Meropenem	Colistin	Streptomycin	Gentamicin	Amikacin	Norfloxacin	Ciprofloxacin	Tetracycline	Doxycycline	Erythromycin	Azithromycin	Minocycline	Clindamycin	Linezolid	Daptomycin	Teicoplanin	Sulfamethoxazole/Trimethoprim	Quinupristin/Dalfopristin	Vancomycin
20N-2028	PES	R	R	S	S	S	S	R	R	I	I	S	R	R	R	S	S	S	S	R	R	R	S	S	R	R	S	R	S	I	S	S
20N-2030	PES	R	R	R	R	S	I	I	I	R	R	I	S	R	R	I	S	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R
20N-2039	PES	R	S	S	S	R	I	S	R	R	S	R	S	R	S	S	S	S	S	R	S	S	S	S	R	R	S	I	S	R	R	S
20N-2041	PES	R	S	S	S	S	S	S	I	S	S	S	S	R	S	S	S	S	S	S	S	R	R	R	S	R	S	R	R	S	S	R
20N-2042	PES	R	S	S	S	S	S	S	I	S	S	S	R	R	S	S	S	S	S	S	S	S	R	S	R	S	S	R	S	I	R	R
20N-2174	PES	R	R	R	R	S	I	I	R	R	I	S	I	S	R	S	S	I	I	R	R	R	R	R	R	S	R	R	S	R	R	R
21N-234	PES	R	R	R	I	I	I	I	I	R	S	S	S	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
21N-235	PES	R	R	R	S	S	S	I	S	R	S	I	S	R	R	R	S	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R
21N-239	PES	R	R	R	I	S	S	I	I	R	S	I	S	R	R	S	S	R	R	R	R	R	S	I	R	R	R	R	R	R	R	R
21N-242	PES	R	R	I	I	S	S	I	I	R	S	I	S	R	R	S	S	R	R	R	R	R	S	I	R	R	R	R	R	R	R	R
20N-1776	PES	R	R	R	R	S	S	S	S	S	S	S	S	R	R	R	I	R	R	R	R	R	S	S	R	R	R	R	R	R	R	R
20N-1777	PES	R	R	R	R	S	S	S	S	S	S	S	S	R	R	R	I	R	R	R	R	R	S	S	R	R	R	R	R	R	R	R
20N-1778	PES	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S	R	R	R	S	S	R	R	R	R	R	R	R	R
20N-1779	PES	R	R	S	I	S	S	S	S	S	S	S	S	S	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
20N-1780	PES	R	S	S	I	R	R	R	R	S	R	I	S	R	R	R	S	I	R	R	S	R	R	R	I	R	R	R	R	S	S	R
20N-1915	PES	R	R	S	R	S	S	I	I	R	S	R	S	S	S	S	S	R	R	R	I	R	S	S	R	R	S	R	S	I	R	I
20N-2037	PES	R	S	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	R	R	S	S	S	R	R	S
21N-2023	AJK	R	R	I	R	S	S	S	S	R	S	S	S	R	R	R	S	R	R	R	R	R	I	S	R	R	S	R	S	R	R	R
20N-2024	PES	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	R	R	R	R	R	I	S	R	S	S	R	S	R	R	R
20N-2422	AJK	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	R	R	R	R	R	I	S	R	S	S	R	S	R	R	R
20N-2423	PES	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	R	R	R	R	R	I	S	R	S	S	R	S	R	R	R
20N-2033	AJK	R	R	I	R	S	S	S	S	R	S	S	S	R	R	R	S	R	R	R	R	R	I	S	R	R	S	R	S	R	R	R
20N-2467	AJK	R	R	I	R	S	S	S	S	R	S	S	S	R	R	R	S	R	R	R	R	R	I	S	R	R	S	R	S	R	R	R
20N-2034	AJK	R	R	R	R	S	R	R	S	R	S	R	S	R	R	R	S	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R
20N-2468	AJK	R	R	R	R	S	R	R	S	R	S	R	S	R	R	R	S	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R
20N-2469	PES	R	R	S	R	I	S	S	I	I	S	I	S	R	R	I	I	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R

Appendix-XXV: AST results of isolates derived from Muzaffarabad, AJK.

