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Prevalence of metallo-β-lactamase among *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated from burn wounds and in vitro activities of antibiotic combinations against these isolates

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Abstract

The prevalence of metallo-β-lactamases (MBLs) produced by isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* and the activities of various antmicrobial combinations against MBL producer strains were investigated. During the period from June 2003 till July 2004, 120 *P. aeruginosa* and 9 *A. baumannii* nonduplicate isolates were obtained from burn wounds. Forty strains (37 *P. aeruginosa*, 3 *A. baumannii*) were selected because of resistance to carbapenems. Screening for MBL production was performed in the latter isolates by the combined disk method which depends on comparing the zones given by disks containing imipenem with and without ethylenediaminetetraacetic acid (EDTA). Of imipenem resistant *P. aeruginosa* strains, 21 and 1 of *A. baumannii* were found metallo-β-lactamase producers. Disk approximation studies were then performed to test for in vitro activities of various antimicrobial combinations. For a total of 21 *P. aeruginosa* strains, synergy was demonstrated predominantly by ciprofloxacin in combination with ceftazidime and imipenem, by ofloxacin in combination with astreonam. Against MBL producer *A. baumannii* strain, synergy was detected only with imipenem-ofloxacin combination. None of the combinations were antagonistic. These results suggest that MBL producing *P. aeruginosa* and *A. baumanni* strains have been introduced into burn centers, and to prevent the further spread of MBL producers, it is essential for carbapenem resistant isolates to be screened for MBLs.

Keywords: *Pseudomonas aeruginosa*; *Acinetobacter baumannii*; Metallo-β-lactamase; Burn wound

1. Introduction

Burns provide a suitable site for bacterial multiplication because of the larger area involved and longer duration of patient stay in the hospital [1]. Infection is one of the most serious complications in burn patients and *P. aeruginosa* is the most important, resistant and dangerous organism in burn patient infections. Although *A. baumanii* is a relatively frequent cause of epidemics in burn units, the therapy of *Acinetobacter* infections is complicated by multidrug resistance: aminoglycosides, extended-spectrum cephalosporins, and fluoroquinolones. Carbapenems have retained better activity against nonfermenting Gram-negative pathogens than other antimicrobial agents [2,3].

The worldwide spread of acquired metallo-β-lactamases (MBLs) in Gram-negative bacilli has become a great concern. MBLs possess a broad hydrolysis profile that includes carbapenems and almost all extended-spectrum β-lactam agents. Early detection of MBL-producing organisms is essential to aid infection control and to prevent the dissemination of these organisms [4]. Based on the fact that the MBL activity is blocked by chelating agents such as ethylenediaminetetraacetic acid (EDTA) and 2-mercaptopropionic acid (2-MPA), several screening methods for the detection of MBL-producing organisms have been developed.

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The IPM-EDTA disk method depends on comparing the zones given by disks containing IPM with and without EDTA, and the microdilution method depends on comparing minimal inhibitory concentrations (MICs) of IPM with and without EDTA. Both methods have been reported to be reliable in the detection of MBLs in carbapenem-resistant *Pseudomonas* and *Acinetobacter* strains [5].

Resistance to carbapenems is an increasing problem among nonfermenting Gram-negative pathogens. The aim of this survey was to determine the prevalence of MBLs produced by *P. aeruginosa* and *A. baumanii* strains isolated from burn wounds and the activities of various antmicrobial combinations against MBLs producer strains.

2. Material and methods

2.1. Bacterial isolates

The prospective study included 120 *Pseudomonas aeruginosa* and 9 *Acinetobacter baumannii* non-replicate isolates recovered from burn wounds of patients in the Burn Unit from June 2003 to July 2004.

