

# Molecular Profile and the Effectiveness of Antimicrobials Drugs Against *Staphylococcus aureus* and *Pseudomonas aeruginosa* in the Diagnostic Approaches of Otitis Infection

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**Background:** Otitis externa and otitis media are two types of ear infections that affect people of all ages, although they are more common in newborns and young children. Antibiotic usage, healthcare, and advanced age all play a role in the development of this illness.

**Methods:** Fifty-eight patients with various kinds of infections of the ears were voluntary patients attending the outpatient clinics of the Prince Mutaib Bin Abdulaziz Hospital in Sakaka, Al Jouf, Saudi Arabia, examined to evaluate the role of bacteria and the likely significance of plasmids in their antibiotic resistance as ear infectious agents.

**Results:** *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the most prevalent bacteria found in ear infections. The greatest number of major bacterial isolates were *S. aureus* (54%), followed by *P. aeruginosa* (13%), whereas a smaller number of isolates (3%) were from *Streptococcus pyogenes*, *Bacillus subtilis*, and *Proteus vulgaris*, respectively. Mixed growth was noted in 3.4% of instances. The isolation rate for Gram-positive organisms was 72%, while the rate for Gram-negative species was 28%. All the isolates had DNA greater than 14 kilobases. *Hind III* analysis of the plasmid DNA extracted from the resistant strains of ear infection demonstrated that antibiotic-resistance plasmids were extensively dispersed. Exotoxin A PCR amplification indicated 396 pb PCR-positive DNA for all identified samples, with the exception of three strains for which no band was observed. Patients in the epidemiological study ranged in number, but all were linked together for the purposes of the study because of their shared epidemiological characteristics.

**Conclusion:** Vancomycin, linezolid, tigecycline, rifampin, and daptomycin are all antibiotics that have been shown to be effective against *S. aureus* and *P. aeruginosa*. Microbiological pattern evaluation and antibiotic sensitivity patterns of the microorganisms providing empirical antibiotics are becoming increasingly crucial to minimize issues and the development of antibiotic-resistant strains.

**Keywords:** bacterial ear infections, otitis externa, otitis media, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, exotoxin A, plasmid, antibiotic resistance, Saudi Arabia

## Introduction

Otitis media (OM) is the medical name for an infection of the tympanic membrane or middle ear, and it is characterized by ear pain or discharge.<sup>1</sup> Infections in the ear canal can be either acute or chronic.<sup>2</sup> An estimated 330 million people throughout the globe suffer from ear infections, with 60% of those individuals losing their hearing.<sup>3</sup> Public health issues

have arisen in both developing and developed nations because of their high prevalence.<sup>4</sup> Adults may become infected with an ear infection as well, although it is more prevalent in children under the age of five. Unnoticed therapy may cause infections to begin in infancy.<sup>1</sup> A long-lasting hole may allow bacteria to enter the middle ear. Children are more susceptible to ear infections than adults because their eustachian tubes are not fully mature.<sup>5</sup> This permits organisms to enter the nasopharynx more easily. In addition, males had a greater rate of infection than women.<sup>6</sup> An ear infection may affect either the inner or outer ear, depending on the source of the infection. Among the three forms are acute otitis media (AOM), otitis media with effusion (OME), and the less common otitis externa, sometimes called swimmer's ear (SE).<sup>7-9</sup> Middle ear tumors, auditory inflammation, and hearing difficulties may all be caused by ear infections.<sup>10-12</sup> Ear infections may vary greatly from area to region owing to regional variances in antibiotic prescribing patterns and the occurrence of drug-resistant bacterial strains.<sup>13</sup> Ear infections are commonly misdiagnosed due to their symptoms, making it difficult for doctors to pinpoint the specific cause of a patient's illness.<sup>14</sup> Antibiotics may be prescribed regardless of the underlying cause of the illness.<sup>15</sup>

