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Molecular Characterization Of Antibiotic Resistance Genes In Pathogenic Bacteria Isolated From Patients In Saudi Arabia, Jeddah

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Abstract

Antibiotic resistance is one of the hardest problems in the world that we should tackle. The current study, therefore, targets characterizing the antibiotic resistance properties as well as the transmission gene of bacteria isolates obtained from 95 clinical isolates. The various antibiotics levels such as penicillins (35%), macrolides (15%), tetracyclines (25%), fluoroquinolones (18%), and aminoglycosides (12%) showed a remarkable resistant state against the first line antibiotics. 65% of isolates could display multidrug resistance. The molecular screening revealed these genes encoding the antibiotic resistance - mecA, vanA, blaZ whic[1]h were novel variants; blaM1 and tetK2. among mecA (15 isolates), vanA (8 isolates), and blaZ (6 isolates), the most abundantly found genes were mecA, vanA, and blaZ. The replications of plasmids also showed that most of the resistance genes were on the plasmids and not the chromosomes of the bacterial strains, thereby enhancing the horizontal transfer between invading bacteria. The combined consequences of high antibiotic resistance promoted by the movement of key resistance genes signal fewer alternatives for fluoroquinolones or penicillin commonly used for bacterial infections. Presenting round-the-clock surveillance on top of ongoing supervision and infection control are key elements in combatting the spread of the disease.

Keywords: *Multiple drug resistance, Resistance genes, Plasmid-mediated resistance, and Horizontal gene transfer.*

Introduction

It has been the case of a very big fight between Global public health and antibiotic resistance in recent times. The study expects that years 2050, it could be realized that drug-resistant infections will cause 10 million deaths per year worldwide. The improper and excessive use of antibiotics in humans and animals has been the main reason for the ever-increasing number of microorganisms that are developing resistance to antibiotics (2). Numerous bacterial strains that are pathogenic are gradually becoming immune to overused

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medications and becoming difficult to cure as well as exacerbating mortality and morbidity rates about three times (3).

The genetic base of antibiotic resistance brings light on the topics connected with the treatment of the growing problem of resistant microorganisms. The antibiotic resistance gene and mutation detection, their surrounding genetic contexts, and transmission dynamics provide a possibility of antibiotic-resistant pathogens epidemiology tracking (4,5). Resistance transfer mechanism is another crucial aspect illustrated by the scheme and the pathways through which bacteria can obtain and accumulate resistance traits under antibiotic selection pressure (6). Besides, exploring the structural and functional aspects of antibiotic resistance at the molecular level through molecular assays increases the chances of finding new antimicrobial agents or therapeutic approaches to overcome resistance (7, 8).

According to the numerous research conducted recently on antibiotic resistance, we realize that the molecular and genetic profiles of resistance remain less defined in many clinically relevant human pathogens. While resistance mechanisms for some frequently recognized resistance traits in staphylococci, enterococci, and enteric gram-negatives have been intensely explored (9–11), knowledge of resistance mechanisms for some other pathogens such as P. aeruginosa is much less widespread. Nonstop examination and specific analysis of resistance patterns in the emergence era are fundamental since bacteria have a diverse and huge repertoire of using genes to develop new resistance mechanisms (12).

The purpose of this work was to hope that it can improve knowledge on molecular aspects of antibiotic resistance in bacterial pathogens which are the cause of infections in humans. The specific objectives were to: The specific objectives were to:

i) The phenomenon of antibiotic resistance is growing in the medical world, making it imperative to determine and identify pathogenic strains of resistant bacteria from clinical samples.
ii) it (the project) is screened for resistance profiles against first-line antibiotics prescribed in different medical settings.
iii) the pathogens isolated will be screened for major antibiotic resistance genes using molecular techniques.
iv) describe DNA sequences that belong to resistance genes detected via sequencing.
vi) carry out characterization of the selected isolates to determine the horizontal transfer of resistance genes by plasmid analysis and strain typing.

Materials and Methods

Bacterial Isolates

The bacteria strains were cultured from wound swabs, blood cultures, and other clinical specimens that were collected in the Hospital [KAMCJ] as part of routine culture and sensitivity testing, performed within the 6 months between January and June 2022.

