Evaluation of Drug Resistance Genes in *Salmonella* Species Isolated from Commercial Poultry of Live Bird Markets in Islamabad Capital Territory

BY

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December, 2021

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CERTIFICATE

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AUTHOR'S DECLARATION

I would like to declare that the data presented in this thesis "Evaluation of Drug Resistance Genes in *Salmonella* Species Isolated from Commercial Poultry of Live Bird Markets in Islamabad Capital Territory" is generated myself from original research work under the supervision of Dr. Muhammad Athar Abbas at National Reference Laboratory for Poultry Diseases (NRLPD), National Agriculture Research Centre, Islamabad, Pakistan. The results and material used in this thesis never presented anywhere else earlier.

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Dedication to

My

FATHER

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My

MOTHER

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LIST OF ABBREVIATION

API	Analytical Profile Index
BGA	Brilliant Green Agar
BPW	Buffered Peptone Water
CDC	Center for Disease Control
CFU	Colony Forming Unit
CLSI	Clinical Laboratory Standards Institute
DNA	Deoxyribonucleic Acid
FAO	Food and Agriculture Organization
GDP	Gross Domestic Product
MDR	Multidrug Resistance
MIC	Minimum Inhibitory Concentration
MKTTn	Muller-Kauffman Tetrathionate-Novobiocin Broth
NMKL71	Nordic Committee on Food Analysis
OECD	Organization of Economic Co-operation and Development
PCR	Polymerase chain Reaction
RDI	Recommended Dietary Intake
RVS	Rappaport-Vassiliadis Soya Broth
rpm	Revolutions per minute
TBE	Tris-borate-EDTA
TSI	Triple Sugar Iron
UNDP	United Nations Development Programme
UN	United Nations
WHO	World Health Organization
WGS	Whole Genome Sequencing
XLD	Xylose Lysine Deoxycholate agar

ABSTRACT

Salmonella is one of most important food-born pathogen transmitted mainly through poultry. Extensive use of antimicrobial agents in animal production systems is considered as a cause of emergence of antimicrobial resistance in *Salmonella* and other pathogens. To analyze this fact, the study was conducted from July 2020 to July 2021 on cecal samples from apparently healthy chicken from live bird markets of different cities of Pakistan. Study was carried out with the objectives to isolate and identify drug resistant among *Salmonella* species from poultry and to evaluate their phenotypic and genotypic antibiotic resistance profile. This was a unique and first study of this kind to evaluate AMR through coordinated surveillance method in healthy poultry across the country. In this regard a total of 763 caecal samples from poultry were processed for the presence of *Salmonella spp.* following standard isolation protocols. Out of 763 samples, 268 (35%) samples were tested positive for *Salmonella spp.* From 268 positive isolates, 173 were analyzed through antimicrobial susceptibility test and all were observed to be resistant to multiple antibiotics with highest level of resistance against Penicillin (100%), Clindamycin (100%), Streptomycin Tetracycline Teicoplanin (100%), Linezolid (100%), Nalidixic Acid (95%), Tetracycline (93%) and Streptomycin (90%). On the other hand, almost all isolates were susceptible to piperacillin/tazobactum. Out of 173 isolates analyzed for phenotypic antimicrobial resistance profile, 24 were further analyzed for genotypic antimicrobial resistance profile for the detection of genes through conventional polymerase chain reaction (PCR) and results were correlated with phenotypic profile. The study concluded that there is high multidrug resistance in *Salmonella* which may be increased with time due to miss-sue and over-use of antibiotics on farm and therapeutic level, poor husbandry practices, and fluctuation in standard recommended dosage of antibiotics. This may be leading to selection pressure on *Salmonella spp.* to acquire and retain resistance genes and causing a major threat. It is hereby recommended to aware farmers and other poultry related stakeholders about controlled use of antibiotics, good husbandry and slaughtering practices to avoid contamination with multidrug resistant *Salmonella spp.,* and to find new mechanisms and solutions to cope with this high level of antibiotic resistance to treat both animals and human food-borne illness.

Key words: *Salmonella*, antimicrobial resistance, isolation and identification, phenotypic antimicrobial resistance profile, genotypic antimicrobial resistance profi

Chapter 1
Introduction

1. INTRODUCTION

1.1 Importance of poultry

Poultry birds fall in avian classification of kingdom Animalia and contribute towards major portion of human diet in the form of meat and eggs. Its importance is widespread with the fact that it is most readily available source of meat for human consumption around the globe. Poultry farming is of prime importance for any country being an economical and accessible source of animal based protein. Poultry industry serves as an efficient way to overcome a country's protein deficiency (Sarwar *et al.*, 2015). Poultry meat and eggs are found to be good source of valuable proteins and are critically required by millions of people living in poverty. It plays a major role in developing countries as these products are easily available and affordable. In rural regions, it aids in terms of food security for poor families by providing them a good protein source as well as a source of income. Commercial poultry industry is growing fast and providing opportunities for employment. Only about 1.7kg feed is required for production of 1kg meat from commercial broiler chicken (Farrell, D. 2013).

In comparison with other livestock, poultry production requires less water usage and has less harmful effects on environment. Chicken meat is healthy as well as cheapest of all type of livestock meat. There are no taboos on consumption of poultry meat as food which is the major advantage (Farrell, 2013). Poultry meat contain low fat percentage than mutton and beef and known as non-fattening good dietary source to prevent from hardening of blood arteries (Ghafoor *et al.*, 2010). Poultry meat is of high quality, contain less saturated fats and rich in essential nutrients and minerals. The chances of several neural and metabolic diseases linked with critical dietary vitamins, amino acids and mineral deficiency can be limited by poultry products which are rich in all essential nutrients (Farrell, 2013).

Consumption of poultry meat is increasing steadily throughout the world reaching to 14.2kg per capita/year according to recent available data. Largest consumers of poultry are western countries which are well developed especially United States of America with 49.8kg per inhabitant/year. The similar pattern of enhanced consumption has been observed for European Union and countries associated with OECD (Organization of Economic Co-operation and Development). Over past thirty

years in France, consumption of poultry meat has raised to double and since 2012, it has acquired the status of second most consumed meat. In 2014, its consumption reached to 26kg per capita after pork with 32.5kg per capita consumption (Rouger *et al.*, 2017).

Poultry domain plays a major role in developing countries and critically required by millions of people living in poverty. According to a survey conducted in various developing poor countries of South Asia and sub-Sahara Africa, 59 percent people in sub-Sahara Africa and 34 percent in South Asia were suffering from energy deficiency. About 67 percent of their total energy was contributed by staple food (cereals, grains) which have only small quantity of low-quality protein. Only 8 percent energy in sub-Sahara Africa comes from animal protein compared with all developed countries and china, where the values are 17 percent and 28 percent respectively (Smith and Wiesman, 2007). Most important amino acids including threonine, lysine, cysteine, methionine and tryptophan for human diet are deficient in cereals while chicken meat is rich with these amino acids. For poultry meat, corresponding annual estimates are 2.9 percent for least developed countries and 1.6 percent for most developed countries between 2005-2016 (Sparks, 2006; Windhorst, 2008).

Certain facts prove the nutritional benefits of poultry meat in comparison to other meat. Chicken meat is devoid of coronary heart disease causing trans fats which are found in lamb and beef in high amount. Values of 8 percent for lamb and 2 to 5 percent for beef have been reported in Canada. Poultry meat is rich in poly-unsaturated fatty acid most importantly omega-3 fatty acid which are essential for health. About 50g of chicken meat per day for infants and 100g for adults is sufficient to meet recommended dietary intake for niacin. About 45µg of meat can provide 23 percent of RDI for folic acid to a pregnant woman (Bingham, 2006). Folic acid deficiency is a major issue that causes neural tube defects with highest rate in Uttar Pradesh, India where it ranges from 3.9 to 8.8/1000 births according to a survey (Cherian *et al.*, 2005).

1.2 Poultry in Pakistan

Importance of poultry is also well known in Pakistan where it is one of most significant segment of agriculture sector contributing up to 19 percent of total meat production of country (Ghafoor *et al.*, 2010). Poultry is Pakistan's second biggest

industry showing 8-10% annual growth with an average investment of almost RS. 750 billion ((Ghafoor *et al.*, 2010; Mukhtar *et al.*, 2012; GoP, 2021). According to 2014 survey of Govt. of Pakistan, poultry is source of employment for almost 1.5 million people (Hussain *et al.*, 2015). Poultry being a good source of economical and nutritious protein contributes in national Gross Domestic Product to about 1.3% (Hussain *et al.*, 2015) and in agricultural GDP to about 4.8% (Ghafoor *et al.*, 2010). Commercial production of poultry started in 1960's in Pakistan and has been major contributor to daily protein uptake of Pakistani population. It is playing major role to fill up gap between demand and supply of protein. According to Govt. of Pakistan survey (2013), the rate of increase in human population in Pakistan is 2.03% per annum and there is a strong correlation of 89.0% between poultry growth and population. In Pakistan, poultry being a well-managed and vibrant sector contributes 26.8% to total meat production, 5.76% to agricultural sector and 1.26% to national GDP (GoP, 2014). According to economic survey of 2010-2011, about 70% of total meat production of country is from Punjab province (GoP, 2011). During early 1960s, commercial poultry farming was started and showed fast growth over years. Government gave special incentives to poultry sector and declared it free of income and sales tax as well as custom duties which promoted its early fast growth and success. Before 1963, the only source of meat and eggs in Pakistan was native chickens raised up as backyard activity which produce an average of 0.769kg meat at age of four months and lay 30 eggs/year (Sahota *et al.*, 2003). A better variety of chicken named Lyallpur Silver Black was introduced during 1965-1966 by Department of Poultry Husbandry at University of Agriculture, Faisalabad by crossing native Desi breed with imported White Leghorn, New Hampshire and White Cornish breeds, being able to survive under severe environmental conditions and produce up to 150 eggs/year (Hussain *et al.*, 2015).

During mid-1960s, first commercial hatchery of Pakistan was established in Karachi by collaboration of Pakistan International Airlines and Shaver Poultry Breeding Farms of Canada and first commercial feed mill for poultry in Rahim Yar Khan (Punjab) by Lever Brothers Pvt. Ltd. (Memon, 2012). In early 1970s, poultry sector showed an annual growth of 20-30%. Industry experienced 177% growth in total bird count, 271% in total production of poultry meat and 297% in egg count during 1971-1980. UNDP/FAO assisted to establish Poultry Research Institute in Rawalpindi

and Karachi in 1978. In 1979, foundation of Federal Poultry Board was laid down to create a link between government and industry . First processing plant for poultry meat (250 birds/h) was established in 1980 at Poultry Research Institute, Rawalpindi as demonstration model (Mukhtar *et al.*, 2012). During 1980s, annual growth rate of poultry sector was observed to be 10-15%. From 2000-2010, growth rate of 127%, 126% and 71% was observed in total birds, total meat production, and total eggs produced respectively (Hussain *et al.*, 2015).

During 2007-2008, production of poultry birds was 518 million and meat production was 601 thousand tons (Ghafoor *et al.*, 2010). According to economic survey of 2010-11, growth rate is 7.8% per annum and live animals of worth $13.95 are being exported (Mukhtar *et al.*, 2012). About 66% of Pakistani population is facing protein deficiency as protein available for consumption is 69.61g per person/day while the requirement is 102.7g per person/day. The gap between demand and availability is 33.09g per person/day which can be narrow down with poultry meat in much better way and in minimum time when compared to other animal protein sources (Maqbool *et al.*, 2005).

1.3 Meat quality and possible contaminants

In regard to worldwide importance and increasing production and consumption, ensuring quality and microbial safety of poultry meat is of prime importance (Rouger *et al.,* 2017). Poultry production is greatly affected by stress of certain environmental, pathological, nutritional and other factors. Environmental concerns are major issue for poultry industry with odors, dust, gases, toxins and microorganism as prominent air pollutants causing respiratory issues. Poor environments make chicken more sensitive to viruses and pathogens by making their defense system weak (Almuhanna *et al.*, 2011). Dust in environment housing poultry farms contain biological toxins and immuno-toxicity factors including fungal glucans and mycotoxins, bacterial endotoxins, plant toxins, volatile odorous compounds and animal venoms (Skóra *et al.*, 2016). Certain bacteria reside in gastro-intestinal track of poultry birds that can be source of contamination and pose a potential risk for consumers. Pseudomonads, *Enterobacteriaceae, Enterococcus* and *Lactobacillus* species are some bacterial contaminants of poultry meat. Contaminants having potential for spoilage of poultry

meat include lactic acid bacteria, B. thermosphacta, *P. fluorescens, and S. putrefaciens* (Zhang *et al.,* 2012). *Salmonella* and Campylobacter are most common pathogens present in gastro-intestinal tract of poultry birds and can be source of contamination for poultry meat (Hue *et al.,* 2011).

Certain environmental, pathological and other factors have imposed a major stress on poultry production. Poultry industry is greatly being affected by environmental contaminants (carbon dioxide, ammonia, dust, air-born microorganisms and toxins) related to air emissions in poultry housing. Ammonia causes reduction in body weight at 25ppm concentration, predisposition to infectious disease, respiratory irritation and cornea/keratoconjunctivis at 50ppm concentration. Poultry dust have been reported to contain Alternaria, Acremonium, Aurobasidium Aspergillus, Cladosporium, Basidiospores, Chrysosporium, Epicoccum, Drechslera, Eurotium, Geomyces, Fusarium, Mucor, Pithomyces, Penicillium, Rhizomucor, Ulocladium and Scopulariopsis mold species. According to Directive 2000/54/EC categorization of potentially hazardous micro-organisms in industrial poultry farms, Group 3 organisms include *Salmonella* choleraesuisvar. Typhi, Chlamydia ornithosis, Bacillusanthracis, and H5N1 virus, while Group 2 include Candida albicans, Listeria monocytogenes, Aspergillusfumigatus, Cryptococcus neoformans, Staphylococcus spp., Mycoplasma spp., Streptococcus spp. (Skóra *et al.*, 2016).

1.4 *Salmonella* as major contaminant of poultry

Salmonella was first discovered in 1855 by Theobald Smith when he isolated it from swine fever infected pigs' intestine and named after his coworker Daniel Elmer Salmon (Hassen, 2020). *Salmonella* are non-spore forming, gram-negative and facultative anaerobic bacteria which make up a large genus of Enterobacteriaceae family. They range from 0.4 to 0.6µm in size (Getenet, 2008). *Salmonella* usually don't require salt concentration but can grow in range from 0.4 to 4 %. Optimum temperature for growth is 35-37 ºC, optimum pH is 6.5-7.5 and water activity range from 0.99 to 0.95. At pH <3.8, temperature <7ºC and water activity <0.94, growth of *Salmonella* is completely inhibited (Pui *et al.*, 2011). The genus *Salmonella* contains two species named *Salmonella enterica* and *Salmonella bongori* from which *S. enterica* has six subspecies called *enterica, salamae, arizonae diarizonae, houtenae* and *indica*

(Threlfall *et al.*, 1999). About 2500 serotypes of *Salmonella* have been identified (Hassen, 2020) from which almost 59% of serotypes belong to *Salmonella enterica* subsp. *enterica* (Brenner, F. *et al.* 2000).

Salmonella are potential pathogens of animals and humans (Fluit, 2005). They are transmitted either through food stuff including dairy products, eggs and meat or via direct contact between animals and human (Olsvik *et al.*, 1985). *Salmonella* cause serious infections that are mostly food-born and result in gastroenteritis (Fluit, 2005). Poultry and livestock are main reservoirs of non-typhoidal *Salmonella* (Dahal *et al.*, 2007). Serotype *Salmonella gallinarum* is highly adapted to poultry. Non-typhiodal strains *Salmonella Typhimurium* and *Salmonella Enteritidis* greatly infect poultry and cattle (Ohl and Miller, 2001).

Salmonella is one of most important food-born pathogen transmitted mainly through poultry. Many countries have reported incidences of *Salmonella* including Belgium, Greece, Malaysia and UK (Domınguez *et al.*, 2002). According to Carramiñana *et al.* (1997), *Salmonella* prevalence in poultry carcasses was studied during their processing in slaughterhouses. Average value of *Salmonella* contamination was 35% with levels ranging from 20% to 70%. Most common serotypes were *S. hadar, S. thyphimurium, S. virchow, S. newport, S. enteritidis, and S. Heidelberg* (Carramiñana *et al.* 1997). *Salmonella* prevalence in poultry meat is reported as high as 20% in US, while 32% in India previously, where as it was reported 5.92% in Saudi Arabia, and 35.5% in Mexico (Dahal *et al.*, 2007; Miranda *et al.*, 2009; Moussa *et al.*, 2010; Adeyanju and Ishola, 2014). In northern Thailand, prevalence of *Salmonella* has been reported to be 57% during 2002-2003, Where as, it was reported to be 14.5% in Kathmandu, Nepal and 42.63% in Vietnam (Bao, 2005; Maharjan *et al.*, 2006; Padungtod and Kaneene, 2006). Studies carried out by Sikder *et al.,* 2005 in Bangladesh found 23.46% sero-prevalence of *Salmonella* in poultry. In South Africa, percentage of *Salmonella* in frozen and fresh poultry products is 19% where as in Spain and Portgal, this value is as high as 49% and 60% respectively (Antunes *et al.*, 2003; Capita *et al.*, 2003; Van Nierop *et al.*, 2005). According to reports of Enter-net surveillance programme, *Salmonella enterica* serotypes Typhimurium and Enteritidis were reported by participating countries to be most dominant organisms making up 80% of all the isolates during 1998-2003 (Fisher, 2004).

Salmonella being most pathogenic microorganism of poultry mediated bacterial food poisoning is of rapidly increasing public health concerns (Panisello *et al.*, 2000). The Food and Agriculture Organization (FAO) of United Nations (UN) has jointly stated with WHO that "illness due to contaminated food was perhaps the most widespread health problem in the contemporary world" (Kaferstein, 2003). *Salmonella* cause acute gastroenteritis in human called salmonellosis (Sockett, 1995) associated generally with subspecies *enterica* of *Salmonella enterica* (Getenet, 2008). *Salmonella spps.* were reported to be most common cause of infection during foodborne outbreaks (Sockett, 1995). During 1970s, poultry-associated *Salmonella virchow* outbreaks were observed in England and Wales, which was increased by 30% during 1981-86 (Humphrey *et al.* 1988). According to Center for Disease Control (CDC) estimates, state health departments reported 50 outbreaks of salmonellosis associated with annually 2,000 cases. Surveillance data reported 40,000 salmonellosis cases annually. According to surveillance data by CDC, true estimates of annual salmonellosis cases were 400,000-4,000,000 humans (Roberts, 1988). Major factor causing *Salmonella* contamination and spread to human is fecal contamination of poultry carcasses during slaughter with gut spill out and handling of carcasses (Panisello *et al.*, 2000; Adeyanju and Ishola, 2014)

Cui *et al.* 2005 has demonstrated that contamination of poultry with *Salmonella* can occur along multiple stages of food chains viz. production, processing, handling, marketing and distribution. In a study conducted by Lahellec *et al.*, in 1986 poultry birds were examined at start and end of rearing period for *Salmonella* contamination. Results indicated that contamination with resident strains was low at day first but increased significantly at the end of period. Serotypes identifies at day first may persist in environment and can be source of contamination at end of rearing period. Walls of poultry houses pose source of *Salmonella* recovery in highest frequencies.

1.5 Antimicrobial resistance in *Salmonella*

Antimicrobials are substances of low molecular weight which have the ability to inhibit bacterial growth or kill the bacteria and currently used in human and veterinary medicine (Schwarz and Chaslus-Dancla, 2001). Antibacterial drugs, antifungal agents, antiviral drugs, and anti-parasitic agents are included in antimicrobials (Frieden 2013).

According to WHO antimicrobial resistance is a phenomenon when antimicrobials and antibiotics are no longer effective against bacteria, fungi, viruses and parasites as they modify themselves with time and don't respond to these medicines. Capability of bacteria to simultaneously resist the effect of multiple antimicrobials is a phenomenon known as multidrug resistance (MDR) (CDC, 2009). Use of antimicrobial agents in animal production systems is thought to cause rise of antimicrobial resistance in *Salmonella* (Alexander *et al.*, 2009). Prophylactic doses or use of antimicrobials at sub-therapeutic level in food animals lead towards the resistant *Salmonella* strains thus increasing health risks to human linked with contaminated meat consumption. (Molla *et al.*, 2003; Molla *et al.*, 2006; Zewdu and Cornelius, 2009).

The spread of resistance by means of food is considered to be a major issue of public health (Lynch *et al.*, 2006). According to Elmadiena *et al.*, 2013 treatment of severe condition of *Salmonella* septicemia that appears in human, has been limited by the emergence of plasmid mediated resistance to previously used drugs for treatment and by chromosomal mediated resistance to fluoroquinolones and quinolones. An important MDR phenotype is resistance of *Salmonella* against chloramphenicol, Ampicillin, Streptomycin, Tetracycline and sulfonamide that shows resistance to 5 classes of clinically important antimicrobials according to CLSI. Resistance to chloramphenicol, Ampicillin, Streptomycin, Tetracycline, sulfonamide, ceftriaxone and amoxicillin-clavulanic acid is another multidrug resistant phenotype (CDC, 2009).

Resistance in *Salmonella* has been assumed to be high up to alarming point during recent years. Where Taiwan, Netherland, India, France, Ethiopia and Canada has reported high prevalence of resistant *Salmonella* isolates. India has certain reports about chicken isolated multidrug resistant (MDR) *Salmonella*. One of such reports showed increase in resistance to antibiotics in *Salmonella* Typhi as well (Gautam *et al.*, 2002; Molla *et al.*, 2003; Van Duijkeren *et al.*, 2003; Mandal *et al.*, 2004; Murugkar *et al.*, 2005; Lauderdale *et al.*, 2006; Poppe *et al.*, 2006; Weill *et al.*, 2006)

The origin of first antimicrobials is thought to be substances of some soil bacteria or fungi which they produce for them to fight for resources and survive in diverse ecology. Thus long before the introduction of antimicrobial for clinical uses, bacteria had been already in contact with antimicrobial substances. Although this

contact was less frequently, still it contributed to exert the selective pressure on microbes to adapt themselves and resist the inhibitory effects of antimicrobials. Bacteria acquire resistance by means of three types of mechanisms. First mechanism involves the resistance genes present in chromosomal DNA of antibiotic producing bacteria for their self-defense. Other bacteria acquire these genes through mobile genetic elements like transposons or plasmids and modify them by mutations to produce resistance domains that are structurally different but functionally homologous (Roberts, 1996). The second mechanism of resistance is to develop stepwise mutation in genes involved in physiological cell metabolic activities. As a result, substrates of metabolic pathways are replaced from metabolites to antimicrobials which are ultimately degraded (Davies, 1994). Another mechanism to develop resistance is modification of structures that are targets of antibiotics by single or multi-step mutations to make them resistant to inhibitory effects exerted by antimicrobial substances (Alekshun, 2000).

1.6 Importance of *Salmonella* testing

Salmonella infections related to contaminated and undercooked poultry products are second highest cause of illness and major cause of hospitalization and death, thus pose high risk to human health (FSIS-USDA). Over the past decade, annually ~45,000 cases and 400 to 600 deaths have been reported to CDC. According to a study conducted in US, salmonellosis account for 95% foodborne illnesses and approximately 30% deaths (Mead *et al.*, 1999).

According to CDC report published first in 2013, antibiotic germs infected about 2 million people and caused 23,000 deaths in US. Antimicrobial resistance causes reduction in productivity leading to 35-billion-dollar economic burden and $20 billion healthcare costs. Foodborne illness due to *Salmonella* that is resistant to antimicrobials leads to longer stay at hospital as a result of treatment failure. These findings emphasize the importance of worldwide surveillance and testing program to cope with issue (Acheson and Hohmann, 2001; CDC, 2013).

ISO 6579:2002 and NMKL 71 (Nordic Committee on Food Analysis) are two Europe approved methods for detection of *Salmonella* in animal feedstuffs and food (Carrique-Mas and Davies, 2008). ISO 6579:2002 is complex but more sensitive

protocol. According to this method, samples are pre-enriched in buffer peptone water and then selective enrichment is performed in (MULLER-KAUFFMANN Tetrathionate Novobiocin) broth (MKTTn) and Rappaport-Vassiliadis soya peptone broth (RVS broth). After 24 hours and 48 hours of incubation, samples from enrichment medium are inoculated on two agar plates one including Xylose Lysine Deoxycholate (XLD) Agar. Up to five colonies must be confirmed in one agar plate for confirmation of up to 40 probable colonies (Ferede, 2014).

The NMKL 71 is relatively simple and inexpensive method established for *Salmonella* isolation in feedstuffs and foods but in comparison to other methods, its sensitivity for faecal samples is limited (Korsak *et al.*, 2004). Samples are added to RVS enrichment media after pre enrichment in BPW and then cultured on XLD media plate (Carrique-Mas and Davies, 2008). Currently, drug susceptibility testing is applied for bacterial pathogens following standardized method (Köser *et al.*, 2012) of either measuring inhibitory zone around an antibiotic disc or minimum inhibitory concentration of an antibiotic by means of two-fold serial dilution (CLSI, 2015; CLSI, 2020). In disc diffusion method, culture media plate is inoculated with selected isolate and then antimicrobial agents are allowed to diffuse over it resulting in zone of growth inhibition. Diameter of this zone corresponds to the minimum inhibitory concentration (MIC) of that antimicrobial agent required to inhibit the growth of that particular microorganism (White, 2019).

Among molecular techniques used for identification of foodborne pathogens, a valuable diagnostic tool is polymerase chain reaction (PCR) (Kapperud *et al.*, 1993; Koch *et al.*, 1993) that is adapted for the rapid *Salmonella* detection in the environment, in foods and in clinical specimens. On the basis of DNA sequence of virulence genes of *Salmonella,* certain PCR tests are developed for identification purposes (Liu *et al.*, 2002).

For the purpose of outbreak detection and public health surveillance, whole genome sequencing (WGS) is highly efficient and economically feasible method in comparison to traditional methods being able to perform high throughput sequencing at low costs for bacterial genome. For whole genome sequencing (WGS), colony is picked up and mechanical lysis is performed with micron glass beads for the purpose

of DNA extraction. Libraries are prepared by amplification through PCR and sequencing is done (Köser *et al.*, 2012; Köser *et al.*, 2014).

For characterization of individual microorganism, WGS propose practical resolution by providing definite information of genotype including complete information of resistance determinants. WGS also has the capability to differentiate between different mechanisms having same pattern of resistance (Liu *et al.*, 2002).

Objectives

The objectives of present study are;

1. To isolate and identify multi-drug resistant *Salmonella* species in commercial poultry from live bird markets
2. To evaluate phenotypic and genotypic antibiotic resistance profile of selected *Salmonella* isolates of commercial poultry obtained from live bird markets.

Chapter 2
Materials and Methods

2. MATERIALS AND METHODS

2.1 Sample collection

The study was designed to analyze multidrug resistance in *Salmonella* isolated from caecal samples of healthy poultry, through AMR Surveillance program, from Islamabad Capital Territory and seven districts of all provinces in Pakistan during the year of 2020-2021. The study was conducted at National Reference Laboratory for Poultry Diseases (NRLPD) of Animal Sciences Institute in National Agricultural Research centre, Islamabad, Pakistan. The sampling criteria set to collect samples was as follow;

- Sample from apparently healthy and disease free birds
- Samples to be collected from live bird market
- 3-5 caeca collected per shop

The samples were collected from Islamabad Capital Territory. Samples from some other cities of Pakistan were also processed for comparative analysis including Rawalpindi and Lahore district of Punjab province, Karachi district of Sindh province, Quetta district of Balochistan province, Peshawar district of Khyber Pakhtunkhwa (KPK) province, Muzaffarabad district of Azad Jammu Kashmir and Gilgit district of Gilgit Baltistan.

Samples were collected during July 2020 to February 2021. Type of sample (caecal), date and time of sample collection, number of caeca, either pooled or un-pooled and sender's ID were already mentioned on sample receiving performa. The samples were allocated with specific sample ID in laboratory before further processing.

2.2 Sample processing

After allocation of sample ID, samples were processed in laboratory for;

1. Isolation and identification
2. Biochemical analysis
3. Antimicrobial susceptibility testing
4. Molecular characterization

2.2.1 Isolation and identification

Isolation and identification of *Salmonella* from caecal samples was carried out according to International Organization of Standardization (ISO-6579, 2002) recommended protocol (appendix I). All media and reagents were prepared following manufacturer's recommendations (appendix II-IX).

