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Carbapenem Resistances among *P. aeruginosa* Isolated in an USA Medical Center: Report of Osteomyelitis Caused by an IMP-15 Producing Strain

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AMENDED ABSTRACT

Background: Acquired carbapenem (CARB) resistance (R) due to metallo-ß-lactamase (MßL) production is increasing rapidly in Asia, Europe and Latin America (LA), but is rarely reported in North America (NA) and particularly in the United States (USA). We evaluated the CARB-R mechanisms among 50 multidrug-R *P. aeruginosa* (PSA) clinical strains (2001-2005) and report one case due to a MßL-producing strain.

Methods: CARB-R (MIC ≥8 μg/ml for imipenem or meropenem) isolates were evaluated. The isolates were tested for susceptibility (S) by Etest and screened for MßLs by Etest MßL strips. MßL screen-positive (inhibited by EDTA) strains were evaluated by PCR for bla_{IMP}, bla_{VIM} and bla_{SPM}, followed by gene sequencing. The role of hyper production of AmpC ß-lactamase, efflux pump and OMP alterations on the R was evaluated on all strains by testing CARBs ± the AmpC (BRL 42715) and efflux pump (MC-207,110) inhibitors, as well as specific PCR tests.

Results: 47 strains showed hyper AmpC production, of which many demonstrated significant decreases (≥four-fold) in CARB MIC values with MC-207,110. One strain was MßL screen-positive, and PCR-positive for bla_{IMP} and gene sequencing demonstrated bla_{IMP-15} (not previously described in NA). The patient was from Mexico where he was hospitalized in March, 2005, for an open femur fracture stabilized with nail fixation. The patient migrated to the USA and was admitted to University of Kentucky HealthCare in August, 2005, with wound drainage. Culture yielded MRSA, *E. faecalis* and the IMP-15 producing PSA. Despite treatment with polymyxin B and other agents, the CARB-R PSA persisted.

Conclusions: CARB-R due to MßL production remains rare in USA hospitals. However, CARB-R strains should be screened for MßL production, especially among patients from endemic geographic regions (LA) to facilitate the implementation of appropriate infection control measures to avoid the dissemination of these important, mobile R mechanisms.

INTRODUCTION

Carbapenems remain the cornerstone of antimicrobial therapy for serious nosocomial infections caused by many antimicrobial-resistant Gram-negative pathogens. However, in recent years, resistance to carbapenems has emerged due to several mechanisms both innate and acquired. *Pseudomonas aeruginosa* can become resistant to imipenem or meropenem by over-production of AmpC ß-lactamases associated with loss of outer membrane porin proteins and/or upregulation of efflux pumps. The production of carbapenemases (serine or metallo-ß-lactamases [MßL]), can also confer high-level resistance to carbapenems.

Acquired carbapenem resistance due to MßL production has increased rapidly in Asia, Europe and Latin America, but remains rare in North America and particularly in the United States (USA). Serine carbapenemase producing strains, however, have been increasingly reported in the USA, mainly among Enterobacteriaceae.

MßLs can be divided into two main groups, those that are mobile, also called acquired MßLs, and those that are normally carried by the host species. Most mobile MßL genes are carried on various genetic elements that aid their movement from one genetic apparatus to another. The first mobile MßL to be characterized outside of *Bacteroides* spp. was IMP-1, which was reported in a *P. aeruginosa* strain from Japan in 1988. IMP-type MßLs have now been observed in at least 12 countries, but only three reports of MßL-producing strains have occurred in the USA: i) a VIM-7 producing *P. aeruginosa* in Houston, Texas; ii) a VIM-2 producing *P. aeruginosa* in Chicago, Illinois and Houston, Texas; and iii) IMP-18 in a *P. aeruginosa* isolate from Las Cruces, New Mexico.

In the present study, we characterized the mechanisms conferring carbapenem resistance in 50 *P. aeruginosa* strains, and report one case of osteomyelitis caused by an IMP-15 producing *P. aeruginosa* strain from Lexington, Kentucky.

MATERIALS AND METHODS

Bacterial isolates and susceptibility testing: A total of 50 *P. aeruginosa* with elevated carbapenem MIC values isolated at the University of Kentucky HealthCare between 2001 and 2005, were referred to JMI Laboratories (North Liberty, IA) for evaluation of resistance mechanisms. The isolates were collected from hospitalized patients and tested for susceptibility by Etest (AB BIODISK, Solna, Sweden) against 10 therapeutically significant antimicrobials. Interpretation of susceptibilities were guided by Clinical and Laboratory Standards Institute (CLSI; M100-S16 document).