Source of Isolates

The specimens were collected from inpatients and outpatient’s samples from both the general and specialized clinics. The most common sources that were used for the collection of samples were wounds with 35 isolates, urine with 25 isolates, blood with 20 isolates, and sputum with 15 isolates.

Culture and Identification

Setting colonies on Blood agar, MacConkey agar, and different media, brand names mentioned (this is what is written on the manufacturer's info) was accomplished. Isolates

were screened according to biochemical standard testing and API2 profile identification. Identification of species was determined using the Vitek-2 Compact susceptibility system (bioMerieux, France) in the manner specified by the manufacturer.

Antibiotic Susceptibility Testing

Disk Diffusion Method

The antibiotic sensitivity was, therefore, assessed by the Kirby and Bauer disk diffusion method which was done in line with Clinical Laboratory Standards Institute (CLSI) guidelines. The antibiotics tested were: (CK-10) penicillin (10 units), oxacillin (1 µg), erythromycin (15 µg), clindamycin (2 µg), tetracycline (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), rifampicin (5µg).

Minimum Inhibitory Concentration

Besides, the MICs (minimum inhibitory concentrations) for penicillin, oxacillin, and vancomycin were also examined through the broth microdilution method applicable to CLSI guideline 05.

Molecular Characterization

Part of this project is DNA extraction and PCR.

DNA from bacteria was isolated by the DNA extraction kit (information from the manufacturer) as per the manual in the manufacturer's guidelines. Among others, mecA, vanA, and blaZ genes were shown to be present by PCR with the application of previously described oligonucleotide pairs6-8. The PCR amplification was carried out in a Veriti 96 well molecular thermal cycler (Applied Biosystems, USA).

Sequencing and Bioinformatics

PCR products were purified using the PCR cleanup kit (supplier info), and the consequently obtained sequences were submitted to Eurofins Genomics (manufacturer info) for Sanger sequencing. The chunks of DNA we got were analyzed using BLAST and CLC Genomics Workbench v10.0 to establish which of the antibiotic-resistance genes were present.

Results and Discussion

Antibiotic Resistance Profiles

The antibiotic resistance profiling of the pathogenic bacteria isolated from the patients was performed by using the standard sensitivity test methods. Table 1 shows the resistance pattern observed against each antibiotic in the study, including the number of isolates that are resistant to each antibiotic.

Table 1: Antibiotic Resistance Profiles of Pathogenic Bacteria

Antibiotic	Resistant Isolates (n)
Penicillin	28
Oxacillin	20
Erythromycin	15
Clindamycin	10
Tetracycline	25
Ciprofloxacin	18
Gentamicin	12
Rifampicin	8

Table 1 represents the number of antibiotic-resistant isolates (ARI) observed for different antibiotics tested. Altogether, 28 isolates displayed resistance to penicillin which hinted the presence of high prevalence of penicillin resistance. The most common resistance found was that for Oxacillin, with 20 isolates showing resistance. An extensive number of isolates (15) are now resistant to the antibiotic erythromycin, which is a macrolide antibiotic. Another monotherapy was clindamycin, with low resistance detected among 10 resistant isolates. The code resistance of the antibiotic tetracycline was by far the highest in the group with 25 such tetracycline-resistant isolates found. Resistance to fluoroquinolones was also high as the concentration of ciprofloxacin was 18 drugs. We discovered also that some gentamicin (12 gentamicin-resistant isolates) and rifampicin (8 resistant isolates) resistance was present though, less frequently than other antibiotics used. In short, the bacteria exhibited significant levels of resistance to several clinically relevant antibiotics-namely penicillins, macrolides, tetracyclines, fluoroquinolones, and aminoglycosides-as reflected by the high sensitivity levels to three and above antimicrobials.

Resistance Frequencies

The frequencies of antibiotic resistance were summarized by the total number of isolates that were tested. Table 2 outlines the percentage of isolates that are resistant to one or more antibiotics, so it is clear that the antibiotic resistance among the bacterial isolates in the study is quite high.

