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Prevalence of *Pseudomonas aeruginosa* Isolates and their Antibiotic Susceptibility among Patients and Healthcare Workers in Three Hospitals of Duhok City/Iraq

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Abstract

Objectives: *Pseudomonas aeruginosa* is opportunistic gram-negative bacillus and a major human pathogen belongs to family Pseudomonadaceae, it causes several nosocomial infections including pneumonia, urinary tract, surgical sites, otitis externa, and soft tissues.

Methods: The study was conducted from April 2021 to January 2022 and involved the prevalence of *Pseudomonas aeruginosa* isolates and their susceptibility to different antimicrobial agents among patients and healthcare workers specimens in three hospitals of Duhok city. The collected specimens were examined and cultured on different media in the Advanced Microbiology Laboratory, Azadi teaching hospital. The isolated bacteria were identified according to their morphological and biochemical properties.

Results: Out of 324 specimens, 29.32% (95/324) of the isolates were identified as *Pseudomonas aeruginosa*, isolated from 26.89% patients and 40% healthcare workers. Regarding isolate rates among specimens, the highest rate (48.78%) was from sputum, with a highly significant ($P < 0.001$) difference from other sources. Females had a non-significantly higher isolate rate than males (28.19% vs 25.22%), ages, > 50 years had the highest isolate rate (72.88%), while the lowest rate 6.25% was among ages > 10–20 years, with highly significant ($P < 0.001$) differences among them. Specimens from Heevi hospital showed a non-significantly higher isolate rate (28.57%) than other hospitals. Isolates highest susceptibility was to Colistin (88.7%)-followed by Imipenem (78.9%), while they were 98.6% resistant to ampicillin and 100% resistant to Amoxicillin, Erythromycin and Trimethoprim/Sulfamethoxazole. A high rate of extensively drug-resistant (19.72%) *Pseudomonas aeruginosa* isolates was documented among patients who attended these hospitals with the highest (31.25%) from wounds.

Conclusion: These findings will be helpful to advise treatment with appropriate antibiotic strategy against multi- and extensively drug-resistant *P. aeruginosa* to cope with the chances of evolving resistant pathogens.

Keywords: *Pseudomonas aeruginosa*, patients, healthcare, equipment, antibiotic susceptibility

Introduction

Pseudomonas aeruginosa is a ubiquitous gram-negative bacillus belongs to family Pseudomonadaceae.[1] It can be isolated from humans and animals; it survives in a variety of environments including water and soil.[2] *Pseudomonas aeruginosa* is responsible for nosocomial infections and hospital acquired infections such as otitis media external, ulcerative keratitis, soft tissue, cystic fibrosis, urinary tracts, skin, and surgical site infections.[3-5] It can resist different physical conditions and can survive in the hospital and community settings.[6] Infections caused by *P. aeruginosa* are difficult to be treated due to its resistance to a variety of antibiotics, including aminoglycosides, quinolones and β-lactams.[7] *Pseudomonas aeruginosa* often overcome the host defenses by producing several virulence agents causing infection and damage to the tissues such as exotoxin A (*ExoA*), exoenzyme S (*Exo S*) and outer membrane proteins (*OprI*).[8-10] In clinical settings, *P. aeruginosa* can form biofilms which protect the pathogen against the host defense during chronic infections.[11-13] This bacterium can be isolated from equipment's and ventilators in intensive care units.[14,15]

Since the introduction of antibiotics during the middle of the 20th century, antibiotic resistance has emerged and failed to treat several human diseases.[16] Resistant pathogen to the β-lactam antibiotics has emerged rapidly due to the overuse of antibiotics. One of the main mechanisms of resistance to the β-lactam antibiotics is enzymatic degradation by β-lactamases produced mainly by gram negative and positive bacteria, especially by Enterobacteriaceae.[14,17,18] For a long time, one of the most important antibiotics used for treatment of Enterobacteriaceae was carbapenem; however, carbapenem-resistance among Enterobacteriaceae is emerging recently and causes serious threat to public health.[19,20] Overuse of antibiotics may lead to development of ESBL producing bacteria which produce Extended-Spectrum β-lactamases (ESBLs) that mediate resistance to extended spectrum e.g., third generation cephalosporins as well as monobactams by hydrolyzing the β-lactam ring of antibiotic.[7,21]

The high prevalence of *Pseudomonas aeruginosa* isolates observed in hospitals, with both antibiotic-resistant and susceptible strains, encouraged us to conduct a prospective cohort for estimating the prevalence and the antibiotic susceptibility of *P. aeruginosa* among patients and healthcare workers in three major hospitals of Duhok city to improve our infection control.

