The Bioscan 5 (2) : 251-253, 2010
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES

INCIDENCE OF METALLO BETA-LACTAMASE PRODUCING *PSEUDOMONAS AERUGINOSA* IN CLINICAL SAMPLES

S. NAGAVENI, H. RAJESHWARI, AJAY KUMAR OLI, S. A. PATIL[1] AND R KELMANI CHANDRAKANTH*
Department of Biotechnology, Gulbarga University, Gulbarga - 585 106, INDIA
[1]Department of Neuromicrobiology, NIMHANS, Bangalore - 650 029, Karnataka, INDIA
E-mail: ckelmani@gmail.com

KEY WORDS
Antibiotic resistance
Metallo beta lactamase
Pseudomonas aeruginosa

Received on :
19.03.2010
Accepted on :
27.05.2010

***Corresponding author**

ABSTRACT
Metallo-beta-lactamases (MBLS) mediated resistance is an emerging threat in hospital isolates of *Pseudomonas aeruginosa*. The present study was undertaken to determine the incidence of MBL producing *P. aeruginosa* in clinical samples and to assess the clinical outcome after antimicrobial treatment. Of the 120 isolates of *P. aeruginosa*, 100% resistantce was found to five antibiotics *i.e.,* ampicillin, ceftazidime, cefepime, ciprofloxacin and norfloxcin. Gentamycin showed 96% resistance. Resistance was moderate for tobramycin (44%) and was found to be low in case of amikacin (25%), 20% to carbenicillin and 32% to Imipenem. 50 (20.8%) were found to be MBL producers confirmed by disc potentiation method. The results of the present study demonstrate that the MBL-producing strains have significantly higher rates of resistance to β-lactam antibiotics.

INTRODUCTION

Pseudomonas aeruginosa is an invasive, predominant pathogen causing serious infections of the lower respiratory tract, the urinary tract, and wounds in hospitalized ill patients. Life threatening infections are usually polymicrobial, however *P. aeruginosa* occurs as a major determinant of these infections in the patients who immunocompromised after chemotherapy for cancer or immunosuppressive therapy for organ transplantation (George *et al*., 2006).

Unfortunately, this pathogen has been developing increased resistance to antimicrobial agents (Livermore, 1995). Due to their broad spectrum of activity and stability to hydrolysis by most beta lactamases, the carbapenems have been the drug of choice for treatment of infections caused by penicillin or cephalosporin-resistant gram-negative bacilli especially, extended spectrum b-lactamase (ESBL) producing gram-negative infections (Mendiratta *et al*., 2005). Since the first isolation of plasmid mediated metallo beta lactamase from *P. aeruginosa* in 1991(Watanabe *et al*., 1991), increasing rates of metallo beta lactamase (MBL) *P. aeruginosa* producing strains have become a serious problem. Such strains are resistant to multiple antibiotics as they hydrolyze all beta-lactams and are insensitive to clinically available inhibitors (Bush, 1998).

Carbapenem, mainly imipenem had been considered the useful agents for the treatment of infections. However, recently resistant producing metallo beta lactamase have been recovered from ICU patients in India (Ami *et al*., 2008; Hemalatha *et al*., 2005). In spite of increasing susceptibility of patients to bacterial infections is well corroborated, the long lasting illness associated with these infections remains poorly understood.

The present study is an attempt to evaluate the incidence of metallo beta lactamase *P. aeruginosa* producing strains in clinical isolates.

MATERIALS AND METHODS

Bacterial strains: In this study, 120 strains of *P. aeruginosa* were isolated from clinical samples during the period from March to October 2008 from three hospital and two diagnostic centers. Bacteria were determined by biochemical tests (Gilardi, 1978) and stored at -20ºC. Standard strain *P. aeruginosa* ATCC 27853 was used as control.

Antimicrobial susceptibility testing: Testing was performed in accordance with the guidelines established by the Clinical and Laboratory Standards Institute, 2009, with the Kirby-Bauer method using nine antibiotic discs including: gentamicin, amikacin, tobramycin, ceftazidime, cefepime, imipenem, ampicillin, carbenicillin, ciprofloxacin and norfloxicn, (Hi-media, Mumbai) on Mueller Hinton agar medium. The plates were incubated at 37ºC for 24h to check the zone of inhibition. Bacterial strains that demonstrated resistance to three or more categories of antibiotics were defined as MDR. The *P. aeruginosa* ATCC 27853 strain were adopted as the standards for quality Control.

Detection of MBLs: MBL producing *P. aeruginosa* was suspected when the isolate was resistant to ceftazidime and imipenem. Various methods have been recommended for screening MBL. These include the modified Hodge test, double disc synergy test using imipenem and EDTA discs or ceftazidime and EDTA discs, EDTA impregnated imipenem discs and EDTA impregnated meropenem discs.