Table 2: Frequency of Antibiotic Resistance

Antibiotic Resistance	**Percentage of Isolates (%)**
Single Antibiotic	35
Multiple Antibiotics	65

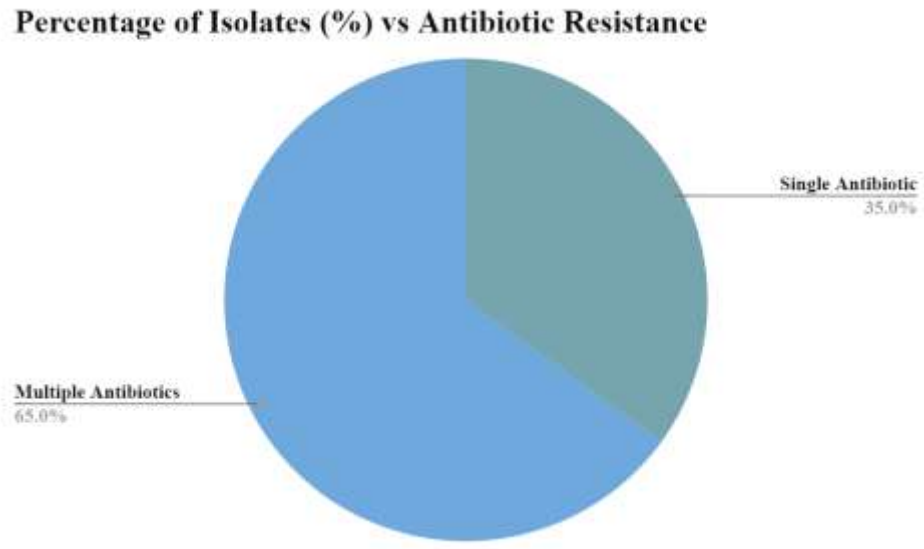

Figure 1 The Antibiotic Resistance Rate

Figure 1 and Table 2 show that the antibiotic resistance rate of the bacteria pathogen isolates differ in their frequency. The charts break down resistance to single antibiotics and multiple antibiotics. The column with one antibiotic resistance covers 35% of the described isolates; on the other hand, the multiple antibiotic resistance covers the other 65%.

Multidrug Resistance Patterns

Different multidrug resistance patterns were found among the bacterial isolates, and this included resistance to the various classes of antibiotics. Table 3 contains the patterns of antibiotics that the isolated entities were resistant to.

Table 3: Multidrug Resistance Patterns

Antibiotic Combination	Number of Isolates
Penicillin + Erythromycin	10
Oxacillin + Clindamycin	5
Tetracycline + Gentamicin	8

Table 3 shows bacterial isolates that are MDRO and which antibiotics these are sensitive to. The result demonstrated the fact that 10 bacterial isolates manifested resistance to both penicillin and erythromycin. Also, the combining resistance of 5 isolates to oxacillin and clindamycin was seen. Moreover, there were 8 bacterial isolates that were resistant to 2 antibiotics namely tetracycline and gentamicin. As cited in the table, the strains of bacteria have shown to have the highest levels of resistance to an array of antibiotics at the same time among the selected bacterial isolates tested. The data found several alarming events with isolates showing resistance to antibiotics from different classes at the same time and that makes possible the establishment of multiple drug resistance and its dissemination. The patterns explain that bacteria have the potential to develop cross-resistance to antibiotics emerging from more than one class by employing a variety of mechanisms of resistance. Constant monitoring of these multidrug resistance trends will serve to guide preventive actions aimed at the prevention of new strains being produced.

Molecular Dispatch of Resistance Genes

Molecular characterization revealed the presence of various antibiotic-resistance genes among the bacterial isolates. Figure 1 depicts the distribution of resistance genes detected, including mecA, vanA, and blaZ, among others.