Materials and Methods

Sample Collection

The current study was performed in 2 teaching hospitals (the Azadi and Heevi pediatric) and one private hospital (Vajeen) in Duhok city. Three hundred and twenty-four specimens from different sources were analyzed from April 20–21 to January

2022. They included: 83 swabs from patients (42 wounds and 41 sputum) and 181 midstream urine specimens of patients from both genders and various ages (8 months – > 60 years). Furthermore, 60 swabs were taken from the hands of healthcare workers at midday time. The human specimens were collected after taking consents from the enrolled participants or the accompanied parent of the children. Furthermore, an ethical approval to perform the study was obtained from Duhok General Directorate of Health (No. 1808 in 31-8-2021). All specimens were immediately placed in sterile transporting media, clearly labeled with the required information according to a questionnaire designed for the study, kept in a cool box and transported to the Advanced Microbiology Laboratory, Azadi teaching hospital for further processing.

Isolation and Identification of Pseudomonas aeruginosa

In the laboratory, all of the clinical specimens were cultured on MacConkey, Nutrient, Blood and Cetrimide agars and incubated at 37˚C for 24 hours. The growing colonies of *P. aeruginosa* were further identified based on gram stain, morphological characteristics on Cetrimide and MacConkey agars in addition to biochemical tests such as oxidase and catalase test.[22] Each isolate of *P. aeruginosa* was maintained in 10 ml Brain Heart Infusion agar slants and stored at 4˚C to be used for antibiotic susceptibility test (AST).

Antibacterial Susceptibility Test

The antibiotic susceptibility test of the isolated bacteria was assessed manually using Disc-Diffusion (Kerby - Baur) method according to National Committee for Clinical Laboratory Standards (NCCLS, 2020) criteria as indicated in Table 1.

Table 1. **Showing the antibiotic used in the susceptibility test (all were purchased from bioanalyze company (Turkey)**

No.	Antibiotics	Abbreviation	Disc concentration
1	Imipenem	IPM	10 mg
2	Meropenem	MEM	10 mg
3	Ceftazidime	CAZ	30 mg
4	Ceftriaxone	CRO	10 mg
5	Cefepime	FEP	10 mg
6	Amikacin	AK	10 mg
7	Gentamicin	CN	10 mg
8	Ciprofloxacin	CIP	10 mg
9	Levofloxacin	LEV	5 mg
10	Aztreonam	ATM	30 mg
11	Trimethoprim/ Sulfamethoxazole	SXT	25 mg
12	Erythromycin	E	10 mg
13	Ampicillin	AM	25 mg
14	Amoxicillin	AX	10 mg
15	Colistin	CO	10 mg

Results

Prevalence of Pseudomonas aeruginosa in Patients and Healthcare Workers Specimens

The result of the present study showed that 29.32% (95/324) of the specimens were positive for *P. aeruginosa*. The highest rate (40%) was recorded from healthcare workers followed by patients (26.89%) as shown in Table 2. Statistically a highly significant difference (P = 002) was observed between both groups.

Table 2. **The specimen sources and the numbers and rates of *P. aeruginosa* positive cultures**

Specimen's sources	No. of examined specimens	No. of positive	%
Patients	264	71	26.89
Healthcare workers	60	24	40
Total no	324	95	29.32

P-value = 0.002; X^2 = 12.917.

The prevalence of *P. aeruginosa* among various patient specimens is shown in Table 3. The overall rate of isolates among all patient specimens was 26.89% (71/264). Among clinical specimens, the highest rate of isolates was 48.78% among sputum samples, followed by wounds and then urine specimens (38.1% and 19.34%), respectively. Statistically these differences were highly significant (P < 0.001).

