DETECTION OF ANTIBIOTIC RESISTANCE IN Pseudomonas aeruginosa ISOLATES WITH SPECIAL REFERENCE TO METALLO B-LACTAMASES FROM A TERTIARY CARE HOSPITAL IN WESTERN INDIA

ANGADI K.M.*, KADAM M.², MODAK M.S.², BHATAVDEKAR S.M.³, DALAL B.A.², JADHAVVAR S.R.², TOLPADI A.G.² THAKKAR V.² AND SHAH S.R.²

1Pad. Dr. D.Y. Patil Medical College and Research Centre, Pimpri, Pune-411018, MS, India.
2Bharati Vidyapeeth Medical College and Hospital, Pune-411043, MS, India.
*Corresponding Author: Email- kalpanaangadi@yahoo.com

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Abstract-

Background: Pseudomonas aeruginosa is an important cause of morbidity and mortality in hospitalised patients. The prevalence of multidrug resistant Pseudomonas aeruginosa strains including resistance to Carbapenems has been an increasing cause of concern. Hence this study was undertaken to know the resistance pattern of Pseudomonas aeruginosa to various anti-pseudomonal antibiotics and also to detect Metallo β-lactamase production in them.

Methodology: Pseudomonas aeruginosa isolates from various clinical samples were tested. Antibiotic sensitivity testing was carried out by Kirby-Bauer method according to CLSI guidelines and detection of Metallo-β-lactamase production was carried out by Imipenem EDTA combined disc method.

Results: 125 clinical isolates of Pseudomonas aeruginosa were tested. The resistance pattern to various antibiotics were: Amikacin (18.4%), Gentamycin (63.2%), Netilmicyn (32.8%), Ceftazidime (74.4%), Ciprofloxacin (60%), Imipenem (21.6%), Piperacillin (54.4%), Piperacillin-tazobactum (45.6%), Polymyxin-B (20%), Colistin (15.2%), Tobramycin (55.2%), Levofloxxacin (41.6%), Carbenicillin (51.2%). 21.6% of the isolates were resistant to Imipenem and 6 out of 27 i.e 22.2% were positive for Metallo β-lactamase production.

Conclusions: Amikacin, Carbapenems, Colistin and Polymyxin are the main drugs to treat multidrug resistant Pseudomonas aeruginosa and Metallo β-lactamase detection has to be done to identify resistance to Carbapenems.

Key words- Pseudomonas aeruginosa, Metallo β-lactamases

Introduction

Pseudomonas aeruginosa (Ps. aeruginosa) is the most common opportunistic pathogen of all Pseudomonas species. The importance of this species derives from the wide spread distribution of its strains in nature, their resistance to many antibacterial compounds and the number of virulence factors produced by them [1]. Pseudomonas are very rich source of plasmids carrying genes for a wide variety of functions, such as resistance to antibiotics and miscellaneous antibacterial agents including chemical agents such as metals and inorganic anions, resistance to bactenophages, bacteriocins. In addition to its resistance to antimicrobial compounds, it is able to produce various virulence properties in the form of enzymes and toxins. The arsenal of the organism has several extracellular proteases among which the most prominent are alkaline proteases, esterase and cytotoxin [2].

In normal, healthy hosts, infection is usually associated with events that disrupt or by pass protection provided by the epidermis e.g. burns, puncture wounds, use of contaminated needles by IV drug abusers, eye trauma with contaminated contact lenses. The result is infection of the skin, bone, heart or eye. Ps. aeruginosa is a notable cause of nosocomial infections of the respiratory and urinary tracts, wounds, blood stream and even the central nervous system [3]. In an immunocompromised patient, such infections are severe and frequently life threatening [1,2].

Ps. aeruginosa shows considerable degree of natural resistance to antibiotics. With the need to treat antibiotic resistant pseudomonas, newer drugs like carbapenem were introduced. These carbapenem are the last resources for treatment of multidrug resistant gram negative infections because of their broad antimicrobial activity and stability against most common beta lactamases [3,4]. Metallo β-
lacrimases (MBL) are the enzymes which can hydrolyze all beta lactam (except Aztreoman) including Carbapenems. They belong to the Class b of Ambler Classification of β lactamases wherein Zn (Zn^{2+}) is used to break the amide bond [4]. Various reports are present wherein variable resistance to the carbapenem have been mentioned [5-14]. Because of the widespread resistance of Pseu-
domonas to various antibiotics including the Carbapenem, this study was undertaken to know the antimicrobial susceptibility pat-
tern with special reference to MBL production by Ps. aeruginosa strains in our hospital.

