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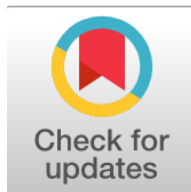
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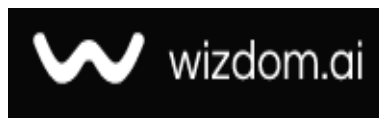
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Antibiotic Resistance Patterns of *Pseudomonas aeruginosa* in Respiratory Infections: Polanya Resistensi Antibiotik *Pseudomonas aeruginosa* pada Infeksi Pernafasan

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Abstract

General Background: Respiratory tract infections remain a major cause of morbidity worldwide and are frequently associated with bacterial pathogens. **Specific Background:** *Pseudomonas aeruginosa* is a prominent hospital-acquired pathogen known for its multidrug resistance, complicating therapeutic management. **Knowledge Gap:** Local and time-specific data on antibiotic susceptibility patterns of *P. aeruginosa* in respiratory infections remain limited. **Aims:** This study aimed to isolate and identify *P. aeruginosa* from sputum samples of patients with respiratory tract infections and to evaluate its antibiotic susceptibility patterns. **Results:** Out of 110 sputum samples, 78 showed bacterial growth, with *P. aeruginosa* identified in 26 samples (33.33%). High resistance rates were observed for cephalosporins, particularly ceftazidime (76.92%) and cefepime (73.07%), as well as levofloxacin (76.92%). Moderate resistance was noted for carbapenems, while higher sensitivity was recorded for amikacin (61.53%) and colistin (53.84%). **Novelty:** The study provides recent localized data on resistance profiles of *P. aeruginosa* in Al-Diwaniyah Governorate. **Implications:** These findings support evidence-based antibiotic selection and highlight the necessity for continuous surveillance to guide effective treatment strategies and limit antimicrobial resistance.

Keywords: *Pseudomonas Aeruginosa*, Respiratory Tract Infections, Antibiotic Resistance, Sputum Samples, Antimicrobial Susceptibility

Key Findings Highlights:

- Pseudomonas aeruginosa* accounted for one-third of culture-positive respiratory samples.
- High resistance was observed against third- and fourth-generation cephalosporins.
- Amikacin and colistin showed comparatively higher susceptibility rates.

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Introduction

Bacterial pathogens that induced respiratory tract infections are common worldwide and raise rates of morbidity and mortality, therefore identification of these bacterial pathogens is important for treatment management and prevention [1]. Both Gram-positive and Gram-negative bacteria, which include a wide variety of bacterial genera, are significant bacterial pathogens of respiratory tract infections such as *Streptococcus pneumoniae*, *Streptococcus agalactiae*, *Staphylococcus aureus*, *Bordetella pertussis*, *Bordetella parapertussis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* [2].

P. aeruginosa is a Gram-negative pathogen that plays a major contributor in causing hospital-acquired infections [3]. Due to its presence and isolation from secretions of patients with bronchitis, asthma and recurrent infections, this bacterium has been identified as a pathogen for respiratory tract infections [4]. One of the most important characteristics of this bacterium resistance to antibiotics and capacity to endure in harsh environmental conditions [5]. Despite the development and discovery of numerous antibiotics, these bacteria are hard to treat, cause health problems, and a high percentage of fatalities because of their widespread resistance to antibiotics [6]. One of the main obstacles its capacity to resist antibiotics, which results from the presence of antibiotic resistance genes [7]. It works by regulating resistance mechanisms, which enable it to harm body tissues and alter its target to eliminate the effects of antibiotics, which is one of the main therapeutic challenges [8].

