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Antimicrobial Resistance Surveillance Pilot in Healthy Food Animals

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Report

Antimicrobial Resistance Surveillance Pilot in Healthy Food Animals

Final Report

The Fleming Fund Country Grant Pakistan
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Collaborating Partners

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List of Acronyms

AHC	Animal Husbandry Commissioner
AJK	Azad Jammu and Kashmir
AMR	Antimicrobial Resistance
AMR-CU	Antimicrobial Resistance Coordination Unit
AMU	Antimicrobial Use
AST	Antibiotic Susceptibility Testing
BIMS	Biorepository Information Management System
CI	Confidence Interval
CLSI	Clinical and Laboratory Standards Institute
ECOFF	Epidemiologic cut-off value
EMRO	Eastern Mediterranean Region Office
FAO	Food and Agriculture Organization of the United Nations
FF	Fleming Fund
GAP	Global Action Plan
GB	Gilgit-Baltistan
GDP	Gross Domestic Product
GLASS	Global Antimicrobial Resistance and Use Surveillance System
ISO	International Organization for Standardization
L&DD	Livestock and Dairy Development Department
MIC	Minimum Inhibitory Concentration
MoNFSR	Ministry of National Food Security & Research
MoNHSR&C	Ministry of National Health Services, Regulations and Coordination
NAP	National Action Plan
NARC	National Agricultural Research Council
NVL	National Veterinary Laboratory
NRL	National Reference Laboratory
NRLPD	National Reference Laboratory for Poultry Diseases
OR	Odds Ratio
PARC	Pakistan Agricultural Research Council
PFP	Provincial Focal Person
PSL	Peripheral Sentinel Laboratory
P Value	Probability Value
SDG	Sustainable Development Goals

SPSS	Statistical Package for the Social Sciences
SS	Sampling Site
UNEP	United Nations Environment Programme
WHA	World Health Assembly
WHO	World Health Organization
WOAH	World Organization for Animal Health

1. Background

Antimicrobial Resistance (AMR) is an emerging global public health threat that can hamper efforts to achieve the 2030 Sustainable Development Goals (SDG). Overuse and misuse of antimicrobials in people and animals, often without professional oversight, contribute to the development of AMR. Antimicrobials are frequently misused to treat viral infections in humans and for growth promotion in food animals. Resistant microbes can be found everywhere, in humans, animals, food and the environment. They can spread between animals and humans, including through food animal products.

Poor infection control practices in health-care settings, inadequate sanitary conditions, and farm-level husbandry practices, which include inappropriate biosecurity and food-handling practices, encourage the spread of AMR. The development and transmission of antimicrobial-resistant pathogens render antimicrobials ineffective, thus depriving us of an essential tool to treat infections.

While the consequences of AMR are acutely experienced in the human health sector, drivers of AMR are dispersed throughout the interconnected ecosystem (i.e., agriculture and the environment). A One Health approach at all levels (global, regional, and national) is required to contain AMR effectively. Such an approach requires human medicine, veterinary medicine, agriculture, finance, the environment, and consumers to work collectively. Quadripartite organizations, i.e., the Food and Agriculture Organization (FAO), the World Organization for Animal Health (WOAH), the United Nations Environment Programme (UNEP), and the World Health Organization (WHO), emphasize the need for strong collaborations among different entities, each with a different mandate and resources to address AMR at the intersection of the human-animal-plant ecosystems. Stakeholders (donor organizations, governments, and private sectors) need to coordinate and work together to streamline processes, prevent duplication, and prevent becoming a burden on under-resourced member states.

AMR is growing and spreading throughout the world. Its impact is expected to be quite extensive in developing countries. Pakistan is the first country of the Eastern Mediterranean Regional Office (EMRO) of the WHO to establish the early implementation of the WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS). Furthermore, in line with the five strategic objectives of the WHO Global Action Plan (GAP) for AMR, the Ministry of National Health Services, Regulations and Coordination (MoNHSR&C) steered the National Strategic Framework's development *for AMR Containment* through a consultative process adopting a One Health approach.

Resultantly, with the involvement and participation of the health, veterinary, agriculture and other sectors at the federal, provincial, and regional levels and in line with the One Health approach, the MoNHSR&C developed the AMR National Action Plan (AMR NAP). The development of AMR NAP signifies the commitments by the Government of Pakistan's to the WHA 68.7 resolution on AMR.

AMR NAP emphasized the animal health sector to initiate AMR surveillance in the animal health sector to determine the burden of antimicrobial-resistant pathogens in the animals. To bridge this gap, the Fleming Fund Country Grant Pakistan provided technical and financial support to the Ministry of National Food Security and Research (MoNFS&R). Under the leadership of the Animal husbandry Commissioner (AHC), MoNFS&R, through a consultative process, the Fleming Fund Country Grant team supported the development of a National Surveillance Strategy for AMR in Healthy Food Animals.[1] Since July 2020 to December 2022, the AHC office coordinated the implementation of a surveillance pilot in healthy food animals involving poultry and large ruminants at slaughterhouses. This pilot has been completed and is one of the big achievements of Fleming Fund Country Grant Pakistan.

[1] AHC 2021. National Surveillance Strategy for Antimicrobial Resistance in healthy Food Animals. Animal Husbandry Commissioner, Livestock Wing, Ministry of National Food Security and Research, Government of Pakistan, Islamabad, Pakistan.

2. Objectives of AMR Surveillance Pilot

The AMR surveillance pilot was aimed to initiate a monitoring and surveillance program for AMR in selected production systems of healthy food animals. The specific objectives were to:

1. Streamline and strengthen all components of the AMR surveillance system for animals, including epidemiological skills (sample design, data analysis, and data reporting), sample collection and processing, laboratory diagnostic capability, and data management.
2. Estimate the prevalence of resistance amongst selected bacteria in poultry and large ruminants (cattle and buffalo) to antibiotics that the WHO has specified as critical for use in humans and other antibiotics of importance to animal health.

The pilot was expected to form the basis for scaling up and expanding AMR surveillance to wider geographic areas and the inclusion of different animal production systems and more bacterial pathogens. This pilot serves as a proof-of-concept national AMR surveillance program in the animal sector for inclusion into the country's AMU/AMR Integrated Surveillance Programme.

3. Salient Characteristics of the AMR Surveillance Pilot Project

The surveillance pilot was simultaneously implemented in poultry and large ruminants.

3.1 Target Population

For the AMR surveillance pilot project, the target populations were healthy "commercial broilers" and "cattle and buffalo" intended for human consumption.

3.2 Source Population

The source population for the pilot was healthy poultry at slaughter shops and slaughtered cattle and buffalo at designated slaughterhouses.

3.3 Target Bacteria Specie

The AMR surveillance pilot focused on two commensal bacteria, i.e., *Escherichia coli (E. coli), and Enterococcus* spp. (*E. faecium* and *E. faecalis*) and one zoonotic foodborne bacterium, i.e., *Salmonella* spp.

3.4 Biological Samples

Considering the ecology and epidemiology of AMR and target bacteria, the required biological samples were the caecal/faecal contents from slaughtered poultry and cattle/buffalo.

3.5 Sampling Sites

The Fleming Fund Country Grant Pakistan supports two federal laboratories, i.e., the National Reference Laboratory for Poultry Diseases (NRLPD) and the National Veterinary Laboratories (NVL), to serve as National Reference Laboratories (NRLs) for AMR in the animal health sector. These NRLs are the lead diagnostic laboratories for the AMR surveillance pilot. Besides, nine Peripheral Sentinel Laboratories (PSL) across Pakistan were engaged in field activities to collect caecal contents from poultry slaughter shops and cattle/buffalo slaughterhouses (Figure 1). The PSLs include:

1. Poultry Research Institute, Karachi
2. Poultry Research Institute, Rawalpindi
3. Poultry Research Institute, Mansehra
4. Provincial Disease Diagnostic Laboratory, Lahore

5. Disease Investigation Laboratory, Peshawar
6. Disease Investigation Laboratory, Muzaffarabad
7. Disease Investigation Laboratory, Quetta
8. Central Veterinary Diagnostic Laboratory, Tando Jam, Hyderabad
9. Gilgit-Baltistan Veterinary Laboratory, Gilgit

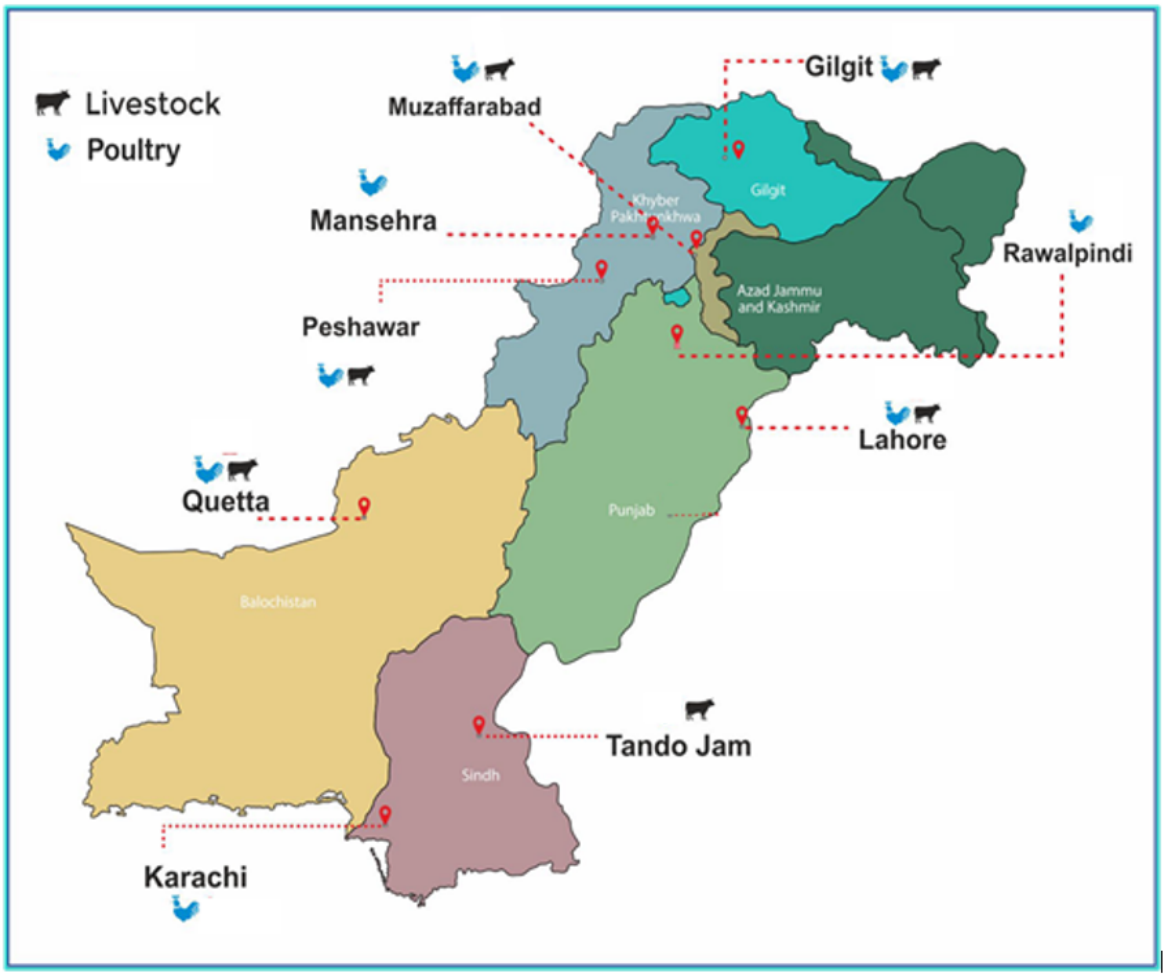

Figure 1. Peripheral sentinel laboratories of AMR surveillance network in the animal health sector

4. Updates on Surveillance

4.1 Focal Persons

To better coordinate AMR and AMU activities in the animal health sector and to foster multisectoral collaborations, the Fleming Fund Country Grant provided support to MoNFS&R in the establishment of the AMR Coordination Unit at the AHC office. In this regard, short term-technical assistance is provided to support activities related to AMR. The MoNFS&R nominated Dr Riasat Wasee Ullah to serve as the AMR Coordination Unit's contact point at the AHC office. Furthermore, Dr Muhammad Abu Bakar (Senior Scientific Officer, NVL) was notified as the National Focal Person of AMR and, Dr Muhammad Athar Abbas (Senior Scientific Officer, NRLPD, Animal Sciences Institute, National Agriculture Research Center (NARC)) was nominated as focal person for AMR of the Pakistan Agricultural Research Council (PARC).

Provincial Focal Persons (PFP) and contact points were nominated by provincial Livestock and Dairy Development Departments (L&DDs), in response to a request from the AHC office. The names of PFPs are as follows:

- Dr Waseem Tahir (Azad Jammu and Kashmir - Poultry and Livestock)
- Dr Muhammad Bilal (Balochistan - Poultry, and Livestock)
- Dr Takbir Ali (GB - Poultry and Livestock)
- Dr Syed Asad Ali Shah (Khyber Pakhtunkhwa - Poultry and Livestock)
- Director (Punjab - Poultry and Livestock)
- Dr Abdul Ahad Soomro (Sindh-Livestock)
- Dr Naeem Aziz Soomro (Sindh-Poultry)
- Dr Farhan Afzal (Punjab - Poultry Research Institute, Rawalpindi)
- Dr Naqash Khal[redacted]Khyber Pakhtunkhwa - Poultry Research Institute, Mansehra)

4.2 Sampling Status

Sample size calculation for the estimation of the prevalence of AMR was a tiered approach. Firstly, the number of isolates required to estimate the prevalence of resistant bacteria was determined. In the second stage, the number of biological samples needed to obtain the required number of bacterial isolates was calculated. The number of isolates needed to estimate the prevalence of resistant bacteria were estimated using the following formula:

$$N = [Z^2 \times (P) \times (1-P)]/e^2$$

Where N = Total bacterial isolates to be tested per year, P = Prevalence of the resistance gene or phenotype, Z = The standard normal deviation, typically set at 95 percent confidence level (Z=1.96) and e = Accepted Error (usually five percent or 0.05).

Assuming 50 percent of the isolates tested per year will be positive for resistant genes [redacted]henotypes, a total of 384 isolates needs to be tested by AST. As recommended by FAO[2] and EFSA[3], five percent missingness and two percent isolate loss should also be factored in. To recover the required number of isolates, the number of samples to be tested were estimated based upon the prevalence of target bacteria in the biological samples.

For isolation of *E. coli, Enterococcus* spp., and *Salmonella* spp., pooled caecal samples were collected from different poultry slaughter shops/slaughterhouses in each selected district. To get one pooled sample, five freshly collected samples were pooled into one sample.

For the surveillance pilot in poultry, NRLPD has received 2317 pooled caecal samples from the freshly slaughtered broiler birds as of November 30th, 2022, from Gilgit, Islamabad, Karachi, Lahore, Mansehra, Muzaffarabad, Peshawar, Rawalpindi, and Quetta. Also, NVL received 3474 individual faecal samples from cattle and buffalo slaughterhouses in Gilgit, Hyderabad, Islamabad, Lahore, Muzaffarabad, Peshawar, and Quetta as of 11.30.2022. In case of cattle and buffalos' individual samples were cultured to obtain *E. coli, Enterococcus and Salmonella* isolates. The details of the isolates obtained from these samples are given in Table 1.

[2] FAO. 2019. Regional Antimicrobial Resistance Monitoring and Surveillance Guidelines – Volume 1. Bangkok

[3] EFSA. 2014. *EFSA Journal,* 12(5): 3686—3719

4.3 Laboratory Diagnostics

NRLs processed the samples received for the isolation of *E. coli, Enterococcus* spp. and *Salmonella* spp. followed by AST according to the country surveillance strategy and testing methodologies described elsewhere[4,5].

4.3.1 Bacterial Isolation

Initially both NRLs started isolating *E. coli* and *Enterococcus* spp. from the biological samples collected from broilers and cattle/buffalo as both NRLs had the capacity to isolate and identify *E. coli* and *Enterococcus* spp. *Salmonella* spp. was included in surveillance in December 2020, and NRLPD initiated the isolation of *Salmonella* spp. in August 2021; while NVL started isolation of *Salmonella* spp. in November 2021.

4.3.2 Antibiotic Sensitivity Testing

Both NRLs initiated AST using disk diffusion method but the preferred technique indicated in the surveillance protocols was microbroth dilution to obtain quantitative data (minimum inhibitory concentration [MIC]. Therefore, both laboratories were provided with training on microbroth dilution technique in November 2022. Both NRLs have standardized the microbroth dilution methods and were able to start generating MIC data. Both NRLs have also been provided with Biorepository Information Management System (BIMS) as e-cataloguing system for a more efficient storage and archival of the isolates. This has markedly improved their used the management of their isolates.

4.3.3 Data management, analysis and reporting

A Microsoft Excel template was developed to collect basic demographic data related to the samples (e.g., slaughter plant or wet market unique identifier, province, species of animal, location, date collected/received/tested), the isolate (e.g., unique isolate identifiers) and microbiological findings (isolate recovered, AST inhibition zone values). The dataset was checked for errors and missing data and AST results were validated (range of inhibition zones and interpretation) prior to analysis. The Microsoft Excel spreadsheet was then imported into IBM SPSS version 26, to obtain basic descriptive statistics. A logistic regression model was used to examine the association between outcome variables (resistant and susceptible) and potential risk factors.

[4] AHC 2021. National Surveillance Strategy for Antimicrobial Resistance in healthy Food Animals. Animal Husbandry Commissioner, Livestock Wing, Ministry of National Food Security and Research, Government of Pakistan, Islamabad, Pakistan.

[5] FAO. 2019. *Monitoring and surveillance of antimicrobial resistance in bacteria from healthy food animals intended for consumption.* Regional Antimicrobial Resistance Monitoring and Surveillance Guidelines – Volume 1. Bangkok.

4.4 Summary Results

4.4.1 Bacterial Isolation

The NVL analysed a total of 3,464 faecal samples and recovered *E. coli* (n = 1,237), *Enterococcus* spp. (n = 560) and *Salmonella* spp. (n = 44) isolates whereas NRLPD obtained *E. coli* (n=1456), *Salmonella* spp. (n = 417) *Salmonella* and *Enterococcus* (n = 300) isolates from poultry samples received from PSLs (Table 1).

National Reference lab receiving samples	Samples received (n)	Animal host specie	Isolates obtained		
			*E. coli, n (%) **	*Salmonella*	*Enterococcus*
National Veterinary Laboratory	3,474	Cattle and buffaloes	1,237 (35%)	44	560
National Reference Lab for Poultry Diseases	2,317	Poultry	1,456 (63%)	417	300

*Table 1: Details of the isolates recovered from faecal and caecal samples collected from ruminants (cattle and buffaloes) and poultry, respectively. *Percentage of bacteria recovered.*

4.4.2 AST Results

The surveillance pilot protocols recommended microbroth dilution methodology to obtain minimum inhibitory concentration (MIC) quantitative values and interpreted using the European Committee for Antimicrobial Susceptibility Testing (AST) was carried out on selected *E. coli*, *Salmonella*, and *Enterococcus* isolates through the disk diffusion method using the CLSI clinical breakpoints, where available (Annexure 1). The microbroth dilution could not be performed due to the limited technical capacity of NRLs. The panel comprised of antimicrobials deemed as "highest priority critically important antimicrobials (HP-CIA's), high priority critically important antimicrobials and highly important antimicrobials according to the World Health Organization's (WHO's) categorization system at the time of the study. Results were described following the European Union Summary Report[6] as: 'rare':<0.1%, 'very low':0.1%–1.0%, 'low':>1%–10.0%, 'moderate':>10.0%–20.0%, 'high':>20.0%–50.0%, 'very high':>50.0%–70.0%, 'extremely high':>70.0%.

4.4.2.1 Percentage of Resistance to Antimicrobials in the Panel Recommended for the Surveillance of *E. coli, Salmonella* and *Enterococcus* Isolates Recovered from Healthy FPAs

The susceptibility of *E. coli* isolates recovered from cattle, buffaloes and poultry to a panel comprised of 9 antimicrobials were determined.

E. coli.

Cattle and buffaloes: The percentage of resistance to 3rd generation cephalosporins occurred at high level (ceftazidime: 41.67) to extremely high level (cefotaxime: 74.51%). Resistance to quinolone occurred at moderate (nalidixic acid: 20.2%) and high level (ciprofloxacin: 49.51%). The highest percentage of resistance detected in *E. coli* isolates recovered from cattle and buffaloes was to ampicillin where extremely high-level resistance was detected (92.65%). In the remaining

[6] EFSA and ECDC. The European Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria for human, animals and food in 2020/2021. https://www.efsa.europa.eu/en/efsajournal/pub/7867

antimicrobials, resistance occurred at low-level (chloramphenicol: 10.92%) to very high level (tetracycline: 56.38%).

Poultry: The percentage of resistance to 3rd generation cephalosporins occurred at moderate (ceftazidime: 19.27%) to high level (cefotaxime: 26.47%). Resistance to quinolones occurred at extremely high-level in both antimicrobials tested (ciprofloxacin: 84.01%; nalidixic acid: 88.34%). The highest resistance observed was to tetracycline (91.19%) (Table 2) and the remaining antimicrobials occurred at high (gentamicin: 39.91%) to extremely high level (ampicillin: 89.37%).

***Salmonella* spp.**

Cattle and buffaloes: Resistance to WHO's HPCIAs also occurred in nontyphoidal *Salmonella* enterica isolated from cattle and buffaloes that ranged from low (<10%: ceftazidime, ciprofloxacin and nalidixic acid) to high (cefotaxime: >40%). In the remaining antimicrobials, resistance was observed from low (<5%: chloramphenicol and trimethoprim) to very high level (>50%: ampicillin). However, a limited number of isolates were recovered from cattle and buffaloes (n = 44, Table 3) for the purposes of estimating AMR levels. More robust data is needed to better estimate the levels of resistance in these animals' host species.

Poultry: Resistance to WHO's HPCIA's were detected at higher level than the ruminants that ranged from moderate (<20%: cefotaxime and ceftazidime) to extremely high levels (>70%: ciprofloxacin, azithromycin and nalidixic acid). In the remaining antimicrobials, resistance occurred from high (gentamicin: 23.11%) to extremely high (>90% tetracycline). As with cattle and buffaloes, there was a limited number of *Salmonella* isolates and was obtained for the purposes of estimating AMR prevalence in *Salmonella.* More robust *Salmonella* spp. data as described elsewhere[7], should be tested as part of the national sampling strategy to get better estimate AMR trends in *Salmonella* isolates.

Enterococcus

Isolates recovered from cattle and buffaloes and poultry were analyzed for 7 and 8 antibiotics, respectively. Of important public health concern, Vancomycin Resistant enterococci (VRE) was detected at high-level (>20%) and teicoplanin detected at moderate level (>10%) in the animal host species under surveillance. Vancomycin (a glycopeptide) and teicoplanin (a lipopolypeptice) are WHO's HP-CIA antimicrobials. Another notable finding was the extremely high erythromycin resistance detected from poultry isolates (92.73%). (Table 4).

[7] AHC 2021. National Surveillance Strategy for Antimicrobial Resistance in healthy Food Animals. Animal Husbandry Commissioner, Livestock Wing, Ministry of National Food Security and Research, Government of Pakistan, Islamabad, Pakistan.

Table 2: Percentage of resistance in *Escherichia coli* isolates recovered from slaughtered cattle, buffaloes and poultry in Pakistan.

Animal host specie	Antimicrobial	Resistant, n	Resistant, %	Intermediate, n	Intermediate, %	Susceptible, n	Susceptible, %	Total Isolates
Cattle and Buffaloes	Ampicillin	189	92.65	4	1.96	11	5.39	204
	Cefotaxime	**152**	**74.51**	**32**	**15.69**	**20**	**9.80**	**204**
	Ceftazidime	**85**	**41.67**	**56**	**27.45**	**63**	**30.88**	**204**
	Chloramphenicol	19	10.92	40	22.99	115	66.09	174
	Ciprofloxacin	**102**	**49.51**	**78**	**37.86**	**26**	**12.62**	**206**
	Nalidixic Acid	**41**	**20.20**	**79**	**38.92**	**83**	**40.89**	**203**
	Tetracycline	84	56.38	16	10.74	49	32.89	149
	Trimethoprim	83	48.54	12	7.02	76	44.44	171
Poultry	Ampicillin	614	89.37	27	3.93	46	6.70	687
	Azithromycin	**414**	**60.53**	**107**	**15.64**	**63**	**9.21**	**684**
	Cefotaxime	**180**	**26.47**	**80**	**11.76**	**420**	**61.76**	**680**
	Ceftazidime	**132**	**19.27**	**105**	**15.33**	**448**	**65.40**	**685**
	Chloramphenicol	543	79.39	14	2.05	127	18.57	684
	Ciprofloxacin	**562**	**84.01**	**41**	**6.13**	**66**	**9.87**	**669**
	Gentamicin	275	39.91	23	3.34	391	56.75	689
	Nalidixic Acid	**606**	**88.34**	**43**	**6.27**	**37**	**5.39**	**686**
	Tetracycline	590	91.19	16	2.47	41	6.34	647
	Trimethoprim	344	84.31	9	2.21	55	13.48	408

Antimicrobials in bold fonts are the World Health Organization's Highest Priority Critically Important Antimicrobials.

Table 3: Percentage of resistance in Salmonella spp. recovered from slaughtered cattle, buffaloes and in Pakistan.

Animal host specie	Antimicrobial	Resistant, n	Resistant, %	Intermediate, n	Intermediate, %	Susceptible, n	Susceptible %	Total Isolates
Cattle and buffaloes	Ampicillin	23	52.27	8	18.18	13	29.55	44
	Cefotaxime	**21**	**48.84**	**11**	**25.58**	**11**	**25.58**	**43**
	Ceftazidime	**1**	**2.27**	**21**	**47.73**	**22**	**50.00**	**44**
	Chloramphenicol	2	4.76	6	14.29	34	80.95	42
	Ciprofloxacin	**3**	**6.82**	**23**	**52.27**	**18**	**40.91**	**44**
	Nalidixic Acid	**3**	**6.82**	**16**	**36.36**	**25**	**56.82**	**44**
	Tetracycline	11	25.00	1	2.27	32	72.73	44
	Trimethoprim	2	4.55	0	0.00	42	95.45	44
Poultry	Ampicillin	124	58.77	13	6.16	74	35.07	211
	Azithromycin	**163**	**76.89**	**19**	**8.96**	**30**	**14.15**	**212**
	Cefotaxime	**30**	**14.15**	**24**	**11.32**	**158**	**74.53**	**212**
	Ceftazidime	**40**	**19.32**	**30**	**14.49**	**137**	**66.18**	**207**
	Chloramphenicol	139	66.51	14	6.70	56	26.79	209
	Ciprofloxacin	**149**	**71.63**	**39**	**18.75**	**20**	**9.62**	**208**
	Gentamicin	49	23.11	27	12.74	136	64.15	212
	Nalidixic Acid	**203**	**96.21**	**5**	**2.37**	**3**	**1.42**	**211**
	Tetracycline	191	91.83	8	3.85	9	4.33	208
	Trimethoprim	147	76.96	4	2.09	40	20.94	191

Antimicrobials in bold fonts are the World Health Organization's Highest Priority Critically Important Antimicrobials.

Table 4: Percentage of resistance in Enterococcus spp. recovered from slaughtered cattle, buffaloes and poultry in Pakistan

Animal host specie	Antimicrobial	Resistant, n	Resistant, %	Intermediate, n	Intermediate, %	Susceptible, n	Susceptible, %	Total Isolates
Cattle and Buffaloes	Ampicillin	194	48.26	0	0.00	208	51.74	402
	Chloramphenicol	59	14.71	86	21.45	256	63.84	401
	Erythromycin	**56**	**14.00**	**265**	**66.25**	**79**	**19.75**	**400**
	Linezolid	178	44.28	47	11.69	177	44.03	402
	Teicoplanin	**51**	**12.75**	**27**	**6.75**	**322**	**80.50**	**400**
	Tetracycline	116	29.07	72	18.05	211	52.88	399
	Vancomycin	**110**	**27.50**	**62**	**15.50**	**228**	**57.00**	**400**
Poultry	Ampicillin	30	51.72	0	0.00	28	48.28	58
	Chloramphenicol	42	71.19	8	13.56	9	15.25	59
	Erythromycin	**51**	**92.73**	**2**	**3.64**	**2**	**3.64**	**55**
	Linezolid	31	53.45	4	6.90	23	39.66	58
	Quinupristin	46	79.31	2	3.45	10	17.24	58
	Teicoplanin	**9**	**18.37**	**4**	**8.16**	**36**	**73.47**	**49**
	Tetracycline	55	96.49	1	1.75	1	1.75	57
	Vancomycin	**15**	**26.32**	**10**	**17.54**	**32**	**56.14**	**57**

4.4.2.2 Percentage of resistance to 10 antimicrobials in *E. coli* from poultry, by province

The series of figures in this section summarizes and compares percentages of resistant, intermediate, and susceptible (RIS) outcomes in *E. coli* from poultry between provinces.

E. coli from caecal samples collected from broiler poultry birds slaughtered in wet bird markets in 7 provinces/regions of Pakistan were tested for susceptibility using disk diffusion method using a panel of 10 antimicrobials as previously described in Tables 2 and 3. The proportion of resistant, intermediate, and susceptible isolates are shown in figures 2-9 by province. Between province variations in the proportion of resistant, intermediate, and susceptible isolates were detected depending on the isolate, however, the *E. coli* isolates recovered from poultry of seven provinces/regions of Pakistan were extremely resistant to ampicillin, ciprofloxacin, nalidixic acid and tetracycline (> 70%). The resistance to trimethoprim in *E. coli* was detected at high-level in all provinces of Pakistan except Punjab (37%). Extremely high percentage of resistance to chloramphenicol (>70%) was observed in Punjab, GB, and AJK.

Of important note, highest percentages of resistance to cefotaxime and ceftazidime were observed in the first 2 provinces (AJK and Balochistan) (Figures 3 and 4) and highest percentage of resistance to ciprofloxacin and nalidixic acid were observed also in Balochistan (Figure 6). The percentage of *E. coli* that exhibited intermediate susceptibility to these WHO's HPCIA antimicrobials ranged from low to moderate and may indicate an emerging trend to these antimicrobials that warrants ongoing surveillance in poultry.

