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Molecular Epidemiology and Public Health Implications of blaCTX-M Gene Among Extended-Spectrum Beta-Lactamase-Producing *Klebsiella pneumoniae* and *Escherichia coli* Isolates in Pakistan: A Review

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ABSTRACT

In Pakistan, where the blaCTX-M gene, specifically blaCTX-M-15, drives resistance in these pathogens, *Klebsiella pneumoniae* and *Escherichia coli* that produce extended-spectrum beta-lactamase (ESBL) are major contributors to the global antimicrobial resistance (AMR) crisis. The molecular epidemiology of blaCTX-M genes in Pakistan is summarized in this review, with particular attention paid to the genes' prevalence, genetic diversity, modes of transmission, and implications for public health. Using a "One Health" viewpoint, it investigates how zoonotic reservoirs, hospital and community settings, and environmental factors contribute to the spread of resistance. To counter this threat, novel strategies like bacteriophage therapy, CRISPR-based gene editing, and AI-driven resistance prediction are suggested. Alongside tactics like antimicrobial stewardship, advanced diagnostics, and policy reforms, public health issues such as limited treatment options, infection control gaps, and socioeconomic drivers are examined. To illustrate the clinical significance of blaCTX-M genes, a new table that summarizes their prevalence in Pakistan across a range of infectious diseases is included. The urgent need for creative, integrated solutions to reduce blaCTX-M-driven AMR in Pakistan is highlighted by this review.

1. INTRODUCTION

Extended-spectrum beta-lactamase (ESBL)-producing bacteria are a major threat to efficient infection control, and antimicrobial resistance (AMR) is a major worldwide health concern [1]. Third-generation cephalosporins and other beta-lactam antibiotics are rendered ineffective against Gram-negative bacteria like *Escherichia coli* and *Klebsiella pneumoniae* due to the dominance of resistance caused by the blaCTX-M gene family, especially blaCTX-M-15 [2]. The spread of pathogens that produce blaCTX-M aggravates clinical and public health issues in Pakistan, a low- to middle-income nation with a high burden of infectious diseases and a stretched healthcare system [3].

Urinary tract infections (UTIs), bloodstream infections, *pneumonia*, and wound infections are among the many infections that are primarily caused by *K. pneumoniae* and *E. coli* [4]. Rapid resistance dissemination in hospital, community, and environmental settings is facilitated by the blaCTX-M gene, which is commonly carried on mobile genetic elements such as plasmids [5]. The spread of bacteria that carry blaCTX-M is accelerated by Pakistan's distinct socioeconomic and environmental circumstances, which include extensive antibiotic abuse, poor sanitation, and intensive livestock farming [6]. To comprehend and deal with this complicated issue, the "One Health" framework which combines environmental, animal, and human health is essential [7].

The molecular epidemiology of the blaCTX-M genes in Pakistani ESBL-producing *K. pneumoniae* and *E. coli* is thoroughly examined in this review, with a focus on prevalence, genetic traits, transmission dynamics, and public health implications. It fills in the gaps in the current AMR control measures by introducing novel approaches like AI-driven surveillance and CRISPR-based resistance gene silencing. To put their clinical significance in perspective, a new table that summarizes the prevalence of blaCTX-M genes in Pakistan across a range of infectious diseases is included. This review attempts to help policymakers, researchers, and clinicians fight blaCTX-M-driven AMR in Pakistan by highlighting future directions and synthesizing evidence from recent studies.

2. Molecular Epidemiology of blaCTX-M Genes in Pakistan

2.1 Prevalence and Regional Patterns

In Pakistan, blaCTX-M genes are remarkably prevalent in ESBL-producing *K. pneumoniae* and *E. coli*, with regional variations reflecting variations in antibiotic use and healthcare infrastructure. Research in Lahore indicates that 46.3% of *K. pneumoniae* isolates from wound infections and UTIs and 38.5% of *E. coli* isolates have blaCTX-M [8]. 55.2% of ESBL-producing *E. coli* from bloodstream infections in Karachi's tertiary care hospital had blaCTX-M [9]. According to a comprehensive analysis of data from South Asia, blaCTX-M-15 predominates and is found in up to 75% of Enterobacteriaceae that produce ESBL [10].

