Volume 3, Number 3, 2026
Pages 1347-1374
Bioscience Insights
ISSN: 3080-3489 (Print)
ISSN: 3080-3497 (Online)
BiosciInsights.com

MOLECULAR EVOLUTION OF ANTIMICROBIAL RESISTANCE AND DRUG RESPONSE IN MULTIDRUG-RESISTANT BACTERIAL PATHOGENS

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https://doi.org/10.5281/zenodo.23939820

Article Information

Article History

Received:	June 19, 2026
Revised:	August 19, 2026
Accepted:	September 07, 2026
Available Online:	October 31, 2026

Keywords:
Antimicrobial resistance; Pakistan; NDM; CTX-M-15; extensively drug-resistant typhoid; molecular epidemiology; One Health

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Abstract

Pakistan carries one of the heaviest antimicrobial resistance burdens in the world, and it is also the setting in which one of the clearest documented events in resistance evolution took place. This review assembles the Pakistani evidence base – national surveillance, published prevalence studies, genomic analyses of Pakistani isolates, and the national policy documents – and asks what it shows about how resistance evolves and spreads in this country specifically.

The picture that emerges differs from the one described in reviews written from North American and European data, and the differences are mechanistic rather than merely epidemiological. Extended-spectrum β-lactamase production, overwhelmingly CTX-M-15, is close to universal among Pakistani Enterobacterales, so any further resistance determinant lands on a genome already primed for β-lactam resistance. Carbapenem resistance here is driven by NDM and OXA-48-like enzymes rather than KPC, which means the β-lactamase inhibitor combinations that transformed treatment elsewhere address a smaller share of the local problem. Colistin and tigecycline resistance are already measurable. Methicillin resistance is reported in roughly half of Staphylococcus aureus isolates, and Pakistan carries one of the world's largest burdens of multidrug-resistant tuberculosis.

The extensively drug-resistant Salmonella Typhi outbreak that began in Hyderabad in 2016 is examined in detail, because its genomics demonstrate the central argument: a multidrug-resistant clone that had circulated locally for years acquired a single conjugative plasmid and became extensively drug-resistant in one step.
We close with the drivers that make these pressures unusually strong in Pakistan – non-prescription dispensing, veterinary and poultry use, untreated effluent – with the limits of the available data, and with what containment measures have so far been evaluated.

INTRODUCTION

Antimicrobial resistance is among the leading causes of death in Pakistan. The Global Research on Antimicrobial Resistance (GRAM) project's country estimates for 2019 attributed 59,200 deaths directly to bacterial AMR and associated a further 221,300 with it, which placed resistant infection third among causes of death nationally, behind cardiovascular disease and maternal and neonatal disorders [1,2]. Revised GRAM modelling for 2021 gives 39,700 deaths attributable (uncertainty interval 31,000–48,300) and 161,000 associated (134,000–187,000) [3]. The two sets differ because the modelling was revised, not because the burden fell; both should be quoted as model estimates with wide intervals rather than as counts.

What is not in doubt is the national direction. On current trends, AMR-associated deaths in Pakistan are projected to reach approximately 168,000 by 2030, against a target of 159,000 implied by the globally agreed 10% reduction [3]. Pakistan also has among the highest AMR mortality rates in the WHO Eastern Mediterranean Region [4].

1.1 Rationale for a country-specific review

Most reviews of resistance mechanisms are written from datasets generated in high-income countries, and their conclusions do not transfer cleanly. Three features make the Pakistani situation mechanistically distinct.

The **resistance gene repertoire differs**. Carbapenem resistance in Pakistani Enterobacterales is dominated by NDM and OXA-48-like enzymes; KPC, which dominates US surveillance and against which avibactam, vaborbactam and relebactam were designed, is comparatively rare here. Treatment algorithms built on that assumption misfire.

The **baseline is already resistant**. CTX-M-15 carriage approaches universality in Pakistani clinical Enterobacterales, which means that an extended-spectrum β-lactamase background is the default genetic context into which any new determinant arrives, rather than an additional step.

The **selective pressures are unusually strong and unusually broad**: antibiotics are widely dispensed without prescription, used extensively in poultry and livestock, and discharged into watercourses with little treatment. Selection therefore acts on the human, animal and environmental compartments at once.

1.2 Scope, sources and limitations

This review uses data generated from Pakistani isolates, Pakistani patients or Pakistani national sources. Where a mechanism is described, it is described as it has been characterised in

Pakistani material. General molecular principles established elsewhere are referred to only where a Pakistani study invokes them.

Literature search. PubMed, PMC, Google Scholar and the WHO and Government of Pakistan document repositories were searched up to November 2026, combining terms for antimicrobial resistance and its mechanisms ("antimicrobial resistance", "ESBL", "CTX-M", "carbapenemase", "NDM", "OXA-48", "mcr", "colistin", "MRSA", "MDR-TB", "XDR typhoid") with "Pakistan" and with the names of its provinces and major cities. Studies were included where the isolates, patients or surveillance data were Pakistani. Preference was given to whole-genome sequencing studies, systematic reviews and meta-analyses, and national surveillance documents over single-centre phenotypic surveys, although the latter are cited where they provide the only available data. No formal quality scoring was applied; this is a narrative rather than a systematic review, and the limitations set out below apply throughout.

The evidence base has real limits, and they shape every figure quoted here. Most published resistance data come from tertiary-care laboratories in Karachi, Lahore, Islamabad and a handful of other cities, which over-represent referred, previously treated and hospitalised patients. National surveillance through the Pakistan Antimicrobial Resistance Surveillance System (PASS), aligned with the WHO GLASS framework since 2018, is still building coverage. Whole-genome sequencing of Pakistani isolates exists but in small numbers, and much of the molecular work rests on PCR detection of a predefined gene panel, which cannot identify determinants that were not looked for. Resistance rates quoted below are therefore prevalence among isolates that reached a laboratory and were tested, and they are likely to overstate resistance in the community while understating the diversity of mechanisms present.

2. The national resistance landscape

Pakistan's National Action Plan on Antimicrobial Resistance 2.0 (2024–2028) identifies five pathogens as responsible for most AMR-associated mortality in the country: *Klebsiella pneumoniae* (34,400 deaths), *Escherichia coli* (31,300), *Staphylococcus aureus* (28,600), *Salmonella* Typhi and *Streptococcus pneumoniae* [5]. The same document reports methicillin resistance in 49% of *S. aureus* isolates reported nationally, and that more than 51% of reported *E. coli* and *K. pneumoniae* isolates were multidrug-resistant, with 2.1% and 6.7% respectively classified as possibly pan-drug-resistant [5].

2.1 Gram-negative resistance rates

A systematic review of Pakistani studies covering 2006–2018 found resistance above 50% to third-generation cephalosporins, fluoroquinolones and co-trimoxazole among hospital isolates of *K. pneumoniae* and *E. coli*, with 30–50% resistance in community and laboratory-based studies, and resistance above 50% in *Acinetobacter* species to both aminoglycosides and carbapenems among hospitalised patients [6].

A more recent systematic review and meta-analysis of Pakistani *K. pneumoniae* studies published between 2015 and 2025 gives the pooled national estimates shown in Table 1 [7].

Table 1. ***Pooled resistance in Pakistani Klebsiella pneumoniae, 2015–2025.***

Agent	Pooled resistance (95% CI)
Meropenem	42% (31–53)
Imipenem	31% (22–41)
Colistin	21%
Polymyxin B	13%
Tigecycline	8%

The pooled prevalence of *K. pneumoniae* among isolates in these studies was 12% (95% CI 10–15) [7]. Carbapenem resistance at 31–42% is an order of magnitude above what is reported in North American surveillance, and places Pakistan among the highest-burden settings globally. Resistance to colistin and tigecycline, the agents reached for when carbapenems fail, is already measurable. The sequence of fallback options is therefore shorter here than treatment guidelines written elsewhere assume.

2.2 ESKAPEE pathogen prevalence

A cross-sectional study of clinical isolates from Hyderabad conducted between August 2023 and August 2024 found that ESKAPEE pathogens accounted for 82.1% of culture-positive clinical isolates, with *E. coli* and *K. pneumoniae* predominating, high resistance to β-lactams, cephalosporins and fluoroquinolones, and substantial carbapenem resistance among the major Gram-negative pathogens [8].

2.3 Methodological caveats

The pooled estimates carry wide confidence intervals and substantial between-study heterogeneity, because they aggregate studies that differ in setting, specimen type, species identification method and susceptibility-testing standard. Some Pakistani studies use CLSI breakpoints, others EUCAST, and some older work used NCCLS criteria, which affects comparability directly. The authors of these reviews conclude, rightly, that empirical therapy should be guided by local antibiograms rather than by national pooled averages, and the first priority for surveillance is standardised testing rather than wider testing [6,7].

3. Extended-spectrum β-lactamases: predominance of CTX-M-15

The single most consistent molecular finding across Pakistani studies is the dominance of CTX-M-15 among extended-spectrum β-lactamases. The proportion of isolates that are ESBL producers varies widely between studies, but the identity of the enzyme does not (Table 2).

