

Molecular Characterization of Antibiotic-Resistant Bacteria in Pregnant Women with Diabetes and UTIs in Tikrit, Iraq

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ABSTRACT

The present study was carried out in Tikrit City, Iraq, to investigate the prevalence and molecular characteristics of antibiotic-resistant bacteria among pregnant women suffering from gestational diabetes and urinary tract infections (UTIs). A total of 77 urine samples were collected and examined, leading to the successful isolation and identification of 14 bacterial isolates, consisting of 7 *Staphylococcus aureus* and 7 *Pseudomonas aeruginosa*. Antimicrobial susceptibility testing revealed a high level of resistance to commonly prescribed antibiotics, particularly ampicillin and Augmentin, with resistance rates of 90% and 95% respectively in *Escherichia coli* and *Klebsiella* spp. isolates. Molecular analysis demonstrated that all *S. aureus* strains harbored the *mecA* gene, confirming their classification as methicillin-resistant *S. aureus* (MRSA). Furthermore, the *oprM* gene was detected in all *P. aeruginosa* isolates, confirming the role of efflux pump mechanisms in their multidrug resistance. These findings highlight a concerning increase in antibiotic-resistant pathogens among high-risk pregnant women in Tikrit, emphasizing the urgent need for effective infection control strategies, rational antibiotic use, and continuous molecular surveillance to prevent further spread and complications in maternal healthcare.

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1. INTRODUCTION

Antimicrobial resistance is one of the most serious public health issues of the 21st century, and it is threatening our ability to treat bacterial infections, many of which were once easily treatable [1]. Antibiotic resistance is one of the World's top ten global health threats according to the World Health Organization, and we must take action to limit its spread and mitigate its effects [2]. This challenge is complicated for vulnerable populations, for example, pregnant women, who are particularly vulnerable to certain types of infections, including urinary tract infections (UTIs). UTIs are among the most common bacterial infections in the world, and they are particularly consequential and prevalent in pregnancy, where they can complicate health for women and risk the health of their fetuses [3]. Pregnancy triggers physiological and hormonal changes in women that predispose them to infection and require careful management of diagnostic and therapeutic approaches after UTI is diagnosed [4]. While complications such as preterm labour, low birth weight, or progression to pyelonephritis may occur if left untreated. UTIs are manageable with common antibiotics, but the rising prevalence of antibiotic resistance in bacteria that cause UTIs is a significant challenge for management. This complexity is compounded in pregnant women with diabetes, who represent a high-risk subset with recurrent and severe urinary tract infections typically caused by resistant bacteria with higher resistance to antibiotics [5].

Elevated blood glucose concentrations modify the urinary tract's microbial environment and inhibit immune responses, making these persons more prone to treatment-resistant infections [6]. Thus, it is important to recognize the patterns of antibiotic resistance in this patient subgroup for improving clinical outcomes. Antimicrobial resistance represents one of the most awkward health crises facing medical systems in the 21st century and is a source of therapeutic failure in bacterial infections. This complex, multi-faceted challenge has emerged, in part, as bacteria have evolved and adapted rapidly and unpredictably in response to selective pressures by overuse or misuse of antibiotics [7]. Therefore, the incidence of infections resistant to antibiotics has increased to a point where many are no longer responsive to conventional treatment. The World Health Organization (WHO) ranks the threat of antibiotic resistance among the ten highest in public health globally. It warns that, without intervention, antibiotic resistance threatens to return humanity to the "pre-antibiotic era," in which minor infections could lead to serious and even lethal consequences [1]. Thus, monitoring resistance patterns and understanding the biological behavior of bacteria and their epidemiology is necessary to implement effective measures to control them.

In this context, both Gram-negative and Gram-positive bacteria can contribute to community- and hospital-acquired infections. Examples of Gram-negative include *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, Gram-positive examples include *Staphylococcus aureus* and *Enterococcus faecium*. These organisms are typically distinguished by their ability to develop endogenous resistance mechanisms, such as the production of β -lactamase, alteration of target sites or targets, and efflux pump activity [8]. Visual representations of the antibiotic resistance patterns of these bacterial species, using heatmaps and graphical plots (i.e. bubble plots), can assist in evaluating currently circulating resistance patterns and the loss of efficacy. This analysis is critical when developing targeted and adaptive treatment regimens, like the examples discussed in this synoptic review, such as amikacin being effective against *P. aeruginosa*, whereas *E. faecium* exhibited broad-spectrum resistance heatmaps help explain drug effectiveness and choose the best treatment, as illustrated with amikacin's activity against *P. aeruginosa* and *E. faecium*'s broad resistance [9]. In terms of antibiotic usage, clinical practice and health policy decisions are key factors contributing to resistance. The unnecessary practices of antibiotics dispensed without prescriptions, the overuse of antibiotic in agriculture and veterinary medicine can contribute to persistently resistant strains and possibly facilitate the spread of resistance through the food chain and environment [10].

Consequently, epidemiological and analytical studies that relate bacterial species to antibiotic susceptibility are vital resources for the establishment of a complete resistance map. Additionally, it is important to realize that antibiotic resistance is a dynamic process that changes over time. Therefore, the collection of local and regional data should be considered on an ongoing basis to assess changes and compare them with worldwide trends. This is important for developing national plans to counter resistance and for timely updates to pharmaceutical policy according to the realities in the field [11]. The purpose of this study is to identify the bacterial pathogens responsible for urinary tract infections in pregnant diabetic women by collecting the urine samples and testing these samples in the laboratory using sophisticated methodology, namely the VITEK 2 Compact system, for accurate identification of the bacteria. More broadly, the current study aims to conduct a thorough assessment of antibiotic resistance patterns among the isolated uropathogenic bacterial strains, confirm the presence of the *oprM* gene as part of the *MexAB-OprM* efflux pump system in *Pseudomonas aeruginosa*, and identify the *mecA* gene responsible for methicillin resistance in *Staphylococcus aureus* using polymerase chain reaction (PCR).

