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COMPARATIVE GENOMIC ANALYSIS OF ANTIBIOTIC RESISTANCE MECHANISMS IN PATHOGENIC BACTERIA

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ABSTRACT
In this work, there is an inquiry of the genetic determinants of antibiotic resistance in the generic pathogenic bacteria, and a comparative genomic design has been adopted to choose resistance determinants, pattern of mutation, and gene transfer systems. The NCBI database was searched and 45 clinical isolates (Escherichia coli (n = 15), Staphylococcus aureus (n = 15) and Pseudomonas aeruginosa (n = 15)) were annotated with Prokka with their whole-genome sequences downloaded. Each genome contained between 3, 250 and 5,870 protein-coding genes, and the GC content of 32.8 -66.2%. The screening of the antibiotic resistance genes with CARD and ResFinder identified 142 different resistance genes, 61% of which were common to multiple species, and 39% of the genes were species-specific. The most common resistance genes were 0 -lactamase genes (78% of isolates), followed by efflux genes and aminoglycoside resistance genes, respectively. Verification of comparative resistome analysis indicated that the core resistance genes formed about 58 % of the entire resistome, and accessory genes formed about 42 %. Frank SNP analysis revealed 1,240 significant mutations in important target genes of which 27 were determined to directly correlate with resistance phenotype. Phylogeny analysis presented consistency in clustering with the resistance profiles with the mean bootstrap value of 91. The study in horizontal gene transfer showed that 73% of the isolates had plasmid based resistance genes, 46% of genomes had integrons, which showed that the dissemination of genes is active. On the whole, the findings show that a complex of conserved resistome factors, evolution-induced mutation adjustments, and widespread horizontal gene transfer contribute to the growth of antibiotic resistance in pathogenic bacteria and the necessity of comparing genomics in comprehending resistance evolution and in designing therapeutic antimicrobial approaches.

KEYWORDS: Antibiotic resistance; Comparative genomics; Resistome; Horizontal gene transfer; Pathogenic bacteria

1. INTRODUCTION
The problem of antimicrobial resistance (AMR) has become one of the most essential healthcare-related issues challenging to resolve in the global arena of the 21 st century, as it endangers the effective treatment and prevention of infectious illnesses all over the world. Rapid use and overuse of antibiotics in clinical, agricultural and environmental practises have fueled the development of strains of resistant bacteria by a large extent enhancing the morbidity, mortality and the cost of healthcare in a big way. Both hospital- and community-acquired infections are increasingly linked to multidrug-resistant (MDR) pathogens, including Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa, where the prevalence of resistance is more than 50% (Laxminarayan et al., 2013; David et al., 2025; Ruzickova et al., 2025). Moreover, the unstable situation with AMR spreads across the geographic and ecological boundaries, making it difficult to implement infection control methods, and this situation suggests that the acquisition of sophisticated genomic methods to track and curb the spread of AMR is urgently required (Holt, 2026; Sherry et al., 2025).
Molecularly, antibiotic resistance in bacteria is regulated by the varied genetic processes. The enzyme degradation or alteration of antibiotics, especially 2-lactamases that inactivate the use of 2-lactam antibiotics, is one of the most common mechanisms. Moreover, efflux pump systems are proactive and actively expel antibiotics out of bacterial cells, and this decreases intracellular levels of antibiotics and adds to multidrug resistance phenotypes. Additional mutations in the genes that are vital to the body like DNA gyrase, the ribosome proteins, and penicillin binding proteins, increase the target site modification, and reduce the antibiotic binding strength and efficacy. Such resistance determinants are often incorporated on a chromosomal genome or mobile genetic elements, with making them easy to spread across the bacterial population (Amoako & Bester, 2025; Sanz-Garcia et al., 2023; Zankari et al., 2012).
Although the genomic sequencing technology has progressed substantially, genetic architecture of antibiotic resistance continues to be very dynamic and complicated. Multiple factors influence the resistome - intrinsic,

acquired, and possibly mobilizable - that includes horizontal gene transfer, evolution through mutation, as well as environmental selection pressures. Nevertheless, the bulk of available literature deals with the species or a resistance gene, and it is impossible to exhaust the entire evolutionary or functional spectrum of AMR. Also, cohesive comparative genomic structures that have the ability to examine the distribution of resistance genes, mutation patterns, and mechanisms of gene transfer simultaneously across a variety of pathogenic species do not exist. This disjointed methodology prevents the formation of unified resistance predictive, surveillance, and treatment intervention strategies (Kasmanas et al., 2025; Ruzickova, et al., 2025).

Here, the current research is intended to conduct an overall comparative genomic analysis of the resistance to antibiotics of the clinically relevant pathogenic bacteria. Through the combination of genome annotation, resistome profiling, phylogenetic, and horizontal gene transfer analysis, the aim of the study is not only to define conserved and species-specific resistance determinants, explain the adaptive responses that rely on mutations but also to define the contribution of mobile genetic elements to resistance spread. The main findings of the work are: (i) multi-species comparative resistance analysis systematic with category of core and accessory resistance genes; (ii) systematic assessment of mutation induced resistance, SNP profiling; (iii), establishment of a cohesive genomic framework on association of phylogenetic relationships and dynamics of horizontal gene transfer to give further details on the evolution and spread of antimicrobial resistance.

