METALLO-β-LACTAMASE-PRODUCING CLINICAL ISOLATES OF ACINETOBACTER SPECIES AND PSEUDOMONAS AERUGINOSA FROM INTENSIVE CARE UNIT PATIENTS OF A TERTIARY CARE HOSPITAL

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Abstract

Prompt detection of metallo-β-lactamase (MBL) producing isolates is necessary to prevent their dissemination. Frequency of MBLs producing strains among multidrug resistant (MDR) Acinetobacter species and Pseudomonas aeruginosa was evaluated in critical care patients using imipenem-EDTA disk method. One hundred MDR Acinetobacter spp. and 42 Pseudomonas aeruginosa were checked for MBL production, from January to June 2001. MBL was produced by 96.6% of imipenem-resistant Acinetobacter isolates, whereas 100% imipenem-resistant Pseudomonas aeruginosa isolates were MBL producers.

Carbapenem resistance in MDR Acinetobacter spp. and Pseudomonas aeruginosa isoletes in this study was due to MBLs. This calls for strict infection control measures to prevent further dissemination. Frequency of imipenem-resistant Acinetobacter isolates in this study was due to MBLs. This calls for strict infection control measures to prevent further dissemination.

Key words: Acinetobacter spp, metallo-β-lactamase, Pseudomonas aeruginosa

Materials and Methods

This study was conducted in the clinical microbiology laboratory of The Aga Khan University Hospital, Karachi, Pakistan. One hundred MDR clinical isolates of Acinetobacter species and 42 Pseudomonas aeruginosa were studied for MBL production. These organisms were isolated from respiratory secretions, wound swabs, urine and blood culture specimens of intensive care unit patients, admitted from January to April 2001. A multidrug resistant isolate was defined as resistance to two or more drugs or drug classes of therapeutic relevance.

The emergence of these MBLs in gram negative bacilli is becoming a therapeutic challenge as these enzymes possess high hydrolytic activity that leads to degradation of higher generation cephalosporins. Moreover, the treatment alternatives are unavailable, or expensive/toxic with poor outcome. Plasmid mediated MBL genes spread rapidly to other species of gram negative bacilli; therefore rapid detection of metallo-β-lactamases production is necessary to modify therapy and to initiate effective infection control to prevent their dissemination. To date there has been one report from Pakistan suggesting presence of MBL producing isolate. There is however need for a systematic study to assess the extent of this form of resistance amongst our isolates. The purpose of this study was to evaluate the metallo-β-lactamase (MBL) production among multidrug resistant (MDR) Acinetobacter spp. and Pseudomonas aeruginosa, isolated from clinical specimens of intensive care unit patients.

Materials and Methods

This study was conducted in the clinical microbiology laboratory of The Aga Khan University Hospital,
Hinton agar. Another disk containing only 750 μg EDTA was also placed as a control. After overnight incubation, the established zone diameter difference of ≥ 7 mm between imipenem disk and imipenem plus EDTA was interpreted as EDTA synergy positive. Figure 1 shows a negative imipenem plus EDTA disk test for ATCC strains of Pseudomonas aeruginosa and positive for MBL producing clinical isolate of Pseudomonas aeruginosa.

Results

Imipenem-resistance by disc diffusion method was found in 90 out of 100 isolates of Acinetobacter spp. Of the 90 imipenem-resistant Acinetobacter spp. 83(96.6%) were metallo-β-lactamase producers. Whereas, remaining 17 isolates, including the 10 imipenem-sensitive isolates, did not show evidence of metallo-β-lactamase production. The average zone diameter difference between imipenem disk and imipenem plus EDTA disk for MBL-positive isolates was 16 mm (Fig. 2a).

Among 42 Pseudomonas aeruginosa isolates, 25 showed resistance against imipenem by disc diffusion method. Imipenem-EDTA disk method showed 100% metallo-β-lactamase production in imipenem resistant Pseudomonas aeruginosa isolates with an average difference of 14 mm in zone diameter between imipenem disk and imipenem plus EDTA disk for MBL-positive isolates. Imipenem sensitive group did not show any metallo-β-lactamase producing isolate (Fig. 2b).

Discussion

By using the Imipenem-EDTA disk method, a very high percentage of imipenem resistant Acinetobacter spp. (97%) and Pseudomonas aeruginosa (100%) isolates showed metallo-β-lactamase activity. This finding is consistent with reports from other tertiary care hospitals,[10] giving the evidence that acquired MBLs can rapidly emerge and establish a condition of endemicity in certain epidemiological settings. A majority of our isolates also showed resistance to other important groups of antibiotics including third generation cephalosporin, aminoglycoside and quinolone, which is a characteristic of majority of metallo-β-lactamases producing isolates.[11] A number of MBLs producers in both organisms showed significant difference between zone of inhibition by imipenem and imipenem plus EDTA disk. When compared with non-MBL producers the average zone difference for the MBL...
producers was 15 mm versus 2 mm for the non-MBL producers, thus making this test reliable for initial screening of the MBL production in clinical isolates.

Reports from various parts of the world showing emergence of metallo-β-lactamase enzymes in Enterobacteriaceae[5] is an evidence for the spread of these enzymes in this family. Emergence of MBLs producing Acinetobacter spp. and Pseudomonas aeruginosa in our clinical strains is alarming and reflects excessive use of carbapenem. Therefore, early detection and prompt instillation of infection control measures is important to prevent further spread of MBLs to other gram negative rods. Additionally, it is also important to follow antibiotic restriction policies to avoid excessive use of carbapenem and other broad spectrum antibiotics. Finally, to understand the epidemiology, there is a need of genetic analysis and also the typing of metallo-β-lactamase enzymes.

In conclusion, by using imipenem-EDTA disk method as a screening test for metallo-β-lactamase production, we found a very high percentage of metallo-β-lactamase producing isolates among multidrug resistant Acinetobacter spp. and Pseudomonas aeruginosa isolates.

References

Source of Support: Nil, Conflict of Interest: None declared.

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