Pooled caecal samples (1g) were added in 10mL of buffered peptone water (OXOID CM0009) for the purpose of pre-enrichment and incubated at 37ºC for 18-24 hours. After pre-enrichment of samples, 1mL from buffered peptone water was transferred to 10mL of tetrathionate broth base Muller-Kauffmann (MKTTn; BIOLABS TMK20500) for the selective enrichment of *Salmonella* and incubated at 37ºC for 24 hours (Carrique-Mas and Davies, 2008; Ferede, 2014).

For isolation and identification of *Salmonella Spp.*, brilliant green agar (BGA; OXOID CM0329) was used as a selective as well as differential media. A loop full of each inoculum from MKTTn broth was cultured on BGA plates using quadrate streaking method (QSM) and incubated at 37ºC for 24 hours. Typical colonies of *Salmonella spp.* appeared pink on BGA after incubation and the color of media was turned red. Well isolated pink colonies (n = 2-3) suspected as *Salmonella spp.* were further inoculated on XLD agar separately using QSM and incubated at 37ºC for 24 hours. Typical *Salmonella spp.* appeared as colorless colonies with black center and color of media turned red. A typical colony from XLD was re-cultured on nutrient agar (OXOID CM003) to obtain purified bacterial isolates in a colony forming units for further processing (Carrique-Mas and Davies, 2008; Abd El-Aziz, 2013; Ferede, 2014).

A single isolated colony from nutrient agar was selected for Gram's staining. A drop of normal saline was placed on glass slide and a well isolated colony was mixed with it well to prepare smear of appropriate size. Slide was heat fixed by passing it over flame for 2-3 times. For staining, crystal violet was added for 1 minute that penetrated into peptidoglycan layer cell wall of bacteria and stained it as purple. Following crystal violet, gram iodine was added for 1 minute that formed macromolecular crystal violet-iodine complex in cell wall. At next step, the stained bacterial smear was decolorized with ethanol for few seconds that washed out crystal violet-iodine complex from thin

peptidoglycan layer of cell wall of gram negative bacteria, but thick peptidoglycan cell walls of gram positive bacteria retain this complex and observed as purple under microscope. As a last step, safranin was added as counter stain to visualize colorless gram negative bacteria as pink stained (Findlay *et al.*, 2018).

2.2.2 Biochemical testing

Biochemical analysis including sugar utilization iron test and citrate utilization test were performed. Slants of Triple Sugar Iron Agar (TSI; OXOID CM0277B) and Simmons' citrate agar (OXOID CM0155) were prepared using10mL prepared media in test tubes. The prepared slants were inoculated with identified colonies and incubated at 37ºC for 24 hours. Change in color of slant, butt, gas and H_2S production was observed in TSI agar tubes and change in color was observed on Simmons' citrate agar. Pink slant with yellow butt and H_2S production indicated the presence of *Salmonella* on TSI. On the other hand, change in color from green to blue on simmons' citrate agar slant was considered positive (Abd El-Aziz, 2013).

2.2.3 Antimicrobial susceptibility test

Antimicrobial susceptibility test of isolates was performed against 36 antibiotics using disc diffusion method according to Clinical Laboratory Standards Institute (CLSI) criteria. For this purpose, well isolated colonies picked from overnight cultured nutrient agar were added to 5mL of ultra-distilled water and mixed well by vortex. Density was checked in densitometer and suspension was compared with 0.5 McFarland standard to obtain 0.5 McFarland units of inoculum. Swabbing method was used to inoculate 0.5 McFarland suspension on sterile Muller-Hinton agar (OXOID CM0337B) plates. Antibiotic discs were applied on inoculated plate at appropriate distance (15-20mm approximately) to prevent overlapping of zone of inhibition. Plates were incubated at 35ºC for 18-24 hours and diameter of zone of inhibition against each antibiotic disc was recorded. The recorded diameter was compared with reference controls and declared as susceptible, intermediate or resistant according to CLSI criteria (CLSI, 2015; Borges *et al.*, 2017; CLSI, 2020). Reference strains of *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC

27853 were used as controls for quality assurance of the tests. Isolates were tested against a panel of 35 antibiotics of different classes which are listed in table 2.1.

Table 2.1: List of antibiotics used for antimicrobial susceptibility test

Class of antibiotics	Antibiotics	Symbol	Concentration
Penicillins	Penicillin	(P)	10 µg
	Ampicillin	(AMP)	10 µg
Penicillin+ Beta-lactamase inhibitor	Amoxicillin-clavulanic acid	(AMC)	30µg
	Ampicillin/Sulbactam	(SAM)	20 µg
Beta-lactamase inhibitor	Tazobactam	(TZP)	100 µg
Macrolide	Azithromycin	(AZM)	15 µg
	Erythromycin	(E)	15 µg
Cephalosporin (1st Generation)	Cephazolin	(KZ)	30 µg
Cephalosporin (2nd Generation)	Cefoxitin	(FOX)	30 µg
Cephalosporin (3rd Generation)	Cefotaxime	(CTX)	30 µg
	Ceftazidime	(CAZ)	30 µg
	Ceftiofur	(EFT)	30 µg
Cephalosporin (4th Generation)	Cefepime	(FEP)	30 µg
Phenicols	Chloramphenicol	(C)	30 µg
	Florfenicol	(FFC)	30 µg
Quinolones (1st Generation)	Nalidixic Acid	(NA)	30 µg
Quinolones (2nd Generation)	Ciprofloxacin	(CIP)	30 µg
	Enrofloxacin	(ENR)	5 µg
Lincomycin	Clindamycin	(DA)	2 µg
Polypeptides cyclic	Colistin sulphate	(CS)	10 µg
Carbapenems	Ertapenem	(ETP)	10 µg
	Imipenem	(IMI)	10 µg
	Meropenem	(MEM)	10 µg
Aminoglycosides	Streptomycin	(S)	10 µg
Aminoglycosides+ Deoxystreptamine	Gentamicin	(CN)	10 µg
	Amikacin	(AK)	30µg
Oxazolidinone	Linezolid	(LNZ)	30 µg
Nitrofurans	Nitrofurantoin	(F)	300 µg
Streptogramin	Quinupristin/Dalfopristin	(QDA)	15 µg
Glycopeptide	Teicoplanin	(TEC)	30 µg
Tetracyclines	Tetracycline	(TE)	30 µg
	Doxycycline	(DO)	30 µg
	Minocycline	(MH)	30 µg

Sulfonamide+ Diaminopyridines	Trimethoprim/sulphamethoxazole	(SXT)	25 µg
Diaminopyridines	Trimethoprim	(TM)	15 µg

2.2.4 Molecular characterization through PCR

A total of 24 isolates, three from each city, were selected for detection of resistance genes in their genome through polymerase chain reaction (PCR).

2.2.4.1 DNA extraction

DNA was extracted by 2 different methods; boiling method for chromosomal DNA and plasmid extraction kit for plasmid DNA. For boiling method, 4-5 colonies of overnight grown culture were added in 200 microliters sterile water in a pre-sterilized Eppendorf and vortexed gently. Eppendorf tubes were boiled at 100ºC for 10 minutes using hot plate. After boiling, tubes were placed in ice box for 5 minutes and then centrifuged at 14000 rpm at 4ºC for 10 minutes. After centrifugation, 150 micro liters of supernatant was separated and pellet was discarded (Noppe-Leclercq *et al.*, 1999; Salehi *et al.*, 2005; Ghoddusi *et al.*, 2015).

For plasmid extraction, FavorPrep™ Plasmid Extraction Mini kit (Favorgen: FAPDE 300) was used. About 3mL of overnight grown bacterial culture was added in centrifuge tube and centrifuged at 11000 × g for 1 minute. Supernatant was discarded and pellet was re-suspended by adding 200 µL FAPD1 buffer in which RNase was added. Then 200 µL FAPD2 buffer was added and tubes were inverted 5-10 times and incubated at room temperature for 2-5 minutes for cell lysis and then neutralized by adding 300 µL FAPD3 buffer and inverting tubes 5-10 times. Supernatant came after centrifugation at full speed for 5 minutes was transferred to FAPD column placed in a collection tube and again centrifuges at 11000× g for 30 seconds. Flow through was discarded and 400µL of WP buffer was added to column. Again centrifuged at 11000× g for 30 seconds and flow through was discarded. Then 700µL of wash buffer was added and column was centrifuged at 11000× g for 30 seconds. Wash through was discarded and column was dried by centrifugation for 3 minutes at full speed. After drying, FAPD column was transferred to new centrifuge tube and 50µL-100µL elusion

buffer was added over its membrane. Column was allowed to stand for one minute and then centrifuged for one minute at full speed to elute the plasmid DNA.

2.2.4.2 Polymerase chain reaction

To perform polymerase chain reaction (PCR) for detection of antibiotic resistance genes in *Salmonella* spp., reaction mixture was prepared with a volume of 25µL containing 12.5µL DreamTaq Green PCR master mix (Thermo scientific), 5µL of nuclease free water, 1µL of both forward and reverse gene-specific primer and 5.5µL of target DNA template (Table 2.2). Primers were used for detection of seven antibiotic resistance genes against selected classes of antibiotics. Primer sequence, target gene and amplicon size is given in table 2.3.

Table 2.2: Composition of PCR reaction mixture for volume of 25µL

Reagents	Volume (µL)
DreamTaq Green PCR master mix	12.5µL
Nuclease free water	5µL
Forward primer	1µL
Reverse primer	1µL
Template DNA	5.5µL
TOTAL	**25µL**

Table 2.3: Primers used for detection of antibiotic resistance genes

Antibiotic class	Target gene	Sequence (5′–3′)	Amplicon size	Reference
Aminoglycoside	acc(3)-II	CTCCGTCAGCGTTTCAGCTA ACTGTGATGGGATACGCGTC	237bp	Yu *et al.*, 2020
Aminoglycoside	aph(3)-II	GAACAAGATGGATTGCACGC GCTCTTCAGCAATATCACGG	688bp	Zhang *et al.*, 2018

Beta-lactamase	bla_{SHV}	AGGTGCTCATCATGGGAAAG CTTTATCGGCCCTCACTCAAA	237bp	Daoud *et al.*, 2015
Beta-lactamase	bla_{TEM}	CGCCGCATACACTATTCTCAGAATGA ACGCTCACCGGCTCCAGATTTAT	444bp	Daoud *et al.*, 2015
Beta-lactamase	bla_{OXA}	GCGTGGTTAAGGATGAACAC CATCAAGTTCAACCCAACCG	438bp	Poirel *et al.*, 2011
Colistin	mcr1	TCGGCAAATTGCGCTTTTGGC ATGCCAGTTTCTTTCGCGTG	502bp	Lescat *et al.*, 2018
Tetracycline	tet A	GCTACATCCTGCTTGCCTTC CATAGATCGCCGTGAAGAGG	210bp	Sun *et al.*, 2019
Tetracycline	tet B	GCTTGGAATACTGAGTGTAA CAGTGCTGTTGTTGTCATTAA	571bp	Adesoji *et al.*, 2015; Ma *et al.*, 2007

For detection of *aac*(3)-II gene against aminoglycosides, amplification process was carried out under following conditions; initial denaturation at 94ºC for 5 minutes, 30 cycles of amplification including 30 seconds of denaturation at 94ºC, 30 seconds of annealing at 60ºC and 1 minute of extension at 72ºC, and a final extension step for 10 minutes at 72ºC (Yu *et al.*, 2020).

For *aph*(3)-II against aminoglycosides, PCR was carried out under following conditions; 1 cycle of initial denaturation at 94ºC for 5 minute, 35 cycles of amplification with denaturation at 94 ºC for 30 seconds, annealing at 58 ºC for 30 seconds, extension at 72 ºC for 50 seconds, and final extension step of 10 minutes at 72 ºC (Zhang *et al.*, 2018).

Amplification for detection of *bla*SHV and *bla*TEM genes for β-Lactamases production was carried out under following conditions; initial denaturation at 94ºC for 15 minutes, 30 cycles of amplification with denaturation at 94ºC for 30 seconds, annealing at 62 ºC for 90 seconds, extension at 72 ºC for 60 seconds, and final extension at 72 ºC for 10 minutes (Daoud *et al.*, 2015).

For bla_{OXA} gene for β-Lactamases production, amplification process was performed under following condition; initial denaturation at 94ºC for 10 minutes, 36 cycles of amplification including 30 seconds of denaturation at 94ºC, 40 seconds of

annealing at 52ºC and 50 second of extension at 72ºC, and final extension for 5 minutes at 72ºC (Poirel *et al.*, 2011).

Amplification conditions for *mcr*-1 gene against Colistin were set as initial denaturation at 94ºC for 4 minutes, 30 cycles of amplification including 5 seconds of denaturation at 94ºC, 20 seconds of annealing at 59ºC and only single final extension for 5 minutes at 72ºC (Lescat et al., 2018).

Tetracycline resistance gene *tet*A was amplified on following conditions; initial denaturation at 95ºC for 10 minutes, 40 cycles of amplification including 15 seconds of denaturation at 95ºC, 1 minute of annealing at 65ºC, followed by extension (Sun *et al.*, 2019)

For *tet*B, amplification conditions were set at 95ºC for 5 minutes for initial denaturation, 35 cycles with denaturation at 94ºC for 30 seconds, annealing at 55 ºC for 30 seconds, extension at 72ºC for 30 seconds. Final extension was carried out for 10 minutes at 72 ºC (Ma *et al.*, 2007; Adesoji *et al.*, 2015).

2.2.4.3 Gel electrophoresis

The amplified DNA products from PCR were analyzed by gel electrophoresis. A 1.5% w/v agarose gel was prepared (procedure given in appendix X) and loaded into tray and wells were produced through comb. After solidification, gel was placed in electrophoretic tank containing 1X TBE buffer (procedure for buffer preparation is given in appendix XI) and 12µL of samples were loaded into each well and a ladder of 100kb was run with samples as standard molecular weight. Gel electrophoresis was carried out at 100V for 40 minutes and visualized under UV light. The band size was recorded and image of the gel was taken using gel documentation system (Vilber Lourmat, France).

Chapter 3
Results

3. RESULTS

3.1 Sample collection

Caecal samples (n=763) were obtained in National Reference Laboratory for Poultry Diseases (NRLPD) through AMR surveillance program from Islamabad Capital Territory and seven other cities of Pakistan including Rawalpindi, Lahore, Karachi, Peshawar, Quetta, Muzaffarabad and Gilgit (Fig. 3.1). Number and percentage of samples received from each district is shown in Fig 3.1.

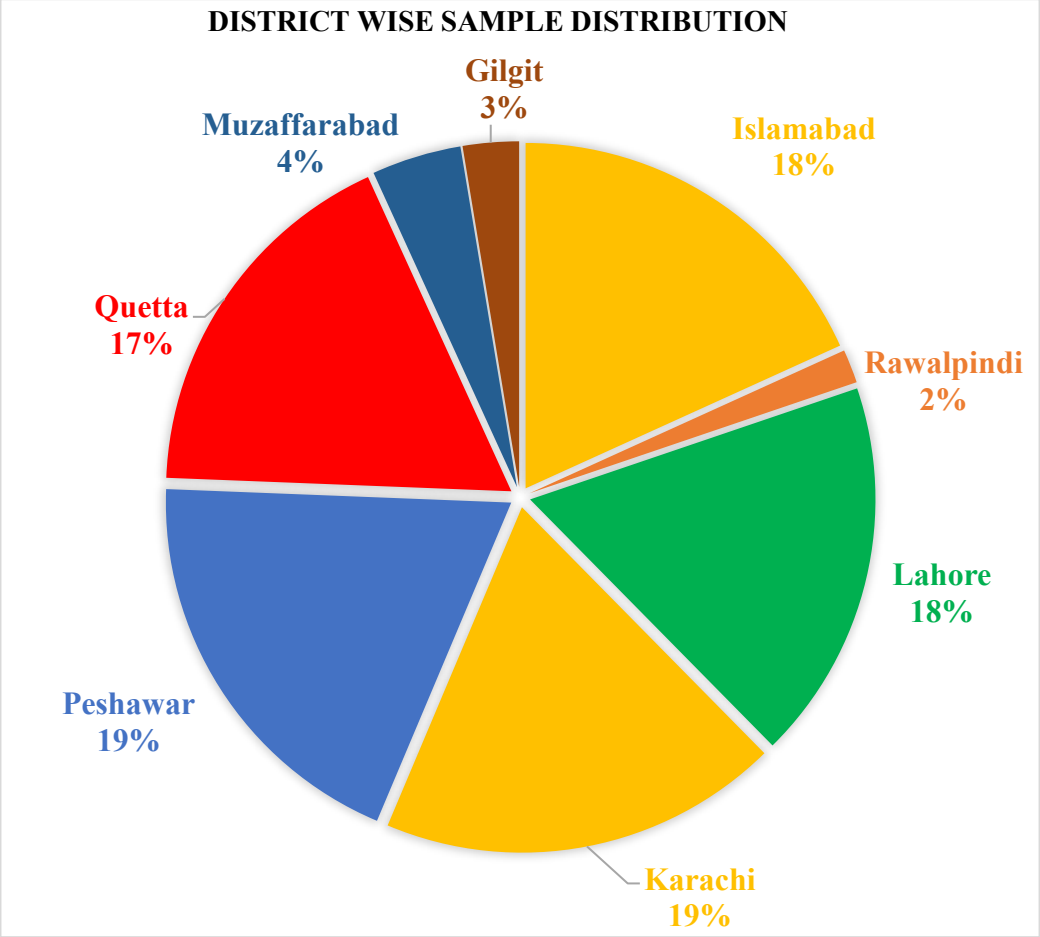


Figure 3.1: Area wise data of Samples received from different regions of the country

3.2 Isolation and identification

Microbiological analysis of caecal samples (n=763) was performed in laboratory for isolation and identification of *Salmonella Spp.* Samples were first inoculated in pre-enrichment BPW (Fig. 3.3) and then in tetrathionate broth for the purpose of selective growth enrichment of *Salmonella spp.* (Fig. 3.4) After pre-enrichment and selective enrichment, samples were cultured on BGA and XLD selective and differential agars to screen the growth for *Salmonella spp*. (Fig. 3.5, 3.6) Pure colonies were cultured on nutrient agar to be further utilized for biochemical analysis (Fig. 3.7).

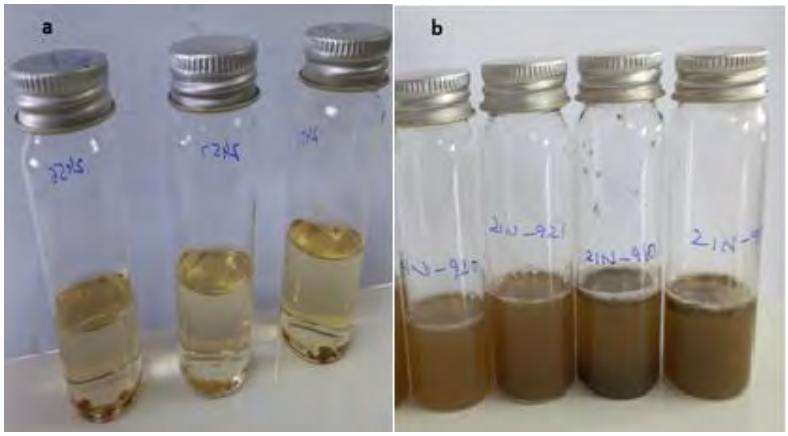


Figure 3.2: Pre-enrichment of caecal samples in buffered peptone water (BPW); (a) BPW before incubation (b) BPW after incubation with turbidity.

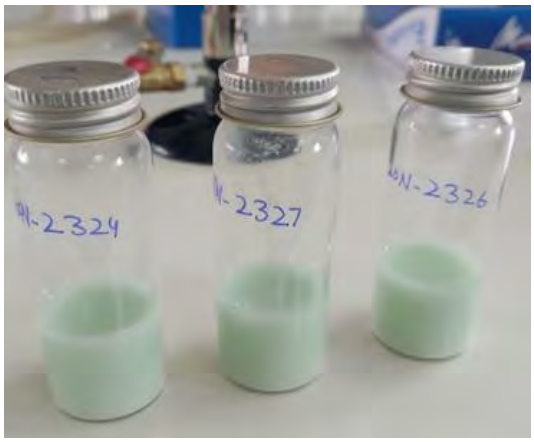


Figure 3.3: Selective enrichment of samples in tetrathionate broth (TTB)

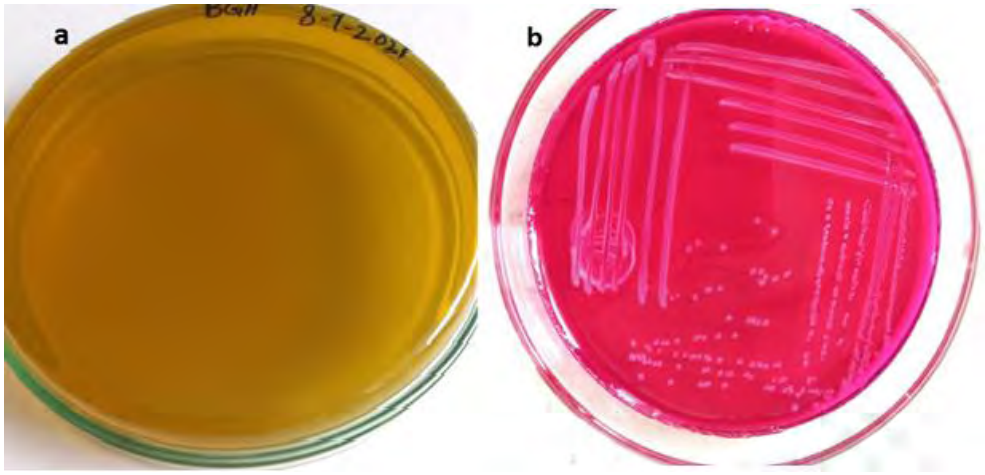


Figure 3.4: Culture growth of *Salmonella* on brilliant green agar; (a) BGA representing negative after incubation with no growth, (b) BGA representing positive *Salmonella* growth with Pink colonies

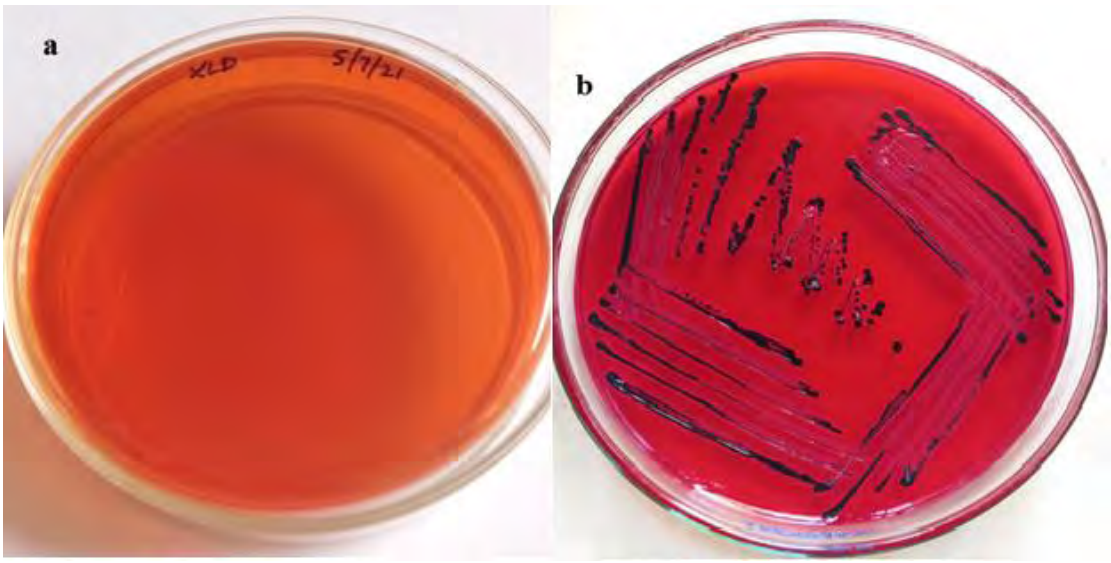


Figure 3.5: Culture growth of *Salmonella* on Xylose lysine deoxycholate *(XLD)* agar; (a) XLD representing negative after incubation with no growth (b) XLD representing positive *Salmonella* growth with colorless colonies having black center

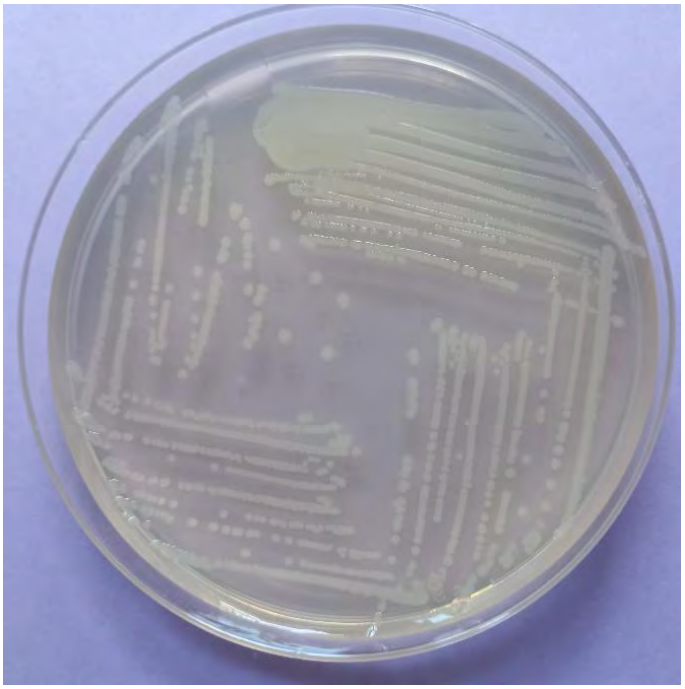


Figure 3.6: Culture growth of *Salmonella spp.* on non-selective media (nutrient agar)

Out of 763 samples tested, 268 (35%) samples were tested positive for *Salmonella* spp. and 495 (65%) samples were found to be negative as shown in figure 3.8 and table 3.2. A total of 46% sample from Islamabad Capital Territory, 41% sample from Rawalpindi, 33% samples from Lahore, 37% samples from Karachi, 32% samples from Quetta, 25% samples from Peshawar, 40% samples from Muzaffarabad and 20% samples from Gilgit were tested positive for *Salmonella spp*. as shown in Table 3.3 and Fig. 3.9. Detail of all isolates from each city along with their identification criteria is given in appendixes from XII to XIX.

Table 3.1: Detail of samples collected and number of isolated *Salmonella spp*

Total number of samples	**Number of *Salmonella* positive isolates**	**Number of negative samples**
763	268	495
Percentage value	35%	65%

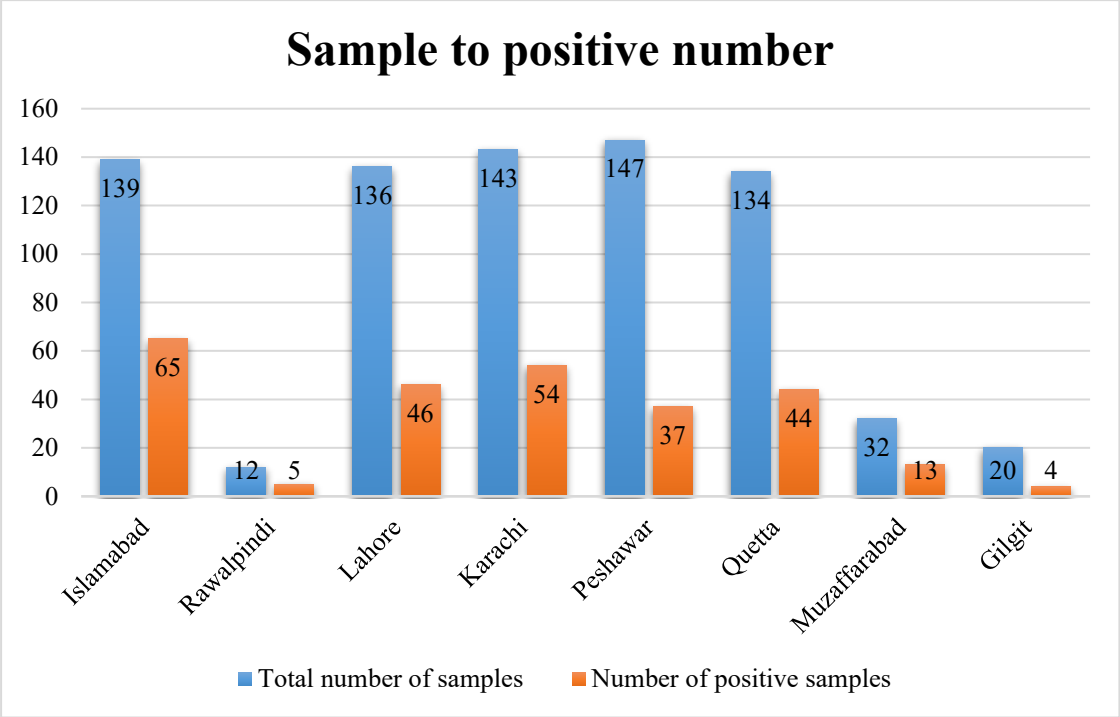


Figure 3.7*:* Area-wise data of *Salmonella* isolates from different regions of country

For gram staining of isolates, slide was prepared by putting a drop of normal saline on slide and mixing a well isolated colony with it to prepare a smear. Slide was heat fixed and stained by adding crystal violet, followed by iodine, decolorized with ethanol and then counter-stained with safranin. After gram staining, a drop of emulsion oil was put over stained slide and observed under 100X power lens of light microscope. All samples appeared as pink rods indicating the presence of gram negative bacteria as shown in Fig 3.10.