Screening for metallo-ß-lactamases (MßL): All isolates resistant to imipenem, meropenem and ceftazidime were screened for potential MßL production using MßL Etest strips (AB BIODISK) according to the manufacturer's instructions. MßL screen-positive (inhibited by EDTA) strains were evaluated by PCR for bla_{IMP}, bla_{VIM} and bla_{SPM}, using generic primers. PCR amplicons were sequenced to determine the type of the MßL. Genetic context of the MßL gene was studied by amplifying the Class 1 integron using primers spanning the conserved regions at the 5' and 3' ends, followed by gene sequencing. Sequences obtained were subjected to NCBI BLAST search.

AmpC and efflux pump screens: The role of hyper production of AmpC ß-lactamase and efflux pump over expression in carbapenem resistance was evaluated using BRL42715 as the AmpC-specific inhibitor, and MC-207,110 as an efflux pump inhibitor. Briefly, the agar dilution method was utilized with imipenem and meropenem alone and in combination with fixed concentrations (4 and 8 μg/ml) of BRL42715, and 20 μg/ml of MC-207,110. A ≥four-fold decrease in the MIC values of imipenem or meropenem in presence of an inhibitor was considered a positive.

Expression of *P. aeruginosa* efflux pumps and OprD: The role of *P. aeruginosa* efflux pump expression and OprD protein was studied by semi-quantitative reverse transcriptase (RT) PCR. Total RNA was extracted from RNAprotect® reagent treated (QIAGEN, Germany) log phase cultures of *P. aeruginosa* using RNeasy® Mini kit (QIAGEN). RNA concentration was determined for each sample and 250ng of RNA was subjected to RT-PCR (OneStep® RT-PCR kit, QIAGEN). The cDNA generated was then amplified using primers specific for *mexAB*, *mexCD*, *mexEF*, *mexXY* as well as *oprD*. Ten μL of each PCR product was separated on 1% agarose gel. Control *P. aeruginosa* PAO1 PCR products obtained simultaneously were also separated by electrophoresis. Intensity of the DNA band was compared to that of the control to determine if a specific gene was up- or down-regulated.

CASE REPORT

A previously healthy 22 years-old Hispanic male was involved in a motor vehicle accident while visiting his family in Mexico, in March 2005. He suffered from a closed right femoral fracture, and underwent open reduction internal fixation at a hospital in Mexico. After the surgery, he returned to Lexington, Kentucky. In July 2005, he noticed pain and swelling with serosanguinous and purulent drainage from the surgical site and visited a local doctor. He was subsequently referred to the University of Kentucky HealthCare for further management.

On August 10, 2005, the patient was taken to the operating room for incision, debridement and removal of hardware. Patient was empirically started on vancomycin plus ampicillin/sulbactam. Bone, tissue and drainage collected from the operation were sent for culture, which both grew methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus faecalis* susceptible to ampicillin and multidrug-resistant (MDR) *P. aeruginosa* (only susceptible to polymyxin B). Blood cultures were negative. The patient developed a fever on the 2nd post-operative day and antimicrobial treatment was changed to polymyxin B, vancomycin and ampicillin based on the culture results. Ampicillin was discontinued on August 22, 2005. Patient subsequently underwent two further incision and debridement procedures, and became afebrile on the 8th day after the first surgery.

On August 24, 2005, patient underwent a final incision and debridement procedure. During this operation, application of a bone external fixation, distal femoral osteotomy and primary wound closure of the right thigh and hip wounds were performed. Bone and tissue specimens were collected for culture, which again grew the same three pathogens. Patient was discharged on August 31, 2005 still receiving polymyxin B and vancomycin for a total of 6-8 weeks.

RESULTS

- The 50 *P. aeruginosa* isolates showed high rates of resistance to all antimicrobial agents tested (Table 1). The most potent (lowest MIC_{50}) antimicrobials were meropenem (MIC_{50}, 24 μg/ml), cefepime (MIC_{50}, 32 μg/ml) and tetracycline (MIC_{50}, 32 μg/ml); while the antimicrobials with the highest susceptibility rates were tobramycin (16%), gentamicin (14%), ciprofloxacin and piperacillin/tazobactam (both 10%).
- Forty-seven of 50 (94%) *P. aeruginosa* demonstrated ≥four-fold reduction in the MIC of imipenem and/or meropenem in presence of BRL42715, indicating a possible contribution of AmpC hyperproduction in carbapenem resistance.

Table 1. Antimicrobial susceptibility of 50 *P. aeruginosa* isolates with elevated carbapenem MIC values (University of Kentucky HealthCare, 2001-2005).

