Microbial Pathogenesis 95 (2016) 62–67

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

First report of novel genetic array *aacA4-bla*$_{IMP-25}$*-oxa30-catB3* and identification of novel metallo-β-lactamase gene *bla*$_{IMP25}$: A Retrospective Study of antibiotic resistance surveillance on *Psuedomonas aeruginosa* in Guangzhou of South China, 2003–2007

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ARTICLE INFO

Article history:
Received 2 February 2016
Received in revised form
19 February 2016
Accepted 26 February 2016
Available online 17 March 2016

Keywords:
P. aeruginosa
Carbapenem-resistant
bla$_{IMP-25}$
aacA4-blaIMP-25-oxa30-catB3
Metallo-β-lactamase

ABSTRACT

Carbapenem, imipenem and meropenem have been broadly prescribed contributing to the global occurrence and prevalence of carbapenem resistance in *Psuedomonas aeruginosa*, and the associated resistance genotypes remains clinically significant. A retrospective surveillance had been conducted on 499 *P. aeruginosa* isolates in South China during 2003–2007, including antimicrobial resistance and characterization of MBLs on carbapenem-resistant strains. One hundred and sixty-four out of 499 strains were carbapenem-resistant, with 11, 4 and 5 strains positive for *bla*$_{IMP-9}$, *bla*$_{IMP-25}$ and *bla*$_{VIM-2}$, respectively. Sixteen out of 20 isolates were positive for *intI1* and contained identical flanking regions (as indicated in KM384735), and all tested isolates containing the *qacE*△*1-sul1* of the typical 3′-conserved region. A novel *bla*$_{IMP-25}$ metallo-β-lactamase and a genetic array of *aacA4-bla*$_{IMP-25}$*-oxa30-catB3* have been discovered from this retrospective surveillance on antimicrobial resistance of *P. aeruginosa*.

1. Background

As a leading pathogen responsible for nosocomial infections, *Pseudomonas aeruginosa* has been recently considered to be a "super bug" due to its natural resistance to various antimicrobial agents and the distinctive capacity (via multiple mechanisms) to virtually engender resistance to all currently available antibiotics. Such factors pose significant clinical and therapeutic challenges for the future management of *Pseudomonas* infections [1]. Despite being regarded as last-resort antimicrobial therapies for such infections, carbapenem (imipenem and meropenem) have been broadly prescribed, which further leads to the global occurrence and prevalence of carbapenem resistance in *P. aeruginosa* [2]. Carbapenem resistance phenotypes are widely associated with expression of Ambler Class B metallo-β-lactamases (MBLs) [3]. In this study, a retrospective surveillance was conducted on *P. aeruginosa* strains isolated from 7 leading tertiary-level teaching hospitals in Guangzhou of South China from 2003 to 2007. Associated antimicrobial resistance tests, phenotypic and molecular detection of MBLs for carbapenem-resistant *P. aeruginosa* isolates, and integron characterization for MBL-positive strains were also performed.

2. Methods

This retrospective surveillance on the antimicrobial resistance of *P. aeruginosa* isolates sampled from 7 leading hospital in Guangzhou of southern China, including Sun Yat-sen Memorial

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http://dx.doi.org/10.1016/j.micpath.2016.02.021
0882-4010/