Table 2. ***Extended-spectrum β-lactamase genes in Pakistani clinical isolates.***

Study setting	Finding
122 clinical *K. pneumoniae*, whole-genome sequenced	*bla*CTX-M-15 in 121 isolates (99.18%); *bla*VEB-5 in 45 (36.89%); 48 distinct acquired resistance genes detected [9]
Teaching hospital, Sindh, clinical *K. pneumoniae*, June 2019 – May 2020	*bla*CTX-M-15 in 60%; *bla*TEM 3%; *bla*SHV 3% [10]

Study setting	Finding
Tertiary care hospitals, Lahore, 148 clinical Gram-negative samples	95 (64%) phenotypic ESBL producers; *bla*CTX-M in 46% of *E. coli* and 50% of *Klebsiella* isolates [11]
Clinical *E. coli*	ESBL prevalence 25%, of which 74.8% carried *bla*CTX-M-15; other Pakistani series report 72% and 76% [12]

3.1 Mechanistic implications of near-universal CTX-M-15

The genomic survey of 122 clinical *K. pneumoniae* isolates is the most informative of these, because sequencing detects what a PCR panel cannot. Finding *bla*CTX-M-15 in 121 of 122 isolates means that in this population an ESBL background is not a resistance event; it is the starting state.

Any subsequently acquired determinant, whether a carbapenemase, a 16S methyltransferase or a *mcr* gene, lands on a genome that is already resistant to penicillins and oxyimino-cephalosporins, so the increment in phenotype from each new acquisition is larger than it would be on a susceptible background. This is the combinatorial accumulation that makes extensively drug-resistant phenotypes reachable in few steps.

Second, because the ESBL is carried on plasmids alongside other determinants, exposure to any one of the linked drug classes maintains the whole cassette. In the same cohort, plasmid-mediated quinolone-resistance determinants were present in 109 of 122 isolates (89.34%) and predicted porin alterations in 63 (51.64%) [9]. Fluoroquinolone use therefore sustains the β-lactam resistance genes it is linked to, and vice versa.

Third, the population structure argues against a single successful clone. The authors report broad genomic diversity, with *bla*NDM-1-positive isolates distributed across four distinct sequence-type, capsule and O-locus backgrounds, and conclude that multiple multidrug-resistant lineages are co-circulating rather than one clone expanding [9]. The resistance is in the mobile gene pool, not in one organism — which matters for control, because infection-control measures aimed at interrupting clonal transmission will not by themselves remove a determinant that is distributed across many lineages.

3.2 CTX-M-15 in animal and environmental isolates

The same enzyme is widespread outside hospitals. Whole-genome sequencing of ESBL-producing *E. coli* recovered from wild migratory birds at wetland sites along the Indus migration route found CTX-M-15 in 92.3% of isolates, with 88.4% showing a multidrug-resistant phenotype [13]. Surveys of Pakistani livestock and companion animals report CTX-M-type enzymes at comparable frequencies, including *bla*CTX-M-15 in 63% of *E. coli* from mastitic cow milk [14].

The Pakistani resistome is therefore not confined to the healthcare system. Wild birds acquire these organisms from the environment, which implicates water and waste rather than clinical transmission, and they then disperse them along migration routes.

4. Carbapenem resistance: predominance of NDM

Pakistan's carbapenemase profile is the sharpest point of divergence from the picture presented in most international reviews. NDM predominates, OXA-48-like enzymes are common, and KPC — the enzyme around which avibactam, vaborbactam and relebactam were designed — is rarely reported (Table 3).

Table 3. Carbapenemase genes in Pakistani carbapenem-resistant isolates.

Study	Setting	Carbapenemase findings
Urinary isolates, Jinnah Postgraduate Medical Centre, Karachi, Jan–Jun 2022	181 Enterobacterales from community- and hospital-acquired UTI	52 (28.7%) carbapenem-resistant; *bla*NDM in 45/52 (86.5%); *bla*OXA-48 in 6/52 (11.5%) [15]
Community carriage study, Rawalpindi	306 community samples, 78 CRE whole-genome sequenced	Carbapenemase-producing Enterobacterales carriage 14.4% (44/306); *bla*NDM in 90%, of which *bla*NDM-5 was the commonest variant (58/70, 82%) [16]
Federal tertiary care hospital, uropathogens	51 CRE, 23 *bla*NDM-positive	*bla*NDM-5 69.6%, *bla*NDM-1 26.1%, *bla*NDM-7 4.3% [17]
Kidney Centre, Rawalpindi	425 clinical isolates	Carbapenemase genes in 61 of 72 carbapenem-resistant isolates; *bla*NDM most prevalent; 12 isolates carried two carbapenemase genes, typically *bla*NDM with *bla*OXA-48 [18]
Critical-care units, Karachi, 2024–2025	Clinical and environmental isolates	Among carbapenem-resistant isolates, *bla*OXA-48-like 52.8%, *bla*NDM 25% [19]
Clinical *K. pneumoniae*, whole-genome sequenced	122 isolates	*bla*NDM-1 in 16 (13.11%), distributed across four distinct ST/capsule/O-locus backgrounds [9]

4.1 Therapeutic implications

NDM is a metallo-β-lactamase. It is not inhibited by avibactam, vaborbactam or relebactam, so the β-lactamase inhibitor combinations that transformed the management of carbapenem-resistant Enterobacterales in settings dominated by KPC do comparatively little here. The agents with activity against metallo-β-lactamase producers are cefiderocol and aztreonam–avibactam, neither of which is widely available or affordable in Pakistan. The therapeutic consequence is that the local fallback from carbapenems is more often colistin or tigecycline — which, as section 2 showed, already face measurable resistance.

The co-occurrence findings sharpen this. Isolates carrying both *bla*NDM and *bla*OXA-48 have been reported repeatedly from Pakistani centres [18], and a strain with that combination is resistant to all β-lactams and to every currently marketed β-lactamase inhibitor combination.

4.2 Community carriage

In the Rawalpindi study, carbapenemase-producing Enterobacterales were carried by 14.4% of community participants rather than hospital inpatients [16]. Carbapenem resistance in most of

the published international literature is a hospital phenomenon, sustained by intensive carbapenem exposure in critical care. A one-in-seven community carriage rate means the reservoir in Pakistan sits in the general population, maintained by something other than hospital prescribing — food, water, and over-the-counter antibiotic use are the obvious candidates.

This changes what containment has to achieve. Hospital infection control can interrupt transmission within wards; it cannot reduce a community reservoir. It also means that a patient presenting with a community-acquired infection may already be colonised with a carbapenemase producer, which undermines the assumption, standard in treatment guidelines written elsewhere, that community-onset infection implies a susceptible organism.

4.3 NDM variants and plasmid vehicles

The variant distribution is informative. *bla*NDM-5 rather than *bla*NDM-1 is the dominant variant in several Pakistani series [16,17] — NDM-5 differs from NDM-1 by two substitutions and has greater carbapenemase activity, so the local population is not merely carrying NDM but carrying a more active version of it. Reported vehicles include IncX3 plasmids, in the first Pakistani description of *bla*NDM-1 on IncX3 in a clinical ST11 *K. pneumoniae* from Islamabad [20], and IncFIB(K) replicons, which were the commonest replicon marker (101/122, 82.79%) in the national genomic survey [9]. IncX3 plasmids are efficient, broad-host-range vehicles for *bla*NDM, and their presence indicates that the gene is spreading horizontally rather than through clonal expansion alone — consistent with the finding that *bla*NDM-1-positive isolates occupy four separate genomic backgrounds [9].

4.4 Comparison with United States and European surveillance

This review is otherwise confined to Pakistani data. One comparison is made here because the paper's central therapeutic claim depends on it: that the β-lactamase inhibitor combinations developed over the past decade address a smaller share of the Pakistani problem than they do elsewhere. That claim can only be evaluated against the distributions those agents were designed for.

Table 4. ***Carbapenemase distribution in Pakistan, the United States and Europe. Comparator data are included only to assess inhibitor-combination coverage.***

Setting	KPC	NDM or other metallo-β-lactamase	OXA-48-like
Pakistan: urinary CRE, Karachi (n = 52)	not detected	86.5%	11.5% [15]
Pakistan: community CPE, Rawalpindi (n = 73)	not reported	90%	not reported [16]
United States: carbapenemase-producing Enterobacterales, 2020–2023	72.3%	17.6%	5.9% [49]
Europe: carbapenem-resistant *K. pneumoniae*, EURECA collection	45–46%	lower proportion, rising	39% [50]

The inversion is close to complete. Where KPC accounts for roughly three-quarters of US carbapenemase production and about half of the European, it is essentially absent from Pakistani series, and NDM accounts for the overwhelming majority here. Avibactam, vaborbactam and relebactam inhibit KPC and OXA-48-like enzymes but not metallo-β-lactamases. Ceftazidime–avibactam, meropenem–vaborbactam and imipenem–relebactam therefore cover most of the US carbapenemase burden, a large part of the European, and a small minority of the Pakistani one. European surveillance is also shifting toward metallo-β-lactamases [50], which suggests the Pakistani pattern is the direction of travel rather than an exception.

These agents retain a role here: OXA-48-like enzymes are common in some Pakistani series [19] and are inhibited by avibactam. The requirement is that the carbapenemase be identified before one of them is chosen, which places the weight on diagnostic capacity rather than on drug procurement alone.