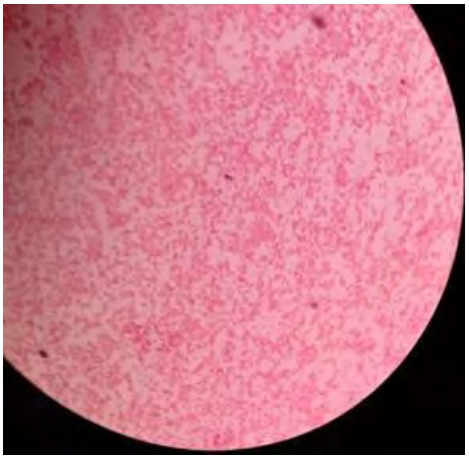


Fig. 3.8: Identification of isolates using gram staining method

3.3 Biochemical analysis

All of 268 isolates were analyzed through biochemical testing for further identification. The results of triple sugar iron (TSI) test and citrate utilization test confirmed *Salmonella spp.* The TSI tubes with alkaline slant (red/pink) and acidic butt (yellow) indicated fermentation of glucose while H_2S was also produced causing blackening of media which are the characteristics of *Salmonella spp*. (Fig. 3.11). Results of citrate utilization test depicted change in color of slant from green to blue (Fig. 3.11).

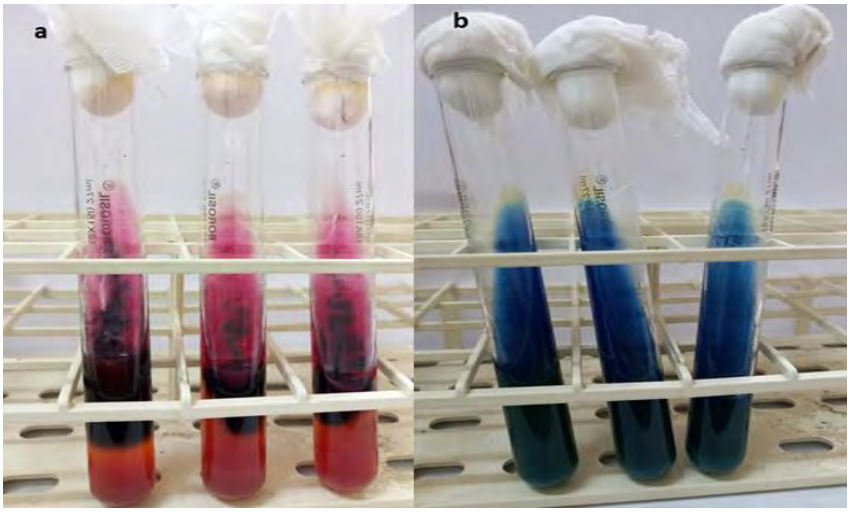


Figure 3.9: Identification of Salmonella through Biochemical analysis (a) Triple sugar iron (TSI) test; pink slant, yellow butt and H_2S production; (b) Citrate utilization test; change in color of slant from green to blue.

3.4 Antimicrobial susceptibility test

After biochemical analysis, 173 samples were selected and antimicrobial susceptibility testing was performed against 35 antibiotics using disc diffusion method. A 0.5 McFarland bacterial suspension was prepared and swabbed on Muller Hinton agar(MHA) and discs were placed by disc dispensers. After incubation for 24 hours at 37ºC, zones of inhibition were measured and results were interpreted according to CLSI criteria (Fig. 3.12). Breakpoints of intermediate values along with sensitivity and resistance values for each antibiotic is shown in appendix (XX). Different patterns of susceptibility and resistance against these antibiotics was observed for different samples. Detail of sensitivity, intermediate and resistance values of all samples against 35 antibiotics is given in appendix (XXI-XXXVI).

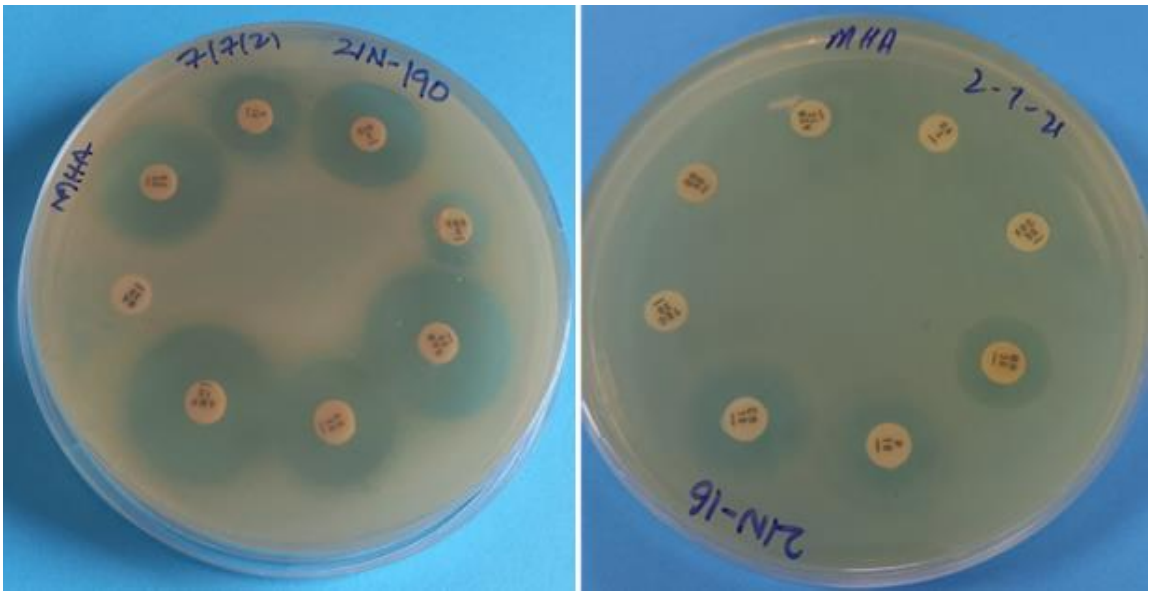


Fig. 3.10: Antimicrobial susceptibility test; zone of inhibition of bacterial growth around antibiotic discs

3.4.1 Phenotypic AMR profiling of isolates obtained from caecal samples

Among 268 *Salmonella* positive isolates, 173 samples were subjected to antimicrobial susceptibility test against thirty-five antibiotics to check their resistance and sensitivity profile. Isolates showed high resistance against most of the antibiotics tested. Penicillin, Clindamycin and Teicoplanin showed 100% resistance against all isolates tested. On second highest level, Linezolid and Quinupristin/Dalfopristin

showed 99% resistance. Erythromycin was 98% resistant followed by Nalidixic Acid 96% resistant, Nitrofurantoin 95% resistant, Doxycycline 94% resistant, Streptomycin 93% resistant, Tetracycline was 91% Enrofloxacin was 82% resistant and Azithromycin was 81% resistant. Colistin and Trimethoprim was 79% resistant against isolates tested. Sulfamethoxazole/trimethoprim was observed to be 77% resistant followed by Minocycline 70% resistant, Chloramphenicol & Ciprofloxacin 65% resistant and Florfenicol 63% resistant. Ampicillin/sulbactam was found to be 56%, Cephazolin was 51%, Imipenem was 40% resistant and Gentamicin was 21% resistant. 18% resistance was observed against Cefotaxime and Ceftazidime, followed by 17% against Ceftiofur, 13% against Meropenem and 12% against Amikacin. Ampicillin was 8% resistant, Cefepime, Cefoxitin, ertapenem was 5% resistant, Piperacillin/tazobactum was 4% and Amoxicillin-clavulanate/Augmentin was only 3% resistant. (Fig. 3.12) (Table. 3.4)

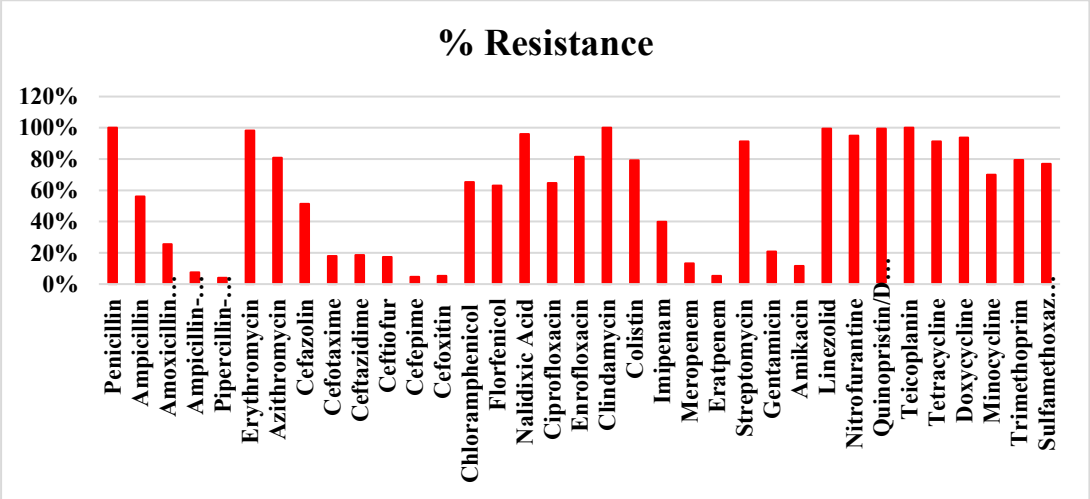


Figure 3.11: AMR profiling of resistance in Salmonella isolates against selected antibiotics

Some isolates also showed intermediate level of sensitivity against some antibiotics. When intermediate profile was observed, highest level of intermediacy was observed against Ciprofloxacin with 35%, followed by Cefazolin 28%, Amoxicillin-clavulanate/Augmentin 27%, imipenem 22%, Ceftiofur & Ampicillin/sulbactam 21% and Enrofloxacin, Cefepime and Minocycline was 17% while Gentamicin and Ceftazidime was 16% and 13% intermediate respectively. Florfenicol & Amikacin showed 12% intermediate profile followed by 11% for Meropenem, 10% for

Cefotaxime, 8% for Piperacillin/tazobactum & Chloramphenicol, 7% for Ampicillin & Cefoxitin and 6% for Streptomycin. Intermediate profile of Tetracycline & Sulfamethoxazole/trimethoprim was 5%, followed by ertapenem 4%, Nalidixic Acid, Nitrofurantoin & Trimethoprim 3%, Erythromycin, Quinupristin/Dalfopristin & Doxycycline 1%. Azithromycin, Clindamycin, Colistin, Linezolid and Teicoplanin showed 0% intermediate profile. (Fig.3.13) (Table. 3.4)

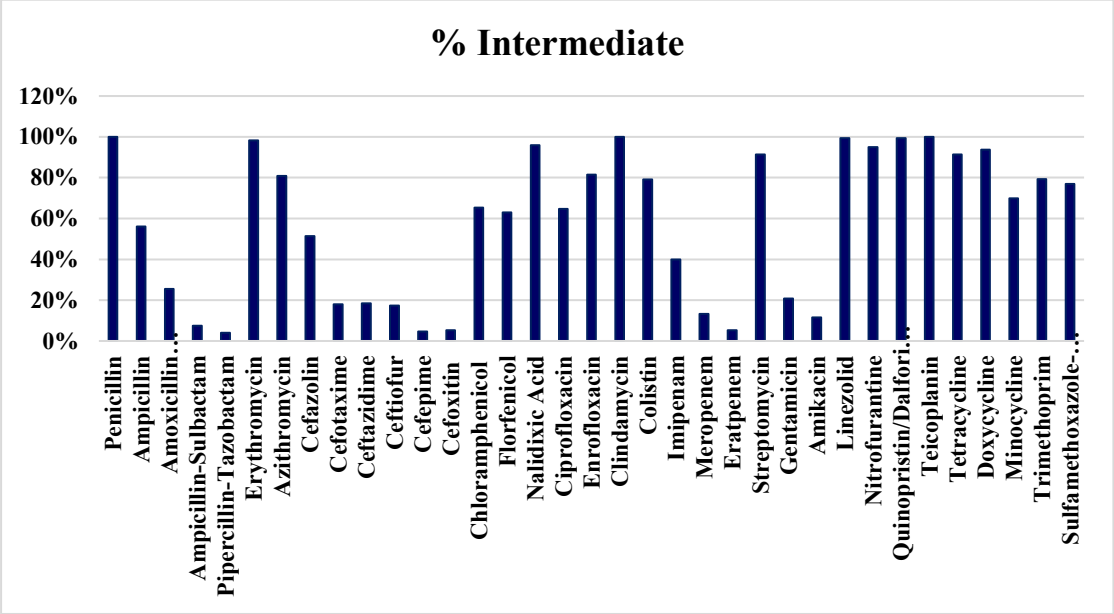


Figure 3.12: AMR profiling of Intermediate sensitivity in Salmonella isolates against selected antibiotics

When sensitivity profile of antibiotics was observed against isolates, Ertapenem was observed to be highly sensitive with 91% sensitivity value, followed by Piperacillin/tazobactum & Cefoxitin 88%, Cefepime 79%, Amikacin 77%, Meropenem 76%, Ampicillin/sulbactam & Cefotaxime 72%, Amoxicillin-clavulanate/Augmentin 71%, Ceftazidime 69%, Gentamicin 64% and Ceftiofur 61% sensitive. Sensitive. Imipenem, Ampicillin, Chloramphenicol, Florfenicol, Colistin and Cefazolin was 38%, 37%, 27%, 25%, 21% and 20% sensitive respectively. Azithromycin was 19% sensitive, Sulfamethoxazole/trimethoprim & Trimethoprim 18%, Minocycline 13%, Doxycycline 6%, Tetracycline 4% and Nitrofurantoin was 3% sensitive. Enrofloxacin, Ciprofloxacin, Nalidixic Acid, Linezolid and Erythromycin was only 1% sensitive while Quinupristin/Dalfopristin, Clindamycin, Streptomycin, Penicillin and

Teicoplanin was 0% sensitive (Fig.3.14) (Table. 3.4). Combined percentages of sensitivity, intermediate and resistance profile of isolates is shown in Fig. 3.15.

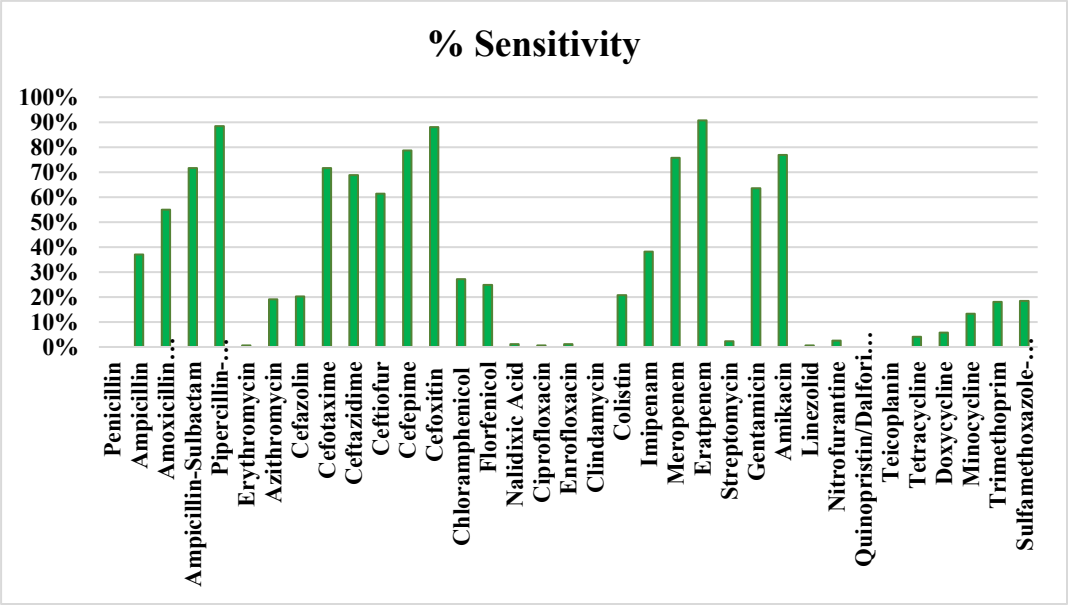


Figure 3.13: AMR profiling of Sensitivity in *Salmonella* isolates against selected antibiotics

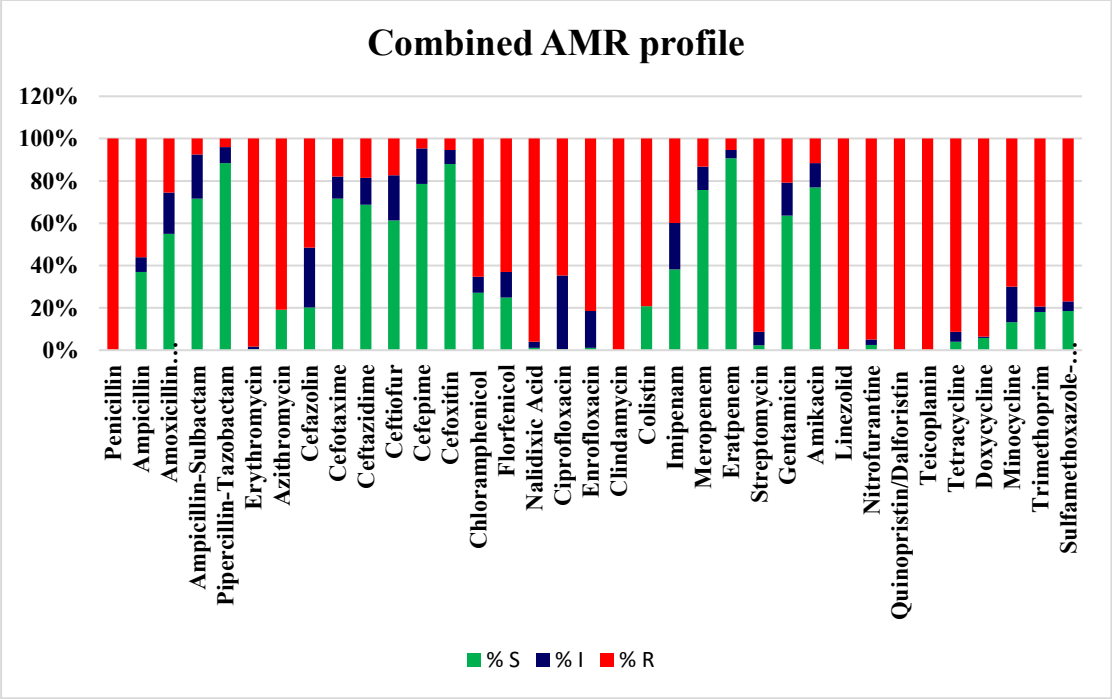


Figure 3.14: Completed AMR profiling of antibiotic tested in Salmonella isolates across the country

Table 3.2: AMR profiling of *Salmonella* isolated from Broilers across the country

Sr. #	Antibiotics	% S	% I	% R	Sr. #	Antibiotics	% S	% I	% R
1	Amikacin	77%	12%	12%	**19**	Erythromycin	1%	1%	98%
2	Amoxicillin Clavulanate/ Augmentin	71%	27%	23%	**20**	Florfenicol	25%	12%	63%
3	Ampicillin	37%	7%	56%	**21**	Gentamicin	64%	16%	21%
4	Ampicillin-Sulbactam	72%	21%	8%	**22**	Imipenem	38%	23%	40%
5	Azithromycin	19%	0%	81%	**23**	Linezolid	1%	0%	99%
6	Cefazolin	20%	28%	51%	**24**	Meropenem	76%	11%	13%
7	Cefepime	79%	17%	5%	**25**	Minocycline	12%	7%	81%
8	Cefotaxime	72%	10%	18%	**26**	Nalidixic Acid	1%	3%	96%
9	Ceftiofur	61%	21%	17%	**27**	Nitrofurantoin	3%	3%	94%
10	Cefoxitin	88%	7%	5%	**28**	Penicillin	0%	0%	100%
11	Ceftazidime	69%	13%	18%	**29**	Piperacillin-Tazobactam	88%	8%	4%
12	Chloramphenicol	27%	8%	65%	**30**	Quinupristin/Dalfopristin	0%	1%	99%
13	Ciprofloxacin	1%	35%	65%	**31**	Streptomycin	0%	7%	93%
1	Clindamycin	0%	0%	100%	**32**	Sulfamethoxazole-Trimethoprim	18%	5%	77%
15	Colistin	21%	0%	79%	**33**	Teicoplanin	0%	0%	100%
16	Doxycycline	6%	1%	94%	**34**	Tetracycline	4%	5%	91%
17	Enrofloxacin	1%	17%	82%	**35**	Trimethoprim	18%	3%	79%
18	Ertapenem	91%	4%	5%					

S= Sensitive; I= Intermediate; R= Resistance

Out of 268 isolates of *Salmonella*, 173 isolates were subjected to antimicrobial susceptibility test (AST) to determine their sensitivity and resistance pattern. Among 173 isolates tested, highest value of sensitivity observed in an isolate was 65% followed by 62% as second highest value of sensitivity profile. A total of 18 isolates out of 173 isolates tested for AST showed sensitivity ≥50%. Three out of 173 isolates showed no sensitivity profile i.e. 0%. Some samples also showed intermediate level of sensitivity with maximum value of 39% shown by two isolates with no isolate having value ≥50% and minimum value of 0% shown by 10 isolates. Highest observed value of resistance was 85% and 129/173 isolates showed resistance profile ≥50%. Lowest value of resistance was observed to be 32% by two samples.

Out of 65 *Salmonella* isolates from Islamabad Capital Territory, 61 were analyzed for antimicrobial susceptibility test against thirty-five antibiotics. Highest value of sensitivity expressed by a sample was 65% and 10/61 isolates showed sensitivity profile ≥50%. Piperacillin/Tazobactum was highly sensitive i.e. 98% against all isolates and Ertapenem was 93% sensitive. Twelve out of 35 antibiotics showed sensitivity profile ≥50% with Ceftazidime being highly sensitive. When intermediate profile was analyzed, maximum value was observed to be 20% intermediate and minimum value was 0% with no isolate having value ≥50%. Maximum value of resistance observed was 85% and 41 out of 61 isolates showed resistance profile ≥50%. Penicillin, Erythromycin, Clindamycin, Linezolid, Quinupristin/Dalfopristin and Teicoplanin showed 100% resistance against all isolates while Nitrofurantoin, Nalidixic Acid and Tetracycline was 98%, 95%, 93% resistant respectively. Twenty-two antibiotics had resistance profile ≥50% while piperacillin/tazobactum and Cefepime was least resistant. These analyses showed high level of resistance in isolates collected from Islamabad Capital Territory. Detailed analysis of percentage in sensitivity, intermediate and resistance profile of antibiotics against 61 isolates obtained from Islamabad is shown in Fig. 3.16 and appendix XXI. Percentage of sensitivity, intermediate and resistance off all antibiotics in each sample is given in appendix XXII.

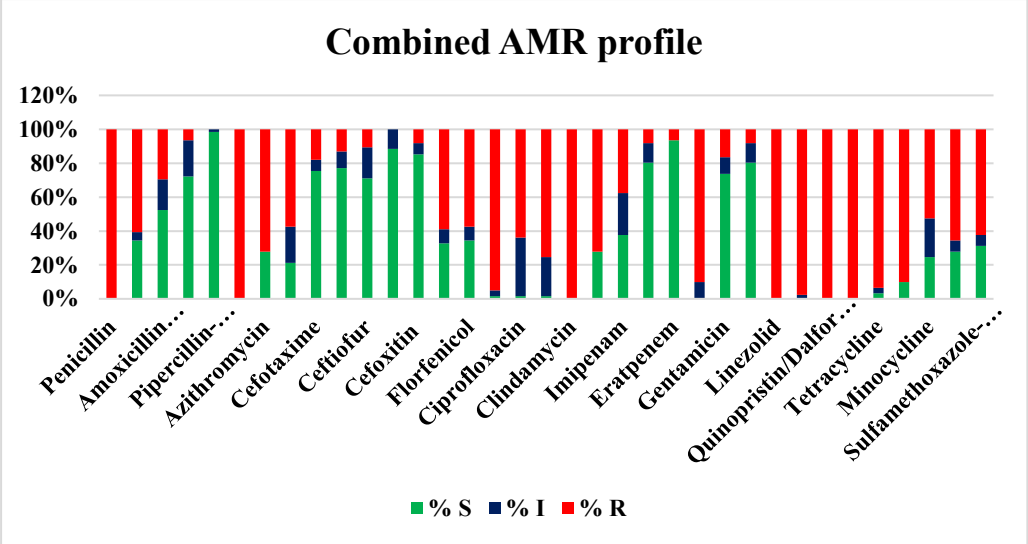


Figure 3.15: Complete AMR profile of *Salmonella* isolates recovered from Islamabad Capital Territory

3.4.2 Area wise Comparative analysis of AMR profile of isolates

A total of 12 samples were collected from Rawalpindi, from which five were tested positive for *Salmonella*. When these isolates were subjected to antimicrobial susceptibility test, maximum value of sensitivity was observed to be 38% and minimum was 21% with no isolate having sensitivity value of ≥50%. Among thirty-five antibiotics tested, Ampicillin/Sulbactam and Ertapenem were 100% sensitive against all isolates and 9/35 showed sensitivity profile ≥50%. Highest observed value of intermediate profile of isolates was 18% and lowest value was on 3% intermediate profile. Maximum value of resistance was seen to be 74% and minimum value of resistance was 47% while 4/5 isolates exhibited resistance profile of ≥50%. Linezolid, Nitrofurantoin, Quinupristin/Dalfopristin, Teicoplanin, Doxycycline, Streptomycin, Clindamycin, Colistin, Erythromycin, Azithromycin and Penicillin showed 100% resistance against all isolates and 22/35 antibiotics had resistance profile of ≥50%. Detailed analysis of percentage in sensitivity, intermediate and resistance profile of isolates obtained from Rawalpindi is shown in Fig. 3.17 and appendix XXIII. Percentage of sensitivity, intermediate and resistance in each sample is also given in appendix XXIV.

