Indian Journal of Science and Technology Vol.2 No. 11 (Nov. 2009) ISSN: 0974- 6846

Studies on multidrug resistant *Pseudomonas aeruginosa* from pediatric population with special reference to extended spectrum beta lactamase

R. Amutha, Padmakrishnan[1], T. Murugan[2] and M.P. Renuga devi[2]
Department of Biotechnology, VELS University, Pallavaram, Chennai-600117, India
[1]*Department of Microbiology, Dr ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai -600 113.*
[2] *Department of Microbiology, SRM college of Arts and Science,Kattankulathur-603203, Tamilnadu, India*
amuthar.bt@gmail.com

Abstract: A total of 53 isolates of *Pseudomonas aeruginosa* were obtained from 250 clinical samples from pediatric populations in Chennai. The isolates were obtained from clinical specimens *viz*. the throat swab, ear swab and urine sample. The isolates of *P. aeruginosa* were subjected to antibiotic sensitivity test. Among various antibiotics tested, the strains showed highest resistance to ampicillin (85%), followed by amikacin (62.2%), norfloxacin (60.3%) and ciprofloxacin (50.9%). The data on ESBL production indicated overall production of 25% among MDR strains. MIC values for the antibiotics ranged from 3.9 µg/ml to 256µg/ml. These data suggest that the prevalence of ESBLs which are very important as these strains may often cause outbreaks in the pediatric population and causes increased morbidity and mortality in patients underlying diseases or limit therapeutic options due to the high degree of multidrug resistance.
Keywords: Multidrug resistant, *Pseudomonas aeruginosa,* ESBL, MIC, India.

Introduction

Pseudomonas aeruginosa is the epitome of an opportunistic pathogen of human. Infection due to *P.aeruginosa* is seldom encountered in healthy adults, but in the last two decades, the organism has become increasingly recognized as the etiological agent in a variety of serious infections in hospitalized patients with impaired immune defenses.

Over the past few years, a notable increase in antibiotic resistance among gram negative bacteria recovered from hospitalized patients has been reported, especially for critically ill patients (Fridkin & Gaynes, 1999). Infections caused by multidrug resistant (MDR) gram negative bacteria, especially MDR *P. aeruginosa* (MDRPa) have been associated with increased morbidity, mortality and costs (Niederman, 2001; Paladino *et al*., 2002). Infection caused by *P. aeruginosa* is particularly challenging because of its resistance to most antimicrobial agents.

P. aeruginosa is inherently resistant to many antibiotics and can mutate to even more resistant strains during therapy. Although numerous resistance mechanisms have been identified, the mutation of porin proteins constitutes the major mechanism of resistance. Penetration of antibiotics in to the Pseudomonad cell is primarily through pores in the outer membrane. If the proteins forming the walls of these pores are altered to restrict flow throw the channels, resistance to many classes of antibiotics can develop. *P. aeruginosa* also produces a number of different beta lactamases that can inactivate many beta lactam antibiotics (eg. penicillins, cepalosporins, and carbapenems).

The extended spectrum of beta lactamases (ESBL) producing organisms are a breed of drug resistant pathogens that are rapidly becoming important globally in the area of hospital acquired infections. ESBL are transferable plasmid encoded, mutated beta lactamases enzymes, that have the capability to hydrolyze third generation cepalosporins. These enzymes are found in a variety of Enterobacteriaceae, most often in *Klebsiella pneumoniae* and *Escherichia coli*. ESBLs have been described in *P. aeruginosa* only recently (Jarlier *et al*., 1988; Nordmann & Naas,1994). The ESBL enzymes described in *P.aeruginosa* belong to various families: the TEM and SHV types which are common among Enterobacteriaceae, the PER type which mostly originates from Turkey (Nordmann & Naas 1994); the VEB type from southeast Asia (Poirel *et al*., 2000) or, more recently, the IBC type and the GES type which have been reported from various parts of the world, including France, Greece, South Africa and Brazil (Castanheir et *al.,* 2004).

Although there are several reports on multidrug resistance and ESBL production from various parts of the globe, there are few reports from this part of the country. The present study was undertaken to find the prevalence of multidrug resistant *P. aeruginosa* in the clinical samples collected from hospitalized and non hospitalized patients in Chennai. This study was an attempt to detect ESBL production and to screen for multi drug resistance *P. aeruginosa* from pediatric populations.

Materials and method

The study group included hospitalized and non hospitalized patients undergoing treatment in various government and private hospitals in Chennai for various clinical ailments. It also included non hospitalized (OP) with a wide variety of illness including wound infection, abscesses, upper respiratory tract infection and urinary tract infection. The various clinical specimens included in the study for the period of 6 months were pus, throat swab, ear swab/discharge and urine. All the specimens were processed according to standard microbiological procedures (Collee *et al.,* 1996). *Pseudomonas* was isolated and speciated based on a battery of biochemical tests. Antibiotic susceptibility was done by Kirby-Bauer disc diffusion method. The panel of antibiotics included: Ampicillin (10mcg), amikacin (30mcg) gentamycin

Indian Journal of Science and Technology Vol.2 No. 11 (Nov. 2009) ISSN: 0974- 6846

(10mcg), cefotaxime (30mcg), ceftriazone (30mcg), ceftazidime (30mcg), nalidixic acid (30 mcg), norfloxacin (10mcg), imipenem (10mcg), meropenem (10mcg), ciprofloxacin (5mcg) and co-trimoxazole (1.25/23.75 mcg). The source of the media, antibiotics and chemicals is HiMedia.