5. Last-line agents: colistin and tigecycline

Because NDM removes the β-lactams, colistin and tigecycline carry much of the therapeutic weight in Pakistan. The published data show two separate resistance mechanisms operating in two separate compartments.

5.1 Chromosomal colistin resistance in clinical isolates

A screen of 545 clinical *E. coli* isolates from Pakistan found colistin susceptibility of 99.27%, with only four isolates (0.73%) resistant — all four carrying *mcr-1*, in what was the first description of an *mcr-1*-bearing IncI2 plasmid in clinical *E. coli* from Islamabad. The four belonged to different sequence types, including ST156 and ST405, indicating multiclonal rather than clonal dissemination [21].

Klebsiella pneumoniae behaves differently. The pooled national estimate for colistin resistance in *K. pneumoniae* is 21% [7], far above the *E. coli* figure, and the mechanism is not usually plasmid-borne. Whole-genome sequencing of colistin-resistant clinical *K. pneumoniae* from a Karachi laboratory strain bank found no *mcr-1*, and instead implicated chromosomal routes — modification of lipid A with 4-amino-4-deoxy-L-arabinose, arising through the *mgrB*, *phoPQ* and *pmrAB* regulatory systems [22].

This distinction matters practically. A PCR panel for *mcr* genes will return negative on most colistin-resistant Pakistani *K. pneumoniae*, because the resistance is chromosomal, regulatory, and arises independently in each patient under colistin exposure rather than being transmitted. Molecular surveillance that looks only for *mcr* will therefore substantially under-report colistin resistance.

5.2 Plasmid-mediated resistance in poultry: *mcr* carriage

The agricultural picture is the reverse, and far worse (Table 5).

Table 5. ***Colistin resistance and mcr carriage in Pakistani food animals.***

Study	Finding
Broiler chickens, three districts of Punjab (Sargodha, Jhang, Toba Tek Singh), 230 visceral samples from 13 farms	*mcr-1* in 42.1% of colistin-resistant *E. coli*; *mcr-2* also detected [23]
Poultry and native chicken faeces, 149 isolates tested	92 (61.7%) colistin-resistant by agar dilution; *mcr-1* in 36.4% of poultry isolates; on the 12 farms with colistin exposure, 46.5% of isolates were *mcr*-positive [24]
Poultry *E. coli*	49.0% carried ESBL genes; 19.0% colistin-resistant carrying *mcr-1* [25]
Integrated poultry production, broiler breeder to retail meat, analysed with 213 publicly available Pakistani *E. coli* genomes	*mcr-1.1* and *tet*(X4) disseminating through the production chain by distinct mechanisms; related lineages detected in wild birds [26]

The last of these findings has the widest implications. *tet*(X4) encodes a tigecycline-inactivating monooxygenase, so its presence in the Pakistani poultry chain means that plasmid-mediated resistance to both remaining last-line options is already circulating in the food system. The authors trace dissemination from breeder flocks through to retail meat and find related lineages in wild birds, which places the farm-to-fork route and the environmental route in the same study [26].

5.3 Implications for the clinical reservoir

Colistin resistance in Pakistani patients currently arises mostly by mutation under treatment; colistin resistance in Pakistani poultry is mostly plasmid-borne and abundant. The second is a reservoir that can seed the first. Colistin has been used extensively as a growth promoter and therapeutic in Pakistani poultry, and the *mcr* prevalence figures track colistin exposure at farm level directly — 46.5% *mcr*-positive isolates on colistin-exposed farms [24]. Restricting veterinary colistin use is therefore not an adjacent agricultural policy question but a direct intervention on the clinical resistance reservoir.

6. Gram-positive pathogens

6.1 MRSA prevalence

A systematic review and meta-analysis of 68 Pakistani studies published between 2015 and 2025 estimated pooled MRSA prevalence at 50% (95% CI 45–54%) among *S. aureus* isolates [27]. This is consistent with the 49% figure reported in the National Action Plan [5], and higher than the equivalent pooled estimate for India of 37% quoted in the same analysis. Pooled prevalence was 51% among general patients and 44% among healthcare personnel, and year-wise estimates ranged from 61% to 31% [27].

The heterogeneity statistic for that meta-analysis is $I^2 = 98.9\%$, which is close to the maximum. That figure should be read as a warning: the included studies differ so much in setting, specimen type and method that the pooled estimate describes the literature rather than the country. A single tertiary-ICU study and a community screening study are not measuring the

same quantity. A separate cross-sectional study of 1,000 clinical specimens from Punjab found *S. aureus* in 10.2% and MRSA in 6.3% of all specimens, corresponding to 61.8% of *S. aureus* isolates [28].

6.2 Clonal structure and SCC*mec* types

Pakistani MRSA is not a single clone. Genotypic characterisation of multidrug-resistant MRSA from a hospital intensive care unit found SCC*mec* type III in 53% of isolates, type IVa in 16% and type V in 5%, with 31% carrying combinations of two or three SCC*mec* types; the commonest *spa* type was t030 and the commonest sequence type ST239 [29]. ST239-SCC*mec*III/t030 is a classic hospital-associated lineage. An earlier study from the same region reported SCC*mec* III in 47% and type IV in 29% [29].

Studies of skin and soft tissue infection show a different and more community-associated picture. Work on MRSA from skin and soft tissue infections at Lady Reading Hospital, Peshawar, in 2021–2022 found multiple highly resistant clones co-circulating with high Panton–Valentine leucocidin carriage, and previous Pakistani work had identified epidemic community-associated lineages including CC8-MRSA-IV (the USA300 background) and ST772-MRSA-V, the Bengal Bay clone [30].

The presence of both a hospital-associated ST239 lineage and community-associated PVL-positive lineages means the two MRSA epidemiologies that are usually described separately are overlapping in Pakistani hospitals. The detection of isolates carrying more than one SCC*mec* type [29] is worth noting in its own right, since SCC*mec* is mobilised by cassette chromosome recombinases and multiple elements in one genome indicate repeated acquisition.

6.3 Glycopeptide susceptibility

Vancomycin, teicoplanin and linezolid were the three most effective agents against MRSA in the Punjab series [28], and this remains the general finding across Pakistani studies. Reports of vancomycin-resistant *S. aureus* from Pakistani intensive care units exist in the literature [27], but such reports require confirmatory MIC testing and genotypic confirmation of *vanA* before they can be accepted, and the global experience is that phenotypic VRSA reports substantially outnumber confirmed cases. On present evidence, glycopeptides remain effective in Pakistan, that reduced-susceptibility phenotypes are plausible given the exposure, and that the country lacks the confirmatory testing capacity to distinguish the two reliably.

7. Drug-resistant tuberculosis

Pakistan is among the handful of countries carrying most of the world's multidrug-resistant tuberculosis burden, and because *M. tuberculosis* acquires resistance only by chromosomal mutation, the Pakistani isolate collections offer an unusually direct view of mutational evolution under treatment.

7.1 Burden of drug-resistant tuberculosis

National estimates put MDR/RR-TB at 3.6% of new TB cases and 18% of previously treated cases, with roughly 11,000 cases reported in 2022 [31]. Case notification has improved

substantially: over 490,000 people (70% of the estimated burden) received care in 2024, against 331,800 (57%) in 2015 [32]. A cross-sectional study of 250 adults with microbiologically confirmed pulmonary TB at a Lahore tertiary hospital between January and June 2024 found MDR-TB in 18.8% (47/250), concentrated among previously treated patients [31]. A retrospective Xpert MTB/RIF series from Khyber Pakhtunkhwa found rifampicin resistance in 29% of cases tested, of which 94.6% were also isoniazid-resistant [33] — confirming that in this setting rifampicin resistance is a reliable surrogate for MDR-TB.

7.2 Resistance profile in sequenced isolates

The largest Pakistani genomic study sequenced 535 *M. tuberculosis* isolates, with the resistance categories in Table 6 and the agent-by-agent profile in Figure 1 [34].

