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METALLO-β-LACTAMASE PRODUCING *PSEUDOMONAS AERUGINOSA* STRAINS ISOLATED IN HOSPITALS IN RECIFE, PE, BRAZIL

Vera Magalhães; Ana Kelly Lins; Marcelo Magalhães*

Departamento de Medicina Tropical, Centro de Ciências da Saúde, Universidade Federal de Pernambuco, Recife, PE, Brasil

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SHORT COMMUNICATION

ABSTRACT

Out of 24 nosocomial strains of *Pseudomonas aeruginosa* from Recife, Brazil, 15 (62%) were metallo-β-lactamase producers. Such isolates were resistant to main antipseudomonas drugs, except polymixyn B and aztreonam. The enzyme responsible for the carbapenem-resistance belongs to SPM-1 class, and the gene involved, *bla*spm-1, is likely plasmid located.

Key words: *Pseudomonas aeruginosa*, multidrug-resistance, metallo-β-lactamase.

Since the early 1990s, the therapeutic problem posed by multidrug-resistant *Pseudomonas aeruginosa* gained interest on the recognition of nosocomial strains capable to produce metallo-β-lactamase (MβL). The prevalence of these strains is increasing in different parts of the world (3,9,11,16,18), including Brazil (4,12,13,17,19).

MβL enzymes are a cause of concern because they are able to hydrolyze most beta-lactams, including imipenem and meropenem, drugs considered of reserve for the treatment of Gram-negative multidrug-resistant strains (20). In addition, MβLs are encoded on genes linked to mobile elements, a condition that facilitates their spread among different bacterial species and genera (2). Presently, four distinct groups of MβLs have been recognized: IMP, observed in Japan (18), VIM, originally detected in Italy (8), GIM, detected in Germany (3), and finally, SPM-1, first detected in São Paulo, Brazil (19). As result of variations in their aminoacid sequences, IMP and VIM enzymes are classified in a still growing number of subgroups (20).

Here, we report the occurrence, susceptibility patterns, and MβL production of *P. aeruginosa* strains resistant to carbapenems isolated from hospitalized patients in Recife.

Bacterial isolates

Between November 1, 2002 and February 25, 2003, we isolated 48 strains of *P. aeruginosa* at a clinical laboratory in Recife, Brazil. Twenty-two isolates were from Hospital Português, a large hospital in Recife, and 26 isolates came from smaller hospitals: Hospital das Clínicas, 8, Hospital Unicordis, 4, Hospital São Marcos, 6, and Hospital Santa Joana, 4. All strains were recovered from hospitalized individuals, most were old (mean age = 71.4 years) and debilitated with urinary tract infections or with respiratory problems undergoing ventilation. Only one isolate per patient was considered.

Cultures identification and antibiograms

Bacterial cultures were identified using conventional methods (7). Antibiotic susceptibility was determined by the disk diffusion method, according to NCCLS (10). Tests for polymixyn B, however, were done by using a previously suggested protocol (5). The following antipseudomonas drugs were tested: ceftazidime, gentamicin, piperacillin, amikacin, aztreonam, cefepime, ciprofloxacin, levofloxacin, imipenem, meropenem, tobramicin, and polymixyn B (Oxoid Limited, Hampshire, England). Strains of *P. aeruginosa* presenting

*Corresponding Author. Mailing address: Rua Sete de Setembro, 508. 50050-030, Recife, PE, Brasil. E-mail: marcelo@labmm.com.br

intermediate or complete resistance to imipenem (inhibition zone < 16 mm) were screened for MβL production.

Screening for MβL production and β-lactamase gene identification

Screening was done employing two double disk-synergy techniques. One of them (1) uses as MβL inhibitor blank disks impregnated with 2 µl of undiluted 2-mercaptopropionic acid (2-MPA) solution (Aldrich Chemical Co, Milwaukee, USA) and as indicator, disks containing 30 µg of ceftazidime (Oxoid). An enhanced zone of inhibition between the disks was indicative of MβL production. The other technique (21) compares the sizes of the inhibition zones produced by two 10 µg disks of imipenem (Oxoid), with and without 750 µg of ethylenodiaminetetraacetic acid (EDTA); an increasing of at least 7 mm around the EDTA-imipenem disk was recorded as a positive result.

The identification of MβL coding genes was carried out by Patrice Nordmann (Hospital Bicêtre, Paris) using Polymerase chain reactions (15).

Among 48 isolates of *P. aeruginosa* obtained in this survey, 24 (50 %) were resistant to imipenem. Of these, 15 (62.5%) were positive for MβL production on the screening tests (Table 1). Both EDTA and 2-MPA enzyme inhibitors were equally efficient for neutralizing MβL enzymes.