2. Experimental Methodology

2.1 Sample collection and Diagnosis.

From February to June 2023, 77 urine samples were taken from pregnant women between the ages of 16-44 years old who had been diagnosed with diabetes and presented with symptoms of a urinary tract infection. Samples were obtained from Tikrit Teaching Hospital and several private laboratories in Tikrit City. A clean-catch midstream urine collection technique was employed to minimize contamination. Clinical and demographic data for each participant were recorded using standardized case report forms. To ensure accurate identification of the causative bacterial agents, all urine samples were cultured and analyzed using the VITEK 2 Compact system (bioMérieux, France), which provides automated identification and confirmation of bacterial isolates based on biochemical profiling.

This study received approval from Tikrit University's Ethics Committee (TUA0044, 10/01/2024) and was executed by the Declaration of Helsinki.

2.2 Antibiotic Susceptibility Testing

Solution polymerization was used in the following manner to carry out the polymerization reaction: After dissolving a sodium saccharin compound (0.01 mol) and acrylic acid (0.01 mol, 0.72 g) in 30 milliliters of benzene, the mixture was degassed by nitrogen gas was purged for N – allyl saccharin formed. After cooling, methanol (25 mL) was added, and the mixture was refluxed for 10 hours with AIBN (0.008 mol, 1.3 g) added. This caused the polymer precipitation to develop and solidify on schedule. According to the following equations:

2.3 Antibiotic Susceptibility Testing

The antibiotic susceptibility of the bacterial isolates was assessed using the Kirby-Bauer disc diffusion method, following the guidelines described by EUCAST (2024) [12]. The medium of choice for this work was Mueller-Hinton agar, with antibiotic discs from Bioanalyse. The bacteria were resuspended in a dilution of 10^{-3} and applied as a surface spread to the agar plates. Antibiotic discs were added at six discs per plate and incubated at 37 °C for 16-18 hours. After incubation, the diameters of zones of inhibition produced by the antibiotic discs were measured and compared to reference values from the Clinical and Laboratory Standards Institute (CLSI) to classify each isolate as susceptible, intermediate, or resistant to test antibiotics.

Table 1: Antibiotics profile

No.	Antibiotics	Symbol	Concentration (µg/disc)
1	Amikacin	AK	30
2	Ampicillin	AM	10
3	Cefotaxime	CTX	30
4	Ceftazidime	CAZ	30
5	Chloramphenicol	C	30
6	Ciprofloxacin	CIP	5
7	Clarithromycin	CLR	15
8	Gentamycin	CN	10
9	Rifampin	RA	5
10	Streptomycin	S	10

2.4 Genomic DNA Extraction

Genomic DNA was isolated from the bacterial isolates using the Genomic DNA Purification Kit (Promega, USA) according to the manufacturer's protocol. This method ensured the isolation of DNA of sufficient quantity and quality for further molecular analyses.

2.5 Screening for Virulence Factor Genes Using Polymerase Chain Reaction (PCR)

The presence of virulence-associated genes in bacterial isolates was highlighted by the polymerase chain reaction (PCR) for detection. This study targeted the *mecA* gene in *Staphylococcus aureus*, which mediates methicillin resistance, and the *oprM* gene from *Pseudomonas aeruginosa*, which is part of the efflux pump *MexAB-OprM* system associated with multidrug resistance. To design primers for both of those genes, we used NCBI resources and designed the sequence that we verified to be the targeted sequence and which we also were able to optimize by Primer3 software. Furthermore, silico PCR was run on the primers, which assured specificity and primers only bind at the intended site; our primers had the intended genomic context. The details of the primers used are shown in Table 2.

Table 2: Design of primers

Gene	Sequence (5'→3')	TM	GC%	Product length
<i>MecA</i>	Forward primer GCCCGTGGATTTAGTCGTGA	60.11	55.00	632 bp
	Reverse primer CGTCGAACTTGAGCTGTTACG	59.62	52.38	
<i>oprM</i>	Forward primer GGTCCTTCCTTCCCTGGC	60.00	63.16	434 bp
	Reverse primer GCCGAAGAGATCGAGTTCCC	60.25	60.00	

2.6 PCR Amplification of *mecA* and *oprM* Genes

Polymerase Chain Reaction (PCR) was used with specific primers to look for the presence of *mecA* gene in *Staphylococcus aureus* and *oprM* gene in *Pseudomonas aeruginosa*. The reactions were carried out using the AccuPower® PCR Premix kit (Bioneer, Korea), which contains all essential components for PCR, including Taq DNA polymerase (1 U), dNTPs (250 µM each of dATP, dTTP, dGTP, and dCTP), 1.5 mM MgCl₂, 30 mM KCl, and 10 mM Tris-HCl (pH 9.0).

Each 20 µL reaction mixture consisted of:

1. 1 µL of forward primer,
2. 1 µL of reverse primer,
3. 2 µL of genomic DNA template,
4. 16 µL of nuclease-free deionized distilled water.

