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Emerging Threat: Antimicrobial Resistance in Pakistan – A Comprehensive Review and Call to Action

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Abstract
Antimicrobials are effective for eradication of pathogens but progressive increase in global trend of antimicrobial resistance (AMR) especially against last resort drugs is worrying and pose a significant threat to human health. Emergence and spread of AMR worldwide is due to reckless use of antibiotics in human, livestock, food and agriculture sector. The worldwide emergence of plasmid borne tigecycline and colistin resistance has raised significant health concerns due to ability of horizontal transfer and being last resort antibiotics. Plasmid mediated colistin resistance (*mcr-1*, *mcr-2*) has been detected in human, poultry and cattle from Pakistan mainly in isolates of *A. bumannii* and *E. coli* highlighting horizontal transfer of AMR genes, the first case of plasmid mediated tigecycline resistance *tet*X has also been reported in clinical isolates of *E. coli* from Pakistan. This emerging resistance against last resort antibiotics colistin, tigecycline, carbapenem along with jeopardizing their efficiency will also restrict the treatment options for medical workers. This situation calls for urgent attention and implementation of effective strategies to combat AMR in Pakistan.

Keywords
Antimicrobial resistance, tigecycline resistance, colistin resistance, last resort antibiotics and Pakistan

Introduction

Antimicrobial resistance (AMR) is a severe concern to global health in the twenty-first century; if not addressed, it could result in 10 million deaths and a global loss of $100 trillion per year by 2050 (Aguilar *et al.*, 2023) https://doi.org/10.1016/j.lana.2023.100561 . According to the CDC (center of disease control), around 2.8 million people are infected with multidrug-resistant (MDR) bacteria, resulting in roughly 700,000 deaths each year, which has a considerable influence on worldwide mortality and morbidity rates (Reygaert, 2018) (Christaki, Marcou, and Tofarides, 2019). The evolution and spread of antimicrobial resistance genes (ARGs) and their associated mobile genetic elements in bacteria is a natural phenomenon that is influenced by various factors, including genetic mutations, horizontal gene transfer, and selection pressure (Irfan, Almotiri, and AlZeyadi, 2022). However, in low- and middle-income countries (LMIC, s), this issue is compounded by various factors, including widespread and inappropriate antibiotic usage, inadequate sanitation, under-resourced healthcare systems with limited diagnostic capabilities, over the counter sale of antibiotics and insufficient access to high-quality antibiotics (Sohaili, Asin, and Thomas, 2024).

Pakistan ranks as the fifth-most populous country in the world and demonstrates a significant consumption of antibiotics. From 2000 to 2015, there was a notable surge in antibiotic consumption, increasing by 65% from 0.8 to 1.3 billion daily defined doses (DDDs) (Klein *et al.*, 2018).. Moreover, during this period, the consumption rate rose by 21%, reaching from 16.2 to 19.6 DDDs per 1000 inhabitants per day. Consequently, Pakistan ranks as the third-highest consumer of antibiotics among low- and middle-income countries, following India and China (Shams *et al.*, 2024)https://doi.org/10.1016/j.chemosphere.2024.141357. This increasing burden of AMR resulted in formation of AMR National Action Plan (NAP). Commonly prescribed antibiotics in the country belong to the classes of fluoroquinolones and β-Lactams, frequently used to treat various diseases (Sharif, Aslam, and Saleem, 2022).. Moreover, during period of 2015 and 2018 about 65% increase was reported in utilization of cephalosporins (Jalil *et al.*, 2023).

