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GENOMIC PROFILING OF ANTIMICROBIAL RESISTANCE AND VIRULENCE DETERMINANTS IN MULTIDRUG-RESISTANT BACTERIAL PATHOGENS

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Abstract

Background: *Multidrug-resistant bacterial infections remain difficult to treat and control. Whole-genome sequencing can reveal acquired resistance genes, resistance-associated mutations, virulence loci, and mobile genetic elements, but the presence of a gene does not necessarily establish its expression or clinical effect.*

Objective: *To elucidate the genomic basis of antimicrobial resistance and virulence among major bacterial pathogens and develop a transparent workflow for comparative genomic investigation.*

Methods: *This narrative synthesis integrates established genomic concepts and proposes an analysis of publicly available bacterial genomes with quality filtering, taxonomic verification, resistance and virulence annotation, sequence typing, plasmid analysis, phylogenetics, and statistical comparisons.*

Results: *Published research supports the importance of beta-lactamases, carbapenemases, efflux systems, target modifications, adhesins, siderophores, capsules, secretion systems, and horizontal gene transfer. Their distributions depend on lineage and species.*

Conclusion: *Integrated resistome–virulome analysis can improve surveillance when genomic predictions are validated against phenotypes and interpreted alongside clinical and epidemiological metadata. A reproducible study should distinguish gene detection from functional resistance, pathogenicity, and patient outcomes.*

1. INTRODUCTION

Antimicrobial resistance threatens effective treatment of bacterial infections and increases pressure on healthcare systems. Global analyses have estimated a substantial burden of deaths associated with bacterial antimicrobial resistance (AMR), with marked variation by pathogen, region, and antibiotic class [1]. Resistance arises through mutation, acquisition of genetic material, and selection under antimicrobial exposure. Genomic surveillance enables investigation of these processes at higher resolution than routine species identification alone [2,3].

A second dimension of bacterial pathogenicity is virulence: the capacity to colonize hosts, obtain nutrients, evade immunity, damage tissues, and persist in challenging environments. Virulence is multifactorial and context-dependent. Genomic loci encoding capsules, adhesins, siderophores, toxins, and secretion systems can contribute to disease, but clinical severity also depends on host susceptibility, infection site, treatment, and healthcare exposure [4,5].

Resistance and virulence can coexist in successful bacterial clones. Yet it is misleading to assume that all resistant organisms are more virulent, or that every detected virulence gene is expressed. Their association can reflect clonal expansion, linked mobile elements, ecological selection, or sampling bias. The key research task is therefore to characterize both sets of determinants while separating genomic co-occurrence from demonstrated biological interaction [2,5,18].

Recent international surveillance frameworks emphasize the need to connect genomic characterization with standardized resistance reporting [15,16]. Population-based genomic investigations further show that hospital transmission and clonal structure shape the distribution of resistant lineages [18].

2. Review scope and evidence selection

This critical narrative review examines genomic antimicrobial resistance and virulence determinants across major bacterial pathogens. It draws on foundational studies, genomic resources, surveillance guidance, and selected recent investigations to compare mechanisms, assess the strength of genomic evidence, and identify priorities for clinical surveillance.

Evidence was selected for its relevance to resistance mechanisms, virulence traits, mobile genetic elements, population structure, genomic methods, and surveillance. The literature was assembled for a narrative synthesis rather than through a documented systematic database search.

Selection and relevance. Sources are discussed when they contribute to at least one of the review's defined themes: antimicrobial resistance mechanisms, virulence-associated loci, mobile genetic elements, population structure, genomic annotation resources, phenotype–genotype concordance, or surveillance applications. Foundational reviews and methodological publications provide biological and analytical context; organism-specific genomic investigations provide examples of observed patterns. Studies of distinct species, specimen types, countries, or sampling frames are not treated as interchangeable. These themes organize the review; they were not used as formal screening criteria.

The synthesis organizes findings by pathogen and mechanism, separating acquired genes from chromosomal variants and gene carriage from demonstrated biological effects. Comparisons consider study setting, sampling, sequencing methods, annotation tools, phenotypic data, and bacterial lineage where reported. Differences in these factors can explain why studies reach different conclusions.

Evidence selection was not based on a registered protocol or formal risk-of-bias assessment. Selective coverage and publication bias may therefore influence the conclusions, particularly when comparing species or regions. Section 7 sets out a reproducible approach for future genomic research.

A systematic extension of this work would document database searches, screening decisions, study-level extraction, and quality assessment.

Table 1 summarizes representative literature used to frame the review. Its entries are illustrative and should be read alongside the narrative rather than as a comprehensive inventory of eligible studies.

Table 1. Representative literature and its relevance to the review.

Source	Evidence type/focus	Contribution and limitation
Murray et al. (2022) [1]	Global AMR burden analysis	Establishes public-health importance; does not profile virulence loci.
Lam et al. (2021) [7]	Klebsiella genomic surveillance framework	Illustrates lineage-aware typing; not a cross-pathogen prevalence estimate.
Alcock et al. (2020) [13]	Resistance database resource	Supports annotation; predictive performance varies by organism and drug.
Russo and Marr (2019) [5]	Hypervirulent *K. pneumoniae* review	Defines virulence context; marker presence is not proof of clinical severity.
David et al. (2019) [18]	European carbapenem-resistant Klebsiella epidemiology	Demonstrates importance of hospital spread; geographically specific.
Liu et al. (2019) [14]	Virulence database resource	Supports candidate locus screening; functional validation remains necessary.

3. Pathogen-specific genomic profiles

3.1. Escherichia coli

Pathogenic *E. coli* includes diverse intestinal and extraintestinal lineages. Extended-spectrum beta-lactamases, notably members of the CTX-M family, and carbapenemases can reduce therapeutic options. Extraintestinal pathogenic strains may carry adhesins, iron-acquisition systems, protectins, and toxins. Interpretation needs to account for pathotype and phylogenetic background rather than treating all *E. coli* virulence markers as equivalent [4].

3.2. Klebsiella pneumoniae

K. pneumoniae is important for the convergence of antimicrobial resistance and hypervirulence. Resistance determinants include ESBL and carbapenemase genes, while capsule-associated loci, siderophore systems such as aerobactin and yersiniabactin, and regulators associated with hypermucoviscosity can contribute to virulence. Genomic screening can flag concerning combinations, but classification as hypervirulent needs careful interpretation and, when possible, phenotypic and clinical corroboration [5,7].

3.3. Acinetobacter baumannii

A. baumannii frequently causes healthcare-associated infections in vulnerable patients. Resistance may involve OXA-type carbapenemases, permeability changes, efflux systems, and other mechanisms. Biofilm formation, surface structures, iron uptake, and persistence-related traits are important to its ecology. Species-complex identification and assembly quality are essential before comparing gene content [8].