21N-295	AJK	R	R	S	R	S	I	I	S	R	S	S	I	R	R	R	S	R	R	R	R	R	R	R	R	I	R	R	R	R	R
21N-296	AJK	R	R	S	S	S	S	S	S	S	I	R	S	S	R	S	S	R	R	R	R	R	R	I	R	S	S	R	R	I	R	R
21N-298	AJK	R	R	S	I	S	S	R	I	R	S	I	S	R	R	S	S	R	S	R	I	I	R	R	R	R	R	R	R	R	R	R
21N-299	AJK	R	R	S	I	I	I	R	I	R	I	I	I	R	R	S	S	R	S	R	I	R	R	R	R	R	R	R	R	R	R	R

Appendix-XIX: Complete AST results of isolates derived from the samples derived from Islamabad

ID number	CITY	Penicillin	Ampicillin (AMP-R µg/disk)	Ampicillin-Sulbactam	Augmentin	pipercillline-tazobactam	Ceftiofur	Cefotaxime	Ceftazidime	Cefazolin	Cefepime	Imipenam	Meropenem	Colistin	Streptomycin	Gentamicin	Amikacin	Chloramphenicol	Florfenicol	nalidixic acid	Enrofloxacin	Ciprofloxacin	Tetracycline	doxycycline	Erythromycin	Azithromycin	Minocycline	Clindamycin	sulfamethoxazole/ trimethoprim	Linezolid	Quinopristin/ Dalfloristin	Teicoplanin
20N-1200	isb	R	R	S	R	S	S	S	I	R	I	R	S	S	S	S	S	R	R	I	R	S	R	R	R	S	I	R	R	R	R	R
20N-1963	isb	R	R	I	I	S	S	R	S	R	I	S	S	R	R	R	S	R	I	I	S	R	R	I	R	R	S	R	S	R	S	R
20N-1964	isb	R	R	S	I	S	S	I	S	I	S	S	S	R	R	S	S	R	R	R	R	R	R	R	R	R	S	R	S	R	S	R
20N-1965	isb	R	R	S	I	S	S	S	S	S	S	R	S	R	R	S	S	R	R	R	S	R	R	I	R	S	S	R	S	R	S	S
20N-1966	isb	R	R	S	S	S	S	S	S	S	S	R	S	R	R	S	S	R	R	R	S	R	R	R	R	S	S	R	S	R	S	R
20N-1967	isb	R	I	S	I	S	S	S	S	I	S	S	S	S	I	S	S	R	R	R	S	S	R	S	R	S	S	R	S	R	S	R
20N-1969	isb	R	R	I	I	S	S	S	S	R	S	S	S	R	R	S	S	R	R	R	R	R	I	S	R	S	I	R	R	R	R	R
20N-1970	isb	R	S	S	S	S	S	S	R	S	I	S	I	S	S	S	I	R	R	R	R	S	S	R	R	R	S	R	S	R	R	R
21N-23	isb	R	R	I	R	S	S	R	R	R	S	R	S	R	R	R	I	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R
21N-26	isb	R	R	I	I	S	I	S	S	R	S	S	S	S	S	R	S	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R
20N-2421	isb	R	R	I	S	S	S	I	S	R	S	S	I	R	R	S	S	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R
20N-2422	isb	R	R	I	R	S	S	S	S	R	S	R	I	R	S	S	S	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R
20N-2424	isb	R	R	I	S	S	S	S	S	R	S	S	S	R	R	S	S	R	R	I	S	R	R	R	R	S	I	R	R	R	R	R
20N-2425	isb	R	R	I	R	S	S	S	S	S	R	R	S	R	S	S	S	R	R	R	R	R	R	I	R	R	I	R	R	R	R	R
20N-2426	isb	R	R	S	S	S	S	S	S	R	R	R	S	R	S	S	S	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R
20N-2427	isb	R	R	S	R	S	S	S	S	S	S	R	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
20N-2428	isb	R	S	S	S	I	S	S	S	R	S	R	S	R	R	S	S	R	R	R	R	R	R	R	R	S	I	R	R	R	R	R

Appendix-XX: Complete AST results of isolates derived from the samples derived from Gilgit.

