In Vitro Sensitivity of *Pseudomonas Aeruginosa* to Piperacillin, Azlocillin, Imipenem and Meropenem

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Abstract

Background and Objective: *Pseudomonas aeruginosa* is widely distributed in nature and it is one of the most significant causes of hospital-acquired infections. It shows resistance to several antibiotics because of production of beta-lactamase enzymes, in addition to its intrinsic and mutational resistance. This study was conducted in Khartoum State to evaluate the *in vitro* activity of anti-microbial agents (piperacillin, azlocillin, imipenem and meropenem) against *Pseudomonas aeruginosa* clinical isolates.

Method: Anti-microbial susceptibility testing was done to all clinical isolates using standardized modified Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI).

Results: The results shows that the most effective antibiotics against *P. aeruginosa* was imipenem which was effective against all isolates(100%), followed by meropenem 97.1 %, piperacillin 74.3 % and azlocillin 70 %. Carbapenems β-lactam antibiotics were found more effective than ureidopenicillins β-lactam antibiotics against *P. aeruginosa*, with mean activity of 98.6 % and 72.2 %, respectively.

Conclusion: Carbapenems β-lactam antibiotic group (imipenem and meropenem) can be used for treatment of infection caused *P. aeruginosa* due to its highly activity against *P. aeruginosa* isolates.

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Introduction

Pseudomonas is a genus of Gram-negative, non-spore forming, rod-shaped bacteria are commonly found in soil, water, and decaying matter and including some species that are plant and animal pathogens.[1] It is a significant opportunistic pathogen and a major cause of nosocomial (hospital - acquired) infections.[2] *P. aeruginosa* is an opportunistic pathogen that can infect almost anybody given that the right predisposing conditions.[3] All infections caused by *P. aeruginosa* are treatable and potentially curable. Acute fulminant infections, such as bacteremic pneumonia, sepsis, burn wound infections, are associated with extremely high mortality rates.[4] *P. aeruginosa* is normally resistant to most commonly employed antimicrobial agents.[5] The development of anti-pseudomonal β-lactam compounds, like azlocillin, ticarcillin, ceftazidime, and aminoglycosides such as gentamicin and tobramycin, in a combination was commonly adopted.[5] The increasing frequency of multi-drug-resistant *P. aeruginosa* (MDRPA) strains is leading to limit the antimicrobial options. The definition of MDRPA was established as isolates that were intermediate or resistant to at least three drugs in the following classes: β-lactams, carbapenems, aminoglycosides, and fluoroquinolones.[6] Development of multi-drug resistance by *P. aeruginosa* (MDRPA) isolates requires several different genetic events that include acquisition of different mutations and/or horizontal transfer of antibiotic resistance genes.[7] The extended spectrum penicillins **(**ureidopenicillins**)** are anti-pseudomonal penicillins include azlocillin, mezlocillin and piperacillin**.** These drugs act in synergy with the aminoglycosides against *P. aeruginosa* and most of the Enterobacteriaceae.[8] Carbapenem, a member of the β-lactam family, has a broad spectrum of activity and is stable to most β-lactamases. These properties make carbapenem to be important therapeutic options for treating serious infections involving resistant strains of Enterobacteriaceae, anaerobes,
P. aeruginosa, and *Acinetobacter spp* [9]

P. aeruginosa was and remain one of the most important Gram-negative bacteria that widely distributed and related to many infections, especially that which infect immunocompromised pa-

tients and which are acquired in hospitals.[10] *P. aeruginosa* isolates susceptibility test should be conducted to investigate the MDRPA newly and un-routinely antibacterial agent. This study tested two types of β-lactam antibacterial agents , the first was the carbapenems group which consisted of imipenem and meropenem, and the second one was the ureidopenicillins group involved piperacillin and azlocillin.

Methodology

Sampling and bacterial identification

This study was an observational descriptive study conducted in Khartoum Teaching Hospital, Police Hospital and the National Health Laboratory, Khartoum, Sudan during the period from November 2008 to April 2009. A total number of 70 *P. aeruginosa* clinical isolates were collected from different clinical samples including urine, ear swab, and wound swab samples. All specimens were inoculated on both blood agar and MacConkey's agar and incubated aerobically at 37ºC for 24 hours. Non-lactose fermenting (yellow colonies) and β-haemolytic colonies were purified by subculture on nutrient agar for subsequence identification. Gram stain was done to each suspected of the *P. aeruginosa* clinical isolates. All Gram-negative bacilli were tested with biochemical tests to identify *P. aeruginosa*. Biochemical tests included Oxidase test, Citrate utilization test, Urease test, cultur on Kligler's iron agar (KIA) and Indole were used to identify *P. aeruginosa*.