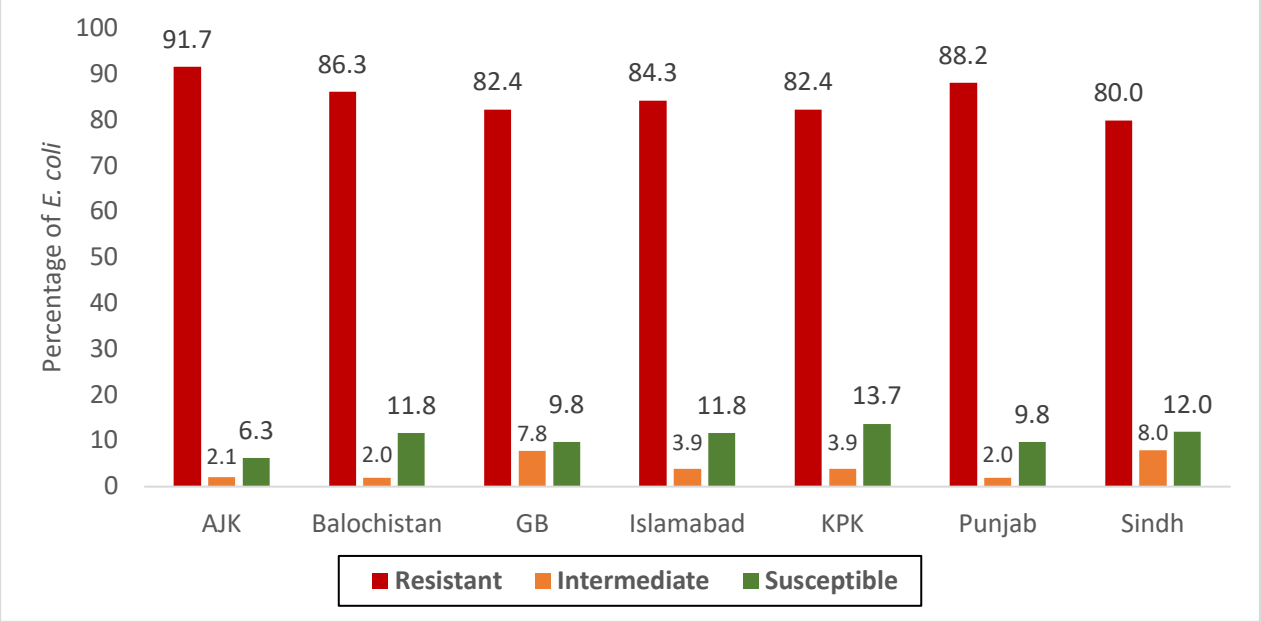

*Figure 2: Percentage of resistant, intermediate, and susceptible E. coli isolates from poultry to **ampicillin** in 7 Provinces of Pakistan.*

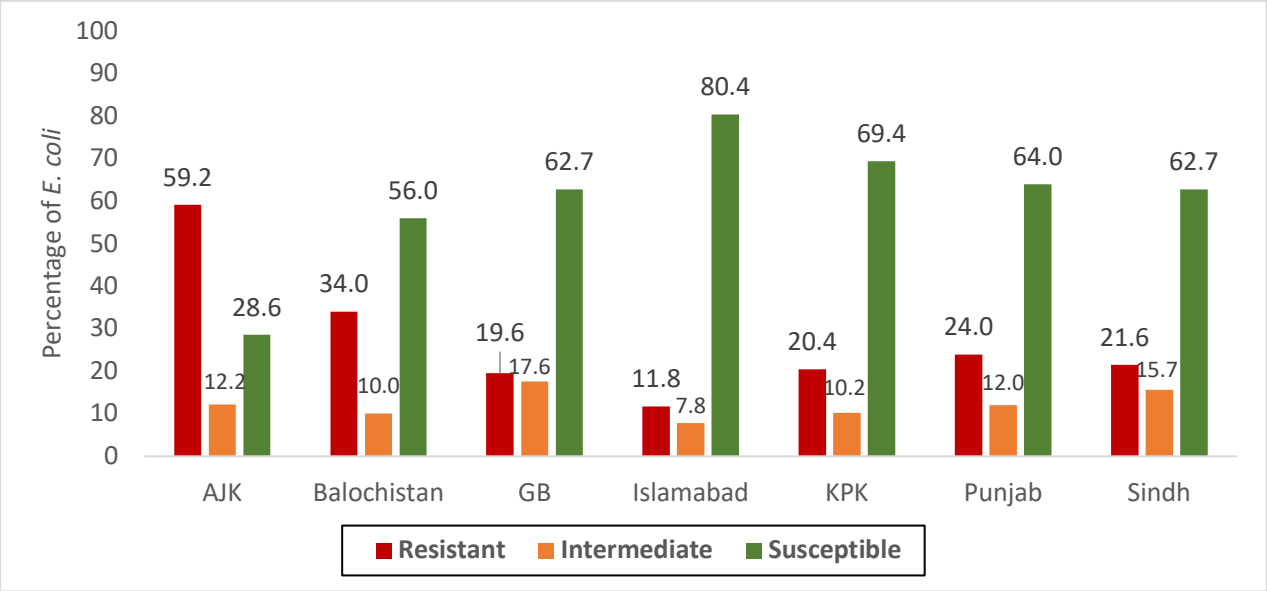

*Figure 3: Percentage of resistant, intermediate, and susceptible E. coli from poultry to **cefotaxime** in 7 provinces of Pakistan.*

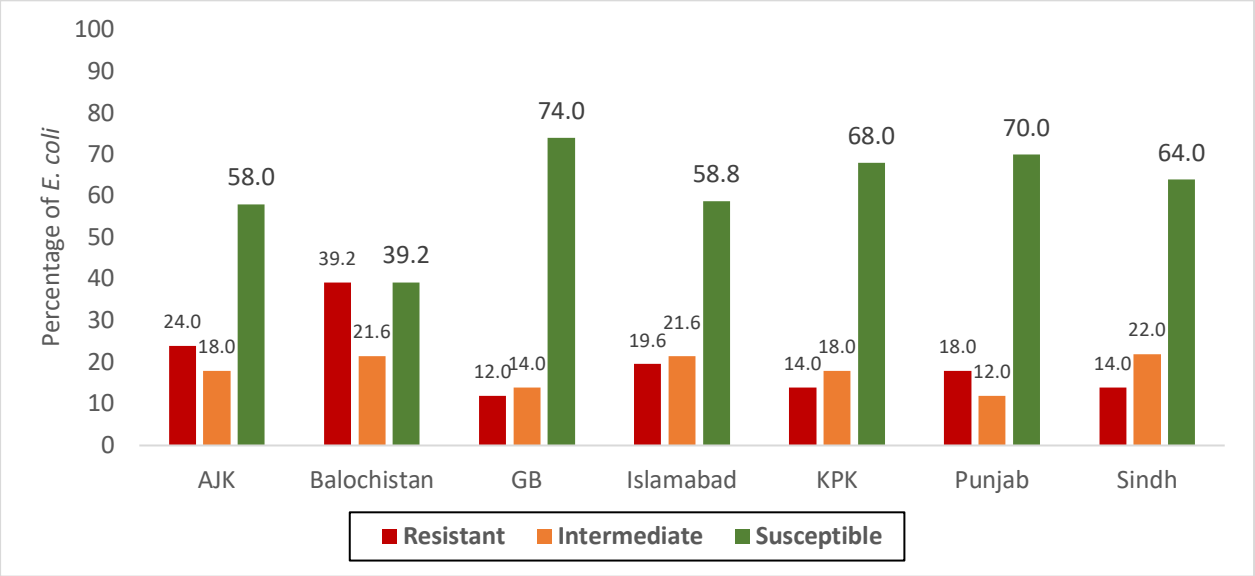

*Figure 4: Percentage of resistant, intermediate, and susceptible E. coli isolates from Poultry to **ceftazidime** in 7 provinces of Pakistan.*

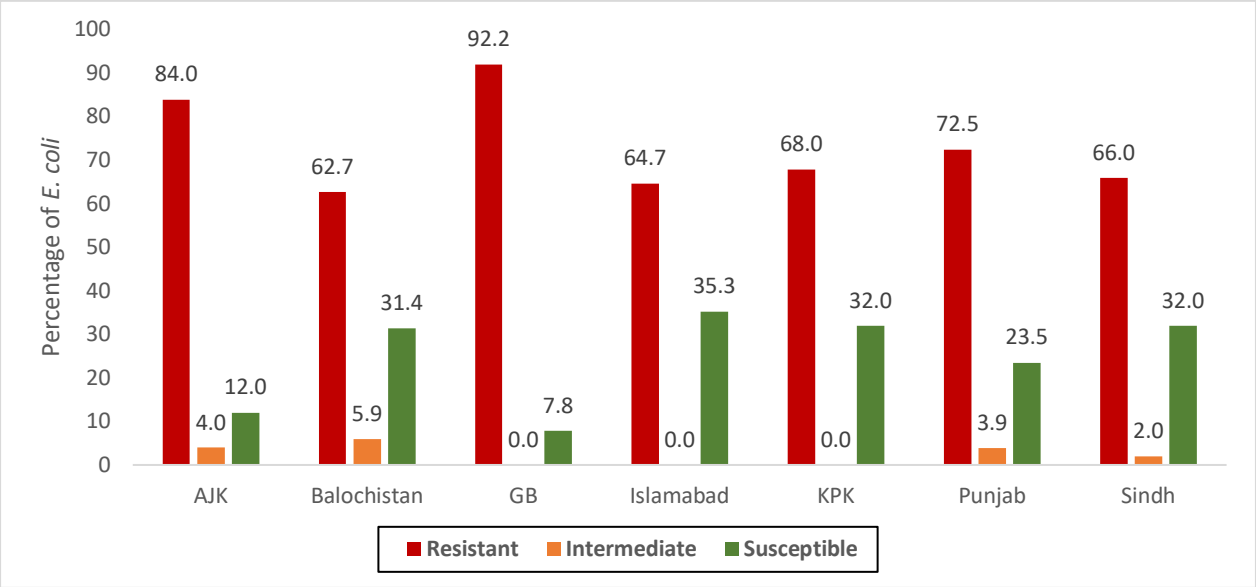

*Figure 5: Percentage of resistant, intermediate, and susceptible E. coli isolates from poultry to **chloramphenicol** in 7 provinces of Pakistan.*

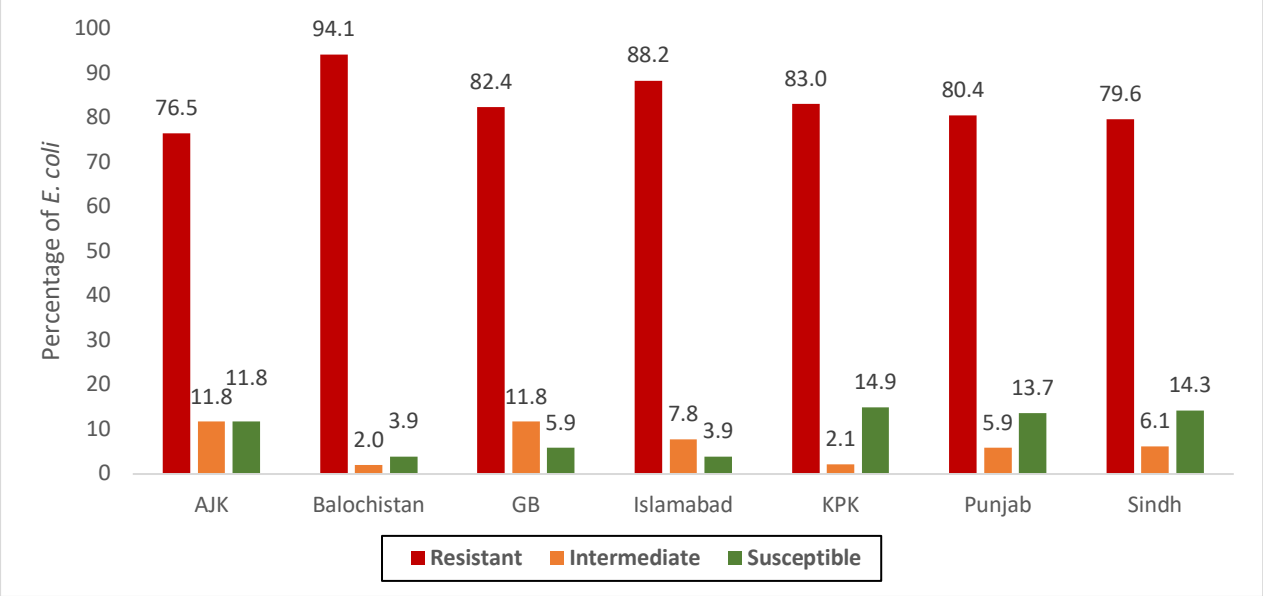

*Figure 6: Percentage of resistant, intermediate, and susceptible E. coli isolates from poultry to **ciprofloxacin** in 7 provinces of Pakistan.*

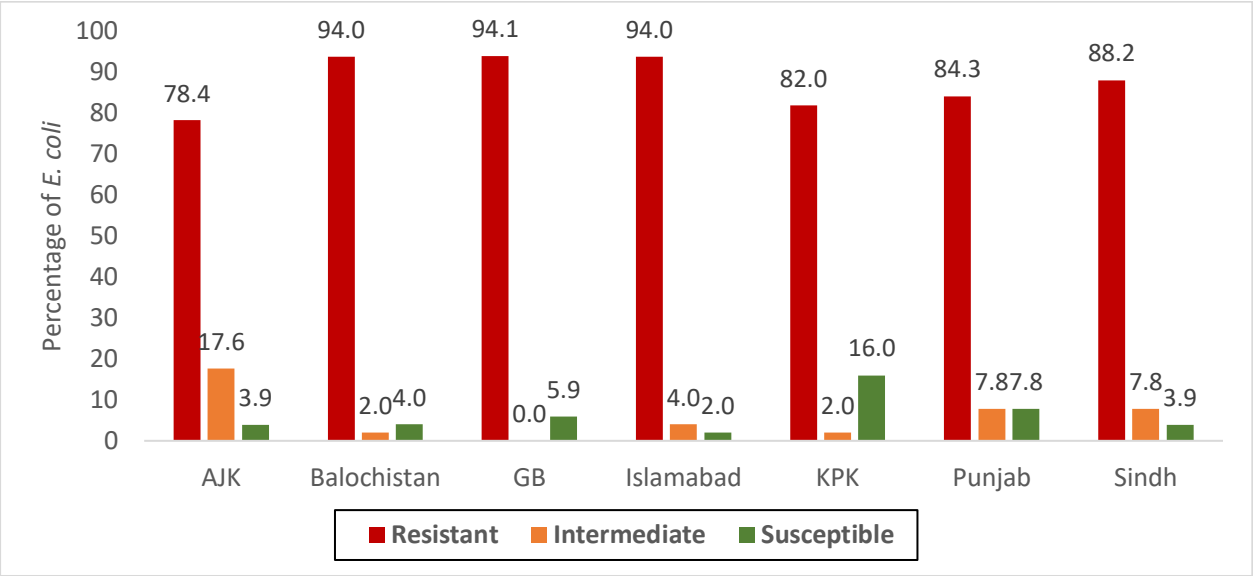

*Figure 7: Percentage of resistant, intermediate, and susceptible E. coli isolates from poultry to **nalidixic acid** in 7 Provinces of Pakistan.*

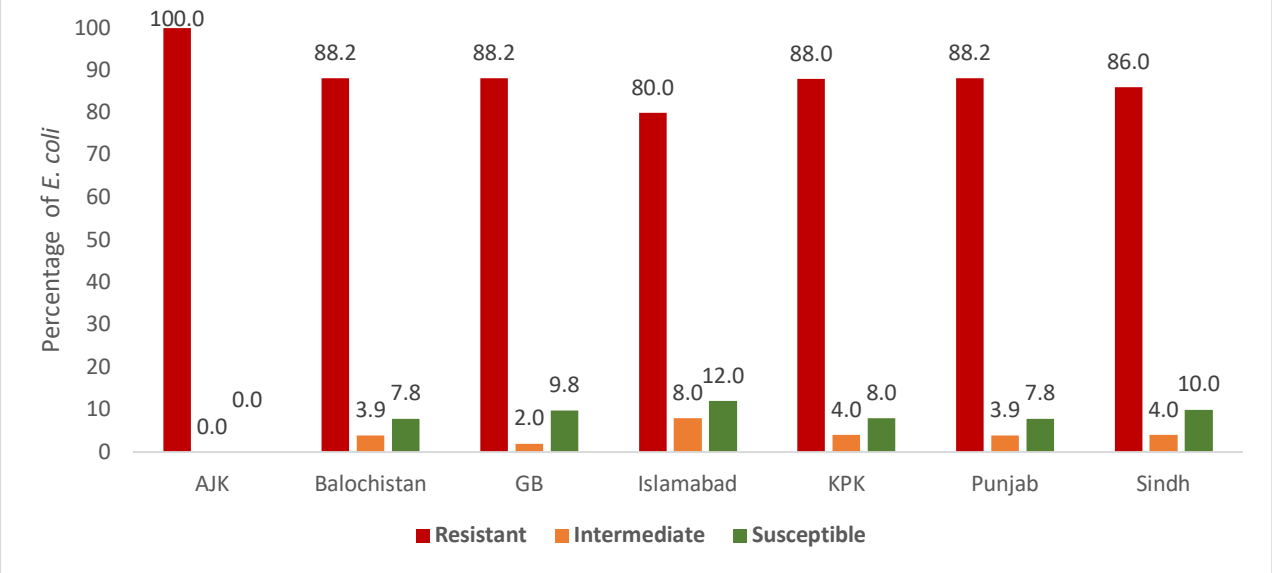

*Figure 8: Percentage of resistant, intermediate, and susceptible E. coli isolates from poultry to **tetracycline** in 7 provinces of Pakistan.*

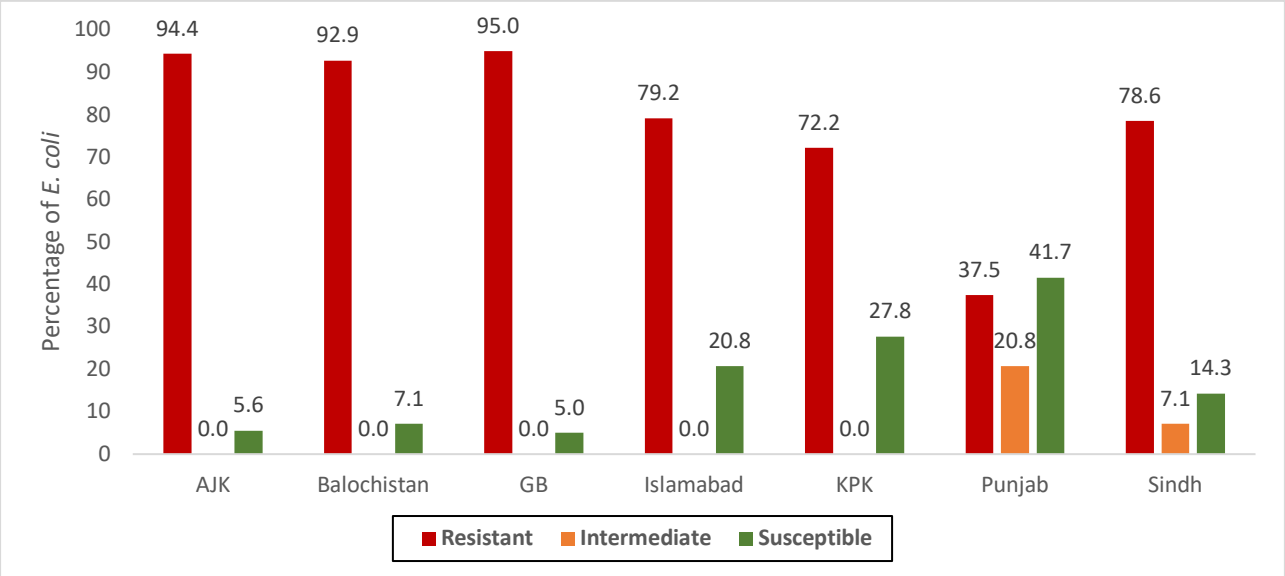

Figure 9: Percentage of resistant, intermediate, and susceptible E. coli isolates of from poultry to ***trimethoprim*** *in 7 provinces of Pakistan.*

4.4.2.3 Percentage of resistance to antimicrobials in *E. coli* isolates from cattle and buffaloes, by province

The series of figures in this section summarizes and compares percentages of RIS outcomes in *E. coli* from cattle and buffaloes between provinces.

E. coli from faecal samples collected from cattle and buffaloes from slaughterhouses located in three provinces of Pakistan were tested for susceptibility to 8 antibiotics and shown in Figures 10-17 by province. There were provincial variations in resistance depending on the antimicrobial. Resistance to ampicillin was detected at extremely high level (>80%) in the three provinces of Pakistan. Very high resistance to cefotaxime was noted in KPK but observed at extremely high-level in Punjab and Sindh provinces (>70). Extremely high-level ciprofloxacin resistance was detected (>60%) concurrent with high nalidixic acid (>20%) resistances also in isolates from Punjab and Sindh provinces. Extremely high-level resistance to tetracycline was detected (>70%) in Sindh province. In all antimicrobials, isolates from KPK exhibited the lowest levels of resistances to the antimicrobials examined.

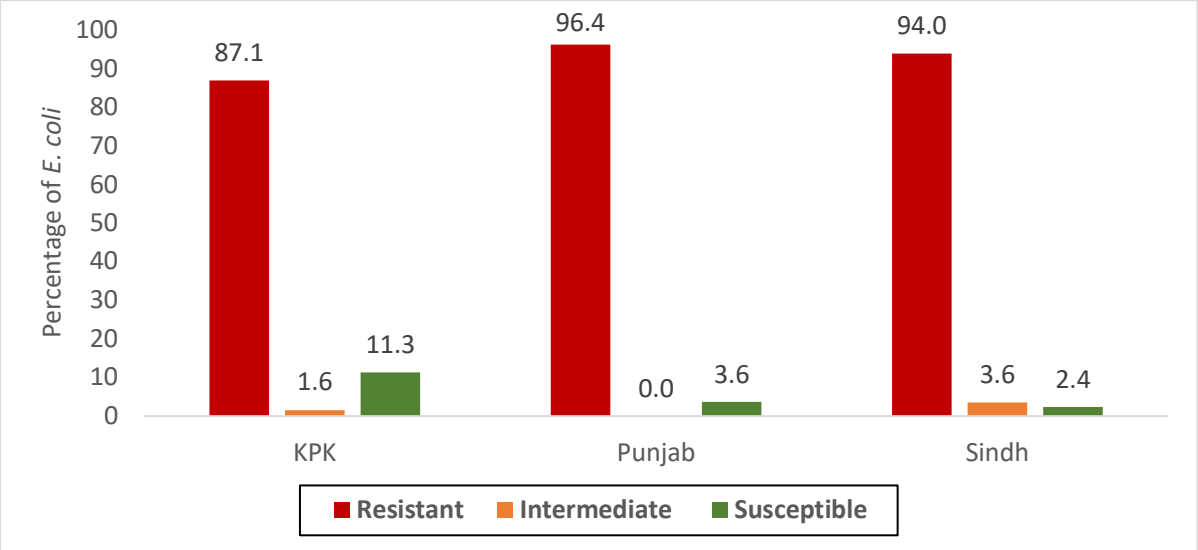

Figure 10: Percentage of resistant, intermediate, and susceptible E. coli isolates from livestock to ampicillin from 3 Provinces of Pakistan

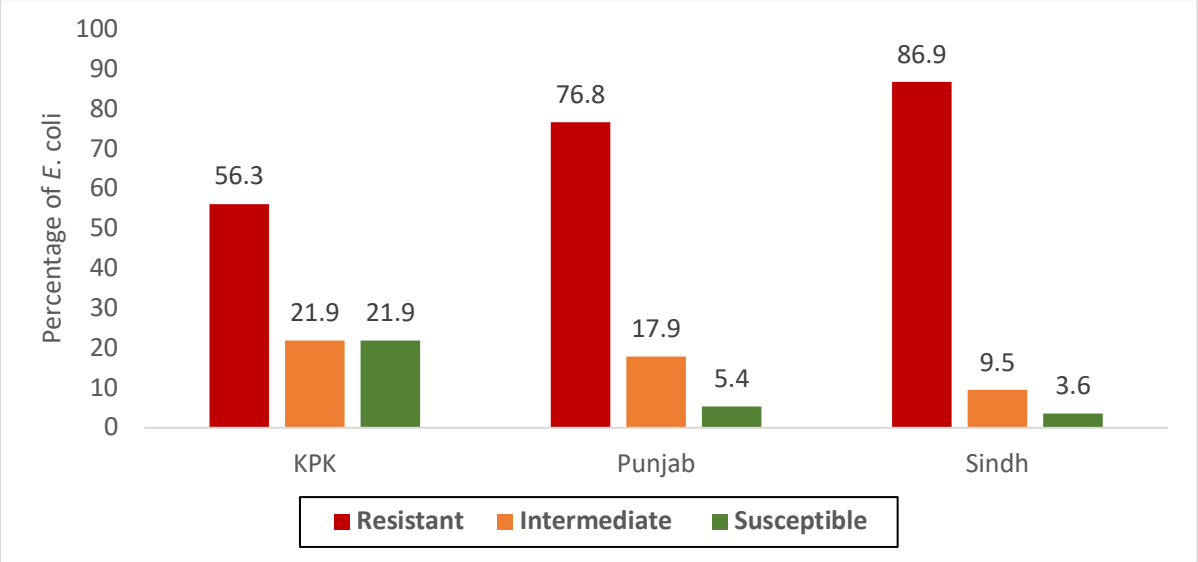

*Figure 11: Percentage of resistant, intermediate, and susceptible E. coli isolates from livestock to **cefotaxime** from 3 provinces of Pakistan*

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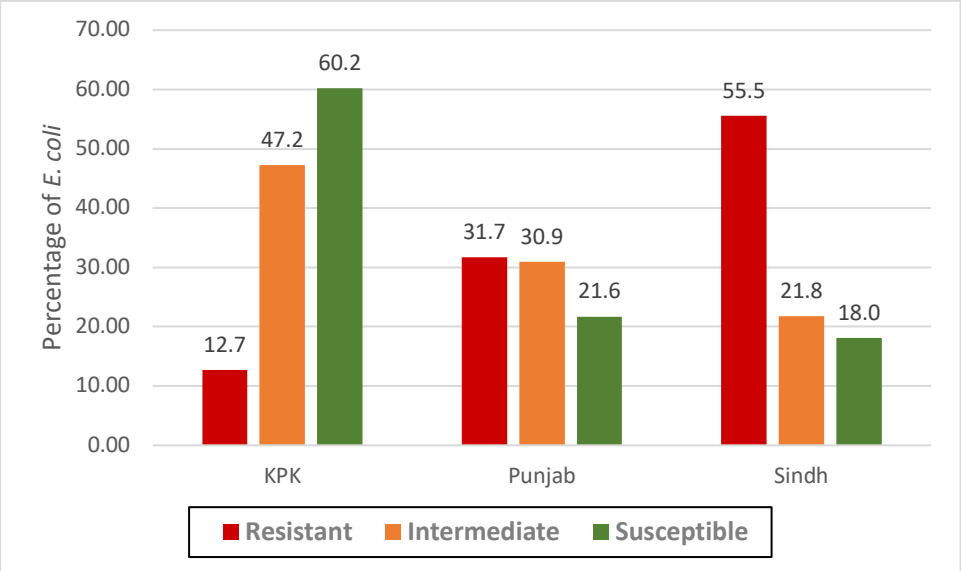

Figure 12: Percentage of resistant, intermediate, and susceptible E. coli isolates from livestock to ceftazidime from 3 Provinces of Pakistan

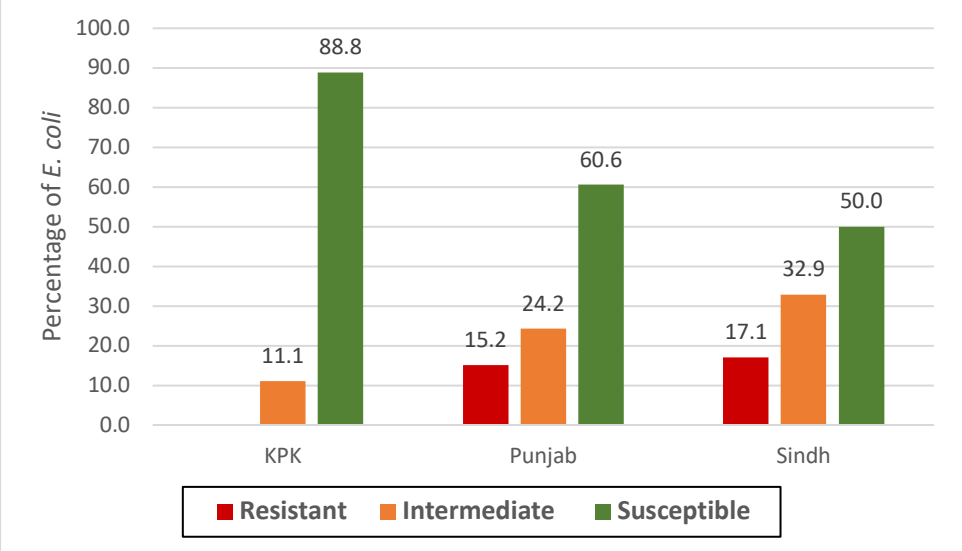

Figure 13: Percentage of resistant, intermediate, and susceptible E. coli isolates from livestock to chloramphenicol from 3 provinces of Pakistan

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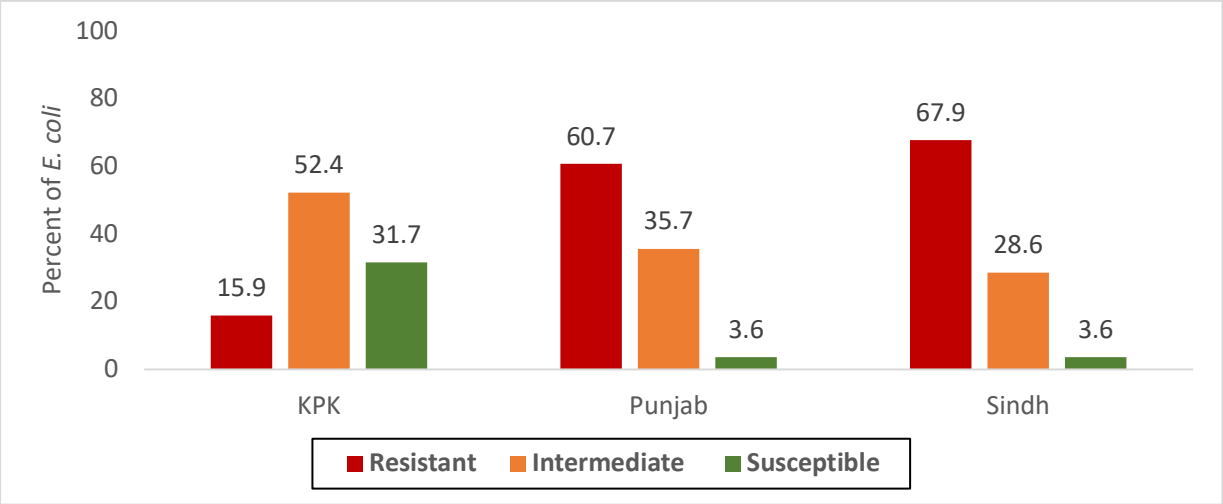

Figure 14: Percentage of resistant, intermediate, and susceptible E. coli isolates from livestock to ciprofloxacin from 3 provinces of Pakistan

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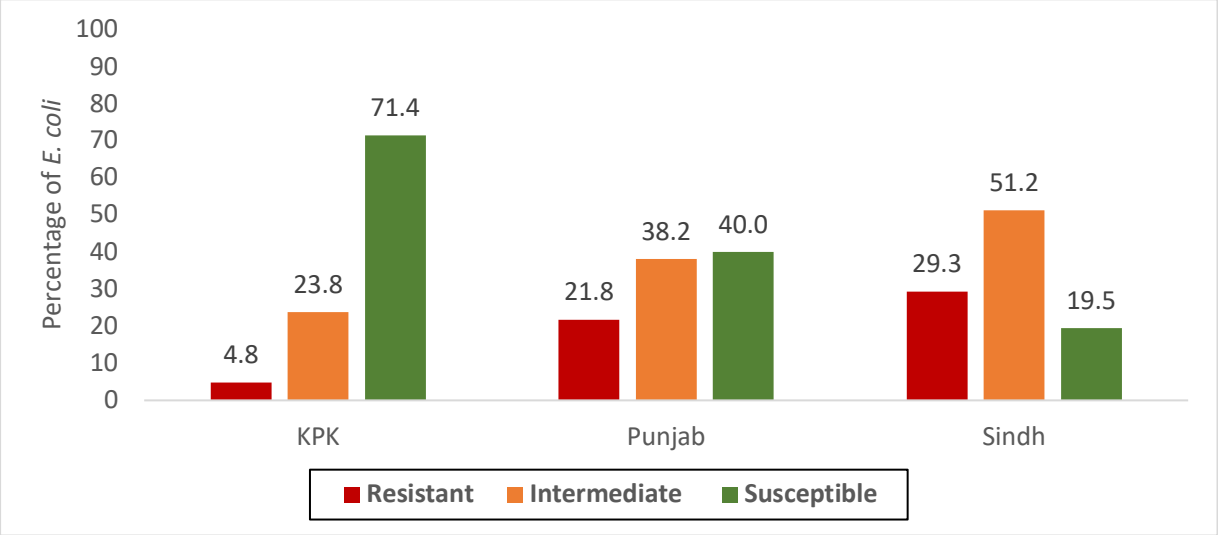

Figure 15: Percentage of resistant, intermediate, and susceptible E. coli isolates from livestock to nalidixic acid from 3 provinces of Pakistan

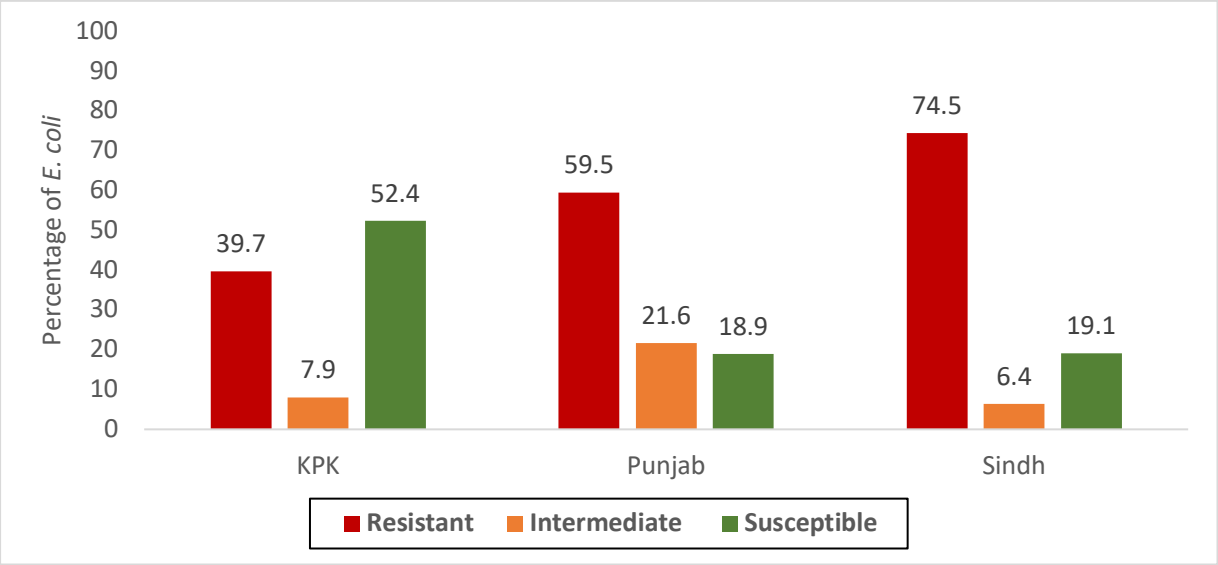

Figure 16: Percentage of resistant, intermediate, and susceptible E. coli isolates from livestock to ***tetracycline*** *from 3 Provinces of Pakistan.*

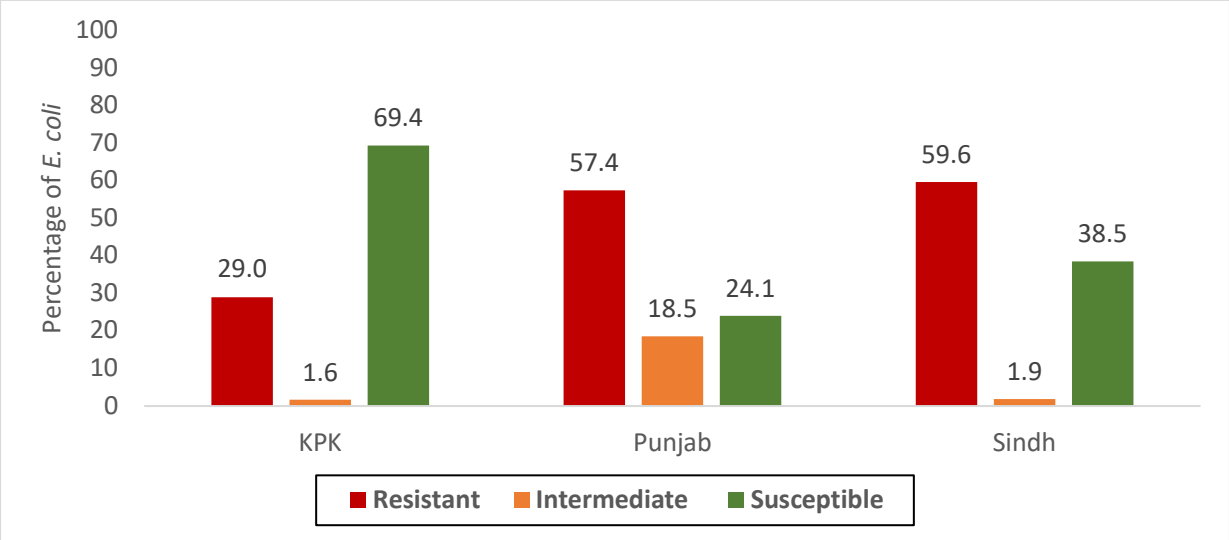

Figure 17: Percentage of resistant, intermediate, and susceptible E. coli isolates from livestock to ***trimethoprim*** *from 3 provinces of Pakistan.*

4.4.2.4 Percentage of resistance to 8 antimicrobials in *Salmonella* from poultry, by province

The series of figures in this section summarizes and compares percentages of RIS outcomes in *Salmonella* from poultry between provinces. Please note that no serotyping/genotyping was done to the isolates, thus the data pertains to nontyphoidal *Salmonella* (NTS) isolated from poultry.

