Research Article

Prevalence of Metallo-β-Lactamase producing *Pseudomonas aeruginosa* in wound infections in Duhok city, Iraq

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ABSTRACT

Background: *Pseudomonas aeruginosa* is common pathogen causing nosocomial infection. Acquired drug resistance and Metallo-β-lactamases (MBL) production have recently emerged as one of the most worrisome resistance mechanism that hydrolyze all beta-lactam antibiotics including penicillins, cephalosporins and carbapenems, with the exception of aztreonam. The aim was to find out the prevalence of multi drug resistant (MDR) and Metallo-β-lactamase (MBL) positive isolates of *P. aeruginosa* in wounds samples which are a serious concern.
Methods: *Pseudomonas aeruginosa* strains were obtained by standard isolation and identification techniques from 307 wound samples of hospital. Strains were then subjected to susceptibility testing for anti-pseudomonas drugs as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Carbapenems resistant strains were selected for the detection of MBL enzyme production by disc potentiation test. Production of MBL was confirmed by enhancement of inhibition zone around imipenem and meropenem discs impregnated with EDTA, as compared to discs without EDTA.
Results: Amongst the 71 isolates of *P. aeruginosa*, 62(87.3%) isolate were imipenem-sensitive, while 9(12.7%) isolates were found to be imipenem resistant and MBL producers. Very high resistance to antibiotics was recorded amongst MBL producers' *P. aeruginosa* compared with non-MBL imipenem-sensitive strains.
Conclusion: Study indicates that, surveillance for the detection of MBL is necessary. The rapid dissemination of MBL producers is worrisome and necessitates the implementation of proper and judicious selection of antibiotics especially carbapenem.

Keywords: Metallo-beta (β)-lactamase, *Pseudomonas aeruginosa*, Imipenem, EDTA

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic pathogen that can cause severe invasive disease in critically ill and immunocompromised patients.[1] This microorganism is an important cause of nosocomial infections, including pneumonia, wound infections, bacteremia, and urinary tract infection.[2] *P. aeruginosa* is a uniquely problematic microorganism because of a combination of inherent resistance to many drug classes and an ability to acquire resistance to all relevant treatments. Thus, carbapenems remain one of the best therapy options, although their use

is threatened by the emergence of carbapenem hydrolyzing, enzyme-producing strains and dissemination of multidrug-resistant clones.[3]

Metallo-β-lactamase (MBL) mediated resistance to carbapenem is an emerging threat in Pseudomonas isolates. Acquired MBL in *Pseudomonas* spp. has recently emerged as one of the most worrisome resistance mechanism.[4] MBL is an enzyme which requires zinc for their catalytic activity. Their activity is inhibited by metal chelators, such as EDTA and THIOL compounds. MBLs spread easily on plasmids and cause nosocomial infections and outbreaks.

Such infections mainly concern patients admitted to Intensive Care Units with several co-morbidities and a history of prolonged administration of antibiotics.[5]

Although, like elsewhere in the world, multi-drug resistant *P. aeruginosa* is an important pathogen in Duhok city, the prevalence and screening of Metallo-β-lactamase (MBL) positive isolates in wounds specimens are not known. To best our knowledge, there is no published data on these aspects. Thus, this study is to be an attempt to find answer to these questions. Therefore, the study was designed to find out the prevalence of multi drug resistant (MDR) and Metallo-β-lactamase (MBL) positive isolates of *P. aeruginosa* in wounds samples which are a serious concern.

METHODS

Study population: This prospective study was conducted at Azadi Teaching Hospital and Research Laboratories at Faculty of Sciences, Biology Dept, Zakho University from January to June 2014. In present study, a total of 307 wound swabs were collected from inpatients admitted Azadi Teaching Hospital in Duhok city; Iraq. All subjects should be provided their oral informed consent before participating in the study.

