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Detection of metallo β-lactamase production and antibiotic resistance with E-test method in pseudomonas, acinetobacter and klebsiella strains, in Turkey

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Abstract The metallo beta-lactamase (MBL) mediated resistance patterns remain unknown in most countries. We aimed to investigate the existence and antimicrobial resistance of MBL-producing strains among carbapenem-resistant Gram-negative bacteria that were isolated from nosocomial infections in patients in an university hospital in Turkey. Fifteen of 52 Pseudomonas aeruginosa strains (29%), 5 of 24 Acinetobacter baumanii strains (21%), and 2 of 2 Klebsiella pneumoniae strains (100%) were found to be metallo enzyme producers, with the Etest MBL technique. The in vitro antibiotic susceptibility of the MBL-positive organisms was investigated by the Etest method. Of the ten drugs tested, isepamicin was the most active agent against the MBL-producing strains. Overall, the rank order of activity of the ten antibiotics, in terms of the percentages of susceptible strains, was: isepamicin, 73%; ciprofloxacin, 64%; amikacin, 59%; aztreonam, 18%; tobramycin, 18%; meropenem, 14%; cefoperazone-sulbactam, 5%; piperacillin-tazobactam, 0%; ticarcillin-clavulanate, 0%; and ceftim, 0%. The meropenem minimum inhibitory concentrations (MICs) of the metallo enzyme-producing and nonproducing carbapenem-resistant strains were compared, and the MBL-producers were found to have higher meropenem MICs than the nonMBL-producing carbapenem-resistant strains. Early preventive measures should be taken against MBL-producing nosocomial pathogens that are associated with wide spread and high antimicrobial resistance.

Key words Pseudomonas s.p. · Acinetobacter s.p. · Klebsiella s.p. · Metallo β-lactamases · Antimicrobial susceptibility · Carbapenems

Introduction

Beta-lactamase-associated resistance is progressively increasing, especially in nosocomial pathogens. One the recently identified group of enzymes is the acquired carbapenemases, a heterogeneous group of beta-lactamases belonging to molecular Ambler classes A, B, and D. The class-B enzymes are the most clinically significant carbapenem-hydrolysing enzymes, and are also called metallo beta-lactamases (MBLs), because their activity depends on some divalent cations such as cadmium or zinc. Metallo enzymes are currently spreading in pseudomonas, acinetobacter, and occasionally in Enterobacteriaceae.

From studies in the 1980s and early 1990s, it has been found that some bacterial species have naturally occurring metallo enzymes. Because these organisms are often found in common physical environments, and most of them have low pathogenic activity for humans, they were considered not to have any significant clinical importance. However, in 1991, the identification a new transferable MBL enzyme, IMP-1, in a Pseudomonas aeruginosa isolate from Japan, caused great concern due to the potential risk of MBLs being disseminated widely to other bacterial species. Subsequent studies from Europe and the Far East have reported the identification of new enzymes in different bacterial strains. VIM type metallo enzymes were described particularly in European studies towards the end of the twentieth century. It has been reported that MBLs, whose genes are plasmid and integron located, hydrolyse penicillins, cephalosporins, and also carbapenems. However, aztreonam is considered the only choice of beta-lactam that can remain stable against some of the MBLs.

An antimicrobial resistance surveillance program has been performed at our medical center since 1996, including methicillin resistance in staphylococcus, low polypeptide susceptibility in enterococcus, extended-spectrum beta-lactamases in Enterobacteriaceae, penicillinase production in fastidious organisms, and multiple antimicrobial resistance in pseudomonas and acinetobacter species. In addi-
tion, a specific surveillance program was initiated in May 2002 after the detection of MBL from a *P. aeruginosa* strain.

We conducted the present study to determine the MBL pattern in Gram-negative bacilli in a Turkish hospital. Also, a second aim was to measure the in vitro activity of some beta-lactam and nonbeta-lactam antibiotics against MBL-positive organisms.

**Patients and methods**

**Resistance screening**

This study was carried out at Firat Medical Center, a 650-bed tertiary-care teaching hospital (which does not carry out organ transplantation). The hospital has a total of 100 beds in surgery, adult, and pediatric intensive care units (ICUs). All clinical isolates were screened for carbapenem resistance by routine disk-diffusion antibiogram test according to the National Committee for Clinical Laboratory Standards (NCCLS) recommendations. When a strain of bacteria is found to be resistant to imipenem or meropenem, or both, metallo enzyme production is screened by the Etest (AB BioDisk, Solna, Sweden) MBL technique. Strains that were positive and negative for MBL production were stored at −40°C for further investigations.

