International Journal for Research in Biology and Pharmacy

GREEN PUBLICATION

ISSN (Online): 2208-2093
Volume10 Issue 02 June 2024
Available on-line at: https://ijrbp.com/index.php/bp/issue/view/551

DOI:*https://doi.org/10.69980/bp.v10i2.2487* *Received: 12-Mar-2024 Accepted:15-May-2024*

GENOMIC SURVEILLANCE OF ANTIMICROBIAL RESISTANCE GENES AND RESISTANCE CLASS DISTRIBUTION IN BACTERIAL ISOLATES

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Abstract
Background: *Antimicrobial resistance (AMR) is a major global health challenge that threatens the effectiveness of antibiotics used to treat bacterial infections. The increasing prevalence of resistant pathogens highlights the need for genomic surveillance to understand the distribution and evolution of AMR genes in bacterial populations.*
Methods: *Genomic records of bacterial isolates were analysed to evaluate AMR gene distribution and resistance class diversity. 50 bacterial genomes were examined for genomic characteristics, including genome length and GC content. Resistance genes were identified and categorised into AMR classes. Resistance burden was assessed using total AMR gene counts and resistance class diversity, supported by descriptive statistical analysis and graphical visualisation.*
Results: *The analysis revealed widespread occurrence of AMR genes across bacterial genomes, with variation in resistance gene counts and resistance class diversity among isolates. Genomes contained multiple resistance determinants linked to different antimicrobial classes, suggesting potential multidrug resistance. Genomes with larger genetic content tended to contain higher numbers of resistance genes.*
Conclusion: *The findings demonstrate that bacterial genomes harbour diverse resistance determinants and multiple resistance classes, emphasising the importance of genomic surveillance in monitoring resistance evolution and supporting strategies to control the global spread of AMR.*

Keywords: *Genomic surveillance; Antimicrobial resistance genes; Bacterial genomes; Multidrug resistance; Resistome analysis*

Introduction

Antimicrobial resistance (AMR) is one of the major global public health issues that poses a threat to the efficacy of antibiotics in the treatment of bacterial infections (Laxminarayan et al., 2013). Antibiotics have played a major role in reducing morbidity and mortality from infectious diseases, but their widespread and inappropriate use in medicine, agricultural practices, and environmental conservation has accelerated the emergence and spread of strains of resistant bacteria. As resistance increases, treatment options have become limited, treatment failures become more frequent, and cost healthcare systems, creating significant challenges to global health systems (Martínez, 2008). The appearance of resistant pathogens at such a quick pace illustrates the urgent need for better surveillance systems and a better understanding of the genetic mechanisms underlying AMR (Torres-Caycedo et al., 2018).

Antibiotic resistance is not a modern phenomenon, but a natural process of evolution that has existed within the community of microorganisms for millions of years. Microorganisms are known to naturally produce antimicrobial compounds as part of ecological competition, and other microbes develop mechanisms that allow them to survive exposure to antimicrobial compounds. As a result, environmental microbial populations are abundant in a wide range of resistance genes that are the source of AMR genes or AMR determinants. These genes can then be found later on pathogenic bacteria through genetic exchange mechanisms, contributing to the resistance trait dissemination (Martinez, 2008; Allen et al., 2010). Findings recovered from ancient bacterial DNA have proven that resistance genes were around before the clinical use of antibiotics, confirming the deep evolutionary origin of the AMR mechanisms (D'Costa et al., 2011).

The whole set of resistance genes found in microbial populations is known as the antibiotic resistome. The resistome comprises inherent resistance mechanisms that are naturally found among species of bacteria, as well as acquired resistance genes that can be transferred between microorganisms. Horizontal gene transfer mechanism such as conjugation, transformation and transduction occurs among bacteria, allowing them to share genetic material and to quickly develop new resistance characteristics. Through these processes, resistance genes are able to be transmitted between bacterial populations as well as between different ecological environments. Environmental microorganisms constitute an important reservoir of resistance determinants that may eventually be expressed in clinically important pathogens (Aminov, 2009; Muteeb et al., 2023). Being able to comprehend the diversity and distribution of the resistome is crucial in order to identify emerging resistance mechanisms and to target the development of new antimicrobial agents (Hobson et al., 2021).

