

Drugs-Resistant *Pseudomonas aeruginosa* Isolated from Various Clinical Specimens in Khartoum, Sudan

MOHAMMED AHMED YAGOUP¹, ABASER ADAM TAHA², AKRAM KHALID MUBARAK³, ABUALGASIM ELGAILI ABDALLA⁴, MOHAMMED EFATIH OURNASSEIR⁵, KHALID OMER ABDALLA ABOSALIF⁶, AYMAN ALI MOHAMMED ALAMEEN⁷, HASAN EJAZ⁸

¹⁻⁶Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Omdurman Islamic University, Omdurman, Sudan.

^{4,6-8}Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Jouf University, Sakaka, Saudi Arabia.

⁷Department of Chemical Pathology, Faculty of Medical Laboratory Science, University of Khartoum, Khartoum, Sudan.

Correspondence to Dr. Abualgasim Elgaili Abdalla, E-mail: gasimmicro@gmail.com; Tel: +966 53 9112018

ABSTRACT

Aim: To identify the drug resistance phenotypes of clinical *Ps. aeruginosa* isolates from various clinical samples in Khartoum state, Sudan.

Methods: This cross-sectional study was carried out during October 2016 up to April 2018. Conventional microbiological tests used for isolation and identification of this organism and Kirby-Bauer disc diffusion technique used to determining antimicrobial resistance patterns.

Results: Out of 150 clinical specimens, 37 (24.7%) were positive for *Ps. aeruginosa*. Sixteen *Ps. aeruginosa* strains (43.24%) were isolated from ear swabs, followed by 15 (41%) from wounds, 3 (8%) from urine, 2 (5%) from sputum and 1 (3%) from eye swabs. *Ps. aeruginosa* isolates were found high resistance to piperacillin (78.4%), followed by cefepime (56.7%), aztreonam (43.2%), ceftazidime (40.5%), gentamicin (18.9%), ciprofloxacin (16.2%) and amikacin (13.5%). However, the isolates were shown low resistance rate to imipenem (8%). Interestingly, we observed that 40.5% of *Ps. aeruginosa* strains were multi-drug resistance.

Conclusion: This study concludes that *Ps. aeruginosa* were resistance to all prescribed anti-pseudomonal drugs and roughly, half of the clinical isolates are multidrug resistance.

Keywords: *Pseudomonas aeruginosa*, resistance, antimicrobials.

INTRODUCTION

Pseudomonas aeruginosa (*Ps. aeruginosa*) is the leading cause of health-care acquired infections. It is most commonly associated with ventilator-associated pneumonia, urinary tract infections, wound infections and eye infections¹. In addition, *Ps. aeruginosa* can be disseminated from the primary site of infection via blood causing serious metastatic infections such as septicemia, meningitis and brain abscess².

Antibiotic resistance is a serious communal health problems since the era of the discovery of antimicrobial drugs. Recently, the emergence of strains with resistance to multiple classes of antibiotics has complicated the decision for the selection of proper drugs³. *Ps. aeruginosa* is an extraordinary pathogen that can develop rapid resistance mechanisms to antimicrobial agents through chromosomal mutations or acquire extra-chromosomal materials from surrounding environments⁴. Multi- and extensive-drugs resistant *Ps. aeruginosa* strains were emerged worldwide and severely reduced treatment options⁵. This problem has arisen due to inappropriate use or discontinuous of antibiotics treatment⁶. Therefore, identification of drug-resistant strains prior to antibiotics exposure is crucial to avoid treatment failure and prevent emerging of drug resistance. This study designed to identify drugs-resistant of *Ps. aeruginosa* strains in Khartoum Hospitals, Sudan.

METHODS

A cross-sectional study was carried out during October

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2016 up to April 2018. The samples were collected from Khartoum Tertiary Hospitals and transferred immediately to the microbiology laboratory at the faculty of Medical Laboratory Sciences, Omdurman Islamic University, Omdurman, Sudan.

One hundred and fifty clinical specimens including swabs (wound, ear and eye), urine and sputum were collected from patients. All patients attended with clinical evidence of infections during the study period were included in this study.

Specimens were cultured on Cetrimide agar, MacConkey agar and Blood agar plates (HiMedia Labs, India), and incubated aerobically at 37°C for 24 hours. Suspected colonies of *Ps. aeruginosa* were identified using colonial morphology, motility testing, Gram's reaction, and conventional biochemical tests.

