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Research Article

Prevalence of Multi Drug Resistant Pseudomonas Species Encoding SPM Metalo Beta Lactamase Gene Collected from Patients with Infected Wound in Khartoum State

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Abstract

Background: Selection of the most appropriate antibiotic is complicated by the ability of *P. aeruginosa* to develop resistance to multiple classes of antibacterial agents, even during the course of treating an infection. As in all genes encoding bacteria, metalloB-Lactamases-producing strains (generally multidrug resistant) cause infections that are difficult to treat and resulting in a high mortality and morbidity rates in the community, an increase in both to extend and diversity of metallo-β- lactamases in *Pseudomonas aeruginosa* severely limits treatment and delay the process of healing the wound of patients suffering from pseudomonal infection, extent the length of hospital stay and chronic care and overall cost of treating the infection, for this reason these study conducted to evaluate the presence of metallo B- lactamase gene encoding Pseudomonas species in Khartoum hospitals.

Aim: To detect Prevalence of Multi Drug Resistant Pseudomonas Species Encoding SPM Metalo Beta Lactamase Gene Collected from Patients with Infected Wound in Khartoum State.

Materials and method: The study was conducted in Khartoum state during the period from February to October 2018-09-22 the aim of the study was to evaluate the prevalence of SPM metalo B-lactamase gene produced by pseudomonades bacteria from infected wound, in Khartoum state. The study includes 380 wound swab collected from Khartoum hospitals. The study showed the prevalence rate of resistance against imipenem was 46 % and after carrying out the phenotypic experiments were identified as of MBL producer. Fourteen species were confirmed by Polymerase Chain Reaction (PCR) method showed positive for Gene SPM-1 among the positive (antibiotic resistant).

Result: A total 380 specimens250 males and 130 females with range from 20-75 years. (Figure-1), from which a total of 70 isolate were identified as *Pseudomonas aeruginosa* from different wound swab samples, collected from patient's attendant a different hospital in Khartoum, rate of resistance against Imipenem was 32(46 %), while the molecular detection of Metalo B –Lactamase encoding genes reveled 14(20%)

Conclusion: This study concludes that *Pseudomonas species* that are considered as represent a serious therapeutic challenge for treatment of both community- acquired and nosocomial infections.

Keywords: *Pseudomonas aeruginosa, metall-β-lactamase, antimicrobial resistance, wound infection, Sudan*

Introduction

Pseudomonas species, particularly Pseudomonas aeruginosa, are gram-negative bacilli commonly found in soil and water. These bacteria are known for their simple nutritional requirements and their ability to survive in various environments, including hospital settings. *P. aeruginosa* is an opportunistic pathogen associated with both community-acquired and nosocomial infections, ranging from mild to severe systemic diseases (1). It is particularly notorious for infecting patients with cystic fibrosis and those with compromised immune systems. The bacterium exhibits a broad range of virulent factors and is inherently resistant to many antibiotics, making infections difficult to treat. Recent studies have documented the emergence of multi- drug resistant (MDR) Pseudomonas strains, particularly those producing metallo-β- lactamases (MBLs), which can hydrolyze a wide spectrum of β-lactam antibiotics, including carbapenems (2). The spread of MBL-producing strains presents a significant public health challenge, as it limits the therapeutic options available for treating infections caused by *P. aeruginosa* (3). The emergence of MBL-producing Pseudomonas species has been reported globally, with various studies highlighting the spread of these resistant strains in hospital settings. In Brazil, for instance, high rates of carbapenem resistance among *P. aeruginosa* isolates were linked to the presence of MBL genes (4). Similarly, studies from Japan and other countries have documented the widespread occurrence of IMP-type MBLs in Pseudomonas and other gram-negative bacteria. In Sudan, the prevalence of MBL-producing Pseudomonas strains and their contribution to MDR profiles in clinical isolates has not been extensively studied (5), making this research crucial for informing local antimicrobial policies and infection control strategies. As it's clearly known thatpseudomonadeseaeruginosarepresents a serious therapeutic challenge for treatment of community-acquired as well as nosocomial infections and selection of the appropriate antibiotic to initiate therapy is essential to optimizing the clinical outcome. Unfortunately; selection of the most appropriate antibiotic is complicated by the ability of *P. aeruginosa* to develop resistance to multiple classes of antibacterial agents, even during the course of treating an infection. As in all genes encoding bacteria, metalloB-Lactamases-producing strains (generally multidrug resistant) cause infections that are difficult to treat and resulting in a high mortality and morbidity ratesin the community, an increase in both to extend and diversity of metallo-β- lactamases in *Pseudomonas aeruginosa* severely limits treatmentand delay the process of healing the wound of patients suffering from pseudomonal infection, extent the length of hospital stay and chronic care and overall cost of treating the infection, for this reason these study conducted to evaluate the presence of metallo B-lactamase gene encoding Pseudomonas species in Khartoum hospitals.