**Material and Methods**

The study was approved by ethical committee of same institute.

**Study Site**

Present study was carried out in Bharati Vidyapeeth’s Medical college and Hospital Pune-Maharashtra India.125 consecutive isolates of Ps. aeruginosa were collected over a period of 6 months from June 2010 to December 2010, from various clinical specimens received in the department of microbiology. All samples were pro-
cessed and identified by standard conventional methods [2,15]. Antibiotic sensitivity testing was done by Kirby- Bauer Disc diffusion method and interpreted according to CLSI (Clinical Laboratory standard institute) guidelines [16]. Antibiotic sensitivity testing was done for the following antibiotics -Gentamycin(10ug), Tobramycin (10ug), Netilmicin(30ug), Amikacin(30ug), Ciprofloxacin(5ug), Levofloxacin(5ug), Cephotaxime, Ceftazidine(30ug), Piperacillin (100ug), Piperacillin /tazobactum(100/10ug), Imipenem(10ug), Colistin(10 ug), Polymyxin B(300ug). Those isolates which exhibit-
ed resistance or reduced susceptibility for Imipenem disc were
selected for further detection and phenotypic confirmation of MBL production by Imipenem-EDTA (Ethylene diamine tetra acetic acid) combined disc test method.

**Detection of Metallo β-lactamases by Imipenem-EDTA Com-
bined Disc Test**

Detection of MBL production by pseudomonal isolates was done by Imipenem EDTA Combined Disc method as described by Lee, et al. [17]. The selection criteria for MBL detection was reduced sus-
ceptibility to Imipenem (inhibition zone diameter less than 16mm). A 0.5 M EDTA solution was prepared by dissolving 186.1gm of disodium EDTA 2H2O in 1000 ml of distilled water and pH was adjusted to 8.0 using NaOH. The mixture was sterilized by auto-
claving. Test organism was inoculated on Muller Hinton agar. Two 10ug Imipenem disc was placed on the surface of the agar plate and appropriate amount of EDTA solution was added to one of the disc to obtain the desired concentration of 750ug. The inhibition zone of Imipenem disc was compared after 16-18 hours of incubation at 35°C. If there was an increase in the inhibi-
tion zone of more than 7mm than the Imipenem disc alone, it was
considered as MBL positive. ATCC 27853 Ps. aeruginosa was
used as negative control.

**Results and Observations**

125 non-repetitive Ps. aeruginosa isolates from various clinical specimens were tested. Out of the 125 specimens, 45 were from Surgical wards, 19 from Medical wards, 18 from the Intensive care units, 21 from combined Orthopaedics, OBGY, ENT and Ophthalm-
ology wards, 17 from OPD and 5 from Paediatric ward (Fig. 1)

**Fig. 1- Distribution of Ps. aeruginosa from various wards**

Others- ENT (13), Ortho, Ophthalmology (3), Burns (4). OBG

The most common source of Ps. aeruginosa were Pus samples from wounds and from urine (Fig. 2)

**Fig. 2- Distribution of Ps. aeruginosa from various clinical samples**

The antibiotic sensitivity testing results indicated the following rates of resistance, Amikacin (18.4%), Imipenem (21.6%), Polymyxin (20%), Colistin (15.2%), Netilmicin (32.8%), Piperacillin (54.4%), Tobramycin (55.2%), Carbenicillin (51.2%), Piperacil-
lin+tazobactum (45.6%), Levofloxacin (41.6%) , Ciprofloxacin (60%), Gentamycin (63.2%), Ceftazidine (74.4%), Cefotaxime (75.2%) which is shown in Fig. 3.

**Fig. 3- Resistance pattern of Ps. aeruginosa to various antibiotics**
Resistance to Imipenem was seen in 21.6% of the isolates. Detection of MBL production was done by Imipenem EDTA Combined Disc method and 6 out of 27 isolates (22.2%) were positive for production of MBL.