We used zone enhancement with EDTA impregnated imipenem discs (Yong *et al*., 2002) for phenotypic determination of MBLs. Test organisms were inoculated on to plates with Mueller Hinton agar as recommended by the NCCLS. A 0.5 M EDTA solution was prepared by dissolving 186.1 g of disodium EDTA. $2H_2O$ in 1000 mL of distilled water and adjusting it to pH 8.0 using NaOH. The mixture was sterilized by autoclaving. EDTA solution was added to ceftazidime discs to obtain a desired concentration of 750 μg. The EDTA impregnated antibiotic discs were dried immediately in an incubator and stored at -20ºC in airtight vials. Then, 10 μg imipenem discs (with and without EDTA) were placed on the surface of an inoculated agar plate. The inhibition zones of imipenem and imipenem EDTA discs were compared after 16-18 h of incubation in air at 35ºC. Strains with enhancement zone in imipenem EDTA discs were recognized as MBL producing *P. aeruginosa*.

RESULTS

Bacterial strains: Out of the 120 isolates of *P. aeruginosa*, 12(9.37%) were isolated from urine, 1 (0.78%) from blood culture and 107 (89.85%) from wound culture.

Antibiotic profile and resistance rate among the *P. aeruginosa* isolates

Based on the resistance pattern the organisms that were resistant to three or more antibiotics were characterized to be multi drug resistant. A number of isolates were also 100% resistant to five antibiotics *i.e.,* ampicillin, ceftazidime, cefepime, ciprofloxacin and norfloxcin. Gentamycin showed 96% resistance. Resistance was moderate for tobramycin (44%) and was found to be low in case of amikacin (25%), 20% to carbenicillin and 32% to Imipenem (Table 1).

Detection of MBLs Of the 120 isolates of *P. aeruginosa*, 32% were found resistant to carbapenems (imipenem) and 50 (20.8%) were found to be MBL producers confirmed by disc potentiation method. The ATCC 27853 *P. aeruginosa* did not exhibit any zone size enhancement with EDTA impregnated imipenem discs.

Table 1: **Incidence of resistance in *Pseudomonas aeruginosa* to three different groups of antibiotics**

Antibiotics	Incidence of resistance No. of Resistant Isolates	Percentage (%)
Aminoglycosides		70.00
Tobramycin (Tb)	54	44.00
Gentamycin	115	96.00
Amikacin (Ak)	32	25.00
ß-Lactams		74.66
Imipenem (I)	38	32.00
Ampicillin (Ap)	120	100.00
Carbencillin (Cb)	20	20.00
Ceftazidime (Ca)	120	100.00
Cefepime (Cp)	120	100.00
Fluoroquinolones		100.00
Ciprofloxacin (Cf)	120	100.00
Norfloxacin (Nx)	120	100.00

DISCUSSION

The emergence of multi drug resistant bacteria is a major concern for health care professionals worldwide. *P. aeruginosa* is a very common cause of gram-negative infections as documented by the CDC independent surveillance programs (Gaynes and Edward, 2005). *P. aeruginosa* is a pathogen associated with numerous nosocomial infections in immunocompromised patients (George *et al*., 2006). The antimicrobial susceptibility results of *P. aeruginosa* against different groups of antibiotics represented the high degree of resistance with beta-lactams (63%) followed by aminoglycosides (55%) and floroquniolones (100%). Bijayini *et al*., (2008) found 64 (70%) were resistant to ceftazidime, 68 (75%) to piperacillin, 54 (59%) to piperacilin/ tazobactam, 81 (89%) to ticarcillin/ clavulinic acid, 75 (82%) to cefoperazone, 67 (74%) to amikacin, 74 (81%) to cefepime, 65 (71%) to levofloxacin, 72 (79%) to ciprofloxacin and 63 (69%) to aztreonam by the disc diffusion method. Similar antibiotic resistance incidence in present study with ceftazidime, cefepime and ciprofloxacin. In recent years, emerging imipenem resistance has been reported in India (Hemalatha *et al*., 2005)

Comparatively the incidence of resistance observed in the present study was higher than the earlier reports. However, the available data indicates the prevalence of resistance among the *P. aeruginosa* isolates varies between countries and difference can be attributed to the variation of resistance to antimicrobials based on extent of exposure to various antibiotics and their differences in prescription patterns and/ or quality of infection control practices. Therefore, our results are moderately correlating with the earlier reported results.

Carbapenems are the drugs of choice for multidrug resistant *P. aeruginosa* and ESBL producing organisms. However, resistance to carbapenems due to reduced uptake of drug leads to imipenem/meropenem resistant isolates (Rie *et al*., 1999). Varying resistance (4-60%) towards imipenem and meropenem been reported worldwide (Mendiratta *et al*., 2005; Alan *et al*., 2002; Vera *et al*., 2005). In this study, we observed a resistance of 32% to imipenem among the *P. aeruginosa,* while 20.8% of screened bacteria were MBL-positive. The results of the present study demonstrate that the MBL-producing strains have significantly higher rates of resistance to β-lactam antibiotics. Therefore, it is important that to monitor closely the situation of drug resistance in *Pseudomonas aeruginosa*.