3.4. Pseudomonas aeruginosa

P. aeruginosa combines intrinsic resistance with acquired resistance and adaptive mutations. AmpC regulation, efflux activity, porin alterations, and acquired carbapenemases can contribute to reduced susceptibility. Its virulence repertoire includes type III secretion effectors, quorum-sensing pathways, motility factors, and biofilm-associated determinants. The organism's large accessory genome and within-host evolution complicate interpretation of cross-sectional genome comparisons [9].

3.5. Staphylococcus aureus and Enterococcus faecium

In Gram-positive pathogens, methicillin resistance in S. aureus is strongly associated with mec-mediated altered penicillin-binding proteins; additional determinants influence resistance to other antibiotic

classes. Adhesins, immune-evasion factors, and toxins vary across lineages. In E. faecium, vancomycin resistance can be mediated by van gene clusters, while hospital-adapted populations may show distinctive accessory genomes. Cross-species comparisons should use mechanism categories, not simply raw gene counts [3,10,41].

In S. aureus, methicillin resistance is principally associated with mecA or mecC carried on staphylococcal cassette chromosome mec (SCCmec), which encodes an altered penicillin-binding protein. This differs from the many determinants of invasive disease, including surface adhesins, immune-evasion proteins, hemolysins, and other toxins. Genomic detection of a toxin locus does not demonstrate expression under clinical conditions, and the prevalence of particular virulence loci can reflect the sampled clonal background rather than the effect of methicillin resistance itself. A sound comparison needs to separate resistance phenotype, sequence type or clonal complex, SCCmec structure, and the clinical syndrome under investigation [12,14,41].

For *E. faecium*, vanA and vanB operons are central to acquired glycopeptide resistance, but the clinical importance of hospital-associated lineages cannot be reduced to vancomycin resistance alone. Accessory genetic elements, surface-associated proteins, and adaptations favoring persistence in healthcare environments may occur alongside antimicrobial resistance. A van operon points to a possible resistance mechanism, but susceptibility testing still matters because expression and phenotype may not always be inferred from an isolated sequence call. Comparisons also require attention to whether isolates represent infection, colonization, or environmental contamination [10,12,13].

The two Gram-positive species thus illustrate different genomic questions. For *S. aureus*, investigators often ask whether resistant and susceptible lineages differ in toxin content or clinical presentation. For *E. faecium*, a major question is how hospital-adapted populations acquire and disseminate resistance while persisting among colonized patients and on healthcare surfaces. Counting virulence genes across species cannot reliably answer either question. Within-species, lineage-adjusted analyses linked to standardized phenotypes and clinical definitions are more informative [10,14].

4. Resistance determinants and genomic interpretation

Beta-lactam resistance is not a single genomic phenotype. Extended-spectrum beta-lactamases can compromise expanded-spectrum cephalosporins, whereas carbapenemase families differ in their substrate profiles and inhibitor susceptibility. In Enterobacterales, blaCTX-M, blaKPC, blaNDM and blaOXA-48-like loci are particularly relevant surveillance targets. Their presence should be interpreted together with porin integrity, gene expression and phenotypic susceptibility instead of being treated as a complete therapeutic prediction [2,6,12,13].

Aminoglycoside-modifying enzymes and 16S rRNA methyltransferases can contribute to distinct resistance spectra. Fluoroquinolone resistance may involve target-site mutations, plasmid-mediated protection, and efflux. Colistin resistance may result from transferable mcr determinants in some Enterobacterales or chromosomal regulatory changes in other species. Because the relevant loci and interpretive rules vary across organisms, a uniform resistance-gene checklist risks false equivalence [2,3,9,40].

The term multidrug-resistant is a phenotypic classification, not a synonym for carrying three resistance genes. A single enzyme may affect multiple antimicrobial agents, while several detected genes may not be expressed or clinically consequential. A reliable study links genomic calls to isolate-level antimicrobial susceptibility testing and clearly identifies isolates lacking those measurements [11].

5. Virulence determinants and host context

Virulence is an emergent property of bacterial genotype, regulation, host susceptibility, and infection site. Capsule production, adhesins, secretion systems, toxins, iron acquisition, and biofilm-associated factors contribute to different stages of infection. A virulence database hit alone is insufficient to demonstrate increased pathogenicity; some loci are common among colonizing strains and others require coordinated regulation or intact gene clusters [5,14].

In K. pneumoniae, aerobactin synthesis, hypermucoidy regulators, and capsule-associated markers warrant special attention when evaluating convergence between resistance and hypervirulence.

Nonetheless, sequence-based markers do not establish clinical hypervirulence without contextual evidence. In *E. coli*, extraintestinal virulence varies by phylogenetic background, with adhesins and siderophores often contributing to urinary tract and bloodstream infection. Comparisons should be lineage-aware rather than restricted to raw virulence-gene totals [5,7].

For A. baumannii and P. aeruginosa, biofilm-related and surface-associated factors influence persistence, but laboratory phenotypes depend strongly on experimental conditions. In S. aureus, toxin repertoire, immune evasion, and adhesion traits vary across clonal complexes; in E. faecium, hospital adaptation and colonization traits require interpretation alongside vancomycin resistance. These examples demonstrate why a cross-species "virulence score" is usually difficult to justify without validation [8–10].

Recent genomic surveillance research emphasizes the importance of One Health sampling and cross-sector metadata in interpreting the circulation of resistance genes [25].

6. Mobile elements, population structure and resistance–virulence relationships

The resistome includes acquired genes and chromosomal variants that may influence antimicrobial susceptibility. The virulome encompasses genomic features implicated in pathogenic processes. Mobile genetic elements, including plasmids, integrative elements, insertion sequences, and transposons, can facilitate the spread of resistance and sometimes virulence traits. Co-location on the same replicon is stronger evidence of physical linkage than detection within the same draft genome, but it does not alone prove co-transfer or expression [2,5].

Potential trade-offs are also important. Resistance may carry a fitness cost under some conditions, while compensatory evolution can reduce that cost. Successful clones may accumulate resistance and virulence features through repeated selection or recombination. Robust inference therefore requires adjustment for population structure and careful distinction between correlations at the isolate level and causal mechanisms [2,5,18].

Plasmids and other mobile genetic elements facilitate horizontal movement of resistance determinants, sometimes alongside virulence-associated loci. However, detecting two genes in the same assembly does not prove that they share a plasmid. Short-read assemblies may split repetitive sequences or collapse homologous regions; plasmid reconstruction and gene linkage are stronger when supported by long reads, hybrid assemblies, read mapping, or experimental transfer evidence [2].

Clonal expansion can create an apparent correlation between resistance and virulence even when the traits are not functionally linked. For example, a successful sequence type may be enriched for both classes of loci because of historical acquisition and transmission. Within-species phylogenies, lineage-adjusted models and sensitivity analyses excluding closely related outbreak isolates help separate these explanations. Recombination and uneven geographic sampling remain potential sources of bias.