ID number	CITY	Penicillin	Ampicillin (AMP-R µg/disk)	Ampicillin-Sulbactam	Augmentin	pipercillline-	Ceftiofur	Cefotaxime	Ceftazidime	Cefazolin	Cefepime	Imipenam	Meropenem	Colistin	Streptomycin	Gentamicin	Amikacin	Chloramphenicol	Florfenicol	nalidixic acid	Enrofloxacin	Ciprofloxacin	Tetracycline	doxycycline	Erythromycin	Azithromycin	Minocycline	Clindamycin	sulfamethoxazole/ trimethoprim	Linezolid	Quinopristin/ Dalforistin	Teicoplanin
21N-190	GB	R	R	S	I	S	S	R	R	R	R	S	S	R	R	S	S	S	S	R	R	R	R	I	R	S	I	R	R	R	R	R
21N-191	GB	R	R	R	R	S	S	S	R	R	R	S	S	S	R	S	S	R	S	R	R	R	S	R	R	S	I	R	S	R	R	R
21N-192	GB	R	R	I	I	I	S	I	I	R	R	S	S	R	R	I	I	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R
21N-193	GB	R	R	S	I	S	S	S	I	R	S	S	S	R	R	S	S	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R
21N-194	GB	R	R	S	I	I	S	S	R	R	S	S	S	R	R	R	S	R	R	R	R	R	R	S	I	S	S	R	S	I	R	R
21N-196	GB	R	R	S	I	S	S	I	I	I	S	S	S	R	R	S	S	R	R	R	R	R	R	R	R	S	S	I	R	R	R	R
21N-197	GB	R	R	I	I	I	I	R	I	R	I	S	S	R	R	I	I	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R
21N-198	GB	R	R	S	S	S	S	I	R	I	S	S	S	R	R	S	S	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R
21N-195	GB	R	R	R	R	I	I	R	I	R	S	S	S	S	R	I	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
21N-199	GB	R	R	R	I	I	R	R	R	R	R	I	S	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
21N-200	GB	R	R	S	R	S	S	I	S	I	S	S	S	R	R	R	S	R	R	R	R	R	R	I	R	S	I	R	R	R	R	R
21N-338	GB	R	R	S	I	S	S	S	S	I	I	R	S	S	R	R	I	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R
21N-339	GB	R	S	S	S	S	S	S	S	S	S	R	R	S	R	R	S	R	R	R	R	R	I	R	R	R	I	R	R	R	R	I
21N-340	GB	R	I	S	I	S	S	I	I	I	S	R	S	S	R	S	I	R	R	R	R	R	I	R	R	S	S	R	R	R	R	R
21N-341	GB	R	I	S	I	S	S	R	I	I	R	R	S	R	R	S	S	R	R	R	R	R	I	I	R	R	R	R	R	R	R	R
21N-344	GB	R	I	S	S	S	S	R	S	I	I	R	S	S	R	R	I	R	R	R	R	R	R	I	R	S	R	R	R	R	R	R
21N-345	GB	R	R	S	R	S	S	S	S	I	I	R	S	S	R	S	I	R	R	R	R	R	R	I	R	R	S	R	R	R	R	R

Appendix-XXI: AST results of isolates derived from Karachi sampling.