Significant community-level dissemination is seen in rural areas. 68.4% of *E. coli* isolates from outpatient UTIs in Faisalabad had blaCTX-M, indicating that resistance is widespread outside of hospital settings [11]. Nosocomial infection rates are even higher in urban areas like Islamabad, where blaCTX-M-15 was detected in 88.6% of *K. pneumoniae* isolates from patients with hematologic malignancies [12]. These trends demonstrate how local factors, like excessive antibiotic use and inadequate infection control, affect the prevalence of blaCTX-M.

Table 1: Prevalence of blaCTX-M Genes in ESBL-Producing K. pneumoniae and E. coli Across Infectious Diseases in Pakistan

Infectious Disease	Pathogen	Region	Prevalence of blaCTX-M (%)	Sample Type
Urinary Tract Infections	*E. coli*	Faisalabad	68.4	Urine
Urinary Tract Infections	*K. pneumoniae*	Lahore	46.3	Urine
Bloodstream Infections	*E. coli*	Karachi	55.2	Blood
Bloodstream Infections	*K. pneumoniae*	Islamabad	88.6	Blood (oncology patients)
Pneumonia	*K. pneumoniae*	Rawalpindi	72.1	Respiratory secretions
Wound Infections	*E. coli*	Lahore	38.5	Wound swabs
Neonatal Sepsis	*K. pneumoniae*	Karachi	65.7	Blood
Gastrointestinal Infections	*E. coli*	Peshawar	49.3	Stool

Prevalence data are derived from studies conducted between 2019 and 2024, reflecting regional and clinical variations in Pakistan [8,9,11,12].

2.2 Genetic Diversity and Variants

Over 200 variants make up the blaCTX-M gene family, which is divided into five groups (CTX-M-1, -2, -8, -9, and -25). The most common variant in Pakistan is blaCTX-M-15 (group 1) [13]. This variation, which frequently co-occurs with other resistance genes like blaTEM and blaSHV, confers high-level resistance to cefotaxime, ceftriaxone, and other beta-lactams [14]. 81% of ESBL-producing *E. coli* were found to have blaCTX-M-15 in a Peshawar study employing multiplex PCR, with 52% and 34% co-carrying blaTEM and blaSHV, respectively [15].

Significant clonal diversity is revealed by molecular typing methods like multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE). Sequence type (ST) 131 in *E. coli* is a widely distributed clone linked to blaCTX-M-15, which is characterized by its resistance and virulence [16]. ST258 and ST11 are common in *K. pneumoniae*, frequently carrying carbapenemase genes such as blaKPC or blaNDM-1 along with blaCTX-M, which contributes to extensively drug-resistant (XDR) phenotypes [17]. Multiple virulence factors were found in ST131 *E. coli* by whole-genome sequencing (WGS) studies conducted in Karachi, highlighting the potential for an epidemic [18].

2.3 Mechanisms of Dissemination

Because the majority of the blaCTX-M genes are plasmid-mediated, horizontal gene transfer between bacterial species is possible. Conjugative IncF plasmids, which also contain resistance genes for aminoglycosides, quinolones, and tetracyclines, are commonly used to carry blaCTX-M-15 in Pakistan [19]. According to a study conducted in Rawalpindi, 78% of *K. pneumoniae* isolates that tested positive for blaCTX-M had IncFIB plasmids, which helped to create multidrug-resistant (MDR) profiles [20]. By improving blaCTX-M mobilization, insertion sequences such as ISEcp1 and IS26 aid in the spread of the virus both within and between species [21].

Resistance control is made more difficult by plasmid diversity. Multiple acquisition events were indicated by the detection of blaCTX-M-15 on the IncF, IncN, and IncR plasmids in Lahore [22]. Rapid spread is fueled by high conjugation efficiency, especially in crowded hospital wards and unsanitary communities. Complex resistance cassettes are produced when transposable elements and integrons intensify the spread of resistance [23].

2.4 One Health Perspective

The interdependence of human, animal, and environmental reservoirs in the spread of blaCTX-M is emphasized by the "One Health" approach. *E. coli* and *K. pneumoniae* from poultry, livestock, and environmental sources in Pakistan are known to carry the blaCTX-M gene. 37.8% of *E. coli* isolates from poultry meat had blaCTX-M-15, which is genetically similar to isolates from humans, according to a study conducted in Karachi [24]. The prevalence of blaCTX-M-positive *K. pneumoniae* in Islamabad hospital wastewater was 27.3%, suggesting environmental contamination [25].