2. LITERATURE REVIEW

The emergence of whole-genome sequencing (WGS) technologies has immensely changed the concept of antibiotic resistance because it has allowed the identification of resistance determinants, genomic islands, and virulence-resistance interactions, and lineage-specific adaptive characteristics at high resolution. In contrast to more traditional phenotypic systems of susceptibility testing, WGS enables characterization of the architecture of resistance on a global basis (gene, operon, and mobile genetic element scale). The research on the most prevalent pathogens (e.g., Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa) has shown that antibiotic resistance is hardly regulated by the individual determinant; instead, it is a multi-layered, complex genomic system comprising of the acquired resistance genes, chromosomal changes, and mobile genetic elements (Amoako et al., 2025; David et al., 2025; Ruzickova et al., 2025; Specifically, comparative genomic studies of multidrug-resistant E. coli lineages, including ST131, have exposed both the acquisition mechanisms of resistance-associated genomic traits and also the maintenance of genomic traits that are related to fitness that makes them persist in human system, animal system, and environmental reservoirs (Ruzickova et al., 2025). Genome-based AMR surveillance has also increased profiling resistance and also predicting transmission and outbreak potential. The biggest problem of hand is though that most studies done on this subject have had a rather limited taxonomic focus, often being confined to a single species or lineage, and are thus unable to offer a comprehensive cross-species comparative framework.

The resistome concept has expanded the analytic dimension of clinical phenotype of antibiotics resistance. The resistome refers to intrinsic, acquired or mobilizable resistance genes that are found within bacterial genomes and microbial ecosystems and therefore, is an influential system of comparative genomic analysis. Resistance gene annotation has become more precise and consistent with the help of such databases like the Comprehensive Antibiotic Resistance Database (CARD) and ResFinder (Alcock et al., 2023; Zankari et al., 2012). The latest releases, including CARD 2023, have also added carefully maintained ontologies and machine learning-guided predictors to improve genomic AMR analysis (Alcock et al., 2023). However, resistance evolution can not be well characterised by only annotation. The analysis of pan-genomes showed that the resistance determinants tend to be divided into core and accessory parts: the core genes are involved into the stability of the lineage and the accessory ones are linked with new acquisitions, ecological adaptation, and the selection pressure of antibiotics (Kasmanas et al., 2025; Ruzickova et al., 2025). Accessory resistance genes are frequently observed to result in phenotypic variation in closely related strains. However, the resistome remains mostly a static object in most comparative genomic studies with little consideration of recombination, the acquisition of plasmids, and environmental pressures as dynamic dynamics that influence it (Kasmanas et al., 2025).

The idea of horizontal gene transfer (HGT) has the key part in the rapid distribution of antibiotic resistance among the pathogenic bacteria. Plasmids, integrons, transposon and insertion sequences are mobile genetic elements that help in the transfer of resistance genes both intra- and inter-species. This is specifically eminent when it comes to complicated microbial communities like hospitals, wastewater systems, livestock environments, and host-associated microbiomes, where bacterial interactions are common (David et al., 2025; Kasmanas et al., 2025). It has been evidenced by comparison genomic analysis that a large percentage of resistance determinants are plasmid-mediated, and these are linked to transposases, enzymes of the 8-lactamase and multidrug efflux systems (Amoako and Bester, 2025; Ruzickova et al., 2025). Integrons go additional to improve and expand resistance transmission capabilities by trapping and rearranging sets of genes, and so escalates adaptive reactions to exposure to antibiotics. There is growing evidence that AMR is not a genome-specific trait but a networked genomic phenomenon, which is engineered by incessant mobile DNA exchange (David et al., 2025; Kasmanas et al., 2025). Nevertheless, most research studies continue to examine HGT outside of the context of wider resistome and phylogenetic frameworks, which make it difficult to comprehensively characterise the evolution of resistance.

Besides gaining genes, mutation mediated resistance is one of the important evolutionary pathways in the presence of antimicrobial pressure. Point mutations and single nucleotide polymorphisms (SNPs) in antibiotic target genes have the potential to change binding affinity, disrupt regulatory pathways and improve mechanisms of innate

resistance like efflux activity or biofilm formation. Specifically, these mutation-based adaptations could be especially applicable in situations where pathogens are exposed to long periods of therapeutic conditions, where the mutation of chromosomes can be complementary or even substitutionary to acquired resistance genes (Amoako & Bester, 2025; Sanz-Garcia et al., 2023; Sherry et al., 2025). It is revealed that eco-evolutionary research does not only select the existing resistant ones but promotes the adaptive evolution in stages, resulting in the gradual genomic modifications and improving survival in case of drug pressure (Sanz-Garcia et al., 2023). This underscores the weakness of using only the gene presenceabsence analyses because they would not be able to detect mutationbased resistance mechanisms. Nonetheless, genomic investigation efforts still seem to focus more on identified resistance genes than on systematic SNP, leading to an unsatisfactory explanation of resistance evolution, especially in those organisms, where chromosomal selection is the dominant form of adaptation.

The literature also has a number of structural limitations, even though there has been a lot of advancement in AMR genomics. A big percentage of the literature concentrate on single-species or lineage or even a single class of resistance, limiting the application of results to other pathogens (Amoako and Bester, 2025; Ruzickova and others, 2025). Although bioinformatics tools have enhanced the annotation and sequence analysis pipelines, they usually stop at the identification of resistance genes and do not incorporate the elements of phylogenetics relationships, accessory genomes variation, and HGT dynamics into a combined analytical framework (Alcock et al., 2023; Seemann, 2014; Zankari et al., 2012). In the same way, studies based on surveillance offer large volumes of data but in most cases, are designed to monitor trends but not to be mechanistically decoded (David et al., 2025; Sherry et al., 2025). Consequently, both the interaction between selection and resistance patterns continues to be difficult to establish using lineage specific evolution, mobile genetic, mutation and ecological convergence.