Injudicious usage of antibiotics as prophylactic and therapeutic agent in the veterinary sector of Pakistan is also contributing as a significant factor in emergence of AMR. Prevalence of resistant bacterial diseases in poultry has surged up to 43.2%, resistance to reserved antibiotics i.e. tetracycline, lincomycin and macrolide is widely reported (Haider *et al.*, 2022). Furthermore, excessive antimicrobial usage (AMU) results in accumulation of antibiotic

residues in meat which fuels AMR development in humans, underscoring concerns of AMR at intersection of animal and human health (Jalil *et al.*, 2023)(Mhondoro *et al.*, 2019) .
Increased reports of resistant genes (tetX4 and tetX3) against tigecycline in recent years have emerged from Pakistan among isolates of zoonotic origin. Moreover, co-existence of colistin resistance was also reported in those isolates (Mohsin *et al.*, 2021). Resistance against carbapenem has also been reported mainly in clinical isolates of *A. bumanii*, *E.coli* and *K. pneumoniae* from Pakistan. (Khurshid *et al.*, 2020) (Shafquat *et al.*, 2019) (Hasan *et al.*, 2013). It highlights the severe threat to clinical utility of reserved antibiotics in Pakistan. This emerging resistance in Gram-negative rods is attributed to their plasmids (Kumarasamy *et al.*, 2010). Occurrence of these resistant genes in environment and particularly in human pathogens is a concern of immense importance (Acman *et al.*) as it can turn common bacterial infections into life threating infections by putting end on antibiotics as therapeutic agents in combating bacterial disease (Sekyere, 2016).

(Ayobola *et al.*) Other than plasmids, efflux pump, chromosomal mutations or jumping elements called transposons, also attribute in acquiring AMR (Mancuso *et al.*) (Alvarez-Uria *et al.*). Bacteria are rapidly developing resistance to broad-spectrum reserved antibiotics i.e. carbapenem, tetracyclines and colistin and are creating serious health hazards by becoming major threats to human health (Ferri *et al.*, 2017).

Pakistan continues to struggle with consistently increasing threat of AMR despite the introduction of NAP, there are some concerns associated with implementation of NAP including lack of resources and personnel being major hurdles in its success (Alam *et al.*, 2023). Self-medication culture, over prescription of antibiotics and readily availability of "Reserved'' antibiotics in market is further exuberating the situation. Antibiotics, since their inception have revolutionized the medical field and have significantly reduced the mortality rate of many fatal disease i.e. meningitis, sepsis, bacterial diarrhea and have increased life expectancy. But unfortunately now this emerging AMR problem is threating these gains (Liu *et al.*, 2016a), reports against last resort antibiotics in South Asia Pakistan are increasing and raising major concerns. It's a high time requiring immediate action to protect current antibiotics by ensuring their appropriate usage by health professionals . This review will highlight current scenario of resistance against these reserved group antibiotics in Pakistan and will create awareness to address this issue by practicing different policies.

Mechanism of AMR

Microorganisms have developed different mechanisms to evade destruction or killing by antimicrobial agents, these can be acquired and intrinsic. Intrinsic or innate resistance results from long term exposure of bacteria to antibiotics or because of mutations which results due to physiological changes in bacterial structure (Christaki, Marcou, and Tofarides, 2020). However, acquired resistance results through mobile genetic elements, plasmids which enable bacteria to carry or transfer resistant genes to different bacterial pathogens. Extrinsic resistance emerges due to selection pressure exerted by antimicrobial agents which favor growth of resistant strains and eliminate sensitive strains, mobilome the mobile genetic elements integron and plasmids play a key role in acquisition of resistance in microbes (Holmes *et al.*, 2016). Furthermore, horizontal gene transfer (HGT) is the mechanism responsible for transfer of resistance genes from one bacteria to different microbes or even to animals and humans, bacteria utilized conjugation process for transfer of resistant genes by utilizing sex pili (Morrison, U. Endoscopy., 2020) (P. Dadgostor, 2019) (Miguel Gueimonde, Borja Sánchez, 2018).