Antibiotic Susceptibility testing

The susceptibility of *P. aeruginosa* clinical isolates was confirmed by using standardized modified Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[11] Inoculum was prepared from all clinical isolates and its turbidity was adjusted by comparing it with MacFarland turbidity standard. Then, Mueller-Hinton agar plates were inoculated with prepared inoculums and antibiotics discs including piperacillin (Pc) 100 mcg, azlocillin (Az) 75 mcg, imipenem (I) 10 mcg and meropenem (Mr) 10 mcg were applied evenly on inoculated medium using sterile forceps and incubated aerobically at 35°C for 18 hours. Suspension of standard strain (*P. aeruginosa* ATCC 27853) was prepared and dealt with as the test organism to assess the validity of the antibiotics and the other conditions. The

diameter of each zone of inhibition (including the diameter of the disc) was measured to the nearest millimeter by using ruler, and then interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS) for the test organism and standard strain (*P. aeruginosa* ATCC 27853). The results were reported as sensitive, intermediate and resistant to antimicrobial discs used in the study.

Results

All *P. aeruginosa* clinical isolates were tested for their antimicrobial susceptibility using modified Kirby-Bauer disc diffusion method. The antimicrobial discs used were piperacillin, azlocillin, imipenem and meropenem (Figure-1, Figure-2). The results showed that the susceptibility of *P. aeruginosa* isolates to imipenem was 100 %, to meropenem was 97%, to pipracillin was 74.3 % and to azlocillin was 70.0 %. These findings revealed that the most effective antimicrobial agents against *P. eruginosa* was imipenem followed by meropenem, piperacillin and azlocillin and their resistance rates were 0 %, 2.9 %, 25.7 % and 30 %, respectively, (Table -1 and Table-2 respectively). The mean susceptibility of isolates to ureidopenicillins (pipracillin and azlocillin) was 72.2 % and to carbapenems (imipenem and meropenem) was 98.6 % (Figure-3) .There was no single multi-drug resistance *P. aeruginosa* (MDRPA) isolated in this study. Forty seven (67 %) of the total clinical isolates were sensitive to all antimicrobial agents used in the study. Semi-multi-drug resistance *P. aeruginosa* (SMDRPA) was found in 18 (26 %) of all clinical isolates examined (Table-3).

Table -1: Explaining the Susceptibility of *P. aeruginosa* isolates to Carbapenems antimicrobial agents

Carbapenems	Sensitive	Resistant	Total
Imipenem	**70 (100%)**	**0 (0%)**	**70**
Meropenem	**68 (97.10%)**	**2 (2.90%)**	**70**

Table -2: Explaning the Susceptibility of *P. aeruginosa* isolates to Ure-idpenicillins antimicrobial agents

Ureidpenicillins	Sensitive	Resistant	Total
Piperacillin	52 (74%)	18 (26%)	70
Azlocillin	49 (70%)	31 (30%	70

Table -3: The Susceptibility of *P. aeruginosa* isolates to all antimicrobial agents

P. aeruginosa isolates	Frequency	Percentage
Multi-drug resistant *P. aeruginosa* isolates (MDRPA) resist to all antimcrobial agents	0	0
Multi-drug susceptible *P. aeruginosa* isolates (MDSPA) sensitive to all antimicrobial agents	47	67%
P. aeruginosa isolates sensitive only to Carbapenems agents	18	26%
P. aeruginosa isolates resistant to one antibiotic	5	7%
Total	70	100%