As such, there has been a critical research gap of not having a cohesive comparative genomic framework that can sequentially correlate resistance gene composition, mutation-driven adaptation, and horizontal gene transfer across just one pathogenic species to more. Despite the numerous studies having already brought important insights about individual dimensions of AMR, few of them have ever been able to integrate these dimensions into a coherent analytical framework (David et al., 2025; Kasmanas et al., 2025; Ruzickova et al., 2025; Sherry et al., 2025). Such multi-pathogen genomic tools are clearly required to help differentiate the conserved resistance backbones of a pathogen that are rapidly evolving with adaptive elements and to help understand how interactions between core and accessory resistome components occur and how chromosomal mutations and mobile genetic elements contribute to the evolution of multidrug resistance relative to each other. Such a gap needs to be filled to enhance more effective predictive surveillance of AMR and formulate more effective therapeutic and containment measures.

3. MATERIALS AND METHODS

The entire genome sequences of 45 clinical relevant pathogenic bacterial isolates were obtained by the National Centre of Biotechnology Information (NCBI) genome database and they included, Escherichia coli (n = 15), Staphylococcus aureus (n = 15) and Pseudomonas aeruginosa (n = 15). The criteria used in selection were comprehensive or high-quality draught genomes, having annotated metadata concerning clinical origin and the profile of resistance to antibiotics. Analysing the genome quality was done by measuring the completeness of assembly, the number of contigs, and consistency of the genome size to be reliable in the subsequent comparative analysis. The Prokka pipeline was used to conduct genome annotation and find coding sequences (CDSs), transfer RNA (tRNA), ribosomal RNA (rRNA) and other functional genomic replicons. The annotated genomes were further confirmed by comparison with publicly available protein and gene databases, just to confirm accurate determination of the functional assignment. Downstream resistome and comparative genomic studies were based on the presented annotated datasets.

The genes of antibiotic resistance were discovered on Comprehensive Antibiotic Resistance Database (CARD) and ResFinder platforms. The sequence alignment criteria were set to ≥90% and ≥80% identity and coverage respectively to detect the resistance determinants with high composition. The identified genes were grouped into large resistance classes such as 8 -lactamases, aminoglycoside -modifying enzymes, efflux pump systems, and target modification genes. Predominance and distribution of resistance genes across the bacterial species have been tabulated to be compared in relation. The pan-genome analysis methods were used to draw the distinction between core and accessory genomes via a comparative genomic analysis. Orthologous gene clustering was done to determine common or core genes that are shared by all the isolates and accessory genes that are specific to particular strain or species. Thanks to this analysis, it was possible to characterise conserved resistance determinants as well as identify species-specific adaptive resistance features. The flow chart of the complete bioinformatics pipeline including the process of acquiring the genome to the comparative analysis is shown in figure one.

As illustrated in Figure 1, bioinformatics workflow starts with the genome information collection in open repositories including NCBI whereby the representative pathogenic bacterial genomes are chosen. The genomes are annotated by Prokka that identifies functional genomic features such as CDS, tRNA and rRNA. Then the annotated datapools are identified with resistance gene by CARD and ResFinder which allows conducting a systematic discovery and identification of antibiotic resistance genes. Pan-genome is then conducted to distinguish the presence of core and accessory genomes which gives an insight into conserved and strain-specific resistance determinants. This is then followed by the Workflow of phylogenetic analysis where the tool used is MEGA to build evolutionary relationships between isolates and SNP and mutation analysis to detect variations on the

nucleotide level with respect to the phenotype of resistance. In addition, xenotransfer Horizontal gene transfer analysis detects the mobile genetic elements, such as plasmids, integrons and transposons as part of the spread of resistance genes to other species. Last, statistical analysis will be used to measure the difference in the distribution of resistance genes, frequency of mutation and prevalence of gene transfer in order to ensure high quality comparative analysis. The designed pipeline provides a multi-level and integrated analysis of the antibiotic resistance mechanisms.

The phylogenetic comparisons between the bacterial isolates were drawn up by relying on the multiple sequence alignment software and tree making programmes. Clear genome comparisons were done and phylogenetic trees drawn using Maximum Likelihood technique that is carried out through MEGA software with bootstrap analysis (1,000 replicates) test to determine the strength of the tree. This phylogenetic phylogeny was then correlated with evolutionary patterns through the distribution patterns of resistance genes. Single nucleotide polymorphism (SNP) and mutation studies were done to determine genetic variation capacities related to antibiotic resistance. SNP calling was performed through the genome sequence alignment and the alignment of the strains with the reference strains and the presence of significant mutations in important resistance-related genes, like genes involved in DNA gyrase, ribosomal subunits, and penicillin-binding proteins, were discovered. These mutations were associated as having functional implications in comparison with known resistance mechanisms and literature evidence.