Pattern of acquiring resistance vary in each bacterial specie including i.e. production of enzymes (carbapenemases, beta-lactamase's), modification of drug target, efflux pump activity, alteration of drug pathways, inhibition of drug deposition in cell (Byarugaba, 2010). The mechanism of acquiring drug resistance in bacteria is shown in **Figure 1**. Overuse of antibiotics in humans and in animals for growth purposes is the biggest driver of AMR which exert selection pressure on microbes and result in emergence of AMR genes (Singhal, 2022). A map highlighting density of AMR genes in different regions of Pakistan is shown in **Figure 2**.

Resistance to carbapenem

Carbapenems, the latest Beta-lactams generation comes under antibiotic of last resort, carbapenem drugs i.e. ertapenem, meropenem, and imipenem were utilized extensively in treatment of multidrug resistant bacterial infections (Nordmann and Poirel, 2019). Therefore, this injudicious usage has resulted in emergence of selected resistant strains among bacteria compromising clinical efficacy of carbapenems, resistance against carbapenems in bacteria mostly develops through expression of carbapenemases enzyme (Sekyere, 2016). However, resistance can also emerge through porins mutation in bacterial membrane which will slow down drug entry and will increase production of beta-lactamase, thus the main resistance mechanisms utilized by bacteria against carbapenems are horizontal gene transfer (HGT) and acquired mutations (Ma *et al.*, 2021). However, carbapenemase production is considered as most important mechanism of resistance from clinical point of view and is divided among four classes i.e. A,B,C,D, all clinically important enzymes GES, IMI and KPC comes under class A while NDM, VIM, and SPM are categorized in class B. OXA-like enzymes are classified under class D, due to low potential of carbapenem hydrolysis class C enzymes are not considered as carbapenemases (Meletis, 2016).

Analysis of research reporting carbapenem resistance showed that approximately 1111 cases were reported during 2015-2021 from Pakistan **(Table 1)**. Furthermore, it was revealed that majority of cases were reported from Punjab province followed by Sindh on second, the most commonly reported carbapenem resistant pathogens were *Acinetobacter baumannii*, *Klebsiella pneumonia* and *Escherichia coli.* However, most reported carbapenem resistant genes were NDM and OXA as described in **Table 1**, carbapenem resistance has been detected in more than 10 bacterial *spp*. from clinical specimens. Due to high rate of mortalities associated with carbapenem resistant infections (CRE) (Xu, Sun, and Ma, 2017) this increasing resistance against one of this last resort antibiotic cannot be neglected, association of CRE with hospitals can result in outbreaks or transfer of these pathogens from hospital environment to local communities significantly increasing mortality rates. Furthermore, detection of carbapenem resistance in *Salmonella typhi* highlights the risk of its transfer through contaminated water and food that will expose many to this deadly pathogen.

Resistance to colistin

Colistin, a polymyxin antibiotic is being used for decades for treating MDR infections and as a growth promoters in food-producing animals (FPAs), colistin was considered among one of those few options available for treating super bugs (Bialvaei and Samadi Kafil, 2015). This drug was approved by US FDA during 1960 for clinical use (Barreto-Santamaría *et al.*, 2021) and it continued to be used in treatment of extensively resistant bacterial infections and in topical formulations for several decades (Barreto-Santamaría *et al.*, 2021). However due to increasing reports of toxicity (Jafari and Elyasi, 2021) (neurotoxicity and nephrotoxicity) associated with its usage this drug was given the status of reserve antibiotic and was shifted to veterinary medicine (Javed *et al.*, 2020). Colistin again attained attention during 1990s due to escalating prevalence of MDR-pathogens especially *K. pneumoniae*, *P. aeruginosa* and *A.*

bumanni and lack of availability of any novel antibiotic, when quinolones, beta-lactams and aminoglycosides become ineffective against XDR-pathogens colistin remains choice of last drug (Dandachi *et al.*, 2018).

