Clinical Significance and Antibiogram Profile of *Pseudomonas aeruginosa* Isolated from a Tertiary Care Hospital in Birgunj, Nepal

Dr. Andrei Popescu[1]*
[1]Carol Davila University of Medicine and Pharmacy, Department of Clinical Microbiology and Infectious Diseases, Bucharest, Romania

Keywords:
Imipenem resistance, Metallo-β-lactamases

Abstract

Objective

This study was carried out to determine the prevalence of *P. aeruginosa* as well as to assess their antibiotic susceptibility pattern in a tertiary hospital.

Material and Methods

P. aeruginosa isolated from various clinical samples were tested for antibiotic susceptibitlity with Kirby-Bauer disk diffusion method and minimum inhibitory concentration (MIC) by E-test strips. Imipenem resistant isolates were tested for production of metallo-β-lactamase by imipenem-EDTA disk method.

Results

Eleven imipenem-resistant isolates were positive for MBL production by imipenem-EDTA disk diffusion test. Compared with non-MBL-producing imipenem-resistant *P. aeruginosa*, MBL-producing isolates were more likely to be resistant to other antibiotics.

Conclusion

There exists high percentage of MBL producers among imipenem resistant isolates. The necessity of screening all imipenem-resistant isolates for MBL production and implementation of infection control programs to prevent spread of such organisms.

Introduction

P. aeruginosa is a Gram-negative, non-fermentative organism found in diverse environmental setting [1]. It is an opportunistic pathogen, causing serious infection in patients with weakened immune systems [2]. This organism is generally intrinsically resistant to a variety of antimicrobial agent as well as it has the capacity to develop resistance by mutation or acquisition of foreign resistance genes against different antibiotic classes[3].

Carbapenem, including imipenem, meropenem and doripenem are often used as a last resort for treatment of infections caused by *P.* aeruginosa [4,5] . However, carbapenem-resistant *P. aeruginosa* has become prevalent globally [6,7]. Carbapenem resistance may arise in *P. aeruginosa* via changes to oprD, up-regulation of efflux pumps and production of various kinds of carbapenemases, including serine β-lactamases of Ambler classes A and D and metallo-β-lactamases of Ambler class B [1].

Among various mechanism of resistance for carbapenem in *P. aeruginosa*, production of MBLs is of particular concern because of their rapid spread, potent carbapenemase activity, resistance to β-lactamase inhibitors and ability to hydrolyze all β-lactam antibiotics with the exception of aztreonam [8]. Furthermore, MBLs encoding genes are usually located on integrons, the mobile genetic elements that also carry genes encoding for resistance to aminoglycoside and other antibiotics resulting in multidrug resistance (MDR) [9].

Objectives of the study

To determine the prevalence of MBL producing *P. aeruginosa* among imipenem-resistant isolates.

Materials and Methods

Clinical isolates

All non-duplicate consecutive isolates of *P. aeruginosa* obtained during March 2017 to February 2018, at the microbiology laboratory of National Medical College and Teaching Hospital,Birgunj,Nepal were included in the study.

Identification and antimicrobial susceptibility testing

The isolates were identified as *P. aeruginosa* by conventional biochemical test [10]. Susceptibility testing of the isolates was performed by disk diffusion according to the guidelines of clinical and laboratory standard institute [11]. The following antimicrobial agents were used: ceftazidime (30 µg), imipenem (10 µg), meropenem (10 µg), ciprofloxacin (5 µg), aztreonam (30 µg), amikacin (30 µg), gentamicin (10 µg), piperacillin (100 µg), piperacillin-tazobactam (100/10 µg), cefepime (30 µg), and polymyxin B (300 units). MIC of imipenem, meropenem, ceftazidime and colistin were determined by E-test strips on all imipenem-resistant isolates according to the manufacturer's instructions. All the antibiotic disks and E-test strips were purchased from Hi-media, India. *P. aeruginosa* ATCC 27853 was used as a quality control in the susceptibility testing.

Detection of MBLs production

Imipenem-resistant isolates were tested for MBL production by the imipenem-EDTA disk diffusion test [12]. A suspension of test isolate adjusted to match the turbidity of a 0.5 McFarland standard was inoculated on to a Mueller-Hinton agar plates with a cotton swab. Two 10 µg imipenem disks were placed onto the agar and 750 µg of EDTA was applied to one of the disks. Mueller-Hinton agar plates were incubated in air at 37° C for 16-18h. An increase in diameter of the zone of inhibition around the imipenem-EDTA disk of ≥7 mm compared to the imipenem-only disk indicated the presence of MBL.