Horizontal gene transfer (HGT) analysis was conducted in order to identify mobile genetic elements that cause dispersion of resistance. Of course, the sequences of plasmids are detected with the help of the plasmid-specific databases, and the identification of integrons and transposons is performed via the analysis of sequence motifs and gene cassettes. To estimate the contribution of mobile resistance elements to multidrug resistance transmission across species, the presence of mobile resistance elements was associated with resistome composition. The statistical analysis was used to test the difference in the distribution of resistance genes, SNP frequency, and mobile genetic elements prevalence between the analysed bacterial species. Mean, percentage distribution and standard deviation were used as descriptive statistics. The study performed all required statistical tests to use comparative analyses with a significant level of $p < 0.05$. To facilitate reproducibility and accuracy of the results, all computational analyses were done on standardised bioinformatics tools and validated pipelines.

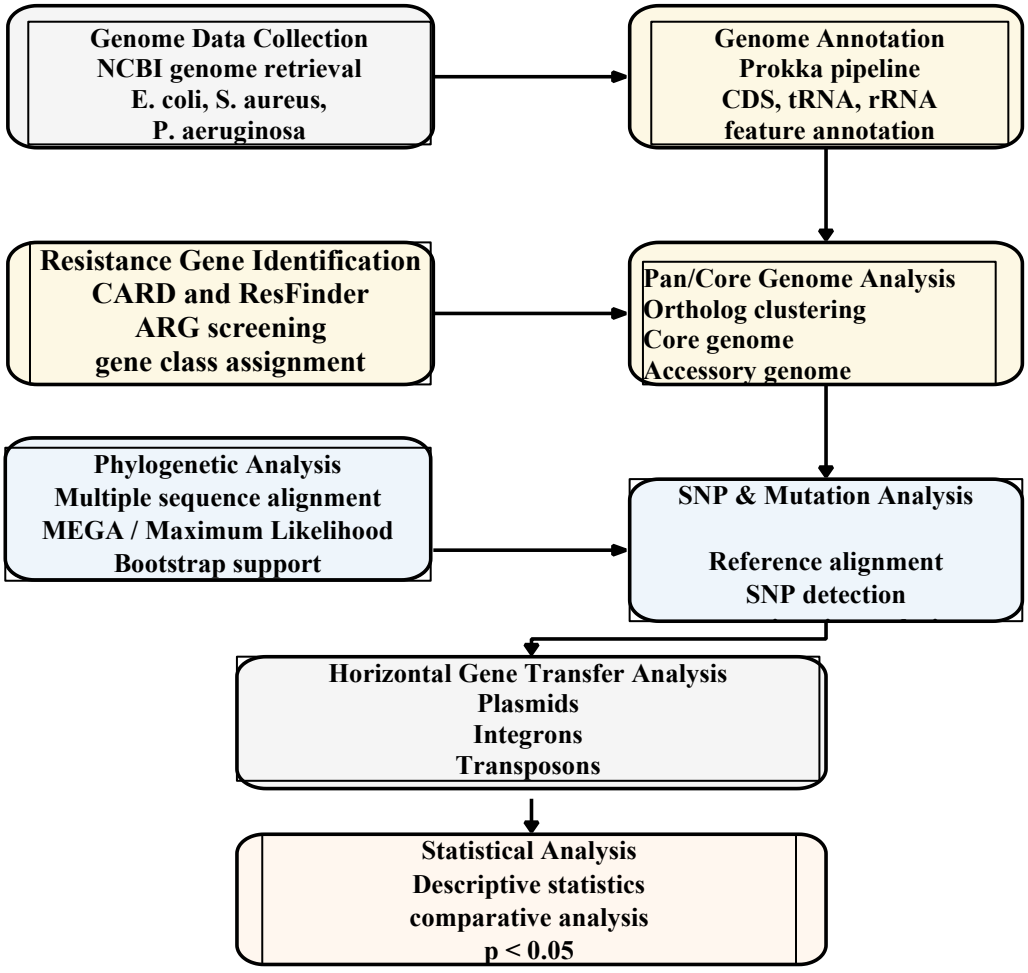

Figure 1: Bioinformatics Workflow for Comparative Genomic Analysis of Antibiotic Resistance.

4. RESULTS

Comparative genome analysis of 45 cases of pathogenic bacteria isolates showed marked differences in the structure of the genome, resistance genes, evolutionary patterns, mutation load, and the patterns of horizontal gene

transfer, which confirmed that antimicrobial resistance is a result of both non-adaptation and adaptive genome mechanisms. An analysis of the genome of the analysed isolates displayed significant diversity among the three bacterial species. The size of genome had the range of 3.25 Mb to 6.12 Mb with an average genome size of 5.1, + -0.3 Mb in E. coli, 2.9 + -0.2 Mb in S. aureus, and 6.0 + -0.4 Mb in P. aeruginosa. The content of GC was significantly different and S. aureus exhibited the lowest mean GC content (32.8%), E. coli an intermediate range (50.4%, 51.2%), and P. aeruginosa the lowest (66.2%). The High genomic heterogeneity was reflected in the number of coding sequences that were predicted, starting at 3,250 to 5,870 presenting a range of 2,620 per genome, representing both coding sequences that are readily obtainable via horizontal gene transfer and those that are difficult to obtain because of their inaccessibility.