Furthermore, intrinsic resistance to colistin that emerge due to chromosomal mutation (*pmrA* and *pmrB*) has been existed for long but it was not worrisome due to its vertical transfer only (Sun *et al.*, 2018). However, the situation has become alarming now because of emergence of mobile plasmid mediated (*mcr*) resistance that has ability for vertical and horizontal transfer against one of last resort antibiotic colistin, the first case of this plasmid resistance was reported in China (Liu *et al.*, 2016b) it has been detected in both animals and humans. Recently this plasmid borne resistance has been detected in Pakistan (Lv *et al.*, 2018) in healthy broilers, poultry and cattle's are considered to be reservoir of this plasmid borne resistance due to injudicious usage of colistin for prophylaxis and prophylactic purposes in FPAs (Javed *et al.*, 2020). The plasmid borne *mcr* resistance has been reported in many *spp*. of bacteria including Enterobacteriaceae, *E. coli, K. Pneumonia, Raoultella* spp., *Enterobacter* spp.,*Klebsiella oxytoca, Citrobacter spp., P. aeruginosa* **(Table 2)** from different regions of Pakistan. Furthermore, *mcr-1* has also been detected in clinical isolates as mentioned in **Table-2** which raise a significant concern as animal to human transfer of *mcr* has been already reported in China (Liu *et al.*, 2016b).

Analysis of research reporting mobile colistin resistance has revealed, to date approximately 331 cases have been reported from Pakistan during 2015-2022 **(Table 2)**, majority of the cases have been reported from KPK followed by Sindh and most reported pathogens are *E. coli, K. pneumoniae*. This emerging mobile resistance in human pathogens against one of last lifesaving drug is a significant threat to human health.

Resistance to Tigecycline

Tigecycline, an important derivative of tetracycline belongs to new class of antibiotics glycylcyclines, has potent activity against gram-negative and gram-positive bacteria. Tigecycline is one of the last resort antibiotics that is active against multi drug resistant gram-negative bacteria and is crucial part of human medicine (Menazea *et al.*),(Menazea, Eid, and Ahmed, 2020). It was initially approved by the US food and drug administration (FDA) in 2008 (Sader *et al.*) due to its minimal organ toxicity. Tigecycline is highly active against Enterobacteriaceae specially against *E.coli*, its antibacterial activity is because of efflux pump of bacteria as they are unable to take up tigecycline when it is present in low concentration (Pankey, 2005).

The occurrence of tigecycline resistance is becoming a global concern now, as it can be transfer to humans from animals via horizontal gene transfer and due to this, its efficacy in human medicines can be restraint. The first case of tigecycline resistant genes was reported in 2007 in *Acinetobacter bumannii* that was a chromosome mediated resistance (Asif, Alvi, and Ur Rehman, 2018), but now plasmid mediated tigecycline resistant genes are emerging. *tet*X4 is one of plasmid mediated tigecycline resistant gene identified in *E.coli* from p47EC plasmid, it was first isolated in China from FPAs in 2019. *tet*X3 and *tet*X4 are the only plasmid mediated tigecycline resistance genes that are identified in *E.coli* (Bai *et al.*, 2019).

Analysis of research reporting tigecycline resistance has shown plasmid mediated resistance *tet*X has been detected in FPAs and in clinical isolates from Pakistan **(Table 3)**, this emerging mobile resistance against tigecycline represents a great threat to human health as tigecycline is the only drug available for treating XDR infections with minimal side effects and this resistant variant *tet*X will significantly restrain the efficacy of this last resort antibiotics. To date approximately 44 cases of mobile tigecycline resistance have been detected from Punjab, Pakistan and these resistant genes were detected in human intestinal pathogen *E. coli* suggesting humans as potential reservoir of *tet*X carrying *E. coli* (Li *et al.*, 2021a).

Alternative strategies to combat AMR

To combat emerging resistance against antimicrobials following alternatives can be considered.