	MIC (μg/ml):				
Antimicrobial agent	50%	90%	Range	% Susceptible[a]	% Resistant[a]
Ertapenem	>32	>32	>32	0	100
Imipenem	>32	>32	2->32	2	94
Meropenem	24	>32	3->32	6	90
Cefepime	32	64	12->256	0	88
Ceftazidime	48	>256	3->256	6	84
Piperacillin/tazobactam	>256	>256	12->256	10	90
Gentamicin	96	>256	0.5->256	14	80
Tobramycin	>256	>256	0.38->256	16	80
Ciprofloxacin	>32	>32	0.25->32	10	88
Tetracycline	32	>256	2->256	2	94

a. Susceptibility categorization per CLSI M100-S16.

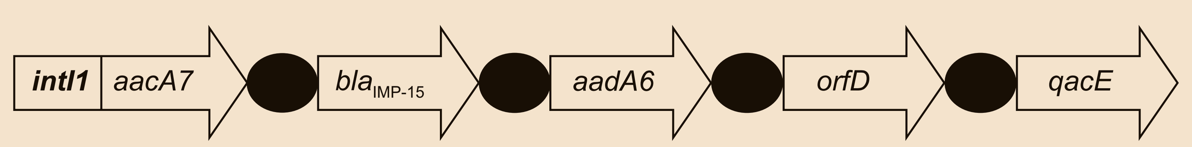
- Only three isolates (6%) showed ≥ four-fold reduction in the imipenem and/or meropenem MIC values in presence of the efflux pump inhibitor MC-207,110 (data not shown).
- Upregulation of *mexXY* and/or *mexCD* was observed in 38 (76%) and three (6%) strains, respectively, while two strains showed upregulation of both efflux genes. Furthermore, reduction of the porin protein OprD was observed in 16 isolates (32%; Table 2).
- Hyperproduction of AmpC was most commonly associated with *mexXY* upregulation (37/50 isolates, 74%), followed by reduction in *oprD* expression (15 isolates, 30%; Table 2).
- All strains (10) with down regulated *oprD* and upregulated *mexXY* also showed hyperproduction of AmpC by phenotypic tests.
- Among 37 isolates screened for MßL production, only one strain (1750J) showed significant reduction of the imipenem MIC value when the carbapenem was combined with EDTA (from >256 to 8 μg/ml).
- PCR screens performed on strain 1750J yielded amplicons with bla_{IMP} as well as class 1 integron primers. Sequencing of the integron revealed bla_{IMP-15} in the integron (position 2) along with aminoglycoside resistance genes *aacA7* and *aadA6* (Figure 1). IMP-15 was originally discovered in *P. aeruginosa* isolates from Thailand (GenBank accession No. AY553333).

Table 2. Distribution of carbapenem resistance mechanisms among 50 *P. aeruginosa* strains (University of Kentucky HealthCare, 2001-2005).

Resistance mechanisms detected:					
AmpC hyperproduction[a]	OprD reduction[b]	*mexXY* upregulation[c]	*mexCD* upregulation[d]	MßL production[e]	No. of isolates
+	+	+			10
+		+	+		2
+	+				5
+		+			25
	+		+		1
+					5
		+			1
				IMP-15	1

a. Hyperproduction of AmpC based on ≥four-fold reduction in the carbapenem MIC (imipenem or meropenem) in presence of BRL42715.
b. Decreased production of outer membrane protein OprD compared to PAO1 control using RT-PCR.
c. Increased expression of *mexXY* operon compared to PAO1 control using RT-PCR.
d. Increased expression of *mexCD* operon compared to PAO1 control using RT-PCR.
e. Metallo-ß-lactamase screens followed by PCR and gene sequencing.

Figure 1. Schematic representation of Class 1 integron carrying bla_{IMP-15} in the index strain 1750J. Antimicrobial resistance genes were aligned on an integron in the sequence shown in the figure. The arrows indicate the direction of transcription while the black ovals represent the 59 base pair (bp) element located between genes.



CONCLUSIONS

- Hyperproduction of AmpC combined with upregulation of the efflux pump *mexXY* represented the most common mechanism of carbapenem resistance among *P. aeruginosa* isolated at University of Kentucky HealthCare.
- This is the fourth report of MßL-producing strains and the first report of an IMP-15-producing strain in the USA.
- Although carbapenem resistance due to MßL production remains rare in USA hospitals, a MßL screen test should be performed on carbapenem-resistant strains, especially among patients returning from endemic geographic regions. The use of these simple tests would facilitate the implementation of appropriate infection control measures and avoid the dissemination of these important, mobile resistance mechanisms.

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