Advances in genomic technologies have been instrumental in greatly enhancing the capacity to study AMR at the molecular level. Whole-genome sequencing (WGS) is a method to thoroughly analyse the genomes of bacteria and to identify the resistance genes to different classes of antimicrobials. Genomic epidemiology approaches offer high-resolution information that can be used to track emerging, transmitting and evolving resistant pathogens both in clinical and environmental settings (Didelot et al., 2017). In clinical microbiology, a genome-based workflow has also been created to predict the pattern of antimicrobial susceptibility directly from the genomic data of the bacteria, which largely enhances the efficiency of the diagnosis and aids in more informed decision-making for the treatment (Cunningham et al., 2020; Tagini et al., 2021).

The explosion in the quantity of genomic data has resulted in the creation of bioinformatics tools and custom databases to aid in the identification and analysis of AMR genes. Databases like the Comprehensive Antibiotic Resistance Database (CARD) gather and curate collections of sequences of resistance genes and their corresponding mechanisms of resistance, which allow one to systematically analyse the resistome in bacterial genomes (Alcock et al., 2020). Bioinformatics-driven analysis has become an integral part of AMR research due to the fact that it offers the possibility of investigating the diversity of resistance genes on a large scale and the genomic surveillance of resistant pathogens (McArthur & Wright, 2015). In addition, genomic technologies are involved in the evolution of better antimicrobial susceptibility testing systems and surveillance strategies to improve the detection and monitoring of resistant bacteria (van Belkum et al., 2019).

Given the rising prevalence of AMR worldwide, genomic surveillance has become an important approach to understanding the dynamics of AMR and guiding public health interventions. Integrating genomic data with epidemiological information is useful for total monitoring of the distribution of resistance genes in different bacterial populations, and for early detection of emerging multidrug-resistant pathogens. At the same time, ensuring the sustained and efficient efficacy of antimicrobials in the longer term demands a balance of antimicrobial conservation and continued innovation of antimicrobial drug development (Laxminarayan, 2014). Research on AMR genes and the distribution of resistance classes in bacterial genomes is intrinsically necessary to understand AMR mechanisms and to design strategies focusing on the global control of AMR.

Materials and Methods

Study Design

The aim of the present study is based on the design of the genomic surveillance analysis for the assessment of the antimicrobial resistance (AMR) genes and the diversity of the resistance classes present in the bacterial isolates. The present study is based on the assessment of the genomic characteristics of the bacterial isolates and the presence of antimicrobial resistance genes in the bacterial genomes. The analytical framework has been utilised for the assessment of the genome attributes, the abundance of the antimicrobial resistance genes, and the assessment of the antimicrobial resistance classes in bacterial genomes. Statistical and computational techniques have been utilised for the analysis of the antimicrobial resistance genes in bacterial genomes.

Data Source
Genomic information used in this study was derived from an openly accessible antimicrobial resistance dataset that contained genomic information of bacterial isolates along with associated metadata. This dataset contained information regarding genome characteristics, resistance gene information, and environmental characteristics associated with each bacterial isolate. Genomic information was used to investigate the prevalence of antimicrobial resistance genes and the variety of resistance classes present in bacterial genomes. This dataset provided structured genomic information that helped in evaluating resistance determinants and resistance patterns among bacterial isolates used in the study (Kulkarni, 2024).

Bacterial Isolates and Genomic Records
Bacterial genomic records were used to assess the distribution of AMR factors amongst the isolates. Each genome was a unique individual bacterial isolate and given a unique identifier to keep track of them throughout the whole process of the analysis. A total of 50 bacterial genomes were included to investigate. The genomic records had information describing the genome characteristics, resistance determinants, and biological metadata associated with each isolate. This information from the genome allowed us to evaluate systematically the AMR gene prevalence and diversity among the isolates. The compiled genomic records gave a framework for patterns of resistance gene profile and resistance class distribution between the bacterial genomes analysed in the study.