Hospital of Sun Yat-sen University (2140-bed and 2.6 million annual admission), Nanfang Hospital (2250-bed and 2.7 million annual admission), Guangdong Hospital of Traditional Chinese Medicine (3000-bed and 6.8 million annual admission), the Third Affiliated Hospital of Sun Yat-sen University (1500-bed and 1.7 million annual admission), First Affiliated Hospital of Guangzhou Medical University (1500-bed and 1.5 million annual admission), First Affiliated Hospital of Guangzhou University of Traditional Chinese Medicine (1250-bed and 2.3 million annual admission) and the First Affiliated Hospital of Sun Yat-sen University (2550-bed and 4.6 million annual admission). A total of 499 consecutive non-duplicated *P. aeruginosa* isolates sampled from various clinical specimens from 2003 to 2007 were recovered and investigated (Table 1). Isolates were identified to the species level with standard procedure, including API strip test and Vitek 2 automated system (Vitek AMS; bioMerieux Vitek Systems Inc., Hazelwood, MO). Antimicrobial susceptibility testing was performed by Vitek (Vitek AMS; bioMerieux Vitek Systems Inc., Hazelwood, MO) and microdilution panels (Microscan gram-negative NMIC30; Dade Behring Canada Inc., Mississauga, Ontario, Canada), including amikacin (AMK), aztreonam (ATM), ceftazidime (CAZ), cefepime (FEP), ciprofloxacin (CIP), gentamicin (GEN), levofloxacin (LVX), meropenem (MEM), piperacillin (PIP), imipenem (IPM), piperacillin/Tazobactam (TZP). Results were interpreted according to criteria established by the Clinical and Laboratory Standards Institute (CLSI) [4], with strains of *Escherichia coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 29213 were used as quality controls. Phenotypic detection of MBLs in imipenem-resistant *P. aeruginosa* was screened using the double-disk synergy and combined-disk tests with 2-mercaptopropionic acid and 100 mM EDTA as MBL inhibitors. Molecular detection for MBL genes were characterized for bla_{VIM}, bla_{IMP}, bla_{SPM-1}, bla_{GIM-1} and bla_{SIM-1} by PCR (Table 2) with conditions of: 5 min at 94 °C; 30 cycles of 45 s at 94 °C, 45 s at 55 °C, and 1 min at 72 °C; 5 min at 72 °C. Amplicons were purified and then sequenced (377 DNA sequence analyzer, ABI). The identity or similarity of both nucleotide and deduced amino acid sequences were further analyzed by DNAStar and BLAST programs. Strains with positive MBL genes were further characterized for possible integrase (including *intI1*, *intI2* and *intI3*) by multiplex-PCR, and *intI1*−positive strains were then characterized for the variable region and the flanking region by inverse-PCR and sequencing, with further sequence analysis using BLAST on GenBank [5–7].

Table 1
Phenotypic characteristics of the 499 *P. aeruginosa* strains.

	P. aeruginosa	Multi-resistant rate
Sample		
Pus	67.9% (399/499)	14.7% (59/399)
Excretion	19.6% (98/499)	8.1% (8/98)
Blood	6.0% (30/499)	6.7% (2/30)
Urine	3.8% (19/499)	5.2% (1/19)
Others	2.7% (13/499)	7.6% (1/13)
Department		
ICU	40.8% (204/499)	15.6% (32/204)
Respiration	35.0% (175/499)	17.1% (30/175)
Neurology	8.6% (43/499)	11.6% (5/43)
Hematology	7.4% (37/499)	8.1% (3/37)
Gastroenterology	3.2% (16/499)	0% (0/0)
Urology	2.0% (10/499)	0% (0/0)
Others	2.8% (14/499)	7.1% (1/14)
Resistance rate, MIC, MIC_{50} and MIC_{90} (μg/ml) of 499 *P. aeruginosa*		14.2% (71/499)
IPM	32.8%, ≥16, 8, 128	
MEM	21.4%, ≥16, 8, 64	
CAZ	27.1%, ≥32, 16, 64	
FEP	28.3%, ≥32, 16, 64	
GEN	42.0%, ≥8, 8, 256	
AMK	36.6%, ≥64, 32, 128	
CIP	7.6%, ≥4, 1, 32	
LVX	8.1%, ≥8, 2, 32	
ATM	20.5%, ≥32, 8, 128	
PIP	8.8%, ≥16, 4, 64	
TZP	8.3%, ≥16/4, 4/1, 64/16	
Resistance rate, MIC, MIC_{50} and MIC_{90} (μg/ml) of 164 carbapenem-resistant *P. aeruginosa*		
IPM	100%, ≥16, 64, 256	
MEM	79.2%, ≥16, 32, 256	
CAZ	83.5%, ≥32, 32, 256	
FEP	81.1%, ≥32, 16, 256	
GEN	84.7%, ≥8, 32, 256	
AMK	71.3%, ≥64, 128, 512	
CIP	50.6%, ≥4, 8, 64	
LVX	63.4%, ≥8, 16, 128	
ATM	49.4%, ≥32, 64, 256	
Antibiogram of 164 carbapenem-resistant *P. aeruginosa*		
AG + CA + CE + MO + QU	28.6%	
AG + CA + CE + QU	15.2%	
AG + CA + CE + MO	12.8%	
AG + CA + CE	10.4%	

Samples: others consisted of skin swabs specimens (from the nares, axilla, groin, or perianal area), as well as swab specimens of the throat, tonsils, eye, ear, and vagina; body liquids; puncture exudates.
Department: others included Obstetrics and Gynecology, Pediatrics, Orthopedics and Dept. infectious disease.
Antibiogram: AG, aminoglycosides; CA, carbapenems; CE, cephalosporins; MO, Monobactam; QU, quinolones.