ID Number of Sample	CITY	Penicillin	Ampicillin (AMP-R µg/disk)	Ampicillin-Sulbactam	Augmentin	pipercillline-tazobactam	Ceftiofur	Cefotaxime	Ceftazidime	Cefazolin	Cefepime	Imipenam	Meropenem	Colistin	Streptomycin	Gentamicin	Amikacin	Chloramphenicol	Florfenicol	nalidixic acid	Enrofloxacin	Ciprofloxacin	Tetracycline	doxycycline	Erythromycin	Azithromycin	Minocycline	Clindamycin	Sulfamethoxazole/ trimethoprim	Linezolid	Quinopristin/ Dalforistin	Teicoplanin
20N-1747	Karachi	R	R	S	S	S	S	I	S	S	S	S	S	S	R	S	S	R	R	R	R	R	I	I	R	S	S	R	R	S	R	R
20N-1748	Karachi	R	R	I	R	S	S	S	S	I	S	S	S	S	I	R	I	R	R	R	R	R	R	R	R	R	I	R	R	S	S	R
20N-1749	Karachi	R	R	I	R	S	S	S	S	S	S	S	S	S	R	S	S	R	S	R	R	R	R	R	R	R	I	R	R	R	R	R
20N-1753	Karachi	R	R	S	I	S	S	S	S	I	S	S	S	S	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R
20N-1908	Karachi	R	R	I	S	S	I	S	R	R	R	S	I	R	R	S	I	S	R	S	R	R	I	S	R	R	S	I	S	R	I	I
20N-1909	Karachi	R	R	S	S	S	I	R	S	R	R	S	S	R	S	S	S	S	S	R	R	R	R	S	R	R	S	S	S	S	S	S
20N-1948	Karachi	R	S	S	S	S	S	I	I	S	R	S	I	R	S	S	S	R	R	R	I	R	R	S	R	S	S	I	S	R	I	S
20N-1952	Karachi	R	S	S	I	R	R	R	R	S	R	I	S	R	S	R	S	I	R	R	S	R	R	R	I	R	R	R	S	I	S	R
20N-1960	Karachi	R	R	S	S	S	S	S	I	S	R	S	S	R	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S
20N-2045	Karachi	R	R	S	S	S	S	S	I	I	S	S	S	R	R	S	S	S	S	R	R	R	R	I	R	R	S	R	I	S	I	S
20N-2047	Karachi	R	S	S	S	S	S	S	I	S	S	R	S	R	S	S	S	S	S	I	S	S	S	S	R	R	S	I	S	S	R	S
20N-2050	Karachi	R	S	S	S	S	S	S	I	I	S	S	S	R	I	S	S	S	S	R	S	S	R	R	R	R	S	I	I	R	R	I
20N-2052	Karachi	R	I	S	S	S	S	S	R	R	S	S	S	R	S	S	S	S	S	R	S	I	S	S	I	S	S	S	S	S	S	I
20N-2054	Karachi	R	I	S	S	S	S	I	R	S	I	S	R	R	S	S	S	S	S	R	I	R	S	S	R	R	S	I	I	S	S	I
20N-2055	Karachi	R	R	S	S	S	S	R	R	S	I	S	R	R	S	S	S	S	S	R	R	R	S	S	R	S	S	I	S	S	S	S
20N-2193	Karachi	R	R	I	I	S	I	R	R	R	I	S	S	R	R	S	R	R	S	R	R	R	R	R	R	S	I	R	R	S	R	R
20N-2197	Karachi	R	R	I	I	S	I	I	I	R	S	S	S	S	R	I	S	S	R	I	R	S	R	R	R	R	S	R	R	R	R	R
20N-2304	Karachi	R	R	S	S	S	S	S	S	I	S	S	S	S	R	R	S	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R
20N-2307	Karachi	R	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S	R	R	R	R	R	R	R	R	S	R	R	R	S	R	R
20N-2311	Karachi	R	R	S	R	S	S	S	S	I	S	S	S	S	R	R	S	R	R	R	R	R	R	R	R	S	I	R	R	R	R	R
20N-2312	Karachi	R	R	S	I	S	S	S	S	S	S	S	S	S	R	R	S	R	R	R	R	R	R	R	R	S	I	R	R	R	R	R
20N-2313	Karachi	R	R	S	R	S	S	S	S	S	S	S	S	S	R	S	S	R	R	R	R	R	R	R	R	S	I	R	R	R	R	R
20N-2315	Karachi	R	R	S	R	S	S	S	S	S	S	S	S	S	R	I	S	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R
20N-2316	Karachi	R	R	S	I	S	S	S	S	R	S	S	S	S	R	R	I	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R
20N-2440	Karachi	R	S	S	S	S	S	S	R	S	S	S	S	R	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S
21N-188	Karachi	R	S	S	S	S	S	S	S	S	S	I	S	S	R	S	S	S	S	R	I	I	R	R	R	S	S	R	R	S	R	R
21N-189	Karachi	R	S	S	S	R	R	S	S	S	R	I	S	R	S	S	S	R	R	R	S	S	R	R	S	S	R	S	R	I	R	R
21N-36	Karachi	R	R	I	I	S	S	I	S	R	I	I	S	R	R	S	S	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R
20N-1794	Karachi	R	S	S	S	S	R	S	S	S	R	S	S	R	S	R	S	S	R	R	I	R	S	R	S	S	I	R	S	R	I	R
21N-179	Karachi	R	R	I	S	S	S	I	S	R	S	S	S	S	I	S	I	R	R	R	R	R	R	I	S	S	S	R	R	S	S	S
21N-186	Karachi	R	R	I	I	I	I	R	I	R	I	S	S	S	R	S	S	R	R	R	R	R	R	R	I	S	I	R	R	I	S	R

