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IMP-18-Producing *Klebsiella oxytoca*, Increasing Genetic Diversity of Metallo-beta-Lactamases in Mexican Hospitals

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SENTRY ANTIMICROBIAL SURVEILLANCE

ABSTRACT

Objectives: To characterize the carbapenem resistance (R) determinant in a *Klebsiella oxytoca* (KOX) strain recovered from a wound infection of an infant patient. IMP-18 was initially described from *P. aeruginosa* (PSA) recovered from southwestern USA and the gene encoding this MBL has been described more recently in PSA from Mexico, Puerto Rico, Brazil, and France carried by distinct genetic contexts.

Methods: 183 Enterobacteriaceae strains collected in Mexican medical centers during 2009 were susceptibility (S) tested by CLSI reference broth microdilution MIC methods. Isolates displaying imipenem (IMI) and/or meropenem (MER) MIC at ≥2 mg/L were screened for carbapenemase production using the Modified Hodge test (MHT) and PCR reactions performed for genes encoding IMP-, VIM-, KPC-, OXA-48, SME-variants, IMI/NMC-A, GES and NDM-1. Amplicons were sequenced. Primers targeting the class 1 integron conserved structures were used to reveal integron structure. S1 nuclease digests were resolved in agarose and hybridized with bla_{IMP-18} probe.

Results: Three (1.6% overall) isolates showed carbapenem-R (one of each: *E. coli*, *K. pneumoniae* and KOX). KOX (imipenem and meropenem MIC values, 4 and 0.5 mg/L, respectively) displayed a positive MHT result and PCR followed by sequencing revealed the presence of bla_{IMP-18}. This isolate was recovered from a 2 y/o female presenting with severe dysuria, latter diagnosed with a bladder rhabdomyosarcoma. After 13 months of chemotherapy a wound infection developed. Vancomycin and meropenem were utilized after poor initial response to ceftriaxone, amikacin and metronidazole. Patient returned to the hospital with fever and bloodculture grew KOX. Prolonged IV infusion of meropenem was initiated and 15 days later the patient was discharged. bla_{IMP-18} was located in an integron with the following cassette arrangement: intI1-bla_{IMP-18}-*aacA4*-*aadA1*-like(K201R and 235E236 insertion)- bla_{OXA-2}(17 nucleotide insertion)-*qacEdelta1/sul1*. This MBL gene was located in a 150-Kb plasmid.

Conclusions: A great variety of MBL enzymes have been described in Mexico (VIM-2, VIM-23 in Enterobacteriaceae and IMP-15 and IMP-18 in PSA). Although IMP-18 was detected in several countries, this gene was only reported in PSA. Our results demonstrate the mobilization of bla_{IMP-18} to Enterobacteriaceae species (KOX) in a unique integron structure, increasing the genetic diversity of MBLs in Mexican hospitals.

INTRODUCTION

The emergence of acquired metallo-β-lactamases (MβLs) among important Gram-negative pathogens, including members of the Enterobacteriaceae family, *Pseudomonas aeruginosa,* and *Acinetobacter* spp. has highlighted this significant clinical problem. MβLs can hydrolyze the vast majority of β-lactam agents available for clinical use and are not inhibited by β-lactamase inhibitors currently marketed or in clinical development.

The metallo-β-lactamase IMP-18 was initially described in 2006 from a *P. aeruginosa* strain collected from a tracheal aspirate specimen recovered from a hospitalized victim of a motorcycle accident. This strain was highly resistant to all antimicrobial agents tested. More recently, IMP-18 has been reported among *P. aeruginosa* strains from Mexico, France, Puerto Rico and Brazil. The gene encoding this enzyme was associated with different class 1 integron structures, including two isolates collected in a Mexican hospital.

In the present study, we report the presence and genetic context of bla_{IMP-18} in a *Klebsiella oxytoca* strain recovered from an infant patient diagnosed with a bladder rhabdomyosarcoma.