Thermal cycling was performed in a thermocycler under optimized conditions to ensure efficient and specific amplification:

For the *mecA* gene:

1. Initial denaturation at 95°C for 5 minutes
2. 35 cycles of:
 - a. Denaturation at 95°C for 30 seconds
 - b. Annealing at 58°C for 30 seconds
 - c. Extension at 72°C for 45 seconds
3. Final extension at 72°C for 5 minutes
4. Hold at 4°C

For the *oprM* gene:

Conditions were identical to those for *mecA*, except the annealing temperature was adjusted to 60°C for 30 seconds. These conditions were selected to provide optimal amplification, efficiency and specificity for the target genes. The results were then visualised by running the PCR sample through agarose gel electrophoresis to visually confirm that amplification was successful.

3. RESULTS AND DISCUSSION

3.1. Identification of Bacterial Isolates

Urine samples from pregnant women were used to identify the pathogenic bacteria with the VITEK 2 Compact system, an automated system to rapidly identify bacteria and develop antibiotic sensitivity profiles. Specialized automated systems such as this allow for the simplified analysis of biochemical and enzymatic reactions interpreted by an algorithm which quickly and accurately (compared to minimally automated analysers) identifies microbes. Automated systems are fundamental for pregnancy-associated infections, as time and accuracy are essential for rapid diagnosis of UTIs and their perturbing effect on both individuals (the mother and fetus). In addition, the VITEK system aids in advancing care more responsibly as it will provide the complete sensitivity pattern on all the isolates collected from all clinical specimens.

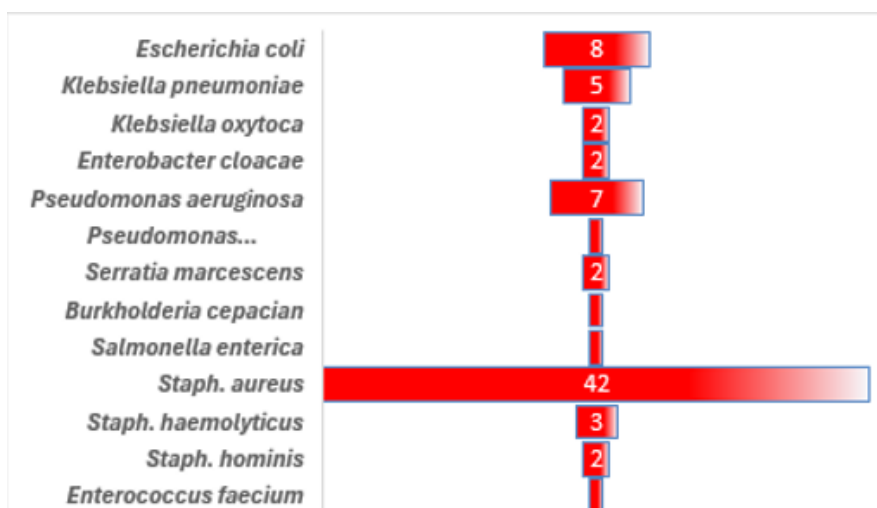


Figure 1: Bacterial species isolated from clinical samples and their distribution. Color coding is only used to improve the visual perception of isolating abundance; it indicates the relative frequency of each isolate within the entire sample set.

In addition to *S. aureus*, other coagulase-negative staphylococci were detected, including *Staphylococcus haemolyticus* (3 isolates) and *Staphylococcus hominis* (2 isolates). This test isolated and identified *Staphylococcus* species from clinical samples. It found *S. aureus* and coagulase-negative staphylococci such as *S. haemolyticus* and *S. hominis*, which were identified biochemically or automatically. Although these species are typically part of the normal skin flora, they can become opportunistic pathogens, particularly in pregnant women, whose immune function and urinary tract physiology are altered. Although these species are normally part of the skin flora, they can become opportunistic infections, especially in pregnant women with altered immune function and urinary tract physiology. In immunocompromised people, especially pregnant women, *Staphylococcus haemolyticus* can cause urinary tract and bloodstream infections. Among enteric bacteria, five isolates of *Escherichia coli* were identified. This species is well-recognized as the most common cause of UTIs in pregnant women, largely due to the anatomical proximity of the rectum to the urethra, facilitating bacterial transmission. Additionally, two isolates, each of *Klebsiella pneumoniae* and *Klebsiella oxytoca* were recovered—both of which are known uropathogens in pregnancy and have been associated with complications such as preterm birth and low birth weight if left untreated [13].

Importantly, seven isolates of *Pseudomonas aeruginosa* were identified. While less commonly associated with UTIs in pregnancy, its presence may reflect complicated or nosocomial infections, especially in cases involving indwelling catheters or underlying chronic conditions. Other species detected included *Enterobacter cloacae*, *Serratia marcescens*, and *Burkholderia cepacia*, all of which exhibit intrinsic resistance to multiple antibiotics and demand careful therapeutic consideration. *Enterococcus faecium* was isolated in one case. As a Gram-positive organism known for its resistance to antibiotics such as vancomycin, its detection raises concerns about treatment difficulty, particularly during pregnancy [14]. Furthermore, a single isolate of *Salmonella enterica* was identified, an unusual finding in urinary samples that may suggest systemic involvement stemming from foodborne transmission. These findings underscore the wide microbial spectrum associated with UTIs in pregnant women, many of which include antibiotic-resistant strains.