Salmonella from caecal samples collected from broiler poultry birds slaughtered in wet bird markets in 7 provinces/regions of Pakistan were tested for susceptibility using disk diffusion method using a panel of 8 antimicrobials. The proportion of resistant, intermediate, and susceptible *Salmonella* isolates are shown in Figures 18-25 by province. There were variations in the proportion of RIS outcomes depending on the antimicrobial. Of important note, resistance to cefotaxime and ceftazidime were detected across all provinces from low to moderate levels (<20%). Ciprofloxacin resistance was detected from very high to extremely high levels (>70%) and highest observed in Balochistan province. In parallel, extremely high-level nalidixic acid resistance was detected across all provinces which is concerning.

Extremely high-level tetracycline (>80%) across all seven provinces/regions of Pakistan were detected. Resistance to chloramphenicol was detected at extremely high level (>70%) in AJK and Sindh provinces. Trimethoprim resistance occurred at very high (>60) to extremely high levels (>90%) across all seven provinces/regions of Pakistan.

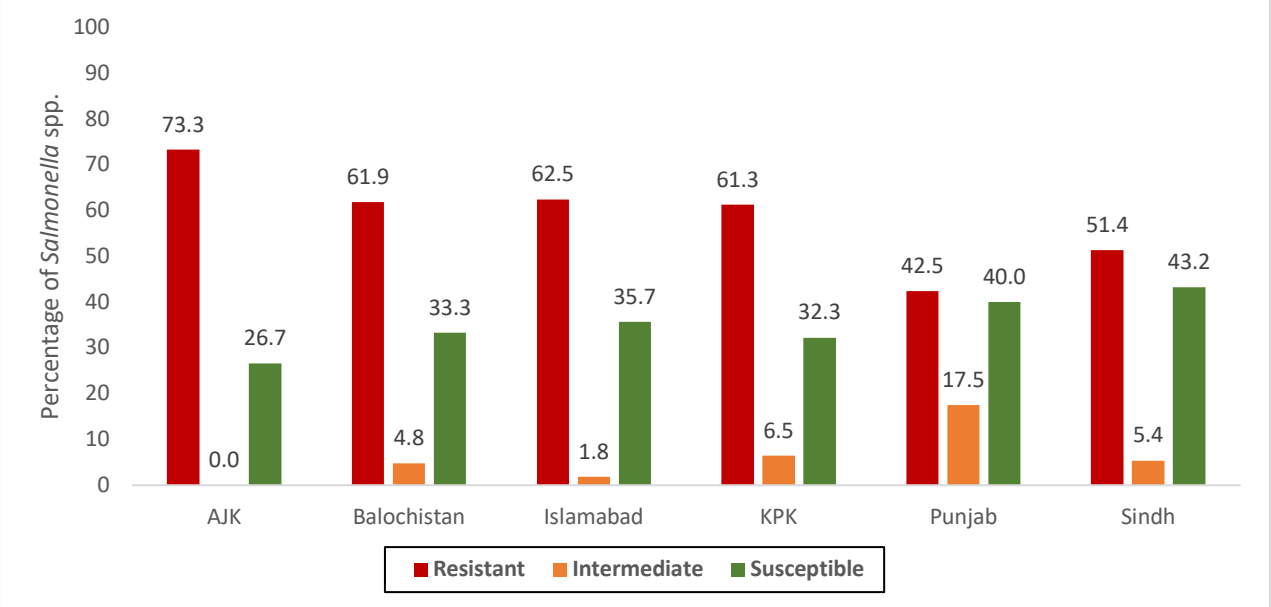

Figure 18: Percentage of resistant, intermediate, and susceptible Salmonella spp. from poultry to ampicillin from all provinces of Pakistan.

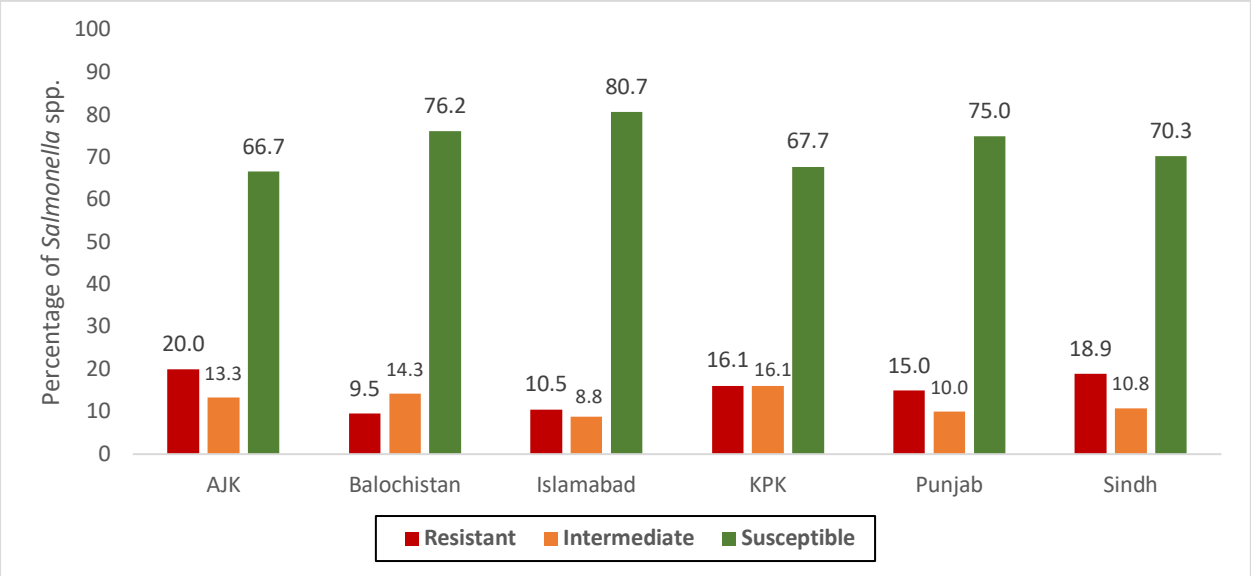

*Figure 19: Percentage of resistant, intermediate, and susceptible Salmonella spp. from poultry to **cefotaxime** from all provinces of Pakistan.*

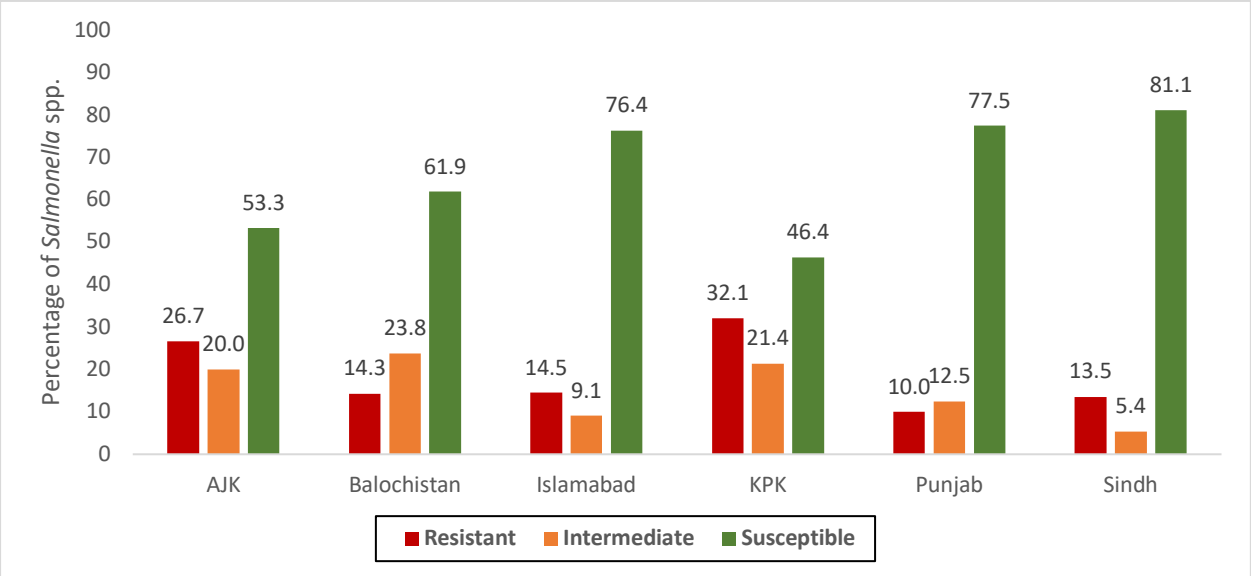

*Figure 20: Percentage of resistant, intermediate, and susceptible Salmonella spp. from poultry to **ceftazidime** from all provinces of Pakistan.*

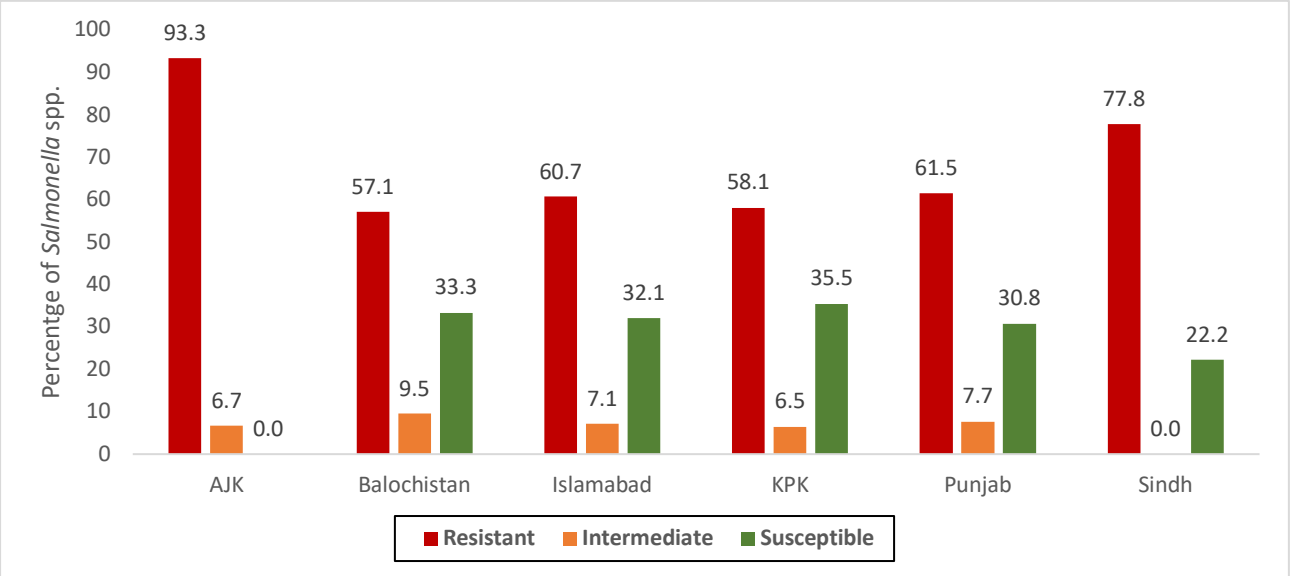

*Figure 21: Percentage of resistant, intermediate, and susceptible Salmonella spp. from poultry to **chloramphenicol** from all provinces of Pakistan.*

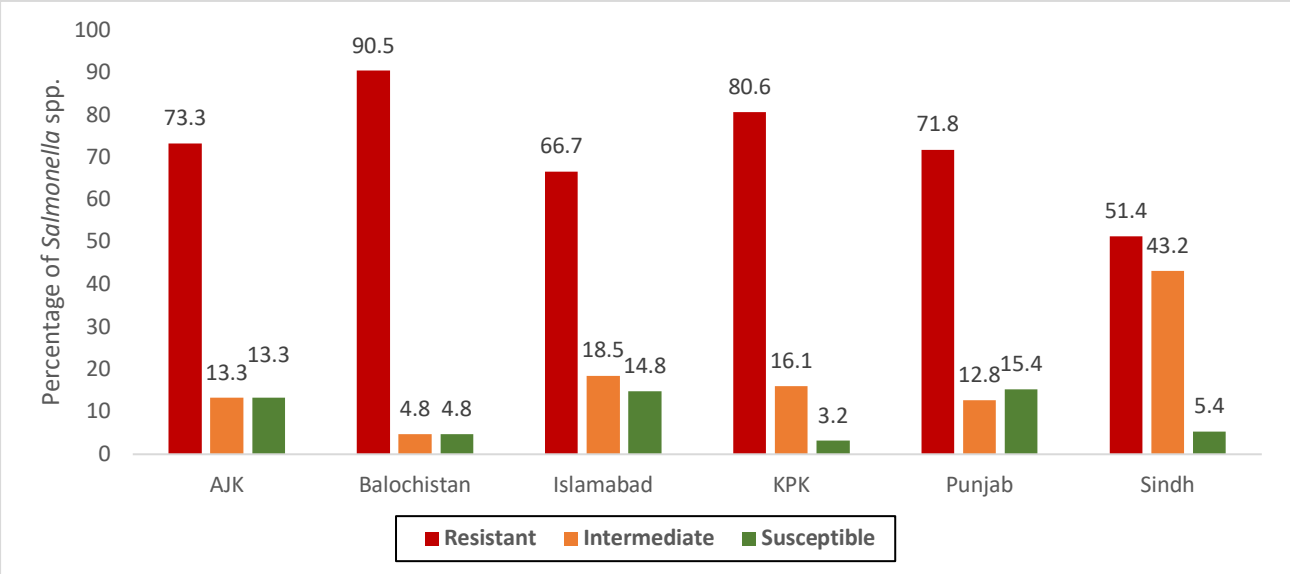

*Figure 22: Percentage of resistant, intermediate, and susceptible Salmonella spp. from poultry to **ciprofloxacin** from all provinces of Pakistan.*

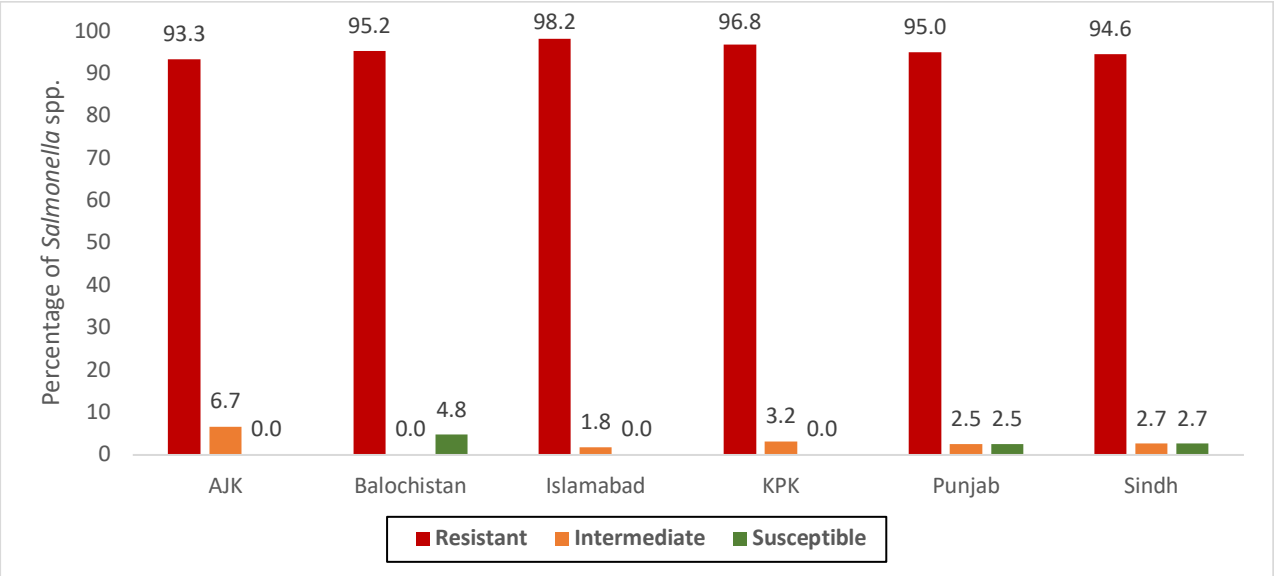

Figure 23: Percentage of resistant, intermediate, and susceptible Salmonella spp. from poultry to ***nalidixic acid*** *from all provinces of Pakistan.*

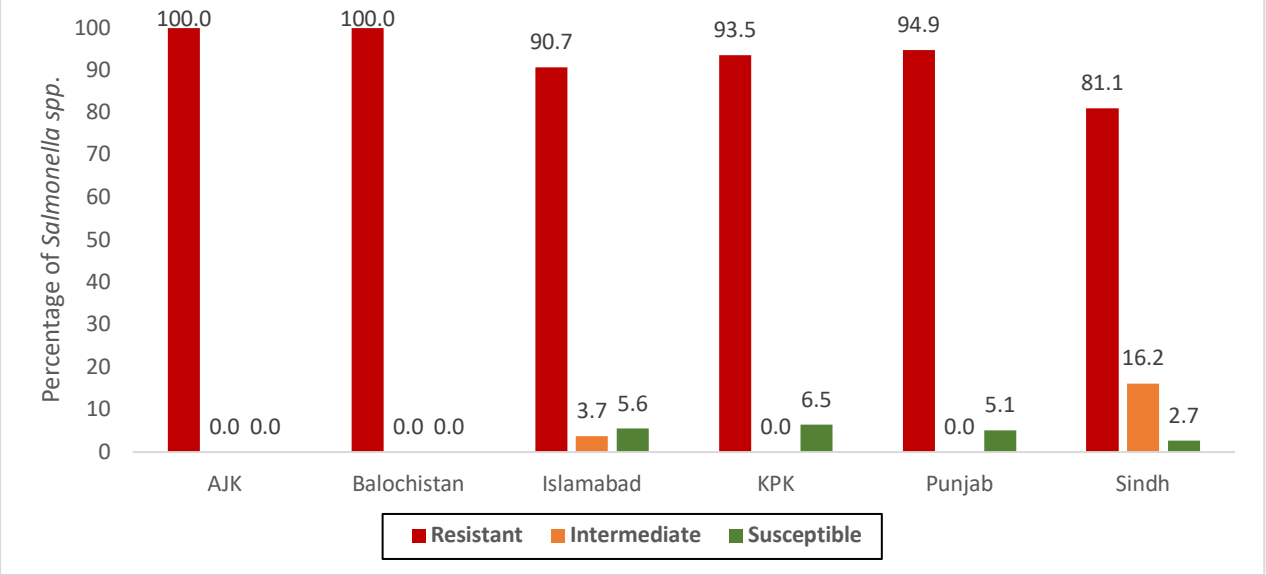

Figure 24: Percentage of resistant, intermediate, and susceptible Salmonella spp. from poultry to ***tetracycline*** *from all provinces of Pakistan.*

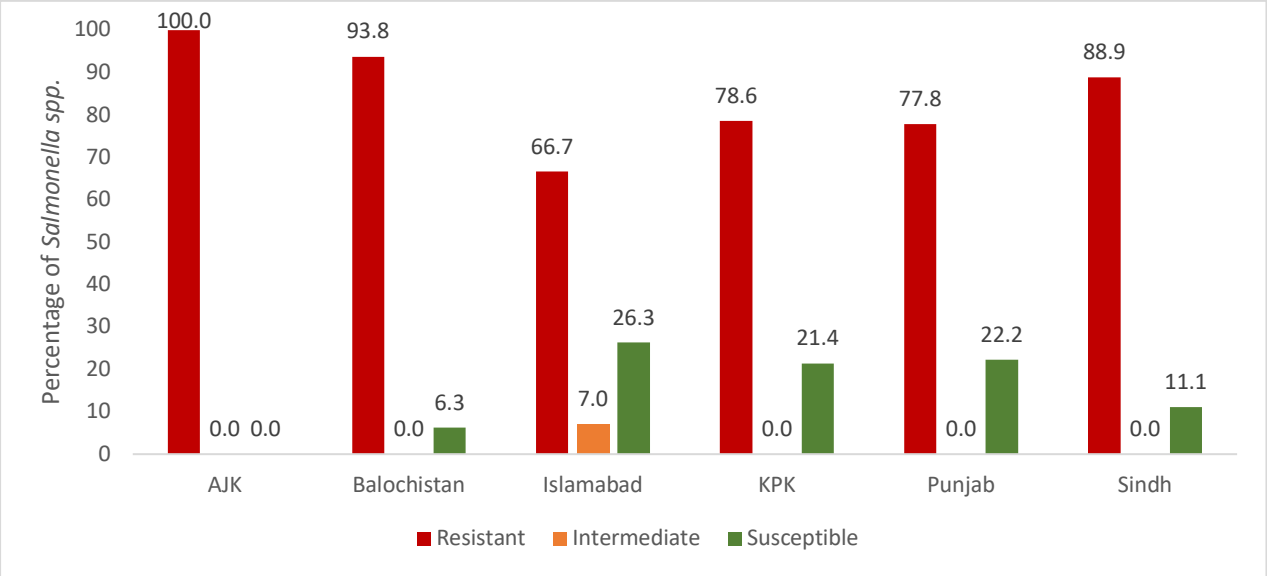

Figure 25: Percentage of resistant, intermediate, and susceptible Salmonella spp. from poultry to ***trimethoprim*** *from all provinces of Pakistan.*

4.4.2.4 Percentage of resistance to 7 antimicrobials in *Enterococcus* spp. from cattle and buffaloes

Enterococcus spp. recovered from fecal samples collected from cattle and buffaloes slaughtered in slaughterhouses of 7 provinces/regions of Pakistan were tested using disk diffusion method using a panel of 7 antimicrobials. The proportion of resistant, intermediate, and susceptible *Enterococcus* spp. to 7 antibiotics is shown in Figures 26-32, by province. Notable observations were the predominance of *Enterococcus* that exhibited intermediate susceptibility to erythromycin, the high to very high occurrence of linezolid across all provinces and the widespread occurrence of VRE and teicoplanin resistant *Enterococcus* spp. across all provinces. Of important concern, > 40% VRE was detected in one province.

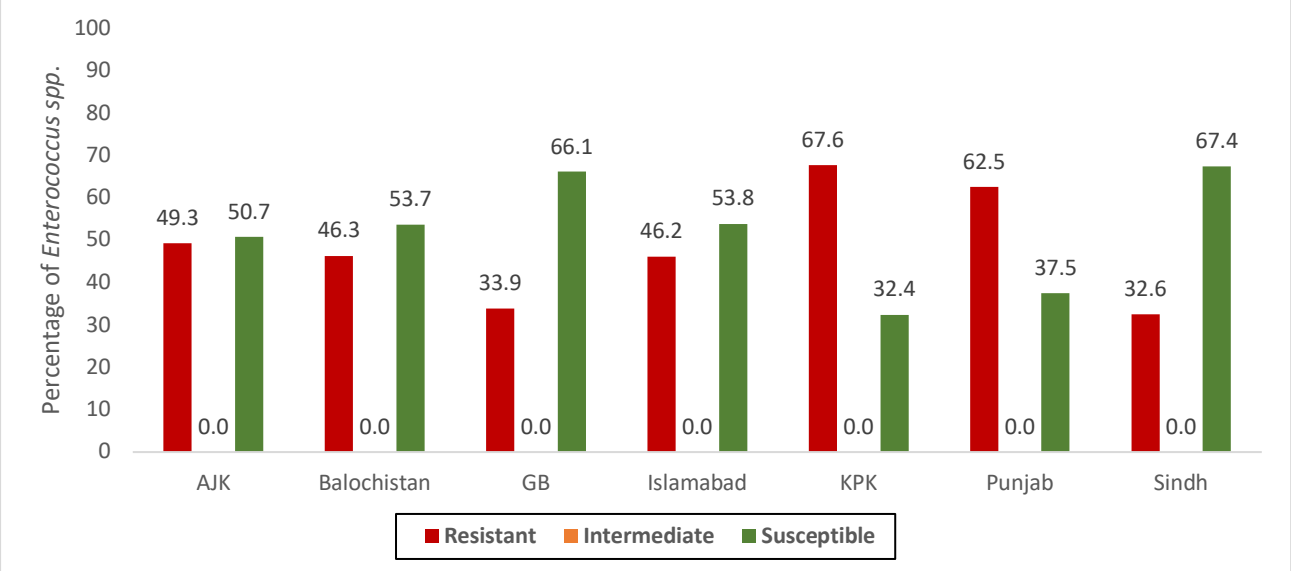

Figure 26: Percentage of resistant, intermediate, and susceptible Enterococcus spp. from livestock to ***ampicillin*** *from all provinces of Pakistan*

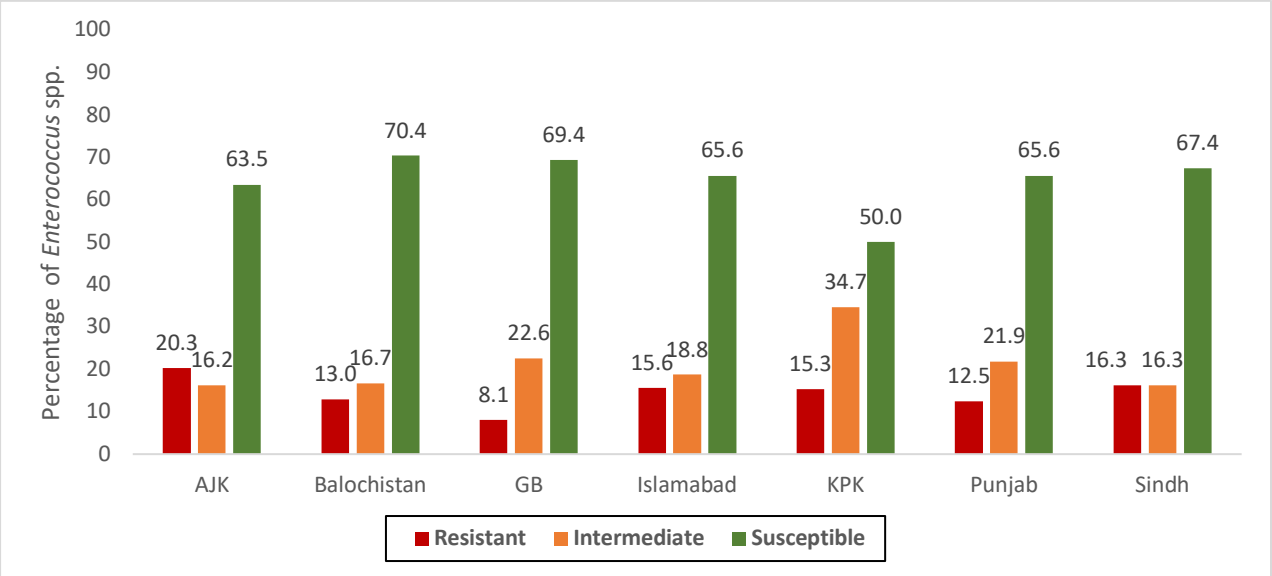

*Figure 27: Percentage of resistant, intermediate, and susceptible Enterococcus spp. from livestock to **chloramphenicol** from all provinces of Pakistan*

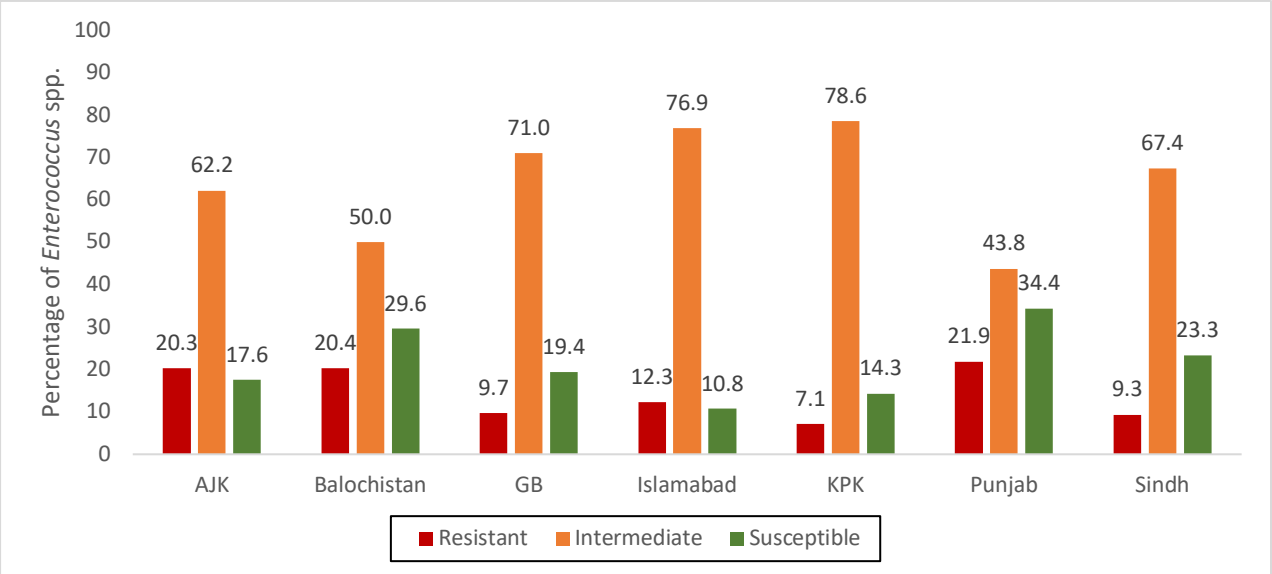

*Figure 28: Percentage of resistant, intermediate, and susceptible Enterococcus spp. from livestock to **erythromycin** from all provinces of Pakistan*

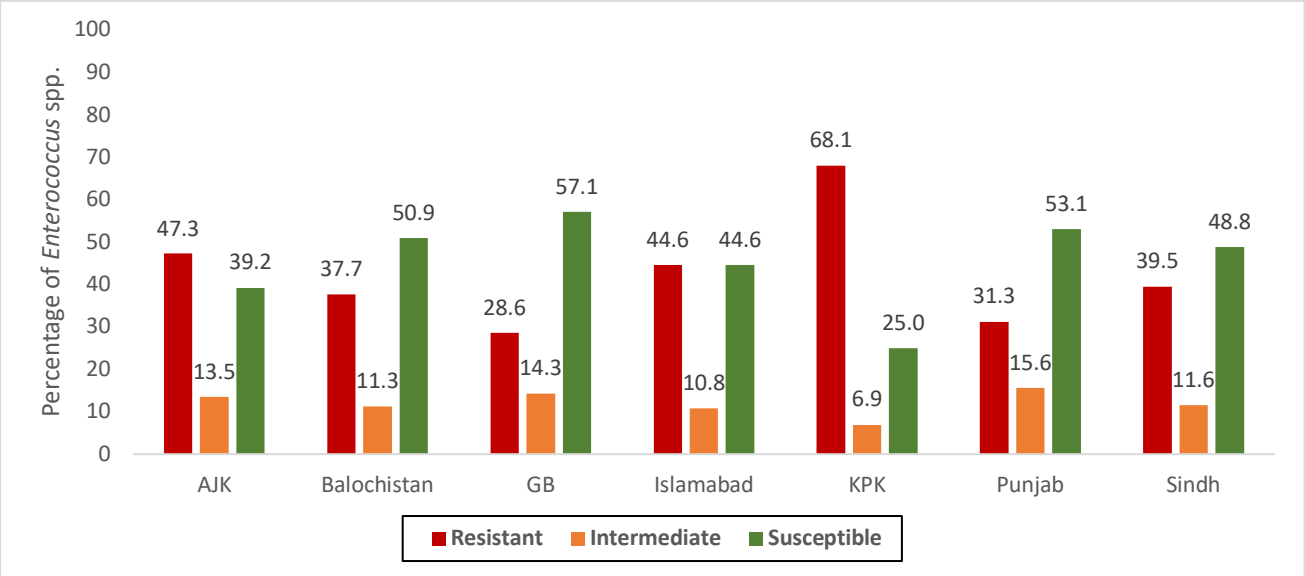

Figure 29: Percentage of resistant, intermediate, and susceptible Enterococcus spp. from livestock to ***linezolid*** *from all provinces of Pakistan*