Ps. aeruginosa isolates were tested for sensitivity to eight different of drugs including ciprofloxacin (5 µg), gentamicin (10 µg), piperacillin (100 µg), amikacin (30 µg), aztreonam (30 µg), ceftazidime (30 µg), cefepime (30 µg) and imipenem (10 µg) (Bioanalyse Ltd., Turkey). Two to three of 24 hours growing colonies were emulsified in sterile normal saline and then, adjusted to 0.5 McFarland's turbidity standard. Next, a sterile cotton swab was immersed in the suspension, excess fluid removed and then, the swab rolled overall the dried surface of Mueller-Hinton agar (MHA) plate. The antibiotic discs were placed on the MHA surface with a sterilized forceps, and subsequently, incubated at 37°C for 16 up to 18 hours and the zone of inhibitions were measured and interpreted according to CLSI recommendations.

Standard microbiological procedures were followed to offer reliable results and reference *Ps. aeruginosa* strain (ATCC 27853) was used to testing the quality of staining, culture, conventional biochemical tests and antibiotic discs. SPSS version 20.0 was used for analyzing the data.

RESULTS

During the study period from October 2016 to April 2018, 150 clinical specimens were collected from patients with clinical evidence of infections and cultured aerobically. Out of them, 37 (24.7%) yielded *Ps. aeruginosa*. The majority 16(43%) were isolated from ear swab, followed by wound swabs 15(41%), urine 3(8%), sputum 2(5%) and eye swabs 1(3%) (Table I). Maximum number of *Ps. aeruginosa* were isolated from elderly patients 22(59%), followed by children 8 (22%) and adults 7(19%) (Table-II).

Table I: Frequency of *Ps. aeruginosa* in different clinical specimens

Sample type	<i>Ps. aeruginosa</i> isolates	
	Number	Percent
Ear swabs	16	43
Wound swabs	15	41
Urine	3	8
Sputum	2	5
Eye swabs	1	3

Table-II: Distribution of *Ps. aeruginosa* among the different age group

Age group	Distribution of <i>Ps. aeruginosa</i>	
	Number	Percent
Children	8	22
Adult	7	19
Elder	22	59
Total	37	100

Table-III: Antibiotics-resistance phenotype of *Ps. aeruginosa*

Antibiotic	Number of resistance	Percent of resistance
Piperacillin	29	78.4
Cefepime	21	56.7
Aztreonam	16	43.2
Ceftazidime	15	40.5
Gentamicin	7	18.9
Ciprofloxacin	6	16.2
Amikacin	5	13.5
Imipenem	3	8

Table-IV: Multi-drug resistance (MDR) rates of *Ps. aeruginosa* isolates (n=37).

Antimicrobial categories	MDR <i>Ps. aeruginosa</i> no. (%)	Resistance phenotype
3	9 (24.3%)	A/B/C*/D*
4	2 (5.4%)	A/B/C**/D**/E
5	3 (8.1%)	A/B/C/D/E
6	1 (2.7%)	A/B/C/D/E/F

Key: A = piperacillin); B = aztreonam; C: cephalosporins (cefepime and ceftazidime); D = aminoglycosides (gentamicin and amikacin); E = ciprofloxacin; F = imipenem.

D* = some isolates resistant to gentamicin. C*: some isolates resistant to cefepime.

C**= some isolates resistant to C; D** = some isolates resistant to D.

The antimicrobial resistance patterns of *Ps. aeruginosa* were analyzed for eight different types of antimicrobial agents as mentioned in the methods section. We found that *Ps. aeruginosa* was highly resistant to

piperacillin 78.4%, followed by cefepime 56.7%, aztreonam 43.2%, ceftazidime 40.5%, gentamicin 18.9%, ciprofloxacin 16.2%, amikacin 13.5% and imipenem 8% as represented in table-III. In addition, 40.5% of *Ps. aeruginosa* isolates were multidrug resistances as displayed in Table-IV.

DISCUSSION

Ps. aeruginosa presents a severe therapeutic challenge and a major health threat globally. Identification and choice of proper antibiotic to initiate therapy is requisite to optimizing the clinical outcome. The present study conducted to investigate the antibiotics resistance phenotypes of clinical isolated *Ps. aeruginosa*.