Isolation and Identification: Several media and tests were used for the isolation; identification and testing the susceptibility of the isolates for commonly used antibiotics. The media used are: Blood agar (with 5-7% defibrinized blood), MacConkey agar, kligler Iron Agar (KIA) and Mueller-Hinton agar. All of the above media and reagents were obtained from (Difco. USA). The media were prepared according to manufacturers instructions in 500 mL bottle and sterilized by autoclaving at 121°C for 20 minutes. All wound swabs collected for bacteriological investigations during the period of this study were treated according to established method of treating wound swabs. Gram stain preparations were made from all the swabs and the plates were incubated at 37°C for 18-24 hour in an incubator. The plates were read the following day but extended to 48 hour if there was no bacterial growth within 24 hours. Isolated colonies were subjected to Gram staining technique and biochemical tests for identification.[6]

Antibiotic susceptibility test: The bacterial strains were isolated and identified as per the standard guidelines. Antimicrobial susceptibility for all *Pseudomonas* isolates was determined by Kirby-Bauer disc diffusion method as per CLSIs guideline.[7] In all imipenem resistant isolates of Pseudomonas spp. MBL detection was done by Imipenem-EDTA combined-disc synergy test (CDST).

Imipenem-EDTA combined-disc test (CDST): The test organisms were inoculated on Mueller Hinton agar as recommended by the CLSIs. A 0.5 M EDTA solution was prepared by dissolving 18.61 gm of EDTA in 100 ml of distilled water and adjusting its pH 8.0 by using NaOH. The

Mixture then sterilize by autoclaving. Two imipenem (10ug) discs were placed on the surface of an agar plate at distance of 30 mm and 4 μl EDTA solution was added to one of them to obtain a desired concentration of 750 μg. The inhibition zones of imipenem and imipenem-EDTA discs can compared after 16 to 18 hours of incubation in air at 37°C. In the combined disc test, if the increase in inhibition zone with the imipenem-EDTA disc is ≥7 mm than the imipenem alone, it is considered as MBL positive.[8]

RESULTS

Three hundred and seven 307 wound swabs were investigated for the presence of *P. aeruginosa*. All swabs were directly transferred to the microbiology laboratory and cultured to the appropriate media (as described in methods).

Table 1 show the isolation rate of *P. aeruginosa* that was 71(23.1%) over total number of wound swabs examined, of them 62(87.3%) isolate were imipenem-sensitive, while 9(12.7%) isolates were found to be imipenem resistant and MBL producers which were confirmed by the disc potentiation method.

Table 1: Isolation rate of imipenem-resistant *P. aeruginosa* and MBL producers.

No. of Isolates (%)	No. of Imipenem Sensitive strains (%)	No. of Imipenem Resistant Strains (%)	No. of MBL producers (%)
71(23.1)	62 (87.3)	9 (12.7)	9 (12.7)

Table 2 reflect the antibiotic resistance patterns of both imipenem-sensitive strains and MBL-positive strains of *P. aeruginosa* against fourteen selected antibiotics; the organism exhibited high resistance rates against all used antibiotic and significantly high percentage of resistance collectively to all used antibiotics were recorded among MBL-positive strains. The resistance rate among imipenem sensitive strains; generally, the organism expressed low to moderate resistance rates and low percentage of resistance collectively to all antibiotics used were measured.

Table 2: Antimicrobial Susceptibility Profile of imipenem-sensitive *P. aeruginosa* and MBL-positive strains.

Antibiotics	Disc potency (μg)	Imipenem-sensitive Strains (%*)	MBL -positive Strains (%*)
Imipenem	30	0	100
Doxycycline	30	77	100
Erythromycin	10	71	100
Chloramphenicol	10	40	100
Vancomycin	30	66	89
Amoxiclav	5	58	89

Tetracycline	30	73	89
Cefixime	30	40	89
Gentamicin	15	46	78
Ceftazidime	30	30	78
Cefotaxime	30	42	78
Piperacillin	100	50	75
Amikacin	30	24	66
Ciprofloxacin	5	11	62
		38%**	100%**

(%*); Resistance rate,
** Percentage of resistance collectively to all used antibiotics