Genomic Characterization
Genomic characteristics of the bacterial isolates were investigated to provide the structural features of each genome. Genome size was calculated on the basis of total genome length in base pairs as the overall genetic content of each bacterial isolate. GC content was determined as a percentage of guanine and cytosine nucleotides of the genome sequence. These attributes of the genome were assessed in order to determine the variation among the genomes of bacteria and to investigate potential links between genome architecture and AMR gene distribution. Examination of genome length and nucleotide composition also gave insight regarding genomic diversity and structural variation of the bacterial isolates included in the investigation.

Attributes of the Biology and Environment
Biological and environmental characteristics linked to each isolate were taken into account when the analysis was performed. Taxonomic classification was applied to discover the bacterial species represented in the genomic records. Temporal attributes referring to the time of sample acquisition included collection year and collection month. Seasonal classification was obtained to add more temporal context. Information detailing the biological origin of the isolates was also assessed using host category and standardised isolation source variables. These attributes were used to give contextual information about the ecological and biological background of the bacterial isolates, enabling descriptive evaluation of the pattern of AMR in different biological environments and collection periods.

Identification of Antimicrobial Resistance Genes
AMR genes could be found within the bacterial genomes by annotating resistance genes to antimicrobial drugs. Each resistance gene corresponded to a particular genetic element that could confer resistance through such mechanisms as enzymatic inactivation, modification of the target or efflux activity. The existence of these genes in each genome was noted as separate genomic features. Identification of resistance genes made it possible to characterise potential resistance capabilities for each bacterial isolate. Resistance gene numbers found in each different genome were calculated to determine the total resistance gene burden that could be found within individual bacterial genomes included in the study.

Classification of Classes of Antimicrobial Resistance
Resistance genes discovered in bacterial genomes were grouped on the basis of AMR classes depending on the antimicrobial agents against which the encoded resistance mechanisms are active. Each resistance gene was related to a particular drug family; the resistance genes permitted their classification into definite AMR categories. The variety of classes of resistance that are represented in each bacterial genome was assessed to determine the variety of antimicrobial groups targeted by resistance determinants. The total number of resistance classes identified in each isolate was calculated to assess the distribution of the breadth of AMR mechanisms present. This classification has allowed us to compare the resistance profiles of the genomes and has so far started in the bacterial genomes studied.

Assessment of Burden of Resistance
Resistance burden at the level of each bacterial genome was evaluated by a set of quantitative indicators on the abundance and diversity of resistance genes. The total number of AMR genes found in the individual isolate was used as an indicator of AMR gene load. In addition, the number of AMR classes represented within each genome was calculated in order to assess the diversity of the resistance mechanisms. These metrics were included to characterise bacterial isolates that had extensive resistance profiles and potential multidrug resistance characteristics. Quantitative evaluation of resistance burden offered a structured way to compare the AMR intensity and diversity among the bacteria in the isolates included in the AMR investigation.

Statistical Analysis

The statistical evaluation was performed to summarise genomic characteristics and AMR gene distribution among bacterial isolates. Descriptive statistical measures were used to analyse the frequency and the distribution of resistance genes and resistance classes. Comparative evaluation was done to study the variation in the numbers of resistance genes and resistance class diversity among isolates. Correlation-based evaluation was performed to investigate possible correlations between genomic attributes, such as genome length and GC content and resistance gene abundance. These analytical procedures permitted systematic interpretation of AMR patterns and aided in the identification of major AMR trends of bacterial genomes analysed in the study.

Visualisation of Resistance Patterns

Graphical visualisation techniques were applied to ease the interpretation of AMR gene distribution in the bacterial isolates. Heatmap representations were built, which showed the occurrence and variation of resistance genes between genomes. Bar charts were created to show the frequency of AMR classes detected between isolates. Additional graphical plots were created to describe variation in the number of resistance genes and resistance classes detected in each genome. These visual representations aided in the comparative assessment of resistance profiles and allowed AMR profiles of the bacterial isolates that were investigated in the study to be clearly depicted.