Out of 150 clinical specimens, *Ps. aeruginosa* were isolated from 37 with a frequent rate of 24.7%. This frequency was similar to that reported in several countries, including Iran (25.7%)⁷, Egypt (21.5%)⁸ and India (20.3%)⁹. However, it was lower than that documented in India (32.1%)¹⁰, and Nigeria (34.23%)¹¹. This variation in the prevalence rate of *Ps. aeruginosa* presented by different studies might be attributed to the type and size of clinical specimens, studied populations, hospitals situations and geographical locations.

Our study showed that 78.4% of *Ps. aeruginosa* were resistant to piperacillin. Previous studies in other countries have been reported similar trends of resistance^{12,13}. Resistance of *Ps. aeruginosa* to cephalosporins antibiotics was ancient fact¹⁴. Cefepime-resistant *Ps. aeruginosa* was secondly ranked in our detectable resistance phenotypes. This consistent with the previous report that cefepime-resistant strains were highly prevalent among clinical isolates^{15,16}. Ceftazidime is one of few anti-pseudomonal with great activity against clinical isolate *Ps. aeruginosa*. However, we found that 40.5% of *Ps. aeruginosa* were resistance to ceftazidime. It was not surprised because the same rate of resistance has been documented previously^{15,17}.

Aztreonam has been approved as an effective treatment for cystic fibrosis patients chronically infected with *Ps. aeruginosa* in 2010¹⁸. Nevertheless, a recent study reported that 59% of *Ps. aeruginosa* were resistance to aztreonam. In the present study, we found that 43.2% of isolated strains were resistant to aztreonam. Similar to our finding, de Oliveira et al. reported a high prevalent 48% of aztreonam-resistant strains among clinical isolates of *Ps. aeruginosa*¹⁹.

Aminoglycoside is crucial for the treatment of various *Ps. aeruginosa* infections²⁰. However, our study showed that 18.9% and 13.5% of *Ps. aeruginosa* strains were resistances to gentamicin and amikacin, respectively. These had tended to be lower than in other countries, such as in China (46.7% and 22.3%)²¹, in Qatar (59% and 67.2%)²² and in Egypt (71.1% and 28.8%)²³ for both gentamicin and amikacin, respectively. Nevertheless, our detected resistance to aminoglycoside was higher than study reported in Saudi Arabia (11.6%) for gentamicin and (7.4%) for amikacin²⁴. This dispute of findings might be attributed to prescribing antibiotics policies and hospitals strategies for management *Ps. aeruginosa* infections²⁵.

Ciprofloxacin has extensively used to treat wide a variety of *Ps. aeruginosa* infections. While, *Ps. aeruginosa* is rapid acquired resistance to ciprofloxacin that creating

therapeutic challenge²⁶. In this study, 16.2% of *Ps. aeruginosa* was found resistance to Ciprofloxacin, which was similar to the resistance rate (16.5%) reported in Saudia Arabia²⁴.

Carbapenems have great bactericidal activity against *Ps. aeruginosa*, while, this notorious pathogen acquisition resistance against these drugs and limited treatment options²⁷. Our isolated strains showed a low rate of resistance against imipenem (8%). This degree of resistance was lower than that documented in several studies²⁷⁻²⁹. This might be attributable to the recent introduction of carbapenems in treatment policy in our hospitals and still low consumable drugs due to their high cost.

MDR *Ps. aeruginosa* strains are increasing all over the world and poses a serious therapeutic dilemma. Our study demonstrated that 40.5% of *Ps. aeruginosa* were MDR. This was roughly similar to the studies documented by Angeletti *et al.*, and Gad *et al.*, showed that prevailing rate of MDR *Ps. aeruginosa* was 36%^{30,31}. The MDR bacterial transmission can be reduced by alcoholic hand rubs and decontamination hospital surfaces^{32,33,34,35}.

CONCLUSION

This study concludes that *Ps. aeruginosa* were resistance to all prescribed anti-pseudomonal drugs. The isolates were comparatively less resistant to imipenem and amikacin. Not surprising, 40.5% of *Ps. aeruginosa* were multi-drug resistance. This data indicating that *Ps. aeruginosa* is unusual drug-resistance bacteria.

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