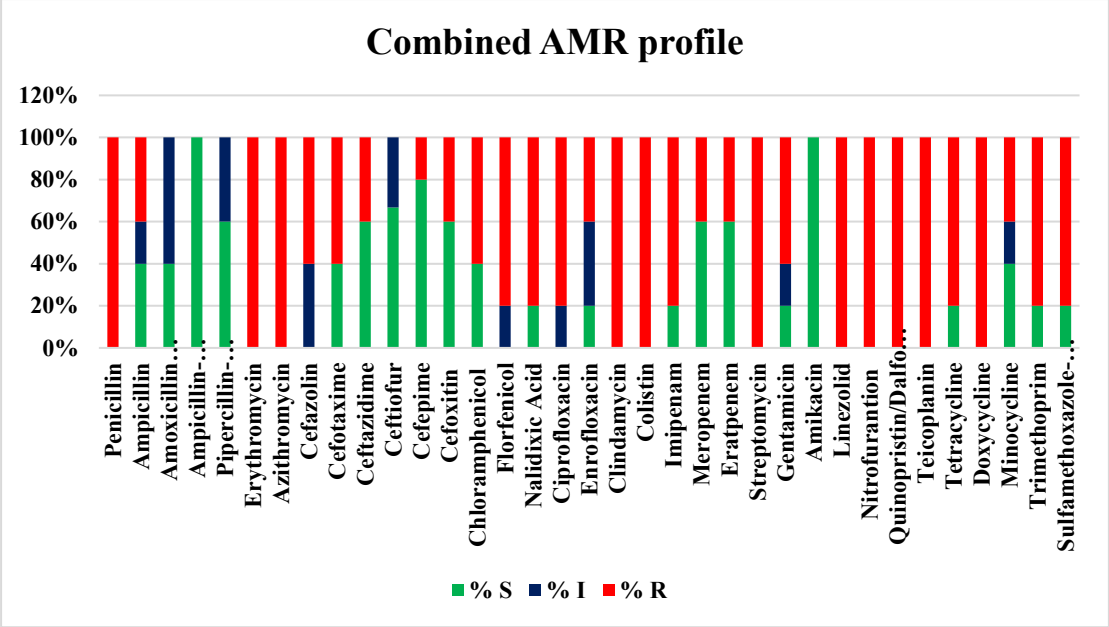


Figure 3.16: AMR profile of *Salmonella* isolates recovered from Rawalpindi

Out of 46 total isolates obtained from Lahore, 20 were subjected to antimicrobial susceptibility test. When sensitivity and resistance profiles of these isolates were observed, highest sensitivity profile observed was 41% and lowest value of sensitivity was 13% with no isolates having value ≥50%. Highest sensitive antibiotic against all isolates was observed to be Cefoxitin that was 94% sensitive followed by Piperacillin/Tazobactum 85% sensitive and Ertapenem 82% sensitive and a total of eleven antibiotics were observed to have sensitivity profile of ≥50%. When intermediate profile of isolates was observed, maximum value was 39% and minimum value was 0% intermediate profile. On the other hand, highest resistance profile was 68% and lowest value of resistance shown by isolate was 47%. Out of 20 isolates tested, 15 showed resistance profile of ≥50%. Twenty-one out of thirty-five antibiotics showed resistance profile of ≥50% with Penicillin, Erythromycin, Clindamycin, Linezolid, Teicoplanin and Quinupristin/Dalfopristin being 100% resistant against all isolates of Lahore. Figure 3.18 and appendix XXV showed detailed analysis of percentage in sensitivity, intermediate and resistance profile of isolates obtained from Lahore. Percentage of sensitivity, intermediate and resistance in each sample is also given in appendix XXVI.

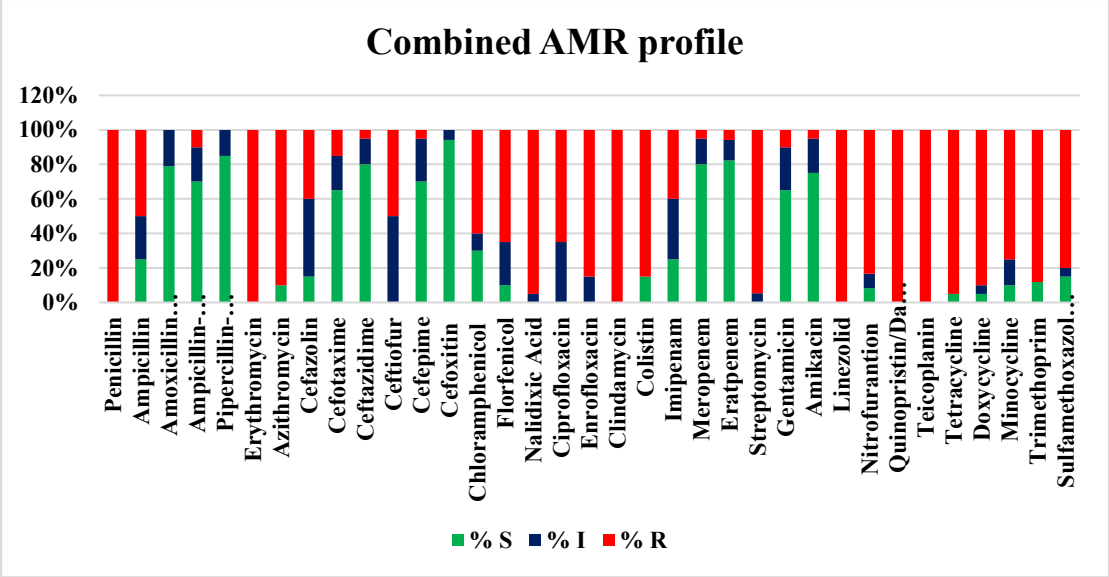


Figure 3.17: AMR profile of *Salmonella* isolates recovered from Lahore

A total of 54 *Salmonella* isolates were obtained from 143 samples collected from Karachi, among which, thirty isolates were subjected to antimicrobial susceptibility test (AST). When results were analyzed, maximum sensitivity value observed was 59% while minimum sensitivity profile of sample was 18%. Four out of 30 isolates showed sensitivity profile ≥50%. Ceftiofur and Amikacin were 100% sensitive against all isolates tested followed by Meropenem and Cefoxitin 97% sensitive, and Ertapenem, Piperacillin/Tazobactam Cefepime 93% sensitive. A total of fourteen antibiotics showed sensitivity profile of ≥50%. Maximum value of intermediate profile was 18% and minimum value was 3%. When resistance profile of samples was observed, highest seen value was 74% resistance and lowest was 38% with 21 isolates having resistance profile ≥50%. Penicillin, Erythromycin, Clindamycin, Nalidixic Acid, Linezolid, Teicoplanin, Quinupristin/Dalfopristin and Doxycycline were 100% resistant against all isolates showing high resistance profile in isolates from Karachi. A total of 19/35 antibiotics was observed to have resistance profile of ≥50%. Detailed analysis of percentage in sensitivity, intermediate and resistance profile of isolates obtained from Karachi is shown in Fig. 3.19 and appendix XXVII Percentage of sensitivity, intermediate and resistance in each sample is also given in appendix XXVIII.

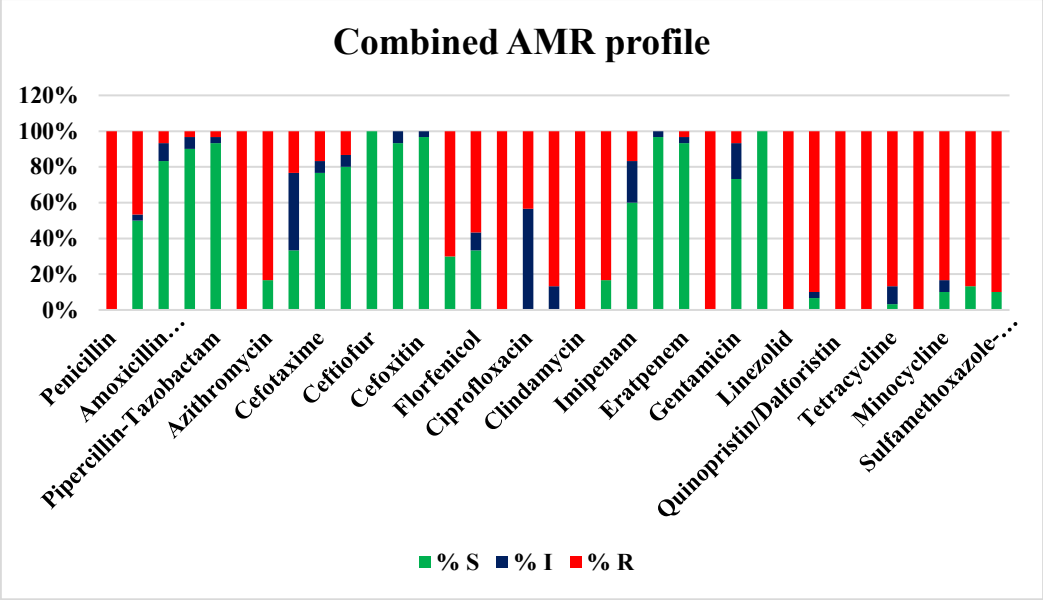


Figure 3.18: AMR profile of *Salmonella* isolates recovered from Karachi

Out of 37 total isolates from Peshawar, 20 were analyzed for antimicrobial susceptibility test. Highest analyzed sensitivity profile of isolate was 52% and lowest was 10% with 3 isolates having sensitivity profile ≥50%. Among all antibiotics, twelve showed sensitivity profile ≥50% and 100% sensitivity profile was observed in Ertapenem followed by Cefoxitin 92% sensitive. Maximum value of intermediate profile of isolates was 23% and lowest value was 3% with no isolate having value ≥50%. On other hand, maximum value of resistance profile of isolates was 77% and minimum value was 35% with 15 out of 20 isolates having resistance value ≥50%. Highly resistant antibiotics showing 100% resistance were Penicillin, Clindamycin, Nitrofurantoin and Teicoplanin. Twenty-two out of 35 antibiotics had resistance profile ≥50%. These results indicated the high level of resistance in isolates obtained from Peshawar. Detailed analysis of percentage in sensitivity, intermediate and resistance profile of isolates obtained from Peshawar is shown in Fig. 3.20 and appendix XXIX. Percentage of sensitivity, intermediate and resistance in each sample is also given in appendix XXX.

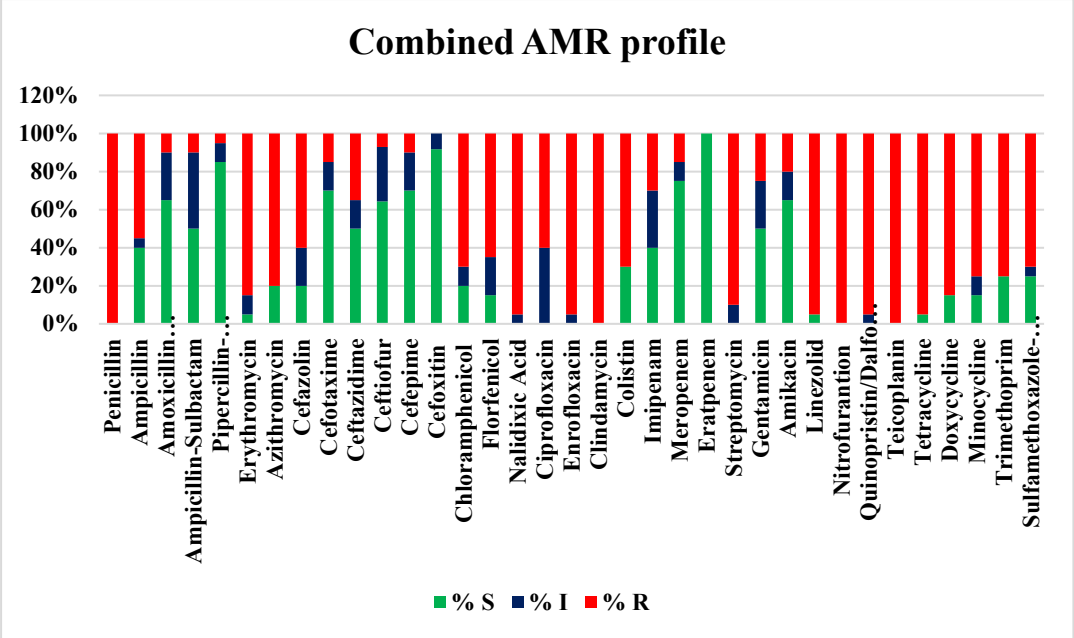


Figure 3.19: AMR profile of *Salmonella* isolates recovered from Peshawar

A total of 134 samples were collected from Quetta from which 44 were tested positive for *Salmonella*. Out of these 44 isolates, 20 were analyzed for antimicrobial susceptibility test (AST) against thirty-five antibiotics. When results were analyzed, maximum value of sensitivity profile of samples was 53% and minimum value was 3% with only one isolate having value ≥50%. Piperacillin/Tazobactam and Ceftiofur showed 100% sensitivity profile and eleven antibiotics showed sensitivity profile ≥50%. Highest value of intermediate profile was 26% and lowest was 0% with no isolate having value ≥50%. Maximum value of resistance profile of isolates was 85% and minimum value observed was 44% with 15 isolates having resistance value of ≥50%. When profile of antibiotics was analyzed, 23/35 antibiotics had resistance profile ≥50% and Penicillin, Erythromycin, Clindamycin, Nalidixic Acid, Streptomycin, Linezolid, Nitrofurantoin, Teicoplanin, Quinupristin/Dalfopristin, Tetracycline, Doxycycline and Trimethoprim were 100% resistant. Figure 3.21 and appendix XXXI showed detailed analysis of percentage in sensitivity, intermediate and resistance profile of isolates obtained from Quetta. Percentage of sensitivity, intermediate and resistance in each sample is also given in appendix XXXII.

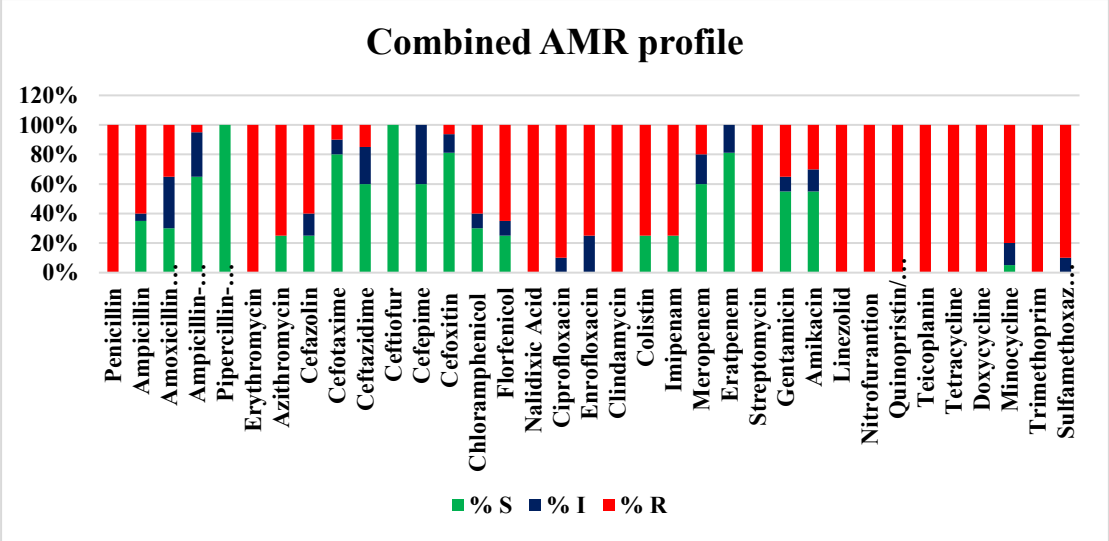


Figure 3.20: AMR profile of *Salmonella* isolates recovered from Quetta

A total of 13 *Salmonella* isolates were obtained from 32 samples received from Muzaffarabad. When antimicrobial susceptibility test was performed of these isolates and results analyzed, highest value of sensitivity profile observed was 38% and lowest value was 0% with no isolate having sensitivity value ≥50%. Ertapenem was 100% sensitive followed by Cefoxitin 88% sensitive while 12/35 showed sensitivity profile ≥50%. When intermediate profile was analyzed, maximum value was 12% and minimum was 0% with isolate having value ≥50% showing a minimum range of intermediate profile. On the other hand, maximum value of resistance profile of these isolates was 81% and all the isolates showed value ≥50% indicating high level of resistance in isolates obtained from Muzaffarabad. Most of the antibiotics including Penicillin, Erythromycin, Azithromycin, Chloramphenicol, Clindamycin, Colistin, Streptomycin, Linezolid, Nitrofurantoin, Teicoplanin, Quinupristin/Dalfopristin, Tetracycline, Doxycycline, Trimethoprim and Sulfamethoxazole/Trimethoprim were 100% resistant against all isolates while 22/35 showed values ≥50% showing high level of resistance in isolates of Muzaffarabad. Detailed analysis of percentage in sensitivity, intermediate and resistance profile of isolates obtained from Muzaffarabad is shown in Fig. 3.22 and appendix XXXIII. Percentage of sensitivity, intermediate and resistance in each sample is also given in appendix XXXIV.

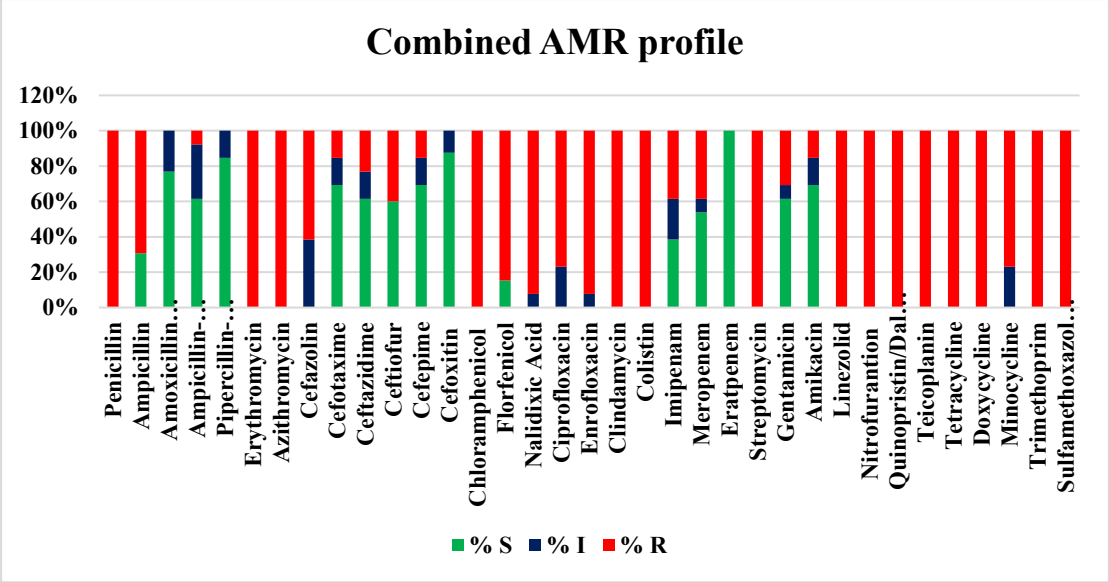


Figure 3.21: AMR profile of *Salmonella* isolates recovered from Muzaffarabad

Four isolates were obtained from 20 samples received from Gilgit region of Gilgit Baltistan. Antimicrobial susceptibility profile of these four isolates was performed and results were analyzed. Maximum value of sensitivity profile of isolates was 24% and minimum was 3% with no isolate having value ≥50%. Seven antibiotics has sensitivity value ≥50% with piperacillin/tazobactum being 100% sensitive. Highest value intermediate profile was 26% and lowest value was observed to be 6% with no isolate having value ≥50%. When resistance profile of isolates was observed, maximum observed value was 77% and minimum value was 68%. All isolates showed resistance value ≥50%. Twenty-eight out of 35 antibiotics had resistance profile of ≥50% among which, most of the antibiotics were 100% resistant including Erythromycin, Azithromycin, Cephazolin, Penicillin, Nalidixic Acid, Enrofloxacin, Clindamycin, Colistin, Linezolid, Nitrofurantoin, Teicoplanin, Quinupristin/Dalfopristin, Doxycycline and Trimethoprim revealing that samples from Gilgit were highly resistant against antibiotics. Detailed analysis of percentage in sensitivity, intermediate and resistance profile of isolates obtained from Gilgit is shown in Fig. 3.23 and appendix XXXV. Percentage of sensitivity, intermediate and resistance in each sample is also given in appendix XXXVI.

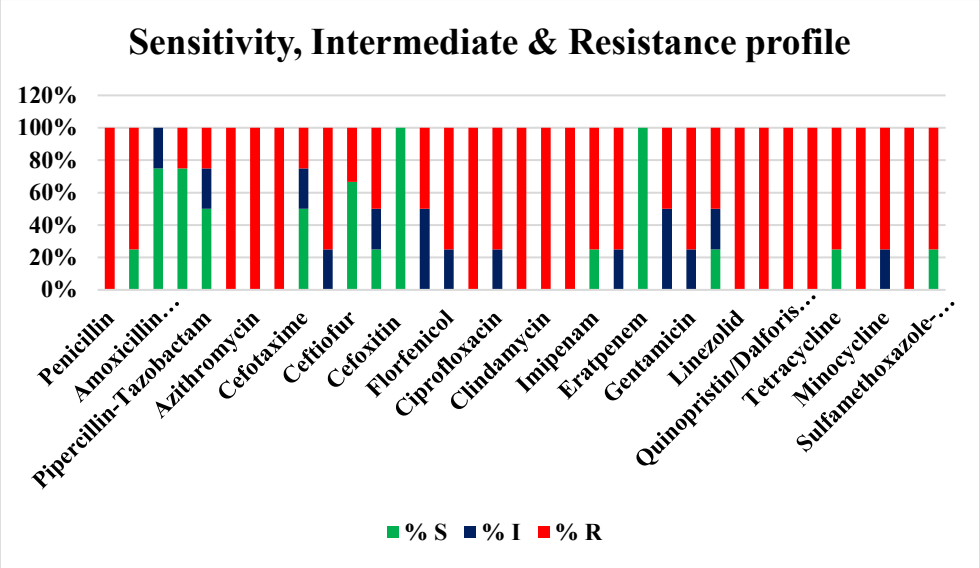


Figure 3.22: AMR profile of *Salmonella* isolates recovered from Gilgit

Antibiotic resistance pattern of isolates of all cities was compared and it was analyzed that Penicillin, Teicoplanin and Clindamycin were 100% resistant in all cities. Erythromycin, Linezolid and quinopristin/dalfopristin was 100% in all cities except in Peshawar where these antibiotics were 85% and 95% resistant respectively. Doxycycline and Streptomycin were 100% in Rawalpindi, Quetta and Muzaffarabad while 90% resistant in Islamabad and Lahore. In Peshawar, these antibiotics were 85% and 95% resistant respectively. Azithromycin and Colistin were 100% resistant in Rawalpindi, Muzaffarabad and Gilgit while their values differed in other cities. Azithromycin was 90%, 83%, 80%, 75% and 72% resistant in Lahore, Karachi, Peshawar, Quetta and Islamabad respectively. Nalidixic Acid showed 100% resistance in Quetta, Karachi and Gilgit, followed by 95% in Islamabad, Lahore and Peshawar, 92% in Muzaffarabad and 80% resistance in Rawalpindi. Least resistance was observed by Amikacin, Ceftiofur and piperacillin/tazobactum (Table 3.5).

When antibiotic sensitivity profile of antibiotics against isolates of all cities was compared, it was observed that piperacillin/tazobactum was 100% sensitive among isolates of Muzaffarabad, Peshawar and Gilgit, while 93% sensitive in Islamabad and Karachi, 82% in Lahore, 81% in Quetta and 60% sensitive in Rawalpindi. Ampicillin/sulbactam was 100% sensitive in Rawalpindi and Karachi, and more than 50% sensitive in Islamabad, Lahore, Muzaffarabad, Peshawar and Quetta but only 25% sensitive in Gilgit. Ertapenem showed more than 50% sensitivity in all cities.

Meropenem was 100% sensitive in Quetta and Karachi, 71% in Islamabad, 67% in Rawalpindi and Gilgit, 64% in Peshawar, 40% in Muzaffarabad but only 0% sensitive in Lahore. Ampicillin, and Sulphamethoxazole, was more than 50% sensitive in all cities except Gilgit where it was 0% sensitive. Amikacin showed least sensitivity in Rawalpindi and highest in Quetta, while Cefepime showed least sensitivity in Rawalpindi and highest in Karachi (Table 3.6).

Table 3.3: Comparative antimicrobial resistance profile of *Salmonella* Isolates recovered from different regions

Antibiotics	RWP	ISB	LHR	QTA	KHI	MZD	PSH	GLT
P-10	100%	100%	100%	100%	100%	100%	100%	100%
AMP-10	40%	61%	50%	60%	47%	69%	50%	75%
AMC-30 / Aug-30	0%	30%	0%	35%	7%	46%	25%	0%
SAM-20	0%	7%	10%	5%	3%	15%	10%	25%
TZP-110/10	0%	0%	0%	0%	3%	8%	20%	25%
E-15	100%	100%	100%	100%	100%	100%	85%	100%
AZM-15	100%	72%	90%	75%	83%	100%	80%	100%
KZ-30	60%	57%	40%	60%	23%	62%	60%	100%
CTX-30	60%	18%	15%	10%	17%	23%	15%	25%
CAZ-30	40%	13%	5%	15%	13%	31%	35%	75%
EFT	0%	11%	50%	0%	0%	60%	7%	33%
FEP-30	20%	0%	5%	0%	0%	15%	10%	50%
aFOX-30	40%	8%	0%	6%	0%	0%	0%	0%
C-30	60%	59%	60%	60%	70%	100%	70%	50%
FFC-30	80%	57%	65%	65%	57%	85%	65%	75%
NA-30	80%	95%	95%	100%	100%	92%	95%	100%
CIP-30	80%	64%	65%	90%	43%	77%	60%	75%
ENR-5	40%	75%	85%	75%	87%	92%	95%	100%
DA-2	100%	100%	100%	100%	100%	100%	100%	100%
CT-10/CS-10	100%	72%	85%	75%	83%	100%	70%	100%
IMI-10 / IPM-10	80%	38%	40%	75%	17%	38%	30%	75%
MEM-10 /MRP-10	40%	8%	5%	20%	0%	38%	15%	75%
ETP-10	40%	7%	6%	0%	3%	0%	0%	0%
S-10	100%	90%	95%	100%	100%	100%	90%	50%
CN-10/GEN-10	60%	16%	10%	35%	7%	31%	25%	75%
AK-30	0%	8%	5%	30%	0%	15%	20%	50%
LNZ-30	100%	100%	100%	100%	100%	100%	95%	100%
F-300	100%	98%	83%	100%	90%	100%	100%	100%
QDA-15	100%	100%	100%	100%	100%	100%	95%	100%
TEC-30	100%	100%	100%	100%	100%	100%	100%	100%
TE-30	80%	93%	95%	100%	87%	100%	95%	75%
DO-30	100%	90%	90%	100%	100%	100%	85%	100%
MH-30	40%	52%	75%	80%	83%	77%	75%	75%
TM-5	80%	66%	88%	100%	87%	100%	75%	100%
SXT-25	80%	62%	80%	90%	90%	100%	70%	75%

RWP= Rawalpindi ISB=Islamabad LHR=Lahore QTA=Quetta KHI=Karachi

MZD=Muzaffarabad PSH=Peshawar GLT=Gilgit

Table 3.4: Comparative antimicrobial sensitivity profile of *Salmonella* Isolates recovered from different regions

Antibiotics	RWP	ISB	LHR	QTA	KHI	MZD	PSH	GLT
P-10	0%	0%	0%	0%	0%	0%	0%	0%
AMP-10	40%	34%	25%	35%	50%	31%	45%	25%
AMC-30 / Aug-30	40%	52%	79%	30%	83%	38%	60%	75%
SAM-20	100%	72%	70%	65%	90%	54%	55%	75%
TZP-110/10	60%	98%	85%	100%	93%	69%	70%	50%
E-15	0%	0%	0%	0%	0%	0%	5%	0%
AZM-15	0%	28%	10%	25%	17%	0%	20%	0%
KZ-30	0%	21%	15%	25%	33%	0%	20%	0%
CTX-30	40%	75%	65%	80%	77%	62%	70%	50%
CAZ-30	60%	77%	80%	60%	80%	54%	50%	0%
EFT	67%	71%	0%	100%	100%	40%	64%	67%
FEP-30	80%	89%	70%	60%	93%	69%	70%	25%
FOX-30	60%	85%	94%	81%	97%	88%	92%	100%
C-30	40%	33%	30%	30%	30%	0%	20%	0%
FFC-30	0%	34%	10%	25%	33%	15%	15%	0%
NA-30	20%	2%	0%	0%	0%	0%	0%	0%
CIP-30	0%	2%	0%	0%	0%	0%	0%	0%
ENR-5	20%	2%	0%	0%	0%	0%	0%	0%
DA-2	0%	0%	0%	0%	0%	0%	0%	0%
CT-10/CS-10	0%	28%	15%	25%	17%	0%	30%	0%
IMI-10 / IPM-10	20%	38%	25%	25%	60%	38%	40%	25%
MEM-10 /MRP-10	60%	80%	80%	60%	97%	54%	75%	0%
ETP-10	60%	93%	82%	81%	93%	100%	100%	100%
S-10	0%	0%	0%	0%	0%	0%	0%	0%
CN-10/GEN-10	20%	74%	65%	55%	73%	62%	50%	0%
AK-30	100%	80%	75%	55%	100%	69%	65%	25%
LNZ-30	0%	0%	0%	0%	0%	0%	5%	0%
F-300	0%	0%	8%	0%	7%	0%	0%	0%
QDA-15	0%	0%	0%	0%	0%	0%	0%	0%
TEC-30	0%	0%	0%	0%	0%	0%	0%	0%
TE-30	20%	3%	5%	0%	3%	0%	5%	25%
DO-30	0%	10%	5%	0%	0%	0%	15%	0%
MH-30	40%	25%	10%	5%	10%	0%	15%	0%
TM-5	20%	28%	12%	0%	13%	0%	25%	0%
SXT-25	20%	31%	15%	0%	10%	0%	25%	25%

