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The Prevalence of Cephalosporins resistance in *Pseudomonas aeruginosa* isolated from clinical specimens in Basra, Iraq

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Abstract- ***Pseudomonas aeruginosa*** **is gram negative rod bacterium.It is commonly found in soil, water, plants and humans. Notably, *P. aeruginosa* has become an emerging opportunistic pathogen in hospitals, which has a major threat to the global health systems, including Iraq. The purpose of this study is to determine the prevalence of cephalosporin resistance in *Pseudomonas aeruginosa* isolated from clinical samples. *Pseudomonas aeruginosa* strains were identified by standard microbiological procedures followed by PCR confirmation. Antibiotic susceptibility testing, for 5 antimicrobials, was conducted according to the Clinical and Laboratory Standard Institute (CLSI) standardized by disk diffusion methodsand VITEK 2 methods. Of the 160 clinical specimens, 81 were positive for *P. aeruginosa.*The results showed that 81 isolates were identified as *Pseudomonasaeruginosa* of which 97.53%,79%, 83%, 81.50 %, 100 and 80.30% were resistant to Cefoxitin, Ceftriaxone, Cefotaxime, Ceftazidime and Cefepime respectively. These findings highlighted a high prevalence rate of cephalosporins antibiotics resistance *P. aeruginosa* at Basra hospitals in Iraq.**

***Keywords*—Pseudomonas aeruginosa, Cephalosporin, antibiotic resistance.**

I. INTRODUCTION

P. aeruginosa is a common nosocomial pathogen. It has been frequently linked to infections, including wound/surgical site, pneumonia, bacteremia, urinary tract and infectionsin people with compromised immune systems and those with burns (Mahmood *et al*., 2020). Although seldom infecting healthy individuals, *P. aeruginosa* is a well know and notorious opportunistic pathogen (Weiner *et al*., 2016;Morin *et al*., 2021), also the most prevalent human opportunistic pathogen associated with nosocomial infections (Pang *et al*., 2019). Indeed, *P. aeruginosa* is capable of evading innate host defence and is highly resistant to a wide spectrum of antimicrobial drugs (Azam & Khan, 2019), making its infections incredibly hard to treat (Pang *et al*., 2019). *P. aeruginosa*is in the "critical" category by WHO "World Health Organization's" priority list of bacterial infections for which new antibiotic research and development are urgently needed (Tacconelli *et al*., 2018;Botelho *et al*., 2019). Because *P. aeruginosa* due to its capacity to rapidly acquire resistance to many types of antibiotics.

Cephalosporins are the most often prescribed antibiotic class, and their structure and pharmacology are comparable to penicillins. It is bactericidal, and its structure includes a beta-lactam ring, similar to penicillin, which interacts with bacterial cell wall formation. Cephalosporins originated from the ancient Acremonium (previously called Cephalosporium). It was discovered in 1945, and scientists have been working to improve the structure of cephalosporins to make them more efficient against a wider spectrum of bacteria. When the structure of cephalosporins changes, a new "generation" of cephalosporins is created. So far, five generations of cephalosporins are available. They are used to treat several organisms and infections. Cephalosporin antibiotics interfere with bacterial cell-wall production, causing the infectious organism to break down. To do this, the antibiotic must penetrate the bacterial cell wall and attach to the penicillin-binding proteins. (Nath *et al*., 2020).

II. MATERIALS AND METHODS

A. SAMPLES COLLECTION

In the present study, 160 samples were collected from different hospitals in Basrah province during the period from 1st November 2021 to 28th February 2022.The samples including burns swabs (106), urine samples (8), wound swabs (16), ICUs (5), Abscess and ulcer swabs (6) and Vagina swabs (19).This study was authorized by the Health Research and the Ethical Commission of Health authorities and consent form was obtained from all participants.

B. Isolation and identification of Pseudomonas aeruginosa

All specimens were investigated by using sterile cotton swabs to collect samples and placed in a tube containing 5 ml of selective media Cetrimide agar, the samples were incubated at 37°C for 24 hours, then cultured by a sterile loop using a streak technique on, *Pseudomonas* chromogenic agar, MacConkey agar, and Nutrient agar, then subjected to microscopic examination and different biochemical tests.

C. Molecular detection of P. aeruginosa

P. aeruginosa strains were identified by standard microbiological procedures followed by PCR confirmation using genus and species-specific primers outer-membrane peptidoglycan-associated lipoprotein (*OprL*) and outer membrane lipoprotein I (*OprI)*. Table 1 showed primer using to identification of *P. aeruginosa.*The total bacterial genome was prepared using a commercial DNA extraction kit (Promega / USA).

