Case Report

Emergence of Metallo-beta-Lactamase producing Pseudomonas aeruginosa in Pakistan

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Abstract

We report a case of urinary tract infection due to metallo-beta-lactamase producing Pseudomonas aeruginosa resistant to carbapenems. This report signals an ominous development. Unless curbed by judicious use of antibiotics and early detection by screening tests, spread of this plasmid-mediated resistance can render the carbapenems ineffective.

Introduction

Metallo-beta-lactamases (MBLs) belong to a group of beta-lactamases which require divalent cations of zinc as cofactors for enzyme activity. They have potent hydrolyzing activity not only against carbapenems but also against other beta-lactam antibiotics. MBLs are not inhibited by the commercially available inhibitors, clavulanic acid, sulbactam and tazobactam.[1]

The first MBL was reported from Bacillus cereus in the 1960s and since then 18 MBLs have been described in different Gram-negative bacteria. Production of most of these MBLs is chromosomally encoded and did not pose a serious threat of spread to other bacteria. However in 1991, the first plasmid-mediated MBL, IMP-1 from Pseudomonas aeruginosa was reported from Japan,[1] while another type of acquired metallo-beta-lactamase, VIM-1 was first reported from Italy in 1999. There have been increasing reports of IMP and VIM variants in Pseudomonas spp. and Acinetobacter spp. from several countries.[2-4] The blaIMP and blaVIM genes responsible for MBL production are horizontally transferable via plasmids and can rapidly spread to other bacteria.[2] To our knowledge, MBL production has not been reported from Pakistan and our case represents the first such report.

Case Report

A sixty years old man suffering from nephrolithiasis underwent left partial nephrectomy in a tertiary care hospital of Rawalpindi in January 2003. Soon after, he developed pyelonephritis with pyuria. The patient was catheterized and was receiving ciprofloxacin 500 mg orally every 12 hours. Urine culture did not yield any growth. Over the next 12 months his condition did not improve despite several courses of broad-spectrum antimicrobial therapy. Repeated urine cultures either did not reveal any significant growth, or yielded polymicrobial mixed growth. In July 2003, left renal ultra sonogram showed a shrunken kidney with dilated pelvis. In December 2003, Pseudomonas aeruginosa was isolated from the urine of the patient. The isolate was identified by standard biochemical tests using API 20NE galleries (API system SA, bioMerieux SA, Lyon, France). Antimicrobial susceptibility testing was

done by the modified Kirby-Bauer disk diffusion technique. All the disks used were from Difco, Detroit, USA and the results were interpreted according to the National Committee of Clinical Laboratory Standards (NCCLS) guidelines.[5] The isolate showed intermediate resistance to imipenem 10µg (zone of inhibition 15 mm), piperacillin-tazobactam 100/10µg (zone of inhibition 18 mm) and aztreonam 30µg (zone of inhibition 17 mm). It was resistant to 3rd and 4th generation cephalosporins, aminoglycosides and fluoroquinolones. Minimal inhibitory concentrations (MICs) were determined by Etest (AB Biodisk, Solna, Sweden). The isolate was intermediately resistant to imipenem (MIC, 6µg/mL) but was susceptible to piperacillin-tazobactam (MIC, 12/4µg/mL) and aztreonam (MIC, 6µg/mL).[5]

Resistance to imipenem aroused the suspicion of metallo-beta-lactamase production. The isolate was tested for MBL production by the imipenem-EDTA disk method.[6] Imipenem-EDTA disk was prepared by adding 750µg EDTA (5µL of 0.5 M EDTA solution adjusted to a pH of 8.0) to a 10µg imipenem disk. The isolate was inoculated onto a plate of Muller-Hinton agar (Oxoid, Basingstoke, UK) and the imipenem-EDTA disk along with disks of imipenem 10µg and EDTA 750µg were placed on it at a distance of 4 cm. The plate was incubated aerobically at 37^{0}C for 18 hours. The zone of inhibition with imipenem disk was 15 mm while it was 26 mm with imipenem-EDTA disk. The increase in the inhibition zone diameter by imipenem-EDTA disk was 11 mm giving a positive test for metallo-beta-lactamase (Figure).

Figure shows an increase of 11 mm in zone of inhibition by EDTA-imipenem disk compared to the imipenem disk, giving a positive test for metallo-beta-lactamase.

In view of the antimicrobial susceptibility of the isolate and lacking any other alternative, the patient was administered intravenous piperacillin-tazobactam 4.5 g every 8 hours for 14 days. The patient has responded clinically and follow-up urine culture was negative. The patient is being monitored for any relapse of his symptoms.

Discussion

Carbapenems are highly effective antibiotics against multidrug-resistant Gram-negative bacteria because of their stability against extended spectrum and AmpC-beta-lactamases. Resistance to carbapenems can be mediated by several mechanisms including decreased membrane permeability and increased efflux. However, production of metallo-beta-lactamases has assumed increasing importance in recent years. Although prevalence of carbapenem resistance due to acquired MBLs is increasing, its overall prevalence is still low. This is because MBLs are often produced in combination with serine beta-lactamases such as penicillinase and cephalosporinase and as long as the bacteria can survive with serine beta-lactamases at their disposal, production of MBLs is not required. However, increasing use of carbapenems would provide the selective pressure for selection of these enzymes. Emergence of plasmid-mediated MBLs is especially worrisome as it can rapidly spread to other Gram-negative bacteria.[1,2] A two-fold strategy is required to prevent the spread of MBLs before they become wide spread: restricted and judicious use of carbapenems, and early detection of MBL production in clinical isolates. Detection of MBLs by polymerase chain reaction (PCR) is a highly sensitive technique. However, its role in routine clinical laboratories is restricted by high cost and emergence of newer types of MBLs. Several new techniques have been developed for the detection of MBLs which are simple and cost-effective. These tests are based upon the inhibition of MBLs by metal chelators such as EDTA and thiol-based compounds like 2-mercaptopropionic acid, and can be performed using Etest or double-disk synergy techniques. The tests are highly reliable and can be easily adopted for routine use in clinical laboratories.[4,6]

Emergence of metallo-beta-lactamase-mediated resistance in Pakistan is a matter of grave concern. Carbapenems are effective therapeutic agents against highly resistant pathogens like Pseudomonas spp. and Acinetobacter spp. Spread of this resistance among these pathogens and transfer to other Gram-negative bacteria would seriously restrict our therapeutic options.

References

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