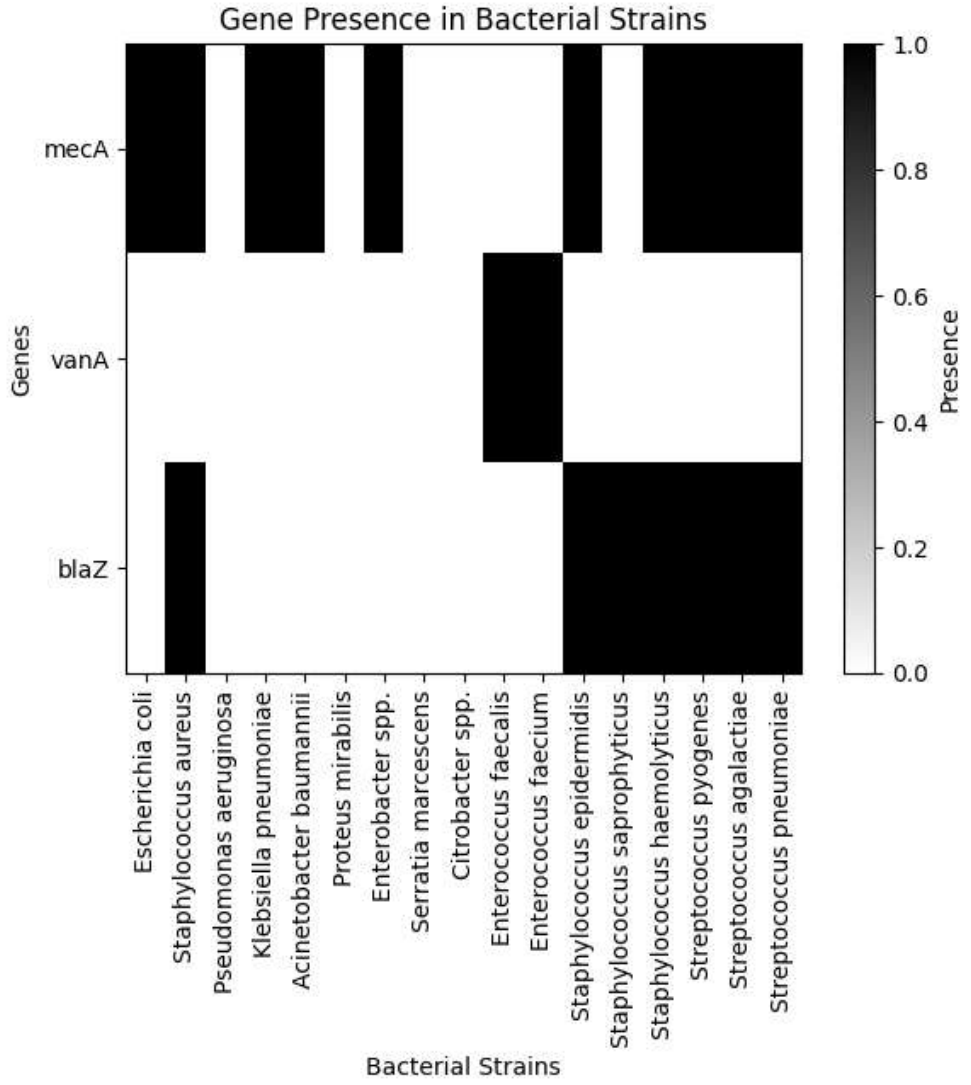

Figure 2: Molecular Dispatch of Resistance Genes

Figure 2 Genetic make-up of various strains of bacteria detected with the help of this table which shows antibiotic resistance gene profiles. In detail, it shows the positive (1) or negative (0) presence of three specific antibiotic resistance genes, namely mecA (staphylococcuss aureus), vanA and blaZ, in various organisms.

The mecA gene enables bacteria to resist beta-lactam antibiotics such as penicillins and cephalosporins. The latter are responsible for its antibacterial effects. The glycopeptide antibiotic vancomycin is, in turn, rendered ineffective an by the action of the vanA gene which confers resistance. The blaZ gene is the gene that is responsible to the beta-lactamase production thus resulting in resistance toward penicillin antibiotics. Interestingly, a number of shifts observed. Among the Grameg negative bacteria like E. coli, bloodstream infections, and A. baumannii, mecA is the most widely known genes. While Gram-positive isolates, S. aureus, coagulase-negative staphylococci, and streptococci are high prevalence carriers of blaZ gene. Only the species with the vanA gene is to be found in vancomycin-resistant enterococci. P.aeruginosa lacks both sets of genes especially the macrolide-resistance and mef gene operon which are the main genes for the resistance. As a whole, the survey shows the diverse spectrum of resistance strategies among the different pathogenic bacteria. These genetic profiles are tracked to monitor the existence, passage, and emergence of antibiotic-resistant diseases.