3.2. Resistance of Bacterial Isolates to Antibiotics

The primary objective of this section was to investigate the relationship between antibiotic resistance and the genetic composition of the bacterial isolates obtained from urine samples of pregnant women with urinary tract infections. Drug susceptibility testing was conducted on seven Gram-positive and Gram-negative bacterial isolates, each subjected to testing with 10 commonly used antibiotics. The antibiotic resistance profiles were determined using the disc diffusion method, in which bacterial cultures were grown on Mueller-Hinton agar, and antibiotic discs were placed on the surface of the inoculated plates. The size of the inhibition zone around each disc was measured after incubation, which directly reflects the bacterial strain's sensitivity or resistance to the respective antibiotic. The resistance levels were calculated based on the diameter of the inhibition zones, and results were categorized as sensitive, intermediate, or resistant.

As shown in Figure 2, the resistance of bacterial isolates from pregnant women with urinary tract infections to various antibiotics is expressed in percentage form. Figure 3 illustrates the antibiotics' effectiveness, highlighting the most potent drugs against the identified bacterial species. The results presented in Figure 2 demonstrate significant variation in the antibiotic resistance profiles of the bacterial isolates, particularly *Escherichia coli*, which exhibited diverse responses to the tested antibiotics. The isolates had extremely high resistance rates for Ampicillin (AM) and Augmentin (AC) at 90% and 88%, respectively. From these results, it is likely that resistance genes to these antibiotics are prevalent among the *E. coli* isolates. In addition, the isolates had high resistance rates for Cefotaxime (CTX) (80%) and Ceftazidime (CAZ) (85%), which could signify ESBL production, a common resistance mechanism for *E. coli* [15]. Moreover, the bacterial isolates showed an intermediate level of resistance to Chloramphenicol (C) (70%) and Clarithromycin (CLR) (60%), which signifies the ability of some strains to produce resistance mechanisms, such as enzymatic modifications and efflux pump mechanisms [16,17]. Resistance observed for Streptomycin (S) (55%) and Gentamicin (GN) (50%) may indicate some degree of reduced effectiveness of these antibiotics. Rifampin (RA) showed 40% resistance, indicating that some isolates remain sensitive.

However, given the previously documented resistance levels by Ciprofloxacin (CIP) (45%) and its history of use in the clinical environment [18], its apparent increase in use in this study should potentially raise more concern. 5 antibiotics tested, Amikacin (AK) had the lowest resistance rate at 20%, supporting its reasonable effectiveness against *E. coli* isolates and providing a justification for the use of aminoglycosides in serious bacterial infections. Keeping in mind, *Klebsiella pneumoniae* and *Klebsiella oxytoca* also showed high resistance, attributed to beta-lactamases by extended-spectrum beta-lactamases (ESBLs) formation, efflux pump activation, and mutations [19].

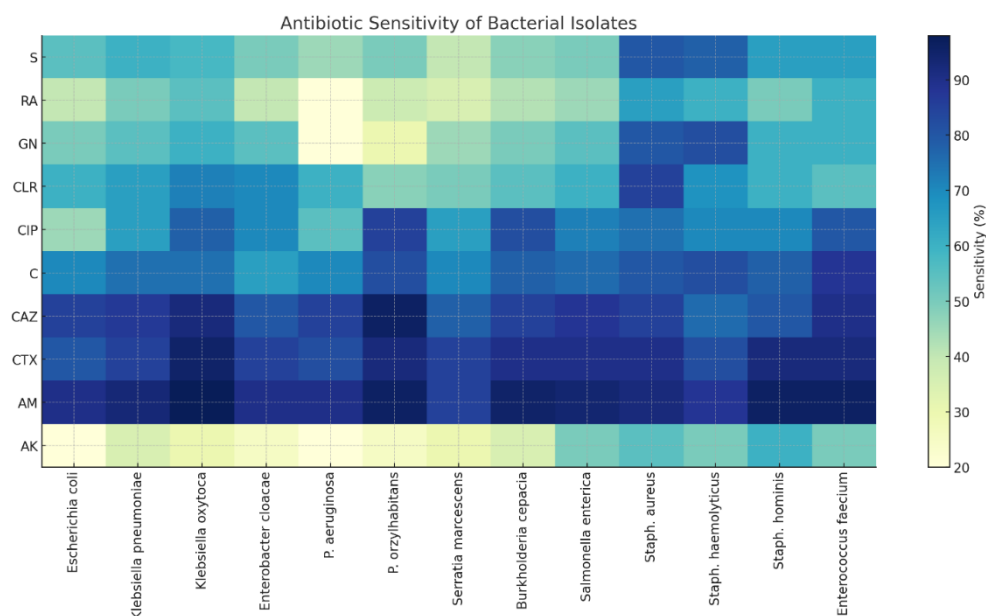


Figure 2: The heatmap shows the sensitivity ratios of different antibiotics against the studied bacterial isolates. Each row represents an antibiotic (e.g., Amikacin, Cefotaxime, Rifampin, etc.), and each column represents a bacterial species. The color ranges from light yellow (low sensitivity) to dark blue (high sensitivity), making it easier to compare the effectiveness of antibiotics on different types of bacteria.

The isolation of both species had very high resistance to Ampicillin (95%) and Augmentin (85%), showing that there is little point in using Ampicillin again as an antibacterial agent. High resistance to Cefotaxime (CTX) (85%) and Ceftazidime (CAZ) (87%) is also attributable to the presence of *bla*_{CTX-M} and *bla*_{SHV} genes [20]. Resistance against the fluoroquinolone, Ciprofloxacin, and Chloramphenicol is about 75%, while Streptomycin and Clarithromycin had increasing resistance from about 60-65%, making it increasingly difficult to treat. The relative resistance against Amikacin is 35%, which means there are still reasonable treatment options with the possibility of some success for infections caused by these species [21].