MATERIALS AND METHODS

Bacterial isolates. A total of 183 Enterobacteriaceae isolates were consecutively collected from medical centers located in Mexico as part of the SENTRY Antimicrobial Surveillance Program (2009). These isolates were recovered from bloodstream, respiratory tract and skin- and skin-structure infections according to defined protocols. Only clinically significant isolates were included in the study; one per patient episode. Species identification was confirmed by standard biochemical tests and use of the Vitek 2 System (bioMérieux; Hazelwood, Missouri, USA), where necessary.

Susceptibility testing. Isolates were susceptibility tested by the reference broth microdilution method using validated panels manufactured by TREK Diagnostics (Cleveland, Ohio, USA). Interpretations of susceptibility testing results were as described in M100-S21 (CLSI, 2011). *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were concurrently tested for quality assurance; all the results were within published ranges.

Screening for carbapenemases. All Enterobacteriaceae isolates with reduced susceptibility to imipenem or meropenem (MIC, ≥2 mg/L) were screened for production of carbapenemases. Modified Hodge test (MHT) was performed to detect carbapenemase production using imipenem and meropenem as substrates.

Isolates were also evaluated for the presence of bla_{IMP}, bla_{VIM}, bla_{NDM-1}, bla_{KPC}, bla_{SME}, bla_{IMI}, bla_{NMC-A}, bla_{GES} and bla_{OXA-48} by PCR. Amplicons were sequenced on both strands and nucleotide sequences obtained were analyzed using Lasergene® software package (DNAStar; Madison, Wisconsin, USA) and compared to available sequences via NCBI BLAST search (http://www.ncbi.nlm.nih.gov/blast/).

Class 1 integron characterization. Primers designed in the 5' and 3' conserved sequence (CS) regions of class 1 integrons were used in combination with the MβL primers to determine the size and structure of the integron. Additional primers targeting the genes detected in the integron were used to complete sequencing. Amplicons were sequenced as described above.

Gene location analysis. Total cellular DNA embedded in 1% agarose plugs was subjected to partial digestion with S1 nuclease. Plasmids were resolved by electrophoresis performed on the CHEF-DR II (BioRad, Richmond, CA), with the following conditions: 0.5 X TBE, 1% agarose, 13°C, 200V, for 6 hours with switch time ramping from 5 to 25 seconds and 14 hours with the switch time from 30 - 45 seconds. DNA gels were transferred to nylon membranes by southern blotting and hybridized with a digoxigenin labeled (Roche Diagnostics GmbH, Mannheim, Germany) bla_{IMP-18}-specific probe.

CLINICAL CASE

A two year old female was admitted in a hospital in Guadalajara (Mexico) in June 2007 with the diagnosis of a rhabdomyosarcoma. The patient was started on anticancer chemotherapy. In November 2008, the tumor was resected and the patient developed a surgical wound infection, which was initially treated with ceftriaxone, amikacin and metronidazol. These antimicrobial agents were subsequently replaced with vancomycin and meropenem after poor initial clinical response. Patient remained stable with ambulatory antimicrobial chemotherapy via central venous catheter.

The patient returned to the hospital in April 2009 with history of fever. Blood culture grew *K. oxytoca* that was later identified as an IMP-18-producer. Meropenem was started, but due to persistence of fever, the central catheter was removed. After removal of the catheter, patient was started on prolonged IV infusion of meropenem. Fifteen days later patient was discharged without fever.