Materials and Methods

Study Design: This descriptive cross-sectional study was conducted in Khartoum state, Sudan, between January and August 2018. The study focused on detecting MBL genes in Pseudomonas species isolated from patients with infected wounds.

Study Area: Wound swabs were collected from patients in Soba Hospital, Fedail Hospital, Ibrahim Malik Teaching Hospital, and Royal Care Hospital. Laboratory work was conducted at the Microbiology Laboratory, Faculty of Medical Laboratory Sciences, International University of Africa.

Study Population: The study population included individuals of all age groups who were treated for infected wounds in the aforementioned hospitals during the study period.

Sample Size: A total of 380 wound swabs were collected, from which 100 isolates were identified as Pseudomonas species.

Data Collection: Clinical data were collected using structured questionnaires and patient records.

Laboratory Procedures

Inoculation and Identification: Wound swabs were cultured on Blood Agar and MacConkey Agar. Non-lactose fermenting colonies were subjected to Gram staining and biochemical tests (Indole, Citrate, Motility, Urease, Kligler Iron Agar, and Oxidase). Confirmation of Pseudomonas species was performed using the API 20 system and PCR.

Antimicrobial Susceptibility Testing: The Kirby-Bauer disk diffusion method was used to assess the antimicrobial susceptibility of the isolates against commonly prescribed antibiotics. The E-test was used for further susceptibility confirmation.

Molecular Identification and MBL Gene Detection:

DNA was extracted using a boiling method, and PCR was conducted to detect MBL genes using specific primers (forward: 5′-CCTACAATCTAACGGCGACC-3′, reverse: 5′-TCGCCGTGTCCAGGTATAAC-3′). PCR products were analyzed by agarose gel electrophoresis.

Quality Control: Sterility of media and accuracy of biochemical tests were monitored using control strains and appropriate aseptic techniques. Ethical consideration The proposal of this study was approved by the medical ethical committee in the stat ministry of health. All the patients or their relatives enrolled in this study were informed about the aim and goals of this study. All patients were also informed about possible publication of needed data. All participants were had a right to delete their data at any part of the study. The Health authority was informed by the processed of this study and the agreement of no patients will get harmed during any part of study was signed.

Results

A total 380 specimens250 males and 130 females with range from 20-75 years. (Figure-1), from which a total of 70 isolate were identified as *Pseudomonas aeruginosa* from different wound swab samples, collected from patient's attendant a different hospital in Khartoum, rate of resistance against Imipenem was 32(46 %), while the molecular detection of Metalo B –Lactamase encoding genes reveled 14(20%).

Table 1. Shows number of specimen's according to the gender.

Age Years	Male No. of Samples	No. of Isolates	Female No. of Samples	No. of Isolates	Total No. of Isolates
20-35	40	7	28	3	10
36-50	92	16	70	12	28
≥50	118	25	32	7	32
Total	250	48 (100%)	130	22(100%)	70(100%)

Table 2. Show the type of wound's.

Wound Type	No. of Samples	Total No. of Isolates
Burns	190	41
Abscess	140	27
Post-Operative	50	2
Total	380	70(100%)

Table 3. Show antibiotic susceptibility

Antibiotics	Resistant %	Intermediate %	Sensitive %
Cefotaxime (ctx)	80	12	8
Chloramphenicol (c)	87	3	10
Cefepime (fep)	89	3	8
Ampicillin (am)	86	2	12
Gentamycin (cn)	67	1	32
Imipenem (ipm)	46	2	42