Genomic epidemiology is strongest when sequence information is integrated with collection date, location, specimen source, and antimicrobial susceptibility. Public repositories frequently underrepresent low-resource settings and overrepresent unusual resistant isolates. The resulting sampling bias limits claims about population prevalence and geographic trends. Reports should therefore describe the sampling frame and use "frequency among analyzed genomes" rather than "regional prevalence" when population representativeness cannot be established.

This evidence can be compared with earlier analyses of plasmid-mediated dissemination [17] and the epidemiological separation of clonal spread from independent gene acquisition [18]. These mechanisms cannot be established from co-carriage alone.

7. Framework for future genomic research

Study design and eligibility

A retrospective comparative genomic study could include publicly deposited whole-genome sequences from clinical isolates of selected bacterial species. Before downloading data, the investigators should register a sampling window, eligible countries, specimen types, sequencing platforms, and minimum metadata [15,16,25]. Records without reliable species identification, clear assembly provenance, or sufficient sequence quality should be excluded using predefined criteria. Duplicate submissions and

multiple isolates from the same patient or outbreak should be identified where metadata permit [15,16,25].

Definition of multidrug resistance

Multidrug resistance (MDR) should follow a recognized organism-specific definition, commonly non-susceptibility to at least one agent in three or more antimicrobial categories [11]. Importantly, genomes alone cannot establish this phenotypic MDR definition unless validated susceptibility data are available. Isolates without antimicrobial susceptibility testing should be labeled genomically predicted resistant or resistance-gene-positive rather than phenotypically MDR [2,5,18].

Sequence quality control and taxonomic verification

For raw reads, perform read-level quality assessment, adapter trimming when needed, contamination checks, and de novo assembly with documented software versions and parameters. For existing assemblies, evaluate length, contiguity, ambiguous bases, completeness, and contamination. Verify species identity using appropriate taxonomic methods and remove obvious misidentifications. Apply consistent inclusion thresholds across cohorts and report exclusions in a study-flow diagram [19,20,21].

Resistome annotation

Screen genomes with a curated resistance resource such as CARD/RGI or AMRFinderPlus [12,13]. Record database versions, detection thresholds, gene identity, coverage, mutation calls, and predicted drug classes. Resolve duplicate or overlapping calls, distinguish acquired genes from intrinsic determinants, and manually review unusual combinations. Compare genotype-based predictions with measured susceptibility where available, reporting sensitivity, specificity, and discordant cases by species and drug class [12,13].

Virulome annotation

Identify virulence-associated loci with a curated resource such as VFDB, supplemented by species-specific typing frameworks where appropriate [14]. Report locus definitions, identity and coverage thresholds, and known limitations. Distinguish intact loci from partial matches. A positive sequence hit should be described as a virulence-associated gene detection, not proof of toxin production, invasion, or increased clinical severity [14].

Population structure and mobile elements

Assign multilocus sequence types using appropriate schemes and construct within-species phylogenies from high-quality core-genome variation. Assess recombination when relevant. Screen for plasmid replicons and mobile-element signatures, recognizing that short-read assemblies may not reliably establish plasmid–gene linkage. Long-read or hybrid assemblies can resolve selected high-priority isolates. Outbreak-related clusters should be analyzed separately to avoid overstating independent observations [7,18,22,24].

Statistical analysis

Report species-specific sample sizes, prevalence with confidence intervals, and missing metadata. Compare gene carriage using contingency analyses or regression as appropriate. For associations between resistance and virulence, fit models accounting for species, lineage, sampling year, geography, specimen source, and clustering where possible. Control false discovery rates when screening multiple loci. Sensitivity analyses should restrict to high-quality assemblies, nonduplicate isolates, and records with phenotypic susceptibility data. Do not pool heterogeneous organisms into a single unadjusted gene-count comparison.

Reproducibility and data governance

Preserve accession identifiers, a version-controlled analysis pipeline, software environments, database snapshots, exclusion logs, and scripts used to create tables and figures. Respect repository terms and avoid

publishing potentially identifying patient metadata. Share aggregate results and code where permissions allow. A reporting checklist should document sampling bias, missingness, database uncertainty, and any deviations from the preregistered protocol [15,16,25].

For implementation, commonly used tools include SPAdes for assembly [20], Unicycler for hybrid assembly [21], Prokka for annotation [19], Roary for pan-genome analysis [24], PlasmidFinder for plasmid replicon screening [23], and population-structure approaches such as fastBAPS [22]. Tool choice, versions, input requirements, and parameter settings would need to be justified and recorded before any original analysis.

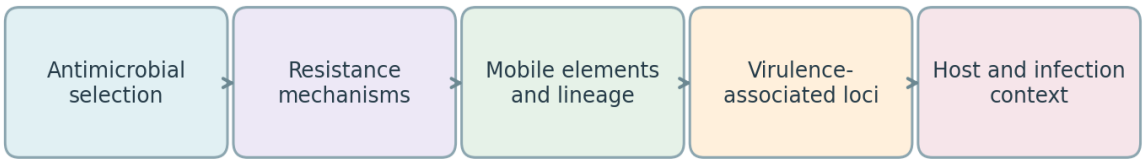


Figure 1. Conceptual relationships; arrows show analytical connections, not established causation.

Figure 1 shows why bacterial population structure belongs in any resistance–virulence analysis. Successful clones can accumulate both categories of determinants and become common in hospital collections through transmission or antibiotic selection. Without lineage-aware comparisons, this shared ancestry can resemble a functional association. Comparative genomic surveillance can therefore generate testable hypotheses, while phenotypic and clinical measurements are needed to establish their significance [2,18].

Figure 1 brings together the pathways discussed here: antimicrobial selection, acquisition or mutation of resistance determinants, virulence-associated traits, bacterial lineage, and the host environment. The arrows indicate potential biological or epidemiological relationships, not effects measured in a shared dataset. In particular, a resistant isolate may carry adhesion or iron-acquisition loci without those features increasing disease severity. This matters when interpreting reports of carbapenem-resistant *K. pneumoniae* with aerobactin-associated loci, because genetic co-carriage does not prove expression, transmission, or a clinical outcome [5,7].

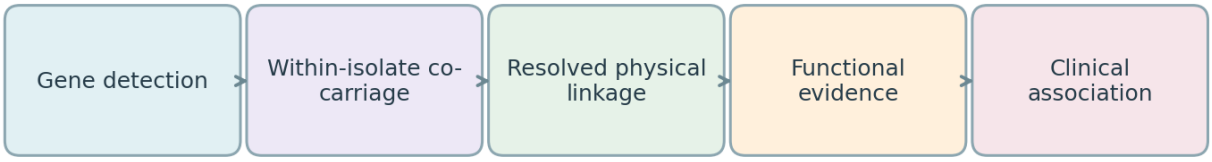


Figure 2. Distinct forms of evidence required to evaluate resistance–virulence convergence.

Experimental conjugation, transcriptional assays, and functional infection models address different questions from genome annotation. Even these results need interpretation within the tested strain, host model, and environmental conditions. Figure 2 is therefore an interpretive guide rather than a quantitative scale, and the transitions between evidence levels should not be treated as automatic proof of causality.