Bacteria, viruses, or fungi may cause ear infections. Bacteria such as *S. aureus*, *Escherichia coli*, *P. aeruginosa*, *Streptococcus pyogenes*, *Protus mirabilis*, *Klebsiella spp.*, and mixed infections are the most common pathogens in ear infections.<sup>16,17</sup> Antibiotics are becoming less effective against AOM-inducing pathogens. The conventional antibiotic treatment for ear infections may need to be re-evaluated after this sighting.<sup>1,18</sup> Recent antibiotic treatment of acute otitis media, children in daycare centers, wintertime infections, and AOM among children under two years of age are all risk factors for resistant bacteria.<sup>19,20</sup> In order to eradicate drug-resistant *Streptococcus pneumoniae*, a greater dose of amoxicillin (80 mg per kg per day) may be necessary.<sup>21</sup> Second-line options include oral cefuroxime or amoxicillin-clavulanate, as well as injectable ceftriaxone.<sup>19</sup> The discovery of the R plasmid demonstrates that bacteria have both innate resistance genes and the ability to acquire resistance in order to protect themselves from environmental threats.<sup>22</sup> Plasmids and transposons, which allow bacteria to acquire and transfer foreign genes, are regarded as key components in the development of resistance.<sup>22-24</sup> Intrinsic resistance in Gram-negative bacteria is linked to the control of outer membrane proteins, such as porins and components of efflux pump systems, on the outer membrane.<sup>25,26</sup> Small metabolites, such as antibiotics, are normally controlled by porins, while efflux systems use an energy-dependent method to remove harmful compounds such as antibiotics via specialized efflux pumps.<sup>27,28</sup> Thus, antimicrobial resistance is a global problem because of mismanagement or abuse of antimicrobial agents. Experts in these places are also unaware of the antibiotic-resistant tendency in their health areas.

As a result, the objective of this investigation is to inspect the incidence of bacterial agents in ear infections and determine the resistance of these organisms to antibiotics. In addition, this study examined the possible link between antibiotic resistance and the presence of plasmids in the bacterial isolates studied. In the present study, isolates of *P. aeruginosa* from outpatients were assessed for epidemiological empathy through exhausting genotyping of DNA. Phenotypic and genotypic data were matched to conclude the truthfulness of antibiotic profiles, and molecular analysis outcomes were matched to create a clonal association between medical isolates of *P. aeruginosa*.

## Materials and Methods

### Study Design

The study was conducted as a cross-sectional survey from December 2020 to February 2022. Fisher's formula was used to appraisal the proper sample size. Data and specimens were collected from 58 patients of all sexes and ages who visited Prince Mutaib Bin Abdulaziz Hospital in Sakaka, Al Jouf, Saudi Arabia throughout the research period. Table 1 exhibitions the distribution of ear specimens by gender, age, and location. Specimens that indicated any evidence of contamination were discarded from the examination.

### Sampling, Isolation, and Identification of Bacteria

All patients hospitalized at Prince Mutaib Bin Abdulaziz Hospital in Sakaka, Al Jouf, Saudi Arabia, suffering from a variety of ear illnesses, volunteered for investigation. Sterilized cotton swabs were used to collect samples from patients with otitis and other illnesses. Separately, 10 mL of alkaline peptone water (5% peptone, pH 8.6) was used to wash the

**Table I** Dissemination of Wound Specimens of Patients by Gender, Age and Locations

	Variable	Number	Percentage (%)
Gender	Male	40	69
	Females	18	31
Age (Years)	< 20	17	29.3
	2–40	22	37.9
	41–60	13	22.4
	> 60	6	10.4
Recurrent infections	Otitis externa	13	22.4
	Otitis media	45	77.6

swabs. One- microliter portions of the bacterial suspensions were distributed into Nutrient agar, Blood agar, MacConkey media, Pseudomonas selective medium, and *Staphylococcus* agar-110. Before enumeration and identification of each plate, the bacteria were incubated face down at 37°C for at least 24–48 hours. Tests on glucose oxidation/fermentation, lactose fermentation, oxygenase, nitrogenase reduction, indole reaction, urease generation and the Voges-Proskauer, arginine and ornithine utilization of pure well-isolated colonies were carried out. The VITEK 2 system (bioMerieux, Marcy l'Etoile, France) assays were used to identify these bacterial species.

### Antibiotic Susceptibility Tests of *S. aureus* and *P. aeruginosa*

The original nutrient agar stock cultures were used in all studies to circumvent the defeat of antibiotic resistance that might ensue when frequently subculturing bacteria. Five µL sterile broth was used to culture *S. aureus*, and *P. aeruginosa* isolates for 18 hours at 37°C. It was then seeded into Müller-Hinton Agar plates with a loopful of 5 µL sterile PBS. After the inoculum had dried, sterile forceps were used to apply antibiotic discs to the infected medium and gently push down to achieve uniform contact. Antibiotic resistance was determined by the width of the inhibitory zones surrounding the antibiotic discs after 48 hours of incubation at 37°C. Oxoid standard antibiotic discs were utilized. After 24 hours of incubation at 37°C, the diameter of clear zones around the antibiotic discs showing bacterial growth inhibition was determined.<sup>29</sup> According to the manufacturer's specifications, bacterial isolates were categorized as "resistant", "inter-mediate", or "sensitive".<sup>30</sup>