Table 6. ***Drug-resistance categories in 535 sequenced Mycobacterium tuberculosis isolates, Pakistan.***

Resistance category	Isolates (%)
MDR	328 (61.3%)
Pre-XDR	47 (8.8%)
Pre-MDR	31 (5.8%)
Pan-sensitive	60 (11.2%)

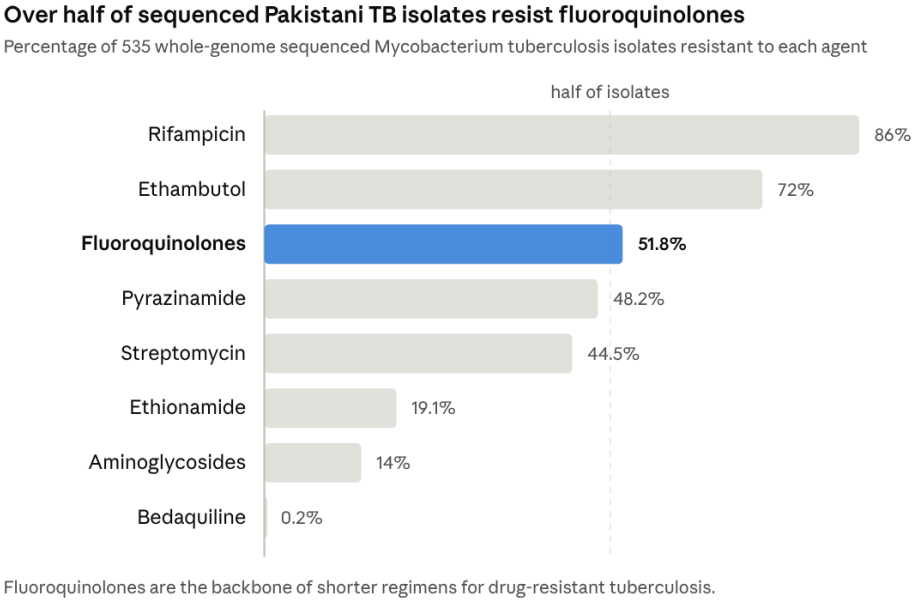


Figure 1. ***Resistance profile of 535 sequenced Mycobacterium tuberculosis isolates, Pakistan. Dashed line marks 50%; fluoroquinolone resistance is highlighted.***

Fluoroquinolone resistance in over half of isolates has immediate clinical weight, because fluoroquinolones form the backbone of shorter MDR-TB regimens. A separate Khyber Pakhtunkhwa series found pre-XDR-TB in 42.9% of isolates and rifampicin resistance in 88.6%, and detected *mmpR5* (*Rv0678*) frameshift mutations (c.140dupA and c.138_139dupTG) in single isolates, conferring bedaquiline and clofazimine resistance [35].

Bedaquiline resistance is therefore present in Pakistan but still rare, which is the window in which it can be protected.

A smaller series reported that 17.6% of pre-MDR isolates already carried fluoroquinolone resistance markers, which the authors attribute to unregulated use of anti-TB drugs [36]. Fluoroquinolones are widely available without prescription in Pakistan and are frequently given for undiagnosed respiratory illness, so patients may arrive at TB diagnosis with fluoroquinolone resistance already selected.

7.3 Mutational spectrum and limits of genotypic testing

Pakistani isolates carry the expected canonical mutations — *rpoB* codon 531 (Ser450Leu in modern numbering) and 513, and *katG* codon 315, with double *rpoB* mutations in 12% of cases in one series and additional *katG* codon 299 changes [37]. Compensatory mutations in *rpoC*, which restore the fitness cost of *rpoB*-mediated rifampicin resistance, have been detected alongside them [38].

The more important finding is how much is not canonical. Whole-genome sequencing of MDR-TB isolates from Khyber Pakhtunkhwa identified 112 *rpoB* mutations, of which only 9 appear in the WHO mutation catalogue and 103 do not, and 24 *katG* mutations of which 3 are catalogued [32,39]. The same work described a novel *rpoB* Ser431Phe variant [39].

This has a direct diagnostic consequence. Genotypic resistance testing — including Xpert MTB/RIF and catalogue-based sequencing pipelines — identifies resistance by matching against a list of known mutations. If most of the *rpoB* variants circulating in a Pakistani province are absent from that list, catalogue-based testing will return false-susceptible results for a meaningful fraction of patients. The authors' conclusion, that genomic surveillance needs to be integrated into routine TB diagnostics regionally rather than relying on globally derived catalogues, follows directly [32].

7.4 Within-host resistance diversity

Sequencing of 51 rifampicin-resistant isolates from Khyber Pakhtunkhwa collected between 2016 and 2019 — 86.3% MDR, 58.8% lineage 3 — found five instances of within-host resistance diversity, including two patients carrying mixtures of MDR strains with different *rpoB*, *katG* and *inhA* promoter mutations, and heteroresistance within a single lineage in two isolates [38]. Standard practice samples one colony from one specimen, so these mixed infections are normally invisible. Where they occur, a susceptibility result describes whichever subpopulation happened to be picked.

8. Case study: extensively drug-resistant typhoid in Sindh

The XDR *Salmonella* Typhi outbreak that began in Hyderabad in November 2016 is the most completely characterised resistance-evolution event documented in Pakistan, and one of the clearest anywhere. It deserves extended treatment because the genomics resolve a question that is usually inferential: how a pathogen moves from multidrug resistance to extensive drug resistance.

8.1 Outbreak description

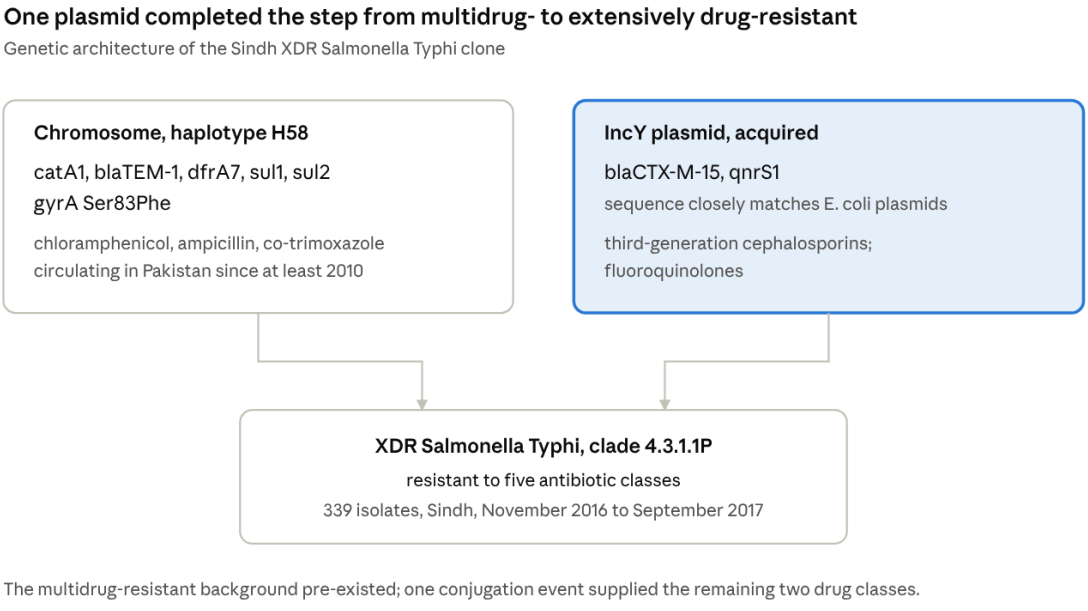
Ceftriaxone-resistant typhoid was first detected in Hyderabad in November 2016 and spread to Karachi. Three hundred and thirty-nine isolates with the same XDR pattern — resistant to chloramphenicol, ampicillin, co-trimoxazole, fluoroquinolones and third-generation cephalosporins — were recovered from Sindh between November 2016 and September 2017, and whole-genome sequencing of over 80 of them showed remarkable clonality and sequence conservation [40]. One laboratory series recorded XDR *S.* Typhi cases rising from 21 in 2016 to 526 in 2017 [41]. Subsequent reporting put the outbreak at 5,274 XDR cases between 2016 and 2018 [42].

8.2 Genetic architecture

Resistance was carried in two layers (Table 7, Figure 2).

Table 7. ***Genetic architecture of the Sindh XDR Salmonella Typhi clone.***

Layer	Determinants	Resistance conferred
Chromosome: H58-associated composite transposon	*catA1*, *bla*TEM-1, *dfrA7*, *sul1*, *sul2*; *gyrA* Ser83Phe	Chloramphenicol, ampicillin, co-trimoxazole; reduced fluoroquinolone susceptibility
Acquired IncY plasmid	*bla*CTX-M-15, *qnrS1*	Third-generation cephalosporins; fluoroquinolones



Sindh XDR S. Typhi · genetic architecture, reference 40

Figure 2. *Genetic architecture of the Sindh XDR Salmonella Typhi clone. A pre-existing H58 chromosomal cassette, circulating since at least 2010, plus one acquired IncY plasmid carrying blaCTX-M-15 and qnrS1 (highlighted), producing clade 4.3.1.1P.*

The isolates belong to haplotype H58, and the Pakistani outbreak strains form a distinct clade designated 4.3.1.1P within that lineage [42]. The same phylogenetic lineage had been circulating in Pakistan since at least 2010 [40].

8.3 Mechanistic significance

The sequence data support four conclusions.

The multidrug-resistant background pre-existed. The chromosomal cassette conferring resistance to the three first-line typhoid drugs was already integrated in H58 strains circulating locally for years. The outbreak did not build resistance from a susceptible organism.

One acquisition did the rest. The step from MDR to XDR was the arrival of a single conjugative plasmid carrying both *bla*CTX-M-15 and *qnrS1*. Two drug classes were lost in one event, pre-assembled. No amount of stepwise mutation under ceftriaxone would have produced this as quickly.

The plasmid came from another organism. The IncY plasmid closely matches plasmids found in *E. coli*, which implies acquisition from a co-resident enterobacterium rather than evolution within *S.* Typhi [40]. Given the near-universal CTX-M-15 carriage in Pakistani Enterobacterales described in section 3, the donor pool was abundant. The gut of a patient or carrier colonised with CTX-M-15-positive *E. coli* is a plausible site for the transfer.