Figure 2 distinguishes levels of evidence for claims about resistance–virulence convergence. Detection of two loci in the same isolate supports co-carriage; their placement on the same assembled sequence

provides more localized linkage evidence; and a closed plasmid assembly can provide stronger evidence of physical co-localization. None of these observations, by itself, demonstrates horizontal transfer, gene expression, or increased pathogenicity. This is especially relevant when short-read assemblies contain fragmented plasmid sequences or repetitive elements [2,21,23].

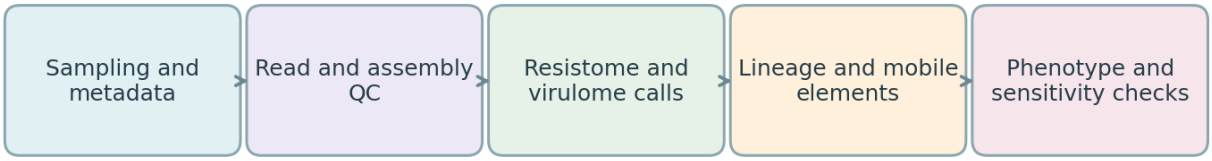


Figure 3. Proposed workflow for a future original genomic study.

Phenotype validation is central to this framework. Genomic resistance predictions should be compared with organism- and drug-specific susceptibility results, with discordant isolates investigated carefully. Virulence loci also need cautious interpretation because gene detection alone cannot establish expression or clinical severity. Figure 3 brings these stages together as a practical research framework.

Figure 3 shows the steps in a reproducible future genomic study, beginning with isolate and metadata selection and continuing through quality control, species confirmation, resistome and virulome annotation, lineage assignment, mobile-element analysis, and statistical synthesis. Each stage can change the interpretation of the final results. For example, excluding low-quality assemblies may reduce false gene calls but can also alter the species or geographic composition of the retained sample [12–14,19–24].

A 2024 investigation of multidrug-resistant *P. aeruginosa* combined whole-genome sequencing with resistance and virulence characterization, illustrating the value of organism-specific interpretation [26]. A report of an extensively drug-resistant ST308 lineage co-producing KPC and NDM further illustrates the clinical relevance of identifying multiple carbapenemase mechanisms [31].

8. Comparative tables and interpretive guidance

The following tables compare representative mechanisms and methodological issues across the bacterial pathogens discussed in this review. They summarize established concepts from the cited literature rather than results of a newly analyzed genomic cohort. They are guides to interpretation, not as estimates of gene prevalence or evidence of causal relationships.

Table 2. Representative resistance determinants and interpretive cautions.

Determinant/mechanism	Example organisms	Interpretive caution
blaCTX-M ESBLs	*E. coli*; *K. pneumoniae*	Allele and coexisting mechanisms affect phenotype
blaKPC, blaNDM, blaOXA-48-like	Enterobacterales	Carbapenemase families differ in inhibitor profile
OXA-type carbapenemases	*A. baumannii*	Expression and insertion sequences matter
mecA/mecC	*S. aureus*	PBP2a-mediated beta-lactam resistance
vanA/vanB	Enterococcus spp.	Phenotype and regulatory context matter
gyrA/parC variants; efflux	Multiple species	Mutation combinations and expression vary

Table 2 shows that resistance cannot be established from a gene name without considering its allele, genomic background and expression. For example, beta-lactamase families differ in substrate specificity, while carbapenem susceptibility may also depend on permeability and other mechanisms. Similarly, mecA/mecC and van gene clusters are interpreted within organism-specific regulatory and phenotypic contexts. The table therefore highlights why sequence-based predictions should be checked against antimicrobial susceptibility testing whenever paired data are available [11–13].

Table 3. Representative virulence-related features across selected pathogens.

Species	Selected features	Biological role/caveat
E. coli	Fimbriae, siderophores, toxins	Host niche and lineage-dependent
K. pneumoniae	Capsule, aerobactin, yersiniabactin, rmpA-associated loci	Markers do not alone prove hypervirulence
A. baumannii	Surface proteins, biofilm-associated loci, iron uptake	Persistence is multifactorial
P. aeruginosa	Type III secretion, elastase, quorum sensing	Regulation shapes expression
S. aureus	Adhesins, toxins, immune-evasion factors	Clonal complex and host context matter
E. faecium	Adhesion and colonization factors	Hospital adaptation not equivalent to invasiveness

Table 3 compares virulence-associated features among the selected species. Capsules and siderophore systems are prominent considerations in K. pneumoniae, whereas adhesins and toxins in E. coli and S. aureus require attention to pathotype or lineage. Biofilm-associated traits and secretion systems are relevant to A. baumannii and P. aeruginosa, but detection of their genes does not prove expression or clinical severity. These differences make it difficult to justify applying a single unvalidated virulence-gene score across species [4,5,8–10,14].

Table 4. Genomic linkage and mobility: strength of evidence.

Observation	What it supports	What it does not establish
Genes found in one isolate	Co-carriage	Same replicon or physical linkage
Genes on same short-read contig	Local sequence adjacency	Complete plasmid structure
Closed hybrid-assembled plasmid	Physical co-localization	Conjugative transfer in vivo
Transfer or expression experiment	Mechanistic evidence under tested conditions	Universal clinical effect

Table 4 is especially useful when discussing apparent convergence of antimicrobial resistance and virulence. Co-carriage within an isolate establishes that both determinants were detected, but not that they reside on the same mobile element. A shared short-read contig offers local linkage evidence, whereas a closed hybrid assembly can support physical co-localization. Experimental transfer or expression studies answer different mechanistic questions and still require careful interpretation before extrapolation to clinical outcomes [2].

Table 5. Proposed analytical outputs for a future original study.

Analysis	Required data	Minimum reporting
Resistome and virulome frequency	QC-passed genomes	Denominators, database versions, thresholds
Phenotype–genotype concordance	Matched AST records	Drug-specific sensitivity, specificity and uncertainty
Lineage structure	Core-genome alignment / MLST	Recombination handling and sampling context
Plasmid linkage	Long-read or hybrid assemblies	Assembly support and mobility caveats
Resistance–virulence association	Genome metadata and phylogeny	Lineage-adjusted estimates and sensitivity analyses

Table 5 translates these considerations into reporting standards for genomic investigations. Resistome and virulome comparisons need clear denominators and annotation thresholds, while genotype–phenotype analyses require matched susceptibility data. Lineage and plasmid studies need adequate sequence resolution and sampling information. Resistance–virulence associations are most informative when they account for related bacterial lineages.

Recent population genomic evidence has investigated iuc3-bearing K. pneumoniae plasmids and the convergence of resistance and virulence determinants across settings [27]. Large multicountry European genomic surveys have also documented carbapenemase-producing K. pneumoniae and E. coli, highlighting the value of standardized surveillance and sampling metadata [28,29]. A large cross-host K. pneumoniae analysis has explored resistance and virulence variation across animal and human-associated reservoirs [36].