RESULTS

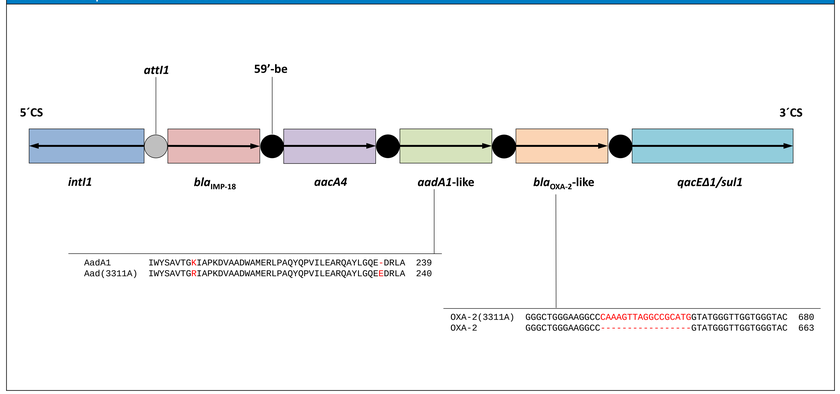
- Among 183 Enterobacteriaceae strains collected in two Mexican hospitals during 2009, three (1.6% overall) showed carbapenem resistance according to current CLSI breakpoints (2011). These isolates were one each of *Escherichia coli*, *Klebsiella pneumoniae* and *K. oxytoca*.
- **Table 1** summarizes the MIC results for the three carbapenem-resistant strains. *K. oxytoca* strain displayed low MIC values for cefepime, ertapenem, meropenem, doripenem and piperacillin/tazobactam (MIC of 0.25, 0.5, 0.5, 2 and 4 mg/L, respectively). The *K. pneumoniae* strain was highly resistant to all β-lactams including carbapenems (MIC results, ≥8 mg/L).
- The *K. oxytoca* strain displayed a positive MHT result; and PCR followed by sequencing revealed the presence of bla_{IMP-18}. The two remaining isolates had negative MHT and negative carbapenemase PCR results.
- bla_{IMP-18} was located in the first position of a class 1 integron and was followed by two aminoglycoside resistance genes encoding for modifying enzymes, *aacA4* and *aadA1* and the ESBL gene, bla_{OXA-2} (**Figure 1**).
- Sequencing of the *aadA1* gene showed the substitution of a lysine for an arginine at position 201 and the insertion of a glutamic acid in the position 236 (**Figure 1**).
- bla_{OXA-2} displayed an insertion of 17 nucleotides(**Figure 1**) that generated a frame shift and the addition of a termination codon.
- Hybridization experiments of S1 nuclease treated preparations showed that bla_{IMP-18} was located in a 150-Kb plasmid.

Table 1. Susceptibility profile of carbapenem resistant Enterobacteriaceae strains collected in Mexican hospitals during 2009 as part as the SENTRY Antimicrobial Surveillance Program.

	MIC results (mg/L)		
Antimicrobial agent	IMP-18-producing *K. oxytoca* 3311A	*E. coli* 12617A	*K. pneumoniae* 1233D
Ampicillin	>16	>16	>16
Ampicillin/sulbactam	>16	>16	>16
Piperacillin/tazobactam	4	>64	>64
Cefoxitin	>16	>16	>16
Ceftazidime	>16	>16	>16
Ceftriaxone	32	>32	>32
Cefepime	0.25	>16	>16
Aztreonam	0.5	>16	>16
Ertapenem	0.5	>8	>8
Doripenem	2	1	>8
Imipenem	4	4	8
Meropenem	0.5	1	>8
Amikacin	32	8	>32
Gentamicin	≤2	≤2	>8
Tobramycin	4	1	>16
Ciprofloxacin	≤0.5	>4	>4
Levofloxacin	≤0.5	>4	>4
Tetracycline	≤2	>8	>8
Tigecycline	0.25	0.5	1
Trim/sulfa[a]	>2	≤0.5	>2
Colistin	≤0.5	≤0.5	≤0.5

a. Trimethoprim/sulfamethoxazole

Figure 1. Schematic representation of class 1 integron carrying bla_{IMP-18}, *aaca4*, *aadA1*-like and bla_{OXA-2}-like from *K. oxytoca* strain 3311A. Partial protein or DNA alignments displaying the alterations (in red) of the genes in the last two gene cassettes and comparison with the ancestor sequence are displayed. Horizontal arrows indicate the gene cassette and their respective translation orientation.



CONCLUSIONS

- The IMP-18-producing *K. oxytoca* displayed low MIC results for various β-lactam agents tested, including cefepime and some carbapenems (**Table 1**). These results are unusual for MβL-producing strains and can pose a challenge for detection of these isolates in clinical microbiology laboratories.
- IMP-18 was previously described in *P. aeruginosa* from Mexico and USA. This report highlights the mobilization of this gene to an Enterobacteriaceae species increasing the diversity of MβL-producing strains in Mexican hospitals.
- Further studies are needed to evaluate the functionality and the spectrum of activity of the *addA1*- and bla_{OXA-2}-like genes also in this *K. oxytoca* isolate.

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