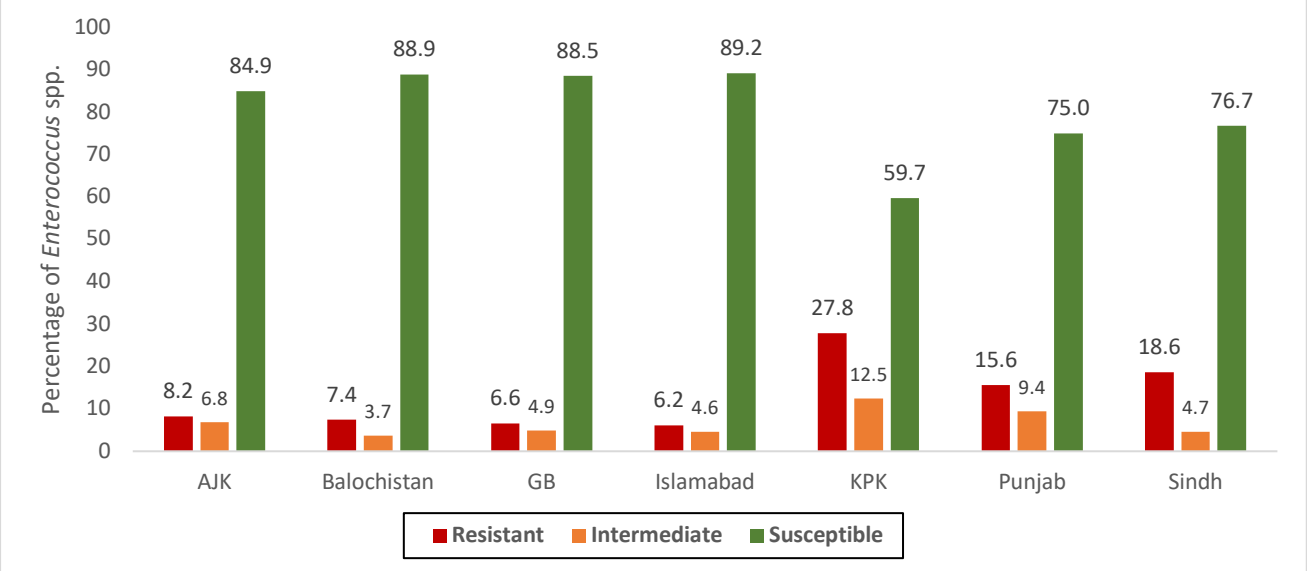

Figure 30: Percentage of resistant, intermediate, and susceptible Enterococcus spp. from livestock to ***teicoplanin*** *from all provinces of Pakistan*

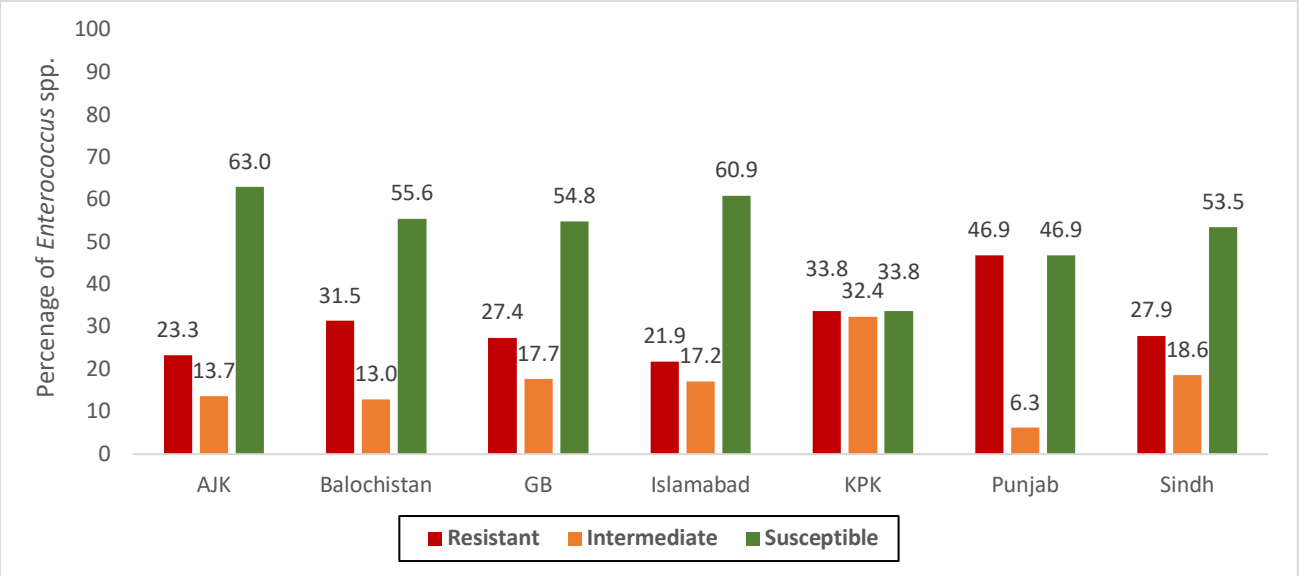

Figure 31: Percentage of resistant, intermediate, and susceptible Enterococcus spp. from livestock to ***tetracycline*** *from all provinces of Pakistan*

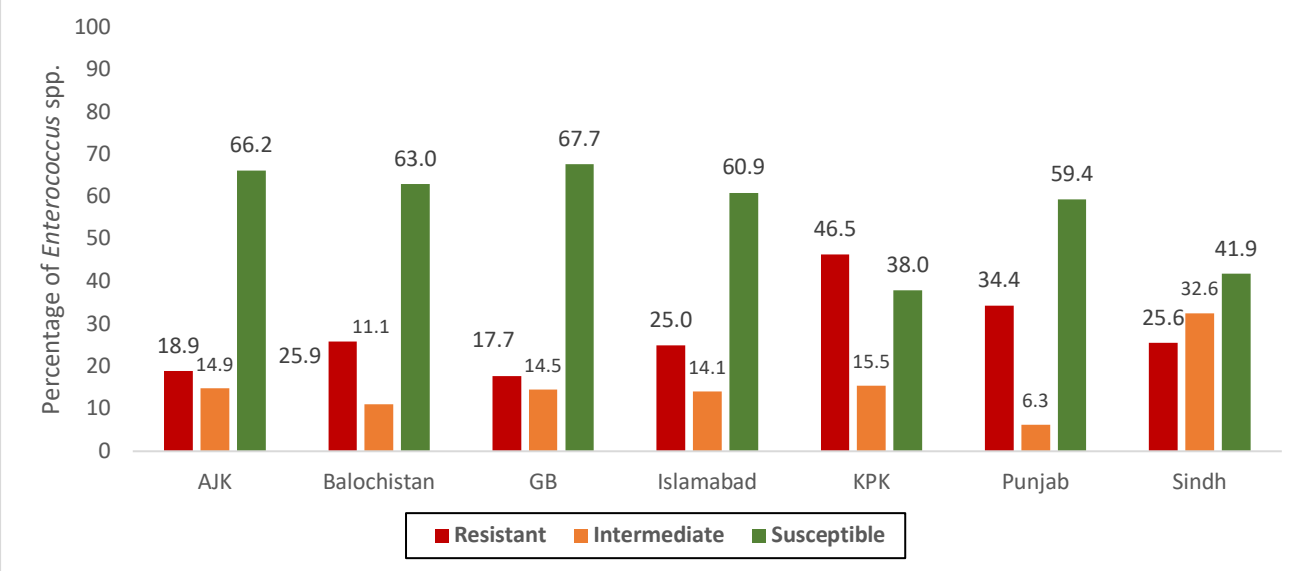

Figure 32: Percentage of resistant, intermediate, and susceptible Enterococcus spp. from livestock to vancomycin from all provinces of Pakistan

4.4.2.5 Percentage of resistance to 8 antimicrobials in *E. coli* from poultry, by season

The series of figures in this section summarizes and compares percentages of RIS outcomes in *E. coli* from poultry *to* recovered from caecal samples collected from poultry birds slaughtered in wet bird markets of 7 provinces/regions of Pakistan *by season (F*igures 33-40*)*. E. coli isolates *exhibited extremely high-level* resistance*s* to ampicillin (86%-94%), chloramphenicol (71%-87%), ciprofloxacin (83%-91%), tetracycline (87%-100%) *and* nalidixic Acid (78%-93%) and *very high to extremely high-level resistance to* trimethoprim (68%-88%) *across all* seasons. *Moderate to high* resistance*s* to cefotaxime (16%-33%) and ceftazidime (11%-33%) *were observed.* As shown in the figures, the relative proportion of RIS was consistent across the seasons in any of the antimicrobials examined except for 1) absence of intermediate resistance to cefotaxime, tetracycline, and trimethoprim in the spring, and 2) relatively similar proportion of RIS (>30%) in ceftazidime results in the spring with a more pronounced levels of ceftazidime susceptible isolates in the remaining 3 seasons (Figure 35).

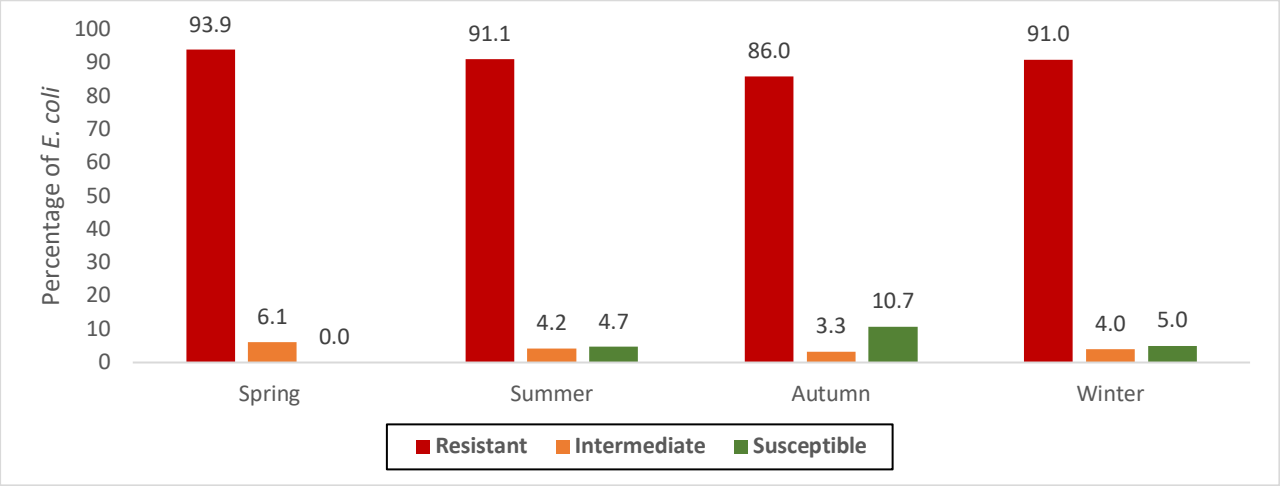

Figure 33: Percentage of resistant, intermediate, and susceptible E. coli. from poultry to ***ampicillin*** *by season.*

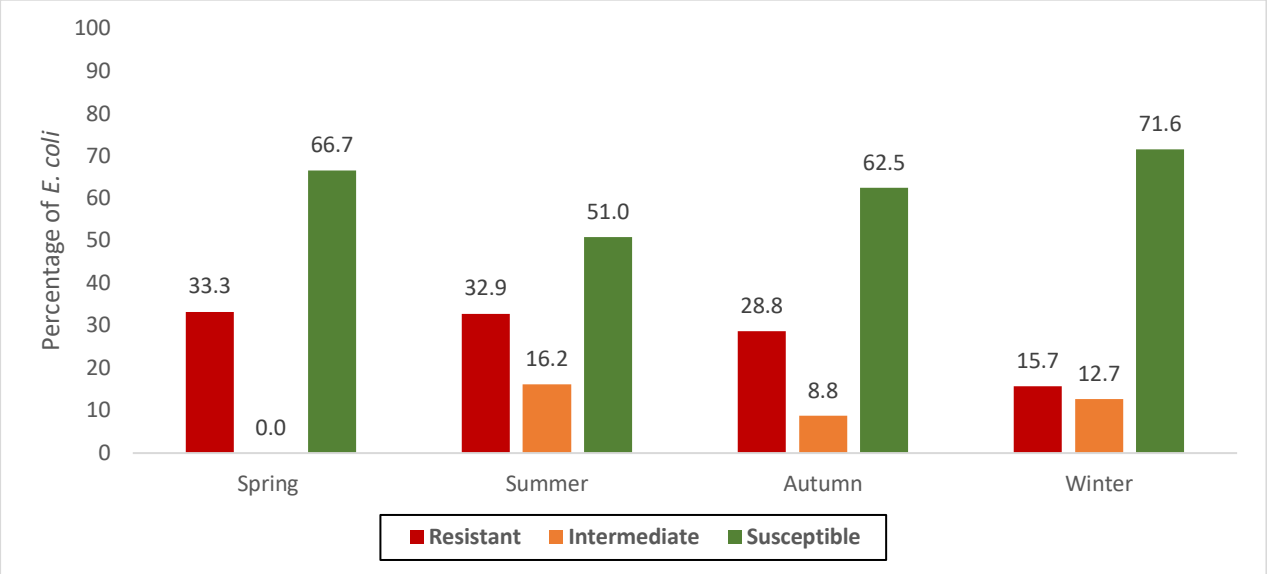

Figure 34: Percentage of resistant, intermediate, and susceptible E. coli. from poultry to ***cefotaxime*** *by season.*

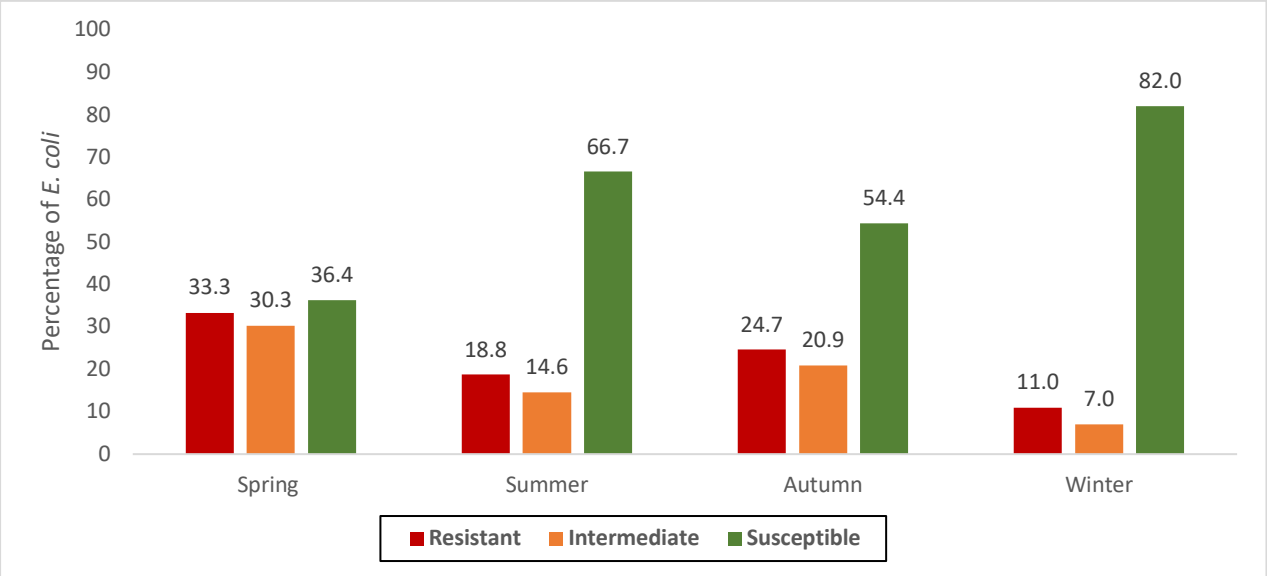

*Figure 35: Percentage of resistant, intermediate, and susceptible E. coli. from poultry to **ceftazidime** by season.*

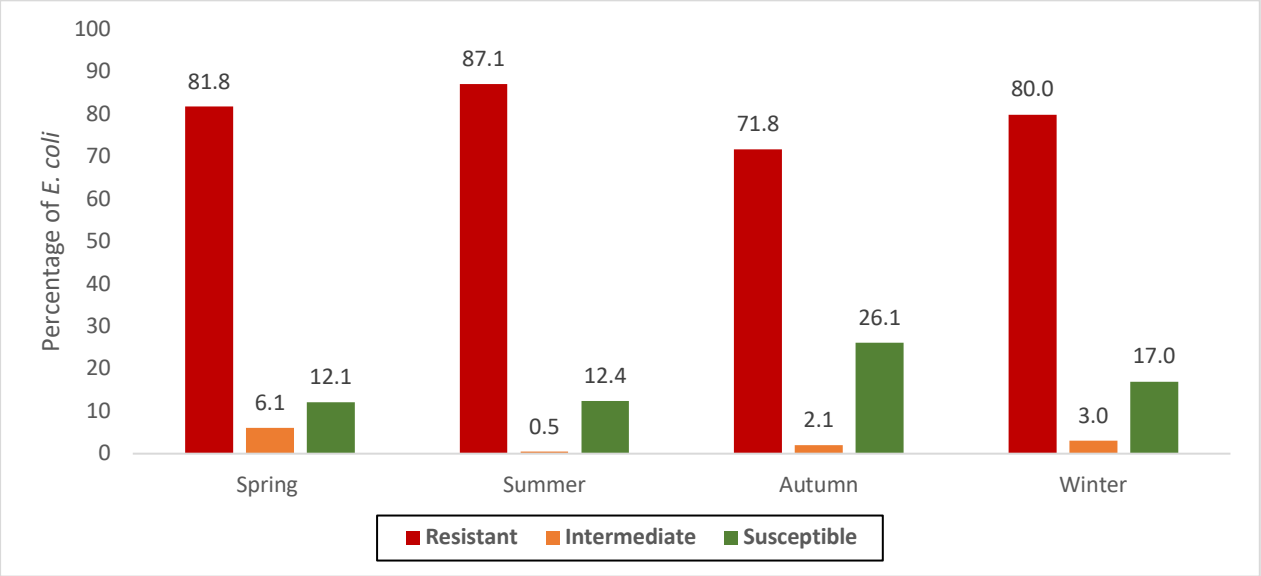

Figure 36: Percentage of resistant, intermediate, and susceptible E. coli. from poultry to chloramphenicol by season.

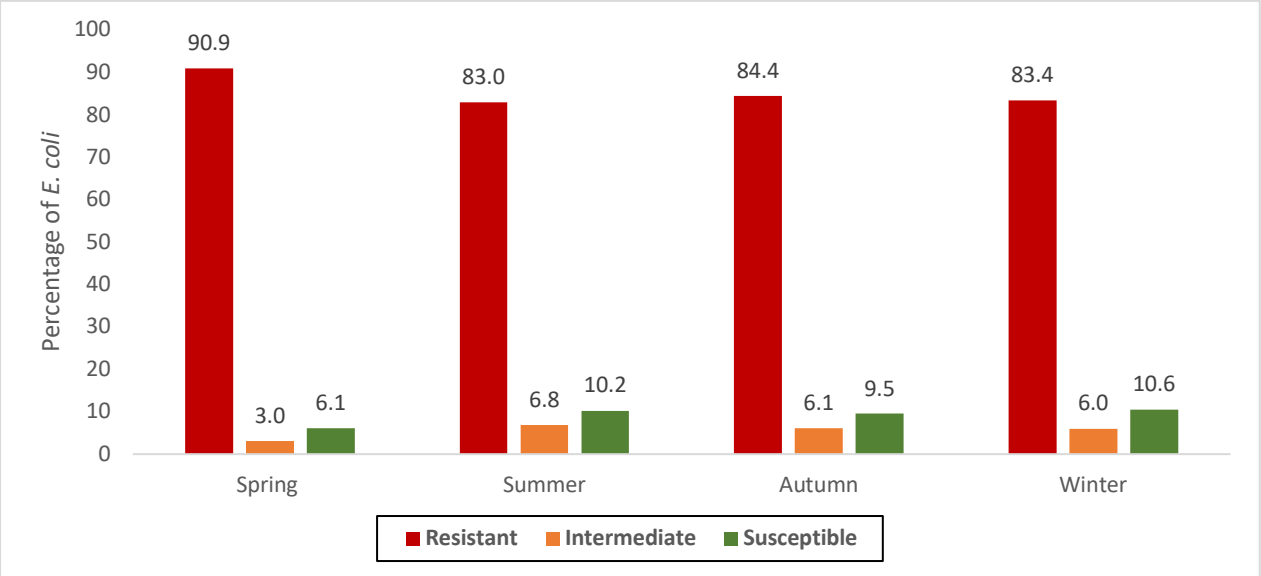

*Figure 37: Percentage of resistant, intermediate, and susceptible E. coli. from poultry to **ciprofloxacin** by season.*

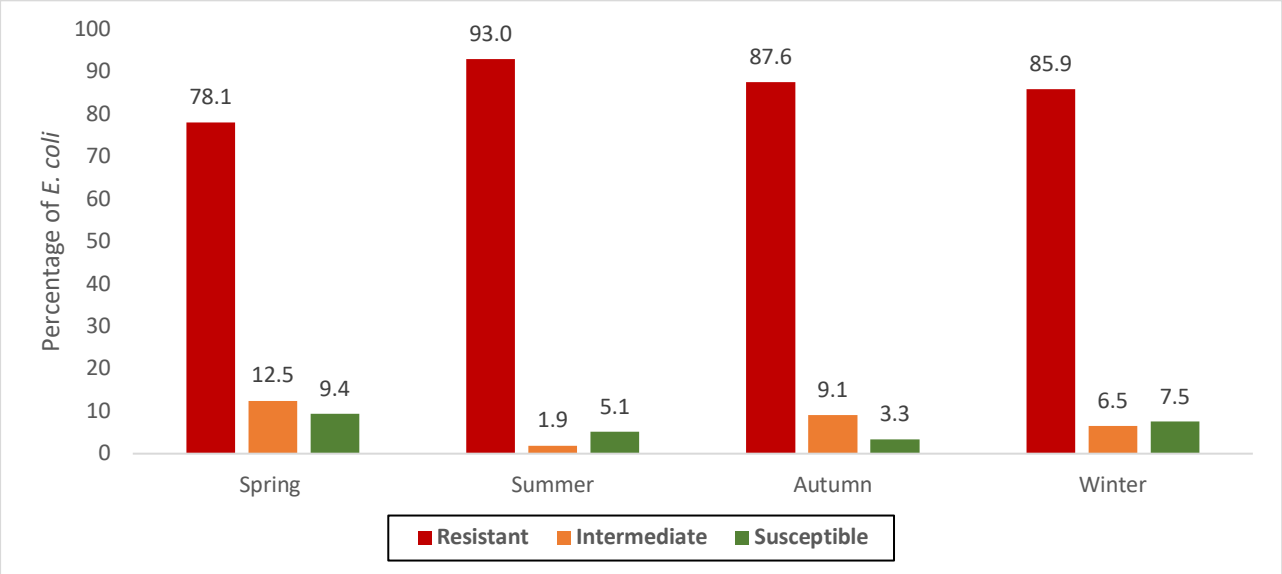

*Figure 38: Percentage of resistant, intermediate, and susceptible E. coli. from poultry to **nalidixic acid** by season.*

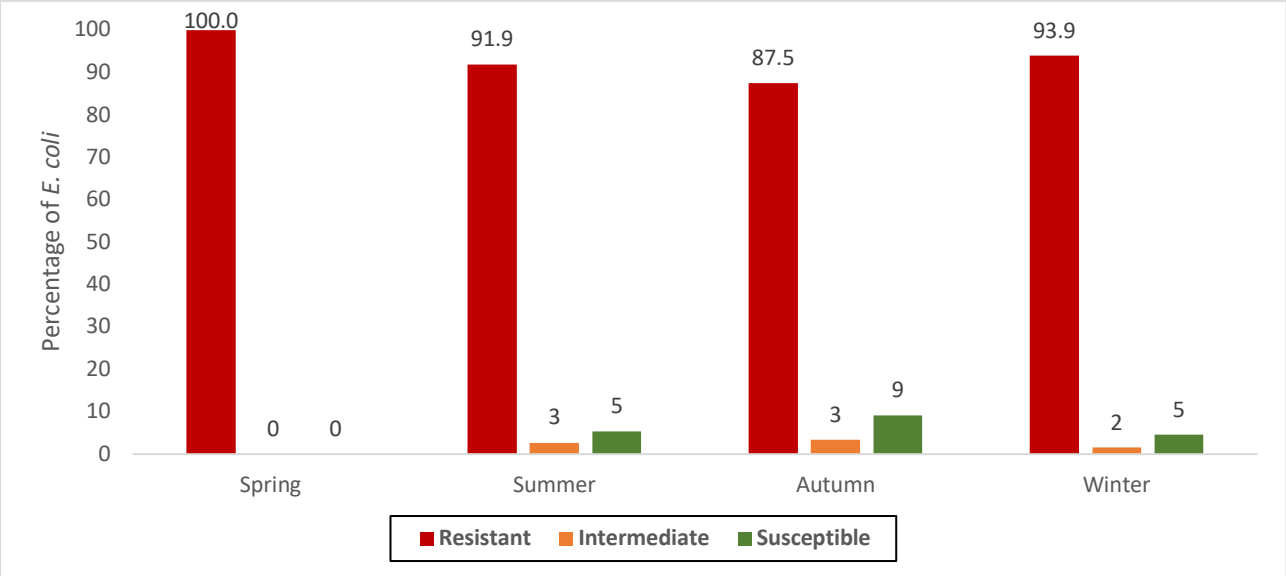

*Figure 39: Percentage of resistant, intermediate, and susceptible E. coli. from poultry to **tetracycline** by season*

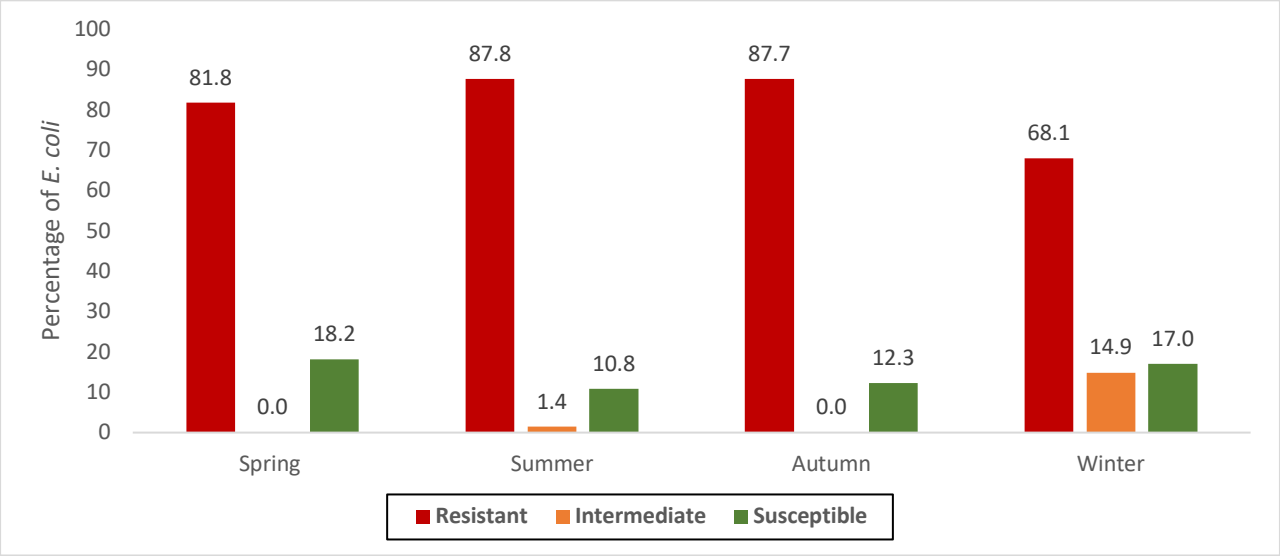

*Figure 40: Percentage of resistant, intermediate, and susceptible E. coli. from poultry to **trimethoprim** by season*

4.4.2.5 Percentage of resistance to 8 antimicrobials in *E. coli* from cattle and buffaloes, by season

The series of figures in this section summarizes and compares percentages of RIS outcomes in E. coli from cattle and buffaloes recovered from faecal samples collected from cattle and buffaloes slaughtered in slaughterhouses of 7 provinces/regions of Pakistan by season (Figures 41-48). The highest percentage of resistance in *E. coli* to ampicillin, cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, tetracycline, and trimethoprim was observed in the winter. Whereas the highest percentage of resistance to nalidixic acid was observed in autumn. The lowest percentage of resistance in *E. coli* isolates to antimicrobials was observed in the summer (chloramphenicol, nalidixic acid, tetracycline, and trimethoprim), spring (ceftazidime and ciprofloxacin) and autumn (ampicillin and cefotaxime). The relative proportions of RIS across the seasons were relatively similar except for the predominantly resistant and zero to low-level intermediate or susceptible isolates in the winter for ampicillin (Figure 41) and cefotaxime (Figure 42) and high proportion of intermediate

chloramphenicol (Figure 44) and nalidixic acid (Figure 46) resistant isolates compared to resistant isolates in the winter. This data warrants further investigation on factors (for example, diseases requiring AMU) impacting seasonality of the resistance observed.

