Antibiogram of Pseudomonas Aeruginosa Isolates from Patients with Diabetic Septic Wounds Attending Two Public Hospitals in Khartoum, Sudan.

Thweaba Hassan Salih Omer
University of Khartoum Faculty of Medicine
Samia Ali Mohammed Mustafa
University of Khartoum Faculty of Medicine
Sagad Omer Obeid Mohamed (s.oom123@yahoo.com)
University of Khartoum Faculty of Medicine https://orcid.org/0000-0003-0597-2397

Research note

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Abstract

Objectives: Treating infections caused by *Pseudomonas aeruginosa* is challenging. In addition to its intrinsic ability to develop resistance to multiple classes of antibiotics, it also produces Extended Spectrum β lactamases (ESBL). This study aimed to determine the antimicrobial susceptibility pattern and to determine the ESBL status among the *P. aeruginosa* isolates from patients at two public military hospitals in Khartoum, Sudan.

Results: A total of 34 isolates of *P. aeruginosa* obtained from patients with diabetic septic foot wounds were tested for their antibiotic sensitivity patterns. Resistance occurred most commonly to Ceftazidime (35%), followed by Ciprofloxacin (20.6%) and Piperacillin (14.7%). We found that 17.6% of the *P. aeruginosa* isolates were ESBL producers and all of these isolates were sensitive to Meropenem. The chi-squared test showed a significant association between the ESBL status and antimicrobial resistance to Amikacin, Ceftazidime, and Piperacillin. The independent T-test showed a significant association between the ESBL status and resistance to Cefepime. The presence of drug-resistant *P. aeruginosa* poses a serious health problem. This study suggests sparing the Carbapenems for the ESBL producing *P. aeruginosa* in our setting. Our findings highlight the need for effective surveillance and antibiogram-guided antibiotic prescription.

Introduction:

Pseudomonas aeruginosa is an opportunistic Gram-negative, rod-shaped bacterium that is frequently implicated in community-acquired and nosocomial infections [1]. It has a natural resistance to different classes of antimicrobial agents, along with the ability to acquire resistance to all other treatment choices [2]. Several studies have reported that *P. aeruginosa* is becoming a multi-drug resistant organism [3–4]. Diabetic patients are highly vulnerable to septic foot wound infection, which is a noteworthy source of morbidity and hospitalization and lower limb amputation [5]. Among those patients, *P. aeruginosa* is a frequently isolated microorganism [6].

Extended-spectrum beta-lactamases (ESBL) enzymes confer resistance to most of the beta- lactam antibiotics, including Penicillins, Cephalosporins, and the Monobactam [7]. Infections with ESBL-producing organisms have been associated with poor outcomes [7]. Antimicrobial resistance threatens the effective prevention and treatment of an ever-increasing range of infections caused by the microorganisms and increases the hospital stay and thus leading to increase economic burden [8]. Nowadays, the problem of resistance to beta-lactamase drugs and extended-spectrum Cephalosporins is becoming a serious problem, and we envisage that shortly, the world will need new antibiotics to replace the existing ones [8].

Antibiograms are often used to assess local susceptibility rates, as an aid in selecting the empiric antibiotic therapy, and in monitoring resistance trends over time within an institution [9]. Continuous update and follow up of the current patterns of the *P. aeruginosa* antibiotic sensitivity is required to cope

with the rate of the MDR emergence. This aim of this study was to assess the current antibiotic sensitivity patterns of *P. aeruginosa* and to determine the prevalence of ESBL among the *P. aeruginosa* isolates from patients with diabetic wound infections.

Methods:

Study design and setting:

A hospital-based cross-sectional study was conducted in September 2016 in Omdurman Military Hospital and Bahri Military hospital in Khartoum state, Sudan. These settings were chosen because they are public tertiary healthcare facilities providing specialized clinical inpatient and outpatient services for a significant number of the Khartoum state population. Patients diagnosed with diabetes mellitus and presented to these hospitals with diabetic septic foot wounds were tested for the presence of *P.aurgenosa*. The wounds were swabbed via sterile swabs in the deepest part of the ulcers after the patients had undergone sharp debridement of all the necrotic and stained tissue until clean granulation tissue was obtained. The recovered *P.aurgenosa* isolates were submitted to the National Laboratory for Public Health in Khartoum for culture and sensitivity testing.