PCR assays were carried out in a 25µl volume comprising (12.5 µl) of master mix (Bioneer master mix), (1 µl) of each primer, (2 µl) of bacterial DNA, and (8.5) Nuclease free water, following a 5minute denaturation at 94ºC for 1 cycle, 30 cycles were performed: (30 seconds) at 94ºC, (30 seconds) at 55ºC, and (1 minute) at 72ºC. A 10minute incubation followed the last cycle at 72ºC. All PCR separation is based on (1%) agarose gels and stained by ethidium bromide using an Ultraviolet light transilluminator.

TABLE 1. PRIMERS USED TO IDENTIFICATION OF *P. AERUGINOSA*

rimers	Sequence 5′ – 3′	Amplicon size (bp)	Re ferences
prI	F ATGAACAACGTTCTGA AATTC TCTGCT	249bp	(D e Vos et al., 1997)
	R CTTGCGGCTGGCTTTTT CCAG		
prL	F ATGGAAATGCTGAAAT TCGGC	504bp	(D e Vos et al., 1997)
	R CTTCTTCAGCTCGACG CGACG		

D. Antibiotic susceptibility test and MICs

Five antibiotics from cephalosporin including Cefoxitin, Ceftriaxone, Cefotaxime, Ceftazidime, and Cefepime were selected to be used in the present study due to the "Clinical and Laboratory Standards Institute" (CLSI 2021). The disc diffusion method was used; few colonies was taken by a sterile loop from a pure culture grown for 24 hours on nutrient agar medium and placed in a plain tube containing (5ml) of normal saline to make the bacterial suspension and compared with a turbidity of prepared McFarland concentration (0.5) added (100µl) of bacterial suspension and spread by the swab of sterile cotton onto the Muller Hinton agar. The plates were leaved to dry before the discs of antibiotic were distributed in the plate using 5 antibiotics. After 24 hours of incubation at (37°C), the diameter of the inhibitory zones was measured of sensitive and resistant bacteria were measured according toCLSI 2021standards and guidelines, as shown in table (2).

The findings of the antibiotic susceptibility test were confirmed using the VITEK 2 system, and the MIC values of the tested antibiotics were calculated using the AST- GN30.

Table 2: Antibiotic zone according CLSI.

Antibiotic disc	**"Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm"**		**M IC µg/ml**
	S	**R**	
Cefotaxime CTX (30 µg)	**≥ 22**	**≤ 18**	**≥ 64**
Ceftazidime CAZ(30 µg)	**≥ 18**	**≤ 14**	**≥ 64**
Ceftriaxone CTR(30 µg)	**≥ 23**	**≤ 17**	**N D***
CefepimeCFP(30 µg)	**≥ 18**	**≤ 14**	**≥ 64**

ND* not determined

E. Statistical analysis

SPSS Version 22 was used for the statistical analysis (Package for Social Sciences).

III. RESULTS AND DISCUSSION

A.PREVALENCE OF P. AERUGINOSAIN CLINICAL SPECIMENS

Out of 160 clinical specimens, 83 (51.9%) *Pseudomonas* strains were isolated and 81 (97.59%) of them were *P. aeruginosa*. The distribution of *P. aeruginosa* (n = 81) isolates among the sample sources shown in figure 1.

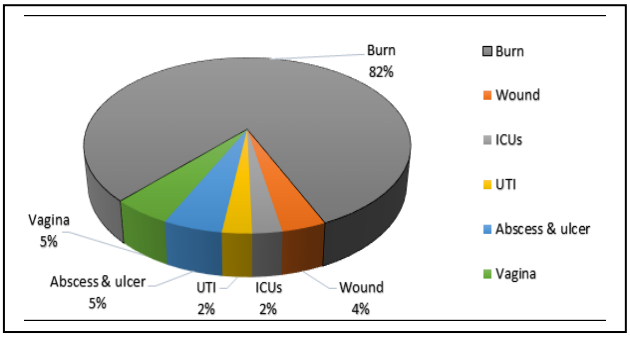

Figure 1: Isolates of *P. aeruginosa* distributed among different source.

In the current research, *P. aeruginosa* was the most predominant species in the isolated strains. *P. aeruginosa* isolates represented 50.6% of clinical *Pseudomonas* isolates. The results have shownthat the prevalence of *P. aeruginosa* isolated from clinical samples was higher than that reported in other local and regional studies (Alkhudhairy & Al-Shammari, 2020; Zarei et al., 2018;Gad et al., 2007).

These results highlight the importance of implementing hygienic strategies and prevention programs in hospital environments, to control the transmission of *P. aeruginosa* in hospital wards because it is one among the most important reasons for nosocomial infections worldwide, including in Iraq, particularly in burn patients.