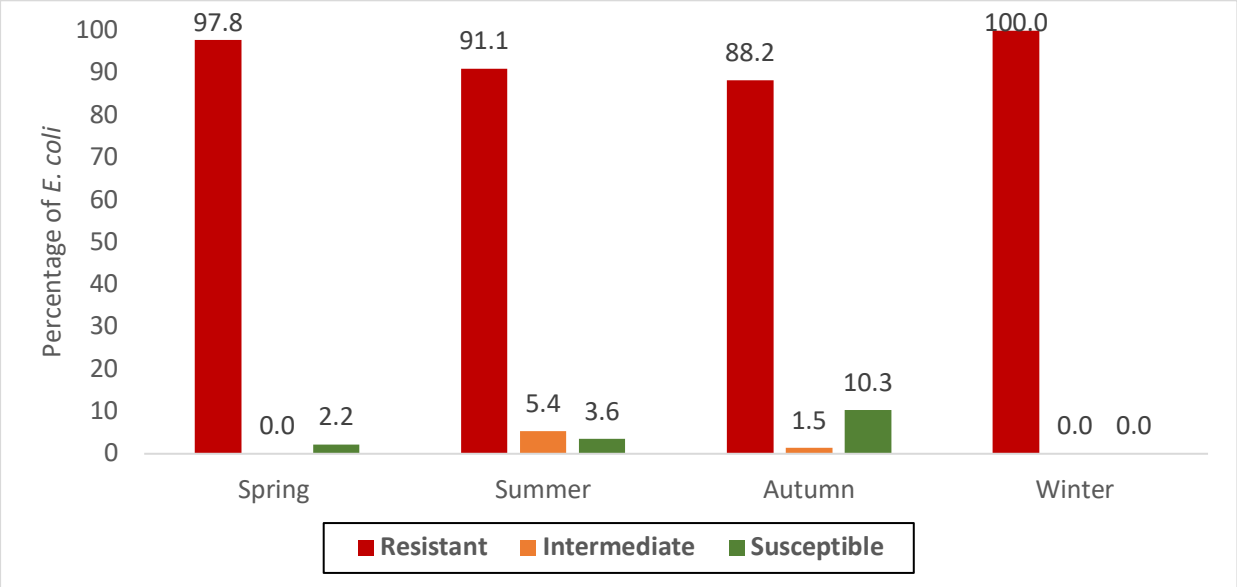

*Figure 41: Percentage of resistant, intermediate, and susceptible E. coli. from livestock to **ampicillin** by season.*

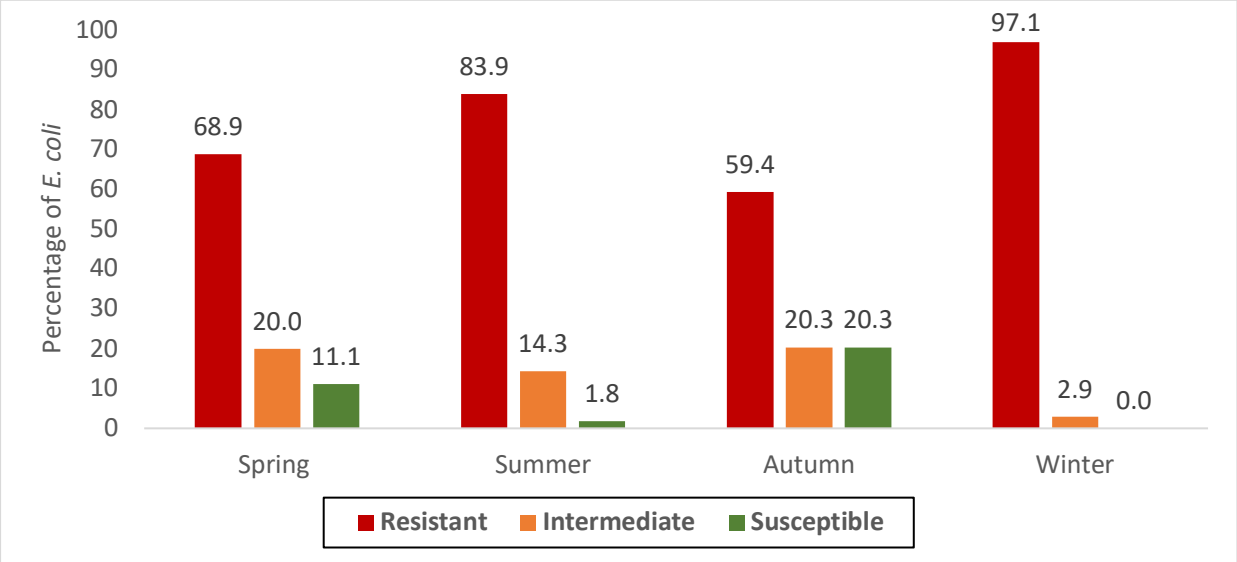

*Figure 42: Percentage of resistant, intermediate, and susceptible E. coli. from livestock to **cefotaxime** by season.*

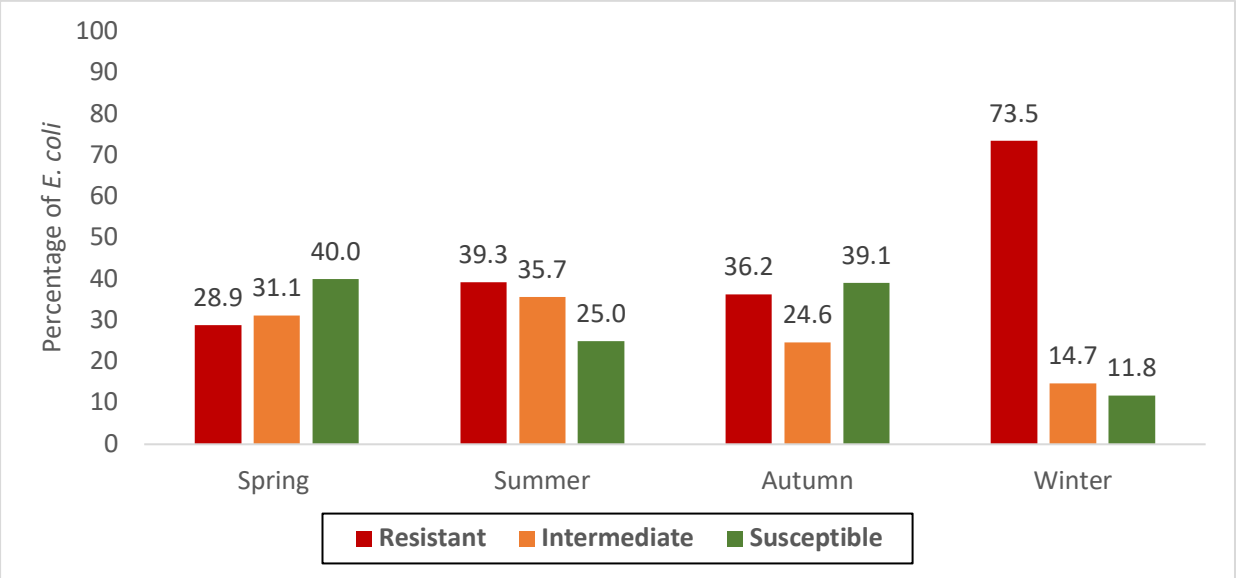

*Figure 43: Percentage of resistant, intermediate, and susceptible E. coli. from livestock to **ceftazidime** by season.*

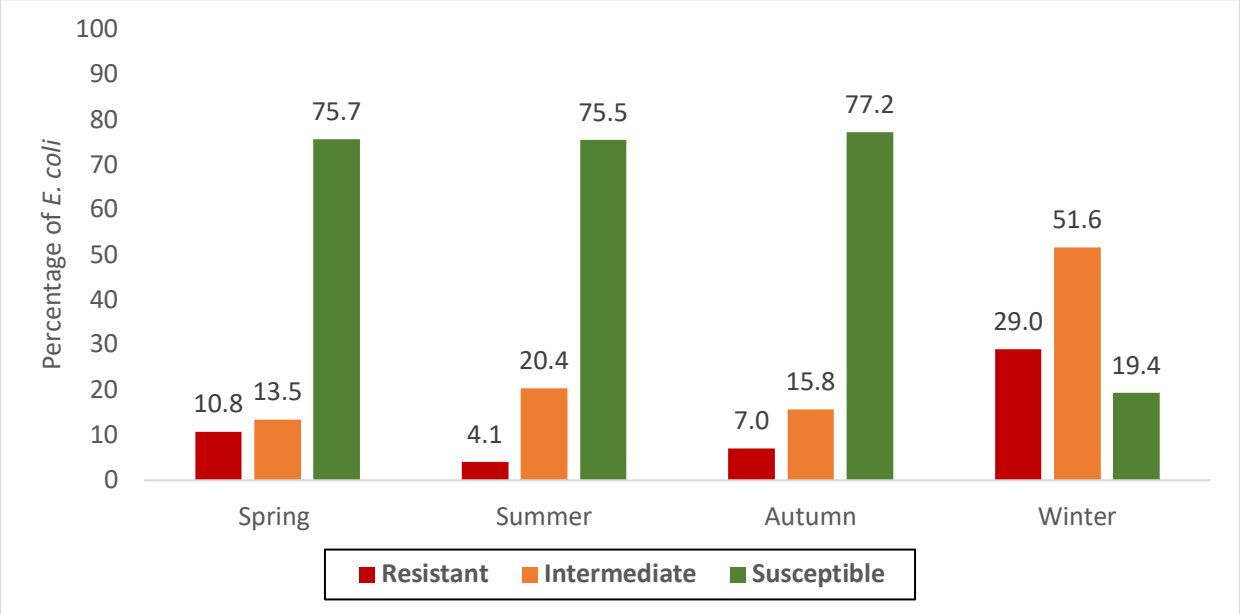

*Figure 44: Percentage of resistant, intermediate, and susceptible E. coli. from livestock to **chloramphenicol** by season*

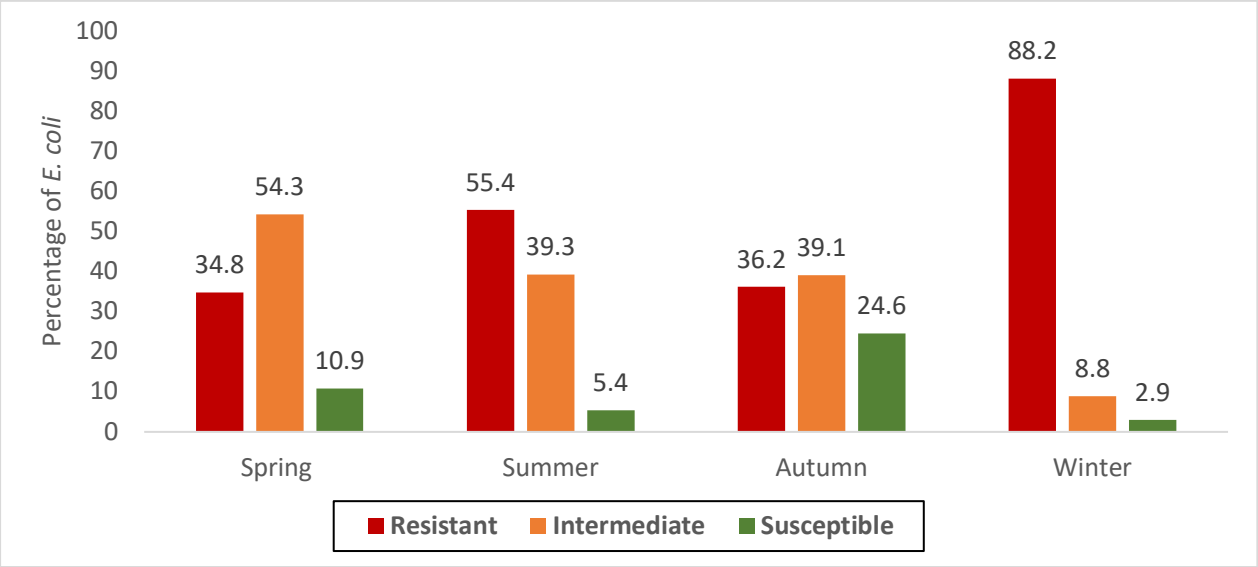

Figure 45: Percentage of resistant, intermediate, and susceptible E. coli. from livestock to ciprofloxacin by season.

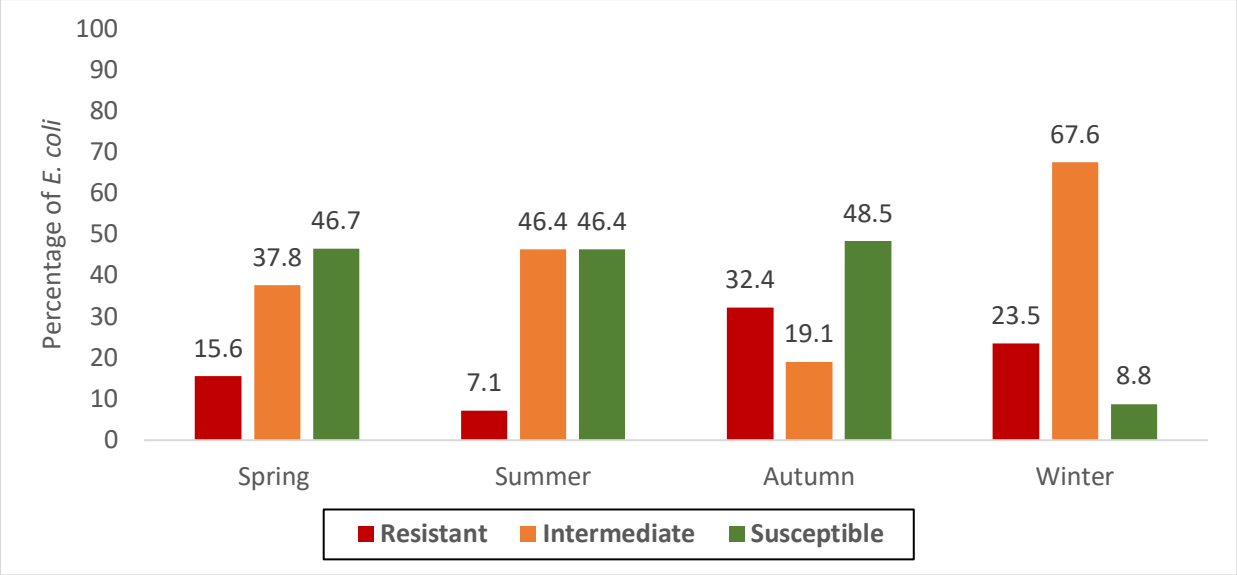

*Figure 46: Percentage of resistant, intermediate, and susceptible E. coli. from livestock to **nalidixic acid** by season.*

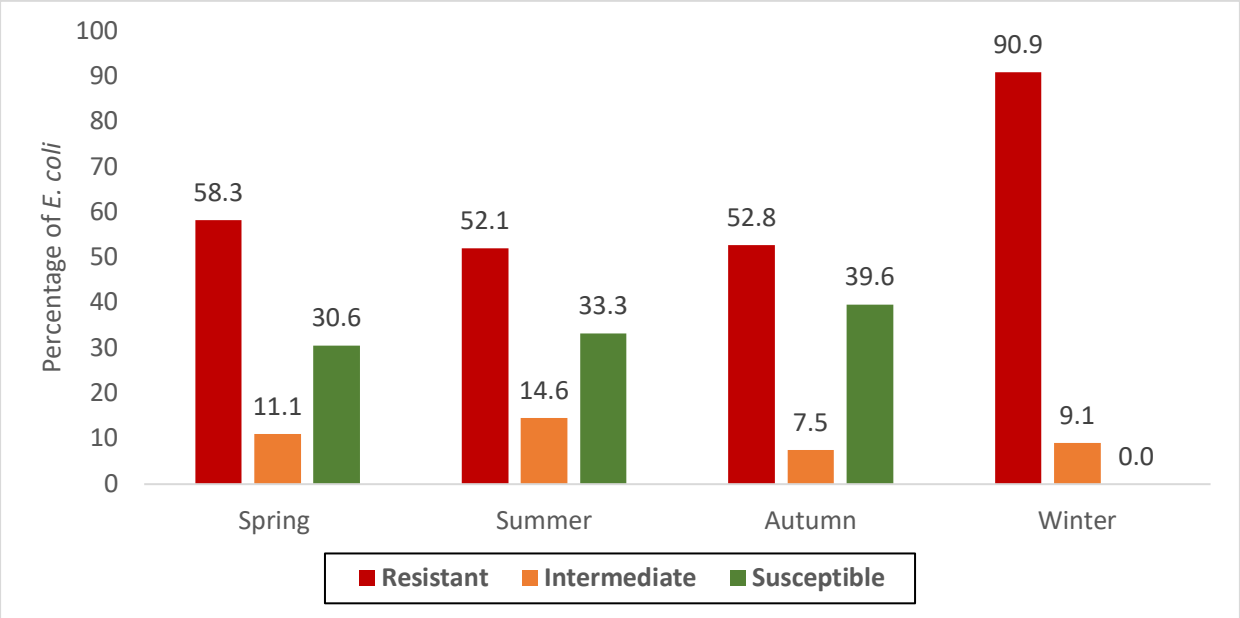

Figure 47: Percentage of resistant, intermediate, and susceptible E. coli. from livestock to ***tetracycline*** *by season.*

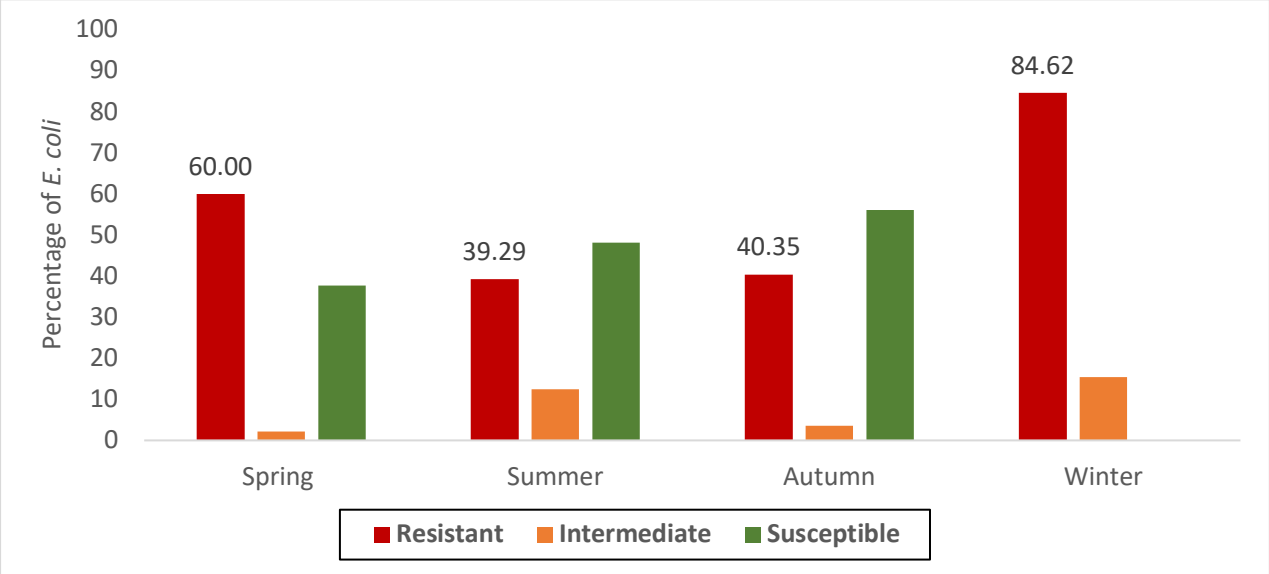

Figure 48: Percentage of resistant, intermediate, and susceptible E. coli. from livestock to ***trimethoprim*** *by season.*

4.4.2.5 Percentage of resistance to 8 antimicrobials in *Salmonella* spp. from poultry, by season

The series of figures in this section summarizes and compares percentages of RIS outcomes in Salmonella spp. isolates recovered from caecal samples collected from poultry birds slaughtered in wet bird markets of 7 provinces/regions of Pakistan, by season (Figures 49-56). The highest resistance percentage observed in *Salmonella* to the 9 antimicrobials under study were distributed in all four seasons. The highest resistance to chloramphenicol, ciprofloxacin, tetracycline, and trimethoprim was observed in autumn whereas in the remaining antimicrobials, highest resistance was noted in spring (ampicillin, cefotaxime and ceftazidime), and winter (nalidixic acid). The relative proportions of RIS were similar across the seasons in most antimicrobials except for the following: 1) similar (29%) proportion of resistant and intermediate in cefotaxime in the spring (Figure 50) and ceftazidime in the summer (Figure 51), and 2) absence of isolates with intermediate resistance and susceptible to

tetracycline and trimethoprim in autumn (Figures 55 and 56). These observations are also suggestive of seasonal variations in resistance to antimicrobials.

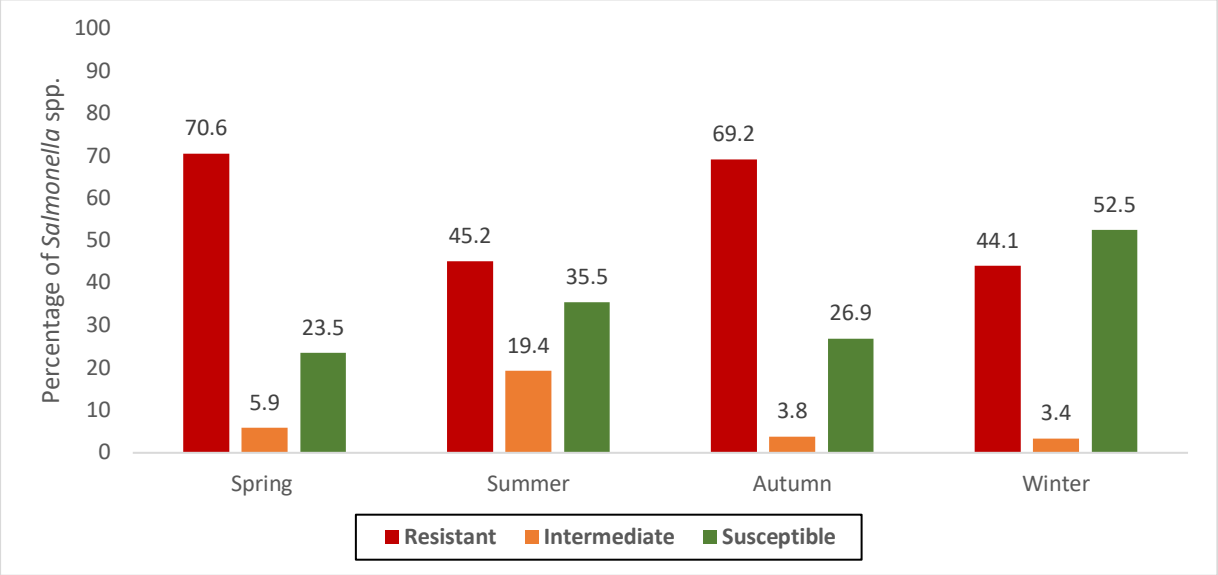

Figure 49: Percentage of resistant, intermediate, and susceptible Salmonella. from poultry to ***ampicillin*** *by season.*

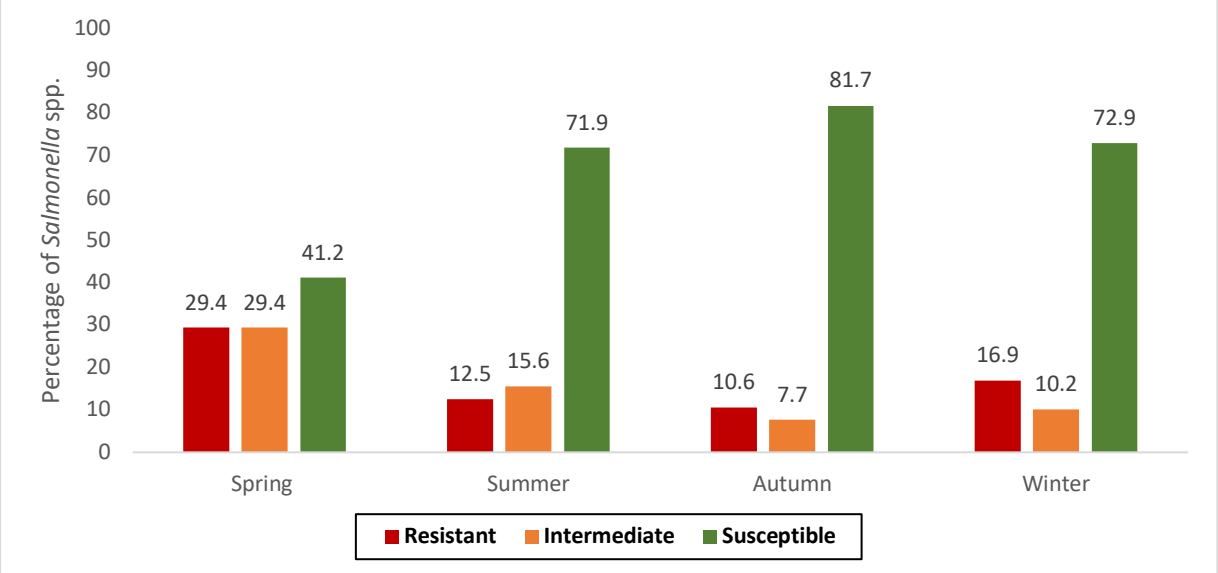

Figure 50: Percentage of resistant, intermediate, and susceptible Salmonella. from poultry to ***cefotaxime*** *by season.*

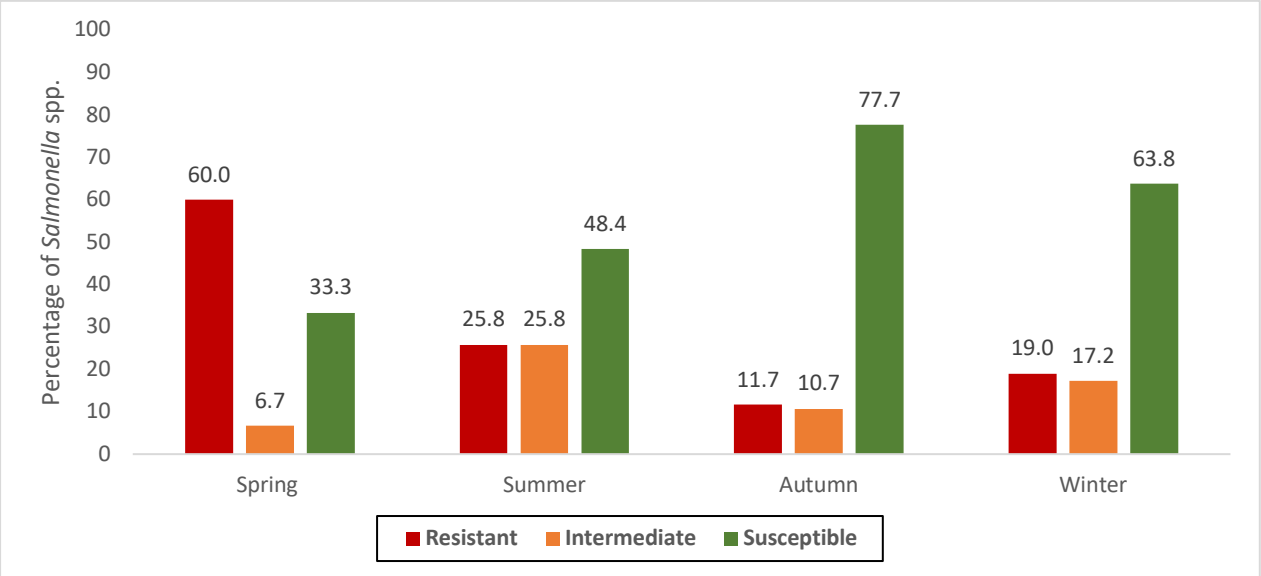

*Figure 51: Percentage of resistant, intermediate, and susceptible Salmonella. from poultry to **ceftazidime** by season.*

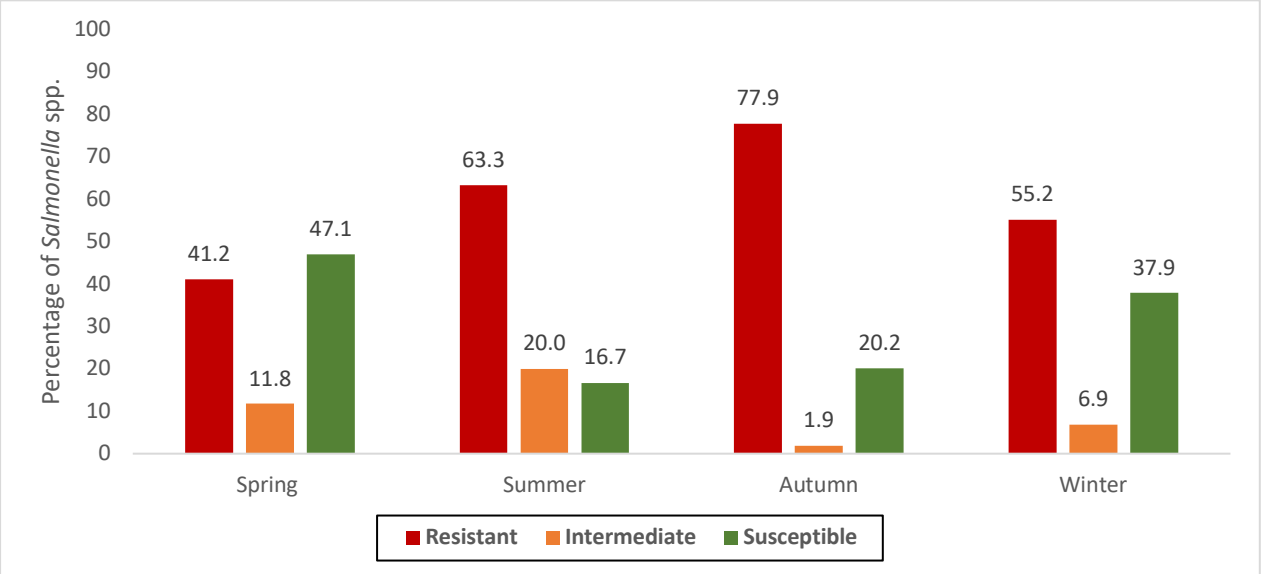

*Figure 52: Percentage of resistant, intermediate, and susceptible Salmonella. from poultry to **chloramphenicol** by season.*

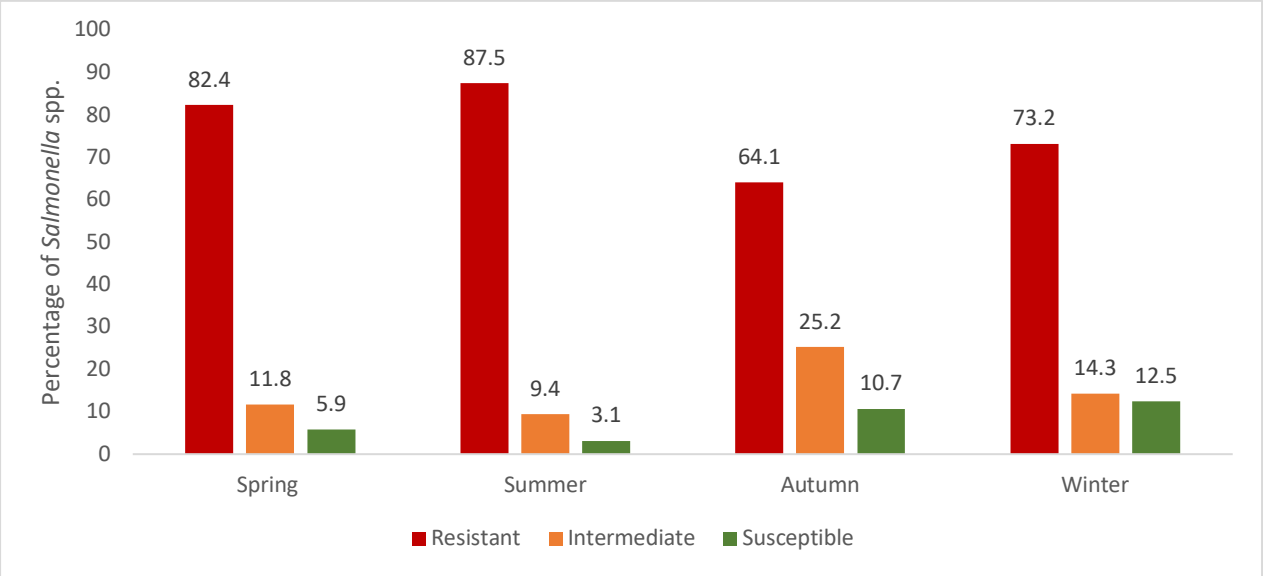

Figure 53: Percentage of resistant, intermediate, and susceptible Salmonella. from poultry to ***ciprofloxacin*** *by season.*

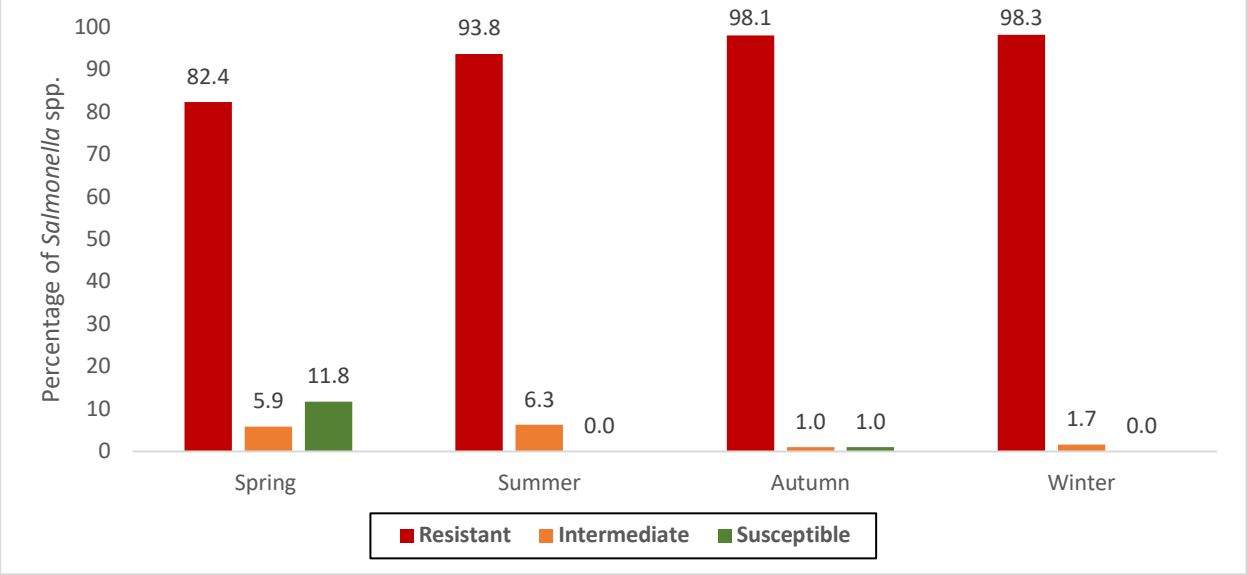

Figure 54: Percentage of resistant, intermediate, and susceptible Salmonella. from poultry to ***nalidixic acid*** *by season.*

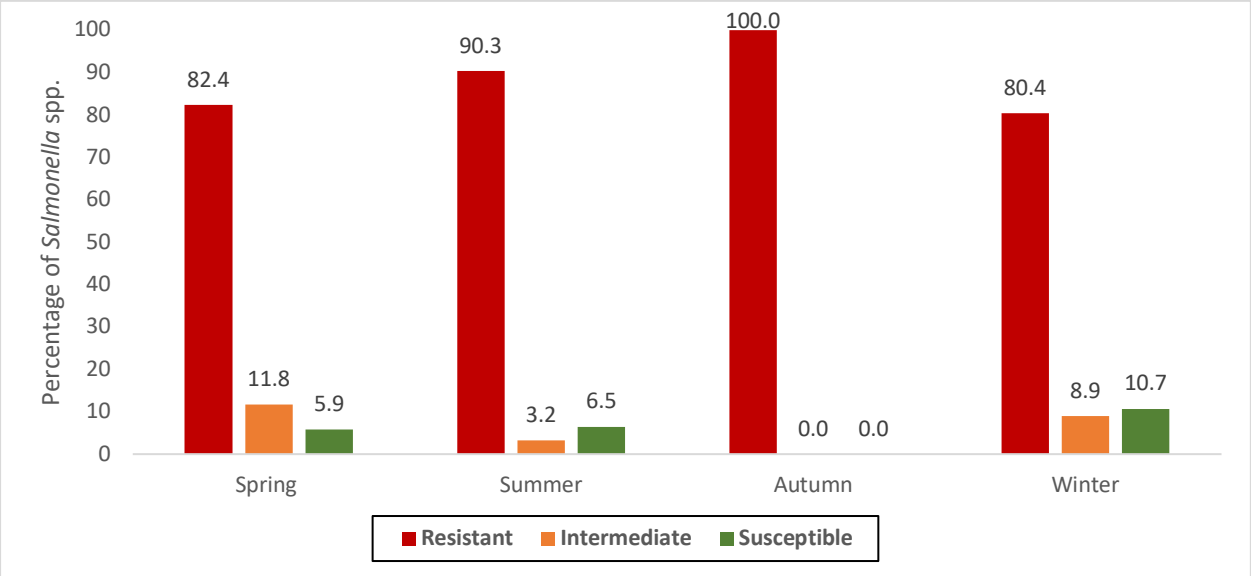

Figure 55: Percentage of resistant, intermediate, and susceptible Salmonella. from poultry to ***tetracycline*** *by season.*

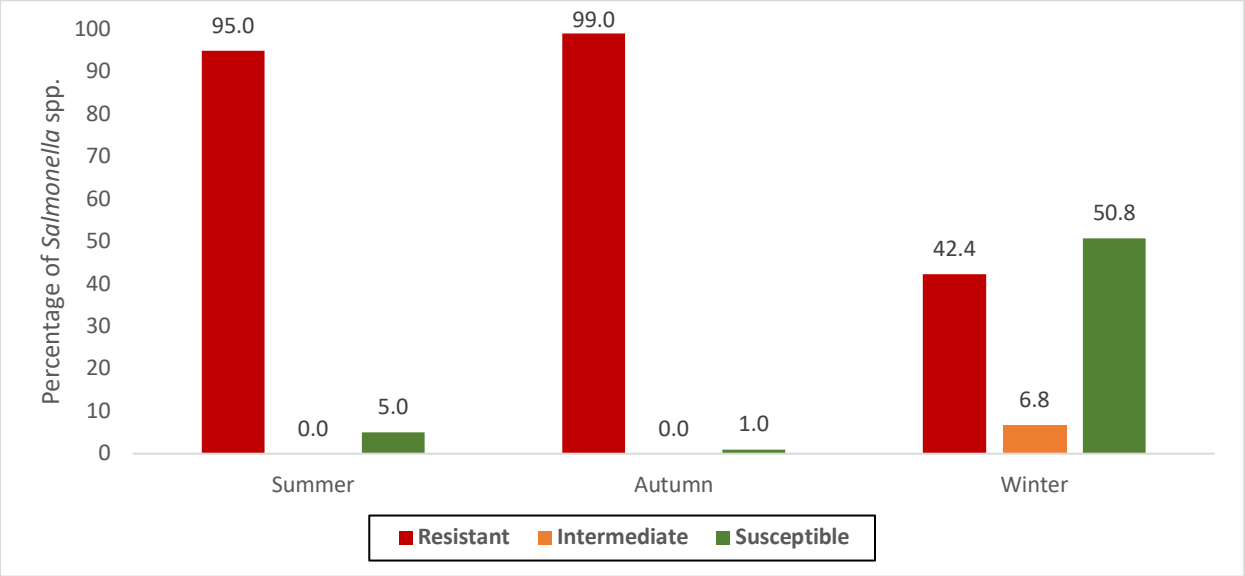

Figure 56: Percentage of resistant, intermediate, and susceptible Salmonella. from poultry to ***trimethoprim*** *by season.*

4.4.2.6 Percentage of resistance to 7 antimicrobials in *Enterococcus* spp. from cattle and buffaloes, by season

The series of figures in this section summarizes and compares percentages of RIS outcomes in *Enterococcus* spp. isolates recovered from faecal samples collected from cattle and buffaloes slaughtered in slaughterhouses of 7 provinces/regions of Pakistan (Figures 62-68). The results indicated that the highest resistance in *Enterococcus* isolates to 7 antimicrobials was observed in the spring (ampicillin, chloramphenicol, erythromycin, teicoplanin, tetracycline and vancomycin) and summer (linezolid). Five of the seven antimicrobials showed lowest resistance in autumn (ampicillin, chloramphenicol, linezolid, teicoplanin, and tetracycline) while the remaining two i.e., erythromycin and vancomycin antimicrobials were observed in the summer and winter, respectively. The relative proportions of RIS across the seasons were similar in most antimicrobials except for the high proportion of intermediate resistant vancomycin during the winter (Figure 68) compared to resistant isolates which were the most common category in all the 3 seasons.