These results indicate an escalation in concern regarding multidrug resistance with urinary tract pathogens, indicating a need for continuous surveillance, rational use of antibiotics, and iterative changes in treatment protocols to manage the dissemination of resistance. In contrast, the findings of this study indicated that *Klebsiella oxytoca* isolates had relatively lower resistance than *Klebsiella pneumoniae* isolates. Resistance to Ampicillin was 92%, and 80% for Augmentin; resistance to Cefotaxime was 75%, and Ceftazidime was 78%, suggesting that *K. oxytoca* has similar (but less severe) resistance mechanisms [22]. Resistance to Ciprofloxacin was 65%, possibly due to mutations within DNA gyrase and topoisomerase IV enzymes, and/or involvement of *qnrA* and *qnrB* genes that are associated with a higher level of fluoroquinolone resistance [23]. The resistance rates for Gentamicin and Amikacin were 45% and 30%, respectively, suggesting aminoglycosides could be a feasible treatment option for infections due to *K. oxytoca* isolates, especially as penicillin and cephalosporins have demonstrated increasing resistance [24]. In addition, moderate resistance to Rifampin (50%) and Chloramphenicol (72%) was seen, suggesting that these antibiotics may still be applicable as treatments for complicated clinical cases, based on susceptibility test findings. On the contrary, *Enterobacter cloacae* isolates had marked resistance to several antibiotics, including 88% resistance to Ampicillin and 85% resistance to Cefotaxime. The high rate of resistance can be attributed to the production of extended-spectrum beta-lactamases; specifically, *AmpC* beta-lactamases, which can inactivate penicillin and cephalosporin antibiotics [25].

The isolates were resistant (100%) to Augmentin, Tetracycline, and Clarithromycin and exhibited multiple resistance mechanisms, including genetic changes to antibiotic-binding sites and the potential activation of efflux pumps that reduce the concentration of antibiotics inside the bacterial cell [26]. The discoveries highlight the therapeutic challenges these bacteria present and the necessity for more complex treatment measures utilizing more targeted antibiotics, as well as monitoring pharmaceutical kinetic effects to track the progression of drug resistance. The bacterial isolates study demonstrated substantial variations in their antibiotic susceptibility. Of the previously mentioned *Enterobacter cloacae* isolates, they showed high susceptibility to Ciprofloxacin (70%) and Chloramphenicol (78%), as well as low resistance to Amikacin (40%), suggesting the respective potential for treating this species. In another part of the study, *Pseudomonas aeruginosa* had resistance to most of the antibiotics tested, particularly Ampicillin (98%) and Cefotaxime (90%), the mechanisms of resistance being mainly attributed to extended-spectrum beta-lactamases (ESBLs) and efflux pump mechanisms. Nevertheless, both Ciprofloxacin (60%) and Amikacin (10%) had some efficacy remaining.

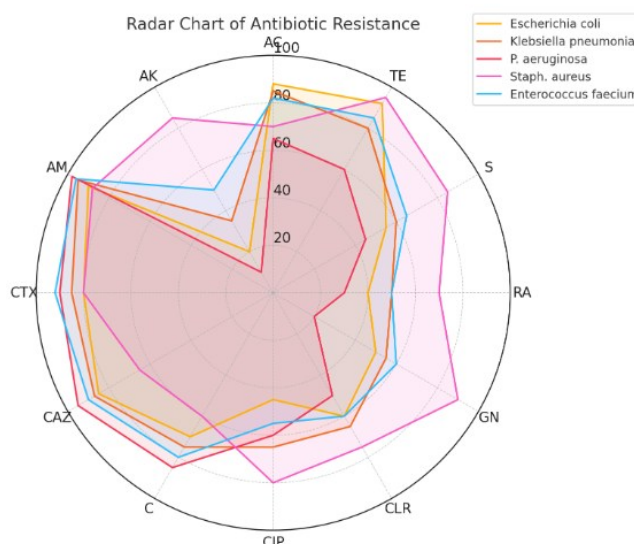


Figure 3: Illustrates the resistance of five types of bacteria to a group of antibiotics, and it shows a distinctive visual shape that helps compare the overall pattern of resistance.

Pseudomonas oryzihabitans showed higher susceptibility, particularly to Ciprofloxacin (85%) and Chloramphenicol (82%), indicating its relative ease of treatment. *Serratia marcescens* exhibited high resistance to beta-lactam antibiotics and tetracyclines, although Amikacin and Rifampin remained acceptable treatment options, with resistance rates of 30-35%. Isolates of *Burkholderia cepacia* and *Salmonella enterica*

also demonstrated high resistance to most antibiotics, particularly cephalosporins and fluoroquinolones, though Amikacin (15-50%) and Rifampin (42-45%) showed potential as therapeutic options. These findings reflect the increasing challenges associated with treating infections caused by Gram-negative bacteria and underscore the importance of laboratory susceptibility testing before selecting an antibiotic regimen. The results further emphasize the need to rationalize antibiotic use and continuously update treatment protocols in response to the growing problem of antimicrobial resistance. Figure 3 illustrates the resistance patterns of five bacterial species, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecium*, to a selected group of antibiotics, providing a clear visual comparison of resistance levels across these species.