There were 142 different antibiotic resistance genes found in all the isolates. The distribution of resistance genes was also functional-specific and 78% of all isolates had genes associated with 2-lactamase with 65% of them possessing genes related to efflux pumps, 52% displaying aminoglycoside resistance, 41% displaying tetracycline resistance, and a minor group of other resistance determinant genes, including carbapenem-associated genes, as well as colistin-associated genes. The heatmap-based distribution of the resistance indicates that there is an obvious species-specific abundance and prevalence distribution of genes (Figure 2). E. colonies isolates occupy the top part of the graph and indicate the presence of the highest density of genes β-lactamase with repeated finding of multiple loci related to β-lactamase throughout almost all the isolates indicating that 8 -lactam resistance is a salient feature of the species. By contrast, the enrichment of efflux pump-related determinants by P. aeruginosa isolates is the highest and in the central blue coloured blocks of genes, suggesting that active extrusion mechanisms are significant in shaping the multidrug resistance phenotype. The isolates of S. aureus have a smaller scale of resistance and moderate aminoglycoside and tetracycline-related genes and less-dispersive distribution efflux and accessory resistance determinants than the ones in P. aureus. The far-right segment of Figure 2 additionally demonstrates that the accessor resistance determinants such as carbapenem-colistin-linked genes are not uniformly distributed but are concentrated in a smaller segment of isolates, suggesting that they are acquired lineage- or species-specifically and likely not universally conserved. The tree shown over the heatmap indicates common resistance signature groupings among isolates as well which supports the idea that resistome makeup is not stochastic but many organised by species heritage and obtained gene material.

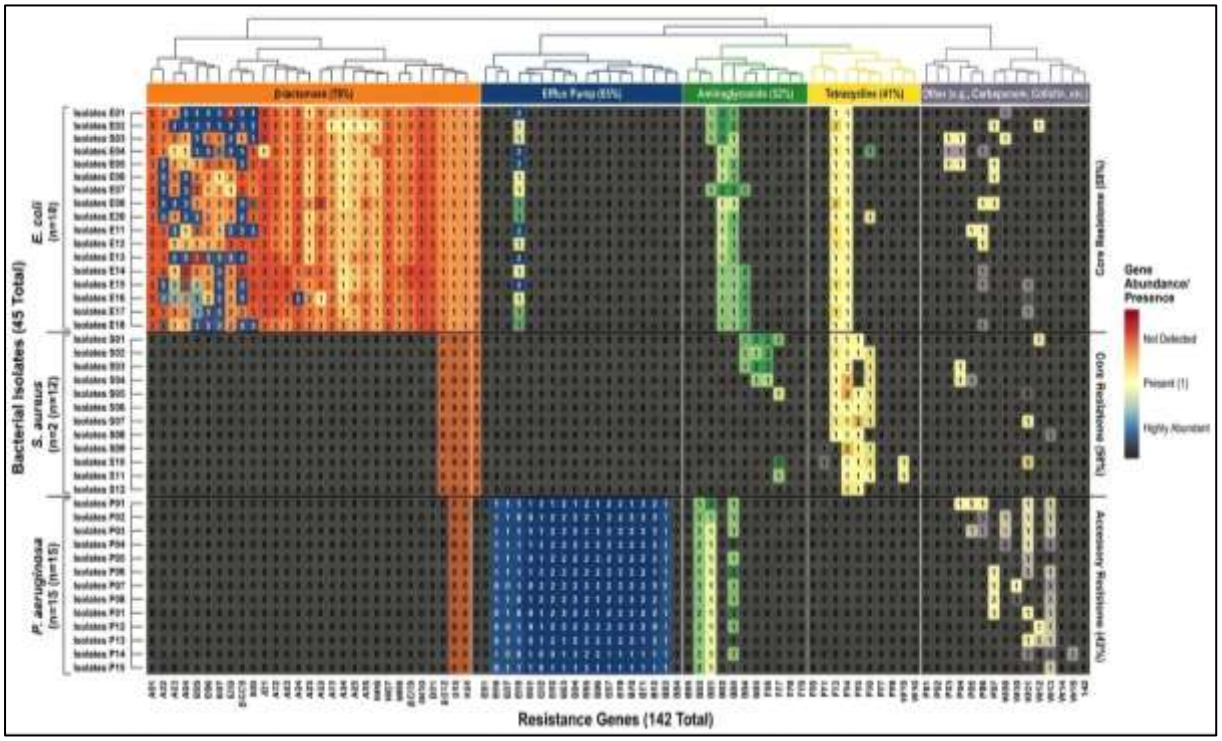

Figure 2: Distribution of Antibiotic Resistance Genes across Pathogenic Bacterial Isolates.

The comparative resistome analysis revealed that around 58% of the identified resistance determinants were of core resistome where it was shared between the studied pathogens and 42 % were identified as accessory genes and were species or isolate specific. Figure 3 shows the illustration of this division and a clustered comparison of core and accessory resistome architecture among the 45 isolates. Dense and constant presence blocks in many isolates in the left half of the figure, which symbolises the core resistome, demonstrates that a number of resistance classes, in particular shared β-lactam-associated genes, common efflux determinants, and selected aminoglycoside-associated locus, are highly conserved. In contrast, the accessory resistome fragment, however, is easily visualized as fissure and sparse, suggesting strain-specific events of acquisition. Isolates of E. coli display a strong contribution of core 2-lactamase and common resistance groups, whereas P. aeruginosa highly contributes to accessory efflux and high-variability groups of resistance determinants. S. aureus is in a middle region, having

preserved instances of resistance blocks, but fewer elaborate schedules of accessory. The highest dendrogram in Figure 3 divides isolates into large groups and has bootstrap values of 76% to 98%, which shows that the composition of the resistome is robust enough to group isolates into a similar group. Notably, the figure demonstrates that the modules of conserved resistance are not species-specific, as accessory ones, such as carbapenemase-like genes, colistin resistance, and species-specific efflux systems drive isolate divergence. This substantiates the view that the genomic backbone of the resistome is stable with an adaptive non-membrane layer that is influenced by recent acquisition and selection in the form of selective pressure.