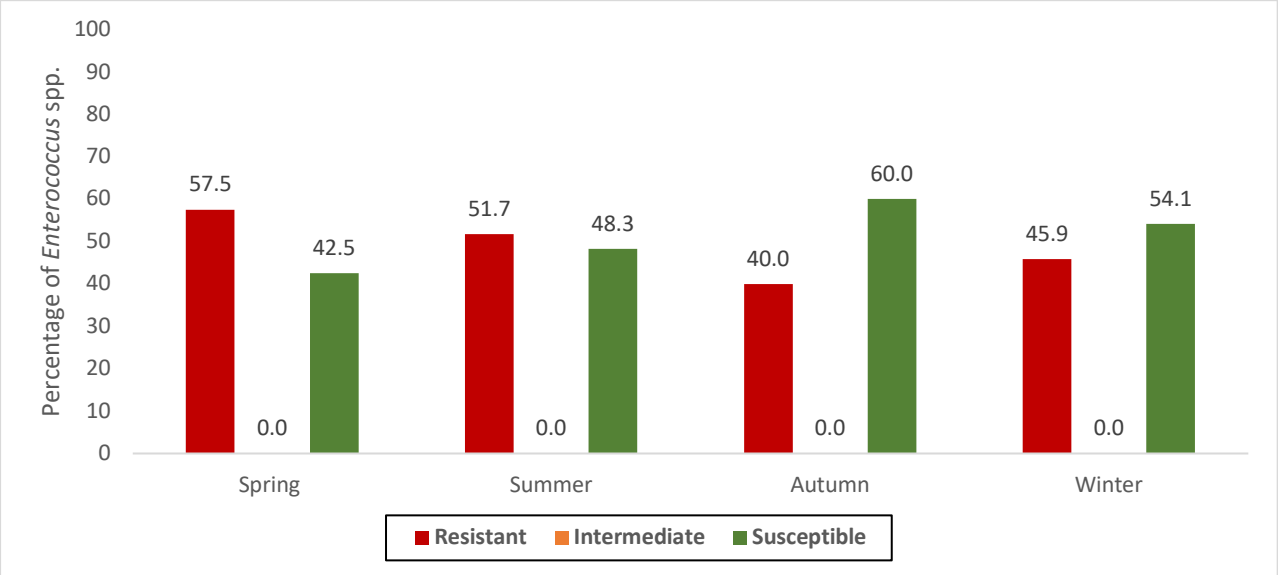

*Figure62: Percentage of resistant, intermediate, and susceptible Enterococcus spp. from livestock to **ampicillin** by season*

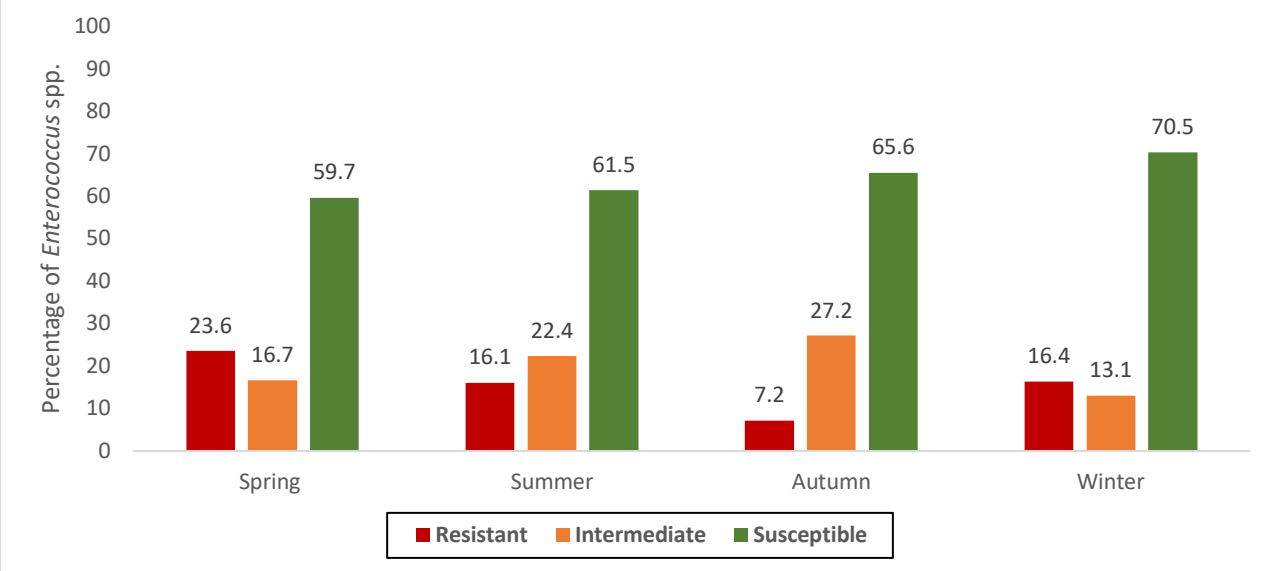

*Figure 63: Percentage of resistant, intermediate, and susceptible Enterococcus spp. from livestock to **chloramphenicol** by season*

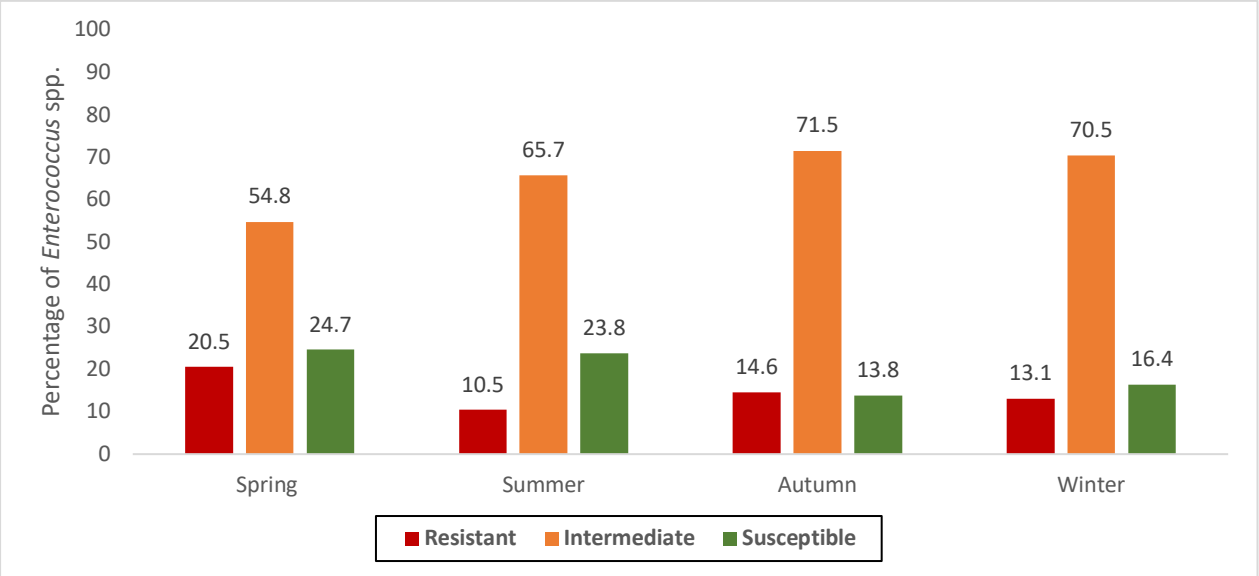

Figure 64: Percentage of resistant, intermediate, and susceptible Enterococcus spp. from livestock to erythromycin by season

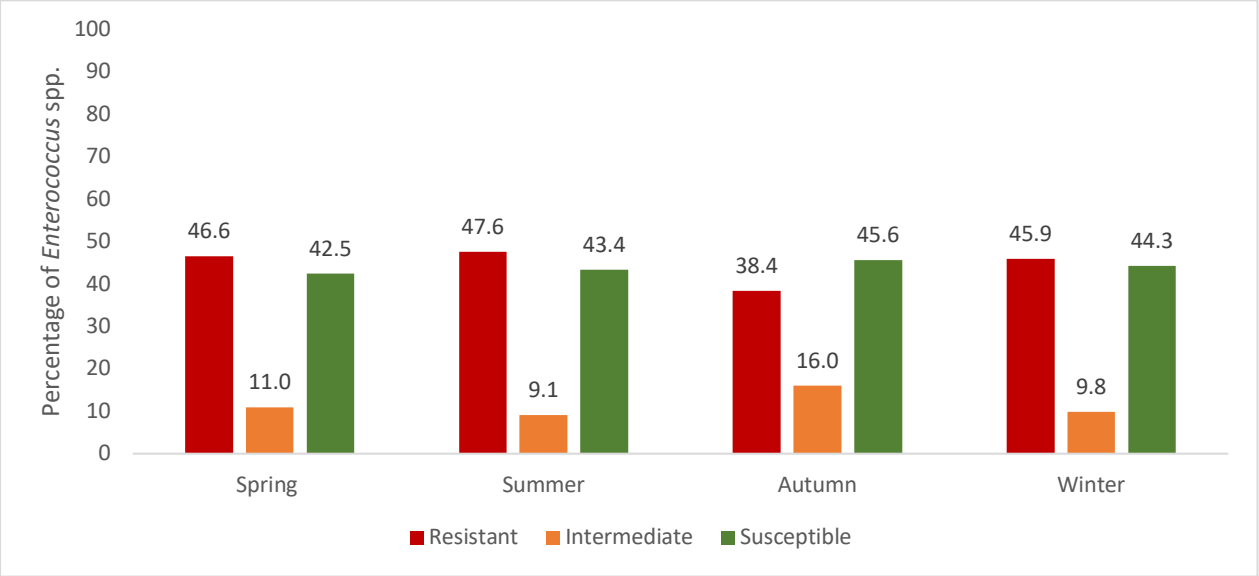

*Figure 65: Percentage of resistant, intermediate, and susceptible Enterococcus spp. from livestock to **linezolid** by season*

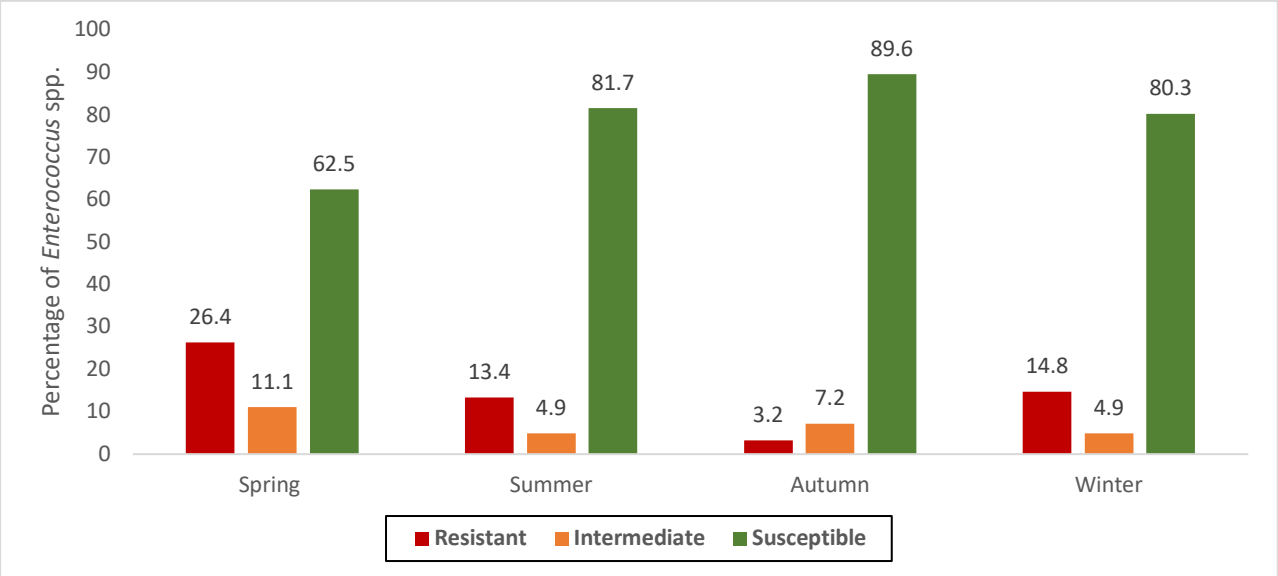

*Figure 66: Percentage of resistant, intermediate, and susceptible Enterococcus spp. from livestock to **teicoplanin** by season*

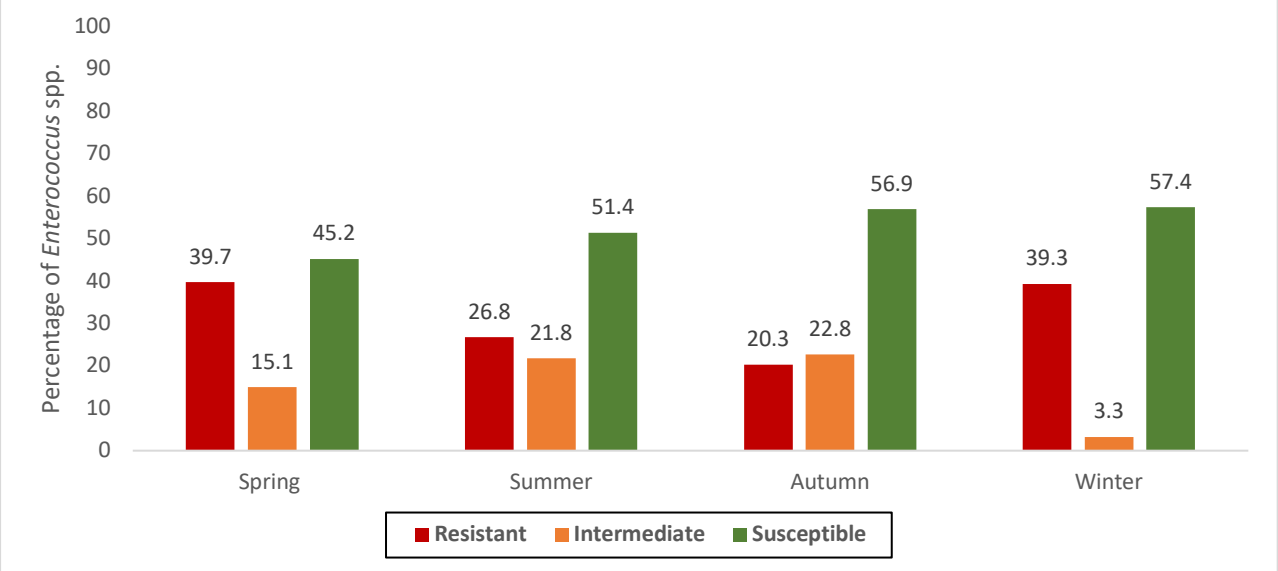

Figure 67: Percentage of resistant, intermediate, and susceptible Enterococcus spp. from livestock to tetracycline by season

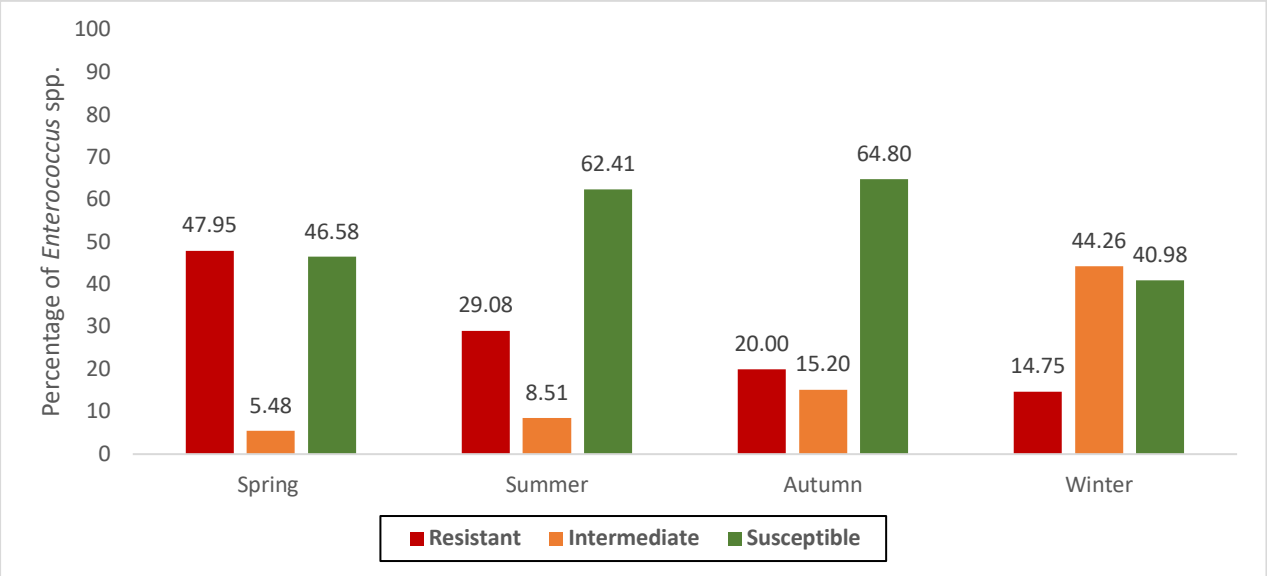

Figure 68: Percentage of resistant, intermediate, and susceptible Enterococcus spp. from livestock to ***vancomycin*** *by season*

5. Statistical Analysis

The series of analyses in this section examined the association between AMR outcome variables and potential risk factors. These are starting point analyses and could be used for future detailed studies.

5.1 Risk Factors Association of Poultry *E. coli* Isolates

5.1.1 Poultry *E. coli* Isolates with Ampicillin

A logistic regression model was used to examine the magnitude of association between the susceptibility of *E. coli* to ampicillin as the outcome variable and the province and season (categorical independent variables) as potential risk factors. For analytic purposes, data were converted to binary outcome measures where isolates that exhibited intermediate resistance were categorized as resistant. The statistical analysis was conducted using software IBM SPSS Statistics. The results were considered statistically significant when P-values were less than 0.05 ($P < 0.05$).

The results of the bivariate model indicated that none of the study provinces are statistically significantly different in their association with susceptibility to ampicillin considering the providence Sindh as the reference (i.e., OR=1). Similarly, observations in the summer or autumn were not statistically different in their susceptibility to ampicillin considering the providence winter as the reference (i.e., OR=1) (Table 5).

Variable	Categories	Odds ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	0.634	0.152	2.648	0.532
	Balochistan	0.877	0.271	2.840	0.826
	Gilgit Baltistan	0.623	0.183	2.122	0.449
	Islamabad	1.315	0.446	3.876	0.62
	Khyber Pakhtunkhwa	0.750	0.257	2.190	0.599
	Punjab	0.823	0.274	2.471	0.729
Season	Winter	1	-	-	-

	Summer	0.931	0.379	2.287	0.876
	Autumn	2.275	1.069	4.840	0.033

Table 5: *The bivariable relationships between the likelihood an E. coli isolate is susceptible to Ampicillin using disc diffusion method and two independent variables (Province and season).*

5.1.2 Poultry *E. coli* Isolates with Azithromycin

A logistic regression model was used to examine the magnitude of association between the susceptibility of *E. coli* to azithromycin as the outcome variable and the province and season as the potential risk factors. For analytic purposes all the isolates classified as intermediate were categorized as resistant.

The results indicated no significant association between susceptibility of poultry *E. coli* isolates to azithromycin (with disc diffusion method) and independent variable province in bivariable and multivariable models considering the province Sindh as the reference (i.e., OR=1). However, a significant relationship was observed between susceptibility of *E. coli* isolates to azithromycin and season in bivariable model considering the season winter as the reference (i.e., OR=1). The *E. coli* isolates recovered in spring, summer and autumn were 5.02, 1.55 and 2.68 times more likely to be resistant to azithromycin compared to winter in bivariable model respectively (Table 6). Similarly, in multivariable model the *E. coli* isolates recovered in spring, summer and autumn were 4.65, 1.56 and 3.22 times more likely to be resistant to azithromycin compared to isolates recovered in winter (Table 7).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	0.906	0.383	2.143	0.822
	Balochistan	1.6	0.768	3.332	0.209
	Gilgit Baltistan	1.023	0.481	2.175	0.953
	Islamabad	1.615	0.78	3.345	0.197
	Khyber Pakhtunkhwa	1.438	0.734	2.818	0.29
	Punjab	1.447	0.722	2.900	0.298
Season	Winter	1	-	-	-
	Spring	0.199	0.059	0.678	0.01
	Summer	0.642	0.419	0.984	0.042
	Autumn	0.372	0.237	0.584	0

Table 6: *The bivariable relationships between the likelihood an E. coli isolate is susceptible to Azithromycin using disc diffusion method and two independent variables (Province and season).*

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	0.851	0.355	2.038	0.717
	Balochistan	2.142	0.988	4.642	0.054
	Gilgit Baltistan	0.962	0.441	2.101	0.923
	Islamabad	1.921	0.911	4.051	0.086
	Khyber Pakhtunkhwa	1.455	0.731	2.896	0.286
	Punjab	1.342	0.658	2.737	0.418
Season	Winter	1	-	-	-
	Spring	0.215	0.062	0.749	0.016
	Summer	0.641	0.413	0.996	0.048
	Autumn	0.31	0.192	0.502	0

Table 7: The multivariable relationships between the likelihood an E. coli isolate is susceptible to Azithromycin using disc diffusion method and two independent variables (Province and season).

5.1.3 Poultry *E. coli* Isolates with Cefotaxime

A logistic regression model was used to examine the magnitude of association between the susceptibility of *E. coli* to cefotaxime as the outcome variable and the province and season as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant.

The results of bivariable model indicated a statistically significant association between *E. coli* isolates recovered from Azad Jammu and Kashmir (AJK) and susceptibility to cefotaxime considering the providence Sindh as the reference (i.e., OR=1). The recovered isolates from AJK were 2.15 times more likely to be resistant to cefotaxime compared to isolates recovered from Sindh. Similarly, observations in summer and autumn were statistically different in their susceptibility to cefotaxime considering the providence winter as the reference (i.e., OR=1). The *E. coli* isolates recovered in summer and autumn were 2.42 and 1.51 times more likely to be resistant to cefotaxime compared to winter in bivariable model respectively (Table 8). The multivariable analysis also indicated a statistically significant association between *E. coli* isolates recovered from province AJK and in seasons summer and autumn with susceptibility to cefotaxime. The *E. coli* isolates recovered from AJK were 2.38 times more likely to be resistant to cefotaxime compared to Sindh. Similarly, the *E. coli* isolates recovered in summer and autumn were 2.46, and 1.63 times more likely to be resistant to cefotaxime compared to isolates recovered in winter (Table 9).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	0.463	0.23	0.935	0.032
	Balochistan	1.389	0.749	2.574	0.297
	Gilgit Baltistan	2	1.087	3.679	0.026
	Islamabad	3.684	1.878	7.228	0
	Khyber Pakhtunkhwa	1.673	0.96	2.915	0.07
	Punjab	2.27	1.26	4.09	0.006
Season	Winter	1	-	-	-
	Spring	0.794	0.362	1.745	0.566
	Summer	0.413	0.274	0.622	0
	Autumn	0.662	0.441	0.993	0.046

***Table 8**: The bivariable relationships between the likelihood an E. coli isolate is susceptible to Cefotaxime using disc diffusion method and two independent variables (Province and season).*

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	0.422	0.206	0.863	0.018
	Balochistan	1.209	0.635	2.3	0.563
	Gilgit Baltistan	1.627	0.863	3.065	0.132
	Islamabad	3.675	1.855	7.281	0
	Khyber Pakhtunkhwa	1.547	0.873	2.74	0.135
	Punjab	1.97	1.081	3.591	0.027
Season	Winter	1	-	-	-
	Spring	0.775	0.341	1.761	0.543
	Summer	0.405	0.263	0.624	0
	Autumn	0.611	0.396	0.945	0.027

***Table 9:** The multivariable relationships between the likelihood an E. coli isolate is susceptible to Cefotaxime using disc diffusion method and two independent variables (Province and season).*

5.1.4 Poultry *E. coli* isolates with Ceftazidime

A logistic regression model was used to examine the magnitude of association between the susceptibility of *E. coli* to ceftazidime as the outcome variable and the province and season as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant.

The results of the bivariable model indicated a statistically significant association between *E. coli* isolates recovered from Balochistan and susceptibility to ceftazidime considering the providence Sindh as the reference (i.e., OR=1). The isolated recovered from Balochistan were 3.89 times more likely to be resistant to ceftazidime compared to isolates recovered from Sindh. Similarly, observations in summer and autumn were statistically different in their susceptibility to ceftazidime considering the providence winter as the reference (i.e., OR=1). The *E. coli* isolates recovered in spring, summer and autumn were 8, 2.27 and 3.81 times more likely to be resistant to ceftazidime compared to winter in bivariable model respectively (Table 10). The multivariable analysis also indicated a statistically significant association between *E. coli* isolates recovered from province Balochistan and in seasons spring, summer, and autumn with susceptibility to ceftazidime. The *E. coli* isolates recovered from Balochistan were 3.71 times more likely to be resistant to ceftazidime compared to Sindh. Similarly, the *E. coli* isolates recovered in spring, summer and autumn were 9.9, 2.70 and 3.44 times more likely to be resistant to ceftazidime compared to isolates recovered in winter (Table 11).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	0.617	0.302	1.258	0.184
	Balochistan	0.257	0.133	0.494	0
	Gilgit Baltistan	0.59	0.312	1.115	0.104
	Islamabad	0.841	0.433	1.633	0.61
	Khyber Pakhtunkhwa	1.211	0.652	2.248	0.544
	Punjab	1.125	0.594	2.13	0.718
Season	Winter	1	-	-	-
	Spring	0.125	0.057	0.278	0
	Summer	0.439	0.277	0.695	0
	Autumn	0.262	0.168	0.407	0

Table 10: *The univariable relationships between the likelihood an E. coli isolate is susceptible to Ceftazidime using disc diffusion method and two independent variables (Province and season).*

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	0.58	0.279	1.207	0.145
	Balochistan	0.269	0.135	0.535	0
	Gilgit Baltistan	0.567	0.288	1.116	0.101
	Islamabad	0.923	0.468	1.819	0.817
	Khyber Pakhtunkhwa	1.374	0.718	2.63	0.337
	Punjab	0.967	0.502	1.863	0.921
Season	Winter	1	-	-	-
	Spring	0.101	0.043	0.235	0
	Summer	0.37	0.229	0.598	0
	Autumn	0.29	0.182	0.462	0

Table 11: *The multivariable relationships between the likelihood an E. coli isolate is susceptible to Ceftazidime using disc diffusion method and two independent variables (Province and season).*

5.1.5 Poultry *E. coli* Isolates with Chloramphenicol

A logistic regression model was used to examine the magnitude of association between the susceptibility of *E. coli* against *Chloramphenicol* as the outcome variable and the province and season as the potential risk factors. For analysis purpose, all the isolates classified as intermediate with disc diffusion method, were categorized as resistant.

The results of the bivariable model indicated a statistically significant association between *E. coli* isolates recovered from Gilgit Baltistan (GB) and Punjab and susceptibility to *Chloramphenicol* considering the providence Sindh as the reference (i.e., OR=1). The isolated recovered from GB and Punjab were 2.84 and 2.04 times more likely to be resistant to *Chloramphenicol* compared to isolates recovered from Sindh respectively. Similarly, observations in spring, summer and autumn were statistically not different in their susceptibility to *Chloramphenicol* considering the providence winter as the reference (i.e., OR=1) (Table 12). The multivariable analysis also indicated a statistically significant association between *E. coli* isolates recovered from GB and Punjab and susceptibility to *Chloramphenicol*. The *E. coli* isolates recovered from GB and Punjab were 2.86 and 2.25 times more likely to be resistant to *Chloramphenicol* compared to Sindh respectively (Table 13).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	0.399	0.156	1.022	0.056
	Balochistan	0.851	0.417	1.736	0.657
	Gilgit Baltistan	0.352	0.157	0.79	0.011
	Islamabad	0.867	0.428	1.757	0.693
	Khyber Pakhtunkhwa	0.713	0.373	1.362	0.305
	Punjab	0.489	0.239	0.998	0.049
Season	Winter	1	-	-	-
	Spring	0.673	0.222	2.041	0.485
	Summer	0.69	0.397	1.198	0.188
	Autumn	1.728	1.083	2.758	0.022

Table 12: *The bivariable relationships between the likelihood an E. coli isolate is susceptible to Chloramphenicol using disc diffusion method and two independent variables (Province and season).*

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	0.395	0.152	1.025	0.056
	Balochistan	0.607	0.289	1.275	0.187
	Gilgit Baltistan	0.349	0.152	0.802	0.013
	Islamabad	0.74	0.359	1.526	0.415
	Khyber Pakhtunkhwa	0.753	0.386	1.468	0.405
	Punjab	0.443	0.214	0.919	0.029
Season	Winter	1	-	-	-
	Spring	0.68	0.217	2.134	0.509
	Summer	0.597	0.339	1.05	0.073
	Autumn	1.562	0.956	2.552	0.075

Table 13*: The multivariable relationships between the likelihood an E. coli isolate is susceptible to Chloramphenicol using disc diffusion method and two independent variables (Province and season).*

5.1.6 Poultry *E. coli* Isolates with Ciprofloxacin

A logistic regression model was used to examine the magnitude of association between the susceptibility of *E. coli* against *Ciprofloxacin* as the outcome variable and the province and season as the potential risk factors. For analysis purpose, all the isolates classified as intermediate with disc diffusion method, were categorized as resistant.