### Plasmid Isolation

Lauryl Broth (LB) tubes and appropriate antibiotics, inoculated with individual bacterial isolates, were incubated in a shaker at 37°C overnight as described by Liu.<sup>31</sup>

### Analysis of Plasmid DNA Restriction

Plasmid DNA was isolated using the density gradient centrifugation method, followed by digestion with the restriction enzyme Hind III (Promega, USA) and electrophoresis on a 0.8% agarose gel to assess the size of the plasmid DNA. Hind III restriction endonuclease purchased from Promega, USA was used to digest the isolated plasmid DNA.

### Exotoxins a Gene Detection

PCR was used to detect *Ps.aeruginosa* from clinical samples by amplifying a 396-bp region of the exotoxin A (ETA) structural gene sequence. PCR amplification of *P. aeruginosa* (Exotoxin A) specific sequence. The primers used in this experiment and conditions are listed in Table 2.

**Table 2** Primers and PCR Condition Used in Detection of Exotoxin a

Primer <sup>a</sup>	ETA1	ETA2
Size	24	24
Region	1001–1024	1373
Sequence	5'-GACAACGCCCTCAGCATCACCAGC-3'	5'-CGCTGGCCCATTCGCTCCAGCGCr-3'
$T_m$ (C°) <sup>b</sup>	73	75

**Notes:** <sup>a</sup>The structural gene extends from position 959 to 2662. <sup>b</sup>The melting temperature ( $T_m$ ) was calculated by using the following formula:  $T_m = [4^{\circ}\text{C} (\text{G}+\text{C} \text{ content of oligonucleotide}) + 2^{\circ}\text{C} (\text{A}+\text{T} \text{ content oligonucleotide})] - 5^{\circ}\text{C}$ .

## Epidemiological Studies

Patients were deliberated to be epidemiological interconnected when they were pickled at the same unit and *P. aeruginosa* was isolated within five days. A relation number (R) was given to each group of associated patients.

## Ethical Considerations

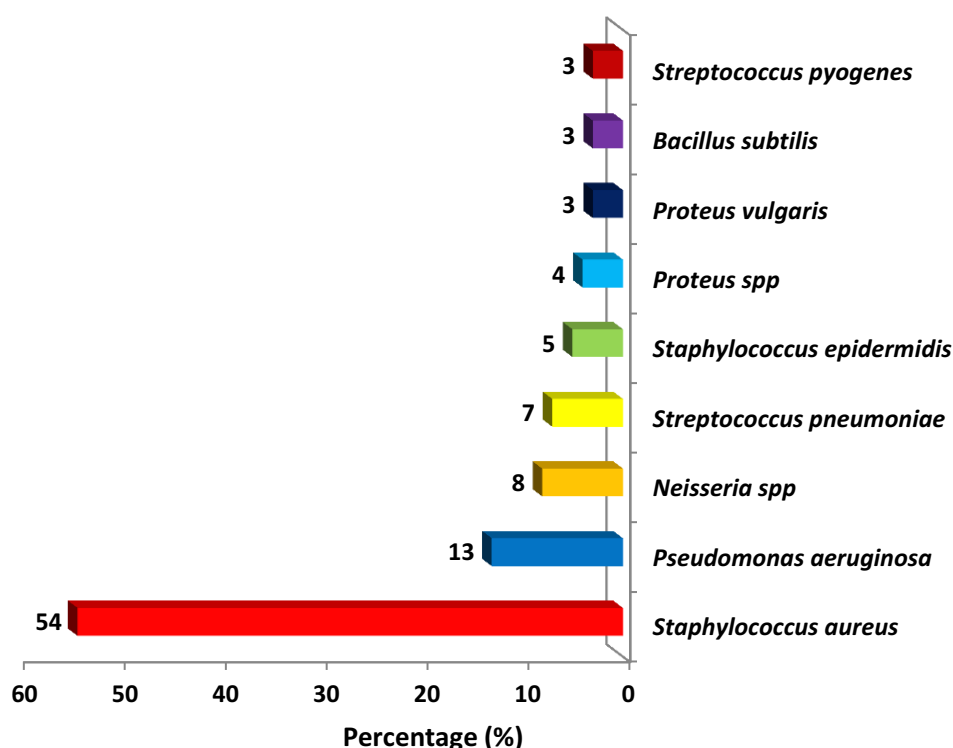
Approval was obtained from the Research Ethics Committee, Jouf University (Ethical Approval No. 3-04-43). All patients provided informed consent, in accordance with the Declaration of Helsinki.