Computational Analysis Framework

Computational analysis was done by means of bioinformatics tools and statistical tools designed for genomic data processing and analysis. Structured analytical setup. Distributed genome attributes and resistance gene annotations were grouped into structured analytical formats to aid in the systematic processing of data. Computational workflows were in place to compute genomic metrics, resistance gene abundance quantification & resistance determinants categorisation as AML of different classes. Analytical procedures were also used to generate graphical visualisations to show patterns of resistance. Structured computational scripts were adopted to ensure a consistent approach to processing the genomic information and a reproducible approach to the AMR gene distribution analysis of the bacterial genomes evaluated in the current study.

Results

Genomic Characteristics of Bacterial Isolates

Genomic characterisation of the bacterial isolates showed variation in the size of the genomes and variation in the composition of nucleotides analysed in the genomes. Genome length demonstrated a measurable difference in genetic design across isolates, suggesting diversity in the genomic architecture. As well as displaying diversity, GC content differed among genomes, showing differences in the composition of nucleotides between bacterial isolates. These variations identify heterogeneity in the genome of the bacterial population under analysis. Despite these differences, all the genomes had recognisable AMR determinants. The detected genomic diversity offered an important framework to look at the distribution of resistance genes and assess how genome structural characteristics may affect the existence and accumulation of AMR genes in bacterial isolates. The genomic features of the analysed bacterial isolates, such as genome length and GC content, are summarised in Table 1.

Table 1: Genomic characteristics of bacterial isolates analysed in the study

Metric	Value
Number of isolates	50
Mean genome length (bp)	5,131,428.32
Minimum genome length (bp)	4,688,502
Maximum genome length (bp)	5,516,786
Mean GC content (%)	50.72
Minimum GC content (%)	50.52
Maximum GC content (%)	50.89

Note: bp: base pairs; GC: guanine–cytosine nucleotide content; GC content (%): percentage of guanine and cytosine nucleotides present in the bacterial genome.

Distribution of Antimicrobial Resistance Genes

Analysis of AMR determinants showed the existence of a few resistance genes distributed throughout the bacterial genomes. A few of the isolates possessed more than one resistance gene, implying the concurrence of a number of resistance mechanisms in individual genomes. The number of resistance genes differed between isolates, showing variation in resistance gene burden. Certain of the isolates had more resistance genes than others, suggesting greater potential for resistance. The overall distribution pattern revealed that AMR determinants were widely present for bacterial isolates that were tested. These results show that resistance genes are widely distributed and could be involved in the adaptive ability of bacterial populations. The difference in AMR gene abundance between the bacterial isolates is shown in Figure 1.