The results of the bivariable model indicated a statistically significant association between *E. coli* isolates recovered from Islamabad and susceptibility to *Ciprofloxacin* considering the providence Sindh as the reference (i.e., OR=1). The *E. coli* isolates recovered from Islamabad were 4.46 times more likely to be resistant to *Ciprofloxacin* compared to Isolates recovered from Sindh. However, observations in spring, summer and autumn were statistically not different in their susceptibility to *Ciprofloxacin* considering the providence winter as the reference (i.e., OR=1) (Table 14). The multivariable analysis also indicated a statistically significant association between *E. coli* isolates recovered from Islamabad and susceptibility to *Ciprofloxacin*. The *E. coli* isolates recovered from Islamabad were 4.71 times more likely to be resistant to *Ciprofloxacin* compared to Sindh (Table 15).

Variable	Variable	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	0.633	0.22	1.822	0.397
	Balochistan	0.36	0.119	1.089	0.07
	Gilgit Baltistan	0.514	0.196	1.348	0.176
	Islamabad	0.224	0.06	0.838	0.026
	Khyber Pakhtunkhwa	0.928	0.416	2.068	0.855
	Punjab	0.766	0.328	1.788	0.538
Season	Winter	1	-	-	-
	Spring	0.547	0.122	2.45	0.43
	Summer	0.962	0.508	1.823	0.906
	Autumn	0.892	0.475	1.676	0.723

Table 14: *The univariable relationships between the likelihood an E. coli isolate is susceptible to Ciprofloxacin using disc diffusion method and two independent variables (Province and season).*

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	0.647	0.224	1.868	0.421
	Balochistan	0.329	0.106	1.023	0.055
	Gilgit Baltistan	0.548	0.205	1.465	0.231
	Islamabad	0.212	0.056	0.801	0.022
	Khyber Pakhtunkhwa	1.007	0.446	2.274	0.987
	Punjab	0.746	0.317	1.758	0.503
Season	Winter	1	-	-	-
	Spring	0.476	0.103	2.193	0.341
	Summer	0.882	0.458	1.698	0.708
	Autumn	1.122	0.58	2.17	0.733

Table 15*: The multivariable relationships between the likelihood an E. coli isolate is susceptible to Ciprofloxacin using disc diffusion method and two independent variables (Province and season).*

5.1.7 Poultry *E. coli* Isolates with Gentamicin

A logistic regression model was used to examine the magnitude of association between the susceptibility of *E. coli* against Gentamicin as the outcome variable and the province and season as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant.

The results of the bivariable model indicated a statistically significant association between *E. coli* isolates recovered from Balochistan and susceptibility to Gentamicin considering the providence Sindh as the reference (i.e., OR=1). The isolated recovered from Balochistan were 2.09 times more likely to be resistant to Gentamicin compared to isolates recovered from Sindh. Similarly, observations in summer were statistically different in their susceptibility to Gentamicin, considering the providence winter as the reference (i.e., OR=1). The *E. coli* isolates recovered in summer were 1.52 times more likely to be resistant to Gentamicin compared to winter in bivariable model (Table 16). The multivariable analysis also indicated a statistically significant association between *E. coli* isolates recovered from province Balochistan and in seasons summer and susceptibility to Gentamicin. The *E. coli* isolates recovered from Balochistan were 2.29 times more likely to be resistant to Gentamicin compared to Sindh. Similarly, the *E. coli* isolates recovered in summer were 1.72 times more likely to be resistant to Gentamicin compared to isolates recovered in winter (Table 17).

Variable	Category	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	0.887	0.445	1.767	0.732
	Balochistan	0.477	0.255	0.891	0.02
	Gilgit Baltistan	0.672	0.366	1.235	0.201
	Islamabad	0.669	0.36	1.246	0.205
	Khyber Pakhtunkhwa	0.996	0.563	1.761	0.988
	Punjab	0.607	0.339	1.088	0.093
Season	Winter	1	-	-	-
	Spring	1.028	0.478	2.209	0.944
	Summer	0.657	0.444	0.973	0.036
	Autumn	0.693	0.473	1.016	0.06

Table 16: *The univariable relationships between the likelihood an E. coli isolate is susceptible to Gentamicin using disc diffusion method and two independent variables (Province and season).*

Variable	Category	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	0.855	0.427	1.713	0.659
	Balochistan	0.436	0.228	0.832	0.012
	Gilgit Baltistan	0.579	0.308	1.085	0.088
	Islamabad	0.655	0.35	1.228	0.187
	Khyber Pakhtunkhwa	0.936	0.524	1.671	0.822
	Punjab	0.547	0.303	1	0.046
Season	Winter	1	-	-	-
	Spring	0.911	0.412	2.011	0.817
	Summer	0.581	0.387	0.872	0.009
	Autumn	0.733	0.49	1.096	0.13

Table 17: *The multivariable relationships between the likelihood an E. coli isolate is susceptible to Gentamicin using disc diffusion method and two independent variables (Province and season).*

5.1.8 Poultry *E. coli* Isolates with Nalidixic Acid

A logistic regression model was used to examine the magnitude of association between the susceptibility of *E. coli* against Nalidixic Acid as the outcome variable and the province and season as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant. The statistical analysis was conducted using software IBM SPSS Statistics.

The results of bivariable model indicated that none of the study provinces is statistically significantly difference in their association with susceptibility to Nalidixic Acid considering the providence Sindh as the reference (i.e., OR=1). Similarly, observations in summer or autumn were not statistically different in their susceptibility to Nalidixic Acid considering the providence winter as the reference (i.e., OR=1) (Table 18).

Variable	Category	Odds ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	0.617	0.109	3.483	0.584
	Balochistan	0.677	0.147	3.124	0.617
	Gilgit Baltistan	1.392	0.393	4.938	0.608
	Islamabad	0.21	0.023	1.922	0.167
	Khyber Pakhtunkhwa	1.475	0.454	4.793	0.518
	Punjab	1.461	0.434	4.915	0.541
Season	Winter	1	-	-	-
	Spring	1.227	0.335	4.493	0.758
	Summer	0.668	0.299	1.491	0.325
	Autumn	0.421	0.175	1.015	0.054

Table 18: *The univariable relationships between the likelihood an E. coli isolate is susceptible to Nalidixic Acid using disc diffusion method and two independent variables (Province and season).*

5.1.9 Poultry *E. coli* Isolates with Tetracycline

A logistic regression model was used to examine the magnitude of association between the susceptibility of *E. coli* against Tetracycline as the outcome variable and the province and season as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant. The statistical analysis was conducted using software IBM SPSS Statistics.

The results of the bivariable model indicated that none of the study provinces are statistically significantly difference in their association with susceptibility to Tetracycline considering the providence Sindh as the reference (i.e., OR=1). Similarly, observations in summer or autumn were not statistically different in their susceptibility to Tetracycline considering the providence winter as the reference (i.e., OR=1) (Table 19).

Variable	Category	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	0.379	0.074	1.948	0.245
	Balochistan	0.698	0.204	2.389	0.567
	Gilgit Baltistan	0.728	0.225	2.357	0.597
	Islamabad	1.295	0.426	3.931	0.649
	Khyber Pakhtunkhwa	0.711	0.229	2.204	0.554
	Punjab	0.692	0.223	2.145	0.523

Season	1	-	-	-	-
	Summer	1.216	0.482	3.063	0.679
	Autumn	2.165	0.973	4.82	0.059

Table 19: *The univariable relationships between the likelihood an E. coli isolate is susceptible to Tetracycline using disc diffusion method and two independent variables (Province and season).*

5.1.10 Poultry *E. coli* isolates with Trimethoprim

A logistic regression model was used to examine the magnitude of association between the susceptibility of *E. coli* against Trimethoprim as the outcome variable and the province and season as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant. The statistical analysis was conducted using software IBM SPSS Statistics.

The results of the bivariable model indicated that none of the study provinces are statistically significantly difference in their association with susceptibility to Trimethoprim considering the providence Sindh as the reference (i.e., OR=1). Similarly, observations in summer or autumn were not statistically different in their susceptibility to Trimethoprim considering the providence winter as the reference (i.e., OR=1) (Table 20).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	0.321	0.031	3.287	0.338
	Balochistan	0.369	0.058	2.351	0.291
	Gilgit Baltistan	1.182	0.272	5.131	0.824
	Islamabad	1.707	0.449	6.486	0.432
	Khyber Pakhtunkhwa	1.444	0.388	5.376	0.583
	Punjab	2.453	0.652	9.232	0.185
Season	Winter	1	-	-	-
	Spring	0.937	0.325	2.701	0.903
	Summer	0.51	0.231	1.125	0.095
	Autumn	0.589	0.279	1.244	0.166

Table 20: The univariable relationships between the likelihood an E. coli isolate is susceptible to Trimethoprim using disc diffusion method and two independent variables (Province and season).

5.2 Risk Factors Association of Cattle and Buffaloes *E. coli* Isolates

5.2.1 Cattle and Buffaloes *E. coli* Isolates with Ampicillin

A logistic regression model was used to examine the magnitude of association between the susceptibility of *E. coli* against *Ampicillin* as the outcome variable and the province and specie as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant. The statistical analysis was conducted using software IBM SPSS Statistics.

The results of the bivariable model indicated that none of the study provinces is statistically significantly difference in their association with susceptibility to *Ampicillin* considering the providence Sindh as the reference (i.e., OR=1). There was also no significant relationship between susceptibility to *Ampicillin* and specie of the animal (Table 21).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-

	Khyber Pakhtunkhwa	5.187	1.039	25.89	0.045
	Punjab	1.537	0.21	11.241	0.672
Specie	Buffalo	1	-	-	-
	Cattle	1.627	0.461	5.741	0.449

Table 21: *The bivariable relationships between the likelihood an E. coli isolate is susceptible to Ampicillin using disc diffusion method and two independent variables (Province and specie).*

5.2.2 Cattle and Buffaloes *E. coli* Isolates with Cefotaxime

A logistic regression model was used to examine the magnitude of association between the susceptibility of *E. coli* against *Cefotaxime* as the outcome variable and the province and specie as the potential risk factors. For analysis purpose, all the isolates classified as intermediate with disc diffusion method, were categorized as resistant. The statistical analysis was conducted using software IBM SPSS Statistics.

The results of the bivariable model indicated a statistically significant association between *E. coli* isolates recovered from KP and susceptibility to *Cefotaxime* considering the providence Sindh as the reference (i.e., OR=1). The isolated recovered from KP were 7.56 times more likely to be susceptible to *Cefotaxime* compared to isolates recovered from Sindh. There was also no significant relationship between susceptibility to *Cefotaxime* and specie of the animal (Table 22).
The multivariable analysis also indicated a statistically significant association between *E. coli* isolates recovered from KP and susceptibility to *Cefotaxime*. The *E. coli* isolates recovered from KP were 9.09 times more likely to be susceptible to *Cefotaxime* compared to Sindh (Table 23).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Khyber Pakhtunkhwa	7.56	2.069	27.624	0.002
	Punjab	1.528	0.297	7.858	0.612
Specie	Buffalo	1	-	-	-
	Cattle	1.096	0.434	2.771	0.846

Table 22*: The bivariable relationships between the likelihood an E. coli isolate is susceptible to Cefotaxime using disc diffusion method and two independent variables (Province and specie).*

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Khyber Pakhtunkhwa	9.098	2.348	35.245	0.001
	Punjab	1.568	0.304	8.084	0.591
Specie	Buffalo	1	-	-	-
	Cattle	0.586	0.209	1.643	0.31

Table 23*: The multivariable relationships between the likelihood an E. coli isolate is susceptible to Cefotaxime using disc diffusion method and two independent variables (Province and specie).*

5.2.3 Cattle and Buffaloes *E. coli* Isolates with Ceftazidime

A logistic regression model was used to examine the magnitude of association between the susceptibility of *E. coli* against Ceftazidime as the outcome variable and the province and specie as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant. The statistical analysis was conducted using software IBM SPSS Statistics.

The results of the bivariable model indicated a statistically significant association between *E. coli* isolates recovered from KP and susceptibility to Ceftazidime considering the providence Sindh as the reference (i.e., OR=1). The isolated recovered from KP were 5.12 times more likely to be susceptible to Ceftazidime compared to isolates recovered from Sindh. Similarly, observations in spring, summer and autumn were not statistically different in their susceptibility to Ceftazidime considering the providence winter as the reference (i.e., OR=1). There was also no significant relationship between susceptibility to Ceftazidime and host specie of the animal (Table 24).
The multivariable analysis indicated a statistically non-significant association between susceptibility of *E. coli* isolates to Ceftazidime and variables: province, season and host specie (Table 25).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Khyber Pakhtunkhwa	5.129	2.462	10.687	0
	Punjab	1.159	0.501	2.682	0.73
Season	Winter	1	-	-	-
	Spring	5	1.503	16.629	0.009
	Summer	2.5	0.749	8.35	0.136
	Autumn	4.821	1.527	15.225	0.007
Specie	Buffalo	1	-	-	-
	Cattle	1.278	0.702	2.324	0.422

Table 24: *The bivariable relationships between the likelihood an E. coli isolate is susceptible to Ceftazidime using disc diffusion method and three independent variables (province, season and specie).*

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh				
	Khyber Pakhtunkhwa	4.295	1.797	10.265	0.001
	Punjab	1.057	0.432	2.586	0.903
Season	Winter				
	Spring	2.018	0.525	7.755	0.307
	Summer	1.915	0.513	7.145	0.334
	Autumn	2.294	0.653	8.062	0.195
Specie	Buffalo	1	-	-	-
	Cattle	0.875	0.43	1.781	0.713

Table 25*: The multivariable relationships between the likelihood an E. coli isolate is susceptible to Ceftazidime using disc diffusion method and three independent variables (province, season and specie).*

5.2.4 Cattle and Buffaloes *E. coli* Isolates with Chloramphenicol

A logistic regression model was used to examine the magnitude of association between the susceptibility of *E. coli* against Chloramphenicol as the outcome variable and the province and host specie as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant. The statistical analysis was conducted using software IBM SPSS Statistics.

The results of the bivariable model indicated a statistically significant association between *E. coli* isolates recovered from KP and susceptibility to Chloramphenicol considering the providence Sindh as the reference (i.e., OR=1). The isolates recovered from KP were 8.35 times more likely to be susceptible to Chloramphenicol compared to isolates recovered from Sindh. The observations in spring, summer and autumn were statistically different in their susceptibility to Chloramphenicol considering the providence winter as the reference (i.e., OR=1). The isolates recovered in spring, summer and autumn were 12.96, 12.84 and 14.10 times more likely to be susceptible to

Chloramphenicol compared to isolates recovered in winter respectively. There was no significant relationship between susceptibility to Chloramphenicol and host specie of the animal (Table 26).
The multivariable analysis indicated a statistically non-significant association between susceptibility of *E. coli* isolates to Chloramphenicol and variables: province and host specie. However, a statistically significant association was observed between the isolates recovered in summer and autumn and their susceptibility to Chloramphenicol. The isolates recovered in summer and autumn were 13.20 and 6.72 times more likely to be susceptible to Chloramphenicol compared to isolates recovered in winter (Table 27).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Khyber Pakhtunkhwa	8.357	3.387	20.621	0
	Punjab	1.579	0.689	3.617	0.28
Season	Winter	1	-	-	-
	Spring	12.963	4.042	41.571	0
	Summer	12.847	4.261	38.733	0
	Autumn	14.103	4.766	41.726	0
Specie	Buffalo	1	-	-	-
	Cattle	0.955	0.508	1.795	0.885

***Table 26**: The bivariable relationships between the likelihood an E. coli isolate is susceptible to Chloramphenicol using disc diffusion method and three independent variables (province, season and host specie).*

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Khyber Pakhtunkhwa	5.782	1.934	17.289	0.002
	Punjab	0.666	0.24	1.845	0.435
Season	Winter	1	-	-	-
	Spring	4.212	1.081	16.41	0.038
	Summer	13.2	3.656	47.657	0
	Autumn	6.724	2.044	22.121	0.002
Specie	Buffalo	1	-	-	-
	Cattle	0.949	0.422	2.137	0.9

Table 27: The multivariable relationships between the likelihood an E. coli isolate is susceptible to Chloramphenicol using disc diffusion method and three independent variables (province, season and specie).

5.2.5 Cattle and Buffaloes *E. coli* Isolates with Ciprofloxacin

A logistic regression model was used to examine the magnitude of association between the susceptibility of *E. coli* against Ciprofloxacin as the outcome variable and the province and specie as the potential risk factors. For analysis purpose, all the isolates classified as intermediate with disc diffusion method, were categorized as resistant. The statistical analysis was conducted using software IBM SPSS Statistics.

The results of the bivariable model indicated a statistically significant association between *E. coli* isolates recovered from KP and susceptibility to Ciprofloxacin considering the providence Sindh as the reference (i.e., OR=1). The isolated recovered from KP were 9.20 times more likely to be susceptible to Ciprofloxacin compared to isolates recovered from Sindh. The observations in spring, summer and autumn were statistically not different in their susceptibility to Ciprofloxacin considering the providence winter as the reference (i.e., OR=1). There was also no significant relationship between susceptibility to Ciprofloxacin and host specie (Table 28).

The multivariable analysis also indicated a statistically significant association between *E. coli* isolates recovered from KP and susceptibility to Ciprofloxacin. The *E. coli* isolates recovered from KP were 9.25 times more likely to be susceptible to Ciprofloxacin compared to Sindh (Table 29).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Khyber Pakhtunkhwa	9.205	2.96	28.624	0
	Punjab	0.736	0.13	4.16	0.729
Season	Winter	1	-	-	-
	Spring	4.024	0.448	36.154	0.214
	Summer	1.833	0.183	18.364	0.606
	Autumn	10.788	1.37	84.936	0.024
Specie	1	-	-	-	-
	Cattle	1.53	0.659	3.554	0.322

***Table 28**: The bivariable relationships between the likelihood an E. coli isolate is susceptible to Ciprofloxacin using disc diffusion method and three independent variables (province, season and specie).*

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Khyber Pakhtunkhwa	9.255	2.441	35.091	0.001
	Punjab	0.849	0.14	5.135	0.859
Season	Winter	1	-	-	-
	Spring	0.752	0.064	8.801	0.82
	Summer	1.031	0.086	12.292	0.981
	Autumn	2.726	0.279	26.675	0.389
Specie	Buffalo	1	-	-	-
	Cattle	0.787	0.288	2.151	0.641

Table 29: The multivariable relationships between the likelihood an E. coli isolate is susceptible to Ciprofloxacin using disc diffusion method and three independent variables (province, season and specie).

5.2.6 Cattle and Buffaloes *E. coli* Isolates with Nalidixic Acid

A logistic regression model was used to examine the magnitude of association between the susceptibility of *E. coli* against Nalidixic Acid as the outcome variable and the province and host specie as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant. The statistical analysis was conducted using software IBM SPSS Statistics.

The results of the bivariable model indicated a statistically significant association between *E. coli* isolates recovered from KP and susceptibility to Nalidixic Acid considering the providence Sindh as the reference (i.e., OR=1). The isolates recovered from KP were 9.91 times more likely to be susceptible to Nalidixic Acid compared to isolates recovered from Sindh. The observations in spring, summer and autumn were statistically different in their susceptibility to Nalidixic Acid considering the providence winter as the reference (i.e., OR=1). The isolates recovered in spring, summer and autumn were 9.04, 8.95 and 9.74 times more likely to be susceptible to Nalidixic Acid compared to isolates recovered in winter (OR=1) respectively. There was no significant relationship between susceptibility to Nalidixic Acid and host specie (Table 30).

The multivariable analysis also indicated a statistically significant association between susceptibility of *E. coli* isolates to Nalidixic Acid and KP province. The isolates recovered from KP were 11.10 times more likely to be susceptible to Nalidixic Acid compared to isolates recovered from Sindh. However,

statistically there was no significant association between susceptibility of *E. coli* isolates to Nalidixic Acid and the variables: season and host specie (Table 31).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Khyber Pakhtunkhwa	9.918	4.616	21.31	0
	Punjab	2.71	1.261	5.822	0.011
Season	Winter	1	-	-	-
	Spring	9.042	2.411	33.911	0.001
	Summer	8.956	2.45	32.737	0.001
	Autumn	9.743	2.717	34.934	0
Specie	Buffalo	1	-	-	-
	Cattle	1.021	0.583	1.788	0.943

Table 30: The bivariable relationships between the likelihood an E. coli isolate is susceptible to Nalidixic Acid using disc diffusion method and three independent variables (Province, season and specie).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Khyber Pakhtunkhwa	11.104	4.306	28.63	0
	Punjab	2.175	0.946	5	0.067
Season	Winter	1	-	-	-
	Spring	2.148	0.493	9.348	0.308
	Summer	4.742	1.197	18.786	0.027
	Autumn	3.144	0.79	12.507	0.104
Specie	Buffalo	1	-	-	-
	Cattle	0.652	0.314	1.354	0.252

Table 31: The multivariable relationships between the likelihood an E. coli isolate is susceptible to Nalidixic Acid using disc diffusion method and three independent variables (province, season and specie).

5.2.7 Cattle and Buffaloes *E. coli* Isolates with Tetracycline

A logistic regression model was used to examine the magnitude of association between the susceptibility of *E. coli* against Tetracycline as the outcome variable and the province and host specie as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant. The statistical analysis was conducted using software IBM SPSS Statistics.

The results of the bivariable model indicated that none of the provinces under study is statistically significantly different in their association with susceptibility to Tetracycline considering the providence Sindh as the reference (i.e., OR=1). There was also no significant relationship between susceptibility to Tetracycline and host specie (Table 32).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Khyber Pakhtunkhwa	4.613	1.923	11.066	0.001
	Punjab	1.011	0.338	3.027	0.984
Specie	Buffalo	1	-	-	-
	Cattle	2.175	1.084	4.363	0.029

Table 32: *The bivariable relationships between the likelihood an E. coli isolate is susceptible to Tetracycline using disc diffusion method and two independent variables (province and specie).*

5.2.8 Cattle and Buffaloes *E. coli* Isolates with Trimethoprim

A logistic regression model was used to examine the magnitude of association between the susceptibility of *E. coli* against Trimethoprim as the outcome variable and the province and host specie as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant. The statistical analysis was conducted using software IBM SPSS Statistics.

The results of the bivariable model indicated that none of the study provinces is statistically significantly difference in their association with susceptibility to Trimethoprim considering the providence Sindh as the reference (i.e., OR=1). There was also no significant relationship between susceptibility to Trimethoprim and host specie (Table 33).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Khyber Pakhtunkhwa	3.547	1.646	7.647	0.001
	Punjab	0.511	0.222	1.176	0.114
Specie	Buffalo	1	-	-	-
	Cattle	1.5	0.816	2.757	0.192

Table 33: The bivariable relationships between the likelihood an E. coli isolate is susceptible to Trimethoprim using disc diffusion method and two independent variables (province and specie).

5.3 Risk Factors Association of Poultry *Salmonella* Isolates

5.3.1 Poultry *Salmonella* Isolates with Ampicillin

A logistic regression model was used to examine the magnitude of association between the susceptibility of *Salmonella* against Ampicillin as the outcome variable and the province and season as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant.

The results indicated no significant association between susceptibility of poultry *Salmonella* isolates against Ampicillin with disc diffusion method and independent variable province in bivariable and multivariable models considering the province Sindh as the reference (i.e., OR=1). A significant relationship was observed between susceptibility of *Salmonella* isolates to Ampicillin and season in bivariable model considering the season winter as the reference (i.e., OR=1). The *Salmonella* isolates recovered in summer and autumn were 3.44 and 4.56 times more likely to be resistant to Ampicillin compared to winter in bivariable model respectively (Table 34). Similarly, in multivariable model the *Salmonella* isolates recovered in summer and autumn were 4.13 and 5.55 times more likely to be resistant to Ampicillin compared to isolates recovered in winter (Table 35).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Balochistan	0.656	0.215	2.004	0.46
	Islamabad	0.729	0.312	1.705	0.466
	Khyber Pakhtunkhwa	0.625	0.231	1.691	0.355
	Punjab	0.875	0.353	2.168	0.773
Season	Winter	1	-	-	-
	Spring	0.306	0.081	1.158	0.081
	Summer	0.29	0.109	0.771	0.013
	Autumn	0.219	0.105	0.455	0

***Table 34**: The bivariable relationships between the likelihood a Salmonella isolate is susceptible to Ampicillin using disc diffusion method and two independent variables (province and season).*

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Balochistan	0.612	0.182	2.051	0.426
	Islamabad	0.43	0.166	1.116	0.083
	Khyber Pakhtunkhwa	0.519	0.178	1.515	0.23
	Punjab	0.792	0.296	2.117	0.642
Season	Winter	1	-	-	-
	Spring	0.263	0.065	1.059	0.06
	Summer	0.242	0.084	0.7	0.009
	Autumn	0.181	0.083	0.396	0

***Table 35:** The multivariable relationships between the likelihood a Salmonella isolate is susceptible to Ampicillin using disc diffusion method and two independent variables (province and season).*

5.3.2 Poultry *Salmonella* Isolates with Azithromycin

A logistic regression model was used to examine the magnitude of association between the susceptibility of *Salmonella* against Azithromycin as the outcome variable and the province and season as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant. The statistical analysis was conducted using software IBM SPSS Statistics.

The results of bivariable model indicated that none of the study provinces are statistically significantly difference in their association with susceptibility to Azithromycin considering the providence Sindh as the reference (i.e., OR=1). Similarly, observations in summer or autumn were not statistically different in their susceptibility to Azithromycin considering the providence winter as the reference (i.e., OR=1) (Table 36).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Balochistan	1.339	0.366	4.905	0.659
	Islamabad	0.504	0.155	1.641	0.255
	Khyber Pakhtunkhwa	1.25	0.385	4.056	0.71
	Punjab	0.476	0.127	1.784	0.271
Season	Winter	1	-	-	-
	Summer	0.337	0.068	1.684	0.185
	Autumn	1.065	0.439	2.581	0.889

***Table 36:** The bivariable relationships between the likelihood a Salmonella isolate is susceptible to Azithromycin using disc diffusion method and two independent variables (Province and season).*

5.3.3 Poultry *Salmonella* Isolates with Cefotaxime

A logistic regression model was used to examine the magnitude of association between the susceptibility of *Salmonella* against Cefotaxime as the outcome variable and the province and season as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant. The statistical analysis was conducted using software IBM SPSS Statistics.

The results of the bivariable model indicated that none of the study provinces are statistically significantly different in their association with susceptibility to Cefotaxime considering the providence Sindh as the reference (i.e., OR=1). Similarly, observations in spring, summer or autumn were not

statistically different in their susceptibility to Cefotaxime considering the providence winter as the reference (i.e., OR=1) (Table 37).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Balochistan	1.354	0.397	4.618	0.628
	Islamabad	1.769	0.675	4.64	0.246
	Khyber Pakhtunkhwa	0.888	0.317	2.492	0.822
	Punjab	1.269	0.465	3.466	0.642
Season	Winter	1	-	-	-
	Spring	0.389	0.107	1.411	0.151
	Summer	1.021	0.367	2.836	0.968
	Autumn	1.556	0.702	3.449	0.277

Table 37: The bivariable relationships between the likelihood a Salmonella isolate is susceptible to Cefotaxime using disc diffusion method and two independent variables (province and season).

5.3.4 Poultry *Salmonella* Isolates with Ceftazidime

A logistic regression model was used to examine the magnitude of association between the susceptibility of *Salmonella* against Ceftazidime as the outcome variable and the province and season as the potential risk factors. For analysis purpose, all the isolates classified as intermediate with disc diffusion method, were categorized as resistant.

The results of the bivariable model indicated a statistically significant association between *Salmonella* isolates recovered from KP and susceptibility to Ceftazidime considering the providence Sindh as the reference (i.e., OR=1). The *Salmonella* isolates recovered from KP were 4.95 times more likely to be resistant to Ceftazidime compared to Isolates recovered from Sindh. However, observations in spring, summer and autumn were statistically not different in their susceptibility to Ceftazidime considering the winter as the reference (i.e., OR=1) (Table 38). The multivariable analysis also indicated a statistically significant association between *Salmonella* isolates recovered from KP and susceptibility to Ceftazidime. The *Salmonella* isolates recovered from KP were 4.31 times more likely to be resistant to Ceftazidime compared to Sindh (Table 39).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Balochistan	0.379	0.114	1.265	0.115
	Islamabad	0.754	0.269	2.115	0.591
	Khyber Pakhtunkhwa	0.202	0.067	0.613	0.005
	Punjab	0.804	0.265	2.434	0.699
Season	Winter	1	-	-	-
	Spring	0.324	0.08	1.315	0.115
	Summer	0.374	0.139	1.005	0.051
	Autumn	1.061	0.473	2.38	0.885

Table 38: *The bivariable relationships between the likelihood a Salmonella isolate is susceptible to Ceftazidime using disc diffusion method and two independent variables (province and season).*

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Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Balochistan	0.552	0.155	1.966	0.359
	Islamabad	0.712	0.245	2.071	0.532
	Khyber Pakhtunkhwa	0.232	0.075	0.72	0.011

	Punjab	1.038	0.325	3.309	0.95
Season	Winter	1	-	-	-
	Spring	0.386	0.084	1.773	0.221
	Summer	0.375	0.126	1.12	0.079
	Autumn	1.054	0.451	2.46	0.904

Table 39: *The multivariable relationships between the likelihood of Salmonella isolate is susceptible to Ceftazidime using disc diffusion method and two independent variables (Province and season)*

5.3.5 Poultry *Salmonella* Isolates with Chloramphenicol

A logistic regression model was used to examine the magnitude of association between the susceptibility of *Salmonella* against Chloramphenicol as the outcome variable and the province and season as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant.

The results indicated no significant association between susceptibility of poultry *Salmonella* isolates against Chloramphenicol with disc diffusion method and independent variable province in bivariable and multivariable models considering the province Sindh as the reference (i.e., OR=1). A significant relationship was observed between susceptibility of *Salmonella* isolates to Chloramphenicol and season in bivariable model considering the season winter as the reference (i.e., OR=1). The *Salmonella* isolates recovered in summer and autumn were 3.58 and 2.87 times more likely to be resistant to Chloramphenicol compared to winter in bivariable model respectively (Table 40). Similarly, in multivariable model the *Salmonella* isolates recovered in summer and autumn were 4.06 and 2.80 times more likely to be resistant to Chloramphenicol compared to isolates recovered in winter (Table 41).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Balochistan	1.75	0.527	5.812	0.361
	Islamabad	1.658	0.631	4.353	0.305
	Khyber Pakhtunkhwa	1.925	0.656	5.648	0.233
	Punjab	1.556	0.55	4.397	0.405
Season	Winter	1	-	-	-
	Spring	2.455	0.652	9.241	0.184
	Summer	0.279	0.091	0.857	0.026
	Autumn	0.348	0.166	0.732	0.005

Table 40*: The bivariable relationships between the likelihood a Salmonella isolate is susceptible to Chloramphenicol using disc diffusion method and two independent variables (Province and season).*

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Balochistan	1.777	0.48	6.585	0.389
	Islamabad	1.455	0.524	4.039	0.471
	Khyber Pakhtunkhwa	1.75	0.559	5.482	0.337
	Punjab	1.729	0.574	5.205	0.33
Season	Winter	1	-	-	-
	Spring	2.321	0.586	9.193	0.231
	Summer	0.246	0.076	0.802	0.02
	Autumn	0.356	0.166	0.763	0.008

Table 41: The multivariable relationships between the likelihood a Salmonella isolate is susceptible to Chloramphenicol using disc diffusion method and two independent variables (province and season).

5.3.6 Poultry *Salmonella* Isolates with Ciprofloxacin

A logistic regression model was used to examine the magnitude of association between the susceptibility of *Salmonella* against Ciprofloxacin as the outcome variable and the province and season as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant. The statistical analysis was conducted using software IBM SPSS Statistics.

The results of the bivariable model indicated that none of the study provinces is statistically significantly different in their association with susceptibility to Ciprofloxacin considering the providence Sindh as the reference (i.e., OR=1). Similarly, observations in spring, summer or autumn were not statistically different in their susceptibility to Ciprofloxacin considering the providence winter as the reference (i.e., OR=1) (Table 42).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Balochistan	0.875	0.075	10.268	0.915
	Islamabad	3.043	0.608	15.236	0.176
	Khyber Pakhtunkhwa	0.583	0.05	6.756	0.666
	Punjab	3.182	0.599	16.893	0.174
Season	Winter	1	-	-	-
	Spring	0.621	0.068	5.715	0.674
	Summer	0.244	0.028	2.139	0.203
	Autumn	0.813	0.277	2.393	0.708

Table 42: *The bivariable relationships between the likelihood a Salmonella isolate is susceptible to Ciprofloxacin using disc diffusion method and two independent variables (province and season).*

5.3.7 Poultry *Salmonella* Isolates with Gentamicin

A logistic regression model was used to examine the magnitude of association between the susceptibility of *Salmonella* against Gentamicin as the outcome variable and the province and season as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant.