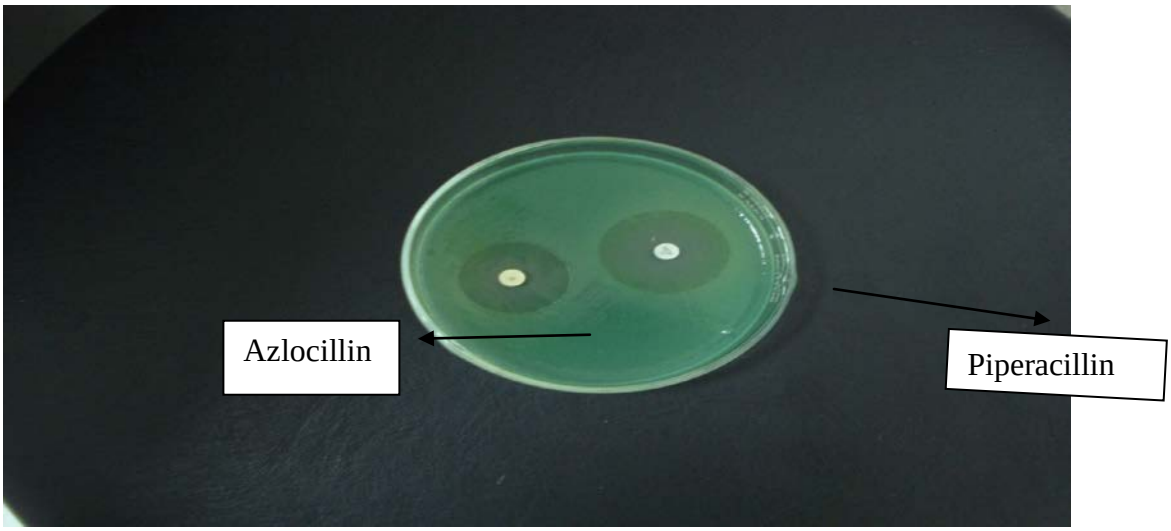

Figure -1: Susceptibility testing of *P. aeruginosa* isolates on Mueller-Hintone agar to Ureidpenicillins agents (piperacillin, azlocillin)

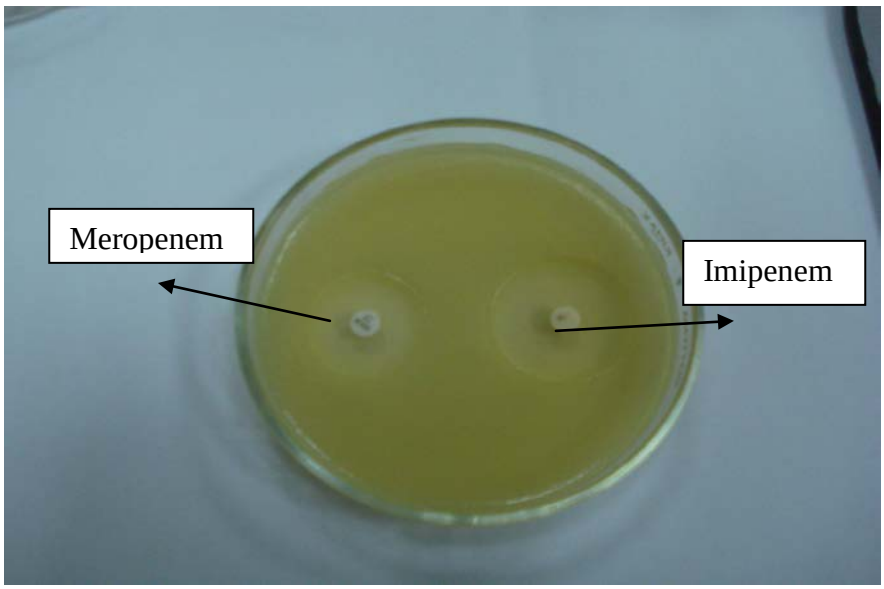

Figure -2: Susceptibility testing of *P. aeruginosa* isolates on Mueller-Hintone agar to Carbapenems agents (imipenem and meropenem).