REFERENCES

Alan, P. G., Chanwit T., Richard, A., Moore, Thomas., Louie, J., Wally, K., David, M., Livermore., Marie., Palepae, I. and Neil, W. 2002. Nosocomial outbreak of carbapenem – resistant *P. aeruginosa* with a new *blaIMP* allele, *blaIMP*-7. *J. antimicrobial agents and chemotherapy*. **46:** 252-258.

Ami, V., Nikhil, K., Manasi, K., Pallavi, B. and Jyotsana, D. 2008. Incidence of metallo beta lactamase producing *Pseudomonas aeruginosa* in ICU patients. *Indian J. Med Res.* **127:** 398-402.

Bijayini, B., Anupam, D., Purva, M. and Arti, K. 2008. High prevalence of carbapenem resistant *Pseudomonas aeruginosa* at a tertiary care centre of north India. Are we under-reporting? *Indian J. Med. Res.* **128:** 324-325.

Bush, K. 1998. Metallo b-lactamase: a class apart. *Clin. Infect Di*. **27**

(Suppl 1): S48-53.

Gaynes, R., and Edwards J. R. 2005. Overview of nosocomial infections caused by gram-negative bacilli. *Clin. Infect. Dis*. **41(6):** 848-54.

George, H. T., John B., John, E. E., David, G., Michael, S. and John G. 2006. Bad Bugs need drugs: An update on the development pipeline from the availability task force of the infectious diseases society of *America. J. Chemotherapy*. **15:** 97-105.

Gilardi, G. L. 1978. Identification of Pseudomonas and related bacteria, In G. L. Gilardi (Ed.), Glucose nonfermenting gram-negative bacteria in clinical microbiology. *CRC Press, Inc*., Boca Raton, Fla. p. 15-44.

Hemalatha, V., Uma, S. and Vijaylakshmi, K. 2005. Detection of metallo betalactamase producing *Pseudomonas aeruginosa* in hospitalized patients. *Indian J. Med. Res.* **122:** 148-152.

Livermore, D. M. 1995. β-lactamases in laboratory and clinical resistance. *Clin. Microbiol. Rev*. **8:** 557-84.

Mendiratta, D. K., Deotale, V. and Narang, P. 2005. Metallo beta lactamase producing *Pseudomonas aeruginosa* in a hospital from rural area. *Indian J. Med. Res*. **121:** 701-3.

Rie, N., Yuka A., Hideaki, I., Koji, Y., Terutaka, H. and Hajme, M. 1999. Carbapenem derivatives as potential inhibitors of various β-lactamases, including class B metallo- β-lactamases. *Antimicrobial agents and chemotherapy*. **43:** 2497-2503.

Vera, M., Kelly, A. and Marcelo, M. 2005. Metallo-β-lactamase producing *Pseudomonas aeruginosa* strains isolated in hospitals in Recife, Pe, Brazil. *Brazilian J. Microbiol.* **36:** 123-125.

Watanabe, M., Shizuko, I., Matsuhisa, Inove. and Susumu, M. 1991. Transferable imipenem resistance in *Pseudomonas aeruginosa*. *J. Antimicrobial agents and chemotherapy*. **35:** 147-151

Yong, D., Lee, K., Yum, J. H., Shin, H. B., Rossolini, G. M. and Chong, Y. 2002. Imipenem-EDTA disk method for differentiation of metallo-beta-lactamase producing clinical isolates of *Pseudomonas spp*. and *Acinetobacter* sp. *J. Clin. Microbiol.* **40:** 3798-801.

Announcing

The Second International Conference of

National Environmentalists Association, India

INTERNATIONAL CONFERENCE ON ENERGY, ENVIRONMENT AND DEVELOPMENT

(from Stokholm to Copenhagen and beyond)

(ICEED 2010)

December 10-12, 2010

Contact

PROF. P. C. MISHRA
D. Sc., FNEA,

Prof. and Head
Department of Environmental Sciences,
Sambalpur University,
Jyoti Vihar, Sambalpur
ORISSA

-:Important dates: -		
Last date of Abstract submission for oral presentation	-	31.08.10
Last date of Full paper submission for proceedings	-	31.08.10
Last date of Registration without late submission charges	-	31.08.10
Organisers will not be responsible for accommodation if not booked in advance		

Web site: www.iceed2010.in

E-mail: pcm_envsu@rediffmail.com; iceed2010@yahoo.in
Mobile no: 99437052301