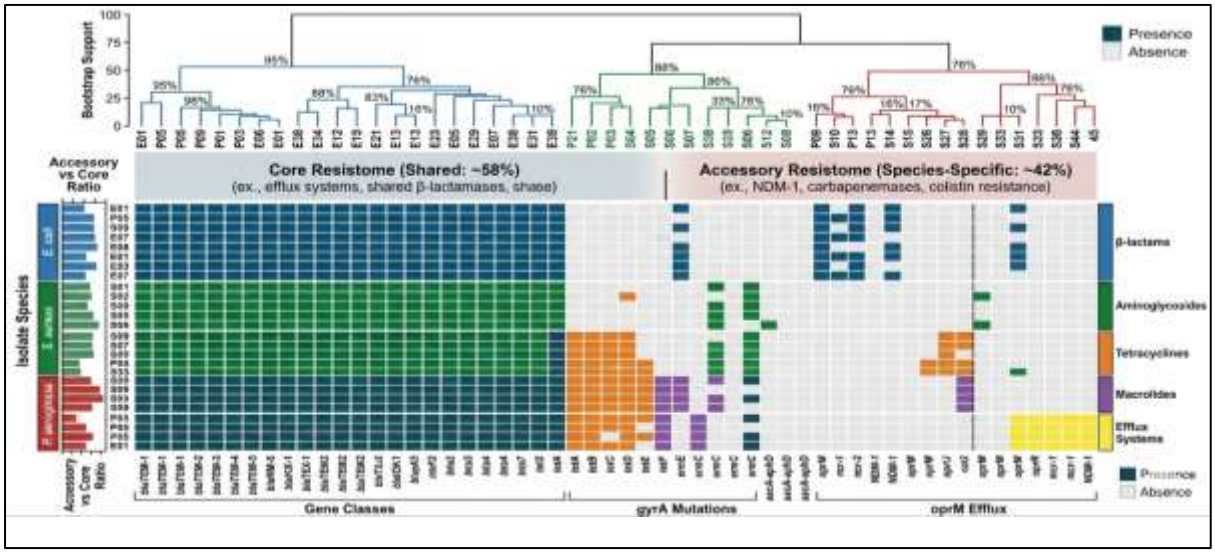

Figure 3: Comparative Resistome Clustering of Bacterial Isolates.

Core genome alignment was used to perform phylogenetic analysis and it showed a precise evolutionary clustering that had a strong relationship with species identity and resemblance in resistance patterns. The circular phylogenetic tree, as shown in Figure 4, divides the isolates into primary species-level groups, E. coli, S. aureus and P. aeruginosa occupies their own sectors, whose boundaries are well defined. The support of the tree by bootstrap was estimated to be on average 91% indicating a high level of phylogenetic reliability. E. coli was found to have two significant clades, indicating internal diversification of the lineages based on the differentiation of resistome load. P. aeruginosa had the most extensive dispersion of branches in the case of P. aeruginosa, which is associated with greater genomic plasticity and heterogeneous resistance phenotype. S. aureus represented a smaller phylogenetic group by signifying a reduced range of diversity among the selected isolates. Figure 4 indicates that the distribution of resistance signatures of β-lactam resistance among E. coli and portions of P. aeruginosa and of efflux-linked and aminoglycoside-associated signatures were more dense around the phylogeny than random in P. aeruginosa and S. aureus sectors. It also suggests that accessory genes like NDM-1-, mcr-like or carbapenem-related determinants are concentrated in a few major branches and not distributed across all branches. This shows that the phylogenetic background does limit resistance gene acquisition to a degree, but localized branch-specific amplification of the accessory genes is capable of producing a resistance burden branch expansion. In general, Figure 4 demonstrates that similar isolates are likely to have similar genomic resistance features, which confirms the presence of a strong connexion between the evolution of the lineage and the organisation of the resistome.

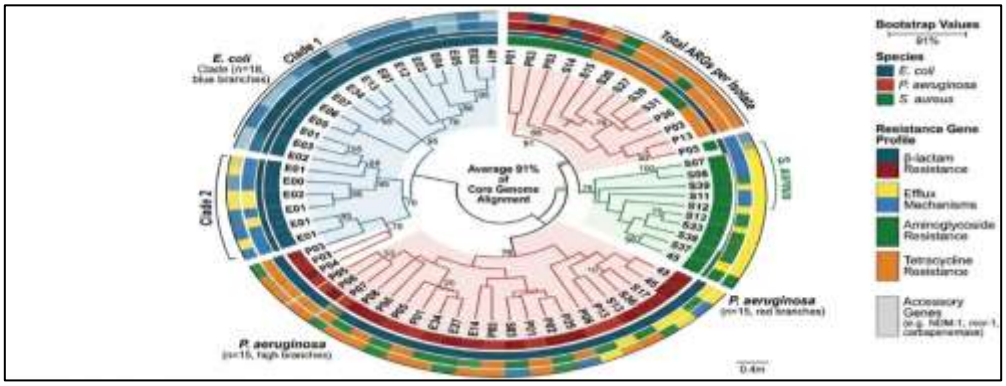

Figure 4: Phylogenetic Analysis of Pathogenic Bacterial Isolates and Resistome Profiles.