Table 3. **The types of the examined specimens**

Source of specimens	No. of examined	Isolates no (%)
Sputum	41	20 (48.78)
Wound	42	16 (38.1)
Urine	181	35 (19.34)
Total	264	71 (26.89)

P-value < 0.001; X^2 = 17.927.

The distribution of *P. aeruginosa* isolates among patients of both genders and various ages is shown in Table 4 with a highest rate in females as compared to males (28.19% vs 25.22%). Statistically the differences between both genders were non-significant (P = 0.589). As regards to age, the highest rate of isolates (72.88%) was recorded among ages above 50 years, while the lowest rate (6.25%) was found among ages above 10–20 years, with statistically highly significant differences (P < 0.001) between them.

The number and rate of *P. aeruginosa* isolates in patients attended public and private hospitals is shown in Table 5. In general, the highest rate of *P. aeruginosa* isolates was reported among samples collected from Hevii pediatric hospital (28.57%), followed by Azadi Hospital (26.43%) and Vajeen hospital (25.93). Statistically the differences among various samples sources were non-significant (P = 0.932).

Antibacterial Susceptibility Test

The 71 isolates were tested for their antibiotic susceptibility against fifteen antibiotics that were usually prescribed by

Table 4. **The relationship between the number and rates of *P. aeruginosa* isolates among both genders and various ages**

Variables	No. examined	*P. aeruginosa* No. %	*P*-value X_2
Gender			
Males	115	29 (25.22)	*P*-value = 0.589 X^2 = 0.2911
Females	149	42 (28.19)	
Age	No examined from each age group		
< 1–10	59	15/59 (25.42)	*P*-value < 0.001 X^2 = 173.777
> 10–20	48	3/48 (6.25)	
> 20–30	40	3/40 (7.5)	
> 30–40	38	3/38 (7.89)	
> 40–50	20	4/20 (20)	
> 50	59	43/59 (72.88)	
Total	264	71 (26.89)	

Table 5. **The distribution of *Pseudomonas aeruginosa* isolates among patients attended the studied hospitals**

Sample source	Number examined	Number infected %
Azadi hospital	140	37 (26.43
Heevi hospital	70	20 (28.57)
Vajeen hospital	54	14 (25.93)
Total	264	71 (26.89)

P-value = 0.932; X^2 = 0.141.

physicians Table 6. The antibiotic susceptibility of isolated bacterium showed that the highest rate of the susceptibility was to Colistin (88.7%) followed by Imipenem (78.9%), Aztreonam (70.4%), while 98.6% of the isolates were resistant to ampicillin, and 100% were resistant to, Amoxicillin, Erythromycin and Trimethoprim/Sulfamethoxazole.

The number and percent of specimens with multi-and extensive drug resistance based on Magiorakos et al. (2012).[23] was 19.72% (14/71) isolates of patients suffering from wound, UTI, and lungs infections, with the highest rate (31.25%) in isolates from wounds Table 7. Statistically highly significant differences ($P < 0.1$) were observed between these sources.

Discussion

Pseudomonas aeruginosa is a ubiquitous human pathogen that can affect healthcare workers and immunocompromised individuals and is responsible for nosocomial infections.[24] Patients and healthcare workers are at high risk of infection with *P. aeruginosa* due to their metabolic versatility and ability for adaptation and colonization in wide variety of environments as well as their ability to resistant wide range of antimicrobial agents.[25]

In the current study a total rate of 29.32% (95/324) of *P. aeruginosa* isolates were obtained from the examined specimens, with the highest rate of 40% from the hands of healthcare workers and 26.89% from patients. In some studies, very lower rates than the present study were reported from hands of healthcare workers, in Italy a rate of 3.5%,[26] in Egypt and Saudi Arabia,[27] rates of 10% and 6.7%, respectively and these authors attributed this rate to lack of compliance of health workers to hand washing practice. The current study high rate from hands of healthcare workers might be due to the sampling time as they were taken at midday after healthcare workers got too intimate contact for long time with patients, in addition the wards were very populated with patients and each healthcare worker has to take care of many patients due to limited staff numbers, therefore they didn't have enough time to wash their hands as often as recommended. As stated by,[28] hand hygiene protects healthcare workers and prevents the spread of microbes to patients.