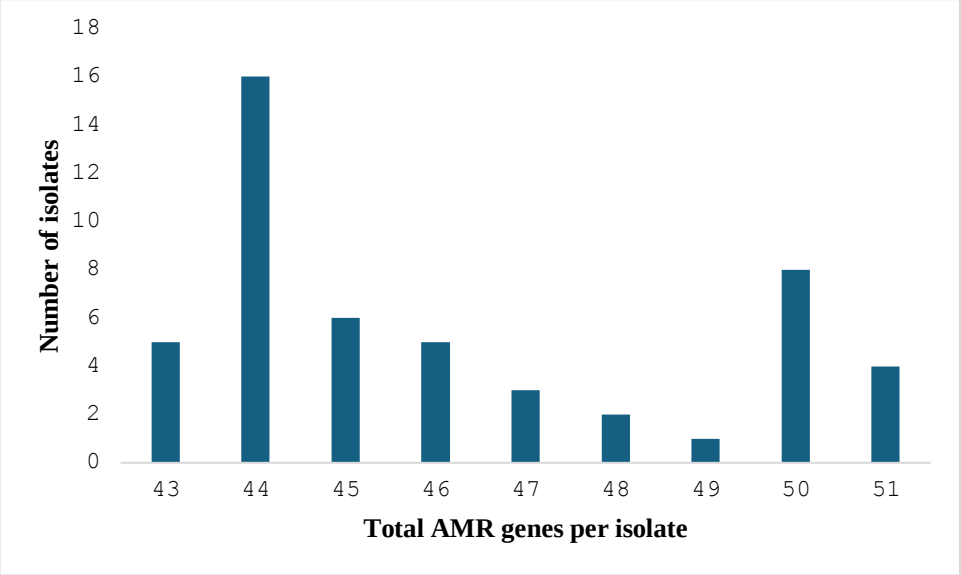

Figure 1: Distribution of AMR gene counts across bacterial isolates

Distribution of Resistance Classes

Resistance genes found among bacterial genomes were linked to AMR classes. The analysis showed that some isolates had resistance determinants against different antimicrobial categories. Some variation was detected in the number of resistance classes detected within individual genomes. A few bacterial isolates showed resistance towards a few classes of antimicrobials, while others showed resistance determinants related to multiple drug categories. This variation reflects variation in the mechanism through which resistance occurs between isolates. The existence of different classes of resistance in individual genomes suggests the possibility of broad-spectrum resistance and raises awareness of the complexity of AMR patterns of bacterial isolates. The prevalence of the most common AMR genes, detected in the bacterial genomes, is shown in Table 2.

Table 2: Most frequently detected AMR genes among bacterial isolates

AMR Gene	Frequency among isolates
CRP	50
Escherichia coli ampH	50
Escherichia coli ampC	50
Escherichia coli acrA	50
H-NS	50
emrK	50
emrB	50
emrA	50
bacA	50
baeR	50

Note: AMR: antimicrobial resistance; CRP: cAMP receptor protein involved in global transcriptional regulation; ampC: beta-lactamase gene conferring resistance to beta-lactam antibiotics; ampH, beta-lactamase-related protein; acrA: component of the AcrAB-TolC multidrug efflux pump; H-NS: histone-like nucleoid structuring protein associated with gene regulation; emrA, emrB, emrK: multidrug efflux transporter proteins; bacA: bacitracin resistance protein; baeR: transcriptional regulator associated with multidrug resistance response.

Multidrug Resistance Patterns

Evaluation of resistance gene abundance and resistance class diversity showed the patterns of multidrug resistance in bacterial isolates. Genomes with more than one resistance gene were frequently found to have resistance determinants linked with more than one antimicrobial class. These isolates showed broadened resistance profiles in comparison with the genomes that were harboring less resistance determinants. The presence of a few resistance mechanisms in the same genome implies the accumulation of genetic elements that provide resistance to different antimicrobial agents. Identification of such isolates suggests the presence of multidrug resistance potential of bacterial genomes, thus underscoring the importance of monitoring the resistance gene diversity and distribution in bacterial populations. The distribution of AMR burden according to the number of resistance genes and the distribution of AMR classes are summarised in Table 3.

Table 3: Distribution of AMR burden among bacterial genomes

Resistance burden category	Number of genomes	Mean AMR genes per genome	Mean resistance classes per genome
Low resistance burden	9	43.3	22.1
Moderate resistance burden	28	45.1	23.2
High resistance burden	13	49.8	24.3

Note: AMR: antimicrobial resistance

Relationship Between Genomic Characteristics and Resistance Burden

Comparative assessment of genomic traits and the number of resistance genes implied the heterogeneity of the resistance burden at the genome level. Differences in genome size and nucleotide composition were associated with variation in the number of AMR genes detected for isolates. Genomes with greater genetic content often had more than one resistance determinant, suggesting that they have a higher potential for harbouring resistance-associated genes. In contrast, some genomes had relatively lower counts of resistance genes for the genomic characteristics. These results show that the accumulation of AMR genes is different among bacterial genomes and may be determined by genomic architecture and genetic acquisition events among bacterial populations. The genome length-AMR gene abundance relationship is shown in Figure 2.