Isolation and Identification of *P. aeruginosa* Isolates:

The clinical specimens were cultured on MacConkey agar and those who were non-lactose fermenters were subcultured in Citrimide agar. *P.aurgenosa* isolates were identified with reference to the colony morphology, production of pyocyanin pigment, negative Gram staining, catalase test, and oxidase test. The isolates of *P. aeruginosa* were evaluated for ESBL production by using the phenotypic confirmatory test. The Clinical and Laboratory Standards Institute (CLSI) guidelines [10] for performing an ESBL confirmatory test involve testing Cefotaxime and Ceftazidime alone and in combination with Clavulanate, which inhibits the activity of the ESBL enzyme and makes the organisms appear more sensitive to the drug + Clavulanate combination. An increase of $\geq$ 5 mm in the zone of inhibition for Ceftazidime + Clavulanic acid compared to Ceftazidime alone was used to confirm the presence of ESBL producers [11].

Antimicrobial Susceptibility Testing:

We used the modified Kirby-Bauer disc diffusion technique on Mueller Hinton agar (HiMedia, India) to perform antimicrobial sensitivity testing of the *P.aurgenosa* isolates according to the guidelines of CLSI [10]. A disc of blotting paper impregnated with a known volume and concentration of an antimicrobial was placed on a plate of susceptibility testing agar uniformly inoculated with the bacterial isolates. The antimicrobial diffuses from the disc into the medium, and the growth of the test organism is inhibited at a distance from the disc in relation to the susceptibility of the organism. Strains susceptible to the antimicrobials were inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grow up to edge of the disc.

We assessed the antimicrobial of *P.aurgenosa* to the commonly used antibiotics for treatment of *P.aurgenosa* in Khartoum, Sudan (Ceftazidime (30 µg), Ciprofloxacin (5 µg), Impenine (30 µg), Piperacillin (100 µg), Amikacin (30 µg), and Cefepime (30 µg)). All of these antibiotics were obtained from (Bioanalyse Laboratories / Ankara, Turkey). The inhibition zones were measured and classified according to the CLSI guidelines into resistant, sensitive, and intermediate.

Data Management and Statistical Analysis:

The Statistical Package for Social Sciences (SPSS) software version of 21 was utilized to analyze the data at hand. Descriptive statistics of SPSS provided frequency tables and the distribution of the variables. A Chi-square test was conducted to determine the relationship between ESBL status and antimicrobial resistance. Since resistance to Cefepime is dose-dependent, the independent T-test was used to determine the relationship between the ESBL status and resistance to Cefepime. The p-value of .05 was set as the significance level of the study.

Results:

A total of 34 specimens of *P. aeruginosa* obtained from patients with diabetic septic foot wounds were analyzed in this study. The antimicrobial resistance occurred most commonly against Ceftazidime (35.3%) and the resistance rates to Ciprofloxacin, Piperacillin, and Meropenem were 14.6%, 20.6%, and 11.8%, respectively. Amikacin was the most potent antibiotic against the isolated *P. aeruginosa* for which more than 97% of the isolates were sensitive to it. Antimicrobial resistance profile of the *P. aeruginosa* isolates is shown in (Table 1).

Table 1
Antibiotic susceptibilities of *P. aeruginosa* isolated at two hospitals in Khartoum:

Antibiotics		No.	Per cent %
Amikacin	Sensitive	33	97.1%
	Intermediate	0	0.0%
	Resistant	1	2.9%
Meropenem	Sensitive	28	82.4%
	Intermediate	2	5.9%
	Resistant	4	11.8%
Ceftazidime	Sensitive	22	64.7%
	Intermediate	0	0.0%
	Resistant	12	35.3%
Piperacillin	Sensitive	18	52.9%
	Intermediate	11	32.4%
	Resistant	5	14.7%
Ciprofloxacin	Sensitive	23	67.6%
	Intermediate	4	11.8%
	Resistant	7	20.6%

Regarding ESBL status, the prevalence of positive ESBL among the isolated *P. aeruginosa* was 17.6%. Chi-square test showed a significant association between the ESBL status and antimicrobial resistance pattern. The positive ESBL *P. aeruginosa* producers were more resistant to Amikacin (P = 0.03), Ceftazidime (P = 0.00), and Piperacillin (P = 0.01). However, all of the positive ESBL *P. aeruginosa* producers were sensitive to Meropenem (Table 2). Independent T- test showed a significant association between ESBL status and resistance to Cefepime (t = 2.046, p = .049).