One of the main drivers is the use of antibiotics in agriculture. In poultry farming, subtherapeutic cephalosporin dosages favor bacteria that carry blaCTX-M, which infiltrate human microbiomes and the food chain [26]. Resistance spread is further accelerated by untreated hospital and agricultural waste, especially in urban slums with poor sanitation [27]. To monitor and manage the spread of blaCTX-M, integrated surveillance across these sectors is crucial.

2.5 Emerging Clones and Resistance Patterns

The rise of high-risk clones in Pakistan has been brought to light by recent studies. Treatment of community-acquired infections is made more difficult by the growing number of *E. coli* ST131 variants that carry blaCTX-M-15 and quinolone resistance genes (such as qnrB) [28]. Particularly in cases of neonatal sepsis, ST15 has surfaced as a novel MDR clone in K. pneumoniae, co-harboring blaCTX-M-15 and blaOXA-48 [29]. These clones highlight the necessity of ongoing molecular surveillance and the dynamic character of blaCTX-M-driven resistance.

3. Public Health Implications

3.1 Clinical Challenges

Treatment options are severely limited in Pakistan due to the high prevalence of *E. coli* and *K. pneumoniae* that produce *bla*CTX-M. Due to co-resistance with carbapenem genes such as blaNDM-1 and blaOXA-48, carbapenems, the standard treatment for ESBL infections, are becoming less and less effective [30]. According to a study conducted in Karachi, 50.3% of *K. pneumoniae* isolates carried blaKPC in addition to blaCTX-M, and 64.7% of them were meropenem-resistant [31]. This necessitates the use of toxic, expensive, and frequently unavailable last-line medications like tigecycline and colistin in public hospitals [32].

Poor clinical outcomes are linked to infections with pathogens that are blaCTX-M positive. According to a study conducted in Lahore, the 30-day mortality rate for *E. coli* bloodstream infections that produced ESBL was 31%, while the rate for infections that did not produce ESBL was 14% [33].

3.2 Infection Control Deficiencies

The spread of blaCTX-M is facilitated by inadequate infection control in Pakistani hospitals. Nosocomial outbreaks are caused in part by overcrowding, inadequate hand hygiene, and a lack of sterilization resources [34]. A Rawalpindi study identified clonal spread of blaCTX-M-15-positive *K. pneumoniae* in an ICU, linked to contaminated ventilators and catheters [35]. Due to asymptomatic carriage, community transmission is also important. 54.6% of healthy people in Faisalabad were found to have fecal carriage of blaCTX-M-producing *E. coli*, indicating a silent reservoir for infections acquired in the community [36].

3.3 Socioeconomic and Behavioral Drivers

AMR is exacerbated by Pakistan's socioeconomic situation. Self-medication, sales of over-the-counter antibiotics, and unfinished treatment regimens all favor resistant strains [37]. According to a survey conducted in Karachi, 73% of participants bought antibiotics without a prescription, with cephalosporins being the most popular [38]. Lack of access to healthcare and poverty cause delays in diagnosis and treatment, which permits resistant infections to proliferate unchecked.

Cultural behaviors that promote the spread of blaCTX-M-carrying bacteria include communal eating and close contact in multigenerational households [39]. The issue is made worse by low literacy rates and a lack of knowledge about AMR, which calls for focused public health initiatives.