RWP= Rawalpindi ISB=Islamabad LHR=Lahore QTA=Quetta KHI=Karachi

MZD=Muzaffarabad PSH=Peshawar GLT=Gilgit

3.5 Genotypic AMR profile of isolates through PCR

A total of 24 isolates were selected for detection of resistance genes in their genome through polymerase chain reaction. PCR was performed for the detection of *aac(*3)-II, *aph*(3)-II genes for aminoglycosides, $bla_{SHV,}$ bla_{TEM}, bla_{OXA}, genes for beta-lactamase, *mcr*1 gene for Colistin and *tet*A, *tetB* genes for Tetracycline with eight primers. All isolates were tested against each primer in conventional PCR. Out of twenty-four isolates, *aac(*3)-II gene was detected in two isolates that was responsible for resistance to aminoglycosides in those isolates (Fig. 3.26). Second gene for aminoglycoside resistance, *aph*(3)-II was detected in only one isolates (Fig. 3.26). Genes for beta-lactamase bla_{SHV} was detected in six isolates (25%) out of twenty-four (Fig. 3.27) while bla_{OXA} was present in only one isolate (Fig. 3.28). Highest prevalent gene in study was found to be bla_{TEM} that was present in 10/24 isolates (41.6%) shown in (Fig. 3.27). Colistin resistance causing gene *mcr*-1 was not detected in any of twenty-four isolates (Fig. 3.26). Two genes *tet*A and *tet*B for Tetracycline resistance was checked, among which, *tet*A was present in 2 isolates while *tet*B was not detected in any of twenty-four isolates (Fig. 3.28). Detail of positive and negative isolates for each gene is given in table 3.5 and Fig. 3.25

Table 3.5: Genotypic profile of selected Salmonella isolates against AMR responsible genes

Sr. #	Antibiotic Class	Gene	Positive isolates	Negative isolates
1	Aminoglycoside	*aac*(3)-II	2	22
2	Aminoglycoside	*aph*(3)-II	1	23
3	Beta-lactamase	bla_{SHV}	6	18
4	Beta-lactamase	bla_{TEM}	10	14
5	Beta-lactamase	bla_{OXA}	1	23
6	Colistin	*mcr*1	0	24
7	Tetracycline	*tet*A	2	22
8	Tetracycline	*tet*B	0	24

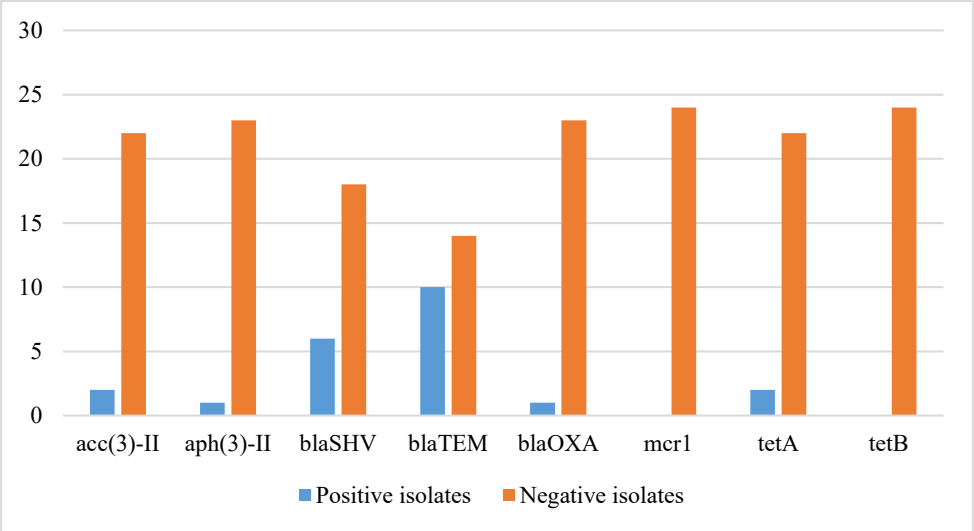


Figure 3.23: Genotypic profile of *Salmonella* isolates for selected genes responsible for AMR

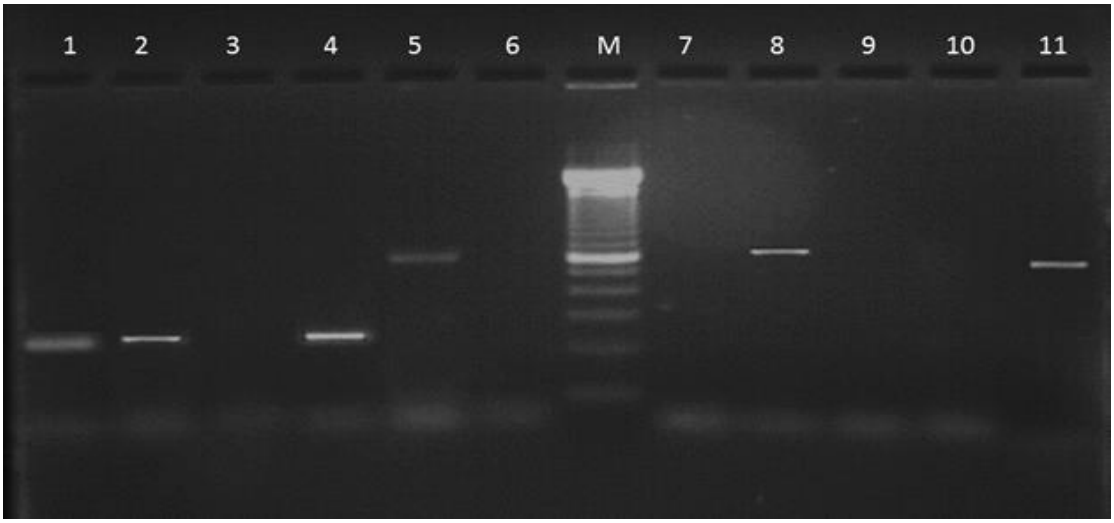


Figure 3.24: PCR amplification of AMR genes (*aac(3)-II, aph(3)-II, bla*SHV, *bla*$_{TEM}$, *bla*$_{OXA}$, mcr*1*, tet*A and* tet*B*); lane M= 100bp DNA ladder, lane 1,2 = *aac* (3)-II positive isolate at 237bp, lane 3= *aac*(3)-II negative isolate, lane 4= *aac*(3)-II standard at 237bp, 5 = *aph*(3)-II positive isolate at 688bp, lane 6,7= *aph*(3)-II negative isolate, lane 8= *aph*(3)-II standard at 688bp, lane 9,10= *mcr*1 negative isolate, lane 11= *mcr*1 standard at 502bp

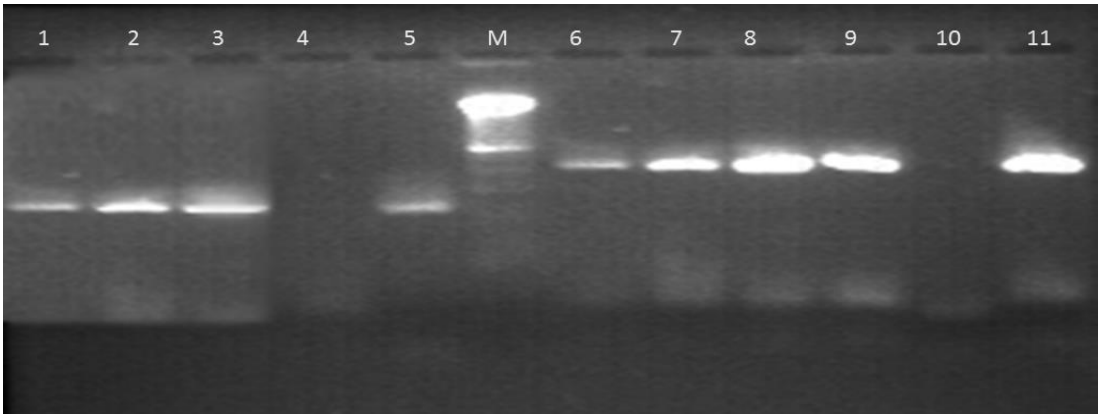


Figure 3.25: PCR amplification of AMR genes (*aac(3)-II, aph(3)-II, bla*SHV, *bla*$_{TEM}$, *bla*$_{OXA}$, mcr*1*, tet*A and* tet*B*); lane M= 100bp DNA ladder, lane 1,2,3 = bla$_{SHV}$ positive isolate at 237bp, lane 4= bla$_{SHV}$ negative isolate, lane 5= bla$_{SHV}$ positive standard at 237bp, lane 6,7,8,9 = bla$_{TEM}$ positive isolates at 444bp, lane 10= bla$_{TEM}$ negative isolate, lane 11= bla$_{TEM}$ positive standard at 444bp.

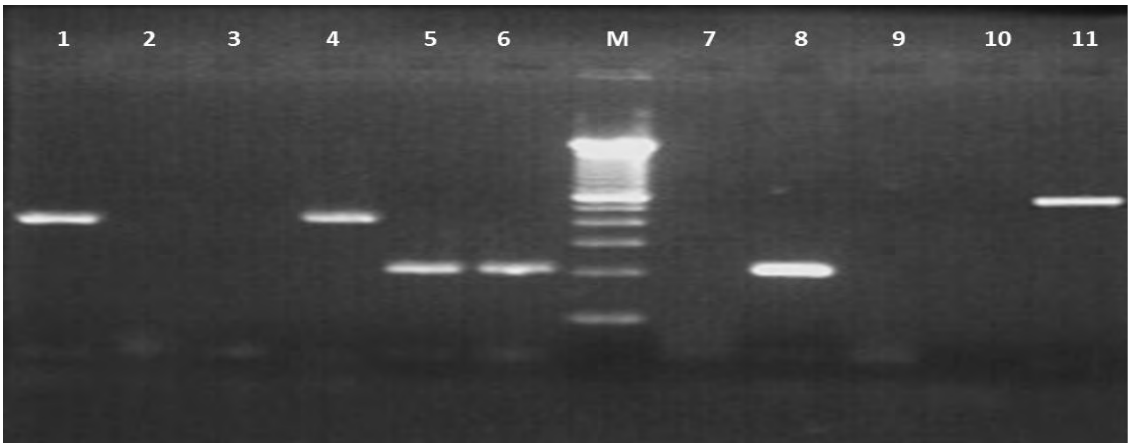


Figure 3.26: *PCR amplification of AMR genes (aac*(3)-II, *aph*(3)-II, bla$_{SHV}$, bla$_{TEM}$, bla$_{OXA}$, *mcr*1, *tet*A and *tet*B*)*; lane M= 100bp DNA ladder, lane 1= bla$_{OXA}$ positive isolate at 438bp, lane 2,3= bla$_{OXA}$ negative isolates, lane 4= bla$_{OXA}$ positive standard at 438bp, lane 5,6= tetA positive isolate at 201bp, lane 7= *tet*A negative isolate, lane 8= tetA positive standard at 201bp, lane 9,10= *tet*B negative isolates, lane 11= *tet*B positive standard at 571bp

Chapter 4
Discussion

4. DISCUSSION

The current study was conducted to evaluate AMR situation in *Salmonella* from healthy poultry in Pakistan with coordinated Surveillance strategies across the country in selected areas. This was the first kind of study conducted at national level involving the provincial coordination mechanism. The study was designed on the guidelines provided by FAO for AMR surveillance in the healthy animals. For this purpose, a total of 763 pooled samples were taken from selected areas (Islamabad, Rawalpindi, Lahore, Karachi, Quetta, Peshawar, Muzaffarabad and Gilgit) from live bird markets during July 2020-Febraury 2021. Out these 268 samples were found positive for *Salmonella spp.* indicating about 35% prevalence of *Salmonella spp* in healthy poultry. Out of 268 isolates 173 were subjected to AST analysis using standard protocols (CLSI, 2015; CLSI, 2020). The results of AST analysis indicated that *Salmonella spp.* from healthy poultry exhibited broad spectrum of resistance to antibiotics used under this study. Maximum resistance up to 85% was observed in *Salmonella* against multiple antimicrobial drugs which was found to be comparably higher than other studies conducted previously reporting 83.3%, 52%, 44.8% and 23.5% multidrug resistant in *Salmonella* isolates (Alemayehu *et al.*, 2003; Endrias, 2004; Molla *et al.*, 2004; Addis *et al.*, 2011). Whereas previous studies conducted in Pakistan reported 16% multi-drug resistance in *Salmonella*e (Aslam *et al.*, 2021), however, this study was conducted in human health sector. Although there was limited information available for AMR analysis in *Salmonellae* from poultry sector in Pakistan. This remarked difference could be possible because of day-by-day increasing level of multi-drug resistance in *Salmonella* due to extensive use of antimicrobial agents as prophylactic doses or at sub-therapeutic level. This extensive use of drugs on farms may lead to the selection of drug resistant strains of *Salmonella* and ultimately increasing human health risks associates with consumption of this poultry meat (Molla *et al.*, 2003; Molla *et al.*, 2006; Zewdu and Cornelius, 2009).

Resistance of *Salmonella* to commonly used antibiotics including Ampicillin, Tetracycline, Streptomycin (Zewdu and Cornelius, 2009), while Nalidixic Acid, Tetracycline and Streptomycin was reported resistant against Salmonella as 63% isolates were found resistant against Tetracycline, followed by 26% and 21% resistant

to Streptomycin and Nalidixic Acid respectively (Velasquez *et al.*, 2018). While in research conducted by Addis *et al.*, 2011, 100% resistance of isolates against Ampicillin, 66.7% against Streptomycin, 58.3% against Nitrofurantoin and 33.3% against Tetracycline was observed. The results of current study showed that out of 173 isolates tested, 96% were resistant to Nalidixic Acid, 93% were resistant to Streptomycin, 91% resistant to Tetracycline and 56% were resistant to Ampicillin indicating values higher than the previous studies with exception of Ampicillin which was found 56% resistant to *Salmonellae*. The current research also showed higher level of resistance in other classes of antibiotics including 100% resistance against Penicillin, Clindamycin and Teicoplanin. Whereas in another study Ciprofloxacin was found 100% susceptible against *Salmonella* (Ferede, 2014) while in current study 65% isolates showed resistance, which was much higher than the previously reported one. This difference in resistance of profile of *Salmonella* isolates with increased level of resistance could be due to difference of husbandry practices and difference in use of antibiotics causing selection pressure in bacteria to maintain resistance genes over the period of time. When antibiotic resistance pattern of *Salmonella* isolates from each city was compared in current study, isolates of Islamabad and Quetta showed maximum resistance profile of 85%, Muzaffarabad & Gilgit, Peshawar, Rawalpindi & Karachi, and Lahore showed maximum resistance profile of 81%, 77%, 74% and 68% respectively. These results indicated that highest levels of resistance against most of antibiotic classes were observed in isolates of Islamabad and Quetta.

Penicillin, Erythromycin, Clindamycin, Linezolid, Quinupristin/Dalfopristin, Teicoplanin and Doxycycline were highly resistant among isolates of all the cities while percentage of resistance against some antibiotics varied from city to city (Table 3.5). Streptomycin was 100% resistant in Rawalpindi, Quetta, and Muzaffarabad, 90% resistant in Islamabad, Lahore, Peshawar and Karachi. Azithromycin was 100% resistant in Rawalpindi, Muzaffarabad and Gilgit, 90% resistant in Lahore, 83% in Karachi, 80% in Peshawar, 75% in Quetta and 72% resistant in Islamabad. Ciprofloxacin was highly resistant in Quetta with 90% profile followed by Rawalpindi, Muzaffarabad, Gilgit, Lahore, Islamabad and Peshawar with 80%, 77%, 75%, 65%, 64% and 60% profile respectively. When resistance profile of Gentamicin was observed, it was more that 50% resistant in Gilgit and Rawalpindi, but less than 40%

resistant in Quetta, Muzaffarabad, Peshawar, Lahore and only 7% resistant in Karachi. Ampicillin was 40% resistant against isolates of Rawalpindi, 47% against isolates of Karachi, 50% in Lahore and Peshawar, 60% against isolates from Quetta, 61% against Islamabad isolates, 69% against Muzaffarabad and 75% resistant in isolates from Gilgit. Cephazolin was 23% in Karachi, 40% resistant in Lahore, 57% resistant in Islamabad, 60% resistant in Rawalpindi, Quetta, Peshawar, 62% in Muzaffarabad while 100% resistant in isolates of Gilgit. Similarly, Ceftazidime showed 75% resistance in Gilgit, 40% in Rawalpindi, 35% in Peshawar, 31% in Muzaffarabad, 15% in Quetta, 31% in Islamabad and Karachi, and only 5% resistance in Lahore. When resistance profile of Carbapenems was observed, they showed highest resistance profile against isolates of Gilgit, followed by Rawalpindi and lowest resistance profile against isolates of Karachi (Table 3.5).

These variations in resistance profile of antibiotics among all cities included in study may be due to fluctuation of general prescribed dosage of medicine and on-farm practices. Furthermore, it was observed that most of antibiotics showed highest resistance profile against isolates of Gilgit thus *Salmonella* present in poultry of Gilgit is highly resistant to most of antibiotic classes. When compared to other cities, resistance profile of isolates of Karachi and Lahore against antibiotics was comparatively low. Furthermore, when origin of poultry sold in live bird market of Islamabad Capital Territory was analyzed, most of birds were coming from either Sargodha or Chakwal thus indicating these regions as main reservoirs of multidrug resistant *Salmonella* isolates from Islamabad.

When sensitivity profile of isolates of different cities was compared, Ampicillin/sulbactam was 100% sensitive in Rawalpindi and Karachi, 80% in Islamabad, 75% in Lahore, 69% in Muzaffarabad, 65% in Peshawar, 55% in Quetta and only 25% sensitive in Gilgit. Meropenem was 100% sensitive in Quetta and more than 60% sensitive in all other cities with the exception of Lahore were it showed 0% sensitivity profile. Piperacillin/tazobactum exhibited more than 80% sensitivity profile in Muzaffarabad, Gilgit, Peshawar, Karachi, Islamabad, Lahore and Quetta and 60% in Rawalpindi. Ceftazidime was 100% sensitive in Quetta, 98% in Islamabad, 93% in Karachi, 70% in Peshawar, 69% in Muzaffarabad, 60% in Rawalpindi, 50% in Gilgit

(Table 3.6). These variations in sensitivity profile of antibiotics among different cities may depend upon husbandry practices which vary from city-to-city.

Among studies previously conducted in Pakistan, only 30.11% studies proceeded towards molecular characterization after microbial identification (Bilal *et al.*, 2021). In current study, when genotypic characterization of few isolates was conducted, their genotypic and phenotypic profile was only partially correlated. Out of twenty four isolates, *aac*(3)-II gene for aminoglycoside class of antibiotics was detected in two isolates (8.3%) which had also previously showed phenotypically resistance to Streptomycin and Gentamicin only. The second gene *aph*(3)-II was detected in one isolate (4.1%) that was phenotypically corresponding to resistant for Streptomycin, Gentamicin and Amikacin. When ESBL linked genes bla_{TEM}, bla_{OXA} and bla_{SHV} were analyzed and correlated with phenotypic profile, bla_{SHV} was detected in 6 out of 24 isolates (25%) while bla_{OXA} was only detected in one isolate (4.1%). In all twenty-four isolates, highly prevalent gene was bla_{TEM} that was detected in genome of 10 isolates. Five out of these bla_{TEM} positive isolates correlate with phenotype as these isolates were phenotypically resistant to Ampicillin when analyzed by disc diffusion method which coincide with previous study by Vélez, D. C. *et al.* (2017) while remaining bla_{TEM} positive isolates were phenotypically susceptible to Ampicillin. This phenomenon of having resistance gene but no phenotypic resistance can be supported by a previous study that reported the possibility of non-expression of corresponding gene for phenotypic profiling (Martineau *et al.*, 2000). The similar kind of observations were also reported previously where isolates having resistant genes but not expressing resistance phenotypically, and thought to be potential to become resistant in future, therefore, these kind of isolates must be regarded as potentially resistant (Biffi *et al.*, 2014). In current study, three isolates found to contain two out of three genes tested for beta-lactamase.

In present study, 23/24 (95.8%) isolates were phenotypically resistant to Colistin. When these isolates were genotypically analyzed for the Colistin resistance causing gene *mcr*1, all of 23 isolates were tested negative for respective gene which was not in correlation with phenotype. These outcomes might be possible due to either any un-known mechanism or effect of some other genes and supported by previous studies by

(Martineau *et al.*, 2000; Peirano *et al.*, 2006; Ribeiro *et al.*, 2011; Biffi *et al.*, 2014). According to Biffi *et al.* (2014), two strains of *Salmonella Typhimurium* were phenotypically resistant to Gentamicin but lack the AadA and AadB genes responsible for resistance to Gentamicin. Similarly, in a research work done by Peirano *et al.,* 2006, 16.3% *Salmonella* isolates were resistant to Ceftiofur, but only 13.6% among them contain bla_{CMY} gene responsible for resistance while remaining were negative for presence of respective gene. This indicated that there may be some other genes which would responsible for resistance in such isolates. Martineau *et al.,* 2000 has also reported such isolates being resistant to Erythromycin but not containing any of four genes tested for resistance thus postulated that there may also be some other mechanisms of resistance which are not known yet. Furthermore, some other studies concluded that it may not be possible always to correlate phenotype and genotype simultaneously where phenomenon of being resistant phenotypically but not genotypically are due to some other mechanism needing further investigations (Peirano *et al.*, 2006; Ribeiro *et al.*, 2011). In Pakistan, most reported gene for Colistin resistance was mcr-1. Thirteen out of 92 phenotypically Colistin resistant isolates were tested positive for *mcr-1* gene in research carried out by Rafique *et al.,* (2020) in *E.coli* from commercial and backyard poultry. Similarly *mcr-1* positive *Escherichia coli* ST6395 from broiler chicken was also reported previously (Mohsin *et al.*, 2019). Whereas, current study revealed that no *mcr-1* gene was involved in Colistin resistance, this indicated that there might be any other gene of *mcr* family causing resistance in the isolates of concern.

In present study, when PCR was performed to analyze resistance genes for Tetracycline, *tet*A was detected in two isolates (9.5%) while *tet*B was not detected in any of Tetracycline resistant isolate. These findings are supported by other studies where it was reported that among twenty-one isolates resistant to Tetracycline, only 15 isolates had *tet*A gene but no single isolate had *tet*B and *tet*C gene (Soufi *et al.*, 2009). Previous studies have reported *tet*A gene as predominant gene among all Tetracycline resistance genes present in *Salmonella* and *E. coli* of animal and livestock origin (Miko *et al.*, 2005; Jouini *et al.*, 2009; Soufi *et al.*, 2009).

Thus overall, this study concluded that there is rapid increase of multidrug resistance in *Salmonella* thus elevating the threat for poultry meat and human health associated with its consumption. Highest resistance was observed in isolates from Islamabad and Quetta followed by AJK, Gilgit, KP, Punjab and Sindh and highly resistant antibiotics against isolates from most of the regions were Penicillin, Streptomycin, Teicoplanin, Quinopristin-Dalfopristin, Tetracycline and Erythromycin. Use of extensive antibiotics should be controlled and new strategies should be designed to cope with the problem.

Future Perspectives:

It would help to understand the emergence of antimicrobial resistance in *Salmonella spp.* associated with poultry and evaluation of AMR containment strategies developed in the country to control its spread to human through food

References

REFERENCES

Abd El-Aziz, D.M., 2013. Detection of Salmonella typhimurium in retail chicken meat and chicken giblets. Asian Pacific journal of tropical biomedicine, 3(9): 678-681.

Acheson, D. and E.L. Hohmann, 2001. Nontyphoidal salmonellosis. Clinical Infectious Diseases, 32(2): 263-269.

Addis, Z., N. Kebede, Z. Sisay, H. Alemayehu, A. Wubetie and T. Kassa, 2011. Prevalence and antimicrobial resistance of Salmonella isolated from lactating cows and in contact humans in dairy farms of Addis Ababa: a cross sectional study. BMC infectious diseases, 11(1): 1-7.

Adesoji, A.T., A.A. Ogunjobi, I.O. Olatoye and D.R. Douglas, 2015. Prevalence of tetracycline resistance genes among multi-drug resistant bacteria from selected water distribution systems in southwestern Nigeria. Annals of Clinical Microbiology and Antimicrobials, 14(1): 35.

Adeyanju, G.T. and O. Ishola, 2014. Salmonella and Escherichia coli contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. Springerplus, 3(1): 139.

Alekshun, M., 2000. Bacterial drug resistance: response to survival threats. Bacterial stress responses.

Alemayehu, D., B. Molla and A. Muckle, 2003. Prevalence and antimicrobial resistance pattern of Salmonella isolates from apparently healthy slaughtered cattle in Ethiopia. Tropical Animal health and production, 35(4): 309-319.

Alexander, K.A., L.D. Warnick and M. Wiedmann, 2009. Antimicrobial resistant Salmonella in dairy cattle in the United States. Veterinary research communications, 33(3): 191-209.

Almuhanna, E., A. Ahmed and Y. Al-Yousif, 2011. Effect of air contaminants on poultry immunological and production performance. Int. J. Poult. Sci, 10(6): 461-470.

Antunes, P.c., C. Réu, J.C. Sousa, L.s. Peixe and N. Pestana, 2003. Incidence of Salmonella from poultry products and their susceptibility to antimicrobial agents. International journal of food microbiology, 82(2): 97-103.

Aslam, A., S.A. Kharal, M. Aslam and A. Raza, 2021. Trends of Antimicrobial Resistance in Typhoidal Strains of Salmonella in a Tertiary Care Hospital in Pakistan. Cureus, 13(1).

Bao, V., 2005. Prevalence of Salmonella and Campylobacter spp. from broiler meat in abattoirs at Ho Chi Minh city, Vietnam. Free University, Berlin dissertation.

Biffi, C., L. Stefani, L. Miletti, C. Matiello, R. Backes, J. Almeida and G. Neves, 2014. Phenotypic and genotypic resistance profile of Salmonella Typhimurium to antimicrobials commonly used in poultry. Brazilian Journal of Poultry Science, 16(2): 93-96.

Bilal, H., M.N. Khan, T. Rehman, M.F. Hameed and X. Yang, 2021. Antibiotic resistance in Pakistan: a systematic review of past decade. BMC infectious diseases, 21(1): 1-19.

Bingham, S., 2006. The fibre–folate debate in colo-rectal cancer. Proceedings of the nutrition society, 65(1): 19-23.

Borges, K.A., T.Q. Furian, S.N. de Souza, R. Menezes, C.T.P. Salle, H.L. de Souza Moraes, E.C. Tondo and V.P. do Nascimento, 2017. Phenotypic and molecular characterization of Salmonella enteritidis SE86 isolated from poultry and salmonellosis outbreaks. Foodborne pathogens and disease, 14(12): 742-754.

Brenner, F., R. Villar, F. Angulo, R. Tauxe and B. Swaminathan, 2000. Salmonella nomenclature. Journal of clinical microbiology, 38(7): 2465-2467.

Capita, R., M. Álvarez-Astorga, C. Alonso-Calleja, B. Moreno and M.a. del Camino García-Fernández, 2003. Occurrence of salmonellae in retail chicken carcasses and their products in Spain. International journal of food microbiology, 81(2): 169-173.

Carramiñana, J.J., J. Yangüela, D. Blanco, C. Rota, A.I. Agustin, A. Ariño and A. Herrera, 1997. Salmonella incidence and distribution of serotypes throughout processing in a Spanish poultry slaughterhouse. Journal of Food Protection, 60(11): 1312-1317.

Carrique-Mas, J. and R. Davies, 2008. Sampling and bacteriological detection of Salmonella in poultry and poultry premises: a review. Revue scientifique et technique, 27(3): 665.

CDC, 2009. National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS): Human Isolates Final Report, 2006. US Department of Health and Human Services, CDC Atlanta, GA.

CDC, 2013. Antibiotic resistance threats in the United States, 2013. CDC (Ed.). U.S. Department of Health and Human Services, CDC, Atlanta, GA, USA.

Cherian, A., S. Seena, R.K. Bullock and A.C. Antony, 2005. Incidence of neural tube defects in the least-developed area of India: a population-based study. The Lancet, 366(9489): 930-931.

CLSI, 2015. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals: CLSI Suppliment, Vet-01S, 3rd Ed. Clinical and Laboratory Standards Institute, Wayne, PA, USA.