Appendix-XXII: AST results for selected isolates derived from samples of Quetta

ID Number of Sample	CITY	Penicillin	Ampicillin (AMP-R µg/disk)	Ampicillin-Sulbactam	Augmentin	pipercillline-tazobactam	Ceftiofur	Cefotaxime	Ceftazidime	Cefazolin	Cefepime	Imipenam	Meropenem	Colistin	Streptomycin	Gentamicin	Amikacin	Chloramphenicol	Florfenicol	nalidixic acid	Enrofloxacin	Ciprofloxacin	Tetracycline	doxycycline	Erythromycin	Azithromycin	Minocycline	Clindamycin	Sulfamethoxazole/ trimethoprim	Linezolid	Quinopristin/ Dalforistin	Teicoplanin
20N-1707	Quetta	R	R	S	R	S	S	S	R	I	S	R	S	S	R	I	I	S	R	R	R	R	R	R	R	R	I	R	S	R	R	R
20N-1708	Quetta	R	R	I	S	S	S	S	S	S	R	I	S	S	R	R	S	R	R	R	I	R	R	R	R	S	I	R	I	R	R	R
20N-1709	Quetta	R	R	S	R	S	S	S	R	I	S	R	S	R	R	I	I	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R
20N-1710	Quetta	R	R	I	R	S	S	S	I	I	S	I	S	S	R	I	I	R	R	R	R	R	R	R	S	R	I	R	R	R	R	R
20N-1711	Quetta	R	R	S	R	S	S	S	S	S	R	I	S	S	R	R	S	R	R	R	I	R	R	R	R	S	S	R	R	R	R	R
20N-1712	Quetta	R	R	S	I	S	S	S	S	I	S	R	S	R	R	S	I	S	R	R	R	R	R	R	R	S	R	R	R	R	R	R
20N-1713	Quetta	R	R	I	R	R	S	R	R	R	S	R	S	R	R	R	I	S	R	I	R	R	R	R	R	R	I	R	R	R	R	R
20N-1714	Quetta	R	R	R	R	S	S	S	I	R	S	R	S	S	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
20N-1715	Quetta	R	R	S	R	S	S	S	S	I	S	R	S	S	R	I	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
20N-1716	Quetta	R	R	S	R	S	S	S	I	I	S	I	S	S	R	R	S	S	R	R	R	R	R	R	R	R	I	R	R	R	R	R
20N-1717	Quetta	R	R	S	R	S	S	S	I	S	S	S	S	R	R	I	I	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R
20N-1718	Quetta	R	S	S	S	R	S	I	R	S	I	R	S	S	R	I	S	S	R	S	S	S	R	S	R	R	S	R	S	I	R	R
20N-1719	Quetta	R	R	S	R	I	S	S	I	I	S	I	S	R	R	I	I	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R
20N-1720	Quetta	R	R	S	S	S	S	S	R	S	R	R	S	R	R	R	S	S	R	R	R	R	R	R	R	S	I	R	S	R	R	R
20N-1721	Quetta	R	R	S	I	S	S	S	I	I	S	I	S	R	R	S	R	R	S	R	R	R	I	R	R	S	I	R	R	R	R	R
20N-1722	Quetta	R	R	I	S	S	S	S	I	I	S	I	I	R	R	S	R	R	S	R	R	R	R	R	R	S	S	R	S	R	R	R
20N-1792	Quetta	R	S	R	S	S	S	S	S	S	S	S	S	R	S	S	S	R	R	R	S	R	R	R	S	S	S	R	S	I	I	S
20N-1794	Quetta	R	R	S	I	S	S	S	R	R	I	S	S	S	S	S	S	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R
20N-1798	Quetta	R	S	R	S	S	S	S	S	R	R	S	S	R	S	S	S	R	R	R	R	S	R	R	S	S	I	R	S	S	I	R
20N-1979	Quetta	R	S	S	S	S	S	S	I	S	I	S	S	R	S	S	S	S	S	R	R	R	S	I	R	R	S	S	S	S	S	R
20N-1991	Quetta	R	R	S	R	S	S	S	R	R	S	R	S	R	R	R	I	I	I	R	R	R	S	S	R	S	S	I	S	I	R	S
20N-2161	Quetta	R	R	I	I	S	I	S	I	R	S	S	R	S	R	S	S	S	R	R	R	R	R	R	R	S	S	R	R	S	R	R
20N-2166	Quetta	R	R	I	I	S	S	S	I	R	S	I	S	S	R	R	S	S	S	R	R	R	R	R	R	S	R	R	R	R	R	R
20N-2169	Quetta	R	R	I	I	I	R	R	R	R	R	S	S	R	R	S	I	S	R	R	R	R	R	R	R	S	I	I	R	R	R	R
20N-2231	Quetta	R	R	S	I	S	S	S	S	R	S	S	S	S	R	R	S	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R
20N-2234	Quetta	R	R	I	I	S	S	S	S	R	S	I	I	S	R	S	S	S	R	R	R	R	R	R	R	S	R	S	R	R	R	R
20N-2415	Quetta	R	R	S	R	S	I	R	R	R	R	S	S	R	R	S	R	S	S	R	I	R	S	R	R	R	S	S	I	R	S	S
20N-2418	Quetta	R	R	S	S	S	S	I	S	R	S	S	S	S	R	S	R	R	R	R	R	R	R	I	R	R	I	R	R	R	R	R
21N-12	Quetta	R	R	S	S	S	S	S	S	I	S	S	S	S	R	S	S	R	R	R	S	S	R	R	R	R	I	R	R	R	R	R