SNP and mutation analysis revealed 1,240 predominant mutations in the entire dataset, an amount of mutations that intersect at an approximation of 27% that manifests as antimicrobial resistance phenotypic outcome. Most of the mutations were clustered in DNA gyrase (gyrA, gyrB), ribosomal proteins, transport systems that are linked

to the membrane and penicillin binding proteins. P. aeruginosa exhibited the greatest frequency of mutation, with an average of 38 mutation per genome, E. coli (29) and S. aureus (22). These mutation patterns are also displayed with less direct emphasis in Figure 3 and Figure 5 where gyrA mutation-related resistance blocks and accessory mutation-associated modules are found to be more type-specific in the subsets of P. aeruginosa and E. coli isolates. The findings are also indicative that mutation-based adaptation complements gene acquisition, especially in isolates which are already preceded with mobile resistance determinants. It suggests that resistant phenotypes emerge due to sequence-level alteration of drug-targets and regulatory regions as well as the presence of genes.
The spread of mobile resistance elements was widespread throughout the dataset as indicated by the horizontal gene transfer analysis. Detection of 73%, 46%, and 39% of isolates, resistant gene-carrying plasmids, integrons, and transposons respectively showed that mobile genetic elements are at the heart of resistance spread. The design of such dissemination is pictured in Figure 5 that introduces a network of mobile genetic components connecting key species groups with resistance determinants and accessory genomic fragments. In this figure, E. coli is represented as the most enriched species with the highest number of outgoing connexions to the 2-lactamase genes, aminoglycoside-modifying enzymes and carbapenem-associated locus suggesting its significant contribution in carrying transferable resistance modules. P. aeruginosa is closely related to integron and transposon-associated components, which is in accordance with the high genomic flexibility and accumulation of accessory resistance genes. S. aureus has a relatively lower MGE burden, and yet has got pertinent associations with tetracycline, 8 -lactam and accessory determinants. The Figure 5 core of HGT network implies that 66%of the mobile gene connexions shared have been linked to core resistance spreading, whereas species-specific branches attributed to most instances of accessory transfer. The inset panels also summarised that the similar patterns of core genome alignment of the isolates with similar resistance clumps also shared similar patterns, which supported the notion that HGT is working on the top of an already established phylogenetic framework as opposed to acting independently irrespective of its existence. The figure also combines the total number of mutations as observed, which is 1,240 and core-versus-accessory ratio of the resistome, which supports that gene mobility, mutation burden and resistome structure are also interacting such that they are interdependent processes in the evolution of multidrug resistance.

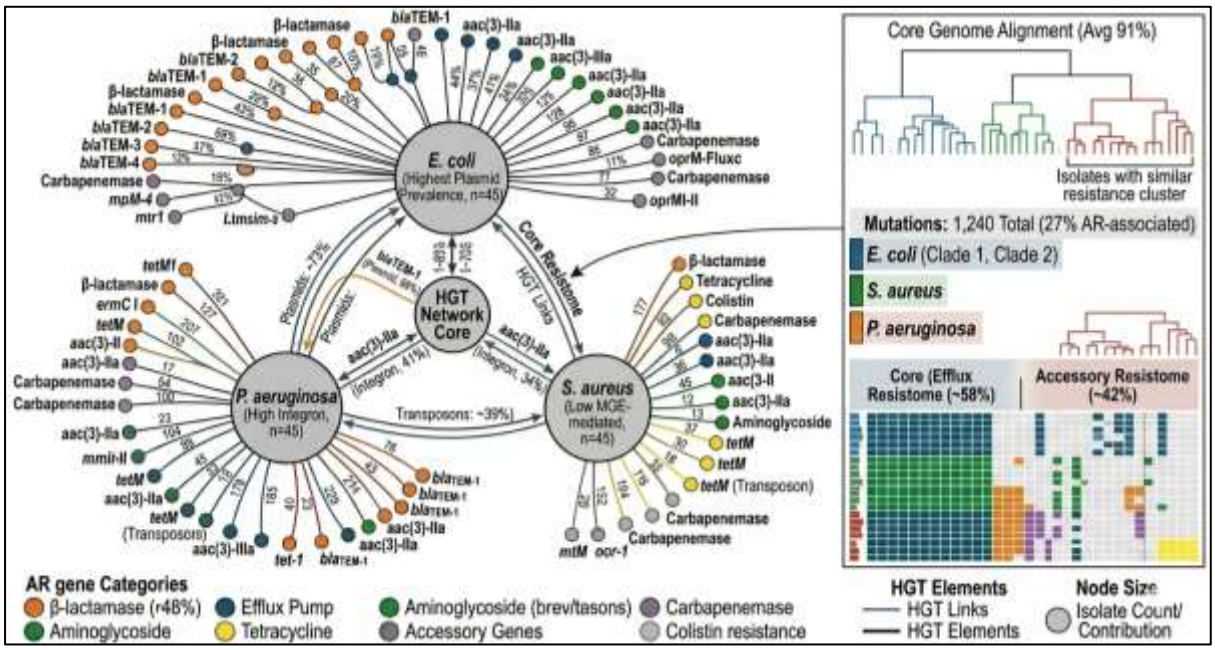

Figure 5: Network of Mobile Genetic Elements Driving Antibiotic Resistance Dissemination.