**Patients and bacterial strains**

During the period from May 2002 to June 2003, a total of 407 pseudomonas, 152 acinetobacter, and 172 klebsiella strains were isolated from nearly 700 inpatients at our institute. Of the 407 pseudomonas strains, 106 strains were recovered from pediatrics, 91 were from nonsurgical clinics (i.e., endocrinology, oncology, cardiology, chest diseases, psychiatry, etc.), 133 were from surgical clinics (i.e., orthopedics, reconstructive surgery, obstetrics-gynecology, general surgery, burns, etc.) and 77 were from (ICUs) (pediatric, adult, surgical). Of the 152 acinetobacter strains, 41 were recovered from pediatric clinics, 20 were from nonsurgical clinics, 53 were from surgical clinics, and 38 were from ICUs. Of the 172 klebsiella strains, 46 were recovered from pediatric clinics, 43 were from nonsurgical clinics, 47 were from surgical clinics, and 36 were from ICUs.

**Identification of the isolates**

All presumptive bacterial strains were identified at the species level by Gram-stained morphologic characteristics, catalase and oxidase tests, carbohydrate utilization, and other conventional biochemical tests. Also, API ID 32 E and API GN System Kits (Bio-Mérieux, Lyon, France) were used to confirm the identifications of the isolates.

**Antimicrobial susceptibility**

Carbapenem susceptibility of the strains was evaluated by the standard disk-diffusion method with antibiotic-containing disks (Oxoid, Hampshire, UK). Any resistance in disk-diffusion to carbapenems was confirmed using the Etest (AB BioDisk) method. Meropenem, ciprofloxacin, amikacin, tobramicin, isepamicin, aztreonam, piperacillin-tazobactam, cefoperazone-sulbactam, ticarcillin-clavulanate, and cefepim were the antibiotics that we used to measure in vitro activity against MBL-producers. We chose these antibiotics due to their high antibacterial activity for routine isolates of pseudomonas, acinetobacter, and also for klebsiella strains. Plates of Mueller–Hinton agar were inoculated with a bacterial suspension equivalent to 0.5 McFarland turbidity and incubated aerobically at 35°C for 18h. Results of the susceptibility tests for each bacterial strain were interpreted according to the criteria of the NCCLS.

**Etest MBL procedure**

Several Etest MBL strips in different configurations were produced, similar to that for Etest strips for the detection of extended-spectrum beta-lactamase (ESBLs). An Etest (AB BioDisk) gradient format was developed for the detection of MBLs based on the reduction of imipenem (IP) MICs in the presence of ethylenediamine tetraacetic acid (EDTA). The Etest MBL strips consisted of a double-sided seven-dilution range of IP with a constant concentration of EDTA. The following formula was used in this method: IP (4 to 256µg/ml) (IP) with IP (1 to 64µg/ml) + EDTA (IPI). The final concentrations of EDTA used in the strip corresponded to 320µg/ml.

**Etest MBL and interpretative criteria**

Individual colonies were picked from 18-h plates and suspended in 0.85% saline to a turbidity of 0.5 McFarland standard. Sterile cotton swabs were used to transfer the inoculum to the plates, which were thoroughly swabbed and dried before the Etest MBL strips were applied. The inoculated plates were incubated for 16 to 20h at 35°C. The MIC endpoints were read where the inhibition ellipses intersected the strip. A reduction of IP MICs by ≥3 fold dilutions in the presence of EDTA was interpreted as being suggestive of MBL production. Equally, the presence of a “phantom” zone between the two gradient sections or deformation of the IP was also indicative of MBL. Metallo enzyme production criteria were based on the manufacturer’s recommendations and on those of Walsh et al.

**Results**

Existence of MBL-producing organisms

From May 2002 to June 2003, 407 pseudomonas, 152 acinetobacter, and 172 klebsiella strains were isolated from patients at our institute. Fifty-two pseudomonas strains (12.7%), 24 acinetobacter strains (15.7%), and 2 klebsiella strains (1.1%) were found to be carbapenem-resistant on