Appendix-XXIII: Complete AST results of isolates derived from the samples of Lahore

ID Number of Sample	CITY	Penicillin	Ampicillin (AMP-R µg/disk)	Ampicillin-Sulbactam	Augmentin	pipercillline-tazobactam	Ceftiofur	Cefotaxime	Ceftazidime	Cefazolin	Cefepime	Imipenam	Meropenem	Colistin	Streptomycin	Gentamicin	Amikacin	Chloramphenicol	Florfenicol	nalidixic acid	Enrofloxacin	Ciprofloxacin	Tetracycline	doxycycline	Erythromycin	Azithromycin	Minocycline	Clindamycin	Sulfamethoxazole/ trimethoprim	Linezolid	Quinopristin/ Dalforistin	Teicoplanin
20N-1884	LHR	R	R	S	S	I	I	S	S	R	R	S	I	R	S	S	S	S	S	I	S	S	I	S	R	R	S	S	S	S	S	I
20N-1888	LHR	R	R	I	I	S	S	I	S	R	S	I	I	S	R	R	S	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R
20N-1894	LHR	R	R	S	I	S	S	I	S	R	S	S	S	S	R	R	S	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R
20N-2009	LHR	R	R	S	S	S	S	R	R	S	R	S	R	R	S	S	S	S	S	R	I	R	S	S	R	R	R	I	S	S	S	S
20N-2014	LHR	R	S	S	S	S	S	I	R	S	I	S	S	R	S	S	S	S	S	I	S	R	S	S	R	R	S	S	S	S	S	S
20N-2016	LHR	R	S	S	S	S	S	I	R	S	I	S	I	R	S	S	S	S	S	I	S	S	I	S	R	R	S	S	I	I	S	S
20N-2146	LHR	R	R	I	I	S	S	S	S	R	I	S	S	S	R	S	S	R	R	I	R	R	R	R	R	R	S	R	R	R	R	R
20N-2148	LHR	R	R	S	I	S	I	R	I	R	I	S	S	R	R	S	I	R	R	R	R	R	R	R	R	S	I	R	R	R	R	R
20N-2151	LHR	R	R	R	R	R	R	S	S	R	R	I	R	R	R	R	I	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R
20N-2216	LHR	R	R	R	R	S	S	I	I	R	I	I	R	S	R	S	S	R	R	R	R	R	R	I	R	R	S	R	R	R	R	R
20N-2217	LHR	R	R	I	R	S	S	S	S	R	S	S	S	R	I	I	I	R	R	R	R	R	R	I	R	S	S	R	R	R	R	R
20N-2228	LHR	R	R	S	R	S	R	R	S	R	R	S	S	R	R	I	S	R	R	R	R	R	R	R	R	R	S	R	S	R	R	R
20N-2240	LHR	R	R	R	R	S	I	I	I	R	I	I	I	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
20N-2337	LHR	R	R	S	I	S	S	I	I	R	S	S	S	R	R	I	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
21N-205	LHR	R	R	R	I	R	R	R	R	R	R	R	I	R	R	S	R	I	I	R	R	R	S	I	R	S	R	R	I	R	R	R
21N-211	LHR	R	S	S	S	S	S	R	S	R	S	I	I	R	R	S	S	R	R	R	R	R	R	I	R	S	I	R	S	R	R	R
21N-213	LHR	R	R	R	S	I	I	R	I	R	I	S	S	R	R	R	R	R	R	R	R	R	R	R	I	S	R	R	R	I	R	R
21N-253	LHR	R	R	I	I	S	S	R	R	R	R	S	S	S	R	S	S	R	R	R	R	R	R	R	R	S	I	R	R	R	R	R
21N-256	LHR	R	R	R	S	S	S	I	R	I	S	S	S	S	R	S	S	R	R	R	R	I	R	R	I	R	R	R	R	S	S	R
21N-257	LHR	R	R	I	R	S	S	R	R	R	I	R	I	R	R	R	S	R	R	R	R	R	R	R	R	S	I	R	R	R	R	R
21N-259	LHR	R	R	S	I	S	S	I	R	I	S	S	S	R	R	S	S	R	S	I	I	R	R	R	R	S	S	R	R	R	R	R
21N-266	LHR	R	R	R	I	S	R	R	I	R	S	S	S	R	R	S	S	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R
21N-269	LHR	R	R	S	R	R	R	S	R	R	R	R	I	R	S	R	S	R	R	I	S	S	R	R	R	R	I	R	S	R	R	R
20N-2158	LHR	R	I	S	S	R	I	S	I	S	I	I	S	R	S	S	S	S	R	R	S	S	R	R	S	S	S	R	R	R	R	R