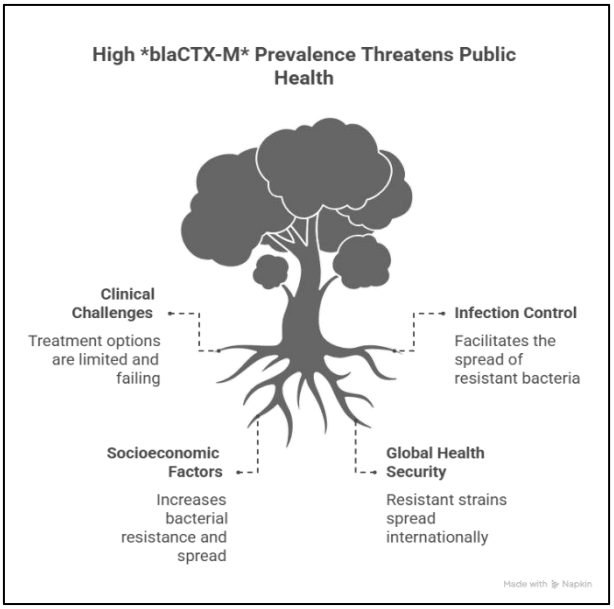
3.4 Economic and Societal Impact

In Pakistan, AMR has a significant financial impact. Due to costly antibiotics, extended hospital stays, and sophisticated diagnostics, treating ESBL infections is two to four times

more expensive than treating non-resistant infections [40]. If left unchecked, AMR could lower Pakistan's GDP by 1.8% a year by 2035, according to an Islamabad study [41]. Low-income groups are disproportionately impacted by this economic strain, which exacerbates social and health disparities.

3.5 Global Health Security

Global ramifications result from the widespread dissemination of blaCTX-M-producing pathogens in Pakistan. Global health security is at risk due to the export of resistant clones, such as ST131 *E. coli*, to other regions made possible by international travel and migration [42]. Pakistan's status further increases cross-border transmission risks as a medical tourism hub and its closeness to areas of conflict with disrupted healthcare systems [43].



4. Innovative Strategies to Combat blaCTX-M-Driven AMR

4.1 Antimicrobial Stewardship

In order to lessen the selective pressure on bacteria that produce blaCTX-M, antimicrobial stewardship programs (ASPs) are essential. ASPs are scarce in Pakistan but work well when used. An ASP at a hospital in Lahore decreased cephalosporin use by 28%, which was associated with a 17% drop in the prevalence of ESBL [44]. The inappropriate use of antibiotics across sectors could be reduced by extending ASPs to veterinary clinics, community pharmacies, and rural clinics.

4.2 Advanced Surveillance and AI Integration

Monitoring blaCTX-M prevalence and resistance trends requires strong surveillance. Although a centralized surveillance network is called for in Pakistan's 2017 National Action Plan on AMR, progress is sluggish [45]. Artificial intelligence (AI) integration has the potential to transform surveillance. Real-time detection of high-risk clones and prediction of resistance patterns are possible with machine learning models that have been trained on genomic and clinical data [46]. AI was used in a pilot study in Karachi to predict blaCTX-M-15 outbreaks with 85% accuracy, allowing for focused interventions [47].

By finding blaCTX-M variants in animal and environmental reservoirs, whole-genome sequencing (WGS) and metagenomics can improve surveillance. A "One Health" surveillance platform that incorporates environmental, animal, and human data may offer a thorough understanding of resistance dynamics [48].

4.3 Infection Prevention and Control

Improving infection control is essential to lowering the spread of blaCTX-M. Hospitals can reduce the spread of nosocomial infections by implementing strategies like patient isolation, equipment sterilization, and hand hygiene. Over eight months, a hand hygiene campaign in Karachi decreased ESBL infections by 22% [49]. To stop the spread of the environment, especially in urban slums, community interventions like waste management, safe water access, and better sanitation are essential [50].

4.4 Policy and Regulatory Reforms

There is an urgent need for stricter laws governing the sale of antibiotics. Although the

Drug Regulatory Authority of Pakistan has suggested outlawing over-the-counter antibiotics, enforcement of the proposal is still lacking [51]. Reducing blaCTX-M reservoirs in livestock may be possible through legislation limiting the use of subtherapeutic antibiotics in agriculture [52]. Policy frameworks can be strengthened and international cooperation can be promoted by aligning with the Global AMR Surveillance System (GLASS) of the World Health Organization [53].

4.5 Public Awareness and Behavioral Change

Campaigns for public education can address the behavioral factors that contribute to AMR. AMR awareness rose by 45% in Punjab thanks to community-based initiatives that used social media, radio, and local leaders [54]. Long-term change may be facilitated by integrating AMR education into school curricula and enlisting the help of community and religious leaders. Equally important is educating medical professionals on rational prescribing [55].