2.2. Identification and antimicrobial susceptibility testing

Bacterial identification was performed using conventional methods and by the API 20 NE system (Becton Dickinson Diagnostic Systems, Sparks, MD). Susceptibility testing was performed for ceftazidime (30 μg), aztreonam (30 μg), imipenem (10 μg), meropenem (10 μg), piperacillin–tazobactam (100/10 μg), cefoperazone–sulbactam (75/30 μg), ofloxacin (5 μg), ciprofloxacin (5 μg), amikacin (30 μg), gentamycin (10 μg) (Oxoid) using the disk diffusion method, according to NCCLS guidelines [6]. Briefly, organisms from an overnight growth plate were suspended in 0.9% saline solution to approximate the density of a 0.5 McFarland standard. This inoculum was spread onto 150 mm plates containing cation-adjusted Mueller–Hinton agar and disks were placed within 5 min. Plates were incubated inverted at 35 °C for 24 h. Disk approximation studies were then performed to test for synergy of ceftazidime, aztreonam imipenem, meropenem, piperacillin–tazobactam and cefoperazone–sulbactam with ofloxacin, ciprofloxacin, amikacin and gentamycin. Organisms were prepared and plates inoculated as described above. Disk combinations were placed at a distance of the sum of the radii of the zone of inhibition for each individual drug, as determined by the earlier diffusion studies. Plates were incubated at 35 °C and read at 24 h. Synergistic activity was defined as an enhancement (≥2 mm) or bridging at the junction of the two zones. Antagonistic activity was defined as truncation at the junction of the two zones of inhibition [7–9].

2.3. Screening of MBL-producing isolates

A 0.5 M EDTA solution was prepared by dissolving 186.1 g of disodium EDTA·$2H_2O$ in 1000 ml of distilled water and adjusting it to pH 8.0 by using NaOH. The mixture was sterilized by autoclaving and EDTA solution was added to 10 μg imipenem disks to obtain a concentration of 750 μg. The disks were dried immediately in an incubator and stored at 4 or −20 °C in airtight vials without desiccant. Test organisms were inoculated onto plates of Mueller–Hinton agar as recommended by the National Committee for Clinical Laboratory Standards. The imipenem and imipenem-EDTA disks were placed on the plate. The inhibition zones of these disks were compared after 16–18 h of incubation in air at 35 °C. The inhibition zones with imipenem-EDTA disks were 14 mm for the MBL-negative isolates, while they were ≥17 mm for the MBL-positive isolates [10].

3. Results

A total of 120 *Pseudomonas aeruginosa* and 9 *Acinetobacter baumannii* strains were isolated from the patients admitted to the Burn Unit of our hospital over a 1-year period. Overall, resistance rates of *P. aeruginosa* strains to the used antimicrobial drugs were as fallows: imipenem, 30.8%; meropenem, 32.5%; ceftazidime, 72.5%; aztreonam, 79.2%; piperacillin/tazobactam, 54.2%; cefoperazone/sul-

Table 1
Combined effects of carbapenems and β-lactam antibiotics or β-lactamase inhibitors against 21 *P. aeruginosa* strains

Antibiotic combination	Synergy, *n* (%)	Antibiotic combination	Synergy, *n* (%)
Astreonam–ofloxacin	7 (33.3)	Ceftazidime–ofloxacin	3 (14.3)
Astreonam–ciprofloxacin	6 (28.6)	Ceftazidime–ciprofloxacin	7 (33.3)
Astreonam–amikacin	1 (4.8)	Ceftazidime–amikacin	2 (9.5)
Astreonam–gentamycin	0	Ceftazidime–gentamycin	2 (9.5)
Imipenem–ofloxacin	2 (9.5)	Meropenem–ofloxacin	3 (14.3)
Imipenem–ciprofloxacin	7 (33.3)	Meropenem–ciprofloxacin	4 (19.1)
Imipenem–amikacin	1 (4.8)	Meropenem–amikacin	0
Imipenem–gentamycin	2 (9.5)	Meropenem–gentamycin	0
Piperacillin/tazobactam–ofloxacin	6 (28.6)	Cefoperazone/sulbactam–ofloxacin	4 (19.1)
Piperacillin/tazobactam–ciprofloxacin	5 (23.8)	Cefoperazone/sulbactam–ciprofloxacin	2 (9.5)
Piperacillin/tazobactam–amikacin	3 (14.3)	Cefoperazone/sulbactam–amikacin	3 (14.3)
Piperacillin/tazobactam–gentamycin	3 (14.3)	Cefoperazone/sulbactam–gentamycin	1 (4.8)