8.1. Evidence from published genomic investigations

The studies below illustrate how species, clinical sampling, resistance mechanisms, and virulence-associated loci affect interpretation. Their reported denominators describe the investigated collections and should be considered in that context. The evidence base is expanded here to include examples from additional bacterial species and genomic surveillance settings.

Klebsiella pneumoniae: A 2024 Egyptian investigation sequenced 19 carbapenemase-producing clinical isolates and combined genomic characterization with antimicrobial susceptibility testing. The most frequently identified carbapenemase determinants included blaNDM-5 and blaOXA-48. Reported high-risk lineage–capsule combinations included ST383-K30, ST147-K64, ST11-K15 and ST14-K2. Some isolates also carried aerobactin-associated iucABCD, rmpA or rmpA2, and peg-344, suggesting possible resistance–virulence convergence. However, the small, selected hospital collection limits generalization, and genomic marker co-carriage does not independently demonstrate enhanced clinical virulence [38].

Acinetobacter baumannii: A longitudinal ICU surveillance investigation in Hangzhou sequenced 518 A. baumannii isolates collected in 2021, of which 419 (80.9%) were carbapenem-resistant. The resistant population was concentrated in ST2/GC2 (213 isolates) and ST164 (206 isolates), whereas the earlier 2019 collection was dominated by GC2. The findings show that changes in clone composition can alter the apparent epidemiology of resistance without necessarily reflecting independent emergence of resistance in every isolate. Its ICU-specific sampling and infection-control context limit extrapolation to other hospitals; clonal turnover is not itself proof of greater virulence [39].

Comparative interpretation: The two studies address different genomic questions. The Egyptian K. pneumoniae investigation identifies carbapenemase genes alongside selected virulence-associated markers in a small clinical collection [38]. The Hangzhou A. baumannii study describes carbapenem resistance and changes in dominant sequence types within an intensive care setting [39]. Their results should not be compared using raw gene counts or treated as estimates of wider prevalence. Differences in species, sampling, and study design call for pathogen-specific interpretation supported by phenotypic and epidemiological data.

Table 6. Representative genomic studies illustrating antimicrobial resistance and virulence-related context across bacterial species.

Study/sample	Resistance findings	Virulence / genomic context	Main limitation
Egypt, *K. pneumoniae*; 19 genomes retained from 46 carbapenem-resistant clinical isolates [38]	All 19 analyzed isolates were extensively drug-resistant or pan-drug-resistant; blaNDM-5 and blaOXA-48 were the most prevalent carbapenemase genes	Seven isolates were classified as putative convergent strains based on iucABCD, rmpA and/or rmpA2, and peg-344; reported lineage–capsule combinations included ST383-K30, ST147-K64, ST11-K15, ST14-K2, and ST709-K9	Small, selected clinical collection; convergence was inferred from genomic markers, not confirmed by functional virulence testing
Hangzhou ICU, *A. baumannii*; 518 isolates sequenced in 2021, including 419 carbapenem-resistant isolates (80.9%) [39]	The carbapenem-resistant population comprised ST2/GC2 (213 isolates) and ST164 (206 isolates); the 2019 collection had been dominated by GC2	Longitudinal population genomics documented a major shift in clone composition; the study was not designed to establish a virulence phenotype	Single-ICU setting and infection-control context limit generalization beyond the sampled hospital and period
England, invasive *S. pneumoniae* surveillance, July 2017–February 2020; 13,749 genomes passed quality control [30]	WGS-based resistance prediction found co-trimoxazole resistance most frequently (17%), followed by tetracycline (8.7%) and beta-lactams (8.4%)	National surveillance described serotype and genomic-cluster composition alongside predicted resistance; it did not test resistance–virulence convergence	National and period-specific findings; resistance was predicted from genomic data rather than uniformly established by isolate-level phenotypic testing in this analysis
P. aeruginosa clinical collection; 48 clinical samples analyzed [26]	Most isolates (77.1%) were multidrug-resistant; genomic findings implicated efflux-mediated resistance and hydrolytic-enzyme genes	Resistance and virulence-associated genes were examined together; toxin-related genes, biofilm variation, and cytotoxic potential were reported	Clinical collection from a single center; gene detection does not by itself establish expression or in-vivo clinical effect
Balochistan, Pakistan, *S. Typhi*; 3 multidrug-resistant strains selected for WGS from 100 patients [32]	Genome analysis characterized resistance determinants in the three sequenced MDR strains	Virulence-associated features included Vi antigen and fimbrial/colonization-associated factors; findings are specific to the selected strains	Only three selected strains were sequenced, so findings do not estimate the prevalence of genomic features in the wider population

Peshawar region, Pakistan, *S. Typhi*; 13 XDR strains sequenced from 65 confirmed *S. Typhi* cases among 200 symptomatic patients [33]	The study characterized resistance genes and genomic features in selected XDR strains; 40 of the 65 confirmed cases were classified as XDR and 25 as MDR	Sequence typing, phylogenetics, pathogenicity islands, and plasmid sequences were assessed	Only 13 XDR strains underwent WGS; a regional clinical sample cannot represent all circulating *S. Typhi*
Punjab, Pakistan, *E. coli* colonization; 5,247 genomes assembled from 1,411 samples from outpatients and community participants [37]	Deep sequencing characterized AMR diversity and found antibiotic use associated with increased prevalence of known MDR clinical lineages	Demonstrates substantial colonization diversity and the effect of sampling depth; this was a colonization-focused study rather than an invasive-infection cohort	Colonization and antibiotic-use findings should not be treated as direct estimates for invasive infections or as evidence of virulence

9. Clinical and public-health implications

In routine microbiology, whole-genome sequencing can complement species identification and susceptibility testing by identifying resistance mechanisms, distinguishing related lineages and investigating suspected transmission clusters. Turnaround time, cost, database maintenance, and analytical expertise determine whether genomic findings can inform real-time treatment. For many infections, phenotypic susceptibility results remain essential because sequence data do not fully capture expression, permeability, inducible resistance, or uncharacterized mechanisms [12,13,15].

At the hospital level, genomic surveillance may identify the emergence of carbapenemase-bearing lineages, possible plasmid transmission, and recurrent outbreaks. Effective implementation requires standard operating procedures for sequencing, contamination control, quality thresholds, data governance, and communication with infection prevention teams. At the regional level, harmonized metadata and shared nomenclature can make surveillance more comparable, provided laboratories document methodological differences.

Future studies should prioritize paired genomic and phenotypic data, representative longitudinal sampling, robust plasmid reconstruction and careful evaluation of clinically meaningful outcomes. Functional validation of selected determinants, including expression assays or isogenic comparisons, is needed before causal claims about virulence or resistance–virulence synergy can be made.

WHO surveillance reporting [15] and its 2024 priority-pathogen framework [16] offer complementary policy contexts for selecting surveillance targets. They do not replace local epidemiological sampling or isolate-level phenotype validation.