B. Antibiotic susceptibility test and MICs

Figure 2 shows the profile of antibiotics resistance distributions for clinical isolates (81 isolates) of *P. aeruginosa*. Among the antibiotics tested 2nd, 3rd and 4th generation of cephalosporins which include, cefoxitin, ceftriaxone, cefotaxime, ceftazidime and cefepime.

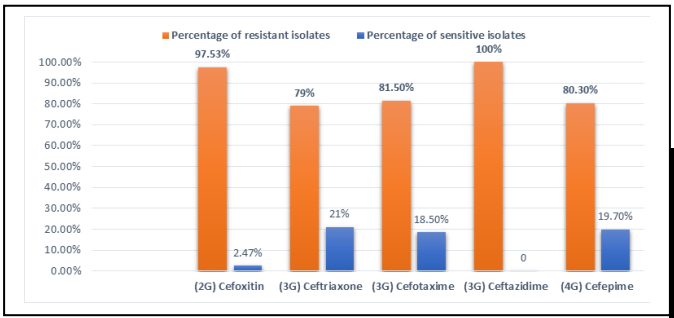

Figure 2: Antibiotic susceptibility profile of isolated *Pseudomonasaeruginosa* strains

Antimicrobial susceptibility tests have shownhigh rates of resistance to antipseudomonal cephalosporines, including 97.53%, 79%, 81.5%, 100%, and 80.3%, were resistant to CX, CRO, CTX, CAZ and CFP respectively, with high value of MICs (≥ 64 µg/ml), most of MIC values exceeded the breakpoint MIC of these antibiotics. The majority of the *P. aeruginosa* strains discovered in this investigation were from clinical sources in Basra and had shown resistance to common antibiotics from cephalosporin.

Resistance to ceftazidime in this study (100%) is highest than in the study carried out by Japoni and colleagues study in Iran (84.3%) and Khosravi and Mihanion their research in Ahwaz, Iran (81%), but resistance to cefotaxime (81.50%) and Ceftriaxone (79%) was much higher than the study carried out by Azeez and Barker in Erbil (16% for all).

The results have showna high prevalence of resistance, (79% to 100%) which is higher than that reported by other studies in Iran (16.5 - 41%), Iraq (12.4%) Brazil (71.4%), andEgypt (70%) (Alkhudhairy & Al-Shammari, 2020;Mirzaei et al., 2020;Ahmadian et al., 2020)(Hamblin, 2016;Kishk et al., 2020),The variation of antibiotic resistance in *P. aeruginosa* could be attributed to improper use of antibiotics in hospitals and communities, accumulating a variety of resistance mechanisms, or could be due the differences in the methods or the sample size used in different research.Regarding the resistance to third- and fourth-generation antipseudomonal cephalosporins observed in our study, they were typically high (74.74 - 98.95%) compared to previous studies in Iraq (41.2%) and many countries in the region including Tunisia (70%), Egypt (68%), Libya (66%), and Yemen (47.1%), but relatively comparable to those reported in Qatar (96.6%) and Bahrain (86%) (Alkhudhairy & Al-Shammari, 2020;Al-Orphaly et al., 2021).

The distribution of antibiotics resistance among the tested isolates according to the type of clinical and environmental samples is shown in table (3). The highest rate of resistance was observed in *P. aeruginosa* isolated from the burn samples.

TABLE 3: DISTRIBUTION OF ANTIBIOTICS RESISTANCE AMONG THE TYPE OF CLINICAL SAMPLES

Antibiotics	Burn	Wound	ICUs	UTI	Abscess & ulcer	Vagina
	n=(66)	n=(3)	n=(2)	n=(2)	n=(4)	n=(4)
Cefoxitin	64 (96.96)	3 (100)	2 (100)	2 (100)	4 (100)	4 (100)
Ceftriaxone	59 (89.39)	0	0	0	4 (100)	1 (25)
Cefotaxime	62 (93.93)	0	0	0	2 (50)	2 (50)
Ceftazidime	66 (100)	3 (100)	2 (100)	2 (100)	4 (100)	4 (100)
Cefepime	58 (87.87)	1 (33.33)	1 (50)	1 (50)	2 (50)	2 (50)

In conclusion, the overall present results showsthat most of these microorganisms have high resistance to cephalosporin antibiotics with rates ranged 79% to 100%. These findings alarm the Health Authority to implement an accurate infection control program and administration of appropriate antibiotic treatment procedures in our hospitals. There were some limitations. The sources and the number of clinical samples were not enough to generalise the conclusions to the entire country. We did not examine all the agents in the all-antibiotic classes to determine the MDR, XDR and pan drug resistance (PDR) isolates. The detection and molecular characterisation of *P. aeruginosa* strains are advised for infection prevention and control, as well as possibly defining the risks of developing severe illnesses and adverse outcomes, in addition to preventing the use of inappropriate antibiotic intake.

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