CLSI, 2020. Performance Standards for Antimicrobial Susceptibility Testing: CLSI Supplement M100, 30th Ed. Clinical and Laboratory Standards Institute, Wayne, PA, USA.

Cui, S., B. Ge, J. Zheng and J. Meng, 2005. Prevalence and antimicrobial resistance of Campylobacter spp. and Salmonella serovars in organic chickens from

Maryland retail stores. Applied and environmental microbiology, 71(7): 4108-4111.

Dahal, N., L. Ellerbroek and N. Poosaran, 2007. Prevalence and antimicrobial resistance of Salmonella in imported chicken carcasses in Bhutan. National Cent Anim Health, 1: 1-92.

Daoud, Z., E. Salem Sokhn, K. Masri, K. Cheaito, N. Haidar-Ahmad, G.M. Matar and S. Doron, 2015. Escherichia coli isolated from urinary tract infections of Lebanese patients between 2005 and 2012: epidemiology and profiles of resistance. Frontiers in medicine, 2: 26.

Davies, J., 1994. Inactivation of antibiotics and the dissemination of resistance genes. Science, 264(5157): 375-382.

Domınguez, C., I. Gomez and J. Zumalacarregui, 2002. Prevalence of Salmonella and Campylobacter in retail chicken meat in Spain. International journal of food microbiology, 72(1-2): 165-168.

Elmadiena, M.M.A.N., A.A. El Hussein, C.A. Muckle, L. Cole, E. Wilkie, K. Mistry and A. Perets, 2013. Antimicrobial susceptibility and multi-drug resistance of Salmonella enterica subspecies enterica serovars in Sudan. Tropical Animal health and production, 45(5): 1113-1118.

Endrias, Z., 2004. Prevalence, distribution and antimicrobial resistance profile of Salmoneclla isolated from food items and workers in Addis Ababa, Ethiopia. In: Fuculty of Veterinary Medicine. Addis Ababa University, , Addis Ababa, Ethiopia: pp: 123.

Farrell, D., 2013. The role of poultry in human nutrition. In: Poultry Development Review, FAO, (Ed.). FAO, Rome, Italy: pp: 1-2.

Ferede, B., 2014. Isolation, identification, antimicrobial susceptibility test and public awareness of Salmonella on raw goat meat at Dire Dawa Municipal Abattoir, eastern Ethiopia. In: Microbiology, Immunology and Veterinary Public Health. Addis Ababa University, Addis Ababa, Ethiopia: pp: 104.

Findlay, L., W. Mondschein and R.R. Modesto, 2018. Gram Stain and Acid-Fast Stain. In: Laboratory exercises in microbiology, R. A. Pollack, L. Findlay, W. Mondschein and R. R. Modesto, (Eds.). John Wiley & Sons, Hoboken, NJ, USA: pp: 53-60.

Fisher, I., 2004. International trends in salmonella serotypes 1998-2003--a surveillance report from the Enter-net international surveillance network. Euro surveillance: bulletin Europeen sur les maladies transmissibles= European communicable disease bulletin, 9(11): 45-47.

Fluit, A.C., 2005. Towards more virulent and antibiotic-resistant Salmonella? FEMS Immunology & Medical Microbiology, 43(1): 1-11.

Gautam, V., N.K. Gupta, U. Chaudhary and D. Arora, 2002. Sensitivity pattern of Salmonella serotypes in Northern India. Brazilian journal of infectious diseases, 6(6): 281-287.

Getenet, B., 2008. Phenotypic and molecular characterizations of Salmonella species in Ethiopia. A PhD thesis on Medical Microbiology presented to the School of Graduate Studies of Addis Ababa University, Ethiopia.

Ghafoor, A., H. Badar, M. Hussain and N. Tariq, 2010. An empirical estimation of the factors affecting demand and supply of poultry meat. Pak. Vet. J, 30(3): 172-174.

Ghoddusi, A., B.N. Fasaei, V. Karimi, I.A. Tamai, Z. Moulana and T.Z. Salehi, 2015. Molecular identification of Salmonella Infantis isolated from backyard chickens and detection of their resistance genesby PCR. Iranian journal of veterinary research, 16(3): 293.

GoP, 2011. Economic Survey of Pakistan. M. o. F. Economic Affair Division, GOP (Ed.). Islamabad: pp: 15-33.

GoP, 2014. Pakistan Economic Survey 2013-2014. M. o. Finance (Ed.). Government of Pakistan, Islamabad.

GoP, 2021. Pakistan Economic Survey 2020-21. G. o. P. Ministry of Finance (Ed.). Economic Adviser's Wing, Finance Division, Islamabad: pp: 17-43.

HASSEN, K.A., Review of Poultry and Dairy Products on Non Typhoid Salmonella and Its Antibiotic Resistance in Ethiopia. International Journal on Integrated Education, 3(12): 373-389.

Hassen, K.A., 2020. Review of Poultry and Dairy Products on Non Typhoid Salmonella and Its Antibiotic Resistance in Ethiopia. International Journal on Integrated Education, 3(12): 373-389.

Hue, O., V. Allain, M.-J. Laisney, S. Le Bouquin, F. Lalande, I. Petetin, S. Rouxel, S. Quesne, P.-Y. Gloaguen and M. Picherot, 2011. Campylobacter contamination of broiler caeca and carcasses at the slaughterhouse and correlation with Salmonella contamination. Food microbiology, 28(5): 862-868.

Humphrey, T., G. Mead and B. Rowe, 1988. Poultry meat as a source of human salmonellosis in England and Wales. Epidemiology & Infection, 100(2): 175-184.

Hussain, J., I. Rabbani, S. Aslam and H. Ahmad, 2015. An overview of poultry industry in Pakistan. World's Poultry Science Journal, 71(4): 689-700.

Jouini, A., K. Ben Slama, Y. Sáenz, N. Klibi, D. Costa, L. Vinué, M. Zarazaga, A. Boudabous and C. Torres, 2009. Detection of multiple-antimicrobial resistance and characterization of the implicated genes in Escherichia coli isolates from foods of animal origin in Tunis. Journal of Food Protection, 72(5): 1082-1088.

Kaferstein, F., 2003. Food safety as a public health issue for developing countries. In: Food Safety in Food Security and Food Trade. International Food Policy Research Institute, Washington, DC, USA.

Kapperud, G., T. Vardund, E. Skjerve, E. Hornes and T. Michaelsen, 1993. Detection of pathogenic Yersinia enterocolitica in foods and water by immunomagnetic separation, nested polymerase chain reactions, and colorimetric detection of amplified DNA. Applied and environmental microbiology, 59(9): 2938-2944.

Koch, W.H., W.L. Payne, B.A. Wentz and T.A. Cebula, 1993. Rapid polymerase chain reaction method for detection of Vibrio cholerae in foods. Applied and environmental microbiology, 59(2): 556-560.

Korsak, N., J.-N. Degeye, G. Etienne, B. China and G. Daube, 2004. Comparison of four different methods for Salmonella detection in fecal samples of porcine origin. Journal of Food Protection, 67(10): 2158-2164.

Köser, C.U., M.J. Ellington, E.J. Cartwright, S.H. Gillespie, N.M. Brown, M. Farrington, M.T. Holden, G. Dougan, S.D. Bentley and J. Parkhill, 2012. Routine use of microbial whole genome sequencing in diagnostic and public health microbiology. PLoS pathogens, 8(8): e1002824.

Köser, C.U., L.J. Fraser, A. Ioannou, J. Becq, M.J. Ellington, M.T. Holden, S. Reuter, M.E. Török, S.D. Bentley and J. Parkhill, 2014. Rapid single-colony whole-genome sequencing of bacterial pathogens. Journal of Antimicrobial Chemotherapy, 69(5): 1275-1281.

Lahellec, C., P. Colin, G. Bennejean, J. Paquin, A. Guillerm and J. Debois, 1986. Influence of resident Salmonella on contamination of broiler flocks. Poultry Science, 65(11): 2034-2039.

Lauderdale, T.-L., F.M. Aarestrup, P.-C. Chen, J.-F. Lai, H.-Y. Wang, Y.-R. Shiau, I.-W. Huang and C.-L. Hung, 2006. Multidrug resistance among different serotypes of clinical Salmonella isolates in Taiwan. Diagnostic microbiology and infectious disease, 55(2): 149-155.

Lescat, M., L. Poirel and P. Nordmann, 2018. Rapid multiplex polymerase chain reaction for detection of mcr-1 to mcr-5 genes. Diagnostic microbiology and infectious disease, 92(4): 267-269.

Liu, T., K. Liljebjelke, E. Bartlett, C. Hofacre, S. Sanchez and J.J. Maurer, 2002. Application of nested polymerase chain reaction to detection of Salmonella in poultry environment. Journal of Food Protection, 65(8): 1227-1232.

Lynch, M., J. Painter, R. Woodruff and C. Braden, 2006. Surveillance for foodborne-disease outbreaks: United States, 1998-2002.

Ma, M., H. Wang, Y. Yu, D. Zhang and S. Liu, 2007. Detection of antimicrobial resistance genes of pathogenic Salmonella from swine with DNA microarray. J Vet Diagn Invest, 19(2): 161-167.

Maharjan, M., V. Joshi, D.D. Joshi and P. Manandhar, 2006. Prevalence of Salmonella species in various raw meat samples of a local market in Kathmandu. Annals of the New York Academy of Sciences, 1081(1): 249-256.

Mandal, S., M. Mandal and N. Pal, 2004. Reduced minimum inhibitory concentration of chloramphenicol for Salmonella enterica serovar typhi. Indian Journal of Medical Sciences, 58(1): 16-23.

Maqbool, A., K. Bakhsh, I. Hassan, M.W.A. Chattha and A.S. Ahmad, 2005. Marketing of commercial poultry in Faisalabad city (Pakistan). Journal of Agriculture and Social Sciences, 1(4): 327-331.

Martineau, F., F.J. Picard, N. Lansac, C. Ménard, P.H. Roy, M. Ouellette and M.G. Bergeron, 2000. Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of Staphylococcus aureus and Staphylococcus epidermidis. Antimicrobial agents and chemotherapy, 44(2): 231-238.

McDermott, P.F., G.H. Tyson, C. Kabera, Y. Chen, C. Li, J.P. Folster, S.L. Ayers, C. Lam, H.P. Tate and S. Zhao, 2016. Whole-genome sequencing for detecting antimicrobial resistance in nontyphoidal Salmonella. Antimicrobial agents and chemotherapy, 60(9): 5515-5520.

Mead, P.S., L. Slutsker, V. Dietz, L.F. McCaig, J.S. Bresee, C. Shapiro, P.M. Griffin and R.V. Tauxe, 1999. Food-related illness and death in the United States. Emerging infectious diseases, 5(5): 607.

Memon, N.A., 2012. Poultry: Country's second-largest industry. Pakistan Journal of Food Science(Novem-Dec): 27-30.

Miko, A., K. Pries, A. Schroeter and R. Helmuth, 2005. Molecular mechanisms of resistance in multidrug-resistant serovars of Salmonella enterica isolated from foods in Germany. Journal of Antimicrobial Chemotherapy, 56(6): 1025-1033.

Miranda, J., A. Mondragón, B. Martinez, M. Guarddon and J. Rodriguez, 2009. Prevalence and antimicrobial resistance patterns of Salmonella from different raw foods in Mexico. Journal of Food Protection, 72(5): 966-971.

Mohsin, M., M. Azam, S. Ur Rahman, F. Esposito, F.P. Sellera, D.F. Monte, L. Cerdeira and N. Lincopan, 2019. Genomic background of a colistin-resistant and highly virulent MCR-1-positive Escherichia coli ST6395 from a broiler chicken in Pakistan. Pathogens and Disease, 77(7): 1-4.

Molla, B., A. Mesfin and D. Alemayehu, 2003. Multiple antimicrobial-resistant Salmonella serotypes isolated from chicken carcass and giblets in Debre Zeit and Addis Ababa, Ethiopia. Ethiopian Journal of Health Development, 17(2): 131-139.

Molla, B., W. Salah, D. Alemayehu and A. Mohammed, 2004. Antimicrobial resistance pattern of Salmonella serotypes isolated from apparently healthy slaughtered camels (Camelus dromedarius) in eastern Ethiopia. Berliner und Munchener Tierarztliche Wochenschrift, 117(1-2): 39-45.

Molla, W., B. Molla, D. Alemayehu, A. Muckle, L. Cole and E. Wilkie, 2006. Occurrence and antimicrobial resistance of Salmonella serovars in apparently healthy slaughtered sheep and goats of central Ethiopia. Tropical Animal health and production, 38(6): 455-462.

Moussa, I., M. Gassem, A. Al-Doss, W. Sadik and A.A. Mawgood, 2010. Using molecular techniques for rapid detection of Salmonella serovars in frozen chicken and chicken products collected from Riyadh, Saudi Arabia. African Journal of Biotechnology, 9(5).

Mukhtar, N., S. Khan and R. Khan, 2012. Structural profile and emerging constraints of developing poultry meat industry in Pakistan. World's Poultry Science Journal, 68(4): 749-757.

Murugkar, H., H. Rahman, A. Kumar and D. Bhattacharyya, 2005. Isolation, phage typing & antibiogram of Salmonella from man & animals in northeastern India. Indian Journal of Medical Research, 122(3): 237.

Noppe-Leclercq, I., F. Wallet, S. Haentjens, R. Courcol and M. Simonet, 1999. PCR detection of aminoglycoside resistance genes: a rapid molecular typing method for Acinetobacter baumannii. Research in microbiology, 150(5): 317-322.

Ohl, M.E. and S.I. Miller, 2001. Salmonella: a model for bacterial pathogenesis. Annual review of medicine, 52(1): 259-274.

Olsvik, O., H. Sørum, K. Birkness, K. Wachsmuth, M. Fjølstad, J. Lassen, K. Fossum and J. Feeley, 1985. Plasmid characterization of Salmonella typhimurium transmitted from animals to humans. Journal of clinical microbiology, 22(3): 336-338.

Padungtod, P. and J.B. Kaneene, 2006. Salmonella in food animals and humans in northern Thailand. International journal of food microbiology, 108(3): 346-354.

Panisello, P.J., R. Rooney, P.C. Quantick and R. Stanwell-Smith, 2000. Application of foodborne disease outbreak data in the development and maintenance of HACCP systems. International journal of food microbiology, 59(3): 221-234.

Peirano, G., Y. Agersø, F.M. Aarestrup, E.M.F. dos Reis and D. dos Prazeres Rodrigues, 2006. Occurrence of integrons and antimicrobial resistance genes among Salmonella enterica from Brazil. Journal of Antimicrobial Chemotherapy, 58(2): 305-309.

Poirel, L., T.R. Walsh, V. Cuvillier and P. Nordmann, 2011. Multiplex PCR for detection of acquired carbapenemase genes. Diagnostic microbiology and infectious disease, 70(1): 119-123.

Poppe, C., L. Martin, A. Muckle, M. Archambault, S. McEwen and E. Weir, 2006. Characterization of antimicrobial resistance of Salmonella Newport isolated from animals, the environment, and animal food products in Canada. Canadian Journal of Veterinary Research, 70(2): 105.

Pui, C., W. Wong, L. Chai, R. Tunung, P. Jeyaletchumi, N. Hidayah, A. Ubong, M. Farinazleen, Y. Cheah and R. Son, 2011. Salmonella: A foodborne pathogen. International Food Research Journal, 18(2): 465-473.

Ribeiro, V.B., N. Lincopan, M. Landgraf, B.D. Franco and M.T. Destro, 2011. Characterization of class 1 integrons and antibiotic resistance genes in multidrug-resistant Salmonella enterica isolates from foodstuff and related sources. Brazilian Journal of Microbiology, 42: 685-692.

Roberts, M.C., 1996. Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility, and distribution. FEMS microbiology reviews, 19(1): 1-24.

Roberts, T., 1988. Salmonellosis control: estimated economic costs. Poultry Science, 67(6): 936-943.

Rouger, A., O. Tresse and M. Zagorec, 2017. Bacterial contaminants of poultry meat: sources, species, and dynamics. Microorganisms, 5(3): 50.

Sahota, A.W., B.M. Bhatti and L.A. Akhtar, 2003. Comparative Productive Performance of Desi Parent Chickens and their First Progeny Maintained on Deep Litter System. Pak. Vet. J, 23(1): 7-10.

Salehi, T.Z., M. Mahzounieh and A. Saeedzadeh, 2005. Detection of invA gene in isolated Salmonella from broilers by PCR method. Int. J. Poult. Sci, 4(8): 557-559.

Sarwar, F., M. Usman, S. Umar, M. Hassan, A. Rehman and A. Rashid, 2015. Some aspects of backyard poultry management practices in rural areas of district Rawalpindi, Pakistan. International Journal of Livestock Research, 5(5): 14-20.

Schwarz, S. and E. Chaslus-Dancla, 2001. Use of antimicrobials in veterinary medicine and mechanisms of resistance. Veterinary research, 32(3-4): 201-225.

Sikder, A., M. Islam, M.M. Rahman and M. Rahman, 2005. Seroprevalence of Salmonella and Mycoplasma gallisepticum infection in the six model breeder

poultry farms at Patuakhali district in Bangladesh. International Journal of Poultry Science, 4(11): 905-910.

Skóra, J., K. Matusiak, P. Wojewódzki, A. Nowak, M. Sulyok, A. Ligocka, M. Okrasa, J. Hermann and B. Gutarowska, 2016. Evaluation of microbiological and chemical contaminants in poultry farms. International journal of environmental research and public health, 13(2): 192.

Smith, L. and D. Wiesman, 2007. Is food security more severe in South Asia or sub-Saharan Africa? International Food Policy Research Institute Discussion Paper, 712, 52.

Sockett, P., 1995. The epidemiology and costs of diseases of public health significance, in relation to meat and meat products. Journal of Food Safety, 15(2): 91-112.

Soufi, L., M.S. Abbassi, Y. Sáenz, L. Vinué, S. Somalo, M. Zarazaga, A. Abbas, R. Dbaya, L. Khanfir and A. Ben Hassen, 2009. Prevalence and diversity of integrons and associated resistance genes in Escherichia coli isolates from poultry meat in Tunisia. Foodborne pathogens and disease, 6(9): 1067-1073.

Sparks, N., 2006. The hen's egg–is its role in human nutrition changing? World's Poultry Science Journal, 62(2): 308-315.

Sun, L., P. Shi, N. He, Q. Zhang and X. Duan, 2019. Antibiotic resistance genes removal and membrane fouling in secondary effluents by combined processes of PAC/BPAC–UF. Journal of water and health, 17(6): 910-920.

Threlfall, J., L. Ward and D. Old, 1999. Changing the nomenclature of Salmonella. Communicable disease and public health, 2(3): 156–157.

Van Duijkeren, E., W. Wannet, D. Houwers and W. Van Pelt, 2003. Antimicrobial susceptibilities of Salmonella strains isolated from humans, cattle, pigs, and chickens in the Netherlands from 1984 to 2001. Journal of Clinical Microbiology, 41(8): 3574-3578.

Van Nierop, W., A. Duse, E. Marais, N. Aithma, N. Thothobolo, M. Kassel, R. Stewart, A. Potgieter, B. Fernandes and J. Galpin, 2005. Contamination of chicken carcasses in Gauteng, South Africa, by Salmonella, Listeria monocytogenes and Campylobacter. International journal of food microbiology, 99(1): 1-6.

Velasquez, C.G., K.S. Macklin, S. Kumar, M. Bailey, P.E. Ebner, H.F. Oliver, F.S. Martin-Gonzalez and M. Singh, 2018. Prevalence and antimicrobial resistance patterns of Salmonella isolated from poultry farms in southeastern United States. Poult Sci, 97(6): 2144-2152.

Weill, F.-X., F. Guesnier, V. Guibert, M. Timinouni, M. Demartin, L. Polomack and P.A. Grimont, 2006. Multidrug resistance in Salmonella enterica serotype Typhimurium from humans in France (1993 to 2003). Journal of Clinical Microbiology, 44(3): 700-708.

White, D., 2019. Laboratory methodologies for bacterial antimicrobial susceptibility testing. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2021, OIE, (Ed.). OIE, Paris, France: pp: 1-10.

Windhorst, H.-W., 2008. A projection of the regional development of egg production until 2015. World's Poultry Science Journal, 64(3): 356-376.

Yu, Z., J. Wang, H. Ho, Y. Wang, S. Huang and R. Han, 2020. Prevalence and antimicrobial-resistance phenotypes and genotypes of Escherichia coli isolated from raw milk samples from mastitis cases in four regions of China. Journal of global antimicrobial resistance, 22: 94-101.

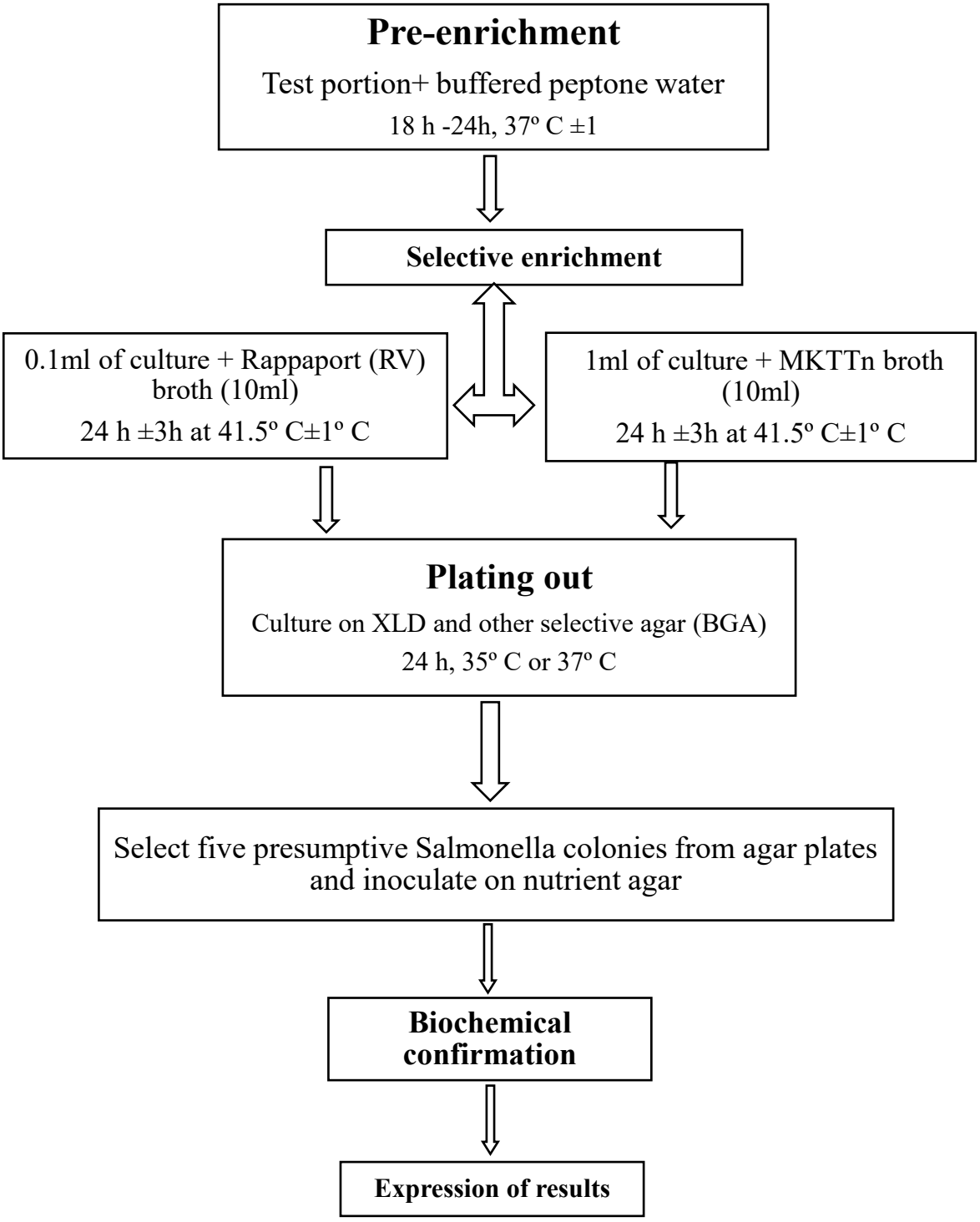
Zewdu, E. and P. Cornelius, 2009. Antimicrobial resistance pattern of Salmonella serotypes isolated from food items and personnel in Addis Ababa, Ethiopia. Tropical Animal health and production, 41(2): 241-249.

Zhang, Q., Y. Han, J. Cao, X. Xu, G. Zhou and W. Zhang, 2012. The spoilage of air-packaged broiler meat during storage at normal and fluctuating storage temperatures. Poultry Science, 91(1): 208-214.

Zhang, S., S. Piepers, R. Shan, L. Cai, S. Mao, J. Zou, T. Ali, S. De Vliegher and B. Han, 2018. Phenotypic and genotypic characterization of antimicrobial resistance profiles in Streptococcus dysgalactiae isolated from bovine clinical mastitis in 5 provinces of China. Journal of dairy science, 101(4): 3344-3355.

Appendixes

APPENDIX I: ISO protocol for the isolation of *Salmonella spp.*



APPENDIX II: Preparation of Buffered Peptone Water

Buffered peptone water (BPW) is a general media used for pre-enrichment of *Salmonella spp.* For preparation of BPW, 15g of BPW (CM0009) base was dissolved in 1liter of distilled water. Mixed well by heating and aseptically dispensed 10mL in each sterile tube. The media in tubes was then autoclaved at 121ºC for 15 minutes to sterilize it.

APPENDIX III: Preparation of Tetrathionate Broth

Tetrathionate broth is a selective medium for *Salmonella spp.* enrichment. To prepare tetrathionate broth, 45g of MKTTn (TMK20500) base was suspended in 500mL of distilled water and mixed well. Medium was heated at low flame with frequent agitation until it boiled well. The medium was cooled to 45-50ºC and 10mL of tetrathionate Iodine-Iodine selective supplement was added. Medium was mixed and dispensed 10mL into sterile tubes aseptically.

For preparation of iodine supplement, 6 grams of iodine and 5grams of potassium iodide is added in 20mL of distilled water and mixed well.

APPENDIX IV: Preparation of Brilliant Green Agar (BGA)

Brilliant green agar is a selective as well as differential media for growth of *Salmonella* spp. For preparation of medium, 52g of BGA (CM0329) was weighed and added in 1liter of distilled water. Medium was heated gently with agitation and boiled to dissolve it completely. Medium was cooled to 50ºC and 25mL was poured in each plate.

APPENDIX V: Preparation of Xylose Lysine Deoxycholate (XLD)

Xylose lysine Deoxycholate is a selective and differential media for isolation of *Salmonella* spp. For preparation of medium, 53g of XLD base (CM0469) was suspended in 1liter distilled water and heated with frequent agitation until boiling. Then medium was sterilized by autoclaving at 121ºC for 15 minutes, then cooled to 50 ºC.

The medium was poured into petri plates and incubated for 24 hours to check sterility of agar plate before usage.

APPENDIX VI: Preparation of Nutrient Agar (NA)

Nutrient agar is a general agar used for growth of bacteria. For preparation of nutrient agar, 28g of NA (CM0003) was dissolved in 1liter of distilled water and boiled to dissolve it completely. Medium was then autoclaved at 121ºC for 15 minutes.

APPENDIX VII: Preparation of Triple Sugar Iron (TSI) agar

Triple sugar iron (CM0277B) agar is used for biochemical analysis of *Salmonella spp.* Medium was prepared by suspending 65g of TSI in 1 liter distilled water, boiled to dissolved completely and dispensed into tubes. Tubes were sterilized by autoclaving at 121ºC for 15 minutes and then set as slope with 2.5cm butt.

APPENDIX VIII: Preparation of Simmons' Citrate Agar

Simmons' citrate is used for citrate utilization test of *Salmonella spp.* For preparation of simmons' citrate agar tubes, 23g of simmons' citrate (CM0155) was suspended in 1 liter distilled water and boiled to dissolve completely. Medium was dispensed into tubes and sterilized at 121ºC for 15 minutes. Tubes were then allowed to set as slants with 2.5cm butt.

APPENDIX IX: Preparation of Muller Hinton Agar (MHA)

Muller Hinton agar (MHA) is used to check antimicrobial susceptibility profile of bacteria. To prepare muller hinton agar, 38g of MHA (CM0337B) was suspended in 1 liter of distilled water and bring to boil by constant agitation to dissolve it completely. Medium was autoclaved at 121ºC for 15 minutes for sterilization, then cooled to 45-50 ºC and poured into sterilize petri plates, 25mL in each.