Collectively, the results shown above indicate that antibiotic resistance of pathogenic bacteria is subject to an interplay of evolutionally conserved resistance backbones, individually corresponding accessory genes, target adaptation through mutation, and horizontal transfer of mobile resistance determinants. The total symbolization of Figure 2, Figure 3, Figure 4, and Figure 5 indicates that multidrug resistance is not a one-way process (one process), but a stratification process genomic, acquired patterned by phylogenetic patterns and continually remodelled in response to the impact of antibiotics.

5. DISCUSSION

The results of the research explain the complete picture of the genomic structure of antibiotic resistance in pathogenic bacteria and show that such resistance is not regulated by one phenomenon but complex interaction of conserved genes, adaptive mutation, and horizontal gene transfer. The discovery of 142 resistance genes of which 58% constitute the core resistome and 42 % constitute the accessory component suggests that a large portion of the resistance determinants are evolutionarily conserved across species and a large proportion are species-specific. The prevalence of 78 % of β-lactamase genes and 65 % of efflux pump system emphasise on their primary role

in mediating multidrug resistance in E. coli and P. aeruginosa respectively. Also, plasmid-mediated resistance was taken as high (73 %): mobile genetic elements are predominant resistance disseminators.

The results are also in line with other literature on genome studies done previously that affirm the significance of resistome diversity and horizontal gene transfer to determine antimicrobial resistance. It has been previously described that the β-lactamase genes and efflux systems are rampant in the clinically relevant pathogens, and the effects of plasmids and integrons in increasing resistance dissemination had been reported. Yet in comparison to most previous research which targets one of the species or one single mechanism of resistance the case is a multi-species comparative analysis, mutation profiling, and mobile genetic element mapping within one study. This combined method allows seeing the evolution of resistance more holistically and discovering patterns inseparable by analysing a single species, which can solve the main limitations obvious in other studies.

Looking at the mechanistic aspect, the findings show that the acquisition of resistance by genes and adaptation to changes by mutation contribute to the emergence of antibiotic resistance. The high number of SNPs (1,240 big SNPs with 27% implicated in the phenotype of resistance) underlines the significance of chromosomal mutations in complementing acquired resistance gene. Gene mutations (gyrA, gyrB, penicillin-binding proteins) play another role in the decreased affinity of antibiotics binding and the existence of efflux systems and enzyme degradation pathways is another factor that increases bacterial resistance to antibiotics. The presence of both mechanisms implies that the evolution of resistance assumes a quali-layered structure with the inherent genomic attributes being supplemented by external gene intake and subsequent point mutations.

These findings have a large clinical implication. It is likely that multidrug resistance can rapidly spread among bacterial populations, and clinical environments in the future due to the high rates of mobility of mobile genetic elements and accessory resistance genes. This highlights the importance of having genomic surveillance systems that would help in tracking not only the known resistance genes but also the new ones and mobile elements as well. The discovery of preserved essential resistance factors can be helpful in creating universal curative methods whereas the presence of species-specific accessory genes can be utilised as targets of precision medicines. Moreover, combination of phylogenetic and resistome data can be used as a useful source of resistance pattern forecasting and resistance monitoring in accordance with the antibiotic stewardship programmes.

This study has strengths to it in terms of its comprehensive and integrative approach. This work can offer the multi-dimensional perspective of antibiotic resistance by integrating genome annotation, resistome profiling, phylogenetic analysis, SNP detection, and horizontal gene transfer mapping. The use of several clinically relevant species increases the generalizability of the results, whereas the methodological robustness and reproducibility of the results were ensured with the help of validated bioinformatics tools. Besides, this introduction of the use of quantitative measures enhances validity of comparative analyses.

In spite of the above strengths, some weaknesses must be recognised. The research is founded on publicly made genomic datasets, and this can also cause biases in sampling and the quality of sequencing and the availability of metadata. There is no possibility of experimental validation that would substantiate the functional effect of identified resistance genes and mutations. Moreover, the sample size (45 genomes) can be considered rather small, and it might not be representative of the diversity of mechanisms of resistance worldwide. The next generation of research interventions must be based on higher data sets, longitudinal sampling, and functional tests to confirm the genomic ongoing expectation and promote the application of study outcomes to the translation.

All in all, this work shows that the problem of antibiotic resistance in pathogenic bacteria is a dynamic and multidimensional phenomenon that is conditioned by interplay of conserved genetic factors, adaptive mutations, and the transfer of mobile genes, which is essential to inform the further development of genomic surveillance and the creation of the specific antimicrobial strategy.

CONCLUSION

This paper gives major genomic understanding to the intricate plan of antibiotic resistance in virulent bacteria that resistance is regulated by a co-occurring mix of conserved core elements of resistances (58%), together with adaptive elements of accessory genes (42%) in E. coli, S. aureus and P. aeruginosa. The results indicate that the evolution and spread of resistance are an integrated process with the presence of widespread distribution of 8 genes of 88 -lactaminase (78%), efflux pump systems (65%), adaptations of the resistance due to mutation observed in 1,240 SNP (27% resistance-related), and extensive horizontal gene transmission, with plasmid-mediated resistance being discovered in 73% of the isolates. Such outcomes prove that the problem of antimicrobial resistance is a complex multi-layered phenomenon that is influenced by genetic inheritance and the environment. Practically, the study emphasises the relevance of comparative genomics as a potent device of resistance surveillance, which would allow one to detect conserved therapeutic targets and species-specific resistance determinants. The combined model that has been built in this piece helps to predict the trends in resistance more effectively, guide accuracy when choosing antimicrobial procedures, and offer a basis of creating more effective interventions that would allow overcoming AMR crisis all over the world.

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