Fitness came first, resistance second. Sporadic ceftriaxone-resistant *S.* Typhi had been reported before without clonal expansion. What distinguished this event was that the recipient was a lineage already successful at transmission in Sindh. The plasmid landed in a genome that could carry it into a population.

8.4 Comparison with ceftriaxone resistance in India

In India, ceftriaxone resistance in *S.* Typhi has arisen repeatedly through different vehicles — IncX3 plasmids carrying *bla*SHV-12 and IncN plasmids carrying *bla*TEM-1B with *bla*DHA-1 — in lineage 4.3.1.2, and these have persisted only transiently without producing sustained clonal spread [42]. The comparison is informative precisely because so much else is shared between the two settings. The same species under comparable drug pressure produced a self-limiting problem in one place and a sustained epidemic in the other, and the difference lay in which plasmid reached which genetic background.

8.5 Public health response

The outbreak prompted the introduction of typhoid conjugate vaccine in Sindh, the first such programmatic deployment in response to drug resistance. Vaccination acts upstream of prescribing: it reduces the number of infections requiring treatment, and therefore both the disease burden and the selective pressure, without depending on an antibiotic remaining effective.

9. Mobile genetic elements in Pakistani isolates

The preceding sections describe determinants; this one describes the vehicles that move them, as characterised in Pakistani material. The recurring finding is that resistance here travels horizontally more than it expands clonally.

9.1 Plasmid replicons

Table 8. ***Plasmid replicons and resistance cargo in Pakistani isolates.***

Replicon	Cargo and context in Pakistani isolates
IncY	*bla*CTX-M-15 with *qnrS1* in the Sindh XDR *S.* Typhi clone; closely related to *E. coli* plasmids [40]
IncX3	*bla*NDM-1 in clinical ST11 *K. pneumoniae*, Islamabad — the first Pakistani report of this combination [20]
IncFIB(K)	Commonest replicon marker in the national *K. pneumoniae* genomic survey, 101/122 isolates (82.79%) [9]
IncI2	*mcr-1* in clinical *E. coli*, Islamabad — first Pakistani description [21]

The replicons listed in Table 8 are efficient, broad-host-range conjugative vehicles. IncX3 in particular is the principal global vehicle for *bla*NDM, and IncI2 for *mcr-1*; their presence in Pakistani isolates means the local gene pool is connected to the international one rather than evolving in isolation.

9.2 Evidence for horizontal spread

Three Pakistani datasets point in the same direction.

In the national genomic survey of 122 clinical *K. pneumoniae*, *bla*NDM-1-positive isolates occurred in four distinct sequence-type, capsule-locus and O-locus backgrounds, and the authors concluded that multiple multidrug-resistant lineages are co-circulating rather than one clone expanding [9].

In the Rawalpindi community study, 78 carbapenem-resistant Enterobacterales were sequenced from community carriage samples, with *bla*NDM in 90% and *bla*NDM-5 the dominant variant — distributed across species and *E. coli* phylogroups rather than confined to one lineage [16].

In the clinical *mcr-1* series, the four positive *E. coli* isolates belonged to different sequence types including ST156 and ST405, which the authors read as multiclonal dissemination [21].

This repeats the point made in section 3. Infection prevention measures that interrupt person-to-person transmission of a particular clone are necessary but not sufficient, because the determinant is distributed across the bacterial population rather than carried by a single organism. Reducing the selective pressure that maintains plasmid carriage is the arm that acts on the gene itself.

9.3 Co-carriage and linkage

Pakistani studies repeatedly report determinants travelling together. Isolates carrying both *bla*NDM and *bla*OXA-48 have been described from Rawalpindi [18]; ESBL and carbapenemase genes co-occur frequently among nosocomial coliforms in Faisalabad [43]; poultry *E. coli* co-harbour *mcr-1* with ESBL genes [25]; and the integrated poultry study found *mcr-1.1* and *tet*(X4) moving through the same production chain [26]. In the national *K. pneumoniae* survey, 48 distinct acquired resistance genes were detected across the cohort, with

plasmid-mediated quinolone resistance determinants in 89.34% of isolates alongside near-universal *bla*CTX-M-15 [9].

Linkage is what makes these determinants difficult to remove. When an ESBL, a quinolone-resistance determinant and a carbapenemase sit on the same element, exposure to any one of those drug classes selects for retention of all three. In a setting where cephalosporins and fluoroquinolones are among the most widely used antibiotics, that linkage sustains carbapenem resistance even where carbapenems themselves are little used.

10. Drivers of selection

The resistance described above is maintained by selection, and in Pakistan that selection acts in three compartments at once.

10.1 Human antibiotic use

Pakistan ranks third among low- and middle-income countries for antibiotic consumption [44]. The dominant route of access is the community pharmacy, and the published figures are consistent across methods and cities (Table 9).

Table 9. ***Non-prescription antibiotic dispensing in Pakistan.***

Study	Method	Finding
National survey of pharmacies and medical stores	Pharmacy survey	96.9% dispensed antibiotics without requiring a prescription [45]
Hazara Division, KPK, 310 pharmacies and drug outlets	Simulated client	Antibiotics dispensed without prescription in 90.5% of visits; only 9.5% declined; commonest agents ciprofloxacin (46.5%) and azithromycin (29.4%) [46]
Lahore, multi-centre	Cross-sectional	95% of antibiotics supplied without a physician's prescription [46]
Community pharmacy staff survey	Questionnaire	81.5% said non-prescribed dispensing is common practice; 51.1% believed themselves authorised to do it; 69.3% agreed it contributes to resistance [44]

These findings do not describe a knowledge deficit. Most of the staff dispensing antibiotics without prescription know it drives resistance and do it anyway, because the economic and social incentives run the other way and because the national drug policy's prohibition is not enforced. Interventions aimed at educating dispensers address the wrong variable.

The agents involved compound the problem. Ciprofloxacin as the single most commonly dispensed non-prescription antibiotic bears directly on two findings reported earlier — fluoroquinolone resistance in 51.8% of sequenced *M. tuberculosis* isolates [34], and fluoroquinolone resistance markers already present in 17.6% of pre-MDR TB isolates [36]. Ciprofloxacin for undiagnosed fever in a country with high TB prevalence means partially treating tuberculosis and selecting fluoroquinolone resistance before the diagnosis is made.

10.2 Antibiotic consumption

Dispensing practice describes how antibiotics are obtained; consumption data quantify how much selective pressure results. Analysis of IQVIA sales data for Pakistan from 2016 to 2023 gives a total of 12.88 billion defined daily doses (DDDs) over the period [51].

Table 10. ***Antibiotic consumption in Pakistan***.

Measure	Value
National consumption, 2015	19.6 DDDs per 1,000 inhabitants per day (DID) [52]
National consumption, 2019	24.8 DID [53]
National consumption, 2021	44.1 DID [53]
Tertiary hospital, Karachi, 2016–2023 (413,596 admissions)	67.3% of admitted patients received an antibiotic; mean 1,128.7 DDDs per 1,000 patient-days [54]
AWaRe distribution, same hospital	Watch 68.8–73.9% of prescriptions; Reserve rose from 3.8% to 5.3% ($p < 0.001$) [54]
Punjab public procurement	Ciprofloxacin alone accounted for 27.2% of procured DDDs [53]

The near doubling of national consumption between 2019 and 2021 covers the COVID-19 period, when azithromycin and other agents were given widely for a viral illness; the increase is therefore partly explained but not thereby harmless, since selection does not distinguish justified from unjustified exposure.

Second, the AWaRe distribution is inverted relative to the WHO target. The target is that at least 60% of consumption should come from the Access category; in Pakistani hospital data the Watch category accounts for around 70% of prescriptions, and Reserve use is rising [53,54]. Watch-category agents are precisely the cephalosporins, fluoroquinolones and macrolides that select for the resistance mechanisms described in sections 3 and 4.

Third, ciprofloxacin's share — 27.2% of procured DDDs in Punjab [53], and the most frequently dispensed non-prescription antibiotic in simulated-client studies [46] — connects the consumption data directly to two findings reported earlier: fluoroquinolone resistance in 51.8% of sequenced *M. tuberculosis* isolates [34] and plasmid-mediated quinolone resistance determinants in 89.3% of sequenced clinical *K. pneumoniae* [9]. The exposure and the resistance are consistent in magnitude as well as direction.

10.3 Animal and poultry use

Section 5 set out the colistin data. The broader picture is that antimicrobials are used extensively in Pakistani poultry and livestock production, including agents classed as critically important for human medicine, and a systematic review and meta-analysis of AMR in Pakistani food animals covering 2016–2020 documented resistant *E. coli*, *Klebsiella*, *Staphylococcus aureus* and *Campylobacter* across production systems [47].

The genomic work makes the farm-to-human link concrete rather than inferential: *mcr-1.1* and *tet*(X4) were traced through integrated poultry production from breeder flocks to retail meat,

with related lineages detected in wild birds [26], and CTX-M-15-producing *E. coli* were recovered from migratory birds at wetland sites along the Indus route, 88.4% of them multidrug-resistant [13].