## Statistical Analysis

All the data were entered and evaluated by exhausting Statistical Platform for Social Science (SPSS) version 24.

## Results

A total of 58 samples were collected comprising of 31% females and 69% males. Most common age group involved was 20–40 years (37.9%) followed by less than 20 years (29.3%) whereas a smaller number of isolates were found in elderly age groups (10.4%) (Table 1). The most predominant bacterial isolates were *S. aureus* (54%) followed by *P. aeruginosa* 13%, whereas a smaller number of isolates (3%) were from *Streptococcus pyogenes*, *Bacillus subtilis* and *Proteus vulgaris* respectively (Figure 1). A single growth of organism was found in 56 (96.6%) and mixed growth was observed in 2(3.4%) cases. Occurrence



**Figure 1** Frequency distribution of different bacterial isolates from ear infection.

of gram-positive organisms was higher than gram negative cases. With gram positive isolation rate was 72% and for gram negative organisms it was 28%.

Table 3 and Table 4 show the frequency of antibiotic susceptible outline of most frequencies ear infection strains, *S. aureus* and *P. aeruginosa*, respectively. The overall resistance rate in *S. aureus* was resistant to ampicillin (100%), cefoxitin (90.9%), erythromycin (65.9%), and imipenem (43.2%). However, they were all susceptible to vancomycin, linezolid, tigecycline, rifampin, and daptomycin (100%) (Table 3).

The overall resistance rate in *P. aeruginosa* was 42.9%, with a range of 0% to 100%. There was a high level of resistance rate in the ampicillin, amoxicillin-clavulanate, cefazolin, cefuroxime, ceftriaxone, ertapenem, colistin and

**Table 3** Frequency of Antibiotic Susceptible Outline of *S. Aureus* (N= 44)

Drug Group	Resistant, n (%)	Susceptible, n (%)
<b>Inhibits Cell Wall Synthesis</b>		
Imipenem	19 (43.2)	25 (56.8)
Cefoxitin	40 (90.9)	4 (9.1)
Ampicillin	44 (100)	0 (0.0)
Oxacillin	4 (9.1)	40 (90.9)
Amoxicillin-Clavulanate	4 (9.1)	40 (90.9)
Vancomycin	0 (0)	44 (100)
<b>Protein Synthesis Inhibitors</b>		
Linezolid	0 (0.0)	44 (100)
Erythromycin	29 (65.9)	15 (34.1)
Mupirocin	4 (9.1)	40 (90.9)
Nitrofurantoin	4 (9.1)	40 (90.9)
Tigecycline	0 (0.0)	44 (100)
<b>DNA Synthesis Inhibitors</b>		
Ciprofloxacin	8 (18.8)	36 (81.2)
<b>RNA synthesis Inhibitors</b>		
Rifampin	0 (0.0)	44 (100)
<b>Folic Acid synthesis inhibitors</b>		
Trimethoprim-Sulfamethoxazole	13 (29.5)	31 (70.5)
<b>Cytoplasmic membrane inhibitors</b>		
Daptomycin	0 (0.0)	44 (100)

**Table 4** Frequency of Antibiotic Susceptible Outline of *P. Aeruginosa* (N= 10)

Drug Group	Resistant, n (%)	Susceptible, n (%)
<b>Inhibits Cell Wall Synthesis</b>		
Imipenem	2 (20%)	8 (80%)
Ampicillin	10 (100%)	0 (0.0)
Meropenem	1 (10%)	9 (90%)
Amoxicillin-Clavulanate	10 (100%)	0 (0.0)
Cefazolin	10 (100%)	0 (0.0)
Cefuroxime	10 (100%)	0 (0.0)
Ceftazidime	0 (0.0)	10 (100%)
Ceftriaxone	10 (100%)	0 (0.0)
Cefepime	0 (0.0)	10 (100%)
Ceftolozane- Tazobactam	0 (0.0)	10 (100%)
Ertapenem	10 (100%)	0 (0.0)
Piperacillin-Tazobactam	0 (0.0)	10 (100%)
Colistin	10 (100%)	0 (0.0)
Aztreonam	1 (10%)	9 (90%)