- Bacteriophage therapy: Phages have been utilized in treatment of bacterial infections since many ages, with the emergence of resistance against antibiotics phage therapy has again gained attention as it can significantly lower the pressure or usage of antibiotics overcoming one of the main driver of AMR (Mulani *et al.*, 2019).
- Combination therapy: it is encouraged to minimize risk of development of resistance against specific class of antibiotic as when different drugs will utilized in combination it will be difficult for bacteria to develop resistance against multiple antibiotics (Mulani *et al.*, 2019).
- Use of antimicrobial peptides (AMPs): AMPs show broad spectrum of activity against different pathogens, they interact with bacterial membrane and physically damage the

bacteria by causing lysis due to which it is difficult for bacteria to develop resistant against AMPs (Pfalzgraff, Brandenburg, and Weindl, 2018).

- Use of antimicrobial alternatives probiotics and prebiotics (Roy *et al.*, 2021)
- Use of silver nano particles (AgNP): silver nano particles has shown significant antimicrobial activity against different bacterial pathogens and their biofilm's, AgNP can be utilized to reduce this emerging microbial resistance (Singh *et al.*)'(Mulani *et al.*, 2019).

Conclusion

Inception of antibiotics was the dawn of relief era for humans suffering from severe bacterial infections, however now injudicious use of antibiotics has resulted in development of AMR genes. Detection of mobile resistance genes against last resort antibiotics especially in developing low middle income countries i.e., Pakistan is raising significant health concerns as they are being detected in clinical pathogens, Pakistan is facing AMR crisis and soon healthcare workers will be out of treatment options if no strict measures will be taken to regulate use of existing antibiotics. These emerging pathogens can jeopardize the efficacy of last resort antibiotics and can put humans back in pre antibiotic era, there is a threat of epidemics that can result due to emergence of plasmid mediated resistance against last resort antibiotics leaving no reserve antibiotic available as a treatment option for MDR infections. One-health strategy by WHO, antimicrobial stewardship, timely detection of infection, proper awareness about AMU or AMR and regulated usage of antimicrobials is recommended to combat this issue.

Conflict of interest

Author declares no conflict of interest.

Authors contribution statement

Ujalla Tanveer did the conceptualization and write the original draft; Kulsoom Ghafar review andedit the manuscript.

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Data Availability statement

Data generated or analysed during this study are provided in full within the published article.

Figure 1:

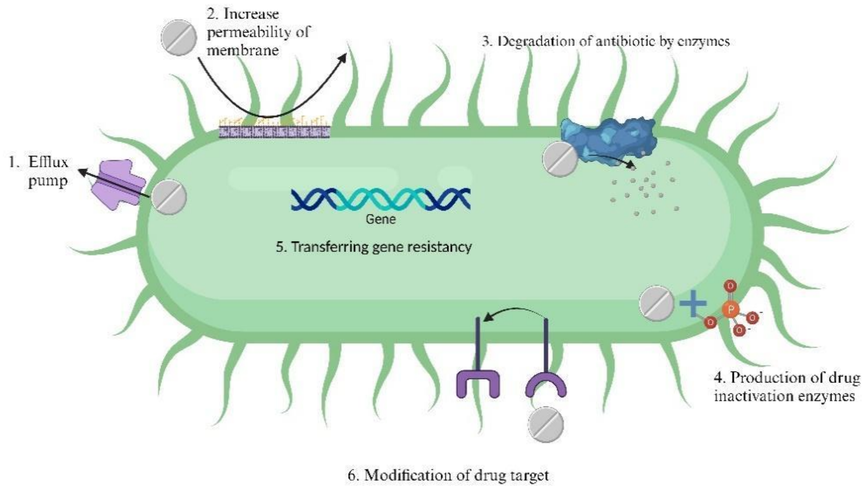

Figure 1. Mechanism of AMR

Figure 2:

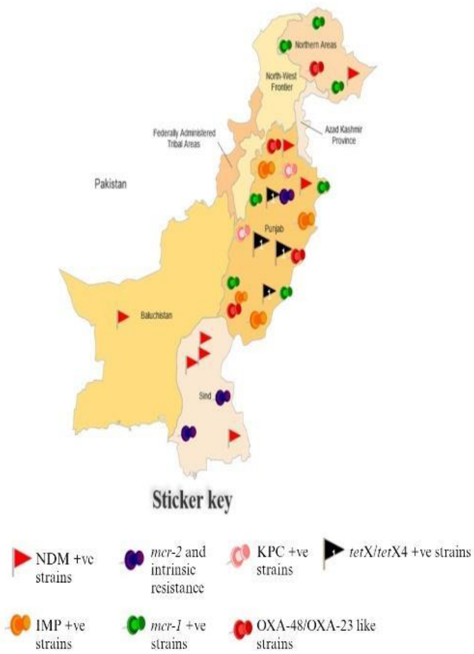

Figure 2. A map highlighting density and distribution of tigecycline, colistin, carbapenem resistant isolates in Pakistan. Resistance to carbapenem is highest in Sindh province followed by Punjab, emerging mobile resistance against colistin (mcr-1,mcr-2) has been increasingly reporting from Punjab and KPK and more worryingly plasmid mediated tigecycline resistant tetX genes have also been reported from Punjab which is raising significant health concerns. Minor resistance was reported from Baluchistan province.

Table 1 Carbapenem resistant genes isolated from different regions of Pakistan.

Year	City (Province)	Bacterial species	Specimens	No. of isolates	Resistant isolates	Carbapenem resistant gene	Reference
2015	Lahore Punjab	*Acinetobacter baumannii*	Urine, blood and tracheal secretions	112	66	Carbapenemase production, MBL producers	(Anwar *et al.*, 2016)
2016	Karachi Sindh	*E. coli* *Klebsiella pneumonia*	Clinical specimens	114	104	blaNDM-1 (104)	(Khan *et al.*, 2016)
2017	Lahore Punjab	*Acinetobacter baumannii*	Tracheal secretions, pus	137	136	*bla-*$_{OXA51}$ *bla-*$_{OXA23}$ *bla-*$_{NDM}$ ISAbA1	(Khurshid *et al.*, 2017)
2018	Lahore Punjab	*A. baumannii, P.aeruginosa , K. Pneumoniae, E. coli, C. Ferundi, P. Vulgaris, E. cloacae*	Pus, urine, blood, tissue, CVC tip, sputum	100	93	*blaIMP (3)* *blaVIM (29)*	(Akhtar *et al.*, 2018)
2018	Lahore Punjab	*Acinetobacter spp., Pseudomonas spp., Klebsiella spp., E. coli*	Clinical samples	924	142	*bla*$_{OXA}$, *bla*$_{IMP-1}$, *bla*$_{VIM}$	(Ain *et al.*, 2018)
2018	Islamabad	*K. pneumoniae*	Neonates, burn patients, pus	271	103	*8 MBL positive bla-*$_{GIM}$, *bla-*$_{IMP}$, *bla-*$_{NDM}$, *bla-*$_{SIM}$, *bla-*$_{SPM}$, *bla-*$_{VIM}$ *bla-*$_{NDM\ (7/8)}$	(Humayun *et al.*, 2018)
2019	Karachi Sindh	Clinical anaerobic isolates	Blood, sterile body fluids, pus	223	39		(Shafquat *et al.*, 2019)