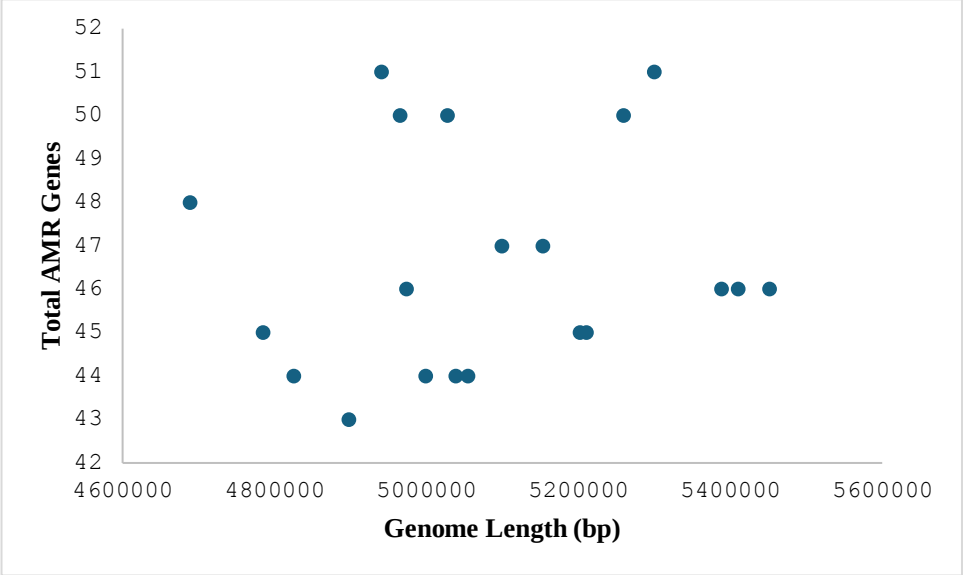

Figure 2: Relationship between genome length and AMR gene burden in bacterial isolates

Temporal and Environmental Distribution of Resistance

Evaluation of temporal and environmental attributes associated with bacterial isolates showed variation of AMR gene occurrence among the collection periods and biological origins. Resistance determinants were found in isolates obtained in various months and seasons, indicating sustained spread of resistance genes over time. Differences in the prevalence of resistance genes were also found between isolates obtained from different hosts and environmental sources. These observations indicate that AMR determinants are spread across a variety of ecological conditions. The occurrence of resistance genes under different temporal and biological conditions can indicate the ubiquity of AMR in bacterial isolates studied in the current study.

Discussion

The current research focused on the genome traits of bacterial isolates and the evaluation of the distribution of AMR genes and resistance classes among genomes. The results showed the bacterial genomes contained some resistance determinants and various classes of resistance, which demonstrated a high burden of resistance genes between isolates. The presence of resistance genes in a large proportion found in this study emphasises the wide distribution of AMR determinants in the bacterial population. Previous studies have also reported similar results of resistance genes being highly shared between environmental bacteria and human pathogens, subsequent to the interconnected nature of the global antibiotic resistome (Forsberg et al., 2012). The existence of some resistance determinants in individual genomes implies that bacterial populations can readily evolve in response to antimicrobial selection pressure by acquisition and maintenance of resistance genes.

Diversity of the resistance classes identified in bacterial isolates indicates the possible development of multidrug resistance. Genomes with higher numbers of resistance genes in the genes often had determinants linked to multiple antimicrobial classes, indicating the accumulation of multiple resistance mechanisms within individual bacterial genomes. Multidrug-resistant bacterial lineages have been more readily reported in genomic studies of bacterial pathogens. The appearance of lineages is usually linked with genetic adaptation and the spread of resistance determinants among

microbial populations. For example, the rise of multidrug-resistant strains of Streptococcus pneumoniae has been attributed to the global dispersal of multidrug-resistant strains and the escalation of resistance to a variety of antimicrobial agents (Lo et al., 2022). The existence of multiple modes of resistance in the bacterial genomes is a major concern from the point of view of clinical treatment and infection control.

