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Co-production of OXA-58 and IMP-1 Carbapenemases in an *Acinetobacter lwoffii*

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SENTRY ANTIMICROBIAL SURVEILLANCE

ABSTRACT

Background: *Acinetobacter* spp. are opportunistic pathogens with ability to spread in the hospital environment and rapidly develop antibiotic resistance. We report the detection of bla_{IMP-1} and bla_{OXA-58} in a single strain of *A. lwoffii*.

Methods: *A. lwoffii* isolate (510-4084W) was recovered from Kanazawa University hospital (April 2007), Japan and tested for susceptibility by CLSI broth microdilution methods. The isolate was screened for metallo-ß-lactamases (MßL) using Etest and imipenem- and meropenem-EDTA double-disk synergy test (DDST). Carbapenem-hydrolyzing oxacillinases (CHCDß) and MßL genes were also screened by PCR, followed by sequencing. bla_{IMP-1}-carrying integron was sequenced. Gene location was performed by Southern blot and hybridization. *catB* was PCR amplified and cloned into pCRII-TOPO, and susceptibility tested. Species identification was confirmed by 16S rRNA sequencing.

Results: *A. lwoffii* was recovered from skin and soft tissue infection in a 58-year-old female. It showed a MIC value of 8 µg/ml for imipenem, meropenem and ertapenem, and ≥32 µg/ml for ceftazidime and ceftriaxone, but remaining susceptible to other antimicrobials. DDST-positive result was obtained, but Etest strip failed to detect the presence of MßL. Further PCR and sequencing confirmed the presence of bla_{IMP-1} and bla_{OXA-58}. bla_{IMP-1} was the third gene in a class 1 integron, also carrying a new *catB* and *aacA4* in the 1st and 2nd position, respectively. The putative CATB protein showed high homology (94.8%) with CATB3, CATB4 and CATB8. *Escherichia coli* carrying the recombinant *catB* gene showed chloramphenicol MIC value 8-fold higher than *E. coli* alone. Specific-bla_{OXA-58} and -bla_{IMP-1} probes showed hybridization signals from the same 27-kb plasmid band.

Conclusions: Although the isolate 510-4084W carried both carbapenemase genes, ß-lactam resistance phenotype was not obvious, which may limit detection and facilitate spread of these genes. This report of co-production of CHCDß and MßL emphasizes the ability of *Acinetobacter* spp. for acquiring resistance genes present in the hospital environment.

INTRODUCTION

Acinetobacter spp. are non-fermentative, Gram-negative rods widely distributed in the environment. These species can be isolated from soil, water and also represent part of the endogenous human bacterial flora, particularly skin, oral cavity and respiratory tract. These pathogens can cause a wide range of opportunistic infections, mainly in elderly and pediatric patients and those with severe underlying disease.

The clinical significance of *Acinetobacter* spp. has escalated lately due to their remarkable ability to develop resistance to several classes of antimicrobial agents. This characteristic in association with the capability of surviving for prolonged periods in the hospital environment provide these microorganisms the great potential for nosocomial spread. *A. baumannii* is considered the main species responsible for hospital outbreaks and treatment complications; while, the remaining species are only occasionally implicated. This study reports the presence of plasmid-encoded IMP-1, OXA-58 and a new CATB in an *A. lwoffii* recovered from Japan.

MATERIALS AND METHODS

Bacterial isolate and species identification. *A. lwoffii* (510-4084W) was collected from Kanazawa University hospital in April, 2007. Species identification was performed by the Vitek System (bioMerieux, Hazelwood, MO) and confirmed by 16S rRNA sequencing analysis. The generated DNA sequence was compared with a DNA library using BIBI, Bioinformatics Bacterial Identification available through the internet (http://pbil.univ-lyon1.fr/bibi).

Antimicrobial susceptibility testing. The isolate was tested for susceptibility using the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI; M7-A7, 2006). Cation-adjusted Mueller-Hinton broth was used in validated panels manufactured by TREK Diagnostics (Cleveland, OH). Etest (AB BIODISK, Solna, Sweden) was also performed according to the manufacture's recommendations. MIC values were interpreted according to the M100-S18 document (CLSI, 2008) for *Acinetobacter* spp. Quality control (QC) was performed using *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853. All QC results were within published ranges.

Screening for MßL and CHCDß. MßL production was screened by disk approximation test (DDST) and Etest MßL strip as recommended by the manufacturer (AB BIODISK).

The isolate was also screened for CHCDß-encoding genes using primers able to detect and distinguish bla_{OXA-23}-, bla_{OXA-24}- and bla_{OXA-58}-like and the intrinsic bla_{OXA-51}-like in a multiplex PCR assay format. MßL screening was performed using generic primers able to detect VIM-, IMP-, SPM-1-, GIM-1-, SIM-1-like-encoding genes in a multiplex real-time platform. Amplicons obtained were sequenced on both strands. The nucleotide sequences and deduced amino acid sequences were analyzed using Lasergene software package (DNASTAR, Madison, WI) and compared with the sequences available through the internet using BLAST (http://www.ncbi.nlm.nih.gov/blast/).

MßL-harboring integron sequencing. Primers targeting the 5' conserved sequence (CS) and the 3' CS of class 1 integrons were used in combination with bla_{IMP-1} primers to amplify and sequence the variable region of the MßL-carrying integron.