(Continued)

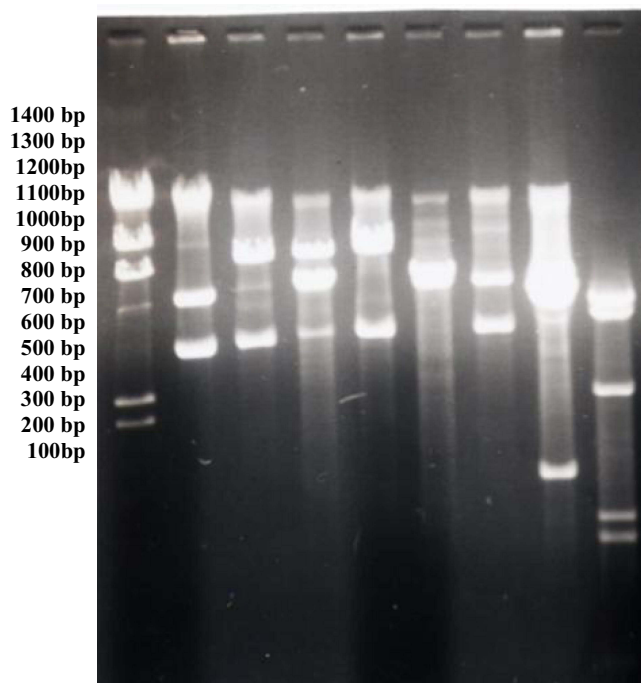
Table 4 (Continued).

Drug Group	Resistant, n (%)	Susceptible, n (%)
<b>Protein Synthesis Inhibitors</b>		
Gentamicin	2 (20%)	8 (80%)
Tigecycline	10 (100%)	0 (0.0)
<b>DNA Synthesis Inhibitors</b>		
Amikacin	0 (0.0)	10 (100%)
Ciprofloxacin	0 (0.0)	10 (100%)
Nitrofurantoin	0 (0.0)	10 (100%)
Levofloxacin	0 (0.0)	10 (100%)
<b>Folic Acid synthesis inhibitors</b>		
Trimethoprim-Sulfamethoxazole	10 (100%)	0 (0.0)

tigecycline among *P. aeruginosa* isolates. However, these isolates were sensitive to ceftazidime, cefepime, piperacillin-tazobactam and all DNA synthesis inhibitors drugs (Table 4).

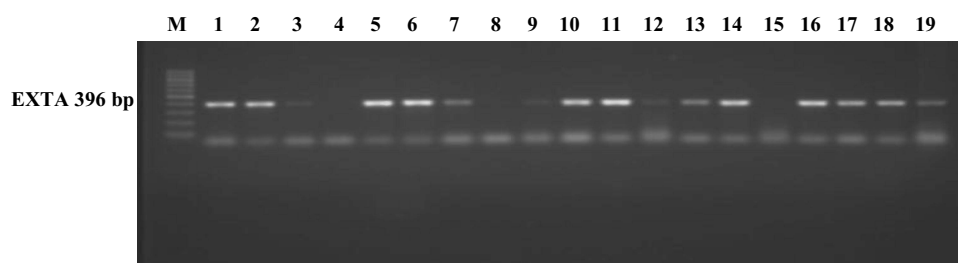
As shown Figure 2 agarose gel profiles of the plasmids after digestion are shown. At least 14 kilobases of plasmid DNA were identified in each one of the isolates. Resistance to ear infection strains can be detected by *Hind III* digestion of their plasmid DNA. A wide variety of antibacterial-resistant plasmids were discovered.

PCR was used to detect *P. aeruginosa* from clinical samples by amplifying a 396-bp region of the exotoxin A (ETA) structural gene sequence. We evaluated the specificity of the PCR assay by testing for amplification of the 396-bp DNA from 19 different strains of *P. aeruginosa* (Figure 3). 84.2% percent of the 19 strains of *P. aeruginosa* were positive for the ETA gene tested by PCR. Two primers, ETA1 and ETA2 (Table 2), were designed on the basis of the nucleotide sequence of the *P. aeruginosa* PA103 upstream region of the ETA gene sequence, which is deleted in strain WR5.