Appendix-XXIV: Complete AST of isolates derived from samples of Peshawar

ID Number of Sample	CITY	Penicillin	Ampicillin (AMP-R µg/disk)	Ampicillin-Sulbactam	Augmentin	pipercillline-tazobactam	Ceftiofur	Cefotaxime	Ceftazidime	Cefazolin	Cefepime	Imipenam	Meropenem	Colistin	Streptomycin	Gentamicin	Amikacin	Chloramphenicol	Florfenicol	nalidixic acid	Enrofloxacin	Ciprofloxacin	Tetracycline	doxycycline	Erythromycin	Azithromycin	Minocycline	Clindamycin	Sulfamethoxazole/ trimethoprim	Linezolid	Quinopristin/ Dalforistin	Teicoplanin
20N-2328	PES	R	S	S	S	S	S	S	R	S	S	S	R	R	S	S	S	S	S	R	I	R	S	S	R	R	S	S	R	S	S	S
20N-2331	PES	R	R	R	I	S	S	S	S	I	S	S	S	S	R	R	S	R	R	R	R	R	R	R	R	R	I	R	I	R	R	R
20N-1786	PES	R	R	S	S	S	S	S	S	R	I	S	S	R	R	S	R	S	S	S	R	R	R	R	R	S	I	R	R	R	R	R
20N-1788	PES	R	R	I	I	S	S	S	S	I	S	S	S	S	R	S	S	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R
20N-2028	PES	R	R	S	S	S	S	R	R	I	I	S	R	R	R	S	S	S	S	R	R	R	S	S	R	R	S	R	S	I	S	S
20N-2030	PES	R	R	R	R	S	I	I	I	R	R	I	S	R	R	I	S	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R
20N-2039	PES	R	S	S	S	R	I	S	R	R	S	R	S	R	S	S	S	S	S	R	S	S	S	S	R	R	S	I	S	R	R	S
20N-2041	PES	R	S	S	S	S	S	S	I	S	S	S	S	R	S	S	S	S	S	S	S	R	R	R	S	R	S	R	R	S	S	R
20N-2042	PES	R	S	S	S	S	S	S	I	S	S	S	R	R	S	S	S	S	S	S	S	S	R	S	R	S	S	R	S	I	R	R
20N-2174	PES	R	R	R	R	S	I	I	R	R	I	S	I	S	R	S	S	I	I	R	R	R	R	R	R	S	R	R	S	R	R	R
21N-234	PES	R	R	R	I	I	I	I	I	R	S	S	S	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
21N-235	PES	R	R	R	S	S	S	I	S	R	S	I	S	R	R	R	S	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R
21N-239	PES	R	R	R	I	S	S	I	I	R	S	I	S	R	R	S	S	R	R	R	R	R	R	R	R	S	I	R	R	R	R	R
21N-242	PES	R	R	I	I	S	S	I	I	I	S	I	I	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
20N-1776	PES	R	R	S	R	S	S	I	S	S	S	S	S	S	R	S	S	R	R	R	R	R	R	I	R	S	S	R	S	R	S	R
20N-1777	PES	R	R	I	R	S	S	S	S	S	S	S	S	S	R	R	I	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R
20N-1778	PES	R	R	I	R	S	S	S	S	S	S	S	S	S	R	S	S	R	S	R	R	R	R	R	R	R	I	R	R	R	R	R
20N-1779	PES	R	R	S	I	S	S	S	S	S	S	S	S	S	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
20N-1780	PES	R	S	S	I	R	R	R	R	S	R	I	S	R	R	R	S	I	R	R	S	R	R	R	I	R	R	R	I	S	S	R
20N-1915	PES	R	R	S	R	S	S	I	I	R	S	R	S	S	S	S	S	R	R	R	I	R	S	S	R	R	S	R	S	I	R	I
20N-2037	PES	R	S	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	R	R	S	S	S	R	R	S
20N-2464	PES	R	S	S	S	S	S	R	R	S	S	S	S	R	S	S	S	R	R	R	R	R	I	S	S	S	S	I	S	R	S	S
20N-2465	PES	R	S	R	I	S	S	S	S	R	I	S	S	S	R	S	S	R	S	R	S	R	R	I	R	S	S	R	S	R	R	S
20N-2467	PES	R	R	S	R	S	S	S	I	S	S	S	S	R	R	I	I	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R
20N-2468	PES	R	S	S	S	R	S	I	R	S	I	R	S	S	R	I	S	S	R	S	S	S	R	S	R	R	S	R	S	I	R	R
20N-2469	PES	R	R	S	R	I	S	S	I	I	S	I	S	R	R	I	I	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R