Pseudomonas aeruginosa exhibited a discrete profile of high resistance to most antibiotics, such as Ceftazidime (CAZ), Cefotaxime (CTX) and Ampicillin (AM), whilst it had lower resistance to Amikacin (AK). The difference in oxide for Amikacin resistance is assumed to occur because *Pseudomonas aeruginosa* resistance mechanisms (efflux pumps and beta-lactamase production) can cause it to remain less resistant to aminoglycosides like the Amikacin group [27]. On the contrary, *Enterococcus faecium* had high and relatively stable resistance against almost all groups of antibiotics, indicating the multiresistant characteristic of such species. For Enterococcal species, resistance is evident from the number of species with resistance genes typically found in health-care settings, such as *vanA* and *vanB* resistance genes that confer vancomycin resistance [28].

Enterococcus faecium also has a WHO classification as a high priority and due to the antibiotic susceptibility of *Enterococcus faecium* [29]. *Escherichia coli* and *Klebsiella pneumoniae* had intermediate resistance with an observed increased resistance against Penicillin and Cephalosporins. Resistance mechanisms of these two groups of species are primarily attributed to Extended-Spectrum Beta-Lactamases (ESBLs) production and the presence of *qnr* genes that confer resistance against quinolones [30].

Staphylococcus aureus had high levels of resistance to Amoxicillin (AM) and Amikacin (AK) suggesting there may be Methicillin-Resistant *Staphylococcus aureus* (MRSA). As MRSA can resist more than a single class of antibiotic and as MRSA can spread more easily through health systems, this is a clinical concern [31]. This visual of the bacterial resistance profile shows a nice, comparative overview of antibiotic resistance and guides the use of appropriate antibiotics and helps in selecting the best treatment options. This can also serve as a means of monitoring resistance tendencies over time and planning intervention strategies in hospital settings.

3.3. DNA Extraction

DNA was extracted from *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacterial strains. With regards to the extraction, seven isolates from each bacterial species were used, selected based on their high resistance to the antibiotics. The results were acceptable, as DNA extractions yielded a high concentration and purity. The concentration of *S. aureus* isolates was in a range of 88 to 234 ng/μL, whereas the purity values were in a range of 1.77 to 1.90.

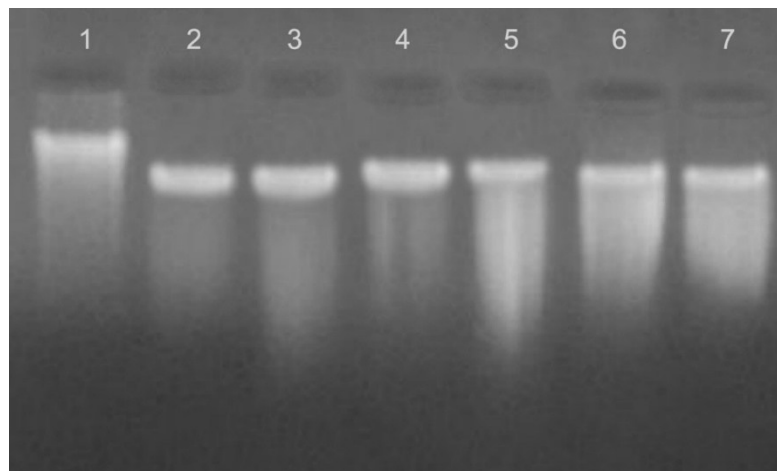


Figure 4: Electrophoresis of genetic content (genomic DNA) extracted using agarose gel1% (45 min, 7 V/cm). (Columns 1 to 7: DNA extracted from *S. aureus* isolates.

For *P. aeruginosa* isolates, the DNA concentration ranged from 79 to 227 ng/μL, with purity values ranging from 1.79 to 1.89. Figures 4 and 5 illustrate the DNA bands extracted from a group of *S. aureus* and *P. aeruginosa* isolates, providing visual confirmation of the quality and integrity of the extracted DNA.

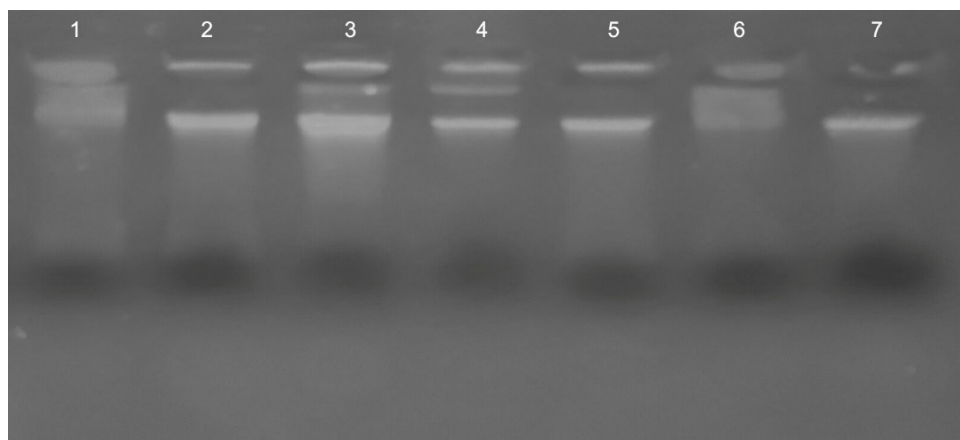


Figure 5: Electrophoresis of genetic content (genomic DNA) extracted using agarose gel 1% (45 min, 7 V/cm). (Columns 1 to 7: DNA extracted from *P. aeruginosa* isolates.