Genomic surveillance can extend beyond the principal ESKAPE pathogens: a national study of invasive pneumococcal disease combined whole-genome sequencing with predicted resistance [30]. Studies of *Salmonella Typhi* from Balochistan and Peshawar demonstrate the relevance of genomic resistance characterization in Pakistan [32,33]. A deep-sequencing study of *E. coli* colonization in Punjab likewise underscores the diversity that may be missed by narrower isolate selection [37].

10. Discussion

Genomic profiling offers a powerful framework for understanding why some bacterial populations combine difficult-to-treat resistance patterns with traits that support transmission or host adaptation. The strongest studies integrate multiple evidence streams: reliable species assignment, high-quality genome assemblies, validated resistance databases, lineage-aware comparisons, susceptibility testing, and clinical metadata. These components help distinguish broad surveillance signals from claims about the biology of individual isolates [2,12–14,18].

An especially important issue is the distinction between co-occurrence and mechanistic linkage. When a resistance gene and virulence determinant appear in the same isolate, they may reside on separate chromosomes or plasmids, reflect shared ancestry, or be independently selected [2,17,18]. Complete assemblies, conjugation studies, transcriptomic experiments, and functional assays provide additional evidence for linkage and biological activity. Epidemiological associations should be framed as hypotheses unless these further lines of evidence exist.

Surveillance priorities will also vary by organism. For K. pneumoniae, convergence of carbapenem resistance with hypervirulence-associated loci deserves careful investigation. In P. aeruginosa, resistance-associated regulatory changes and chromosomal mutations can be as important as acquired genes. In A. baumannii, clonal expansion and healthcare transmission can dominate observed distributions. A single universal annotation threshold or virulence score is unlikely to capture these differences reliably.

The clinical value of genomic data depends on how they are used. Rapid sequencing may support outbreak investigation, recognition of emerging lineages, and targeted laboratory follow-up. However, genotype-based predictions should not replace standardized phenotypic antimicrobial susceptibility testing without adequate validation for the relevant organism and antibiotic. Similarly, virulence gene detection cannot be used alone to predict an individual patient's prognosis.

This review distinguishes genomic detection, biological function and clinical effect. However, the cited evidence spans different organisms, sampling designs and genomic platforms, making numerical cross-study comparisons inappropriate. Some studies emphasize acquired carbapenemase dissemination whereas others emphasize clonal transmission or regulatory resistance mechanisms. A future empirical study would need matched phenotype data and within-species analyses to distinguish these explanations. One Health sequencing investigations in Palestine and environmental sewage surveillance in China show how genome-based methods can support analysis across human, animal, and environmental sources, although sampling designs and species differ [34,35].

Comparisons between published studies are complicated by different research questions and sampling frames. David and colleagues examined more than 1,700 K. pneumoniae genomes from 244 European hospitals and found that carbapenemase-positive isolates clustered strongly within several successful lineages, emphasizing healthcare transmission [18]. In contrast, Lam and colleagues developed a genomic classification framework that combines resistance and virulence markers, but explicitly cautioned that its genotype scores do not establish phenotypic resistance or clinical virulence [7]. These results are complementary rather than contradictory: one evaluates population spread, whereas the other standardizes genomic characterization. Neither alone demonstrates that resistance genes cause greater virulence.

The longitudinal A. baumannii investigation provides a useful counterpoint to cross-sectional reports. In a single intensive care unit, 419 of 518 isolates were carbapenem-resistant in 2021, with a substantial ST164 population carrying blaNDM-1 and blaOXA-23 [39]. This shift from the earlier predominance of global clone 2 illustrates how local clone replacement can change observed resistance patterns. It does not show a global change in clone frequencies, nor can the association of a clone with resistance be taken as evidence of greater pathogenicity. Geographic scope, patient mix, environmental sampling, and infection-control interventions differ from those in broader multicentre studies.

Differences in findings about resistance–virulence convergence also depend on the markers measured. Klebsiella studies may assess aerobactin, yersiniabactin and rmp-associated loci, whereas P. aeruginosa studies often emphasize chromosomal regulatory mechanisms, OprD loss, efflux, and type III secretion factors [7,9,26]. A low prevalence of a marker in one organism cannot be interpreted as evidence of reduced virulence relative to another organism. Likewise, detecting bla genes and virulence loci in the same draft assembly establishes co-carriage, not co-transfer or coordinated expression [2,23].

Study designs also differ in their treatment of genotype–phenotype discordance. Resistance databases and curated annotation tools support standardized screening, but differences in database versions, identity thresholds, expression, permeability, and antibiotic-specific breakpoints can produce discordant classifications [12–14]. Studies reporting gene presence alone should not be combined numerically with studies that verify isolate-level susceptibility. For that reason, the comparisons in Tables 1 and 6 are qualitative; they do not support pooled prevalence estimates or causal effect sizes.

Finally, published genome collections may be enriched for unusual resistant isolates, particular hospitals, or outbreak investigations. Such ascertainment can exaggerate the apparent concentration of resistance and virulence determinants. The evidence considered here is therefore strongest for identifying plausible mechanisms and surveillance priorities, not for estimating population prevalence or predicting patient-level outcomes [18,25,39]. Future comparative work should use harmonized sampling, complete metadata, lineage-aware models, and phenotypic validation.

Gram-positive findings require an additional layer of interpretation. In S. aureus, the presence of mec-associated methicillin resistance and a toxin gene in the same genome does not prove that resistance increases toxin production or disease severity. Both characteristics can be concentrated within particular clonal complexes, and sampling invasive infections rather than colonization can alter their observed frequency. Studies that adjust for lineage and infection source may therefore reach different conclusions from unadjusted surveys. Genome annotation should be supplemented by susceptibility testing and, where the question concerns virulence, expression or functional evidence [12,14,41].

In *E. faecium*, the main interpretive challenge is different: hospital adaptation, vancomycin resistance, and persistence can overlap without being interchangeable outcomes. A study sampling rectal carriage may capture the reservoir of van-bearing lineages, whereas a bloodstream-infection series selects for organisms that have crossed additional host and clinical barriers. These sampling frames can produce apparently conflicting associations between resistance determinants and invasive disease even when both studies are internally valid. Longitudinal sampling and epidemiologically linked isolates are needed to distinguish acquisition, clonal spread, and repeated introduction [10,13].

Overall, the Gram-positive evidence cautions against presenting resistance–virulence convergence as a uniform process across the six pathogens. For *S. aureus*, toxin expression and lineage structure deserve particular scrutiny; for *E. faecium*, colonization pressure, hospital-associated populations, and mobile vancomycin-resistance determinants are prominent considerations. These are mechanistic and surveillance distinctions, not proof that either organism has a predictable clinical outcome based on genome sequence alone. The review therefore identifies a relative evidence gap: its published study-level comparisons remain richer for selected Gram-negative species than for these two Gram-positive pathogens.