Appendix-XXV: AST results of isolates derived from Muzaffarabad, AJK.

ID Number of Sample	CITY	Penicillin	Ampicillin (AMP-R µg/disk)	Ampicillin-Sulbactam	Augmentin	pipercillline-tazobactam	Ceftiofur	Cefotaxime	Ceftazidime	Cefazolin	Cefepime	Imipenam	Meropenem	Colistin	Streptomycin	Gentamicin	Amikacin	Chloramphenicol	Florfenicol	nalidixic acid	Enrofloxacin	Ciprofloxacin	Tetracycline	doxycycline	Erythromycin	Azithromycin	Minocycline	Clindamycin	Sulfamethoxazole/ trimethoprim	Linezolid	Quinopristin/ Dalforistin	Teicoplanin
21N-289	AJK	R	R	I	R	S	S	R	R	R	S	R	R	R	R	R	S	R	R	R	R	R	R	I	R	R	S	R	R	R	R	R
21N-292	AJK	R	S	S	S	S	S	S	S	I	S	R	S	S	R	S	S	R	R	R	R	R	R	I	R	S	S	R	R	R	R	R
21N-293	AJK	R	R	I	R	S	S	I	S	R	S	I	I	R	R	S	S	R	R	R	R	R	R	I	R	R	S	R	R	R	R	R
21N-294	AJK	R	R	R	R	S	R	R	S	R	S	R	I	R	R	R	S	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R
21N-295	AJK	R	R	S	R	S	I	I	S	R	S	S	I	R	R	R	S	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R
21N-296	AJK	R	R	S	S	S	S	S	S	S	I	R	S	S	R	S	S	R	R	R	R	R	R	I	R	S	S	R	R	I	R	R
21N-298	AJK	R	R	S	I	S	S	R	I	R	S	I	S	R	R	S	S	R	S	R	I	I	R	R	R	R	R	R	R	R	R	R
21N-299	AJK	R	R	S	I	I	I	R	I	R	I	I	I	R	R	S	S	R	S	R	I	R	R	R	R	R	R	R	R	R	R	R

Thesis

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