**Figure 2** Electrophoresis agarose gel of plasmids digested by *HindIII* endonucleases. There are a variety of DNA differences can be seen in the digestion products of plasmids from various ear infection isolates in lanes 1–10 (from 100–100 bp). More genetic variety may be seen in all of the bands.





**Figure 3** Representing positive pattern to Exotoxin A (EXTA) Lane M: DNA (ladder) marker (100b). These result showed a 396-bp PCR- Positive DNA for the samples in lanes 2, 3, 4, 6, 7, 8, 10, 11, 12, 13, 14, 17, 18, 19 and negative in lanes 5, 9 and 16 that mean the *P. aeruginosa* have no Exotoxin A as virulence factors in those three isolates.

## Discussion

Every day, OM is a prevalent illness encountered however, it is a non-fatal condition that may cause hearing loss, facial paralysis, and other neurological issues.<sup>32</sup> As a result of the low socioeconomic position of persons with limited affordability, the discharging ear is neglected, resulting in insufficient and incorrect treatment. Early detection and adequate treatment are essential to preventing issues.<sup>33,34</sup> On the basis of their effectiveness, resistance pattern, cost, and side effects, antibiotics are often prescribed. In order to get the most out of therapy, it is critical to know the antibiotic sensitivity of this disease.<sup>35,36</sup> Our findings in our study, 58 samples were obtained comprising 31% females and 69% males. The most common age group involved was 20–40 years (37.9%), followed by 0–20 yrs (29.3%). Related results originated in a study by other investigations who, in their respective studies, found that the common age group involved was 21–30 years, with female predominance (62% and 64.35%) respectively. However, in a study by Wasihun,<sup>37</sup> it was seen that the pediatric age group (6–10 yrs) was most commonly involved with male predominance (64.8%).

The most common bacterial isolates were found to be *S. aureus* (54%) followed by *P. aeruginosa* 13%, according to our examination. Wasihun et al<sup>37</sup> and Agarwal et al<sup>38</sup> reported that *S. aureus* was the most prevalent organism (37.6% and 28.4%, respectively), followed by *P. aeruginosa* (32.8% and 16.7%) in their investigation; single growth organism was 96.6% in our examination, whereas 3.4% showed mixed growth. Similarly, Agarwal et al<sup>38</sup> found monomicrobial bacterial isolates in 80% of patients, whereas only 8% showed mixed growth. *Staphylococcus* species can be treated effectively with vancomycin, linezolid tigecycline, rifampin, and daptomycin.<sup>39</sup> All the cell wall synthesis inhibitors, including ceftazidime, cefepime, piperacillin-tazobactam, and others, had a stronger impact on *P. aeruginosa*.<sup>40</sup> In the current study, the overall resistance rate in *S. aureus* was resistant to ampicillin (100%), cefoxitin (90.9%), erythromycin (65.9%), and imipenem (43.2%). *S. aureus* isolates in a similar investigation by Tadesse et al showed the highest level of antimicrobial resistance to ampicillin (100%), clindamycin (63.3%), cephalothin (59.5%), tetracycline (57%), cotrimoxazole and bacitracin (53.2%) and erythromycin (51.9%).<sup>41</sup> These results are also in agreement with the reports of Mama et al,<sup>42</sup> Soltani et al<sup>43</sup> and Okwu et al.<sup>44</sup>

This investigation found that ear discharge bacterial isolates were MDR (95%). MDR bacteria are on the rise, posing a severe threat to healthcare workers and the general public throughout the world.<sup>45</sup> With limited diagnostic resources, insufficient patient education, and widespread non-human antimicrobial use, MDR burdens developing nations more than other places in the world.<sup>46</sup> This is especially true in places where there is no formal antimicrobial stewardship policy in place.<sup>47</sup> There may be an increased MDR rate in gram negative bacteria owing to the presence of carbapenems enzymes in isolates.<sup>48</sup> According to a recent analysis research, are ESL producers, and most of them are classed as MDR because they generally have genes for resistance.<sup>49–53</sup>