3.4. Molecular Investigation of Virulence Factors of the Bacterial Isolates Under Study

3.4.1. Investigation of the *mecA* Gene in *Staphylococcus aureus*

Antibiotic resistance remains a significant global challenge, and *Staphylococcus aureus* has been identified by the World Health Organization as a critical pathogen due to its ability to colonize nearly one-third of the global population. This bacterium is a leading cause of both hospital- and community-acquired infections. The accurate detection of methicillin resistance is essential, as methicillin is a key antibiotic for treating *Staphylococcus aureus* infections. The *mecA* gene is the principal determinant of methicillin resistance in *S. aureus*, as it encodes a penicillin-binding protein (PBP2a) with little affinity for methicillin or other beta-lactam antibiotics. Thus, when detecting the *mecA* gene, there is a reliable molecular marker and identification of methicillin-resistant *S. aureus* (MRSA) strains, which may assist in treatment decisions and in limiting the spread of resistant strains. In this study, the *mecA* gene was studied to determine if methicillin resistance could be detected in *Staphylococcus aureus* isolates at the molecular level. DNA was extracted from seven bacterial isolates that were determined to be resistant to the antibiotics in standard laboratory assays (the phenotypic diagnosis). Polymerase chain reaction (PCR) analysis results confirmed that the *mecA* gene was present in all seven isolates, yielding a 632-base pair (bp) amplification product seen in Figure 4-13.

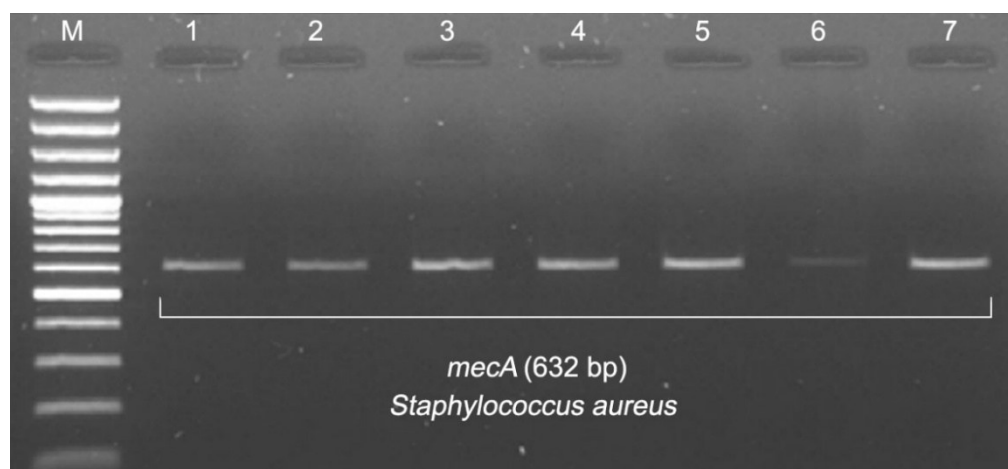


Figure 6: Gel electrophoresis of PCR reaction products for gene detection of *mecA* using an agarose gel 2%, 60 min, 7 V/cm. Column M is a 1500 bp DNA standard, columns 1 to 7 are *Staphylococcus aureus* samples.

This product size corresponds to the expected relative size of the *mecA* gene when amplified with the designed primers. The congruency between the phenotypic diagnosis and molecular diagnosis confirms the validity and efficacy of the *mecA* gene as a molecular marker, and the mechanism of antibiotic resistance in *Staphylococcus aureus*. This molecular method has several advantages over conventional tests, including speed and specificity, and will serve as an important method of diagnosis in treating infections appropriately.

Effectiveness of PCR for rapid and accurate *mecA* gene detection for determination of methicillin resistance compared with conventional laboratory methods in Brazil. *mecA* encodes a penicillin-binding protein (PBP2a) with low affinity. affinity for methicillin and other beta-lactam antibiotics. These antibiotics usually inhibit the synthesis of the bacterial cell wall by binding to PBPs PBP2a, produced by the *mecA* gene, prevents beta-lactam antibiotics from binding effectively, allowing bacteria to continue cell wall synthesis and survive in the presence of these drugs [32]. Researchers explored how PBP2a contributes to methicillin resistance by altering the structure of the antibiotic-binding site, which reduces the binding affinity and enables the growth of bacteria in the presence of methicillin. highlighted the benefits of targeting PBP2a with specific inhibitors to reverse methicillin resistance in *S. aureus*, suggesting PBP2a as an important therapeutic target [33]. Identifying the *mecA* gene is important for determining if *S. aureus* is methicillin-resistant and providing treatment to minimize the dissemination of MRSA strains. The importance of molecular tests for the prompt detection of the *mecA* gene indicated to improve patient outcomes and mitigate the risk of complications [18]. Likewise, Early identification of MRSA strains makes isolation practices easier to implement, limiting the dissemination of resistant strains in hospitals and the community [34].

3.4.2. Investigation of the *oprM* Gene of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is one of the most prevalent pathogenic species, particularly in hospital-acquired Infections. These bacteria are resistant to several different types of antibiotics due to their effective efflux *MexAB-OprM* system, which significantly reduces antibiotic accumulation inside bacterial cells. In this study the presence of the *oprM* gene was characterized in various *Pseudomonas aeruginosa* isolates, both from clinical sources and from pregnant women with urinary tract infections. DNA was extracted from 7 antibiotic-resistant isolates, identified through phenotypic testing. Polymerase chain reaction (PCR) was then performed to confirm that the *oprM* gene is present specially designed primers to detect its genetic sequence. PCR analysis showed that all the isolates had the *oprM* gene, suggesting its widespread distribution among resistant strains.