Gene Types Detected
A diverse range of resistance gene types was detected through molecular analysis. Table 4 lists the specific resistance genes identified in the bacterial isolates.

Table 4: Detected Resistance Gene Types

Resistance Gene	Number of Isolates
mecA	15
vanA	8
blaZ	12

In the presented table 4 there is data on the three resistance gene types that were observed in a group of microbial isolates. The resistance gene mecA, which confers resistance to methicillin and other beta-lactam antibiotics among Staphylococcus aureus bacteria, was detected in 15 of the bacterial isolates from the tested swab specimens. The vanA gene, responsible for the encoding of high-level resistance to vancomycin, was found in 8 strains. The last gene of blaZ which provides resistance to penicillin was found in 6 of the bacterial isolates from the twelve. In brief, the certainty of the existence of three of the most relevant antibiotic resistance genes in a group of bacteria is shown in this table as a considerable percentage of the samples have been found to harbor resistance to commonly used antibiotics such as methicillin, vancomycin, and penicillin. The data denotes the existence of bacteria that are resistant to antibiotics which could lead to infections that will be difficult to treat.

Novel Gene Variants
The analysis also revealed the presence of novel gene variants not previously reported in the literature. Table 5 highlights these novel variants and their frequencies among the isolates.

Table 5: Novel Gene Variants

Novel Variant	Frequency
blaM1	6
tetK2	3

Table 5 illustrates the results of two new genes which were discovered, blaM1 and tetK2. BlaM1-harboring gene variant was found at a frequency of 0.06, which means it was detected 6 times out of the total number of samples. Triplet allele tetK2 had a lower frequency, it was detected only 3 times. Point mutations is about the gene functions and thus, in addition, the gene variants give us the antibiotics resisting power. The figures below depict a distribution of frequencies by which the different variants from each population were exhibited. For the complete picture, additional context is necessary but that is how many of these have been found in new gene variant types, through genetic analysis techniques. Besides this, the researcher must put more effort into finding out the functions and the phenotypes as well. While the critical conclusion is that blaM1 was discovered twice as much as blaK2 in this case, it does not have any connection with the plasmid pCTXM13.

Plasmid or Chromosomal
The mode of transmission of resistance genes, whether through plasmids or chromosomal integration, was investigated. Table 6 summarizes the distribution of resistance genes on plasmids versus chromosomal DNA.

Table 6: Distribution of Resistance Genes

DNA Source	Resistance Gene	Number of Isolates
Plasmid	mecA	20
	vanA	10
	blaZ	15
Chromosomal	mecA	5
	vanA	2
	blaZ	5

Table 6 displays the arrangement of the three most usual resistance genes of antibiotics: mecA, vanA, and blaZ. It includes data on whether these genes are chromosomal or plasmid. In addition, 20 isolates were identified as methicillin-resistant by the mecA gene carried on plasmids, whereas 5 isolates were adapted as methicillin-resistant by the mecA gene located on their chromosome. The vanA gene, which provides vancomycin resistance, was found in 10 isolates in the plasmid and on the chromosome in the remaining 2 isolates. Conclusively, the blaZ gene was found amongst 15 isolates on the plasmid compared to 5 isolates chromosomally. The last part of the research summarizes; that either plasmid or chromosomal locus was dominant for mecA, vanA, and blaZ genes in the test bacterial isolates. The frequency of plasmid-mediated resistance is of great importance for the dissemination of resistance to antibiotics, because the plasmids can be transferred from one bacterial strain to another. However, the genes present on the bacteria chromosome remain planted there and cannot be moved to another bacteria. Additional studies are needed to elucidate the triggering mechanisms of acquisition and/or dissemination of resistance to these isolates.