11. Limitations

The narrative approach allows comparison of diverse genomic mechanisms, but study selection may favor well-characterized pathogens, widely cited research, and regions with stronger sequencing capacity. Differences in sampling and reporting also make direct numerical comparisons difficult. These limitations are particularly relevant when interpreting the uneven evidence available across the six pathogens.

Sequence data also leave important biological questions unresolved. Short-read assemblies may not resolve plasmid structure or show whether resistance and virulence determinants occupy the same transferable element. Gene carriage does not guarantee expression, while clinical outcomes depend on infection site, underlying illness, treatment, and exposure history. Stronger conclusions require linked susceptibility, functional, and clinical evidence.

Comparisons are also limited by differences in clinical setting, specimen source, sampling period, antimicrobial susceptibility methods, sequencing platforms, assembly quality, database releases, and gene-calling thresholds. A resistance determinant identified in one species may not carry the same predictive value in another. Likewise, a virulence-associated locus may be conserved among colonizing strains or may require additional regulatory elements to produce a measurable phenotype. The review therefore avoids pooled gene frequencies, cross-species virulence rankings, and causal estimates based on heterogeneous published cohorts.

12. Future research directions

Future genomic investigations should use transparent, preregistered eligibility criteria and preserve accession lists, metadata dictionaries, quality-control exclusions, software versions, and resistance/virulence database releases. Representative, longitudinal collections spanning different hospitals and specimen types would help distinguish local outbreaks from persistent lineage-level patterns.

Investigators should report the denominator for each analysis and avoid treating repeated isolates from the same patient or outbreak as independent observations.

Long-read and hybrid sequencing deserves priority for selected isolates in which resistance and virulence determinants appear to converge, especially when plasmid linkage is central to the research question. Closed assemblies, transfer experiments, transcriptional measurements, and appropriate functional models can test progressively stronger mechanistic hypotheses. Multi-centre surveillance in underrepresented regions, including South Asia, would improve geographic coverage, but claims about regional prevalence should still depend on a defensible sampling frame. Finally, prospective studies should evaluate whether genomic information changes infection-control decisions or improves patient outcomes rather than assuming clinical benefit from gene detection alone.

The most useful studies will combine whole-genome sequencing with matched antimicrobial susceptibility measurements and relevant clinical metadata. Organism- and drug-specific genotype–phenotype concordance should be reported alongside confidence intervals and careful review of discordant cases. Within-species analyses should account for phylogeny, sequence type, year, geography, and specimen source, with sensitivity analyses addressing missing data and enrichment for unusually resistant isolates. These steps can help distinguish true associations from patterns caused by clonal expansion or selective sampling.

Studies vary in sampling frame, sequence quality, AST availability, resistance-gene databases, virulence-locus definitions, and lineage composition. Comparisons should therefore focus on direction and plausibility of mechanisms rather than pooled gene frequencies. Particularly, gene co-carriage does not demonstrate co-expression, physical linkage, or a clinically important phenotype.

The cited literature offers the most detailed isolate-level genomic evidence for K. pneumoniae and A. baumannii. E. coli is supported by genomic surveillance and extraintestinal-pathogenicity literature, but direct paired resistance–virulence analyses are less developed in this review. P. aeruginosa is represented by mechanistic and selected clinical genomic studies. S. aureus and E. faecium are discussed mainly through foundational pathogen biology and therefore remain comparatively underrepresented in the study-level synthesis. This uneven coverage prevents strong cross-species comparisons and should be stated explicitly rather than implying equivalent evidence across all six organisms.

13. Conclusion

The studies discussed here suggest that antimicrobial resistance and bacterial virulence are best understood as related but distinct genomic and biological dimensions. Resistance may arise through acquired enzymes, target changes, altered permeability, and efflux regulation, while virulence-associated loci contribute to colonization, nutrient acquisition, immune evasion, and tissue injury in organism-specific ways. Their co-occurrence can be clinically concerning, particularly in successful hospital-associated lineages, but it is not by itself proof of increased virulence or a direct molecular interaction.

Genomic surveillance is most useful when sequence-derived predictions are interpreted alongside susceptibility testing, sampling information, and relevant clinical evidence. High-quality assemblies, curated annotation, and lineage-aware analysis provide a reliable foundation. Future work should test specific questions about gene linkage, expression, and clinical relevance using integrated genomic, laboratory, and epidemiological data.

Comparisons across *K. pneumoniae*, *E. coli*, *A. baumannii*, *P. aeruginosa*, *S. aureus*, and *E. faecium* underscore the importance of bacterial population structure and methodological context. The published examples considered in this review emphasize different processes, including carbapenemase acquisition, lineage replacement, chromosomal resistance mechanisms, and mobile-element carriage. These differences explain why studies can report apparently conflicting resistance–virulence patterns without necessarily contradicting one another. A single gene-count score or unadjusted cross-species comparison would miss these important differences.

REFERENCES

[1] Murray CJL, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022;399:629–655. doi:10.1016/S0140-6736(21)02724-0.

[2] Partridge SR, Kwong SM, Firth N, Jensen SO. Mobile genetic elements associated with antimicrobial resistance. Clin Microbiol Rev. 2018;31:e00088-17. doi:10.1128/CMR.00088-17.

[3] McArthur AG, et al. The comprehensive antibiotic resistance database. Antimicrob Agents Chemother. 2013;57:3348–3357. doi:10.1128/AAC.00419-13.

[4] Johnson JR, Russo TA. Extraintestinal pathogenic Escherichia coli: the other bad E coli. J Lab Clin Med. 2002;139:155–162.

[5] Russo TA, Marr CM. Hypervirulent Klebsiella pneumoniae. Clin Microbiol Rev. 2019;32:e00001-19. doi:10.1128/CMR.00001-19.

[6] Pitout JDD, Peirano G, Kock MM, Strydom KA, Matsumura Y. The global ascendency of OXA-48-type carbapenemases. Clin Microbiol Rev. 2019;33:e00102-19. doi:10.1128/CMR.00102-19.

[7] Lam MMC, Wick RR, Watts SC, Cerdeira LT, Wyres KL, Holt KE. A genomic surveillance framework and genotyping tool for Klebsiella pneumoniae and its related species complex. Nat Commun. 2021;12:4188. doi:10.1038/s41467-021-24448-3.

[8] Peleg AY, Seifert H, Paterson DL. Acinetobacter baumannii: emergence of a successful pathogen. Clin Microbiol Rev. 2008;21:538–582. doi:10.1128/CMR.00058-07.

[9] Pang Z, Raudonis R, Glick BR, Lin TJ, Cheng Z. Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies. Biotechnol Adv. 2019;37:177–192. doi:10.1016/j.biotechadv.2018.11.013.

[10] Arias CA, Murray BE. The rise of the Enterococcus: beyond vancomycin resistance. Nat Rev Microbiol. 2012;10:266–278. doi:10.1038/nrmicro2761.

[11] Magiorakos AP, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18:268–281. doi:10.1111/j.1469-0691.2011.03570.x.