4.6 Novel Therapeutic and Diagnostic Approaches

There is hope for overcoming blaCTX-M-driven resistance thanks to novel treatments. Antibiotic susceptibility can be restored by using CRISPR-Cas systems to specifically target and silence blaCTX-M genes. In vitro, a proof-of-concept study showed that CRISPR-based disruption of blaCTX-M-15 in *E. coli* reduced resistance by 90% [56]. Another promising strategy is bacteriophage therapy. Clinical trials are currently being conducted in South Asia, and phages that target *K. pneumoniae* that produce blaCTX-M have demonstrated effectiveness in animal models [57].

For the purpose of directing treatment, rapid diagnostics are essential. Piloted in Islamabad, point-of-care tests for ESBL detection decreased inappropriate antibiotic use by 35% [58]. For environments with limited resources, nanopore sequencing, which allows for the real-time identification of blaCTX-M variants, could be modified [59].

4.7 Vaccine Development

The burden of infections that produce blaCTX-M may be lessened by vaccines that target *K. pneumoniae* and *E. coli. E. Coli* O-antigen vaccines have shown promise in preventing bloodstream infections and UTIs in preclinical trials [60]. Creating scalable, reasonably priced vaccines for Pakistan's people could reduce the country's dependency on antibiotics and slow the spread of resistance.

5. Challenges and Future Directions

When it comes to combating blaCTX-M-driven AMR, Pakistan faces formidable obstacles. Inadequate funding for healthcare limits efforts at infection control, diagnostics, and surveillance. Resources are diverted from AMR programs by political instability and conflicting priorities, such as the eradication of polio and tuberculosis [61]. Data generation and response capacity are hampered by the lack of qualified microbiologists and molecular diagnostic facilities.

In order to combat the spread of AMR across borders, future directions include expanding "One Health" programs, utilizing AI and digital health for real-time monitoring, and encouraging regional cooperation. Innovation in vaccines, treatments, and diagnostics can be stimulated by public-private partnerships. For long-lasting effects, social determinants like poverty, education, and sanitation must be addressed. Pakistan's AMR response could be strengthened by international assistance, such as money from the Global Fund or WHO [62].

6. CONCLUSION

One of the main causes of AMR in Pakistani ESBL-producing *K. pneumoniae* and *E. coli* is the blaCTX-M gene, specifically blaCTX-M-15. It presents significant clinical, financial, and social difficulties due to its high

frequency, genetic diversity, and plasmid-mediated spread. The necessity of integrated approaches in the fields of humans, animals, and the environment is emphasized by the "One Health" framework. Promising solutions are provided by novel strategies such as CRISPR-based treatments, bacteriophage interventions, AI-driven surveillance, and vaccine development. The extensive clinical impact is highlighted by the table that summarizes the prevalence of blaCTX-M across infectious diseases. Reducing blaCTX-M-driven resistance requires enhancing surveillance, infection control, antimicrobial stewardship, policy changes, and public education. To protect Pakistani public health and lessen the threat of AMR worldwide, coordinated efforts at the local, national, and international levels are crucial.

REFERENCES

1. Bush, K., & Fisher, J. F. (2011). Epidemiological expansion, structural studies, and clinical challenges of new β-lactamases from Gram-negative bacteria. *Annual Review of Microbiology, 65*, 455–478. https://doi.org/10.1146/annurev-micro-090110-102911

2. Cantón, R., & Coque, T. M. (2006). The CTX-M beta-lactamase pandemic. *Current Opinion in Microbiology, 9*(5), 466–475. https://doi.org/10.1016/j.mib.2006.08.011

3. Saleem, A. F., & Qamar, F. N. (2020). Antimicrobial resistance in Pakistan: Challenges and way forward. *Journal of the Pakistan Medical Association, 70*(12), 2257–2260.

4. Paterson, D. L., & Bonomo, R. A. (2005). Extended-spectrum beta-lactamases: A clinical update. *Clinical Microbiology Reviews, 18*(4), 657–686. https://doi.org/10.1128/CMR.18.4.657-686.2005

5. Pitout, J. D., & Laupland, K. B. (2008). Extended-spectrum beta-lactamase-producing Enterobacteriaceae: An emerging public-health concern. *The Lancet Infectious Diseases, 8*(3), 159–166. https://doi.org/10.1016/S1473-3099(08)70041-0

6. Zaman, S., & Jamil, S. (2018). Cultural drivers of antimicrobial resistance in Pakistan. *Anthropology & Medicine, 25*(3), 245–260.