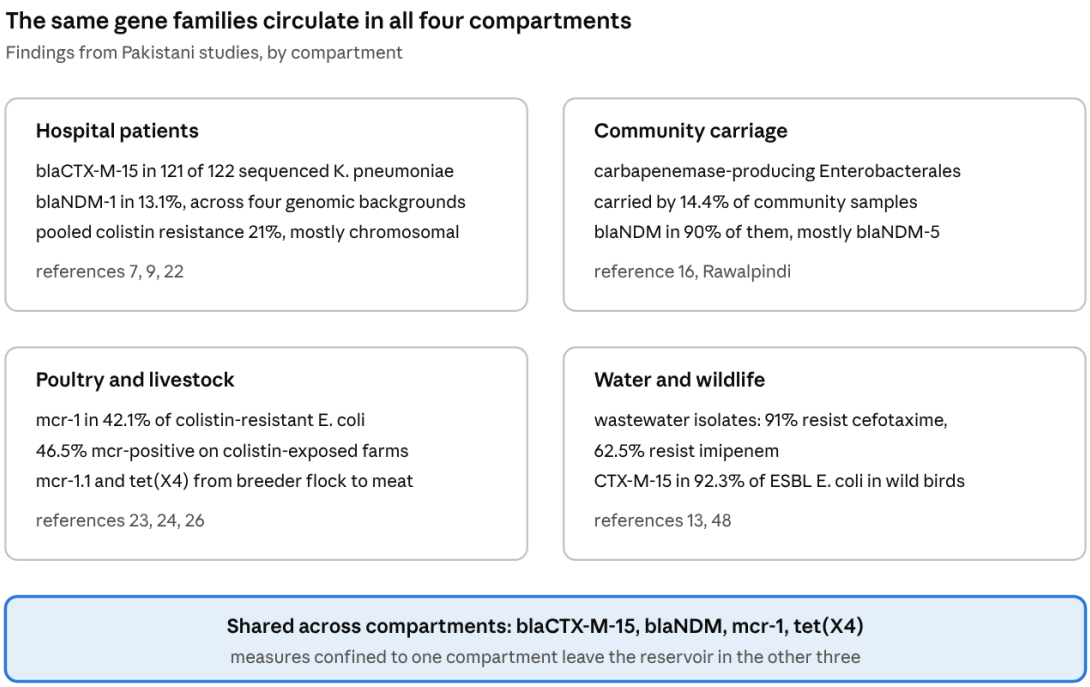
10.4 Environmental contamination

Whole-genome sequencing of bacteria isolated from Pakistani wastewater found resistance at levels comparable to clinical isolates: 91% of isolates resistant to cefotaxime, 75% to ciprofloxacin and 62.5% to imipenem, with 31% non-susceptible to gentamicin [48]. The resistance gene complement was correspondingly broad — *sul* genes in 72% and *dfrA* in 61% of isolates tested, *aadA* variants including *aadA5* and *aadA2*, macrolide resistance via *mph(A)* in 83%, and chromosomal *gyrA*, *parC* and *parE* quinolone-resistance mutations [48].

Carbapenem resistance in 62.5% of wastewater isolates is difficult to account for by clinical exposure. Wastewater is not a clinical compartment under carbapenem selection; those organisms are there because they were excreted, and they persist because the water is not treated. This closes the loop with the community carriage finding in section 4: a 14.4% community carriage rate for carbapenemase-producing Enterobacterales [16] and a heavily contaminated water environment are two measurements of the same circulation.

10.5 Interdependence of the three compartments

The same gene families appear in all three compartments, and in community carriage alongside them (Figure 3): CTX-M-15 in patients, in poultry, in wild birds and in wastewater; *mcr* in poultry and in patients; *bla*NDM in hospital isolates, in community carriage and in wastewater. Restricting hospital prescribing while colistin remains in poultry feed and untreated effluent enters watercourses will reduce the pressure in one compartment while leaving the reservoir intact in the other two. This is the substantive case for the One Health framing adopted in Pakistan's national plan, and it rests on the genomic evidence rather than on principle.



Pakistani studies by compartment · references as marked

Figure 3. ***Shared resistance determinants across four compartments in Pakistan: blaCTX-M-15, blaNDM, mcr-1 and tet(X4).***

11. Surveillance and diagnostic capacity

Every number in this review is a function of what was measured, and in Pakistan the measurement system is the limiting factor.

11.1 Existing surveillance infrastructure

Pakistan launched national AMR surveillance aligned with the WHO GLASS framework in 2018, following the National Action Plan of that year [6]. National surveillance now draws on a network of laboratories, and three National Point Prevalence Surveys of antimicrobial use covering major tertiary care hospitals were completed in 2019, 2023 and 2025 [5]. The AMR Secretariat at the National Institute of Health has conducted a situational analysis synthesising GLASS reporting, antimicrobial consumption data and the Tripartite AMR Country Self-Assessment Survey, and is tracking resistance trends against blood pathogens [5].

11.2 Effect of capacity gaps on the data

Four limitations recur across the studies cited in this review, and each biases the figures in a predictable direction.

Referral bias. Most published data come from tertiary centres in Karachi, Lahore, Islamabad, Rawalpindi and Peshawar. These receive patients who have already failed treatment elsewhere, so resistance prevalence is inflated relative to the community — except where, as with the

Rawalpindi carriage study [16], community sampling was done deliberately and found resistance anyway.

Method heterogeneity. Pakistani studies variously use CLSI, EUCAST and older NCCLS breakpoints, disc diffusion, broth microdilution and automated systems. The MRSA meta-analysis reports $I^2 = 98.9\%$ [27] and the *K. pneumoniae* meta-analysis reports wide confidence intervals [7]; much of that heterogeneity is methodological rather than biological, which means pooled national estimates should not be used as though they were measurements.

Gene-panel blindness. Most molecular characterisation in Pakistan uses PCR against a predefined panel. This cannot detect determinants not on the panel, and it systematically under-reports mechanisms that are regulatory or chromosomal. The colistin data illustrate this directly: *mcr* panels return negative on *K. pneumoniae* whose resistance arises through *mgrB*, *phoPQ* or *pmrAB* [22]. The TB data illustrate the converse problem — 103 of 112 *rpoB* mutations found in one Pakistani province are absent from the WHO catalogue [32,39], so catalogue-matching will miss them.

Diagnostic access. Resistance can only be recorded where a culture was taken. Where empirical treatment is given without microbiology — which is the norm outside tertiary centres, and universal for antibiotics bought over the counter — neither the infection nor its resistance enters any dataset.

11.3 Priority investments

Three investments would do more than additional prevalence studies. Standardised susceptibility testing with a single breakpoint standard and external quality assessment would make existing data comparable, which is a prerequisite for the trend analysis the national plan requires. Routine whole-genome sequencing in a small number of reference laboratories would replace gene-panel PCR for mechanism characterisation, and the TB data show what it detects that panels miss. And blood-culture capacity at secondary level would move surveillance out of the tertiary referral bias that currently shapes every national figure.

12. Containment

12.1 National Action Plan

Pakistan's National Action Plan on Antimicrobial Resistance 2.0 covers 2024–2028 and succeeds the 2018 plan, adopting a One Health structure across human health, animal health and the environment [5]. Its stated priorities include strengthening laboratory systems, expanding GLASS reporting, improving stewardship and increasing the share of 'Access' category antibiotics in total consumption.

The plan's own situational analysis identifies the principal weaknesses candidly: there is no regular surveillance system in the environmental sector, roughly half of reporting laboratories lack the systems to generate reliable data, and enforcement of existing regulation is limited [5]. A plan that correctly identifies its own gaps is more useful than one that does not, but the gaps named are the ones that matter most.

12.2 Evaluated interventions

Typhoid conjugate vaccine. The strongest evidence for any Pakistani containment measure concerns vaccination. The Sindh XDR outbreak prompted introduction of typhoid conjugate vaccine, an intervention that acts upstream of antibiotic use: fewer typhoid infections means both less disease and less ceftriaxone exposure, and the vaccine's effectiveness does not degrade as resistance evolves.

Stewardship. Hospital antimicrobial stewardship programmes exist in major Pakistani centres and the point prevalence surveys have documented irrational hospital prescribing repeatedly [5]. What is largely absent from the Pakistani literature is evaluation — studies measuring whether a stewardship intervention changed resistance rates, rather than describing prescribing patterns.

Regulation of dispensing. Non-prescription sale of antibiotics is already prohibited under the national drug policy [44]. The 90–97% dispensing figures [45,46] measure the gap between regulation and enforcement, not an absence of rules.

Veterinary colistin restriction. The *mcr* prevalence data tracking farm-level colistin exposure [24] make this the agricultural intervention with the clearest supporting evidence, and several countries have demonstrated rapid falls in *mcr* prevalence after withdrawing colistin from feed.

12.3 Access to active agents

For a patient in Pakistan with an infection caused by an NDM-producing organism, the agents with reliable activity are cefiderocol and aztreonam–avibactam. Neither is widely available or affordable here. Stewardship, infection control and surveillance all reduce how often that situation arises; none of them helps the patient who is already in it. Access to newer agents is therefore not a separate commercial question but part of the same problem, and it is the part least addressed by the available Pakistani literature.