Table 2
Antibiotic susceptibilities of the positive ESBL *P. aeruginosa* isolates:

Antibiotics		ESBL status		X^2, p value
		negative ESBL	positive ESBL	
Amikacin	Sensitive	100.0%	83.3%	4.808,0.028
	Resistant	0.0%	16.7%	
Meropenem	Sensitive	78.6%	100.0%	1.561, 0.458
	Intermediate	7.1%	0.0%	
	Resistant	14.3%	0.0%	
Ceftazidime	Sensitive	78.6%	0.0%	13.357, 0.000
	Resistant	21.4%	100.0%	
Piperacillin	Sensitive	64.3%	0.0%	8.228,0.016
	Intermediate	25.0%	66.7%	
	Resistant	10.7%	33.3%	
Ciprofloxacin	Sensitive	75.0%	33.3%	4.478, 0.107
	Intermediate	10.7%	16.7%	
	Resistant	14.3%	50.0%	

Discussion:

The development of antimicrobial resistance to commonly used antibiotics is largely due to drug misuse, which will lead to the occurrence of more bacterial mutations over time. Updated knowledge of antimicrobial susceptibility profiles of clinical isolates could assist in designing the most appropriate treatment schedule against diabetic wounds infected with *P. aeruginosa* and help in curbing the expanding menace of drug resistance. To the best of our knowledge, this is the first study that determined the ESBL status among the *P. aeruginosa* isolates among the patients in Sudan. The current study showed variable levels of resistance against different common classes of antibiotics used for the treatment of *P. aeruginosa* infection.

In contrary to the findings of Alsammani et al. [12], we found that *P. aeruginosa* was to be sensitive to many antibiotics. Alsammani et al. showed a higher resistance rate to Ciprofloxacin (70%) compared to our finding (20.6%). The difference may be attributed to the different hospital settings, study population, and consumption of the drugs. Also, they reported resistance rates against other antibiotics such as Cephalexin (81.2%), Co-trimoxazole (62%), and Ceftriaxone (43.8%) [12].

In this study, the antibiogram showed a resistance rate of 35.3% to Ceftazidime, which is a lower rate than the one reported by Peshattiwar et al. and Ibukun et al. [13–14]. In this study, Meropenem retained a good anti-pseudomonas activity in this study and as reported by Aziz et al. [13], and *P. aeruginosa* was most sensitive to Amikacin with a resistance rate of only 2.9%. The prevalence of the ESBL among the isolates was 17.6%, which is close to that reported by Peshattiwar et al. and Aziz et al. [13–14].

All of the positive ESBL *P. aeruginosa* isolates were sensitive to Meropenem. Similarly, it has been reported that Carbapenems are the best antimicrobial agent for infections caused by ESBL producers [15]. The association between the antimicrobial resistance and the ESBL status could be attributed to the plasmids, which are responsible for ESBL production [17].

We recommend routine surveillance on antibiotic resistance in the hospitals and we suggest sparing the Carbapenems for the ESBL producing bacteria, and recommend that ESBL testing should be a routine practice done in conjugation with the antibiotic sensitivity, which includes the Cephalosporins. In conclusion, *P. aeruginosa* isolates exhibited variable levels of resistance against different common classes of antibiotics. Resistance occurred most commonly to Ceftazidime and less frequently to Amikacin. The susceptibility data from this study may be worth consideration while implementing empiric treatment strategies for diabetic wounds infected with *P. aeruginosa*.

Limitations:

The main limitations of this study are the nature of a cross-sectional study done in one site and the few numbers of isolates that we could find which could limit results generalization for all settings in the country. Also, there are scarce similar studies to compare the findings of this study with them.

Abbreviations

ESBL: Extended Spectrum β lactamases; CLSI: Clinical and Laboratory Standards Institute

Declarations

- Ethical approval and consent to participate:

Permission for conducting this research was granted by the institutional review board of the faculty of Medicine, University of Khartoum prior to study initiation, and from general directors of hospitals, Khartoum, Sudan. Ethical approval was obtained from the State Ministry of Health in Khartoum state, Sudan. Each respondent's informed written consent was obtained prior to participation.

- Consent to publish:

not applicable

- Availability of data and materials:

The datasets used during the current study are available from the corresponding author on request.

- Competing interests:

The authors declare that they have no conflicts of interests.

- Funding:

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- Authors' contribution:

(THSO) conceptualized the research idea and undertook data collection; (THSO and SOOM) undertook data analysis; (THSO, SAMM, and SOOM) interpreted the results and drafted the manuscript. All authors revised and approved the final manuscript.

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