Discussion

Ps. aeruginosa is currently one of the most frequently isolated nosocomial pathogen and the infections due to this organism are often difficult to treat due to resistance to various antibiotics. The general resistance is due to a combination of factors. It is intrinsically resistant to antimicrobial agents due to low permeability of its cell wall. It has the genetic capacity to express a wide repertoire of resistance mechanism. It has become resistant through mutation in chromosomal genes which regulate resistance genes and it can acquire additional resistance genes from other organisms via plasmids, transposons and bacteriophages [18].

In the present study, the resistant pattern of clinical isolates of Ps. aeruginosa studied showed lower resistance to Amikacin (18.4%), Imipenem (21.6%), Colistin (15.2%), Polymyxin (20%) and increasing resistance to Netilmicyn (32.8%) Piperacillin+tazbactom (45.6%) and high resistance to Quinolones (40-60%) and third generation Cephalosporins (>70%). Among the aminoglycosides, resistance to Amikacin was seen in 18.4% of the isolates in our study, while lower rate of resistance were reported from Pakistan, 6.73% by Nadeem, et al. [19] and 8% by Nadeem and Qasmi, et al. [20], a resistance of 21% was reported by Farida, et al. (2010) [21]. Jamshaid, et al. (2008) reported 24% resistance [18]. 25% in Nagaveni, et al. also reported similar finding in 2010 [10]. Relative findings also reported from various studies which showed higher rates of resistance i.e. 42.8% by Murugan, et al. [13], 50% by Viren, et al. study [23] and 73% in Tehran by Horeih Saderi, et al. [9], 74% in Behera, et al. study [24,25]. In 2012 higher rate of resistance has been reported by Madhu Sharma, et al. i.e. 91.2% [26], 96.6% by Awari, et al. [12] and 97% by Bhale Rao, et al. [27].

Our study showed 63.2% resistance to Gentamycin, while the study by Nadeem, et al. showed lower resistance i.e.12.9% [19] and 21.6% by Paul, et al. in their studies [28].

In 2010 Murugan, et al. study showed moderate resistance i.e.42.8% to Gentamycin [13], comparatively we found that India, Pakistan and Iran studies showed higher rate of resistance to Gentamycin- aminoglycosides [9,10,12,21,23,26,27,29], while in aminoglycoside-Netilmicyn showed fair susceptibility rates i.e. 67.2% in our study and similar finding has been reported from Pakistan by Nadeem, et al. i.e. 90% [19] but higher rate of resistance were reported by Viren, et al. from India (60%) [23]. Resistance to Tobramycyn was seen in 55.2% of isolates in our study, while lower rates of resistance was observed with Nicholas, et al. 1% [30]. Farida, et al. 30% [21], Paul, et al. I 25.5% [28], 44% in Nagaveni, et al. study [10], higher rates of 66% was seen in study by Viren, et al. [23] and Pittout, et al. [14], 71.4% in Murugan, et al. study [13] and 98% in Awari, et al. study [12].

Resistance to the third generation Cephalosporins (Cefotaxime and Cefazidime) was seen 75% in our study. Lower rates were observed by Nicholas, et al. (6%), Nadeem, et al. (10.9%), Nadeem and Qasmi, et al. (16%), Paul, et al. (19.6%), Farida, et al. (38%) [19,20,21,28,30]. Higher resistance to third generation cephalosporins has been reported by Pittout, et al. (59%), Viren, et al. (67.86%), Prajapathi, et al. (68%) and (70%) by Behera, et al. [14, 23,25,29]. In 2010 from India very high rate of resistance to third generation cephalosporins have been recorded (>90%) by Bhale Rao, et al. and (96-100%) by Awari, et al. [12,27]. Higher rate of susceptibility to third generation cephalosporins were reported by Nadeem, et al. (68.7%), Farida, et al. (73%), by Nadeem and Qasmi, et al. (89%), Nicholas, et al. (85%), Paul, et al. (76.5%) [19,21,28,30]. We detected comparatively lower resistance to Levofloxacin (41.6%) in India than other reported studies i.e. (52.5%) by Viren, et al., (71%) Prajapathi, et al. and Behera, et al. in both in their studies showed (57.1%) by Murugan, et al. [13,23,25,29].