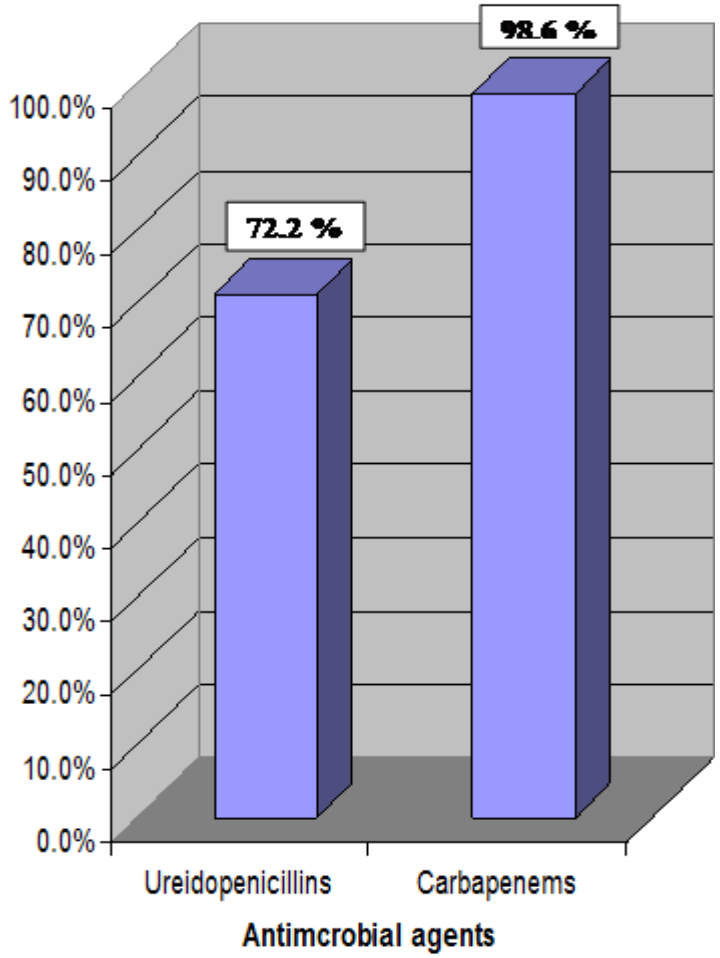

Figure -3: Mean of activity of ureidopenicillins and carbapenems antimicrobial agent against *P. aeruginosa* isolates.

Discussion

Infections with *P. aeruginosa* were and remain a serious problem due to its high spread in hospital environments and to its innate (intrinsic) resistance to the most antimicrobial agents. Therefore, it is very important to study this bacterium and its response to new and non-routinely used antimicrobial agents in order to monitor the emergence of new resistant strains and prevent multi-drug resistance phenomenon. This study was conducted to test the in vitro activity of pipe-

racillin, azlocillin, imipenem and meropenem against *P. aeruginosa* isolated from different clinical specimens using modified Kirby-Bauer disc diffusion technique. The results showed that the susceptibility of *P. aeruginosa* isolates to imipenem was 100 % and to meropenem was 97.1 %. These results were close to those obtained by Dinic *et al* who found that the sensitivity of *P. aeruginosa* to meropenem was 96. 13% and to the imipenem was 95.13% for clinical isolates from outpatients.[12] Also another study was done in New Delhi found that the most active antimicrobial agents against *P. aeruginosa* was imipenem (90% susceptible).[13] However, the results were not in accordance with those reported by Baumgart *et al* who reported the resistance rates to imipenem and meropenem were 45.09% and 40 %, respectively, and also with those reported by Khuntayaporn *et al* who found the resistance rates to imipenem and meropenem were 44.44% and 65.5 %, respectively.[14, 15] Our study showed that the susceptibility of *P. aeruginosa* isolates to pipracillin was 74.3 % and to azlocillin was 70.0 %. This result was in conformance with those observed by NagKumar *et al* who reported 73.7 % sensitivity to piperacillin in his study .[16] However, the results were in discordance with those obtained by Meradji *et al* who reported the resistance rate of *P. aeruginosa* to piperacillin as 36.25%.[17] The results of present study showed that the most effective antimicrobial agents against *P. aeruginosa* were imipenem followed by meropenem, piperacillin and azlocillin and their resistance rates were 0 %, 2.9 %, 25.7 % and 30 %, respectively. The high resistance rate to piperacillin and azlocillin obtained in this study might, possibly, due to their susceptibility to β-lactamase enzyme produced by *P. aeruginosa.* The results of susceptibility testing among the antibiotic groups showed the mean of susceptibility of isolates to ureidopenicillins (pipracillin and azlocillin) was 72.2 % and to carbapenems (imipenem and meropenem) was 98.6 % which were close to those obtained by Saxena *et al* who determined the susceptibility of *p. aeruginosa* isolates to carbapenem group as 89%, but not in agreement with those found by Varaiya *et al* , who reported 74 % mean sensitivity of *P. aeruginosa* to carbapenems antimicrobial agents (imipenem and meropenem).[18, 19] According to our study, there was no single multi-drug resistance *P. aeruginosa* (MDRPA) isolate. However, the results were in discordance with those obtained by Sheikh *et al*, who found 44.4% of P. *aeruginosa* isolates were MDRPA.[9] [20] These differences, could, possibly, be due to the difference of the isolated strains of *P.aeruginosa* in different geographical areas and environmental conditions during the study performance (e.g. sample size).

In conclusion, Carbapenems β-lactam antibiotic group (imipenem and meropenem) can be used for treatment of infection caused *P. aeruginosa* due to its highly activity against *P. aeruginosa* isolates. Although, carbapenems were the most effective anti-pseudomonal anti-microbial agent, they should be taken as a second line antimicrobial agent used only for multi-drug resistant strains (MDRS) and in sever case to avoid development of carbapenems resistance *P. aeruginosa* strains (CRPAS).

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