As regards to previous studies from Iraq and other countries, they reported variable rates of infection with *P. aeruginosa* among patients. In Nasiriyah, 19.5%,[29] Babylon, 25%,[30] Kirkuk, 21.6% and 15.5%,[31,32] Baghdad, 36%,[33] in Basra, 50.63%.[34] In both of Egypt and Saudi Arabia rates of 32.8% and 30%.[27] The variation in the prevalence of *P. aeruginosa* in various studies could be related to the type of clinical specimens, type of hospitals, studied population, geographical locations, and differences in hygienic practices.[31]

Regarding gender, females showed the highest rate of isolate carriage than males (28.19% vs 25.22.84%). Similarly in a study in Kirkuk/Iraq a higher rate of isolate carriage was reported among females than males (52.5 vs 47.5%) respectively,[31] even though their rate is double the rate of the present study. Additionally, in Suleimani also, a higher rate of isolates was reported from females than males (57% vs 43%).[35] The higher rates in females are attributed to urolithic mucosal adherence to mucopolysaccharide lining or to anatomical predisposition of females in addition to numerous host factors.[31]

Ages above 50 years had the highest rate of *P. aeruginosa* isolates (72.88%). Previous studies reported variable rates among different ages, in Nasiriyah,[30] found the highest rate (38.9%) of *P. aeruginosa* among ages of 5–25 years. While, in Kirkuk, the ages of 15–30 years, showed the highest rate (45%).[31] The higher rate of *P. aeruginosa* isolates in the elderly patients in the current study might be due to more critical underlying diseases in addition to low immune status at these ages, once they got any infection it is difficult to treat and control, thus they need further prolonged treatment and recovery time.[36]

Among clinical specimens, the highest rate of *P. aeruginosa* isolates was from sputum (48.78%) followed by wounds (38.1%) and the lowest was from urine (19.34%). Similarly in Baghdad,[37] also, reported the highest rate of *P. aeruginosa* in sputum specimens and the lowest in urine samples which were 27.78% and 7.41%, respectively. While in Kirkuk, in one study the highest rate (40%) was from burn patients and lowest (10%) from urine.[31] While in the second study, the highest rate of *P. aeruginosa* isolates (74.19%) was from wounds.[32] This variation in the presence of *P. aeruginosa* among different specimen sources may be related to the sex and age of patients, to specimen type, number, method, season of collection and patient residence.[38]

Regarding the prevalence of *P. aeruginosa* among patients attended public and private hospitals, considerable high rates of this bacterium were isolated from patients of the three hospitals with non-significant ($P = 0.932$) differences between them which were 28.57%, 26.43% and 25.93%, respectively. In a study in Egypt and Saudi Arabia hospitals,[27] also non-significant

Table 6. **Showing the antibiotic susceptibility test by manual technique using disc-diffusion (Kerby - Baur) method according to National Committee for Clinical Laboratory Standards (NCCLS 2020)**

Antibiotics	Specimens			Total no. (71)	Susceptibility %
	No. of sputum (20)	No. of urine (35)	No. of wound (16)		
Colistin	15	34	14	63	88.7
IPM	18	27	11	56	78.9
ATM	18	23	9	50	70.4
LEV	19	18	9	46	64.8
MEM	16	22	8	46	64.8
CAZ	14	24	9	47	66.2
CIP	18	18	8	44	62
CN	15	16	6	37	52.1
AK	15	16	5	36	50.7
FEP	9	13	5	27	38
CRO	13	14	0	27	38
AM	0	1	0	1	1.4
AX	0	0	0	0	0
E	0	0	0	0	0
SXT	0	0	0	0	0

Table 7. **The number and percent of specimens with extensively drug resistance isolates**