13. Implications for practice

The evidence assembled here supports a small number of specific actions, ordered below by the strength of the Pakistani evidence behind them rather than by ease of implementation.

For clinicians and hospitals

1. *Treat community-onset infection as potentially resistant.* A 14.4% community carriage rate for carbapenemase-producing Enterobacterales [16] invalidates the usual inference that community-acquired means susceptible. Severity, prior antibiotic exposure and prior hospitalisation should drive empirical choice, not setting of onset alone.
2. *Use local antibiograms, not national averages.* The heterogeneity across Pakistani studies (section 2.3) means pooled national figures describe the literature rather than any individual hospital's organisms. Every centre treating serious infection should produce and update its own antibiogram.
3. *Identify the carbapenemase before choosing an inhibitor combination.* Because NDM predominates here and is not inhibited by avibactam, vaborbactam or relebactam (Table 4), empirical use of these agents will fail in the majority of carbapenem-resistant cases.

Where molecular testing is unavailable, that fact should inform the expectation of failure.

For laboratories

4. *Adopt a single breakpoint standard with external quality assessment.* Mixed CLSI, EUCAST and legacy NCCLS reporting is the largest single obstacle to interpreting Pakistani resistance data, and standardisation costs less than expanding testing volume.
5. *Do not rely on* mcr *panels to detect colistin resistance.* Most colistin resistance in Pakistani *K. pneumoniae* is chromosomal, arising through *mgrB*, *phoPQ* or *pmrAB* [22], and will be missed by plasmid-gene panels.
6. *Treat catalogue-based tuberculosis genotyping as incomplete.* With 103 of 112 *rpoB* mutations in one Pakistani province absent from the WHO catalogue [32,39], a negative molecular result does not exclude rifampicin resistance, and phenotypic confirmation retains a role.

For policy

7. *Enforce the existing prohibition on non-prescription sale rather than legislating further.* Non-prescription dispensing is already unlawful; the 90–97% dispensing rates [45,46] measure non-enforcement, and most dispensers already know the practice drives resistance [44].
8. *Restrict veterinary colistin.* The correlation between farm-level colistin exposure and *mcr* carriage — 46.5% of isolates positive on colistin-exposed farms [24] — makes this the agricultural intervention with the clearest supporting evidence, and international experience shows *mcr* prevalence falls quickly after withdrawal.
9. *Shift procurement toward the Access category.* Watch-category agents account for roughly 70% of hospital prescribing against a 60% Access target [53,54], and ciprofloxacin alone for 27.2% of procured DDDs in Punjab [53]. Procurement is a lever that acts without requiring prescriber behaviour to change first.
10. *Sustain typhoid conjugate vaccination and evaluate it.* It is the only intervention here that removes infections rather than redistributing antibiotic pressure, and the Sindh programme offers an opportunity to measure effect on resistance that has not yet been taken.

The first three of these can be implemented by individual clinicians this week; the last four require decisions at provincial or federal level. The ordering reflects evidential strength, and the gap between those two groups is where most of the available benefit currently sits unclaimed.

14. Research gaps

The gaps set out below are specific to the Pakistani evidence base, and all are addressable with existing capacity.

Genomic depth. Pakistan has produced a handful of whole-genome studies — 122 clinical *K. pneumoniae* [9], 78 community CRE [16], 535 *M. tuberculosis* [34], and the Sindh *S.* Typhi outbreak isolates [40] — against a population of over 240 million. These are reference points,

not surveillance. Sustained sequencing of blood-culture isolates at even a few sites would allow lineages and plasmids to be tracked rather than inferred.

Plasmid-level resolution. Most Pakistani molecular studies report which genes are present, not which element carries them. Long-read sequencing resolves plasmid structure and would answer the question that matters for containment: whether a determinant is spreading as a clone, as a plasmid, or as a transposon moving between plasmids.

Outcome data. Pakistani studies overwhelmingly report prevalence. Very few link resistance to clinical outcome in Pakistani patients, whether mortality, length of stay or treatment failure. Without that, the national burden estimates rest on modelled parameters derived elsewhere.

Intervention evaluation. Stewardship programmes, infection prevention measures and the typhoid vaccine campaign have been implemented; their effects on resistance rates have largely not been measured. A before-and-after study of *mcr* prevalence following any restriction of veterinary colistin would be among the most informative pieces of work available to Pakistani researchers.

Non-genetic resistance. No Pakistani data exist on tolerance, persistence or heteroresistance, which are invisible to standard susceptibility testing and plausibly contribute to the treatment failure observed in this setting. Population analysis profiling requires no sequencing and could be done in existing laboratories.

The environmental compartment. The national plan concedes there is no regular environmental surveillance [5]. Given wastewater carbapenem resistance at 62.5% [48] and community carriage at 14.4% [16], this is the gap with the largest ratio of importance to cost.

15. Conclusion

The Pakistani evidence base is now large enough to support a coherent account of how antimicrobial resistance evolves in this country, and that account differs from the standard one in ways that matter clinically.

The baseline is already resistant. CTX-M-15 is present in 99% of sequenced clinical *K. pneumoniae*, which means an extended-spectrum β-lactamase background is the genetic starting state rather than an acquired step. Every subsequent determinant lands on a genome that is already resistant to the penicillins and oxyimino-cephalosporins, so each acquisition yields a larger phenotypic increment than it would elsewhere, and extensively drug-resistant phenotypes are reachable in few steps.

The mechanisms are the ones current therapy does not cover. Carbapenem resistance here is NDM and OXA-48-like, not KPC. The β-lactamase inhibitor combinations developed over the last decade address the KPC problem and do comparatively little for this one, while cefiderocol and aztreonam–avibactam, which would cover it, are not accessible at scale. The fallback agents, colistin and tigecycline, already face measurable resistance, and plasmid-borne determinants against both are circulating in the poultry chain.

The reservoir is not confined to hospitals. Carbapenemase-producing Enterobacterales were carried by 14.4% of community participants in Rawalpindi; wastewater isolates show 62.5% carbapenem resistance; CTX-M-15 is in migratory birds and in cattle milk; *mcr* genes track colistin exposure on poultry farms. Hospital infection control cannot reach any of that.

Resistance travels horizontally more than clonally. NDM-1 across four genomic backgrounds in one cohort, *mcr-1* across multiple sequence types, community CRE distributed across species and phylogroups — these are signatures of gene movement, not clonal expansion, and they mean that reducing selective pressure acts on the problem in a way that transmission control alone does not.

And the Sindh typhoid outbreak shows the whole process in a single documented event: a locally successful multidrug-resistant lineage, circulating since at least 2010, acquiring one plasmid from a co-resident enterobacterium and becoming extensively drug-resistant in one step, in a setting where the donor pool for that plasmid was everywhere. The response, typhoid conjugate vaccination, is also the only intervention discussed here that acts upstream of the entire mechanism.

These conclusions rest on the data rather than on general principle. Empirical therapy should follow local antibiograms, not national averages assembled from methodologically incompatible studies. Molecular diagnostics calibrated on globally derived mutation catalogues will under-call resistance in Pakistani *M. tuberculosis* and in colistin-resistant *K. pneumoniae*. Enforcement of existing dispensing regulation would do more than further education of dispensers, most of whom already know what they are contributing to. And because the same gene families circulate in patients, poultry, wild birds and wastewater, measures confined to one compartment will leave the reservoir in the others untouched.

References

1. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022;399(10325):629–55. doi:10.1016/S0140-6736(21)02724-0. Country estimates for Pakistan.
2. Institute for Health Metrics and Evaluation. The burden of antimicrobial resistance (AMR) in Pakistan: country profile. Seattle: IHME; 2023. Available from: https://www.healthdata.org/sites/default/files/2023-09/Pakistan.pdf
3. GBD 2021 Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050. Lancet. 2024;404(10459):1199–226. doi:10.1016/S0140-6736(24)01867-1
4. GBD 2021 Antimicrobial Resistance Collaborators. The burden of bacterial antimicrobial resistance in the WHO Eastern Mediterranean Region, 1990–2021. Lancet Public Health. 2025. doi:10.1016/S2468-2667(25)00201-4
5. Government of Pakistan, Ministry of National Health Services, Regulations and Coordination. National Action Plan on Antimicrobial Resistance 2.0, 2024–2028. Islamabad: Government of Pakistan; 2024. Available from: https://cdn.who.int/media/docs/default-source/antimicrobial-resistance/amr-spc-npm/nap-library/pakistan_nap-amr-2.0_2024-2028.pdf