Public health is threatened by the rise of antibiotic-resistant pathogens.<sup>54</sup> A broad variety of bacterial species in the environment have antibiotic resistance genes, therefore they are not restricted to the clinic.<sup>28</sup> If we want a better understanding of antibiotic resistance, we need to take into account all or most non-producer environmental bacteria's resistance genes, which may contain determinants that transmit resistance to themselves, as well as those encoding intrinsic resistance mechanisms.<sup>55–59</sup> Antibiotic resistance continues to offer severe public health risks for *S. aureus*. Genes involved in antibiotic resistance in this bacterium include the common ERY resistance genes (*ermA*, *ermB*, *ermC*), and the TET resistance genes (*tetL*, *tetK*, *tetM*, *tetO*). Transformation (the exchange of nucleic acid), transduction (the insertion into bacteria of a resistance gene mediated by a bacteriophage), conjugation with plasmids or transposons, and

mutation are all methods of acquiring resistance. Resistances to high levels of antibiotics have been associated in most instances to the presence of plasmids.<sup>60,61</sup> A threat to human health does not arise from the presence of resistance determinants in soil and environmental bacteria; however, when these determinants are mobilized to new hosts and expressed in various contexts, such as plasmids and integrons in pathogenic bacteria, they may become an enormous problem.<sup>62–64</sup> Current research has linked antimicrobial-resistant *Staphylococci* to plasmids (R plasmids) that can facilitate the development of drug inactivated enzymes, such as  $\beta$ -lactamase, and other related functions. Plasmids facilitate the transfer of genetic material, particularly antimicrobial resistance genes, across bacteria of different species and genera.<sup>65</sup> Detection of particular strains with possible variations in plasmid content using plasmid profiles is a critical step in epidemiological studies because it is the earliest DNA-based tool for determining serotype-specific reference patterns.<sup>66</sup>

In the Enterobacteriaceae family, plasmid-mediated antibiotic resistance is well-known. To better understand antibiotic resistance, it has been suggested that examining the plasmid types prevalent in pathogenic bacteria is a vital first step. To examine genetic polymorphism, restriction mapping is one of the most accurate procedures available. Plasmid DNA variation has a distinct restriction nucleotide sequence. It is also possible to identify the original plasmid by looking at the distinctive patterns of DNA bands that appear on agarose after treatment with a single restriction endonuclease. All isolated infection isolates were found to have distinct plasmid patterns. These ten designs were highly diverse. Plasmid variety rises as the number of DNA bands proliferations. Pathogenic bacteria that are isolated from one kind of infection show considerable variance in plasmid. Antibiotic resistance in this disease has been shown through a variety of plasmid restriction maps. Bacterial isolates in this examination were found to be extremely diverse in the DNA of their plasmids.<sup>62–64</sup>

Endonucleases *HindIII*, which detect and degrade plasmid DNA molecules at every 5'-AAGCTT-3', were used to treat the plasmids recovered from pathogenic bacteria that causes ear infection in the present investigation. *HindIII* is not sensitive to DNA methylation, were chosen in the double digesting procedure for this purpose because of their ability to degrade DNA. Ploidy detection may be accomplished by several approaches, although the majority depend on amplifying certain sequences or genes that are only found in certain kinds of plasmids. Such approaches are used to determine whether a plasmid is present in the bacterium by finding some of the plasmid's distinctive genes. Because of the large variety of plasmids that identified, restriction mapping was used to identify six-base pair long DNA sequence repeats (restriction sites) in this exertion. Since the *HindIII* restriction sequences was simple to estimate their distribution here, presenting us with an obvious perspective of plasmid DNA polymorphism.<sup>63</sup>

There are several kinds of antibiotics now in clinical use that are resistant to plasmid-encoded antimicrobial resistance.<sup>22</sup> Cephalosporins, fluoroquinolones, and aminoglycosides are notable examples of these classes of antibiotics.<sup>67</sup> In order to facilitate cell-to-cell DNA transfer, many resistance plasmids are conjugative, which encodes the functionalities essential to facilitate their own transfer.<sup>68,69</sup> Others may be mobilized with the aid of a cell-resident conjugative plasmid. Genes that allow cells to link prior to DNA transfer are not found in mobilizable plasmids (which are given by the conjugative plasmid), but the genes that encode the activities necessary for the transfer of their own DNA. Thus, whereas conjugative plasmids tend to be much larger than mobilizable resistance plasmids, they are typically less than 10kb in size and encode only a few genes, including the resistance gene(s).<sup>70,71</sup> This is because the conjugation functions that allow cell-to-cell coupling, particularly between Gram-negative bacteria, require a substantial amount of DNA (20–30 kb) to encode.<sup>72,73</sup> Sex pilus, an exterior filamentous appendage that serves as a grappling hook to unite donor and recipient cells and an envelope-to-envelope contact point when a DNA transfer pore develops to link the cytoplasmic compartments of conjoined cells, mediates this kind of coupling.<sup>74</sup> Conjugative plasmids in Gram-positive bacteria tend to be smaller than those in Gram-negative bacteria, suggesting a somewhat different process of cell-to-cell coupling, which necessitates a lower genetic information storage space.<sup>75–77</sup> Both the donor and recipient cells get a copy of the plasmid after conjugation, which is a replication event.<sup>22</sup>