Detection of ESBL production

Double disk synergy test (DDST) was done by the method of Subha and Ananthan (2002). In the DDST, synergy was determined between a disk of Augmentin (20µg amoxicillin and 10µg clavulanic acid) and 30µg disk each 3 GC test antibiotics viz. cefotaxime, ceftriaxone and ceftazidime placed at a distance of 30mm apart on a lawn culture of the MDR isolates under test on Muller Hinton agar. The test organism was considered to produce ESBL, if the zone size around the test antibiotic disc increased towards the Augmentin disc after overnight incubation at 37˚C.

In the disc approximation test (DAT), the Muller Hinton agar plates were inoculated with the test organism in the same manner as for the disc diffusion tests. third generation cephalosporin disc (ceftazidine 30 µg) was placed at a distance of 20mm from ceftazidime (30µg) + clavulanic acid (10µg) combination disc on MHA plates. After overnight incubation at 37˚C, zone diameter around the 3GC ceftazidime was measured with and without clavulanate. A zone size increase > 5mm in antibiotic with clavulanate indicated the presence of ESBL.

Determination of minimal inhibitory and minimum bactericidal concentrations

The broth micro dilution is a quantitative technique for determining the MIC of antimicrobial agent (µg or units/ml), which will inhibit the growth of the organisms in vitro. Pure powder form of amikacin, ciprofloxacin and gentamycin were obtained from HiMedia laboratory, ceftazidime, ceftriaxone and cefoperazone were obtained from Orchid chemicals and pharmaceuticals. MIC were determined by an broth dilution techniques on Muller Hinton broth medium.MIC was the highest dilution of the antimicrobial agent which showed clear fluid with no development of turbidity. Sub culture all wells which was not showed any visible growth. The sub culture may show no growth- if the whole inoculum has been killed. The highest dilution showing al least 99% inhibition, on plate was taken as MBC.

Results

Isolation of P. aeruginosa

A total of 53 isolates were obtained from 250 clinical samples collected from patients having pyogenic infection, upper respiratory tract infection, otitis media and urinary tract infection. Amongst the clinical isolates, 20 (37.7%) were from pus sample, 17 (32.1%) from throat swab, 11 (20.7%) from ear swab / discharge and 5 (9.4 %) from urine samples.

Antibiotic susceptibility

Screening for MDR isolates showed that 24/53 (45.2%) isolates were MDR strains showing resistance to at least 3 antibiotics and to third generation cephalosporin. Antibiotic susceptibility testing of the isolates showed varying degree of sensitivity to the antibiotics tested. Highest resistance was seen for ampicillin (85%) followed by amikacin (62.2%), norfloxacin (60.3%) and ciprofloxacin (50.9%) (Fig.1).

MIC against the MDR isolates

MIC studies for amikacin, cefoperazone, ciprofloxacin, ceftazidime, ceftriaxone & gentamycin showed that the MIC values of the representative isolates for antibiotics tested fell in the range of 3.9µg/ml to 250µg/ml.

ESBL production

13/53 (25%) isolates showed ESBL production when tested by DAT & DDST method. Out of 24 MDR isolates 9 isolates were resistant to meropenem out of which only three were resistant to imipenem.

Discussion

In the present study a total of 250 samples were collected from which 53 isolates of *P. aeruginosa* were obtained. Of these 20 were from pus sample,17 from throat swab,11 from ear swab/discharge & 5 from urine sample. Agniohotri *et al.*, (2004) reported an isolation rate of 59% for *P. aeruginosa* from patients with wound infection. Mehta *et al.*, (2001) isolated 381 *P.aeruginosa* from pus & other body fluids during 2 years of study period. In the present study, all the 24 MDR isolates (100%) showed resistance to at least 1 third generation cephalosporins. Subha and Ananthan (2002) reported 95% resistance to at least 1 third generation cephalosporin's amongst the MDR isolates.

Antibiotic susceptibility testing by disk diffusion assay showed that the *P. aeruginosa* isolates were resistant to most of the β - lactam, all tested amino glycosides & fluoroquinolones. Similar findings were reported by Poirel *et al.*, in (2002) in which *P. aeruginosa* isolates were resistant to most of the antibiotics tested by disk diffusion method. MIC studies for amikacin, cefoperazone, ciprofloxacin, ceftazidime, ceftriaxone and gentamycin showed that the MIC values of the representative isolates for antibiotics tested fell in the range of 3.9 µg/ml to> 250µg/ml. Veenu Gupta *et al.*, (2000) reported that the MIC values against resistant strains varied from 60 to 3840µg/ml for amikacin, 60 to 1920 µg/ml for ceftazidime & cefriaxone,60 to 480µg/ml for ceftriaxone,10 to 80 µg/ml for cefoperazone and 20 to > 2560µg/ml for gentamycin.