2019	Lahore Punjab	*Klebsiella pneumoniae Acinetobacter baumannii,*	Clinical samples	117	72	*bla-$_{NDM-1}$*	(Qamar *et al.*, 2019)
2019	Peshawar KPK	*46E. coli, 10Enterobacter spp. ,6 K. pneumoniae, 2 Alcaligenes faecalis and 1 Citrobacter freundii.*	Blood, urine, pus, bronchial lavage	200 65 (G -ve)	38	*bla-$_{NDM-1}$ (33)* *bla-$_{OXA181}$ (5)* *bla-$_{OXA48}$ (1)* *bla-$_{OXA232}$ (1)*	(Masseron *et al.*, 2019)
2019	Quetta Baluchistan	*Morganella morganii Enterobacter cloacae Citrobacter freundii*	Pus samples	300	5	*bla-$_{NDM-1}$*	(Din *et al.*, 2019)
2020	Lahore Punjab	*Acinetobacter baumannii*	UTI infections, catheter tips, wound infections	156	139	*bla-$_{oxa51}$* *bla-$_{oxa23}$* *ISAbA1-bla$_{oxa51}$ like* *ISAbA1-bla$_{oxa23}$ like*	(Khurshid *et al.*, 2020)
2020	Islamabad Rawalpindi	*K. pneumoniae*	Blood, urine, pus, catheter	200	72	*bla$_{NDM-1\ (39\%)}$* *bla$_{KPC}$ (5%)* *bla$_{OXA-48\ (24\%)}$*	(Imtiaz *et al.*, 2021a)
2020	Faisalabad Punjab	*K. pneumoniae*	Clinical samples, environmental samples	1946	334	*bla$_{KPC}$ (6)*	(Aslam *et al.*, 2020)
2020	Faisalabad Punjab	*K. pneumoniae*	Veterinary samples	138	13	*bla$_{NDM-1\ (12)}$* *bla$_{OXA-48\ (11)}$*	(Chaudhry *et al.*, 2020)
2020	Islamabad	*P. aeruginosa*	Wound infection, bacteremia, burn infections	108	88	*bla-VIM (2)* *bla-NDM (3)*	(Saleem and Bokhari, 2020)
2020	Lahore Punjab	*K. pneumoniae*		227	117	*bla*$_{NDM-1}$ *bla*$_{OXA-48}$ *bla*$_{VIM}$	(Gondal *et al.*, 2020)
2021	Rawalpindi	*A.bumannii, K. pneumoniae, E. coli, P. aeruginosa , E. cloacae,*	Throat swabs, Foley catheter, wound swabs,	44	44	*bla-$_{CTX-M15}$* *bla-$_{TEM206}$* *bla-$_{NDM7}$*	(Hadjadj *et al.*, 2021)

		Klebsiella oxytoca, *Achromobacter xylosoxidans*				*bla-$_{OXA48}$* *bla*$_{\text{OXA-181}}$, *bla*$_{\text{NDM-4}}$ *bla*$_{\text{NDM-5}}$ *bla-$_{OXA23}$* *bla-$_{OXA72}$* *bla*$_{\text{OXA-181}}$, *bla*$_{\text{NDM-4}}$	
2021	Punjab	*Salmonella typhi*	Blood samples	4543	458	*bla*$_{\text{VIM (14)}}$	(Qamar *et al.*, 2021)
2021	Lahore Punjab	*Acinetobacter baumannii*	Wound, blood, pus	593	90	*bla$_{OXA-23}$ (63)* *bla$_{OXA-51}$ (81)* *bla$_{OXA-40}$ (58)* *bla$_{NDM1}$ (83)* *bla$_{IMP}$ (81)* *bla$_{VIM}$ (36)*	(Zahra *et al.*, 2021)
2021	Karachi Sindh	*E. cloacae (40)* *K. pneumonia (69)* *E. coli (84)*	Clinical specimens	238	52	*bla$_{NDM}$* $_{(43)}$	(Uddin *et al.*, 2021)
2021	Lahore Punjab	*A. baumannii*	Clinical isolates	174	113	*bla*$_{\text{OXA-23}}$ *bla*$_{\text{NDM-1}}$ *bla*$_{\text{OXA-51}}$	(Ejaz *et al.*, 2021a)

Table 2 Colistin resistance reported from different regions of Pakistan.