One of the most widely used treatments for bacterial respiratory tract infections is antibiotics, because antibiotics have multiple mechanisms against bacterial pathogens [1]. Beta-lactam antibiotics, which are frequently used to treat infections brought on by microbes all over the world, are among the most significant of these treatments [9]. Its mode of action involves using penicillin-binding proteins (PBPs) to stop the bacterial cell wall from forming, these include four main types penicillins, cephalosporins, carbapenems and monobactams, which differ in their effectiveness and uses [10]. A beta-lactam antibiotic called Piperacillin is used to treat severe *P. aeruginosa* infections and inflammations, in addition to Tazobactam, which is an inhibitory beta-lactam antibiotic [11]. Also many infections brought on by Gram-negative bacteria are treated with Avibactam, a broad-spectrum, inhibitory beta-lactam antibiotic [12].

Another class of antibiotics that works well against Gram-negative bacteria such as *P. aeruginosa* are cephalosporins, it has been discovered that the third and fourth generation Cephalosporins, Cefotaxime and Cefepime are broad-spectrum antibiotics against bacteria, it has also been discovered that contemporary cephalosporins like Ceftolozane are effective against bacteria [13].

Additionally, it was discovered that among the first-line therapies for infections brought on by the *P. aeruginosa* bacteria are carbapenem antibiotics, because of their high effectiveness against the bacteria and wide range of activity [14]. It targets the cell wall by preventing the transport of peptides that are vital to the bacterial cell wall, It is a bactericidal antimicrobial and is used for Gram-negative bacteria such as *P. aeruginosa* [15]. Among the most widely used carbapenems are Imipenem and Meropenem [16]. Aminoglycosides are effective antibiotics in treating respiratory infections like pneumonia brought on by the *P. aeruginosa* [17]. Like the antibiotics Gentamicin and Amikacin, which prevent the synthesis of proteins, they also interfere with translation by attaching to the bacterial 30S ribosomal subunit, thus having a bactericidal effect [18]. Ciprofloxacin and levofloxacin are among the class of antibiotics known as fluoroquinolones, which have a bactericidal effect, by inhibiting DNA gyrase enzyme, they stop DNA replication in Gram-negative bacteria [19]. Colistin a narrow-spectrum antibiotic that works well against Gram-negative bacteria, is one of the useful medications used to treat *P. aeruginosa* infections [20], it is an polymyxin antibiotic that works by inhibiting the cell membrane and endotoxins of bacteria [21].

Because *P. aeruginosa* a pathogenic agent of respiratory tract infections and exhibits distinct patterns of interaction with antibiotics, this study sought to isolate and diagnose *P. aeruginosa* from patients with respiratory tract infections and examine the bacterium's reaction to antibiotics. Periodic examination and ongoing monitoring of the patterns of response to antibiotics of this bacterium are crucial for effectively managing treatment for the infection because the nature of the effect of antibiotics varies from time to time.

Work Methods

Ethical approval

In order to protect patient's physical and psychology well-being general ethics were adhered to when interacting with them and when collecting samples in this study, which is consistent with instructions of the ethics committees at Al-Furat Al-Awsat Technical University.

Sample Collection

Samples were taken from patients who visited Al-Diwaniyah Teaching Hospital in Al-Diwaniyah Governorate with respiratory infections from 1/3/2025 to 1/7/2025. According to the specialist physician's diagnosis 110 sputum samples were collected, patients were instructed to cough deeply to collect sputum sample by using sterile, sealed plastic containers and the samples were brought to the laboratory, to perform laboratory culture.

Sample Culture

Sputum samples were cultivated on solid culture media, specifically blood agar and MacConkey agar, prepare in accordance with the manufacturer's guidelines (Himedia/India). Using sterile cotton swabs, the samples were striated into plates for culture. The plates were then incubated for a full day 24 hour at 37°C [22].

Isolation and Morphological Diagnosis

Colony size and shape on culture media, hemolytic activity on blood agar, and lactose fermentation on MacConkey agar were used to identify bacterial isolates. purified bacterial colonies were subsequently smeared onto sterile glass slides for microscopic analysis to determine the morphology of bacterial cells and colonies as well as their response to Gram stain, [22]. Catalase, oxidase, and urea tests were among biochemical tests used to identify isolates. In addition to the IMVIC test suite, which comprised the indole, methyl reed, Voges-Proskaur, and Simmons' citrate growth test (identifying a shift in the medium's color from green to blue) [23]. The VITEK test, which is a crucial, precise, and confirmatory test, was then used to confirm diagnosis of *P. aeruginosa* bacterial isolates, the test was conducted in accordance with the guidelines on the prepared kit.