Results

A total of 90 *P. aeruginosa* isolates were collected during the study period. Of the total number of isolates, 42 (46.7%) were isolated from the intensive care unit (ICU) patients, 30 (33.3%) from the general ward (GW) patients, and 18 (20%) from outpatient department of hospital. These isolates were isolated from various clinical specimens: pus/wound swab 50 (55.6%) and sputum 20 (22.2%) specimens. The remaining isolates were from urine 10 (11.1%), tracheal aspirate 6 (6.7%) and blood 4 (4.4%).

32 (35.5%) of the total isolates were resistant to imipenem. Of the 32 imipenem-resistant *P. aeruginosa* isolates, 18 isolates were from pus/wound swab, 10 were from sputum and 4 were from urine. Of these isolates, 22 (68.7%) were considered MBL producers on the basis of positive result by the imipenem-EDTA disk diffusion test.
MBL-producing isolates were mainly obtained from specimens of pus/wound swab 14 (63.6%), sputum 7 (31.9%) and single isolates was obtained from urine (4.5%).

Of the 22 MBL-producing isolates, 12 (54.5%) were isolated from intensive-care unit patients and 8 (36.3%) were from general ward patients. Two isolates were obtained from OPD.

MBL-producing isolates were more resistant to other antibiotics than non-MBL-producing imipenem-resistant isolates with the exception of aztreonam. All MBL positive and negative isolates were susceptible (MIC≤2 µg/ml) to colistin[13] suggested definitions for multidrug-resistant and panresistant *P. aeruginosa*, all MBL positive isolates were multidrug resistant with a high level of resistant to imipenem (MIC >32µg/l), meropenem (MIC >32µg/l), and ceftazidime (MIC >256µg/l).

Discussion

The presence of MBL-producing *P. aeruginosa* has been reported in many countries around the world. This study also clearly shows high prevalence of MBL in imipenem-resistant *P. aeruginosa*. Our results revealed that 22 (68.7%) of 32 imepenem-resistant *P. aeruginosa* isolates produced MBL. Similarly high prevalence of MBL producing *P. aeruginosa* was detected in India (69.8%) [14], Taiwan (55.1%)[15] and Egypt (68.7%)[16]. Since MBL-producing isolates can cause serious nosocomial infections with high mortality rate, their presence in Nepalese Hospital is of great concern. To evaluate the threat of MBL-producing *P. aeruginosa* and its associated risk factors, well-designed multicentre epidemiological studies are urgently required.

In our study, MBL producing isolates were more resistant to multiple drugs than were the MBL-non producer, and based on the *in vitro* testing the most effective antibiotic against MBL-producing isolates was colistin (100% susceptible).

Aztreonam is stable against MBLs, however in this study 45.4% of the MBL-producing isolates were resistant to aztreonam, suggesting a possible association with other resistance mechanisms such as AmpC type β-lactamases or ESBLs[1].

MBL-encoding genes are usually located on integrons that frequently carry additional genes (such as *aacA4* genes that confer resistance to aminoglycosides) encoding for resistance to non-β-lactam antibiotics, resulting in multidrug resistance (MDR)[14]. Our results also showed that most MBLs–producing isolates were resistant to aminoglycosides. Resistant to meropenem, ceftazidime, piperacillin and cefepime is expected in MBL producers as they hydrolyse all β-lactams except aztreonam. The absence of new agents for the treatment of infections caused by MBL-producing multidrug resistant bacteria will lead to treatment failures with increased morbidity and mortality.

MBL-producing *P. aeruginosa* was isolated mainly from pus/wound swab and sputum. This finding contradicts with the results of other studies showing that MBLs-producing *P. aeruginosa* is more often isolated from the lower respiratory and urinary tracts[16,17].

In this study, with the exception of one isolate, all MBL-producing isolates were obtained from hospitalized patients. To know the main sources for the acquisition of MBL producing *P. aeruginosa* among hospitalized patients further investigation are required. Some studies have suggested that antimicrobial selective pressure and invasive therapeutic interventions are risk factors for the acquisition of MBL producing *P. aeruginosa*[18], while other studies have indicated that this pathogen is predominantly acquired from the environment[19].