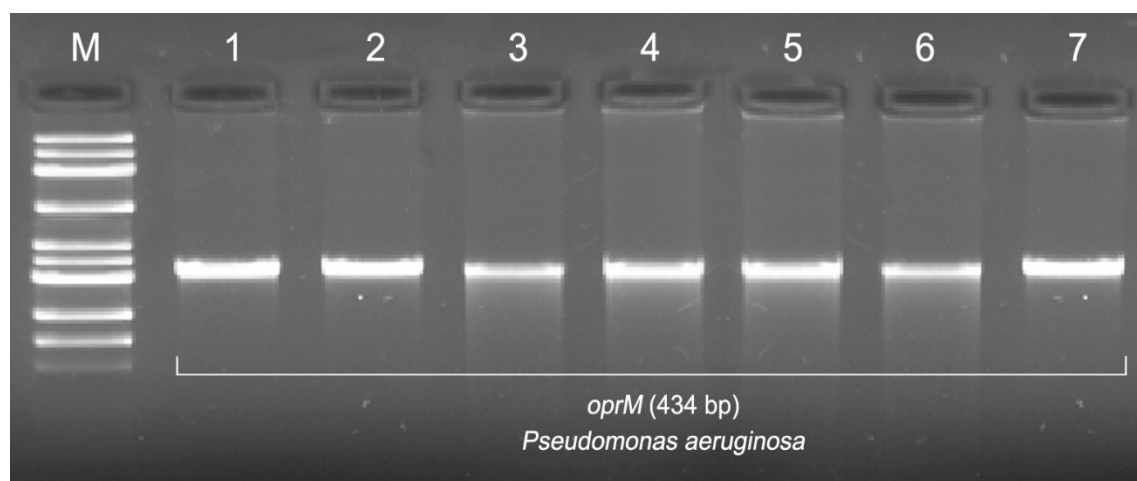


Figure 7: Gel electrophoresis of PCR reaction products for gene detection *oprM* using an agarose gel a 2%, 60 min, 7 V/cm. Column M is a 100 bp DNA standard; columns 1 to 7 are *Pseudomonas aeruginosa* samples.

The amplification product measured 434 base pairs, which is in line with the reported average size of this gene (Figure 7). This finding is consistent with a recent study published in the Journal of Antimicrobial Chemotherapy, which showed that the *oprM* gene was expressed in 95% of antibiotic-resistant clinical isolates from hospitals in China [35].

The *oprM* gene is responsible for the *MexAB-OprM* efflux system, in which antibiotics pass through an external channel to be pumped out of the bacterial cell (porous membrane), ensuring that antibiotics do not accumulate and deplete antibiotic potency. It has been demonstrated through various investigations that the presence of the *oprM* gene correlates with the increased resistance of bacterial species to a broad range of antibiotics, ranging from quinolones to beta-lactams to aminoglycosides [36]. Moreover, expression of this gene is often elevated in response to environmental stresses, such as repeated exposure to antibiotics. Some previous research shows that mutations in gene expression regulators (*mexR* and *nalC*) can lead to overexpression of the *oprM* gene, thereby enhancing antibiotic resistance [38].

A study by Yoshida et al. (2019) [37] also revealed that inhibiting the *MexAB-OprM* pump system could improve *Pseudomonas aeruginosa's* response to conventional antibiotics, positioning *oprM* as a promising therapeutic target. The *oprM* gene is one of the keys affecting this evolution, these findings suggest. *Pseudomonas aeruginosa* resistance, it is important to develop strategies for its control as well as the development of inhibitors targeting the multi-resistance pump systems, or combination therapies for inhibition and/or suppression of the *oprM* gene activity. Isolate serology routine surveillance for the presence of *oprM* could also provide valuable information for therapeutic decisions and the design of more effective control strategies. This study adds further evidence for the role of the *oprM* in a growing literature. The gene involved in the antibiotic resistance of *Pseudomonas aeruginosa* echoes a need for continued research to address the potential impact of resistant pump systems through novel therapeutic approaches as well as better practices in clinical care [39]. Based on the growing numbers of antibiotic-resistant bacteria, including MRSA (*mecA*-positive) and multidrug-resistant *Pseudomonas aeruginosa* (*oprM*-positive), we report the alarming spread of these bacteria among pregnant women with diabetes and urinary tract infections in Tikrit City, Iraq. Here we underscore the importance of genetic mechanisms in resistance and emphasize the need for use of advanced molecular diagnostic techniques, such as PCR, for rapid detection of resistance genes. Rationalisation of antibiotic use, especially in children against resistant strains, and switching to more effective treatments. Amikacin is critical. Strengthening surveillance programs and exploring therapies targeting bacterial resistance mechanisms, like efflux pumps, are vital steps to combat this growing health challenge [40].

4. CONCLUSION

Based on the growing numbers of antibiotic-resistant bacteria, including MRSA (*mecA*-positive) and multidrug-resistant *Pseudomonas aeruginosa* (*oprM*-positive), we report the alarming spread of these bacteria among pregnant women with diabetes and urinary tract infections in Tikrit City, Iraq. Here we underscore the importance of genetic mechanisms in resistance and emphasize the need for the use of advanced molecular diagnostic techniques, such as PCR, for rapid detection of resistance genes. Rationalization of antibiotic use, especially in children, to resistant strains, and switching to more effective treatments. amikacin are critical. Strengthening surveillance programs and exploring therapies targeting bacterial resistance mechanisms, like efflux pumps, are vital steps to combat this growing health challenge

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