Resistance to Piperacillin was seen in (54.4%) isolates in our study and lower rate of resistance were reported in other studies by Nicholas, et al. (4%), Nadeem, et al. (10.8%) and (15.7%) in Paul, et al. (20%) by Pittout, et al. (35%) by Farida, et al. study [14,19,21,28,30], whereas higher rates of resistance was seen with Behera, et al. study75% [24] 73% in Horeih Saderi, et al. study [9], 85% in Awari, et al. study [12], 88.8% in Madhu Sharma, et al. study [26], 100% in Murugan, et al. study [13]. Resistance to Piperacillin+ tazobactom combination was seen in 45.6% of our isolates, lower rates of 9.4% was observed in Nadeem, et al. [19] and a slightly higher resistance i.e 54% was seen in Prajapathi, et al. study [29] and 59% in Behera, et al. study [25]. Resistance to the Imipenem were seen in (21.6%) of isolates in our study, while lower rates were seen with Farida, et al. (3%), Nicholas, et al. (9%), Paul, et al. (9.8%), Nadeem, et al. (9.9%) [19,21,28,30] while higher rate of resistance were reported by Nagaveni, et al. (32%), (71.4%) by Murugan, et al., Prajapathi, et al. (59%), (69%) Horeih Saderi, et al., (55%) by Awari, et al. in their studies [9,10,12,13,29].

Among all isolated imipenem resistance strains of Ps. aeruginosa (n=27) from present study, we found (n=7 i.e. 22.2%) strains were MBL producers, detected by phenotypic detection method of MBL production. We found reasonably similar rate of MBL producer’s strains of Ps. aeruginosa from other studies i.e. (20.8%) by Nagaveni, et al. [24] and (28%) by Anuradha, et al. [25] from India and (24.2%) by Nam Hee Ryoo, et al. from Korea [26]. Varsha Gupta, et al. reported MBL production (84%), Bhale Rao, et al. (67%) and 72% by Fereshteh shaheraghi, et al. [31,27,32]. 16% of the Imipenem resistant isolates were positive for MBL production in Rajput Anuradha, et al. study [33]. MBL production was seen in 50% of Ps. aeruginosa isolates in Dey, et al. study [5]. MBL production was seen in 20.8% of the isolates in Nagaveni, et al. study [10]. 70% were positive for MBL in Murugan, et al. study [13], 46% were positive for MBL production in Pittout, et al. study [14]. In Manoharan, et al. study 42.6% were found to be MBL positive [35] and in Deeba Bashir, et al. study 13.42% of Ps. aeruginosa were resistant to Imipenem and 11.66% were positive for MBL production [36].

Prevalence of MBL producing clinical isolates of Pseudomonas species have been continuously reported globally with some disparity in the rates of resistance. As MBL producing Pseudomonas species poses therapeutic problems in hospitals, it is better to understand the mechanism and spread of such multidrug resistant strains. This is our initial step towards controlling the spread of MDR (Multidrugresistant) strains by detecting their incidence in our hospital.

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Limitations of this study is that this is a retrospective study so we could not implement some statistical analysis for finding some qualitative data, we analyzed this data from clinical samples received in microbiology laboratory from various wards and OPD so is subject to sampling bias and phenotypic confirmation of MBL should be further characterized by molecular detection methods by which we can find predominant MDR strain of *Ps. aeruginosa*. To defeat our limitations, we are implementing genotypic characterization of such MDR isolates and also collecting appropriate demographic data and clinical information concerning such isolates from our isolates by which we can locate out the source of infection as well as determine the mechanism of resistance of MDR strains.

**Conclusion**

Increase in antibacterial resistance in *Ps. aeruginosa* is a cause of concern. So, continuous monitoring of bacterial resistance trends should be done and therapy should be based on antibacterial susceptibility results. Infection control programme and policies should be vigorously pursued in our health care facilities as well as antibiotic susceptibility results. Infection control programme and policies should be done and therapy should be based on antibacterial susceptibility. So, continuous monitoring of bacterial resistance trends and also collecting appropriate demographic data and clinical information concerning such isolates from our isolates by which we can locate out the source of infection as well as determine the mechanism of resistance of MDR strains.

**References**