6. Antimicrobial resistance among GLASS priority pathogens from Pakistan: 2006–2018. BMC Infect Dis. 2021. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8650393/
7. Mehmood MS, Saddique MN, Iqbal MU, et al. Prevalence and antimicrobial resistance of *Klebsiella pneumoniae* in Pakistan, 2015–2025: a systematic review and meta-analysis. Curr Clin Microbiol Rep. 2026. doi:10.1007/s40588-026-00281-4. PROSPERO registration CRD420251154194.
8. Prevalence and antimicrobial resistance trends of ESKAPEE pathogens in clinical isolates from Hyderabad, Pakistan: a cross-sectional study. 2025. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC13499059/
9. Genomic epidemiology of multidrug-resistant clinical *Klebsiella pneumoniae* from Pakistan. 2025. PMID: 42634252. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC13500998
10. Molecular epidemiological surveillance of CTX-M-15-producing *Klebsiella pneumoniae* from patients of a teaching hospital in Sindh, Pakistan. 2021. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8596178/
11. Phenotypic and molecular characterization of CTX-M type β-lactamases in Gram-negative bacterial strains isolated from hospitals, Lahore, Pakistan. 2022. PMID: 35661068.
12. Molecular epidemiology of *mcr-1*, *bla*KPC-2 and *bla*NDM-1 harbouring clinically isolated *Escherichia coli* from Pakistan. Infect Drug Resist. 2021. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8057800/
13. High prevalence of CTX-M-15-type ESBL-producing *Escherichia coli* from migratory avian species in Pakistan. Front Microbiol. 2017. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5733096/
14. Extended-spectrum β-lactamases (ESBL): challenges and opportunities. 2022. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10669213/. Source of tabulated Pakistani livestock and companion-animal ESBL prevalence; underlying primary studies should be cited directly.
15. Molecular characterization of *bla*NDM and other carbapenemase genes among carbapenem-resistant Enterobacterales from community- and hospital-acquired urinary tract infections. 2025. PMID: 41068624.
16. Habib A, Lo S, Villageois-Tran K, Petitjean M, Malik SA, Armand-Lefèvre L, et al. Dissemination of carbapenemase-producing Enterobacterales in the community of Rawalpindi, Pakistan. PLoS One. 2022;17(7):e0270707. doi:10.1371/journal.pone.0270707
17. Phylogenetic analysis of *bla*NDM genes of carbapenem-resistant uropathogens isolated from a federal tertiary care hospital, Pakistan: insights into the evolution and dissemination of drug resistance. 2025. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC12606371/
18. Prevalence of carbapenemase-producing organisms at the Kidney Centre of Rawalpindi (Pakistan) and evaluation of an advanced molecular microarray-based carbapenemase assay. 2018.
19. Patients and surfaces: integrated clinical–environmental surveillance of MDR Gram-negative bacteria in critical-care units, Karachi, 2024–2025. 2026. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC12735442/

20. First report of *bla*NDM-1-bearing IncX3 plasmid in clinically isolated ST11 *Klebsiella pneumoniae* from Pakistan. Microorganisms. 2021;9(5):951. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8146611/
21. Dissemination of clinical *Escherichia coli* harbouring the *mcr-1* gene in Pakistan. 2024. See also reference 12.
22. Lipid A-Ara4N as an alternate pathway for colistin resistance in *Klebsiella pneumoniae* isolates in Pakistan. BMC Res Notes. 2021. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8670247/
23. Detection and risk factor analysis of avian colibacillosis associated with colistin-resistant *Escherichia coli* and *Klebsiella pneumoniae*. Front Vet Sci. 2025. doi:10.3389/fvets.2025.1612542
24. Colistin resistance and *mcr* gene carriage in poultry and native chicken faeces in Pakistan. Sci Rep. 2020;10. doi:10.1038/s41598-020-74402-4
25. Genetic diversity of *Escherichia coli* co-harbouring *mcr-1* and extended-spectrum β-lactamases from poultry. 2022. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC9556198/
26. Integrated poultry production as a reservoir of *tet*(X4) and *mcr-1.1* encoding *Escherichia coli* in Pakistan. Microb Genom. 2025. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC13431907/
27. The prevalence of methicillin-resistant *Staphylococcus aureus* in clinical settings of Pakistan: a systematic review and meta-analysis. 2026. PMID: 42159817. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC13269595/
28. Prevalence of *mecA*- and *mecC*-associated methicillin-resistant *Staphylococcus aureus* in clinical specimens, Punjab, Pakistan. 2023. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10045897/
29. Genotypic characterization of clinical isolates of *Staphylococcus aureus* from Pakistan. Pathogens. 2021;10(8):918. doi:10.3390/pathogens10080918
30. Multiple highly resistant clones of MRSA circulating among patients with skin and soft tissue infection, Peshawar, Pakistan, 2021–2022. 2025. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC12529422/
31. Prevalence of multidrug-resistant tuberculosis and associated risk factors among adult pulmonary tuberculosis patients at a tertiary care hospital, Lahore, Pakistan, January–June 2024. 2025.
32. Genetic characterization of first-line drug-resistance mutations in multidrug-resistant *Mycobacterium tuberculosis* from Khyber Pakhtunkhwa, Pakistan. Pathogens. 2026;15(5):455. Available from: https://www.mdpi.com/2076-0817/15/5/455
33. Rifampicin resistance mutations in the 81 bp RRDR of the *rpoB* gene in *Mycobacterium tuberculosis* clinical isolates using Xpert MTB/RIF in Khyber Pakhtunkhwa, Pakistan: a retrospective study. 2016. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4983047/
34. Characterisation of drug-resistant *Mycobacterium tuberculosis* mutations and transmission in Pakistan. 2021. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC9095715/
35. Whole-genome analysis of drug resistance and transmission patterns in *Mycobacterium tuberculosis* from Khyber Pakhtunkhwa, Pakistan. 2025. Preprint; confirm publication status before submission.

36. Fluoroquinolone resistance markers among pre-MDR *Mycobacterium tuberculosis* isolates from north-west Pakistan. Reported in reference 35.
37. Early detection of MDR *Mycobacterium tuberculosis* mutations in Pakistan. Sci Rep. 2021;11. doi:10.1038/s41598-021-96116-x
38. Characterization of rifampicin-resistant *Mycobacterium tuberculosis* in Khyber Pakhtunkhwa, Pakistan. Sci Rep. 2021. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8270973/
39. Emerging rifampicin resistance in *Mycobacterium tuberculosis*: first report on characterization of a novel *rpoB* (Ser431Phe) mutation and phylogenetic lineages in Khyber Pakhtunkhwa, Pakistan. 2025. Preprint; confirm publication status before submission.
40. Klemm EJ, Shakoor S, Page AJ, Qamar FN, Judge K, Saeed DK, et al. Emergence of an extensively drug-resistant *Salmonella enterica* serovar Typhi clone harbouring a promiscuous plasmid encoding resistance to fluoroquinolones and third-generation cephalosporins. mBio. 2018;9(1):e00105-18. doi:10.1128/mBio.00105-18
41. Extraintestinal seeding of *Salmonella enterica* serotype Typhi, Pakistan. Emerg Infect Dis. 2021;27(3). Available from: https://wwwnc.cdc.gov/eid/article/27/3/20-0464_article
42. *Salmonella* Typhi acquires diverse plasmids from other Enterobacteriaceae to develop cephalosporin resistance. Genomics. 2021. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8276774
43. High frequency of simultaneous presence of ESBL and carbapenemase producers among nosocomial coliform isolates in Faisalabad, Pakistan. 2021. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7794153/
44. Dispensing of non-prescribed antibiotics from community pharmacies of Pakistan: a cross-sectional survey of pharmacy staff's opinion. Antibiotics (Basel). 2021. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8143445/
45. Saleem Z, et al. Point prevalence of non-prescription antibiotic dispensing in Pakistani community pharmacies. 2020. Cited for the 96–96.9% figure; the primary study should be traced and cited directly in place of this entry.
46. Assessment of without-prescription antibiotic dispensing at community pharmacies in Hazara Division, Pakistan: a simulated client's study. PLoS One. 2022;17(2):e0263756. doi:10.1371/journal.pone.0263756
47. Status of antimicrobial resistance in food animals in Pakistan (2016–2020): a systematic review and meta-analysis. J Adv Vet Anim Res. 2025;12(2):668–79. doi:10.5455/javar.2025.l930
48. Analyzing antibiotic resistance in bacteria from wastewater in Pakistan using whole-genome sequencing. 2024. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC11504851/
49. Activity of cefiderocol against carbapenem-non-susceptible and carbapenemase-producing Enterobacterales from United States medical centres, SENTRY Antimicrobial Surveillance Program 2020–2023. Poster, IDWeek 2024. Available from: https://jmilabs.com/data/posters/IDWeek2024_23-SHI-09_A4_Enteric_IDWeek2024.pdf. Comparator data only.

50. International and regional spread of carbapenem-resistant *Klebsiella pneumoniae* in Europe. Nat Commun. 2024;15. doi:10.1038/s41467-024-49349-z. EURECA and EuSCAPE collections; comparator data only.
51. Trends in antimicrobial consumption in Pakistan (2016–2028): retrospective observational study with forecasting. JMIR Public Health Surveill. 2026;12:e81288. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC13124083/
52. Continuous rise in cephalosporin and fluoroquinolone consumption in Pakistan: a 5 year analysis (2014–18). JAC Antimicrob Resist. 2019;1(3):dlz063. doi:10.1093/jacamr/dlz063
53. Unequal budgeting and limited WHO AWaRe Access antibiotics in Punjab: procurement policy gaps in Pakistan's National Action Plan against antimicrobial resistance. Arch Public Health. 2025. doi:10.1186/s13690-025-01720-2
54. Antibiotic consumption and prescription patterns in a tertiary care hospital in Karachi, Pakistan: a cross-sectional study. 2026. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC13162518/