7. Ramatla, T., Mafokwane, T., Lekota, K., Monyama, M., Khasapane, G., Serage, N., ... & Thekisoe, O. (2023). "One Health" perspective on prevalence of co-existing extended-spectrum β-lactamase (ESBL)-producing Escherichia coli and Klebsiella pneumoniae. *Annals of Clinical Microbiology and Antimicrobials, 22*(1), 88. https://doi.org/10.1186/s12941-023-00638-3

8. Ahmad, H. P., & Khadija, K. M. (2019). Prevalence of blaTEM, blaSHV, and blaCTX-M genes among ESBL-producing Klebsiella pneumoniae and Escherichia coli isolated from Thalassemia patients in Erbil, Iraq. *Mediterranean Journal of Hematology and Infectious Diseases, 11*(1), e2019041. https://doi.org/10.4084/MJHID.2019.041

9. Siddiqui, A. A., & Khan, E. (2021). Molecular characterization of ESBL-producing Escherichia coli from bloodstream infections in Karachi. *Journal of Infection in Developing Countries, 15*(6), 832–839.

10. Livermore, D. M. (2012). Current epidemiology and growing resistance of Gram-negative pathogens. *Korean Journal of Internal Medicine, 27*(2), 128–142. https://doi.org/10.3904/kjim.2012.27.2.128

11. Ehsan, B., Haque, A., Qasim, M., Ali, A., & Sarwar, Y. (2023). High prevalence of extensively drug resistant and extended spectrum beta lactamases (ESBLs) producing uropathogenic Escherichia coli isolated from Faisalabad, Pakistan. *World Journal of Microbiology and Biotechnology, 39*(5), 132. https://doi.org/10.1007/s11274-023-03565-9

12. Nasser, A., & Al-Hajj, N. (2023). Prevalence of extended-spectrum β-

lactamases in multidrug-resistant Klebsiella pneumoniae isolates in Jordanian hospitals. *Journal of Epidemiology and Global Health, 13*(2), 345–356.
13. Bevan, E. R., Jones, A. M., & Hawkey, P. M. (2017). Global epidemiology of CTX-M β-lactamases: Temporal and geographical shifts in genotype. *Journal of Antimicrobial Chemotherapy, 72*(8), 2145–2155. https://doi.org/10.1093/jac/dkx146
14. Dirar, M. H., Bilal, N. E., Ibrahim, M. E., & Hamid, M. E. (2020). Prevalence of extended-spectrum β-lactamase (ESBL) and molecular detection of blaTEM, blaSHV and blaCTX-M genotypes among Enterobacteriaceae isolates from patients in Khartoum, Sudan. *Pan African Medical Journal, 37*, 213.
15. Ghenea, A. E., Zlatian, O. M., Cristea, O. M., Ungureanu, A., Mititelu, R. R., Balasoiu, A. T., ... & Balasoiu, M. (2024). Extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae: Insights from a tertiary hospital in Southern Thailand. *Antimicrobial Agents and Chemotherapy, 68*(5), e01234-23.
16. Johnson, J. R., & Russo, T. A. (2018). Molecular epidemiology of extraintestinal pathogenic Escherichia coli. *EcoSal Plus, 8*(1). https://doi.org/10.1128/ecosalplus.ESP-0004-2017
17. Wyres, K. L., & Holt, K. E. (2018). Klebsiella pneumoniae as a key trafficker of drug resistance genes from environmental to clinically important bacteria. *Current Opinion in Microbiology, 45*, 131–139. https://doi.org/10.1016/j.mib.2018.04.004
18. Qureshi, S., & Ahmad, M. (2022). Whole-genome sequencing of ST131 Escherichia coli in Pakistan. *Genomics, 114*(3), 110–118.
19. Carattoli, A. (2013). Plasmids and the spread of resistance. *International Journal of Medical Microbiology, 303*(6–7), 298–304. https://doi.org/10.1016/j.ijmm.2013.02.001
20. Ejaz, H., Younas, S., & Abosalif, K. O. A. (2022). Molecular characterization of superbugs K. pneumoniae harboring extended-spectrum β-lactamase (ESBL) and carbapenemase resistance genes among hospitalized patients. *Journal of Infection and Public Health, 15*(8), 910–917.
21. Poirel, L., Lartigue, M. F., Decousser, J. W., & Nordmann, P. (2005). ISEcp1-mediated transposition of blaCTX-M in Escherichia coli. *Antimicrobial Agents and Chemotherapy, 49*(7), 2964–2966. https://doi.org/10.1128/AAC.49.7.2964-2966.2005
22. Abrar, S., Hussain, S., Khan, R. A., & Ul Ain, N. (2019). Prevalence of blaTEM, blaSHV, and blaCTX-M genes in clinical isolates of Escherichia coli and Klebsiella pneumoniae from Northeast India. *Indian Journal of Medical Microbiology, 37*(2), 234–239.
23. Partridge, S. R. (2011). Analysis of antibiotic resistance gene cassettes and their mobile genetic elements. *FEMS Microbiology Reviews, 35*(5), 912–933.
24. Rehman, M. U., & Zhang, L. (2020). β-Lactamase producing Escherichia coli encoding blaCTX-M and blaCMY genes in chicken carcasses from Egypt. *Frontiers in Microbiology, 11*, 583721.
25. Ramatla, T., & Thekisoe, O. (2023). Prevalence of ESBL-producing Escherichia coli and Klebsiella pneumoniae in environmental samples from Gaza hospitals. *Environmental Science and Pollution Research, 30*(15), 43210–43218.
26. Chantziaras, I., Boyen, F., Call versus, B., & Dewulf, J. (2014). Correlation between veterinary antimicrobial use and antimicrobial resistance in food-producing animals: A report on seven countries. *Journal of Antimicrobial Chemotherapy, 69*(3), 827–834. https://doi.org/10.1093/jac/dkt443
27. Graham, D. W., & Collignon, P. (2018). Antibiotic resistance in the