DISCUSSION

Metallo-beta-lactamase (MBL) enzyme is an emerging threat and cause of concern for physician. The MBL are plasmid mediated, so the resistance can be spread among hospital pathogen and will cause problems in treating infections namely wounds and burns. Hence, detection of MBLs among *P. aeruginosa* is crucial for the optimal treatment of patients and to control spread of resistance. In present study, all isolates of *P. aeruginosa* 71(23.1%) were obtained from samples of wound swabs and attempt was made to detect MBL production. Of 71 isolates of *P aeruginosa*, 9 (12.6%) were resistant to imipenem and all 9 isolates were found to be MBLs producers. Our results were close to results obtained by Mehul SC et al who found 8 (5%) *P. aeruginosa* isolates, recovered from wound swabs, were resistance to imipenem and all 8 isolates were found to be MBL producers.[9] Our investigation accords with other studies.[10, 11, 12] Our data deviates from reports of two studies by El-Kholy et al[13] and Pitout et al[14] issued higher rates of MBLs among *P. aeruginosa* that were (62%) and (46%) respectively. MBLs among *P. aeruginosa* have been increasingly recognized from clinical isolates worldwide; for example (55.2%) in Egypt and (66.7%) in Saudi Arabia,[15] in Brazil (76.7%),[16] in France (46%),[17] in India (13.6%),[18] in Iran (20%),[19] and in Korea(24.2%).[20] These reports on MBL-producing *P. aeruginosa* isolates are increasing globally due to the increased beta-lactam usage and emergence of resistant bacteria under antibiotic pressure. Moreover, dissemination of MBLs population pose a therapeutic challenge concern for treating physicians as it can hydrolyze carbapenems which are given as a last resort to the patient having infection. Thus, the early and rapid detection of MBL producing is necessary to aid infection control and avoid the future spread.

Regarding antibiotic resistance patterns among imipenem-sensitive and MBL producing *P. aeruginosa* isolates of the present study illustrated that MBL – positive were pan-resistant to all used antimicrobial agents than the imipenem-sensitive non-MBL isolates. The level of MBL production among multi-drug resistance (MDR) strains is in substantial rates in our study. The present work revealed that no single antibiotic showed 100% sensitivity to all MBL-positive strains but multi-drug resistance was shown. Moreover, the majority of MBL positive isolates have expressed absolute resistance 100% towards antibiotics like doxycycline, erythromycin and chloramphenicol. On another hand, high resistance rates (75-89%) found with remains antibiotics such as vancomycin, tetracycline, gentamicin, cefotaxime, amoxiclav, cefixime and pipericillin. This is already reported in other studies.[10, 14, 15, 21] This co-resistance might be due to MBLs encoding is largely association with mobile genetic elements that often carry other resistance genes, resulting in multidrug resistance (MDR).[22] The later observation insight great importance and implies that these antibiotics are no longer be effectively used as empirical therapy for these so-called "superbugs" MBL producing *P. aeruginosa* infections and reflects a threat limiting the treatment options in our hospitals. The lowest activity of these antibiotics can be attributed in part to earlier and recurrent exposure of the isolates to these drugs which may have enhanced resistant development. This assertion can further be strengthened by the high level of antibiotic abuse in our locality. Antibiotics like ciprofloxacin followed by amikacin considered acceptable drugs and exhibited moderate resistance (62% and 66% respectively) among overall isolates of MBL-producers. This is accords with some previous reports[10, 23] and comparable with the others studies[18, 24] Thus, these two drugs are still effective and could be considered as alternative options in the empirical treatment of MBL producing *P. aeruginosa* infections in our area. Resistance to all used antibiotics in present study was rather less and uncommon in imipenem-sensitive non-MBL producing strains of *P. aeruginosa*. This data is already similar to other data.[10,14,15] As a general, results from present study indicate that infections associated with multi-resistant MBL-producing *P. aeruginosa* in wounded patients might be associated with significantly greater morbidity, mortality and cost of care in study area.

CONCLUSION

The present study emphasizes high prevalence of multidrug resistant *Pseudomonas aeruginosa* producing MBL in our region that is at an alarming rate and is an infection control issue. To combat these problems, epidemiological studies should be undertaken in hospital settings to monitor the source of infection. Furthermore, strict antibiotic policies and measures to limit the indiscriminative use of cephalosporins and carbapenems in the hospital environment should be undertaken to minimize the emergence of this multiple resistant pathogen whose spread would leave no other option to treat nosocomial infections. Early detection of these isolates in a routine laboratory could help to avoid treatment failure and dissemination. Further genetic confirmation by PCR and analysis of the genetic context and relatedness of the MBL-producers would be mandatory for isolates screened positive by phenotypic tests.

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