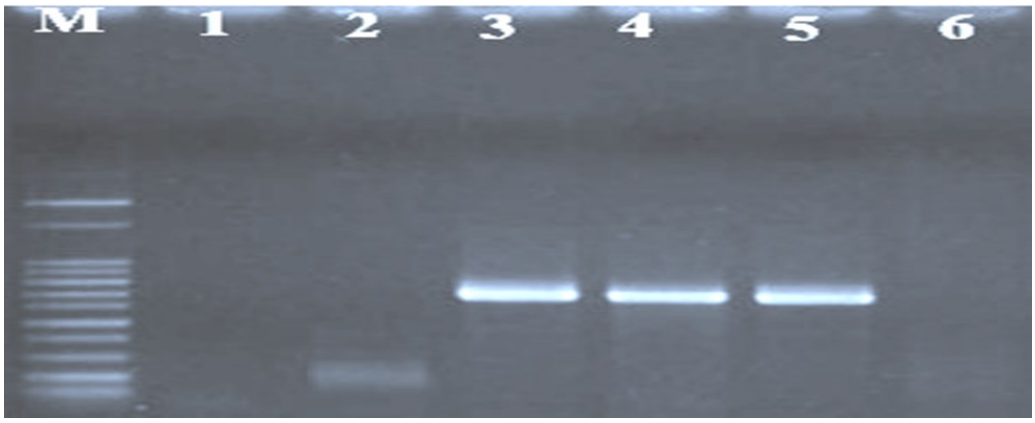

Figure 1*. SPM-1 gene DNA results (770 bp) on % agarose gel. Lane M shows 100 bp DNA marker, lane 1 and 2 shows negative control, lane 3 shows positive control, lanes 4 and 5and shows positive results and lane 6 show negative results.*

Discussion

Wound infection is considered as one of the major health problems in the world, especially when it developed by a multi drug resistant strains as pseudomonas specie. The results of this research showed a high resistance of pseudomonas bacteria to some of the antibiotic's which used in this study, the rate of resistance of imipenem after carrying out the phenotypic experiments were identified as metalo B- lactamase gene producer ,the spread of the P. aeruginosa SPM metalo gene ,among hospitals situated near each other may be explained by the transfer of infected patients , or sharing of common healthcare staff material in hospitals, which result in resistance mechanism. However, this explanation is unlikely for hospitals distant from each other. Selection of the appropriate antibiotic to initiate therapy is essential to optimizing the clinical outcome. Unfortunately, selection of the most appropriate antibiotic is complicated by the ability of Pseudomonas to develop resistance to multiple classes of antibacterial agents, even during the course of treating an infection, latterly an accelerated increase in frequency of multidrug-resistant clinical strains has severely limited the availability of therapeutic options. Pseudomonas bacteria are Multi drug resist organism (MDR) because it resists to different, class of antibiotic which are. Cefotaxime- chloramphenicol- cefepime- ampicillin- gentamicin- imipenem. MBLs are emerging worldwide as an important mechanism of carbapenem resistance among non-fermentative Gram-negative isolates. In this study, the production of SPM-metalo B-lactamase was associated with broad-spectrum B-lactam resistance, including carbapenem resistanceand there was high resistance to the imipenem (from carbapenem group) this is one of the most effective drugs for treatment Multi drug resistance Gram negative bacteria which is metalo gene (MBL) producer (6), (7). And this may due to the long duration of stay in the ICU for patients also have contributed to the high rate of MDR pseudomonas. In this study also we detect that the mechanism of resistance to antibiotic by pseudomonas is production of metalo B-lactamase gene, and this finding was agree with study conducted in Brazil our results indicate that carbapenem resistance among pseudomonades aeruginosa isolates has increased because of both the emergence of resistant strains under antimicrobial selective pressure and the dissemination of epidemic clones.

Conclusion

This study concludes that Pseudomonas species that are considered as represent a serious therapeutic challenge for treatment of both community-acquired and nosocomial infections, Pseudomonas bacteria are Multi drug resistant (MDR) because it resists to different, class of antibiotic which are. Cefotaxime- chloramphenicol- cefepime- ampicillin- gentamicin- imipenem and there was high resistance to the imipenem (from carbapenem group) this is one of the most effective drugs for treatment Multi drug resistance Gram negative bacteria which is metalo gene (MBL) producer.

Recommendation

Other types of clinical specimen, and large sample size should be tested to cover wider range of isolates. Infectious control program should be performed at hospitals, to prevent the spread of pseudomonas infection.

Use of antibiotics especially broad spectrum antibiotic should be controlled to decrease the emergence of antibiotic resistance and to decrease the emergence of new resistance mechanisms. The spread of pseudomonades Species can best be controlled by observing proper isolation procedures, aseptic technique, and careful cleaning and monitoring of respirators, catheters, and other instruments. Topical therapy of burn wounds with antibacterial agents, has dramatically reduced the incidence of pseudomonades species sepsis in burn patients. Proper sterilization method for theatre and surgical room should be applied before surgery and after surgery. Proper sterilization for surgery instrument, and lab benches every day to avoid contamination of this organism, also there should be proper awareness to the population about how to use antibiotic, and they shouldn't use without prescription from their doctor. And PCR is best confirmatory procedure for diagnosing of pseudomonas resistance to antibiotic.

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