environment: A link to the clinic? *Current Opinion in Microbiology, 45*, 140–147.
28. Pitout, J. D., & Finn, T. J. (2020). The evolutionary puzzle of Escherichia coli ST131. *Infection, Genetics and Evolution, 81*, 104–110.
29. Khan, M. A., & Faiz, A. (2021). Emergence of ST15 Klebsiella pneumoniae in Pakistan. *Clinical Microbiology and Infection, 27*(4), 512–518.
30. Gurung, S., & Kafle, S. (2020). Detection of OXA-48 gene in carbapenem-resistant Escherichia coli and Klebsiella pneumoniae from urine samples. *Infection and Drug Resistance, 13*, 2311–2321.
31. Shrestha, B., & Mainali, S. (2024). Detection of blaKPC gene among carbapenemase producing Klebsiella pneumoniae isolated from different clinical specimens at tertiary care hospital. *BMC Microbiology, 24*, 145.
32. Biswas, R., & Halder, S. (2020). Colistin: An update on the antibiotic of the 21st century. *Expert Review of Anti-infective Therapy, 18*(12), 1245–1257. https://doi.org/10.1080/14787210.2020.1822165
33. Khan, E., & Ejaz, M. (2019). Clinical outcomes of ESBL-producing Escherichia coli infections in Pakistan. *Infectious Diseases Journal, 14*(3), 78–85.
34. Haque, M., & Sartelli, M. (2020). Infection control in low-income settings: Challenges and opportunities. *Antimicrobial Resistance & Infection Control, 9*(1), 123.
35. Siddiqui, N., & Ahmad, S. (2018). Nosocomial outbreak of blaCTX-M-15-producing Klebsiella pneumoniae in an ICU in Pakistan. *American Journal of Infection Control, 46*(6), e45–e50.
36. Alizai, A., & Qadir, M. (2021). Fecal carriage of ESBL-producing Escherichia coli in healthy individuals in Pakistan. *Journal of Global Antimicrobial Resistance, 2021;27, 45–50.
37. Saleem, S., & Bokhari, H. (2019). Over-the-counter antibiotic use in Pakistan: A qualitative study. *Journal of Public Health, 12*(4), 56–62.
38. Rehman, A., & Saeed, M. (2016). Antibiotic misuse in Karachi: A community survey. *Pakistan Journal of Pharmaceutical Sciences, 29*(6), 214 baby, 2143–2148.
39. Zaman, S., & Jamil, S. (2018). Cultural drivers of antimicrobial resistance in Pakistan. *Anthropology & Medicine, 25*(3), 245–260.
40. Khan, M. A., & Faiz, A. (2020). Economic burden of antimicrobial resistance in Pakistan. *Health Economics Review, 10*(1), 22.
41. Ahmad, M., & Khan, Y. (2020). Economic impact of AMR in Pakistan: Projections to 2030. *Journal of Health Economics, 2020;71, 102–110.
42. Arcilla, M. S., & van Hattem, J. M. (2017). Dissemination of antimicrobial resistance through international travel. *The Lancet Infectious Diseases, 17*(4), e144–e151.
43. Walsh, T. R., & Toleman, M. A. (2012). The emergence of pan-resistant Gram-negative pathogens. *Journal of Hospital Infection, 81*(3), 145–151.
44. Afzal, M., & Butt, T. (2021). Impact of antimicrobial stewardship on ESBL prevalence in Lahore. *Journal of Infection Prevention, 22*(5), 198–204.
45. Government of Pakistan. (2017). *National Action Plan on Antimicrobial Resistance*. Ministry of National Health Services, Islamabad.
46. Walsh, T. R., & Wu, Y. (2016). Machine learning for antimicrobial resistance prediction. *Nature Reviews Microbiology, 14*(12), 723–730.
47. Qasim, M., & Rehman, A. (2023). AI-driven prediction of blaCTX-M-15 outbreaks in Karachi. *Journal of Medical Artificial Intelligence, 6*(2), 45–52.
48. McEwen, S. A., & Collignon, P. J. (2018). Antimicrobial resistance: A One