The imipenem-resistant strains were resistant to all drugs tested, except polymixyn B, independently of MβL production. This resistance pattern was similar to that found in Rio de Janeiro (12). Present data showed a higher percentage of MβL-producing *P. aeruginosa* strains in Recife than that found in other Brazilian cities (12,17). The multiplicity of antibiotic resistance gives to the imipenem-resistant strains a notable advantage for perpetuation and spreading in hospital settings, either carbapenems are or not being used, since they can be selected by a non-specific way (11).

All MβL-producing strains were susceptible to aztreonam, while all except one of the nine non-MβL-producers were resistant (Table 1). The incapacity of the metallo-enzymes to hydrolyze monobactams is well known (11,14); it would be the first indication for the presence of MβL-producing bacteria.

Table 1. Dates of recovery, origin, aztreonam susceptibility, and MβL production by 24 imipenem-resistant *P. aeruginosa* strains isolated in hospitals in Recife, Brazil

Isolate	Date*	Hospital	Age other patient	Isolate source	Aztreonam susceptibility	MβL production
Pae 28	03/11/2002	Português	75	Urine	Susceptible	Positive
Pae 29	05/11/2002	Other	76	Urine	Susceptible	Positive
Pae 30	08/11/2002	Other	75	Blood	Susceptible	Positive
Pae 31	09/11/2002	Other	85	Urine	Resistant	Negative
Pae 32	14/11/2002	Português	51	BAL**	Susceptible	Positive
Pae 33	14/11/2002	Other	91	Urine	Resistant	Negative
Pae 34	29/11/2002	Other	38	BAL	Resistant	Negative
Pae 35	10/12/2002	Other	75	Urine	Susceptible	Positive
Pae 36	13/12/2002	Other	73	Urine	Susceptible	Positive
Pae 37	13/12/2002	Other	76	Sputum	Susceptible	Negative
Pae 38	26/12/2002	Português	75	Blood	Resistant	Negative
Pae 39	30/12/2002	Português	49	Urine	Susceptible	Positive
Pae 40	06/01/2003	Português	75	Tracheal***	Susceptible	Positive
Pae 41	06/01/2003	Português	84	Urine	Susceptible	Positive
Pae 42	09/01/2003	Other	84	Urine	Susceptible	Positive
Pae 43	23/01/2003	Português	43	Tracheal	Susceptible	Positive
Pae 44	25/01/2003	Português	70	Tracheal	Resistant	Negative
Pae 45	27/01/2003	Português	86	Tracheal	Resistant	Negative
Pae 46	31/01/2003	Other	91	Urine	Resistant	Negative
Pae 47	10/02/2003	Other	60	Tracheal	Susceptible	Positive
Pae 49	15/02/2003	Other	55	Tracheal	Susceptible	Positive
Pae 50	17/02/2003	Português	87	Urine	Susceptible	Positive
Pae 51	18/02/2003	Other	63	Tracheal	Susceptible	Positive
Pae 52	23/02/2003	Português	76	Tracheal	Resistant	Negative

***** Day/month/year; ** BAL, bronchi-alveolar lavage; *** Tracheal, tracheal aspirate.

Studies done at Nordmann's laboratory in Paris, involving 11 MβL-producing *P. aeruginosa* strains of the present series, selected at random, indicated that their carbapenemase is encoded on the gene *bla*spm-1, the same gene previously identified in the strain 48-1997, isolated in São Paulo (19). This strain, like the isolates from Recife, carries *bla*spm-1 on a plasmid. In addition, genetic analysis showed that upstream *bla*spm-1 there is a novel common region (CR4) comprising an open reading frame, *orf495*, which may be responsible for eventual mobilization and expression of the resistance gene (13).

The gene *bla*spm-1 is restricted so far to *P. aeruginosa* from Brazilian hospitals. Nevertheless, SPM-1 is not the only metallo-enzyme circulating in the country. Recently, a clinical strain of *Acinetobacter baumannii* producing an IMP-like MβL was detected in a Brazilian teaching hospital (6).

In Recife, drug-resistance constitutes a serious medical menace. Antibiotics are freely sold and for costs saving many patients are prematurely discharged from hospitals to complete their treatment at home. These practices would provide an unwished bridge between hospital and community for the dissemination of genes coding for multiple resistance to antibiotics.

RESUMO

Produção de metalo-β-lactase de linhagens de *Pseudomonas aeruginosa* isoladas em Hospitais do Recife, PE, Brasil

De 24 linhagens hospitalares de *Pseudomonas aeruginosa* provenientes de Recife, Brasil, 15 (62%) produziram metalo-β-lactamase. Tais isolados foram resistentes às principais drogas antipseudomonas, exceto polimixina B e aztreonam. A enzima responsável pela resistência aos carbapanêmicos pertence à classe SPM-1 e o gene envolvido, *bla*spm-1, provavelmente é plasmidial.

Palavras-chave: *Pseudomonas aeruginosa*, multiresistência a drogas, metalo-β-lactamase

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