Genomic approaches have become the key to studying the dynamics of AMR. Whole-genome sequencing (WGS) allows for the wide-ranging identification of resistance determinants and more detailed information about the genetic diversity/evolution of the bacteria. Sequencing-based methods have enhanced the capacity to analyse the diversity of resistance genes and examine mechanisms that cause the spread of AMR among bacterial populations (Boolchandani et al. 2019). In addition, genomic analysis can provide high-resolution information that can be used to track transmission of bacteria and investigate outbreaks of resistant pathogens in healthcare settings. Whole-genome sequencing is increasingly being used to analyse outbreaks and provide epidemiological surveillance on resistant bacteria (Quainoo et al., 2017).

Environmental and ecological factors are also important factors in the evolution and spread of AMR. Environmental microbial communities serve as a reservoir of resistance genes, which can be transferred to pathogenic bacteria in the future by horizontal gene transfer. Resistance genes move throughout the environmental, animal and human microbiome environment, forming interconnected networks that promote the spread of resistance (Baquero et al., 2019). Environmental reservoirs make a substantial contribution to the worldwide dissemination of AMR determinants and are potential sources for the emergence of resistant pathogens (Bengtsson-Palme & Larsson, 2015).

Global studies of antimicrobial resistance genes show a broader distribution of resistance genes from across a range of ecological environments. Metagenomic analyses of environmental samples, including the human sewage collected in the cities, have shown the existence of huge reservoirs of resistance genes circulating in microbial populations. These researchers jot down the importance of environmental monitoring in understanding the global distribution of resistance determinants (Hendriksen et al., 2019). Environmental surveillance complements clinical surveillance and can help in the early detection of new emerging threats of resistance.

Genomic surveillance has emerged as a key approach to AMR in public health systems. Genome-based monitoring helps researchers to determine the pathways of transmission of resistant pathogens and assess the spread of multidrug-resistant strains in healthcare settings. For example, genomic surveillance has been applied to quantifying transmission of resistant pathogens like Enterococcus faecium and understanding more about the dynamics of outbreaks in hospitals (Gouliouris et al., 2021). Integrating genomic data with epidemiological information increases the capacity to identify resistant lineages and design blonde intervention to tackle resistant lineages.

The existence of various types of resistance genes throughout the bacterial genomes is also a reflection of the molecular mechanisms by which bacteria can evade the antimicrobial action. Mechanisms such as enzymatic drug inactivation, modification of targets and efflux-mediated resistance help bacteria to survive exposure to antibiotics and persist in different environments (Wright, 2011). Advances in genomic technologies have helped strengthen AMR surveillance across the world through the ability to rapidly detect resistance genes and monitor resistant pathogens. Integration of genomic data with public health surveillance systems has aided in enhancing the ability to trace the spread of microbes that have acquired resistance to medical drugs and understand their distribution across the globe (Baker et al., 2023).

Conclusion

This study focused on the genomic features of bacterial isolates and assessed the distribution of AMR genes and class resistance on genome. The results showed that there are some resistance determinants in bacterial genomes and diverse resistance classes, suggesting that there is a significant burden of resistance genes and possible multidrug resistance among the isolates. The widespread occurrence of resistance genes shows the increasing importance of resistance to antimicrobial drugs and the importance of monitoring the occurrence of resistance determinants in bacterial populations in a continuous manner. Genomic analysis offers useful information about the diversity and distribution of resistance genes and an in-depth examination of resistance mechanisms. Integrating the genomic approaches in AMR surveillance can enhance the identification of new resistant strains and provide an effective infection control approach. Genomic surveillance of resistance genes plays a role in the better understanding of the dynamics of AMR and the development of evidence-based strategies for the management and control of the extent of spread of resistant bacterial pathogens around the globe.

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