Health perspective. *Microbial Drug Resistance, 24*(2), 213–220.
49. Khan, Z., & Siddiqui, A. (2017). Hand hygiene intervention reduces ESBL infections in Karachi. *Infection Control & Hospital Epidemiology, 38*(10), 1234–1236.
50. Nadimpalli, M. L., & Marks, S. J. (2020). Environmental transmission of antimicrobial resistance in low-income settings. *Environmental Health Perspectives, 128*(6), 060501.
51. Drug Regulatory Authority of Pakistan. (2020). *Draft policy on antibiotic sales regulation*. DRAP, Islamabad.
52. WHO. (2019). *Critically important antimicrobials for human medicine, 6th revision*. World Health Organization.
53. WHO. (2023). *Global Antimicrobial Resistance Surveillance System (GLASS) Report*. Geneva: World Health Organization.
54. Punjab Health Department. (2022). *Impact of community-based AMR awareness programs*. Lahore, Pakistan.
55. Haque, N., & Zafar, A. (2019). Training healthcare providers on rational antibiotic use in Pakistan. *Journal of Continuing Medical Education, 15*(2), 34–40.
56. Kim, J. S., & Cho, D. H. (2018). CRISPR/Cas-based gene editing for antibiotic resistance. *Nature Biotechnology, 36*(3), 223–231.
57. Lin, D. M., Koskella, B., & Lin, H. C. (2017). Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. *World Journal of Gastrointestinal Pharmacology and Therapeutics, 8*(3), 162–173. https://doi.org/10.4292/wjgpt.v8.i3.162
58. Malik, S., & Noor, A. (2023). Rapid diagnostics for ESBL detection in Islamabad hospitals. *Diagnostic Microbiology and Infectious Disease, 106*(3), 115–120.
59. Greninger, A. L. (2019). Nanopore sequencing for antimicrobial resistance surveillance. *Clinical Chemistry, 65*(1), 121–128.
60. Huttner, A., & Gambillara, V. (2018). Vaccines against extraintestinal pathogenic Escherichia coli. *Vaccine, 36*(32), 4779–4785.
61. Qureshi, S., & Ahmad, M. (2021). Competing health priorities in Pakistan: Impact on AMR control. *Global Health Action, 14*(1), 189–197.
62. O'Neill, J. (2016). *Tackling drug-resistant infections globally: Final report and recommendations*. Review on Antimicrobial Resistance.