Year	City (Province)	Bacterial species	Specimens	No. of isolates	Resistant isolates	Colistin resistant gene	Reference
2021	Islamabad Rawalpindi	*K. pneumoniae*	Blood, urine, puss, catheter, body fluids, nasal swabs, sputum, tissue	200	12	*mcr-1* *mcr-2*	(Imtiaz *et al.*, 2021b)
2021	Sindh Karachi	*K. pneumoniae*	Sputum, blood, urine, pus, tissue, tracheal aspirates CSF	34	34	Mutations in lipid-A and Ara-4 N pathways	(Masood *et al.*, 2021)
2018	Punjab Faisalabad	*E. coli*	Healthy broilers	100	8	*mcr-1*	(Lv *et al.*, 2018)
2019	KPK Peshawar	*A. baumannii (n = 62)* *P. aeruginosa (n = 84)*	Clinical isolates	146	6/62 10/84	*mcr-1*	(Hameed *et al.*, 2019)
2022	KPK Peshawar	*E. coli*	Fecal of food producing animals (FPAs)	250	29	*mcr-1*	(Shafiq *et al.*, 2022)
2017	Sindh Karachi	*E. coli* *K. pneumonia* *Raoultella* spp. *Enterobacter* spp. *Klebsiella oxytoca* *Citrobacter spp*.	Clinical isolates	251	40	Intrinsic resistance	(Qamar *et al.*, 2017)
2021	KPK Peshawar	*E. coli* (120)	Clinical isolates	180	28/120 24/60	*mcr-1* (10)	(Hameed *et al.*, 2020)

		K. pneumoniae (60)					
2021	Punjab Multan Islamabad Rawalpindi Muzaffargarh Abbottabad Okara Toba Tek Singh	*Bacillus Shigella* *Escherichia* *Lysinibacillus* *Staphylococcus* *Pseudomonas* *Actinobacteria* *Stenotrophomonas* *Arthrobacter* *Enterobacter* *Exiguobacterium* *Klebsiella* *Luteimonas* *Glutamicibacter* *Microbacterium* *Rhodococcus* *Kurthia*	FPA	51	23%	*mcr-1*	(Ali *et al.*, 2021)
2018	Sindh Karachi	*K. pneumoniae*	Blood, urine, wound	10	7	Chromosome mediated resistance (*mgrB* and *pmrB)*	(Lomonaco *et al.*, 2018)
2022	KPK Peshawar	*E.coli* *Pseudomonas* *Aeruginosa* *Klebsiella* *Pneumoniae*	Clinical isolates (urine)	2000 (281 showed growth)	55	----------	(Arif *et al.*, 2022)
2020	KPK Peshawar	*K. pneumoniae*	Clinical isolates	298	4	*mcr-1*	(Bilal *et al.*, 2020)
2021	Islamabad	*E. coli*	Stool, blood, urine, wound	545	4	*mcr-1*	(Bilal *et al.*, 2021)
2021	Punjab	*E. coli*	Clinical isolates	100	4	*mcr-1*	(Li *et al.*, 2021b)

	Faisalabad						
2022	Punjab Faisalabad	*E. coli*	FPAs (chickens)	100	36	*mcr-1*	(Li *et al.*, 2022)
2021	Punjab Faisalabad Lahore	*E. coli* *K. pneumoniae* *A. baumannii* *P. aeruginosa*	Clinical specimens	718	19	*mcr-1 (18)* *mcr-2 (1)*	(Ejaz *et al.*, 2021b)

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Table 3 Tigecycline resistance reported from different regions of Pakistan.

Year	City (Province)	Bacterial species	Specimens	No. of isolates	Resistant isolates	Tigecycline resistant gene	Reference
2021	Punjab Faisalabad	*E. coli*	Clinical Animals Environmental sources	1100	4	*tet*X4	(Mohsin *et al.*, 2021)
2021	Punjab Faisalabad	*E. coli*	Clinical isolates	100	4	*tet*X	(Li *et al.*, 2021b)
2022	Punjab Faisalabad	*E. coli*	FPAs (chickens)	100	36	*tet*X4	(Li *et al.*, 2022)

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