The results of the bivariable model indicated a statistically significant association between *Salmonella* isolates recovered from KP and susceptibility to Gentamicin considering the providence Sindh as the reference (i.e., OR=1). The isolates recovered from KP were 4.40 times more likely to be resistant to Gentamicin compared to isolates recovered from Sindh. Similarly, observations in summer were statistically different in their susceptibility to Gentamicin considering the winter as the reference (i.e., OR=1). The *Salmonella* isolates recovered in spring were 3.38 times more likely to be resistant to Gentamicin compared to winter in bivariable model respectively (Table 43). The multivariable analysis also indicated a statistically significant association between *Salmonella* isolates recovered from province KP and their susceptibility to Gentamicin. The *Salmonella* isolates recovered from KP were 4.25 times more likely to be resistant to Gentamicin compared to Sindh (Table 44).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Balochistan	0.368	0.115	1.18	0.093
	Islamabad	0.847	0.315	2.276	0.742
	Khyber Pakhtunkhwa	0.227	0.079	0.652	0.006
	Punjab	0.46	0.167	1.264	0.132

Season	Winter	1	-	-	-
	Spring	0.442	0.118	1.652	0.225
	Summer	0.295	0.111	0.782	0.014
	Autumn	0.652	0.299	1.419	0.281

Table 43: *The bivariable relationships between the likelihood a Salmonella isolate is susceptible to Gentamicin using disc diffusion method and two independent variables (province and season).*

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Balochistan	0.445	0.132	1.497	0.191
	Islamabad	0.772	0.281	2.123	0.616
	Khyber Pakhtunkhwa	0.235	0.08	0.689	0.008
	Punjab	0.524	0.185	1.484	0.223
Season	Winter	1	-	-	-
	Spring	0.6	0.149	2.421	0.473
	Summer	0.356	0.126	1.008	0.052
	Autumn	**0.654**	**0.29**	**1.471**	**0.304**

Table 44*: The multivariable relationships between the likelihood a Salmonella isolate is susceptible to Gentamicin using disc diffusion method and two independent variables (province and season).*

5.3.8 Poultry *Salmonella* Isolates with Trimethoprim

A logistic regression model was used to examine the magnitude of association between the susceptibility of *Salmonella* against Trimethoprim as the outcome variable and the province and season as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant.

The results indicated no significant association between susceptibility of poultry *Salmonella* isolates against Trimethoprim with disc diffusion method and independent variable province in bivariable and multivariable models considering the province Sindh as the reference (i.e., OR=1). A significant relationship was observed between susceptibility of *Salmonella* isolates to Trimethoprim and season in bivariable model considering the season winter as the reference (i.e., OR=1). The *Salmonella* isolates recovered in summer and autumn were 17.54 and 83.33 times more likely to be resistant to Trimethoprim compared to winter in bivariable model respectively (Table 45). Similarly, in multivariable model the *Salmonella* isolates recovered in summer and autumn were 20 and 90.90 times more likely to be resistant to Trimethoprim compared to isolates recovered in winter (Table 46).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Islamabad	2.857	0.865	9.439	0.085
	Khyber Pakhtunkhwa	2.182	0.551	8.644	0.267
	Punjab	2.286	0.621	8.412	0.214
Season	Winter	1	-	-	-
	Summer	0.057	0.007	0.459	0.007
	Autumn	0.012	0.001	0.089	0

Table 45: *The bivariable relationships between the likelihood a Salmonella isolate is susceptible to Trimethoprim using disc diffusion method and two independent variables (province and season).*

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Islamabad	3.173	0.54	18.65	0.201

	Khyber Pakhtunkhwa	2.924	0.358	23.886	0.317
	Punjab	4.744	0.618	36.387	0.134
Season	Winter	1	-	-	-
	Summer	0.05	0.006	0.447	0.007
	Autumn	0.011	0.001	0.09	0

Table 46: *The multivariable relationships between the likelihood a Salmonella isolate is susceptible to Trimethoprim using disc diffusion method and two independent variables (province and season).*

A table summarizing the susceptibility of poultry *E. coli* and *Salmonella* isolates with the list of antibiotics and significant factors is given in Annexure 2.

5.4 Risk Factors Association of Cattle and Buffaloes *Enterococcus* Isolates

5.4.1 Cattle and Buffaloes *Enterococcus* Isolates with Ampicillin

A logistic regression model was used to examine the magnitude of association between the susceptibility of *Enterococcus* against Ampicillin as the outcome variable and the province, season and specie as the potential risk factors. For analysis purpose, all the isolates classified as intermediate with disc diffusion method, were categorized as resistant.

The results of the bivariable model indicated a statistically significant association between *Enterococcus* isolates recovered from Khyber Pakhtunkhwa (KP) and Punjab and susceptibility to Ampicillin considering the providence Sindh as the reference (i.e., OR=1). The isolated recovered from KP and Punjab were 4.40 and 3.44 times more likely to be resistant to Ampicillin compared to isolates recovered from Sindh. The observations in spring, summer and autumn were statistically not different in their susceptibility to Ampicillin considering the providence winter as the reference (i.e., OR=1). However, the isolates recovered from cattle were 1.85 times more likely to be resistant to Ampicillin compared to isolates recovered from buffalo (Table 47).
The multivariable analysis also indicated a statistically significant association between *Enterococcus* isolates recovered from KP and Punjab and susceptibility to Ampicillin. The *Enterococcus* isolates recovered from KP, and Punjab were 3.43 and 2.85 times more likely to be resistant to Ampicillin compared to Sindh. There was no significant relationship between susceptibility to Ampicillin and variables: province and specie of the animal (Table 48).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	0.538	0.246	1.179	0.121
	Balochistan	0.56	0.244	1.287	0.172
	Gilgit Baltistan	0.943	0.412	2.154	0.888
	Islamabad	0.563	0.252	1.257	0.161
	Khyber Pakhtunkhwa	0.227	0.101	0.508	0
	Punjab	0.29	0.111	0.755	0.011
Season	Winter	1	-	-	-
	Spring	0.626	0.316	1.242	0.18
	Summer	0.791	0.434	1.443	0.445
	Autumn	1.273	0.686	2.361	0.444
Specie	Buffalo	1	-	-	-
	Cattle	**0.538**	**0.359**	**0.806**	**0.003**

Table 47*: The bivariable relationships between the likelihood an Enterococcus isolate is susceptible to Ampicillin using disc diffusion method and three independent variables (province, season and specie).*

Variable	Categories	Odds Ratio	(95% CI)	P value

Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	0.699	0.299	1.635	0.409
	Balochistan	0.758	0.307	1.87	0.548
	Gilgit Baltistan	0.972	0.422	2.238	0.948
	Islamabad	0.645	0.283	1.472	0.298
	Khyber Pakhtunkhwa	0.291	0.124	0.684	0.005
	Punjab	0.35	0.132	0.932	0.036
Season	Winter	1	-	-	-
	Spring	0.667	0.326	1.362	0.266
	Summer	0.836	0.448	1.561	0.574
	Autumn	1.263	0.665	2.396	0.476
Specie	Buffalo	1	-	-	-
	Cattle	0.666	0.407	1.088	0.104

Table 48: *The multivariable relationships between the likelihood an Enterococcus isolate is susceptible to Ampicillin using disc diffusion method and three independent variables (Province, season and specie).*

5.4.2 Cattle and Buffaloes *Enterococcus* isolates with Chloramphenicol

A logistic regression model was used to examine the magnitude of association between the susceptibility of *Enterococcus* against Chloramphenicol as the outcome variable and the province, season and specie as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant. The statistical analysis was conducted using software IBM SPSS Statistics.

The results of the bivariable model indicated that none of the study provinces is statistically significantly difference in their association with susceptibility to Chloramphenicol considering the province Sindh as the reference (i.e., OR=1). Similarly, observations in spring, summer and autumn were not statistically different in their susceptibility to Chloramphenicol considering the winter as the reference (i.e., OR=1). There was also no significant relationship between susceptibility to Chloramphenicol and specie of the animal (Table 49).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	0.84	0.38	1.86	0.668
	Balochistan	1.147	0.483	2.723	0.757
	Gilgit Baltistan	1.093	0.474	2.52	0.836
	Islamabad	0.922	0.406	2.093	0.845
	Khyber Pakhtunkhwa	0.483	0.22	1.061	0.07
	Punjab	0.922	0.35	2.429	0.869
Season	Winter	1	-	-	-
	Spring	0.621	0.301	1.281	0.197
	Summer	0.67	0.351	1.277	0.223
	Autumn	0.798	0.412	1.548	0.505
Specie	Buffalo	1	-	-	-
	Cattle	0.734	0.485	1.112	0.145

Table 49*: The bivariable relationships between the likelihood an Enterococcus isolate is susceptible to Chloramphenicol using disc diffusion method and three independent variables (province, season and specie)*

5.4.3 Cattle and buffaloes *Enterococcus* isolates with Erythromycin

A logistic regression model was used to examine the magnitude of association between the susceptibility of *Enterococcus* against Erythromycin as the outcome variable and the province, season and specie as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant. The statistical analysis was conducted using software IBM SPSS Statistics.

The results of the bivariable model indicated that none of the study provinces is statistically significantly different in their association with susceptibility to Erythromycin considering the providence Sindh as the reference (i.e., OR=1). Similarly, observations in spring, summer and autumn were not statistically different in their susceptibility to Erythromycin considering the providence winter as the reference (i.e., OR=1). However, there was a statistically significant relationship between specie of the animal and susceptibility of *Enterococcus* isolates to Erythromycin. The isolates recovered from cattle were 2.40 times more likely to be resistant to Erythromycin compared to isolates recovered from buffalo (Table 50).
The multivariable analysis indicated no statistically significant association between susceptibility to Erythromycin and variables: province, season and specie (Table 51).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	0.703	0.278	1.777	0.457
	Balochistan	1.389	0.555	3.478	0.482
	Gilgit Baltistan	0.792	0.307	2.042	0.629
	Islamabad	0.398	0.139	1.145	0.088
	Khyber Pakhtunkhwa	0.55	0.208	1.457	0.229
	Punjab	1.729	0.626	4.776	0.291
Season	Winter	1	-	-	-
	Spring	1.669	0.705	3.952	0.244
	Summer	1.591	0.73	3.469	0.243
	Autumn	0.818	0.35	1.913	0.643
Specie	Buffalo	1	-	-	-
	Cattle	0.415	0.237	0.727	0.002

Table 50: The bivariable relationships between the likelihood an Enterococcus isolate is susceptible to Erythromycin using disc diffusion method and three independent variables (province, season and specie).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	1.304	0.483	3.522	0.601
	Balochistan	2.902	1.047	8.044	0.041
	Gilgit Baltistan	0.741	0.284	1.934	0.54
	Islamabad	0.498	0.17	1.459	0.204
	Khyber Pakhtunkhwa	0.874	0.313	2.441	0.797
	Punjab	2.092	0.727	6.019	0.171
Season	Winter	1	-	-	-
	Spring	1.674	0.678	4.134	0.264
	Summer	1.546	0.687	3.479	0.292
	Autumn	0.764	0.317	1.838	0.548
Specie	Buffalo	1	-	-	-
	Cattle	0.281	0.145	0.545	0

Table 51: The multivariable relationships between the likelihood an Enterococcus isolate is susceptible to Erythromycin using disc diffusion method and three independent variables (province, season and specie).

5.4.4 Cattle and Buffaloes *Enterococcus* isolates with Linezolid

A logistic regression model was used to examine the magnitude of association between the susceptibility of *Enterococcus* against Linezolid as the outcome variable and the province, season and specie as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant.

The results of the bivariable model indicated a statistically significant association between *Enterococcus* isolates recovered from KP and susceptibility to Linezolid considering the providence Sindh as the reference (i.e., OR=1). The isolates recovered from KP were 2.86 times more likely to be resistant to Linezolid compared to isolates recovered from Sindh. The observations in spring, summer and autumn were statistically not different in their susceptibility to Linezolid considering the providence winter as the reference (i.e., OR=1). However, the isolates recovered from cattle were 1.79 times more likely to be resistant to Linezolid compared to isolates recovered from buffalo (Table 52).
The multivariable analysis indicated no statistically significant association between susceptibility to Linezolid and variables: province, season and specie (Table 53).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	0.675	0.316	1.441	0.31
	Balochistan	1.128	0.506	2.515	0.768
	Gilgit Baltistan	1.358	0.622	2.965	0.442
	Islamabad	0.844	0.39	1.827	0.667
	Khyber Pakhtunkhwa	0.349	0.157	0.778	0.01
	Punjab	1.187	0.475	2.968	0.713
Season	Winter	1	-	-	-
	Spring	0.929	0.468	1.846	0.834
	Summer	0.964	0.527	1.763	0.905
	Autumn	1.056	0.57	1.954	0.863
Specie	Buffalo	1	-	-	-
	Cattle	0.558	0.37	0.842	0.005

Table 52: The bivariable relationships between the likelihood an Enterococcus isolate is susceptible to Linezolid using disc diffusion method and three independent variables (province, season and specie).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	0.901	0.397	2.045	0.803
	Balochistan	1.559	0.652	3.729	0.319
	Gilgit Baltistan	1.365	0.624	2.985	0.436
	Islamabad	0.969	0.441	2.132	0.938
	Khyber Pakhtunkhwa	0.449	0.193	1.042	0.062
	Punjab	1.387	0.544	3.54	0.493
Season	Winter	1	-	-	-
	Spring	0.887	0.434	1.812	0.743
	Summer	0.964	0.516	1.8	0.908
	Autumn	0.989	0.524	1.867	0.973

Specie	Buffalo	1	-	-	-
	Cattle	1.6	0.973	2.629	0.064

***Table 53**: The multivariable relationships between the likelihood an Enterococcus isolate is susceptible to Linezolid using disc diffusion method and three independent variables (province, season and specie).*

5.4.5 Cattle and Buffaloes *Enterococcus* Isolates with Teicoplanin

A logistic regression model was used to examine the magnitude of association between the susceptibility of *Enterococcus* against Teicoplanin as the outcome variable and the province, season and specie as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant.

The results of the bivariable model indicated that none of the study provinces is statistically significantly different in their association with susceptibility to Teicoplanin considering the providence Sindh as the reference (i.e., OR=1). The observations in summer were statistically different in their susceptibility to Teicoplanin considering winter as the reference (i.e., OR=1). The *Enterococcus* isolates recovered in spring were 2.45 times more likely to be resistant to Teicoplanin compared to winter in bivariable model. Similarly, the isolates recovered from cattle were 1.94 times more likely to be resistant to Teicoplanin compared to isolates recovered from buffalo (Table 54).
The multivariable analysis also indicated a statistically significant association between *Enterococcus* isolates recovered in spring and susceptibility to Teicoplanin. The *Enterococcus* isolates recovered in spring were 2.77 times more likely to be resistant to Teicoplanin compared to winter. There was also statistically significant relationship between specie of the animal and susceptibility to Teicoplanin. The isolates recovered from cattle were 2.02 times more likely to be resistant to Teicoplanin compared to buffalo (Table 55).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	1.708	0.657	4.438	0.272
	Balochistan	2.424	0.803	7.319	0.116
	Gilgit Baltistan	2.338	0.811	6.737	0.116
	Islamabad	2.511	0.873	7.22	0.088
	Khyber Pakhtunkhwa	0.449	0.192	1.051	0.065
	Punjab	0.909	0.312	2.645	0.861
Season	Winter	1	-	-	-
	Spring	0.408	0.185	0.901	0.026
	Summer	1.093	0.51	2.339	0.82
	Autumn	2.11	0.899	4.953	0.086
Specie	Buffalo	1	-	-	-
	Cattle	0.515	0.313	0.848	0.009

***Table 54:** The bivariable relationships between the likelihood an Enterococcus isolate is susceptible to Teicoplanin using disc diffusion method and three independent variables (province, season and specie).*

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	2.728	0.921	8.081	0.07
	Balochistan	4.816	1.375	16.863	0.014
	Gilgit Baltistan	2.596	0.868	7.762	0.088
	Islamabad	3.81	1.212	11.975	0.022
	Khyber Pakhtunkhwa	0.69	0.262	1.817	0.453

	Punjab	1.419	0.45	4.475	0.551
Season	Winter	1	-	-	-
	Spring	0.361	0.153	0.849	0.02
	Summer	1.103	0.492	2.47	0.812
	Autumn	1.967	0.805	4.806	0.138
Specie	Buffalo	1	-	-	-
	Cattle	0.493	0.259	0.938	0.014

***Table 55**: The multivariable relationships between the likelihood an Enterococcus isolate is susceptible to Teicoplanin using disc diffusion method and three independent variables (province, season and specie).*

5.4.6 Cattle and buffaloes *Enterococcus* isolates with Tetracycline

A logistic regression model was used to examine the magnitude of association between the susceptibility of *Enterococcus* against Tetracycline as the outcome variable and the province, season and specie as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant.

The results of the bivariable model indicated a statistically significant association between *Enterococcus* isolates recovered from KP and susceptibility to Tetracycline considering the province Sindh as the reference (i.e., OR=1). The isolates recovered from KP province were 2.25 times more likely to be resistant to Tetracycline compared to isolates recovered from Sindh. The observations in spring, summer and autumn were statistically not different in their susceptibility to Tetracycline considering winter as the reference (i.e., OR=1). Similarly, there was not a statistically significant association between specie of the animals and susceptibility to Tetracycline (Table 56).
The multivariable analysis indicated no statistically significant association between susceptibility to Tetracycline and variables: province, season and specie exists (Table 57).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	1.481	0.69	3.183	0.314
	Balochistan	1.087	0.486	2.43	0.839
	Gilgit Baltistan	1.056	0.484	2.305	0.891
	Islamabad	1.357	0.621	2.965	0.445
	Khyber Pakhtunkhwa	0.444	0.205	0.964	0.04
	Punjab	0.767	0.307	1.92	0.571
Season	Winter	1	-	-	-
	Spring	0.613	0.309	1.216	0.162
	Summer	0.786	0.429	1.439	0.435
	Autumn	0.981	0.528	1.825	0.952
Specie	Buffalo	1	-	-	-
	Cattle	0.683	0.456	1.023	0.064

***Table 56:** The bivariable relationships between the likelihood an Enterococcus isolate is susceptible to Tetracycline using disc diffusion method and three independent variables (province, season and specie).*

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	2.021	0.877	4.657	0.099
	Balochistan	1.562	0.649	3.762	0.32
	Gilgit Baltistan	1.086	0.495	2.38	0.837
	Islamabad	1.627	0.728	3.637	0.236
	Khyber Pakhtunkhwa	0.597	0.262	1.36	0.219

	Punjab	0.946	0.37	2.419	0.908
Season	Winter	1	-	-	-
	Spring	0.645	0.317	1.312	0.227
	Summer	0.801	0.429	1.494	0.485
	Autumn	0.949	0.501	1.795	0.872
Specie	Buffalo	1	-	-	-
	Cattle	0.617	0.376	1.012	0.056

***Table 57**: The multivariable relationships between the likelihood an Enterococcus isolate is susceptible to Tetracycline using disc diffusion method and three independent variables (province, season and specie).*

5.4.7 Cattle and Buffaloes *Enterococcus* Isolates with Vancomycin

A logistic regression model was used to examine the magnitude of association between the susceptibility of *Enterococcus* against Vancomycin as the outcome variable and the province, season and specie as the potential risk factors. For analysis purpose all the isolates classified as intermediate with disc diffusion method were categorized as resistant.

The results of the bivariable model indicated that none of the study provinces is statistically significantly different in their association with susceptibility to Vancomycin considering the providence Sindh as the reference (i.e., OR=1). Similarly, observations in spring, summer and autumn were not statistically different in their susceptibility to Vancomycin considering the winter as the reference (i.e., OR=1). However, there was a statistically significant relationship between specie of the animal and susceptibility of *Enterococcus* isolates to Vancomycin. The isolates recovered from cattle were 1.82 times more likely to be resistant to Vancomycin compared to isolates recovered from buffaloes (Table 58).

The multivariable analysis indicated no statistically significant association between susceptibility to Vancomycin and variables: province and season. However, there was a statistically significant relationship between specie of the animal and susceptibility to Vancomycin. The isolates recovered from cattle were 2.33 times more likely to be resistant to Vancomycin compared to buffalo (Table 59).

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-
	Azad Jammu and Kashmir	2.722	1.255	5.903	0.011
	Balochistan	2.361	1.04	5.36	0.04
	Gilgit Baltistan	2.917	1.302	6.534	0.009
	Islamabad	2.167	0.986	4.759	0.054
	Khyber Pakhtunkhwa	0.852	0.394	1.845	0.685
	Punjab	2.03	0.801	5.144	0.136
Season	Winter	1	-	-	-
	Spring	1.255	0.632	2.495	0.516
	Summer	2.391	1.294	4.416	0.005
	Autumn	2.651	1.414	4.97	0.002
Specie	Buffalo	1	-	-	-
	Cattle	0.549	0.365	0.824	0.004

***Table 58**: The bivariable relationships between the likelihood an Enterococcus isolate is susceptible to Vancomycin using disc diffusion method and three independent variables (province, season, and specie).*

Variable	Categories	Odds Ratio	(95% CI)		P value
Province	Sindh	1	-	-	-

	Azad Jammu and Kashmir	4.848	1.982	11.635	0
	Balochistan	4.32	1.713	10.892	0.002
	Gilgit Baltistan	2.971	1.302	6.78	0.01
	Islamabad	2.804	1.224	6.426	0.015
	Khyber Pakhtunkhwa	1.287	0.555	2.982	0.557
	Punjab	2.683	1.013	7.104	0.047
Season	Winter	1	-	-	-
	Spring	1.309	0.633	2.706	0.468
	Summer	2.511	1.318	4.784	0.005
	Autumn	2.671	1.384	5.153	0.003
Specie	Buffalo	1	-	-	-
	Cattle	0.429	0.256	0.72	0.001

***Table 59**: The multivariable relationships between the likelihood an Enterococcus isolate is susceptible to Vancomycin using disc diffusion method and three independent variables (province, season and specie).*

A table summarizing the susceptibility of poultry *E. coli* and *Salmonella* isolates with the list of antibiotics and significant factors is given in Annexure 3.

5.5 Association of Seasonality and Geographical Location with AMR

In this study, AMR percentages in *E. coli, Salmonella* and *Enterococcus* isolates to a number of antimicrobials tested has been found to be associated with the seasons. The AMR prevalence in *E. coli, Salmonella* and *Enterococcus* isolates from different antimicrobials showed seasonal variation. This seasonal variation may be attributed to the use of antimicrobials. The use of antimicrobials could vary depending on the occurrence of bacterial pathogens during certain seasons of the year. For example, *Salmonella* is more prevalent in the summer compared to winter[8,,9] Diseases occurring more frequently in the summer months may drive the use of antimicrobials leading to selection pressure in enteric bacteria.

The results of this study also suggested regional/geographical influence on the occurrences of resistance to certain antimicrobials. These differences may be due to variation in prevalence of diseases in different provinces/regions of the country. The difference in prevalence of animal diseases may influence the choice and quantity of antimicrobials used. Previous studies have indicated that prevalence of livestock diseases varies with geographical location due to differences in farming practices, animal management systems and environmental conditions[10, 11, 12]. In addition, regional variations in disease surveillance and control programs and farmer access to veterinary services (e.g., prescribing practices or lack of veterinary oversight) may also influence the occurrent of livestock diseases and need for antimicrobials in different regions. These factors were not considered in the current pilot AMR surveillance. Therefore, there is a need to further investigate disease pressures and drivers for use of antimicrobials and other farm-level factors in the country. A concurrent monitoring of antimicrobial use is necessary to monitor quantity of use and the classes of antimicrobials used in each animal sector.

[8] Zdragas, A., K. Mazaraki, G. Vafeas, et al., 2012. Prevalence, seasonal occurrence and antimicrobial resistance of *Salmonella* in poultry retail products in Greece. Letters in Applied Microbiology. 55(4):308-13.

[9] Wottlin, L. R., T. S. Edrington and R. C. Anderson, 2022. *Salmonella* Carriage in Peripheral Lymph Nodes and Feces of Cattle at Slaughter Is Affected by Cattle Type, Region, and Season. Frontiers in Animal Science, 3:1-9.

[10] Zdragas, A., K. Mazaraki, G. Vafeas, et al., 2012. Prevalence, seasonal occurrence and antimicrobial resistance of *Salmonella* in poultry retail products in Greece. Letters in Applied Microbiology. 55(4):308-13.

[11] Wottlin, L. R., T. S. Edrington and R. C. Anderson, 2022. *Salmonella* Carriage in Peripheral Lymph Nodes and Feces of Cattle at Slaughter Is Affected by Cattle Type, Region, and Season. Frontiers in Animal Science, 3:1-9.

[12] Islam, M. Z., A. Musekawa, K. Islam et al., 2014. Regional Variation in the Prevalence of *E. coli* O157 in Cattle: A Meta-Analysis and Meta-Regression, PLOS ONE, 9(4):1-15.

The results also indicated that *E. coli*, *Salmonella* and *Enterococcus* isolates recovered in this study showed resistance to **highest** priority critically important antimicrobials (nalidixic acid, ciprofloxacin, azithromycin, cefotaxime and ceftazidime), **high** priority critically important antimicrobials (ampicillin and gentamicin) and **highly** important antimicrobials (chloramphenicol, tetracycline and trimethoprim) indicating that AMR is an issue in Pakistan. The high to extremely high levels of resistance across the bacteria examined, particularly to the WHO's HPCIA's are very concerning.

6. Conclusion

In Pakistan National AMR surveillance guidelines for healthy food animals were developed with the help of Fleming Fund Country Grant Pakistan in collaboration with Animal Husbandry Commissioner and other relevant stakeholders. These guidelines were implemented through AMR pilot in healthy animals of Pakistan, thus served as a proof of concept for a sustainable AMR surveillance in animal sector that the country could implement and progressively expand (other specie, other bacteria of interest) beyond this country grant Two NRLs and 9 peripheral labs were established for AMR surveillance. Staff of NRLs and the 9 peripheral labs were trained for sample collection, bacterial isolation (*E. coli*, *Salmonella* and *Enterococcus*), identification, and AST, as per the relevant CLSI guidelines and ISO standards. The results of the pilot AMR surveillance indicated an association between AMR *E. coli*, *Salmonella* and *Enterococcus* isolates with season and geographical location. This association may be due to variation in the quantity and classes of antimicrobials used, disease pressures, differences in animal production systems, provision of veterinary services, environmental conditions, and other risk factors. Therefore, **there is a need to expand the AMR surveillance to other animal production systems and geographical areas of Pakistan to get a clear understanding of risk factors and the epidemiology of AMR in the country. All *E. coil, Salmonella* and *Enterococcus* isolates were resistant to at least one antimicrobial, indicating the predominance of multidrug resistant bacteria in animal populations raised for human consumption. The results also indicated that *E. coli, Salmonella* and *Enterococcus* isolates showed resistance to WHO'S HPCIA's, high priority critically important antimicrobials and highly important antimicrobials indicating that food animals may represent a source of transmission of these resistant bacteria to humans.** This again highlights the need for establishment of AMR surveillance and control program at national level in Pakistan to combat this emerging issue of AMR.

7. Challenges Faced

Technical challenges can be categorized into two main groups: 1) quality of samples and epidemiological data and 2) capacity of NRLs to perform the recommended diagnostics. To ensure good quality samples and complete epidemiological data, the Fleming Fund Country Grant team provided continuous virtual backstopping.

Regarding capacity strengthening of NRLs, the Fleming Fund Country Grant Pakistan has invested in improving NRL capacity for bacterial isolation, identification, and AST, as per the relevant CLSI guidelines and ISO standards and both NRLs can perform isolation and identification of *E. coli, Salmonella* and *Enterococcus* as per SOPs. In terms of the NRL technical capacity, training on isolation of *Campylobacter* and microbroth dilution was rendered to NRLPD and NVL to ensure that NRLs perform bacterial isolation and AST according to the protocols established in the surveillance pilot.

The successful implementation of the AMR surveillance pilot faced several logistic and technical challenges. Logistically, the flooding across Pakistan posed a serious impediment to field activities. Despite these challenges, PFPs, in collaboration with NRL counterparts and the Fleming Fund Country Grant Pakistan team, have made tremendous efforts to continue the field activities.

8. Way forward

Some of the potential next steps are:

- Currently AMR surveillance pilot was limited to slaughtered animals (poultry, cattle, and buffaloes). The AMR surveillance activities should be expanded to include different livestock production systems and production phases (e.g., farm-level and retail at least periodically) to obtain a more accurate picture of AMR drivers and trends at national level.
- Initiation of AMR surveillance pilot by Provincial Disease Diagnostic Lab Lahore and Disease Investigation Laboratory Peshawar involving the same strategy followed at federal level. Both labs will share the AMR surveillance data to AHC on monthly basis, thus, a harmonized approach for data collection (standardized template such as the spreadsheet used in this pilot) data storage and data validation are necessary to generate a reliable AMR data intended for reporting, communication, and advocacy.
- Share animal health AMR surveillance outcomes including those not determined in this pilot study and analysis (e.g., resistance to ≥1, 3, or 5 classes of antimicrobials, resistance patterns, distribution of inhibition zones) with the public health counterparts to foster a One Health approach and initiate an integrated AMU/AMR surveillance program.
- Government of Pakistan should allocate funds at national and provincial level to sustain AMR surveillance activities for livestock sector in Pakistan.

Annexure 1

The disc potency and zone diameter for various antibiotics for *E. coli* and *Salmonella* isolates[13]

Antibiotic	Potency	Susceptible (mm)	Intermediate (mm)	Resistant (mm)
Ampicillin	10µg	≥ 17	14-16	≤ 13
Cefotaxime	30µg	≥ 26	23-25	≤ 22
Ceftazidime	30µg	≥ 21	18-20	≤ 17
Chloramphenicol	30µg	≥ 18	13-17	≤ 12
Ciprofloxacin	5µg	≥ 31	21-30	≤ 20
Nalidixic Acid	30µg	≥ 19	14-18	≤ 13
Tetracycline	30µg	≥ 15	12-14	≤ 11
Trimethoprim	5µg	≥ 16	11-15	≥ 10
Azithromycin	15µg	≥ 13	-	≤ 12
Gentamicin	10µg	≥ 15	13-14	≤ 12

The disc potency and zone diameter for various antibiotics for *Enterococcus spp.*[13]

Antibiotic	Potency	Susceptible (mm)	Intermediate (mm)	Resistant (mm)
Ampicillin	10µg	≥17	-	≤16
Chloramphenicol	30µg	≥18	13-17	≤12
Erythromycin	15µg	≥23	14-22	≤10
Linezolid	30µg	≥23	21-22	≤20
Teicoplanin	30µg	≥14	11-13	≤10
Tetracycline	30µg	≥19	15-18	≤14
Vancomycin	30µg	≥17	15-16	≤14

[13] Clinical and Laboratory Standards Institute. 2021. Performance standards for antimicrobial susceptibility testing; CLSI M100, edition 31. Clinical and Laboratory Standards Institute, Wayne, PA.

Annexure 2

Bacterial Specie	Antibiotic	Significant categories/factors	Odds Ratio	95% CI		P-value
E. coli	Azithromycin	Spring	0.215	0.062	0.749	0.016
		Summer	0.641	0.413	0.996	0.048
		Autumn	0.310	0.192	0.502	0.000
	Cefotaxime	Azad Jammu and Kashmir	0.422	0.206	0.863	0.018
		Summer	0.405	0.263	0.624	0.000
		Autumn	0.611	0.396	0.945	0.027
	Ceftazidime	Balochistan	0.269	0.135	0.535	0.000
		Spring	0.101	0.043	0.235	0.000
		Summer	0.370	0.229	0.598	0.000
		Autumn	0.290	0.182	0.462	0.000
	Chloramphenicol	Gilgit Baltistan	0.349	0.152	0.802	0.013
		Punjab	0.443	0.214	0.919	0.029
	Ciprofloxacin	Islamabad	0.212	0.056	0.801	0.022
	Gentamicin	Balochistan	0.436	0.228	0.832	0.012
		Summer	0.581	0.387	0.872	0.009
Salmonella	Ampicillin	Summer	0.242	0.084	0.700	0.009
		Autumn	0.181	0.083	0.396	0.000
	Ceftazidime	Khyber Pakhtunkhwa	0.232	0.075	0.720	0.011
	Chloramphenicol	Summer	0.246	0.076	0.802	0.020
		Autumn	0.356	0.166	0.763	0.008
	Gentamicin	Khyber Pakhtunkhwa	0.235	0.080	0.689	0.008
	Trimethoprim	Summer	0.050	0.006	0.447	0.007
		Autumn	0.011	0.001	0.090	0.000

A table summarizing the susceptibility of poultry *E. coli* and *Salmonella* isolates with the list of antibiotics and significant factors.

Annexure 3

Bacterial Specie	Antibiotic	Significant categories/factors	Odds Ratio	95% CI		P-value
E. coli	Cefotaxime	Khyber Pakhtunkhwa	9.09	2.34	35.24	0.001
	Chloramphenicol	Summer	13.2	3.65	47.65	0
		Autumn	6.72	2.04	22.12	0.002
	Ciprofloxacin	Khyber Pakhtunkhwa	9.25	2.44	35.09	0.001
	Nalidixic acid	Khyber Pakhtunkhwa	11.1	4.3	28.63	0
Enterococcus	Ampicillin	Khyber Pakhtunkhwa	0.291	0.124	0.684	0.005
		Punjab	0.35	0.132	0.932	0.036
	Teicoplanin	Spring	0.361	0.153	0.849	0.02
		Cattle	0.493	0.259	0.938	0.014
	Vancomycin	Cattle	0.429	0.256	0.72	0.001

A table summarizing the susceptibility of *E. coli* and *Enterococcus* isolates recovered from apparently healthy cattle and buffaloes with the list of antibiotics and significant factors.

Suggested Citation:

AHC 2023. Antimicrobial Resistance Surveillance Pilot in Healthy Food Animals. Animal Husbandry Commissioner, Ministry of National Food Security and Research, Government of Pakistan, Islamabad, Pakistan.