APPENDIX X: Preparation of Agarose Gel

1.8g agarose gel was suspended in 120mL of 1X TBE buffer and dissolved completely by heating in microwave. Then 10 micro liter ethidium bromide was added.

APPENDIX XI: Preparation of 1X TBE

For preparation of 1L of 1X TBE buffer, 200mL of 5X TBE was added in 800mL of distilled water.

APPENDIX XII: *Salmonella* isolates of samples received from Islamabad

Sample ID	Date of sample	City	Isolate	Growth on BGA	Growth on XLD	TSI			Citrate	Microscopy
						Butt	Slant	H_2S		
N-1200	6/7/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1201	6/7/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1204	6/7/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1233	22/07/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1235	22/07/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1236	22/07/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1377	21/08/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1379	21/08/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1380	21/08/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1381	21/08/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1682	4/9/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1684	4/9/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1685	4/9/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1686	4/9/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1687	4/9/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1689	4/9/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1699	11/9/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1720	21/9/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1723	21/9/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1724	21/9/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1725	21/9/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1726	21/9/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR

Sample ID	Date of sample	City	Isolate	Growth on BGA	Growth on XLD	TSI			Citrate	Microscopy
						Butt	Slant	H_2S		
N-1730	21/9/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1745	22/9/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1747	22/9/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1750	22/9/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1963	22/10/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1964	22/10/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1967	22/10/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1970	22/10/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1971	22/10/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1995	27/10/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1996	27/10/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1997	27/10/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1998	27/10/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2000	27/10/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2001	27/10/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2003	27/10/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2133	9/11/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2134	9/11/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2137	9/11/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2138	9/11/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2142	9/11/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2290	30/11/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2291	30/11/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2292	30/11/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR

Sample ID	Date of sample	City	Isolate	Growth on BGA	Growth on XLD	TSI			Citrate	Microscopy
						Butt	Slant	H_2S		
N-2293	30/11/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2295	30/11/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2427	16/12/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2428	16/12/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2429	16/12/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2430	16/12/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2616	31/12/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2618	31/12/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2619	31/12/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2621	31/12/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2623	31/12/2020	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-24	13/01/2021	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-27	13/01/2021	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-28	13/01/2021	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-30	13/01/2021	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-349	25/2/2021	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-350	25/2/2021	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-352	25/2/2021	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-354	25/2/2021	Islamabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR

CBC= Colorless with Black Center

H2S + = H2S produced and cause blackening in media

Citrate + = slant turn blue from green

GNR= Gram Negative Rod

APPENDIX XIII: *Salmonella* isolates of samples received from Rawalpindi

Sample ID	Date of sample	City	Isolate	Growth on BGA	Growth on XLD	TSI			Citrate	Microscopy
						Butt	Slant	H_2S		
N-1156	25/06/2020	Rawalpindi	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1157	25/06/2020	Rawalpindi	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1551	26/08/2020	Rawalpindi	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1554	26/08/2020	Rawalpindi	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1555	26/08/2020	Rawalpindi	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR

CBC= Colorless with Black Center

H2S + = H2S produced and cause blackening in media

Citrate + = slant turn blue from green

GNR= Gram Negative Rod

APPENDIX XIV: *Salmonella* isolates of samples received from Gilgit

Sample ID	Date of sample	City	Isolate	Growth on BGA	Growth on XLD	TSI			Citrate	Microscopy
						Butt	Slant	H2S		
N-191	25/1/2021	Gilgit	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-339	24/2/2021	Gilgit	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-344	24/2/2021	Gilgit	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-346	24/2/2021	Gilgit	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR

CBC= Colorless with Black Center

H2S + = H2S produced and cause blackening in media

Citrate + = slant turn blue from green

GNR= Gram Negative Rod

APPENDIX XV: *Salmonella* isolates of samples received from Peshawar

Sample ID	Date of sample	City	Isolate	Growth on BGA	Growth on XLD	TSI			Citrate	Microscopy
						Butt	Slant	H2S		
N-1778	29/09/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1779	29/09/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1782	29/09/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1784	29/09/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1789	29/09/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1914	13/10/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1915	13/10/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1916	13/10/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1917	13/10/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1918	13/10/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1921	13/10/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1923	13/10/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-1933	20/10/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2028	2/11/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2030	2/11/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2032	2/11/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2037	2/11/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2039	2/11/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2042	2/11/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR

N-2188	24/11/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2190	24/11/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2191	24/11/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2320	7/12/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2324	7/12/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2326	7/12/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2327	7/12/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2328	7/12/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2334	7/12/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2337	7/12/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2456	22/12/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2459	22/12/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2463	22/12/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-2467	22/12/2020	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-235	1/2/2021	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-240	1/2/2021	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-241	1/2/2021	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-243	1/2/2021	Peshawar	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR

CBC= Colorless with Black Center

H2S + = H2S produced and cause blackening in media

Citrate + = slant turn blue from green

GNR= Gram Negative Rod

APPENDIX XVI: *Salmonella* isolates of samples received from Lahore

Sample ID	Date of sample	City	Isolate	Growth on BGA	Growth on XLD	TSI			Citrate	Microscopy
						Butt	Slant	H2S		
N-1884	12/10/20	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1885	12/10/20	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1886	12/10/20	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1887	12/10/20	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1888	12/10/20	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1889	12/10/20	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1891	12/10/20	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1893	12/10/20	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1895	12/10/20	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1896	12/10/20	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1897	12/10/20	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2009	27/10/2020	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2012	27/10/2020	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2014	27/10/2020	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2021	27/10/2020	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2146	12/11/20	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2148	12/11/20	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2150	12/11/20	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2151	12/11/20	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2152	12/11/20	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2153	12/11/20	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2159	12/11/20	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2218	26/11/2020	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR

N-2219	26/11/2020	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2221	26/11/2020	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2222	26/11/2020	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2223	26/11/2020	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2225	26/11/2020	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2226	26/11/2020	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2227	26/11/2020	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2229	26/11/2020	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2337	8/12/2020	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2340	8/12/2020	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2341	8/12/2020	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2345	8/12/2020	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2346	8/12/2020	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2349	8/12/2020	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2587	29/12/2020	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2589	29/12/2020	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2590	29/12/2020	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2594	29/12/2020	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2599	29/12/2020	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-208	25/1/2021	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-255	2/2/2021	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-259	2/2/2021	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-264	2/2/2021	Lahore	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR

CBC= Colorless with Black Center

H2S + = H2S produced and cause blackening in media

Citrate + = slant turn blue from green

GNR= Gram Negative Rod

APPENDIX XVII: *Salmonella* isolates of samples received from Karachi

Sample ID	Date of sample	City	Isolate	Growth on BGA	Growth on XLD	TSI			Citrate	Microscopy
						Butt	Slant	H_2S		
N-1747	21/09/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1750	21/09/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1899	12/10/20	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1900	12/10/20	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1901	12/10/20	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1902	12/10/20	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1903	12/10/20	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1906	12/10/20	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1908	12/10/20	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1909	12/10/20	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1910	12/10/20	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1911	12/10/20	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1912	12/10/20	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1947	20/10/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1949	20/10/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1951	20/10/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1952	20/10/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1953	20/10/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1954	20/10/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1955	20/10/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1956	20/10/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1957	20/10/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1959	20/10/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR

N-2043	2/11/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2046	2/11/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2049	2/11/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2051	2/11/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2053	2/11/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2193	23/11/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2194	23/11/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2195	23/11/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2198	23/11/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2199	23/11/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2202	23/11/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2302	2/12/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2311	2/12/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2316	2/12/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2432	15/12/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2433	15/12/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2434	15/12/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2435	15/12/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2438	15/12/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2442	15/12/2020	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-31	11/1/2021	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-33	11/1/2021	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-39	11/1/2021	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-179	25/1/2021	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-182	25/1/2021	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-187	25/1/2021	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-189	25/1/2021	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-190	25/1/2021	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR

N-326	23/2/2021	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-331	23/2/2021	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-334	23/2/2021	Karachi	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR

CBC= Colorless with Black Center

H2S + = H2S produced and cause blackening in media

Citrate + = slant turn blue from green

GNR= Gram Negative Rod

APPENDIX XVIII: *Salmonella* isolates of samples received from Quetta

Sample ID	Date of sample	City	Isolate	Growth on BGA	Growth on XLD	TSI			Citrate	Microscopy
						Butt	Slant	H_2S		
N-1794	28/09/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1795	28/09/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1796	28/09/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1797	28/09/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1801	28/09/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1978	26/10/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1980	26/10/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1981	26/10/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1983	26/10/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1984	26/10/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1985	26/10/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1986	26/10/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1988	26/10/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1989	26/10/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1990	26/10/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-1991	26/10/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2161	18/11/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2166	18/11/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2167	18/11/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2168	18/11/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2169	18/11/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2170	18/11/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2171	18/11/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2172	18/11/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR

N-2173	18/11/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2235	29/11/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2408	13/12/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2409	13/12/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2410	13/12/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2411	13/12/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2412	13/12/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2414	13/12/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2415	13/12/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-2416	13/12/2020	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-5	4/1/2021	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-7	4/1/2021	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-12	4/1/2021	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-16	4/1/2021	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-77	17/01/2021	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-79	17/01/2021	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-306	22/2/2021	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-311	22/2/2021	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-312	22/2/2021	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR
N-316	22/2/2021	Quetta	*Salmonella*	pink	CBC	yellow	pink	+	+	GNR

CBC= Colorless with Black Center

H2S + = H2S produced and cause blackening in media

Citrate + = slant turn blue from green

GNR= Gram Negative Rod

APPENDIX XIX: *Salmonella* isolates of samples received from Muzaffarabad

Sample ID	Date of sample	City	Isolate	Growth on BGA	Growth on XLD	TSI			Citrate	Microscopy
						Butt	Slant	H2S		
N-166	25/1/2021	Muzaffarabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-170	25/1/2021	Muzaffarabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-171	25/1/2021	Muzaffarabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-172	25/1/2021	Muzaffarabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-173	25/1/2021	Muzaffarabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-174	25/1/2021	Muzaffarabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-175	25/1/2021	Muzaffarabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-178	25/1/2021	Muzaffarabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-287	15/2/2021	Muzaffarabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-290	15/2/2021	Muzaffarabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-293	15/2/2021	Muzaffarabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-295	15/2/2021	Muzaffarabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-296	15/2/2021	Muzaffarabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-191	25/1/2021	Muzaffarabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-339	24/2/2021	Muzaffarabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-344	24/2/2021	Muzaffarabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR
N-346	24/2/2021	Muzaffarabad	*Salmonella*	Pink	CBC	Yellow	Pink	+	+	GNR

CBC= Colorless with Black Center

H2S + = H2S produced and cause blackening in media

Citrate + = slant turn blue from green

GNR= Gram Negative Rod

APPENDIX XX: Intermediate breakpoints, sensitivity and resistance values for all antibiotics against which isolates were tested

Sr. #	Antibiotics	Symbol	Concentration	Sensitivity value	Intermediate breakpoint	Resistance value
1	Penicillin	(P)	10 µg	≥29	-	≤28
2	Ampicillin	(AMP)	10 µg	≥17	14-16	≤13
3	Amoxicillin-clavulanic acid	(AMC)	30µg	≥18	14-17	≤13
4	Ampicillin/Sulbactam	(SAM)	20 µg	≥15	12-14	≤11
5	Tazobactam	(TZP)	100 µg	≥21	18-20	≤17
6	Azithromycin	(AZM)	15 µg	≥13	-	≤12
7	Erythromycin	(E)	15 µg	≥23	14-22	≤13
8	Cephazolin	(KZ)	30 µg	≥23	20-22	≤19
9	Cefotaxime	(CTX)	30 µg	≥26	23-25	≤22
10	Ceftazidime	(CAZ)	30 µg	≥21	18-20	≤17
11	Ceftiofur	(EFT)	30 µg	≥21	18-20	≤17
12	Cefepime	(FEP)	30 µg	≥25	19-24	≤18
13	Cefoxitin	(FOX)	30 µg	≥18	15-17	≤14
14	Chloramphenicol	(C)	30 µg	≥18	13-17	≤12
15	Florfenicol	(FFC)	30 µg	≥18	15-18	≤14
16	Nalidixic Acid	(NA)	30 µg	≥19	14-18	≤13
17	Ciprofloxacin	(CIP)	30 µg	≥31	21-30	≤20
18	Enrofloxacin	(ENR)	5 µg	≥23	17-22	≤16
19	Clindamycin	(DA)	2 µg	≥21	15-20	≤14
20	Colistin sulphate	(CS)	10 µg	≥11	-	≤10
21	Ertapenem	(ETP)	10 µg	≥22	19-21	≤18
22	Imipenem	(IMI)	10 µg	≥23	20-22	≤19
23	Meropenem	(MEM)	10 µg	≥23	20-22	≤19

24	Streptomycin	(S)	10 µg	≥15	12-14	≤11
25	Gentamicin	(CN)	10 µg	≥15	13-14	≤12
26	Amikacin	(AK)	30µg	≥17	15-16	≤14
27	Linezolid	(LNZ)	30 µg	≥21	-	≤20
28	Nitrofurantoin	(F)	300 µg	≥17	15-16	≤14
29	Quinupristin/Dalfopristin	(QDA)	15 µg	≥19	16-18	≤17
30	Teicoplanin	(TEC)	30 µg	≥14	11-13	≤10
31	Tetracycline	(TE)	30 µg	≥15	12-14	≤11
32	Doxycycline	(DO)	30 µg	≥14	11-13	≤10
33	Minocycline	(MH)	30 µg	≥16	13-15	≤12
34	Trimethoprim/sulphamethoxazole	(SXT)	25 µg	≥16	11-15	≤10
35	Trimethoprim	(TM)	15 µg	≥16	11-15	≤10

APPENDIX XXI: AMR profile of isolates obtained from Islamabad Capital Territory

Antibiotics	% S	% I	% R	Antibiotics	% S	% I	% R
Penicillin	0%	0%	100%	Clindamycin	0%	0%	100%
Ampicillin	34%	5%	61%	Colistin	28%	0%	72%
Amoxicillin Clavulanate/ Augmentin	52%	18%	30%	Imipenem	38%	25%	38%
Ampicillin-Sulbactam	72%	21%	7%	Meropenem	80%	11%	8%
Piperacillin-Tazobactam	98%	2%	0%	Ertapenem	93%	0%	7%
Erythromycin	0%	0%	100%	Streptomycin	0%	10%	90%
Azithromycin	28%	0%	72%	Gentamicin	74%	10%	16%
Cefazolin	21%	21%	57%	Amikacin	80%	11%	8%
Cefotaxime	75%	7%	18%	Linezolid	0%	0%	100%
Ceftazidime	77%	10%	13%	Nitrofurantoin	0%	2%	98%
Cefepime	89%	11%	0%	Quinupristin/Dalfopristin	0%	0%	100%
Cefoxitin	85%	7%	8%	Teicoplanin	0%	0%	100%
Chloramphenicol	33%	8%	59%	Tetracycline	3%	3%	93%
Florfenicol	34%	8%	57%	Doxycycline	10%	0%	90%
Nalidixic Acid	2%	3%	95%	Minocycline	25%	23%	52%
Ciprofloxacin	2%	34%	64%	Trimethoprim	28%	7%	66%
Enrofloxacin	2%	23%	75%	Sulfamethoxazole-Trimethoprim	31%	7%	62%
Ceftiofur	71%	18%	11%				

S= Sensitive; I= Intermediate; R= Resistance

APPENDIX XXII: Percentage of sensitivity, intermediate and resistance of isolates received from Islamabad

Sample ID	N-1200	N-1201	N-1204	N-1233	N-1235	N-1236	N-1377	N-1379	N-1380	N-1381	N-1682	N-1684	N-1685	N-1686	N-1687	N-1689	N-1699	N-1720	N-1723	N-1724
City	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB
P-10	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
AMP-10	R	R	R	R	R	R	S	S	R	S	R	I	R	R	R	R	R	R	S	S
AMC-30 / Aug-30	R	S	S	S	S	S	S	S	S	S	S	S	I	I	S	S	S	S	S	S
SAM-20	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
TZP-110/10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
E-15	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
AZM-15	S	S	S	R	S	R	S	S	R	S	S	S	R	S	S	S	R	S	S	R
KZ-30	S	S	R	S	S	S	S	S	S	I	I	S	I	R	R	S	I	R	I	R
CTX-30	R	S	R	R	S	R	R	R	S	R	R	S	S	S	R	S	S	S	S	S
CAZ-30	S	R	S	S	R	S	S	S	I	I	S	R	I	S	R	I	S	S	S	S
EFT	S	I	S	S	S	I	S	S	S	S	R	I	S	S	S	S	S	S	S	S
FEP-30	S	S	S	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S
FOX-30	S	R	S	S	S	S	S	S	R	S	S	I	S	S	R	S	S	S	S	S
C-30	S	R	R	R	S	R	R	S	R	S	R	S	S	R	R	S	R	S	S	S
FFC-30	S	S	R	R	S	R	S	S	R	S	S	S	R	S	R	S	R	I	S	S
NA-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R
CIP-30	I	R	R	R	I	R	R	R	I	I	I	R	R	R	R	R	I	I	R	I
ENR-5	I	I	R	I	I	I	I	I	I	I	S	I	I	R	R	I	I	R	R	R
DA-2	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
CT-10/CS-10	S	S	S	R	S	R	S	S	S	S	S	S	S	S	S	S	R	S	R	R
IMI-10 / IPM-10	S	R	S	I	S	I	S	S	I	S	S	S	S	S	R	S	S	S	I	R
MEM-10 /MRP-10	S	R	S	S	S	S	S	S	S	S	S	S	S	I	R	S	S	S	S	S
ETP-10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S
S-10	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	I
CN-10/GEN-10	S	R	S	R	S	R	S	S	S	S	S	S	S	R	S	R	S	S	S	S
AK-30	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S
LNZ-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
F-300	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R	R	R
QDA-15	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
TEC-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
TE-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
DO-30	R	R	R	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R
MH-30	S	I	R	S	S	S	S	S	S	S	S	S	I	R	R	I	R	I	S	I
TM-5	R	R	R	R	S	R	S	S	R	S	R	S	S	S	R	S	R	S	S	I
SXT-25	R	R	S	R	R	R	S	S	R	S	R	I	S	S	R	I	R	S	S	S
% Sensitive	50%	32%	41%	38%	59%	35%	62%	65%	35%	56%	44%	50%	44%	44%	26%	50%	38%	54%	54%	46%
% Intermediate	6%	9%	0%	6%	6%	9%	3%	3%	15%	12%	9%	15%	15%	6%	0%	12%	9%	9%	9%	11%
% Resistant	44%	59%	59%	56%	35%	56%	35%	32%	50%	32%	47%	35%	41%	50%	74%	38%	53%	37%	37%	43%

Sample ID	N-1725	N-1726	N-1730	N-1745	N-1747	N-1750	N-1963	N-1964	N-1967	N-1970	N-1971	N-1995	N-1996	N-1997	N-1998	N-2000	N-2001	N-2003	N-2133	N-2134
City	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB
P-10	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
AMP-10	S	S	S	S	I	S	R	R	R	R	R	I	S	S	S	R	S	S	S	R
AMC-30 / Aug-30	I	S	I	S	S	S	R	R	S	S	I	R	S	S	S	I	R	S	I	R
SAM-20	S	S	S	S	S	S	I	S	I	I	I	I	I	R	I	R	I	I	S	I
TZP-110/10	S	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S
E-15	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
AZM-15	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
KZ-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R
CTX-30	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	I
CAZ-30	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R
EFT	S	S	S	S	S	S	S	S	S	S	S	R	I	I	R	I	I	R	-	-
FEP-30	S	S	S	I	S	S	S	S	I	S	S	S	I	S	S	S	S	S	S	I
FOX-30	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	I
C-30	S	S	I	S	S	R	I	I	R	R	R	R	S	S	S	I	S	R	I	R
FFC-30	S	S	I	S	I	R	R	S	R	R	R	R	I	S	S	I	S	R	R	R
NA-30	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
CIP-30	I	I	I	S	I	I	R	R	I	I	I	R	R	I	R	R	R	R	R	R
ENR-5	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
DA-2	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
CT-10/CS-10	R	R	R	R	S	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R
IMI-10 / IPM-10	I	I	I	R	I	I	R	R	S	S	S	I	S	S	R	R	R	S	R	R
MEM-10 /MRP-10	S	I	S	I	S	S	I	I	S	S	S	S	S	S	S	S	S	S	I	S
ETP-10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S-10	I	I	I	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
CN-10/GEN-10	S	S	S	S	S	S	S	S	S	S	R	I	S	S	S	S	S	S	I	R
AK-30	S	I	S	S	R	S	I	S	S	I	I	S	S	S	S	S	S	S	R	I
LNZ-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
F-300	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
QDA-15	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
TEC-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
TE-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R
DO-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
MH-30	S	I	S	I	S	I	R	R	R	R	R	I	I	I	R	R	I	I	R	R
TM-5	S	S	I	I	S	R	R	R	R	R	R	R	S	S	S	R	R	R	R	R
SXT-25	S	S	S	S	S	R	R	R	R	R	R	R	S	S	S	I	S	R	R	R
% Sensitive	51%	46%	40%	43%	43%	37%	20%	31%	31%	31%	26%	23%	40%	46%	43%	26%	37%	34%	24%	9%
% Intermediate	11%	17%	20%	14%	14%	9%	14%	6%	9%	9%	11%	14%	14%	9%	3%	14%	9%	6%	18%	15%
% Resistant	37%	37%	40%	43%	43%	54%	66%	63%	60%	60%	63%	63%	46%	46%	54%	60%	54%	60%	59%	76%

Sample ID	N-2137	N-2138	N-2142	N-2290	N-2291	N-2292	N-2293	N-2295	N-2427	N-2428	N-2429	N-2430	N-2616	N-2618	N-2619	N-2621	N-2623	N-24	N-27	N-28	N-30
City	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB	ISB
P-10	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
AMP-10	R	R	S	R	R	S	R	R	R	R	R	R	R	S	R	R	R	S	S	R	R
AMC-30 / Aug-30	R	R	S	I	R	R	R	R	S	R	S	I	R	R	R	R	R	S	I	S	I
SAM-20	S	I	S	S	R	S	I	S	S	S	S	S	S	I	S	S	S	S	S	S	S
TZP-110/10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
E-15	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
AZM-15	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R
KZ-30	R	R	S	R	R	R	S	R	R	R	I	I	I	R	I	I	R	S	I	R	I
CTX-30	I	I	S	S	S	S	S	S	S	R	S	I	S	S	S	S	S	S	S	S	S
CAZ-30	R	I	S	S	S	S	I	R	S	R	S	S	S	S	S	S	S	S	S	S	S
FEP-30	I	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
FOX-30	I	R	S	S	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S
C-30	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S
FFC-30	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S
NA-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I
CIP-30	R	R	R	R	R	R	R	R	R	R	I	I	R	R	R	R	R	I	R	R	I
ENR-5	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I
DA-2	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
CT-10/CS-10	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
IMI-10 / IPM-10	R	R	R	R	R	R	R	R	R	R	R	R	I	I	S	I	S	I	I	S	S
MEM-10 /MRP-10	R	R	S	S	I	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S
ETP-10	R	R	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S
S-10	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
CN-10/GEN-10	R	R	S	S	R	S	S	S	S	I	S	I	S	S	S	S	S	S	I	I	S
AK-30	R	R	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
LNZ-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
F-300	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I
QDA-15	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
TEC-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
TE-30	R	R	R	I	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	S
DO-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S
MH-30	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S
TM-5	R	R	I	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R
SXT-25	R	R	S	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R
% Sensitive	6%	3%	47%	29%	18%	32%	24%	26%	35%	21%	29%	24%	29%	29%	32%	26%	32%	41%	29%	32%	47%
% Intermediate	9%	12%	3%	6%	9%	0%	6%	0%	3%	6%	6%	15%	6%	6%	3%	6%	0%	6%	12%	3%	18%
% Resistant	85%	85%	50%	65%	74%	68%	71%	74%	62%	74%	65%	62%	65%	65%	65%	68%	68%	53%	59%	65%	35%

APPENDIX XXIII: AMR profile of isolates obtained from Rawalpindi

Antibiotics	% S	% I	% R	Antibiotics	% S	% I	% R
Penicillin	0%	0%	100%	Clindamycin	0%	0%	100%
Ampicillin	40%	20%	40%	Colistin	0%	0%	100%
Amoxicillin Clavulanate/ Augmentin	40%	60%	0%	Imipenem	20%	0%	80%
Ampicillin-Sulbactam	100%	0%	0%	Meropenem	60%	0%	40%
Piperacillin-Tazobactam	60%	40%	0%	Ertapenem	60%	0%	40%
Erythromycin	0%	0%	100%	Streptomycin	0%	0%	100%
Azithromycin	0%	0%	100%	Gentamicin	20%	20%	60%
Cefazolin	0%	40%	60%	Amikacin	100%	0%	0%
Cefotaxime	40%	0%	60%	Linezolid	0%	0%	100%
Ceftazidime	60%	0%	40%	Nitrofurantoin	0%	0%	100%
Cefepime	80%	0%	20%	Quinupristin/Dalfopristin	0%	0%	100%
Cefoxitin	60%	0%	40%	Teicoplanin	0%	0%	100%
Chloramphenicol	40%	0%	60%	Tetracycline	20%	0%	80%
Florfenicol	0%	20%	80%	Doxycycline	0%	0%	100%
Nalidixic Acid	20%	0%	80%	Minocycline	40%	20%	40%
Ciprofloxacin	0%	20%	80%	Trimethoprim	20%	0%	80%
Enrofloxacin	20%	40%	40%	Sulfamethoxazole-Trimethoprim	20%	0%	80%
Ceftiofur	67%	33%	0%				

S= Sensitive; I= Intermediate; R= Resistance

APPENDIX XXIV: Percentage of sensitivity, intermediate and resistance of isolates received from Rawalpindi

Sample ID	N-1156	N-1157	N-1551	N-1554	N-1555
Date	25/06/2020	25/06/2020	26/08/2020	26/08/2020	26/08/2020
City	Rawalpindi	Rawalpindi	Rawalpindi	Rawalpindi	Rawalpindi
P-10	R	R	R	R	R
AMP-10	R	R	S	S	I
AMC-30 / Aug-30	I	I	S	I	S
SAM-20	S	S	S	S	S
TZP-110/10	S	S	I	S	I
E-15	R	R	R	R	R
AZM-15	R	R	R	R	R
KZ-30	R	R	I	I	R
CTX-30	R	R	R	S	S
CAZ-30	S	S	S	R	R
EFT	-	-	S	S	I
FEP-30	S	S	S	S	R
FOX-30	S	S	S	R	R
C-30	R	R	R	S	S
FFC-30	R	R	R	I	R
NA-30	R	R	R	S	R
CIP-30	R	R	I	R	R
ENR-5	R	R	I	I	S
DA-2	R	R	R	R	R
CT-10/CS-10	R	R	R	R	R
IMI-10 / IPM-10	R	R	S	R	R
MEM-10 /MRP-10	S	S	S	R	R
ETP-10	S	S	S	R	R
S-10	R	R	R	R	R
CN-10/GEN-10	R	R	S	I	R
AK-30	S	S	S	S	S
LNZ-30	R	R	R	R	R
F-300	R	R	-	-	-
QDA-15	R	R	R	R	R
TEC-30	R	R	R	R	R
TE-30	R	R	R	S	R
DO-30	R	R	R	R	R
MH-30	R	R	S	I	S
TM-5	R	R	R	S	R
SXT-25	R	R	R	S	R
% Sensitive	24%	24%	38%	35%	21%
% Intermediate	3%	3%	12%	18%	9%
% Resistant	74%	74%	50%	47%	71%