Sensitivity Test of Antibiotics

The antibiotic tablets and dose in microgram used in the study are listed in table (1). The test was conducted by filling tubes with a bacterial suspension prepar of a saline solution containing purified bacterial colonies until the turbidity of the solution in the tubes matched the standard turbidity constant solution (McFarland standard). The culture plates containing Muller-Hilton medium were then inoculated with bacterial suspension using sterile cotton swabs. The antibiotic tablets were then applied to the plates using sterile forceps, and plates were incubated for 24 hours at 37°C. After that, circumference of the antibiotic-resistant and inhibited regions was measured and compared to standard values using [24].

Antibiotic Name	Symbol	Dose
Piperacillin	PRL	100
Avibactam	AVI	20
Tazobactam	TZ	10
Cefepime	FEP	10
Ceftazidime	CAZ	30
Ceftolozane	CTZ	30
Imipenem	IPM	10
Meropenem	MEM	10
Amikacin	AK	30
Gentamicin	CN	10
Ciprofloxacin	CIP	10
Levofloxacin	LEV	5
Colistin	CT	10

Table 1. **Table (1) Antibiotic t ablets m ade by Himedia**
Analysis of Statistical

SPSS version 32 was used for the statistical analysis, and the chi-square test was used to analyze the findings. Because it is statistically significant, a p-value of less than 0.05 was employed [25].

Results

Isolation and Diagnosis

110 sputum samples from patients suffering from respiratory tract infections were gathered for this study investigation. According to table (2), which displays the results of bacterial culture on culture media, 78 samples (70.9%) showed a positive result for growth, while 32 samples (29.1%) showed a negative result and did not show bacterial growth on culture media.

Culture results	Number	Percentage
Cases of positive culture	78	70.9%
Cases of culture negative	32	29.1%
Total	110	100%
X2		38.47
P value		<0.0001

Table 2. **Table (2) Distribution of samples based on bacterial growth on culture media**

The morphological traits of bacterial isolates, such as color and shape of the colonies on culture media, were used to identify the bacterial isolates that were causing respiratory tract infections in patients. Since the goal was to isolate *P. aeruginosa* the focus was on this bacterium. They were discovered to be hemolytic colonies on blood agar medium, encircled by a transparent halo and non-lactose fermenting colonies on MacConkey agar medium, appearing as small, pale-colored colonies. Under a microscope examination, bacterial cells shape revealed that they were rod-shaped and Gram-negative cells. As for the biochemical tests, it was discovered that adding drops of hydrogen peroxide to the colonies produced air bubbles, which indicated a positive result for the catalase test. Additionally, oxidase test yielded a positive result because the colonies turned purple 10 to 30 seconds after drops of hydrogen peroxide reagent were added. These bacteria showed a

negative result for the indole test, as no red ring appeared at the top of the medium following the addition of kovacs reagent, similarly methyl red test yielded a negative result, as the medium did not turn color from yellow to red after adding the methyl red reagent. The results demonstrated that this bacterium is agile for growth testing on simmons citrate agar, because medium's color changed from green to blue, indicating the use of citrate as a source of carbon. The diagnostic results also demonstrated that *P. aeruginosa* were negative for the urease test after being cultivated on urea agar, because medium did not change color from yellow to pink, which is a result of urease production [22; 23]. As shown in Table (3), which shows the results of the tests for this bacterium. Additionally, since the VITEK device is one of the tests that provides accurate results, the bacterial isolates were diagnosed using it to confirm diagnosis. The findings demonstrated that 99% of the isolates were related to *P. aeruginosa* bacteria.

Bacteria	Tests	Result
<i>P. aeruginosa</i>	Gram stain	-