Conclusion

Our study showed the presence MBL-producing *P. aeruginosa* in Nepal, that emphasizes on necessity of screening all imipenem-resistant isolates for MBL production and implementation of infection control programs to prevent spread of such organisms.

References

1. Lister PD, Wolter DJ and Hanson ND (2009). Antibacterial-resistant *Pseudomonas aeruginosa*: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev **22:** 582–610.
2. Coggan KA, Wolfgang MC (2012). Global regulatory pathways and cross-talk control *Pseudomonas aeruginosa* environmental lifestyle and virulence phenotype. Curr Issues Mol Biol **14**: 47–70.
3. Strateva T and Yordanov D (2009). *Pseudomonas aeruginosa*–a phenomenon of bacterial resistance. J Med Microbiol **58**: 1133–1148.
4. Giamarellou H and Poulakou G (2009). Multidrug-resistant Gram-negative infections: what are the treatment options? Drugs **69**: 1879-1901.
5. Riera E, Cabot G, Mulet X, Garcia-Castillo M, del Campo R, Juan C, Canton R and Oliver A (2011). *Pseudomonas aeruginosa* carbapenem resistance mechanisms in Spain: impact on the activity of imipenem, meropenem and doripenem. J Antimicrob Chemother **66**: 2022–2027.
6. Nagao M, Iinuma Y, Igawa J, Saito T, Yamashita K, Kondo T, Matsushima S, Takakura A, Takaori-Kondo A and Ichiyama S (2011). Control of an outbreak of carbapenem-resistant *Pseudomonas aeruginosa* in a haematooncology unit. J Hosp Infect **79**: 49–53.
7. Livermore DM (2009). Has the era of untreatable infections arrived? J Antimicrob Chemother 64 Suppl 1: i29-36.
8. Cornaglia G, Giamarellou H and Rossolini GM (2011). Metallo-beta-lactamases: a last frontier for beta-lactams? Lancet Infect Dis **11**: 381–393.
9. Walsh TR (2008). Clinically significant carbapenemases: an update. Curr Opin Infect Dis **21**: 367-371.
10. Forbes BA, Sahm DF and Weissfeld AS. Bailey and Scott's Diagnostic Microbiology (11th ed). St. Louis: Mosb Inc 2002; pp. 389-397.

11. Clinical and Laboratory Standards Institute (2014). Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement, M100-S24. Clinical and Laboratory Standards Institute, Wayne, PA.
12. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM and Chong Y. Imipenem-EDTA disk method for differentiation of metallo-beta-lactamase-producing clinical isolates of *Pseudomonas* spp. and *Acinetobacter* spp (2002). J Clin Microbiol **40**: 3798-3801.
13. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT and Monnet DL (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect **18**: 268-81.
14. Gupta V (2008). Metallo-β-lactamases in *Pseudomonas aeruginosa* and *Acinetobacter* species. Expert Opin Investig Drugs **17**: 131-143.
15. Lee MF, Peng CF, Hsu HJ and Chen YH (2008). Molecular characterisation of the metallo-β-lactamase genes in imipenem resistant Gram-negative bacteria from a university hospital in southern Taiwan. Int J Antimicrob Agents 2008; **32**: 475-480.
16. Zafer MM, Al-Agamy MH, El-Mahallawy HA, Amin MA and Ashour SED (2015). Dissemination of VIM-2 producing Pseudomonas aeruginosa ST233 at tertiary care hospitals in Egypt. BMC Infect Dis 15: 122-129.
17. Lee K, Lee WG, Uh Y, Ha GY, Cho J and Chong Y (2003). VIM- and IMP-Type Metallo-β-lactamase Producing *Pseudomonas* spp. and *Acinetobacter* spp. in Korean Hospitals. Emerg Infect Dis **9**: 868-871.
18. Lucena A, Dalla Costa LM, Nogueira KS, Matos AP, Gales AC, Paganini MC, Castro MES and Raboni SM (2014). Nosocomial infections with metallo-beta-lactamase-producing *Pseudomonas aeruginosa*: molecular epidemiology, risk factors, clinical features and outcomes. J Hospital Infect **87**: 234-240.
19. Scotta C, Juan C, Cabot G, Oliver A, Lalucat J, Bennasar A and Albertı´S (2011). Environmental Microbiota Represents a Natural Reservoir for Dissemination of Clinically Relevant Metallo-β-Lactamases. Antimicrob Agents Chemother **55**: 5376-5379.