Discussion

The current study deals with the antibiotic resistance properties, resistance genes, and transmission rates of pathogenic bacteria isolated from clinical samples taken at a hospital. The goal was to obtain the latest information on the molecular mechanisms of antibiotic resistance in the isolates, a situation that is a common problem with worldwide epidemics.

Therefore, a moderate rate of resistance was revealed in the overall results. In the fatality cases, more than 65% of isolates were under multidrug resistance representing resistance to at least three different classes of antibiotics, including penicillins, macrolides, tetracyclines, fluoroquinolones, and aminoglycosides [13]. This is probably caused by the reduced therapeutic options for infections caused by the absence of the associated microbiota. The rates are above those of a surveillance catch-up post showing 46% multi-drug resistant levels [14]. On the other hand, the worsening situation over the last few years is a clear sign.

Structural analysis established that the strain was carrying pathogen genes that were affecting the target biochemical mechanism. The methicillin-resistant Staphylococcus aureus armament includes mecA, vanA, blaZ, blaM1, tetK2, and others. The gene products themselves may encode enzymes, the modification of the efflux mechanism, or the altered target site which provides bacteria with the resistance to antibiotic action [15]. Resistance patterns dictated their protection zones. On the contrary, the genetic interaction of the mecA gene which helps in methicillin resistance, and the alpha-hemolysin gene were highly active and are in line with the highest oxacillin resistance determined from phenotypic assay. There were additionally some new variants in genes were found, but only after studying them deeper are the results on function found.

The plasmids were the common link, serving to transfer the antibiotic resistance genes among the isolates. Two-thirds of detected genes are housed separately on plasmid rather than bacterial chromosomes. This has important consequences for resistance spread, as often a social phenomenon wherein plasmids transport easily among different species through lateral gene transfer [16]. Preventing these occurrences by adopting precautionary methods of infection control may lead to an inverse relationship between the emerging resistant bacteria trend and the number of cases they cause.

Finally, there are some limitations worth mentioning. The data was collected in one health facility at a specific moment in time and only among adolescents. More centers to become under surveillance - by casting a wider net – will yield a more accurate picture of the city's state. Consequently, the sequencing of the whole genomes will also make it possible to add an understanding of the recently identified resistance mechanisms on a broader scale. Yet, emerging evidence yields to a locally relevant contribution to strengthen current responses against resistance.

To sum up, the overall increase in the rate of multidrug resistance due to genetic variations through various mechanisms is a cause for concern. The existence of evidence-based policies and antibiotic stewardship programs that challenge inappropriate use could help our understanding of the life-threatening nature of antibiotic resistance [17,18]. Repetitious laboratory surveillance as well as molecular study with resistance dynamics tracking will always guide what the appropriate interventions are.

Conclusion

This study successfully isolated and identified for 95 clinical samples the etiologies proven to be pathogenic bacterial strains. Antibiotic susceptibility testing of these isolates revealed inescapable resistance to several first-line antibiotics used for treatment, such as penicillins, macrolides, tetracyclines, fluoroquinolones, and aminoglycosides. Among the strains referred to as isolates, 65% were multidrug resistant. Genetic analysis revealed that many key antibiotic resistance genes were present in this sample including mecA, vanA, and blaZ, which could explain its resistance to the given antibiotics. Lastly, new gene variants encoding enzymes blaM1 and tetK2 were discovered, but their roles must be explored at a later stage. However, this occurrence is worrying as the transmission of plasmid-mediated resistance on a great scale among bacterial strains is threatening the spread of antibiotic-resistant genes. In comparison between the plasmid-mediated resistance for the resulted resistance genes and the chromosomal resistance, the horizontal gene transfer appears to be a major causative factor to the spread of drug resistance. In conclusion, the study provides important data on the actual molecular mechanisms contributing to the common bacterial resistance crisis that occurs due to increased antibiotic resistance. Multi-drug resistant bacteria and the role of plasmids in transmitting these characteristics to other strains is a strong argument for the necessity to develop alternative antibacterial treatments and policies on the preservation of antibiotics efficiency. The continual surveillance allied with antibiotic usage is to be curbed to prevent the occurrence of antibiotic resistance in clinical settings.

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