In the present study 24 of 53 isolates were MDR of which 13 (25%) isolates showed ESBL production screened using ceftazidime in combination with clavulanic acid. Gaind 2003 reported 40.9% of *P.aeruginosa* isolates to be ESBL producers when tested using ceftazidime & Cefotaxime alone and in combination with clavulanic acid. 9 (17%) were resistant to Meropenem alone. Only 3 of the Meropenem resistant strains were resistant to imipenem. Navaneeth *et al.*, (2002) similarly reported 12% carbapenem resistance among 50 strains

Indian Journal of Science and Technology Vol.2 No. 11 (Nov. 2009) ISSN: 0974- 6846

of *P.aeruginosa*. Gladstone *et al.,* (2005) reported 24/56 (42.8%) of *P.aeruginosa* to be resistant to imipenem and meropenem.

This study indicates a prevalence of *P.aeruginosa* producing ESBL which are very important as these strains may often cause outbreaks in the pediatric population and causes increased morbidity and mortality in patients underlying diseases or limit therapeutic options due to the high degree of multidrug resistance.

References

1. Agnihotri N, Gupta V and Joshi RM (2004) Aerobic bacterial isolates from burn wound infections and their antibiogram-a five year study. *Burns*. 30, 241-243.
2. Castanheira M, Toleman MA and Jones RN (2004) Molecular characterization of beta-lactamase gene, blagim-I, encoding a new subclass of metallo-beta-lactamase. *Antimicrobial Agents Chemotherapy.* 48, 4564-4661.
3. Colle JG, Miles RS and Wan B (1996) Tests for the identification of bacteria. In: Mackie and Mc Cartney Practical Medical Microbiology. Eds. Collee JG, Fraser AG, Marmion BP & Simmons A. 14th ed. Edinburg: Churchill Livingstone. pp: 131-50.
4. Fridkin SK and Gaynes RP (1999) Antimicrobial resistance in intensive care units. *Clinical Chest Med*. 20, 303-316.
5. Gladstone P, Rajendran P and Brahmadathan KN (2005) Incidence of carbapenem resistance nonfermenting gram negative bacilli from patients with respiratory infections in the intensive care units. *Indian J. Med. Microbiol*. 23 (3),189-191
6. Jarlier V, Nicolas MH and Fourneir G (1998) Extended spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in *Enterobacteriaceae*: Hospital prevalence and susceptibility patterns. *Rev. Infect. Dis.*10, 867-878.
7. Mehta M, Punia JN and Joshi RM (2001) Antibiotic resistance in *Pseudomonas aeruginosa* strains isolated from various clinical specimen- a retrospective study. *Indian J. Med. Microbiol*. 19(4), 232.
8. Navaneeth BV, Sridaran D, Sahay D and Belwadi MR (2002) A preliminary study on metallo -beta-lactamase producing *Pseudomonas aeruginosa* in hospitalized patients. *Indian J. Med. Res*. 116, 264-267.
9. Niederman MS (2001) Impact of antibiotic resistance on clinical outcomes and the cost of care. *Critical Care Med*. 29, N114-N120.
10. Nordmann P and Naas T (1994) Sequence analysis of PER-1 extended spectrum beta-lactamase from *Pseudomonas aeruginosa* and comparison with class A beta lactamase. *Antimicro Agents Chemotheraphy*. 38,104-114.
11. Paladino JA, Sunderlin JL, Price CS and Schentag J (2002) Economic consequences of antimicrobial resistance. *Surg. Infect. (Larchmont)*. 3, 259-267.
12. Poirel L, Weldhagen GF, De Champs C and Nordmann P (2002) A nosocomial outbreak of *Pseudomonas aeruginosa* isolates expressing the extended spectrum beta-lactamase GES-2 in South Africa. *J. Antimicrobial.Chemotheraphy*. 49, 561-565.
13. Poirel L, Le Thomas I, Naas T, Karim A and Nordmann P (2000) Biochemical sequence analysis of GES-1, a novel class A extended-spectrum beta-lactamase and the class 1 integron In52 from *Klebsiella pneumonia*. *Antimicrob. Agents Chemotherapy*. 44, 622-632.
14. Subha A and Ananthan S (2002) Extended spectrum β-lactamase mediated resistance to third generation cephalosporins among *Klebsiella pneumoniae* in Chennai. *Indian J. Med. Microbiol*. 20, 92-95.
15. Veenu Gupta, Rama Sehgal and Arora DR (2002) Mic values of antimicrobial agents against non-fermenting gram negative bacilli. 97 (12), 458-459.

Fig 1. Antibiotic susceptibility pattern of P.aeruginosa isolated from various clinical samples of pediatric population