The host range of a conjugative plasmid might be wide or confined, when it comes to the latter, transmission is often confined to and between a few closely related bacterial species.<sup>78,79</sup> There are plasmids from Gram-negative bacteria that can transfer between different bacterial species with a broad host range, and these plasmids appear to have no host limitation within the division; and using genetic constructs assembled in the test tube, they can transfer but do not survive



in Gram-positive bacteria and unicellular eukaryotic microbes such as yeast.<sup>80–82</sup> The resistance plasmid RP1 (also known as RP4 and RK4) was initially discovered in a clinical strain of *P. aeruginosa* and has since been found in a wide spectrum of bacteria.<sup>83,84</sup> Most, if not all, Gram-negative bacteria seem to be able to accept this plasmid. There are other unrelated resistance plasmids with wide host ranges as well.<sup>85</sup> Host range is not well understood, but one theory represents the kind of surface receptor required for the plasmid's specific conjugation machinery in a possible recipient cell.<sup>86</sup> This structure must be present in the recipient cell for plasmid transfer to take place. The plasmid's host range will be restricted if the receptor is only widely distributed. Another scenario is that the recipient cell is unable to sustain the plasmid's replication, even if the transfer was successful.<sup>22</sup> Wide and restricted host range plasmids are not uncommon, although they are not mutually exclusive. Plasmids have been found in the vast majority of bacterial species that have been studied so far, indicating the existence of a sizable reservoir of genetic material that is mobile.<sup>28,75</sup> Furthermore, the presence of several plasmids is not at all unusual. In conclusion, the management of this infection will be greatly aided by culture and sensitivity tests, as well as ongoing surveillance to assess antimicrobial resistance and advise antibiotic therapy.

*P. aeruginosa*'s pathogenicity is influenced by a number of factors; the identification of distinct virulence genes in *P. aeruginosa* isolates implies that these isolates might be associated with varying degrees of intrinsic virulence and infection propensity. In spite of this, it is important to remember that virulence and other phenotypic features, such as resistance genes, can aid in organism survival, illness dissemination, and severity. Among the several extracellular proteins (LasA and LasB elastases, alkaline protease, protease IV, hemolytic and non-hemolytic phospholipase C, exoenzyme S, and cytotoxin) generated by the opportunistic pathogen *P. aeruginosa*, the most dangerous is exotoxin A (ETA). ETA is a kind of bacterial toxin that transfers the nicotinamide adenine dinucleotide cation (NAD+adenosine)'s diphosphate (ADP)—ribose moiety to its intended target proteins in eukaryotic cells. Toxins like ETA follow a common A–B structure–function paradigm, where the toxin's A domain is enzymatically active and the toxin's B domain binds to a particular receptor on the surface of target cells.<sup>87</sup>

## Conclusion

A single species of microorganism infection, notably *S. aureus* and *P. aeruginosa*, was the most prevalent in our investigation. Vancomycin, linezolid, tigecycline, rifampin, and daptomycin were effective against *S. aureus*. *P. aeruginosa* is becoming less susceptible to routinely used antimicrobials, such as ampicillin, amoxicillin-clavulanate, cefazolin, cefuroxime, ceftriaxone, ertapenem, colistin and tigecycline. In order to avoid difficulties and the development of antibiotic-resistant strains, it is becoming more important to evaluate the microbiological pattern and the antibiotic sensitivity pattern of the microorganisms to provide empirical antibiotics. In order to prevent the emergence of antibiotic-resistant bacteria and their accompanying consequences, we implore antimicrobial monitoring and rigorous adherence to antibiotic usage guidelines. Further studies are required to find strains that are resistant to antibiotics utilizing molecular approaches. Most, if not all, kinds of antibiotics currently in clinical use, including resistance to many that are at the forefront of antibiotic therapy, are encoded on plasmids. Plasmids are found in the majority of bacterial species studied to far, revealing a mobile reservoir of transferable genetic material. *P. aeruginosa* most lethal virulence factor is exotoxin A, which causes intoxication and destroy host cells.

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## Disclosure

The authors report no conflicts of interest in this work

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