[12] Feldgarden M, et al. Validating the AMRFinder tool and resistance gene database by using antimicrobial resistance genotype-phenotype correlations in a collection of isolates. Antimicrob Agents Chemother. 2019;63:e00483-19. doi:10.1128/AAC.00483-19.

[13] Alcock BP, et al. CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Res. 2020;48:D517–D525. doi:10.1093/nar/gkz935.

[14] Liu B, Zheng D, Jin Q, Chen L, Yang J. VFDB 2019: a comparative pathogenomic platform with an interactive web interface. Nucleic Acids Res. 2019;47:D687–D692. doi:10.1093/nar/gky1080.

[15] World Health Organization. Global antimicrobial resistance and use surveillance system (GLASS) report 2022. Geneva: WHO; 2022.

[16] World Health Organization. WHO bacterial priority pathogens list, 2024: bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. Geneva: WHO; 2024.

[17] Wyres KL, Holt KE. Klebsiella pneumoniae as a key trafficker of drug resistance genes from environmental to clinically important bacteria. Curr Opin Microbiol. 2018;45:131–139.

[18] David S, Reuter S, Harris SR, et al. Epidemic of carbapenem-resistant Klebsiella pneumoniae in Europe is driven by nosocomial spread. Nat Microbiol. 2019;4:1919–1929.

[19] Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics. 2014;30:2068–2069.

[20] Bankevich A, Nurk S, Antipov D, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 2012;19:455–477.

[21] Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol. 2017;13:e1005595.

[22] Tonkin-Hill G, Lees JA, Bentley SD, Frost SDW, Corander J. Fast hierarchical Bayesian analysis of population structure. Nucleic Acids Res. 2019;47:5539–5549.

[23] Carattoli A, Zankari E, García-Fernández A, et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother. 2014;58:3895–3903.

[24] Page AJ, Cummins CA, Hunt M, et al. Roary: rapid large-scale prokaryote pan genome analysis. Bioinformatics. 2015;31:3691–3693.
[25] Djordjevic SP, Jarocki VM, Seemann T, et al. Genomic surveillance for antimicrobial resistance—a One Health perspective. Nat Rev Genet. 2024;25:142–157. doi:10.1038/s41576-023-00649-y.
[26] Almaghrabi RS, Macori G, Sheridan F, et al. Whole genome sequencing of resistance and virulence genes in multi-drug resistant Pseudomonas aeruginosa. J Infect Public Health. 2024;17:299–307. doi:10.1016/j.jiph.2023.12.012.
[27] Gibbon MJ, Couto N, Cozens K, et al. Convergence and global molecular epidemiology of Klebsiella pneumoniae plasmids harbouring the iuc3 virulence locus: a population genomic analysis. Lancet Microbe. 2026;7:101236. doi:10.1016/j.lanmic.2025.101236.
[28] Fröding I, et al. Persistent spread of carbapenemase-producing Klebsiella pneumoniae in acute care hospitals in 36 European countries (the CCRE survey): a prospective, multicentre, cross-sectional, epidemiological, microbiological, and genomic surveillance study. Lancet Microbe. 2026;7:101320. doi:10.1016/j.lanmic.2025.101320.
[29] David S, et al. Emergence of carbapenemase-producing Escherichia coli in acute care hospitals in 32 European countries (the CCRE survey): a prospective, multicentre, cross-sectional, epidemiological, microbiological, and genomic surveillance study. Lancet Microbe. 2026;7:101321. doi:10.1016/j.lanmic.2025.101321.
[30] D'Aeth JC, et al. Whole-genome sequencing, strain composition, and predicted antimicrobial resistance of Streptococcus pneumoniae causing invasive disease in England in 2017–20: a prospective national surveillance study. Lancet Microbe. 2025;6:101102. doi:10.1016/j.lanmic.2025.101102.
[31] Nurjadi D, et al. Emergence of extensively drug-resistant Pseudomonas aeruginosa ST308 co-producing Klebsiella pneumoniae carbapenemase and New Delhi metallo-β-lactamase in Viet Nam. Lancet Microbe. 2025;6:100958. doi:10.1016/j.lanmic.2024.100958.
[32] Fatima S, et al. Whole-genome sequencing of multidrug resistance Salmonella Typhi clinical strains isolated from Balochistan, Pakistan. Front Public Health. 2023;11:1151805. doi:10.3389/fpubh.2023.1151805.
[33] Mumtaz MN, et al. Whole-genome sequencing of extensively drug-resistant Salmonella enterica serovar Typhi clinical isolates from the Peshawar region of Pakistan. J Infect Public Health. 2024;17:271–282. doi:10.1016/j.jiph.2023.12.002.
[34] Abukhattab S, et al. Whole-genome sequencing for One Health surveillance of antimicrobial resistance in conflict zones: a case study of Salmonella spp. and Campylobacter spp. in the West Bank, Palestine. Appl Environ Microbiol. 2023;89:e00658-23. doi:10.1128/aem.00658-23.
[35] Su B, et al. Antimicrobial resistance analysis and whole-genome sequencing of Salmonella isolates from environmental sewage: Guangzhou City, Guangdong Province, China, 2022–2023. China CDC Wkly. 2024;6:254–260. doi:10.46234/ccdcw2024.050.
[36] Huang X, Yao X, Hou Y, et al. Global trends of antimicrobial resistance and virulence of Klebsiella pneumoniae from different host sources. Commun Med (Lond). 2025;5:383. doi:10.1038/s43856-025-01112-1.
[37] Khawaja T, Mäklin T, Kallonen T, et al. Deep sequencing of Escherichia coli exposes colonisation diversity and impact of antibiotics in Punjab, Pakistan. Nat Commun. 2024;15:5196. doi:10.1038/s41467-024-49591-5.
[38] Abdelsalam NA, ElBanna SA, Mouftah SF, et al. Genomic dynamics of high-risk carbapenem-resistant Klebsiella pneumoniae clones carrying hypervirulence determinants in Egyptian clinical settings. BMC Infect Dis. 2024;24:1193. doi:10.1186/s12879-024-10056-1.
[39] Liu H, Moran RA, Doughty EL, et al. Longitudinal genomics reveals carbapenem-resistant Acinetobacter baumannii population changes with emergence of highly resistant ST164 clone. Nat Commun. 2024;15:9483. doi:10.1038/s41467-024-53817-x.
[40] Binsker U, Käsbohrer A, Hammerl JA. Global colistin use: a review of the emergence of resistant Enterobacterales and the impact on their genetic basis. FEMS Microbiol Rev. 2022;46(1):fuab049. doi:10.1093/femsre/fuab049.

[41] Liu J, Chen D, Peters BM, Li L, Li B, Xu Z, Shirliff ME. Staphylococcal chromosomal cassettes mec (SCCmec): a mobile genetic element in methicillin-resistant Staphylococcus aureus. Microb Pathog. 2016;101:56–67. doi:10.1016/j.micpath.2016.10.028.