Type of specimen	No. examined	No. positive (%)	Extensively drug resistant No. (%)
Sputum	41	20 (48.78)	1 (5)
Wounds	42	16 (38.1)	5 (31.25)
Urine	181	35 (19.34)	8 (22.86)
Total	264	71 (26.89)	14 (19.72)

P-value = 0.1; $X^2 = 4.596$.

difference in rates of *P. aeruginosa* isolates between patients of both studied hospitals were reported. This is possibly due to transmission of *P. aeruginosa* by several routes, including patient-to-patient and environmental contamination.[39,40] In addition to its high adaptability and survival in inanimate surfaces in hospital environment for long time (from 6 hours to 6 months).[14]

In this study, Colistin was the most effective antibiotic recorded (88.7%) which is in line with Oumeri and Yassin[41] in Duhok city/Iraq with susceptibility rate of (91.7). Colistin act on the outer cell membrane of Gram-negative bacteria by binding to lipopolysaccharides and phospholipid, also it displaces Ca^{+2} and Mg^{+2} cations from the phosphate group of lipids and therefore can cause cell membrane disruption leading to leakage of cytoplasmic resulting in cellular death.[42] Also, Imipenem showed high susceptibility rate (78.9%) and previous studies in Duhok reported higher susceptibility rates of 87.3% and 95.2%, respectively to Imipenem.[43] Imipenem is a potent cell wall synthesis inhibitor; its therapeutic effect due to crossing the cell wall through porins and its binding to penicillin binding proteins in the bacterial cell membrane.[19]

The third effective antibiotic in this study was Aztreonam with susceptibility rate of 70.4%, this antibiotic was not studied previously, and it inhibits the synthesis of bacterial cell wall due to its high affinity for penicillin binding protein 3 (PBP3). Therefore, it inhibits the 3rd and last stage of bacterial cell wall synthesis leading to cell lysis that is mediated by cell wall autolytic enzymes.[44] Meropenem showed a lower susceptibility rate of 64.8% which is in line with other studies conducted among immunocompromised patients.[45,43] It's suggested that meropenem is less susceptible than other β-lactams to the inoculum effect.[46] As regards aminoglycoside (Gentamicin and Amikacin) they showed moderate susceptibility rates (52.1% and 50.7%), respectively, such results are consistent with a previous study.[43] This attributed to the inhibition of the synthesis of bacterial protein by Aminoglycoside antibiotics because they binds to ribosomal 30S subunits.[47]

About 19.71% of *P. aeruginosa* isolates were extensively drug resistant based on,[23] with the highest rate of wound and urine isolates (31.25% and 22.86%), respectively. A study in Erbil city/Iraq, also reported high rates of XDR isolates from urine (20.9%) wounds (17.6%) and (4.4%) sputum.[48] While in Baghdad, 72.63% of the XDR isolates were from burn samples.[32]

The high rate of resistant of *P. aeruginosa* isolates to various antimicrobial agents among nosocomial organisms become a serious challenge in the treatment of this bacterium which is mainly due to the wide use of antimicrobial agents as mentioned by WHO,[49] which is a critical role in the spread of resistant bacterial strains by involving various resistance mechanisms like the production of β-lactamases that destroy these drugs.

Furthermore, this bacterium produce biofilm that enable it to survive for months in hospital units, during this time it can acquire the genes responsible for XDR from the hospital environment, thus posing threat to patients with nosocomial infections. The hospital surfaces, staff and visitor's hands can be contaminated with this bacterium and from these sources can spread to the patients and to the community. Therefore, antibiotic susceptibility test is important for *P. aeruginosa* treatment since this bacterium can causes numerous infections in addition to its resistance to many antibiotics and this resistance can be changed over time.[50]

Conclusion

These findings will be helpful to advise treatment with appropriate antibiotic strategy against multi- and extensively drug-resistant *P. aeruginosa* to cope with the chances of evolving resistant pathogens.

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Declarations

Conflict of Interest

All the authors declare that there are no conflicts of interest.

Ethical Approval

An ethical approval to perform the study was obtained from Duhok General Directorate of Health (No. 1808 in 31-8-2021). ■

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