APPENDIX XXV: AMR profile of isolates obtained from Lahore

Antibiotics	% S	% I	% R	Antibiotics	% S	% I	% R
Penicillin	0%	0%	100%	Clindamycin	0%	0%	100%
Ampicillin	25%	25%	50%	Colistin	15%	0%	85%
Amoxicillin Clavulanate/ Augmentin	55%	20%	25%	Imipenem	25%	35%	40%
Ampicillin-Sulbactam	70%	20%	10%	Meropenem	80%	15%	5%
Piperacillin-Tazobactam	85%	15%	0%	Ertapenem	82%	12%	6%
Erythromycin	0%	0%	100%	Streptomycin	0%	5%	95%
Azithromycin	10%	0%	90%	Gentamicin	65%	25%	10%
Cefazolin	15%	45%	40%	Amikacin	75%	20%	5%
Cefotaxime	65%	20%	15%	Linezolid	0%	0%	100%
Ceftazidime	80%	15%	5%	Nitrofurantoin	8%	8%	83%
Cefepime	70%	25%	5%	Quinupristin/Dalfopristin	0%	0%	100%
Cefoxitin	94%	6%	0%	Teicoplanin	0%	0%	100%
Chloramphenicol	30%	10%	60%	Tetracycline	5%	0%	95%
Florfenicol	10%	25%	65%	Doxycycline	5%	5%	90%
Nalidixic Acid	0%	5%	95%	Minocycline	10%	15%	75%
Ciprofloxacin	0%	35%	65%	Trimethoprim	12%	0%	88%
Enrofloxacin	0%	15%	85%	Sulfamethoxazole-Trimethoprim	15%	5%	80%
Ceftiofur	0%	50%	50%				

S= Sensitive; I= Intermediate; R= Resistance

APPENDIX XXVI: Percentage of sensitive, intermediate and resistance of isolates received from Lahore

Sample ID	N-1884	N-1885	N-1886	N-1887	N-1888	N-1889	N-1891	N-1893	N-1895	N-1896	N-1897	N-2012	N-2014	N-2021	N-2340	N-2341	N-2345	N-2346	N-2349	N-208
	LHR	LHR	LHR	LHR	LHR	LHR	LHR	LHR	LHR	LHR	LHR	LHR	LHR	LHR	LHR	LHR	LHR	LHR	LHR	LHR
P-10	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
AMP-10	R	S	R	R	R	S	S	R	R	R	R	I	I	I	R	I	S	I	S	R
AMC-30 / Aug-30	R	R	R	R	R	S	I	S	I	S	I	S	S	S	S	S	S	I	S	S
SAM-20	S	I	S	S	S	S	S	S	S	S	S	S	S	S	I	R	I	I	R	S
TZP-110/10	S	S	S	S	S	S	S	S	S	S	S	I	I	I	S	S	S	S	S	S
E-15	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
AZM-15	R	R	R	R	R	S	R	R	R	R	R	R	R	R	S	R	R	R	R	R
KZ-30	I	R	S	I	R	S	I	R	I	I	I	I	I	I	R	R	R	R	R	S
CTX-30	S	S	S	S	S	S	S	S	S	S	I	I	R	R	S	I	R	I	S	S
CAZ-30	S	S	S	S	S	S	S	S	S	S	S	I	I	I	S	R	S	S	S	S
EFT	-	-	-	-	-	-	-	-	-	-	-	I	R	I	I	R	R	R	I	-
FEP-30	S	S	S	S	S	S	S	S	S	S	S	I	I	I	S	R	I	S	I	S
FOX-30	S	S	S	S	S	S	S	S	S	S	S	-	-	-	S	S	S	I	S	S
C-30	R	R	R	R	R	R	R	R	S	R	R	S	I	S	S	S	S	R	I	R
FFC-30	R	R	R	R	R	R	R	R	R	R	R	I	I	I	S	S	I	R	I	R
NA-30	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R
CIP-30	R	R	R	R	R	I	R	R	I	I	I	R	R	I	I	I	R	R	R	R
ENR-5	R	R	R	R	R	R	R	R	R	R	R	I	I	I	R	R	R	R	R	R
DA-2	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
CT-10/CS-10	R	R	R	R	R	R	R	R	R	R	R	S	R	R	S	R	S	R	R	R
IMI-10 / IPM-10	I	I	I	I	I	I	I	S	S	R	R	R	R	R	S	S	R	R	R	S
MEM-10 /MRP-10	S	S	S	R	S	S	S	S	S	S	S	I	I	I	S	S	S	S	S	S
ETP-10	S	S	S	S	S	S	S	S	S	I	I	-	-	-	S	R	S	S	S	S
S-10	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R	R
CN-10/GEN-10	S	S	S	S	S	S	I	I	S	I	S	S	R	I	S	S	S	R	I	S
AK-30	S	S	S	S	S	S	S	S	S	S	S	I	R	I	I	S	S	I	S	S
LNZ-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
F-300	R	R	R	R	R	R	R	R	S	I	R	-	-	-	-	-	-	-	-	R
QDA-15	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
TEC-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
TE-30	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R
DO-30	R	R	R	R	R	R	R	R	R	R	R	R	I	S	R	R	R	R	R	R
MH-30	R	R	R	R	R	R	R	R	S	R	R	R	I	R	I	R	S	I	R	R
TM-5	R	R	R	R	R	R	R	R	R	R	R	-	-	-	R	S	S	R	R	R
SXT-25	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	S	S	R	I	R
% Sensitivity	29%	29%	32%	26%	29%	41%	29%	32%	41%	26%	24%	16%	13%	13%	41%	35%	41%	15%	26%	38%
% Intermediate	6%	6%	3%	6%	3%	6%	12%	3%	12%	15%	15%	35%	39%	39%	15%	9%	9%	21%	18%	0%
% Resistant	65%	65%	65%	68%	68%	53%	59%	65%	47%	59%	62%	48%	48%	48%	44%	56%	50%	65%	56%	62%

APPENDIX XXVII: AMR profile of isolates obtained from Karachi

Antibiotics	% S	% I	% R	Antibiotics	% S	% I	% R
Penicillin	0%	0%	100%	Clindamycin	0%	0%	100%
Ampicillin	50%	3%	47%	Colistin	17%	0%	83%
Amoxicillin Clavulanate/ Augmentin	83%	10%	7%	Imipenem	60%	23%	17%
Ampicillin-Sulbactam	90%	7%	3%	Meropenem	97%	3%	0%
Piperacillin-Tazobactam	93%	3%	3%	Ertapenem	93%	3%	3%
Erythromycin	0%	0%	100%	Streptomycin	0%	0%	100%
Azithromycin	17%	0%	83%	Gentamicin	73%	20%	7%
Cefazolin	33%	43%	23%	Amikacin	100%	0%	0%
Cefotaxime	77%	7%	17%	Linezolid	0%	0%	100%
Ceftazidime	80%	7%	13%	Nitrofurantoin	7%	3%	90%
Cefepime	93%	7%	0%	Quinupristin/Dalfopristin	0%	0%	100%
Cefoxitin	97%	3%	0%	Teicoplanin	0%	0%	100%
Chloramphenicol	30%	0%	70%	Tetracycline	3%	10%	87%
Florfenicol	33%	10%	57%	Doxycycline	0%	0%	100%
Nalidixic Acid	0%	0%	100%	Minocycline	10%	7%	83%
Ciprofloxacin	0%	57%	43%	Trimethoprim	13%	0%	87%
Enrofloxacin	0%	13%	87%	Sulfamethoxazole-Trimethoprim	10%	0%	90%
Ceftiofur	100%	0%	0%				

S= Sensitive; I= Intermediate; R= Resistance

APPENDIX XXVIII: Percentage of sensitivity, intermediate and resistance of isolates received from Karachi

Sample ID	N-1899	N-1900	N-1901	N-1902	N-1903	N-1906	N-1908	N-1909	N-1910	N-1911	N-1947	N-1949	N-1951	N-1952	N-1953	N-1954	N-1955	N-1956	N-1957	N-1959	N-2043	N-2046	N-2049	N-2051	N-2053	N-179	N-182	N-187	N-189	N-190
P-10	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
AMP-10	R	R	S	S	S	R	R	R	R	S	S	S	R	R	S	S	S	S	R	I	R	R	S	R	R	R	S	S	S	S
AMC-30 / Aug-30	R	I	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	I	S	S	S	S	S
SAM-20	I	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S
TZP-110/10	S	S	S	R	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
E-15	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
AZM-15	R	R	R	R	R	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R
KZ-30	R	R	I	S	S	I	I	S	I	S	I	I	R	R	I	S	S	I	I	I	R	R	S	I	I	R	S	S	S	I
CTX-30	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	R	S	S	R	I	S	I	R	S	S	S	S
CAZ-30	R	R	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S	I	S	R	S	R	S	S	S	S	S	S	S
EFT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	S	S
FEP-30	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S
FOX-30	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
C-30	R	R	R	R	R	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	S	S	S
FFC-30	R	R	R	I	R	S	S	S	I	S	R	R	I	R	R	S	S	S	R	R	R	R	R	R	R	R	R	S	S	S
NA-30	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
CIP-30	R	I	I	I	R	R	R	I	R	I	I	I	I	I	I	I	R	R	R	R	R	R	R	I	I	I	I	I	R	I
ENR-5	R	R	R	R	R	I	R	I	I	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
DA-2	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
CT-10/CS-10	R	R	R	R	R	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
IMI-10 / IPM-10	S	S	I	I	S	S	S	S	S	S	I	S	S	S	S	I	S	S	I	I	R	R	R	R	R	S	S	I	S	S
MEM-10 /MRP-10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S
ETP-10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	I	S	S	S	S	S
S-10	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
CN-10/GEN-10	R	I	I	I	S	S	S	I	S	S	S	S	S	S	S	S	S	S	R	S	S	S	I	S	I	S	S	S	S	S
AK-30	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
LNZ-30	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
F-300	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	I	R	R	R	R	S	R
QDA-15	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
TEC-30	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
TE-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	I	I	R	R	R	R	S	R	R	R	R	R	R	R
DO-30	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
MH-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	I	S	S	S
TM-5	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	S	S	S
SXT-25	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	S	S	S
% Sensitive	24%	24%	32%	32%	38%	47%	47%	44%	44%	50%	35%	38%	26%	29%	38%	41%	47%	41%	18%	32%	26%	32%	35%	29%	21%	32%	41%	53%	59%	53%
% Intermediate	3%	12%	12%	12%	3%	6%	3%	12%	9%	6%	9%	6%	12%	6%	6%	9%	6%	6%	15%	9%	0%	0%	6%	9%	18%	3%	6%	9%	3%	9%
% resistant	74%	65%	56%	56%	59%	47%	50%	44%	47%	44%	56%	56%	62%	65%	56%	50%	47%	53%	68%	59%	74%	68%	59%	62%	62%	65%	53%	38%	38%	38%

APPENDIX XXIX: AMR profile of isolates obtained from Peshawar

Antibiotics	% S	% I	% R	Antibiotics	% S	% I	% R
Penicillin	0%	0%	100%	Clindamycin	0%	0%	100%
Ampicillin	40%	5%	55%	Colistin	30%	0%	70%
Amoxicillin Clavulanate/ Augmentin	65%	25%	10%	Imipenem	40%	30%	30%
Ampicillin-Sulbactam	50%	40%	10%	Meropenem	75%	10%	15%
Piperacillin-Tazobactam	85%	10%	5%	Ertapenem	100%	0%	0%
Erythromycin	5%	10%	85%	Streptomycin	0%	10%	90%
Azithromycin	20%	0%	80%	Gentamicin	50%	25%	25%
Cefazolin	20%	20%	60%	Amikacin	65%	15%	20%
Cefotaxime	70%	15%	15%	Linezolid	5%	0%	95%
Ceftazidime	50%	15%	35%	Nitrofurantoin	0%	0%	100%
Cefepime	70%	20%	10%	Quinupristin/Dalfopristin	0%	5%	95%
Cefoxitin	92%	8%	0%	Teicoplanin	0%	0%	100%
Chloramphenicol	20%	10%	70%	Tetracycline	5%	0%	95%
Florfenicol	15%	20%	65%	Doxycycline	15%	0%	85%
Nalidixic Acid	0%	5%	95%	Minocycline	15%	10%	75%
Ciprofloxacin	0%	40%	60%	Trimethoprim	25%	0%	75%
Enrofloxacin	0%	5%	95%	Sulfamethoxazole-Trimethoprim	25%	5%	70%
Ceftiofur	64%	29%	7%				

S= Sensitive; I= Intermediate; R= Resistance

APPENDIX XXX: Percentage of sensitivity, intermediate and resistance of isolates received from Peshawar

Sample ID	N-1789	N-1778	N-1779	N-1782	N-1784	N-2032	N-2037	N-2320	N-2324	N-2326	N-2327	N-2328	N-2334	N-2337	N-2456	N-2459	N-2463	N-2467	N-241	N-243
P-10	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
AMP-10	S	R	R	S	S	S	R	I	S	S	R	S	S	S	R	R	R	R	R	R
AMC-30 / Aug-30	S	R	I	S	S	S	R	R	S	S	S	R	S	S	R	I	I	S	S	S
SAM-20	S	I	I	S	S	S	S	I	S	I	S	I	I	R	I	I	S	S	S	S
TZP-110/10	R	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	I	S	I	R
E-15	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	I	I
AZM-15	R	R	R	R	R	S	R	R	R	R	R	R	R	S	R	R	R	S	R	S
KZ-30	S	R	R	I	S	S	I	I	R	R	R	R	R	R	R	R	R	S	I	R
CTX-30	S	S	S	S	S	I	I	S	R	S	S	S	S	S	I	S	S	S	R	I
CAZ-30	S	R	R	S	S	I	R	S	S	S	S	S	S	I	S	R	R	S	I	R
EFT	-	-	-	-	-	S	I	S	S	S	S	S	I	R	S	S	S	S	I	I
FEP-30	S	S	S	S	S	S	S	S	I	S	S	S	S	S	R	R	I	S	I	I
FOX-30	S	S	S	S	S	-	-	I	S	S	S	S	S	S	I	-	-	-	-	-
C-30	R	R	R	R	R	R	R	R	S	S	I	S	R	S	R	R	R	I	R	R
FFC-30	R	R	R	R	I	R	R	R	I	S	I	S	R	S	R	R	R	I	R	R
NA-30	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R
CIP-30	I	I	I	I	I	I	R	R	R	I	R	R	R	I	R	R	R	R	R	R
ENR-5	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R
DA-2	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
CT-10/CS-10	R	R	R	R	R	R	S	R	S	R	R	R	R	S	R	R	R	S	S	S
IMI-10 / IPM-10	S	S	S	I	I	S	I	I	S	S	R	R	S	S	I	R	R	R	I	I
MEM-10 /MRP-10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	I	R	I	S	R
ETP-10	S	S	S	S	S	-	-	S	S	S	S	S	S	S	I	-	-	-	-	-
S-10	R	R	R	R	R	I	I	R	R	R	R	R	R	R	R	R	R	R	R	R
CN-10/GEN-10	S	R	I	R	I	S	R	I	S	S	S	S	S	S	I	I	S	S	R	R
AK-30	S	S	S	S	S	S	S	I	S	S	I	S	S	S	S	R	I	S	R	R
LNZ-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R
F-300	R	R	R	R	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
QDA-15	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R
TEC-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
TE-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R
DO-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S
MH-30	R	R	R	R	R	I	I	R	R	R	R	R	R	R	R	R	R	S	S	S
TM-5	R	R	R	R	R	-	-	R	S	S	R	R	R	S	-	-	-	-	-	-
SXT-25	R	R	R	R	R	S	R	R	S	S	I	S	R	S	R	R	R	R	R	R
% Sensitivity	38%	24%	24%	32%	32%	42%	19%	21%	47%	50%	32%	41%	35%	50%	12%	10%	13%	52%	23%	19%
% Intermediate	3%	6%	12%	9%	12%	23%	19%	21%	6%	6%	12%	3%	6%	6%	18%	13%	13%	13%	23%	16%
% Resistant	59%	71%	65%	59%	56%	35%	61%	59%	47%	44%	56%	56%	59%	44%	70%	77%	74%	35%	55%	65%

APPENDIX XXXI: AMR profile of isolates obtained from Quetta

Antibiotics	% S	% I	% R	Antibiotics	% S	% I	% R
Penicillin	0%	0%	100%	Clindamycin	0%	0%	100%
Ampicillin	35%	5%	60%	Colistin	25%	0%	75%
Amoxicillin Clavulanate/ Augmentin	30%	35%	35%	Imipenem	25%	0%	75%
Ampicillin-Sulbactam	65%	30%	5%	Meropenem	60%	20%	20%
Piperacillin-Tazobactam	100%	0%	0%	Ertapenem	81%	19%	0%
Erythromycin	0%	0%	100%	Streptomycin	0%	0%	100%
Azithromycin	25%	0%	75%	Gentamicin	55%	10%	35%
Cefazolin	25%	15%	60%	Amikacin	55%	15%	30%
Cefotaxime	80%	10%	10%	Linezolid	0%	0%	100%
Ceftazidime	60%	25%	15%	Nitrofurantoin	0%	0%	100%
Cefepime	60%	40%	0%	Quinupristin/Dalfopristin	0%	0%	100%
Cefoxitin	81%	13%	6%	Teicoplanin	0%	0%	100%
Chloramphenicol	30%	10%	60%	Tetracycline	0%	0%	100%
Florfenicol	25%	10%	65%	Doxycycline	0%	0%	100%
Nalidixic Acid	0%	0%	100%	Minocycline	5%	15%	80%
Ciprofloxacin	0%	10%	90%	Trimethoprim	0%	0%	100%
Enrofloxacin	0%	25%	75%	Sulfamethoxazole-Trimethoprim	0%	10%	90%
Ceftiofur	100%	0%	0%				

S= Sensitive; I= Intermediate; R= Resistance

APPENDIX XXXII: Percentage of sensitivity, intermediate and resistance of isolates received from Quetta

Sample ID	N-1794	N-1795	N-1796	N-1797	N-1801	N-1978	N-1980	N-1981	N-1983	N-1984	N-1985	N-1986	N-1988	N-1989	N-1990	N-1991	N-5	N-7	N-12	N-16
P-10	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
AMP-10	R	R	R	R	S	R	R	S	R	R	R	S	R	R	S	R	S	S	I	S
AMC-30 / Aug-30	S	S	I	S	S	I	I	I	R	R	R	S	I	R	R	R	I	I	R	S
SAM-20	S	S	S	S	S	I	S	S	I	I	I	S	S	R	S	I	S	S	I	S
TZP-110/10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
E-15	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
AZM-15	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
KZ-30	I	I	S	S	S	R	R	I	R	R	R	S	R	R	R	S	R	R	R	R
CTX-30	S	S	S	S	S	S	S	S	I	R	S	S	S	S	S	S	S	I	R	S
CAZ-30	S	I	S	S	S	S	S	S	R	R	I	S	S	S	S	I	I	I	R	S
EFT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	S	S	S
FEP-30	S	S	S	S	S	S	S	S	I	I	I	S	S	I	S	S	I	I	I	I
FOX-30	S	S	S	S	S	S	S	S	I	I	R	S	S	S	S	S	-	-	-	-
C-30	S	S	S	S	S	R	R	I	R	R	R	S	R	R	R	R	I	R	R	R
FFC-30	S	S	S	I	S	R	R	R	R	R	R	S	R	R	R	R	I	R	R	R
NA-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
CIP-30	R	I	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
ENR-5	I	I	I	I	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
DA-2	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
CT-10/CS-10	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
IMI-10 / IPM-10	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
MEM-10 /MRP-10	S	S	S	S	S	S	S	I	S	R	R	I	S	I	S	S	R	I	R	S
ETP-10	S	S	S	S	S	S	S	S	S	I	I	S	S	I	S	S	-	-	-	-
S-10	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
CN-10/GEN-10	S	S	S	S	S	R	R	S	R	R	R	S	S	R	S	S	I	I	R	S
AK-30	S	S	S	S	S	S	S	R	I	R	R	S	S	I	S	R	I	S	R	R
LNZ-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
F-300	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	-	-	-	-
QDA-15	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
TEC-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
TE-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
DO-30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
MH-30	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	S	I	R	I
TM-5	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	-	-	-	-
SXT-25	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	I	R	R	R
% Sensitive	47%	44%	47%	47%	53%	24%	26%	26%	9%	3%	6%	41%	29%	12%	32%	24%	19%	16%	6%	29%
% Intermediate	6%	12%	9%	6%	3%	6%	3%	12%	15%	12%	12%	6%	3%	15%	0%	6%	26%	23%	10%	6%
% Resistant	47%	44%	44%	47%	44%	71%	71%	62%	76%	85%	82%	53%	68%	74%	68%	71%	55%	61%	84%	65%

APPENDIX XXXIII: AMR profile of isolates obtained from Muzaffarabad

Antibiotics	% S	% I	% R	Antibiotics	% S	% I	% R
Penicillin	0%	0%	100%	Clindamycin	0%	0%	100%
Ampicillin	31%	0%	69%	Colistin	0%	0%	100%
Amoxicillin Clavulanate/ Augmentin	77%	23%	0%	Imipenem	38%	23%	38%
Ampicillin-Sulbactam	62%	31%	8%	Meropenem	54%	8%	38%
Piperacillin-Tazobactam	85%	15%	8%	Ertapenem	100%	0%	0%
Erythromycin	0%	0%	100%	Streptomycin	0%	0%	100%
Azithromycin	0%	0%	100%	Gentamicin	62%	8%	31%
Cefazolin	0%	38%	62%	Amikacin	69%	15%	15%
Cefotaxime	69%	15%	15%	Linezolid	0%	0%	100%
Ceftazidime	62%	15%	23%	Nitrofurantoin	0%	0%	100%
Cefepime	69%	15%	15%	Quinupristin/Dalfopristin	0%	0%	100%
Cefoxitin	88%	13%	0%	Teicoplanin	0%	0%	100%
Chloramphenicol	0%	0%	100%	Tetracycline	0%	0%	100%
Florfenicol	15%	0%	85%	Doxycycline	0%	0%	100%
Nalidixic Acid	0%	8%	92%	Minocycline	0%	23%	77%
Ciprofloxacin	0%	23%	77%	Trimethoprim	0%	0%	100%
Enrofloxacin	0%	8%	92%	Sulfamethoxazole-Trimethoprim	0%	0%	100%
Ceftiofur	60%	0%	40%				

S= Sensitive; I= Intermediate; R= Resistance

APPENDIX XXXIV: Percentage of sensitivity, intermediate and resistance of isolates received from Muzaffarabad

Sample ID	N-166	N-170	N-171	N-172	N-173	N-174	N-175	N-178	N-287	N-290	N-293	N-295	N-296
P-10	R	R	R	R	R	R	R	R	R	R	R	R	R
AMP-10	R	R	S	R	R	S	S	R	R	S	R	R	R
AMC-30 / Aug-30	S	S	S	R	S	S	S	S	R	R	R	I	I
SAM-20	S	R	S	I	S	S	S	I	S	I	I	S	S
TZP-110/10	S	S	S	I	I	S	S	S	S	S	S	S	S
E-15	R	R	R	R	R	R	R	R	R	R	R	R	R
AZM-15	R	R	R	R	R	R	R	R	R	R	R	R	R
KZ-30	R	R	R	R	R	I	I	R	I	R	I	I	R
CTX-30	R	S	S	I	I	S	S	R	S	S	S	S	S
CAZ-30	S	R	R	R	I	S	S	I	S	S	S	S	S
EFT	R	S	S	R	S	-	-	-	-	-	-	-	-
FEP-30	R	R	S	I	I	S	S	S	S	S	S	S	S
FOX-30	S	S	-	-	-	S	S	I	S	S	S	S	S
C-30	R	R	R	R	R	R	R	R	R	R	R	R	R
FFC-30	S	S	R	R	R	R	R	S	R	R	R	R	R
NA-30	R	I	R	R	R	R	R	R	R	R	R	R	R
CIP-30	R	R	R	R	R	I	I	I	R	R	R	R	R
ENR-5	R	I	R	R	R	R	R	R	R	R	R	R	R
DA-2	R	R	R	R	R	R	R	R	R	R	R	R	R
CT-10/CS-10	R	R	R	R	R	R	R	R	R	R	R	R	R
IMI-10 / IPM-10	R	I	R	R	R	S	S	S	I	I	S	I	S
MEM-10 /MRP-10	R	S	I	R	R	S	S	S	S	S	S	R	S
ETP-10	S	S	-	-	-	S	S	S	S	S	S	S	S
S-10	R	R	R	R	R	R	R	R	R	R	R	R	R
CN-10/GEN-10	I	R	I	R	R	S	S	S	S	S	S	S	S
AK-30	S	S	R	I	I	S	S	S	S	S	S	S	S
LNZ-30	R	R	R	R	R	R	R	R	R	R	R	R	R
F-300	-	-	-	-	-	R	R	R	R	R	R	R	R
QDA-15	R	R	R	R	R	R	R	R	R	R	R	R	R
TEC-30	R	R	R	R	R	R	R	R	R	R	R	R	R
TE-30	R	R	R	R	R	R	R	R	R	R	R	R	R
DO-30	R	R	R	R	R	R	R	R	R	R	R	R	R
MH-30	R	R	I	I	I	R	R	R	R	R	R	R	R
TM-5	-	-	-	-	-	R	R	R	R	R	R	R	R
SXT-25	R	R	R	R	R	R	R	R	R	R	R	R	R
% Sensitive	**24%**	**21%**	**23%**	**0%**	**10%**	**38%**	**38%**	**26%**	**29%**	**29%**	**29%**	**26%**	**32%**
% Intermediate	**3%**	**15%**	**10%**	**19%**	**19%**	**6%**	**6%**	**12%**	**6%**	**6%**	**6%**	**9%**	**3%**
% Resistant	**73%**	**64%**	**68%**	**81%**	**71%**	**56%**	**56%**	**62%**	**65%**	**65%**	**65%**	**65%**	**65%**

APPENDIX XXXV: AMR profile of isolates obtained from Gilgit

Antibiotics	% S	% I	% R	Antibiotics	% S	% I	% R
Amikacin	25%	25%	50%	Erythromycin	0%	0%	100%
Amoxicillin Clavulanate/ Augmentin	50%	25%	25%	Florfenicol	0%	25%	75%
Ampicillin	25%	0%	75%	Gentamicin	0%	25%	75%
Ampicillin-Sulbactam	75%	0%	25%	Imipenem	25%	0%	75%
Azithromycin	0%	0%	100%	Linezolid	0%	0%	100%
Cefazolin	0%	0%	100%	Meropenem	0%	25%	75%
Cefepime	25%	25%	50%	Minocycline	0%	0%	100%
Cefotaxime	50%	25%	25%	Nalidixic Acid	0%	0%	100%
Ceftiofur	67%	%	33%	Nitrofurantoin	0%	0%	100%
Cefoxitin	100%	0%	0%	Penicillin	0%	0%	100%
Ceftazidime	0%	25%	75%	Piperacillin-Tazobactam	50%	25%	25%
Chloramphenicol	0%	50%	50%	Quinupristin/Dalfopristin	0%	%	100%
Ciprofloxacin	0%	0%	100%	Streptomycin	0%	50%	50%
Clindamycin	0%	0%	100%	Sulfamethoxazole-Trimethoprim	25%	0%	75%
Colistin	0%	0%	100%	Teicoplanin	0%	0%	100%
Doxycycline	0%	0%	100%	Tetracycline	25%	0%	75%
Enrofloxacin	0%	0%	100%	Trimethoprim	0%	0%	100%
Ertapenem	100%	0%	0%				

S= Sensitive; I= Intermediate; R= Resistance

APPENDIX XXXVI: Percentage of sensitivity, Intermediate and resistance of each isolate received from Gilgit

Sample ID	N-191	N-339	N-344	N-346
Date	25/1/2021	24/2/2021	24/2/2021	24/2/2021
City	Gilgit	Gilgit	Gilgit	Gilgit
P-10	R	R	R	R
AMP-10	R	R	S	R
AMC-30 / Aug-30	S	S	R	I
SAM-20	S	S	S	R
TZP-110/10	S	S	S	I
E-15	R	R	R	R
AZM-15	R	R	R	R
KZ-30	R	R	R	R
CTX-30	R	S	S	I
CAZ-30	I	R	R	R
EFT	-	S	S	R
FEP-30	S	R	I	R
FOX-30	S	-	-	-
C-30	R	I	R	I
FFC-30	R	R	R	I
NA-30	R	R	R	R
CIP-30	R	R	I	R
ENR-5	R	R	R	R
DA-2	R	R	R	R
CT-10/CS-10	R	R	R	R
IMI-10 / IPM-10	S	R	R	R
MEM-10 /MRP-10	I	R	R	R
ETP-10	S	-	-	-
S-10	I	R	R	I
CN-10/GEN-10	R	R	R	I
AK-30	S	I	R	R
LNZ-30	R	R	R	R
F-300	R	-	-	-
QDA-15	R	R	R	R
TEC-30	R	R	R	R
TE-30	R	R	S	R
DO-30	R	R	R	R
MH-30	R	R	R	I
TM-5	R	-	-	-
SXT-25	R	R	R	S
% Sensitive	24%	13%	19%	3%
% Intermediate	9%	6%	6%	26%
% Resistant	68%	81%	74%	71%

Thesis

Rafia Sameen (AGB, PIASA,NARC)

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