Plasmid analysis and CHCDß- and MßL-encoding gene location. Plasmid DNA was extracted using the Plasmid DNA Midi Kit (Qiagen GmbH, Hilden, Germany) and separated on 1% agarose gel in TAE buffer on a Criterion Sub-cell GT system (Bio-Rad, Hercules, CA). Plasmid sizes were determined using plasmid bands from *E. coli* NCTC 50192 as standard references. Total DNA from *A. lwoffii* was digested with I-Ceu-I and DNA fragments and plasmids were separated and transferred onto a nylon membrane by Southern blot. Specific labeled probes for bla_{IMP-1}, bla_{OXA-58} and 16S rRNA were used for hybridization.

Cloning of CATB-encoding gene. Amplicons containing the complete sequence of *catB* were cloned into pCRII-TOPO (Invitrogen, Carlsbad, CA). The colonies obtained after transformation in TOP10 chemically competent *E. coli* were selected on plates containing kanamicin (50 µg/ml). The presence and orientation of insert was confirmed by sequencing.

RESULTS

- *A. lwoffii* (510-4084W) was recovered from a skin and skin struture infection in a 58-year-old female patient hospitalized in Kanazawa University Hospital in April, 2007. The isolate showed MIC values of 8 µg/ml for imipenem, meropenem and ertapenem, ≥32 µg/ml for ceftazidime and ceftriaxone, and 16 µg/ml for cefepime, but remained susceptible to the other antimicrobial agents tested (Table 1).
- A DDST-positive result was obtained, but the Etest strip failed to detect MßL production. Further PCR and sequencing confirmed the presence of bla_{IMP-1} and bla_{OXA-58}.
- bla_{IMP-1} was the third gene cassette detected as part of a class 1 integron, which also carried a new *catB* and *aacA4* in the first and second position, respectively (Figure 1).
- The putative CATB protein showed high homology (94.9%) with CATB4 and CATB8 (Figure 2), and *E. coli* carrying *catB* showed chloramphenicol MIC value 8-fold higher than the *E. coli* carrying only the pCRII-TOPO plasmid vector (Table 2).
- Plasmid analysis revealed presence of several plasmid bands, and hybridization signals using bla_{OXA-58} and bla_{IMP-1}-specific probes were noted from the same plasmid band (approximately 27-kb). Hybridization signals with bla_{OXA-58} and bla_{IMP-1} probes from the I-Ceu-I-digested chromosomal DNA were not observed.

Table 1. Antimicrobial susceptibility profile of an *A. lwoffii* (510-4084) clinical isolate.

Antimicrobial agent	MIC value (µg/ml)
Ampicillin/sulbactam	≤2
Piperacillin/tazobactam	≤0.5
Aztreonam	8
Ceftriaxone	32
Ceftazidime	128
Cefepime	16
Ertapenem	8
Imipenem	8
Meropenem	8
Ciprofloxacin	0.06
Amikacin	1
Gentamicin	4
Tobramycin	4
Polymyxin B	≤0.5

Table 2. MIC values for chloramphenicol tested against *A. lwoffii* clinical isolate and *E. coli* (TOP10) harboring *catB* recombinant plasmid and *E. coli* harboring the plasmid vector with no insert.

Microorganism	Chloramphenicol MIC (µg/ml)
A. lwoffii (510-4084W)	2
E. coli (TOP10 – pCRII-TOPO + *catB*)	16
E. coli (TOP10 – pCRII-TOPO)	2

Figure 1. Schematic representation of bla_{IMP-1}-carrying integron detected in *A. lwoffii* isolate recovered from Kanazawa University hospital. The 3-kb integron possessed a *catB*, an *aacA4* and bla_{IMP-1} gene cassettes as part of the variable region of a class 1 integron. Filled circles represent the 59-be recombination sites.

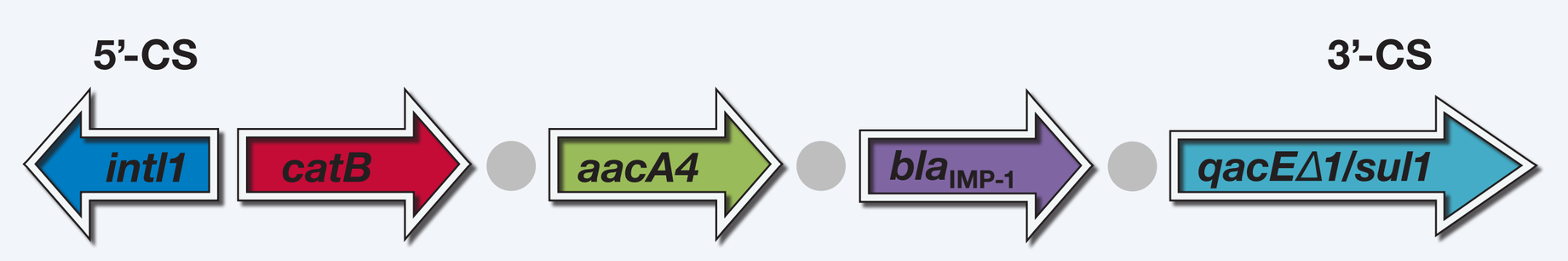

Figure 2. A. ClustalW amino acid alignment of chloramphenicol acetyl transferase encoded by *catB* gene cassette found in the bla_{IMP-1}-carrying class 1 integron with those most similar proteins. Differences in the amino acid sequences are noted by insertion of a single letter representing the amino acid change within that particular sequence. Accession numbers of CATB3, CATB4, CATB7 and CTAB8 are as follows: YP190213, AAK54951, AAD02068 and AAX14005; B. Percent of identity and divergence values of each CATB amino acid sequence pair obtained with ClustalW alignment.

Figure 2A

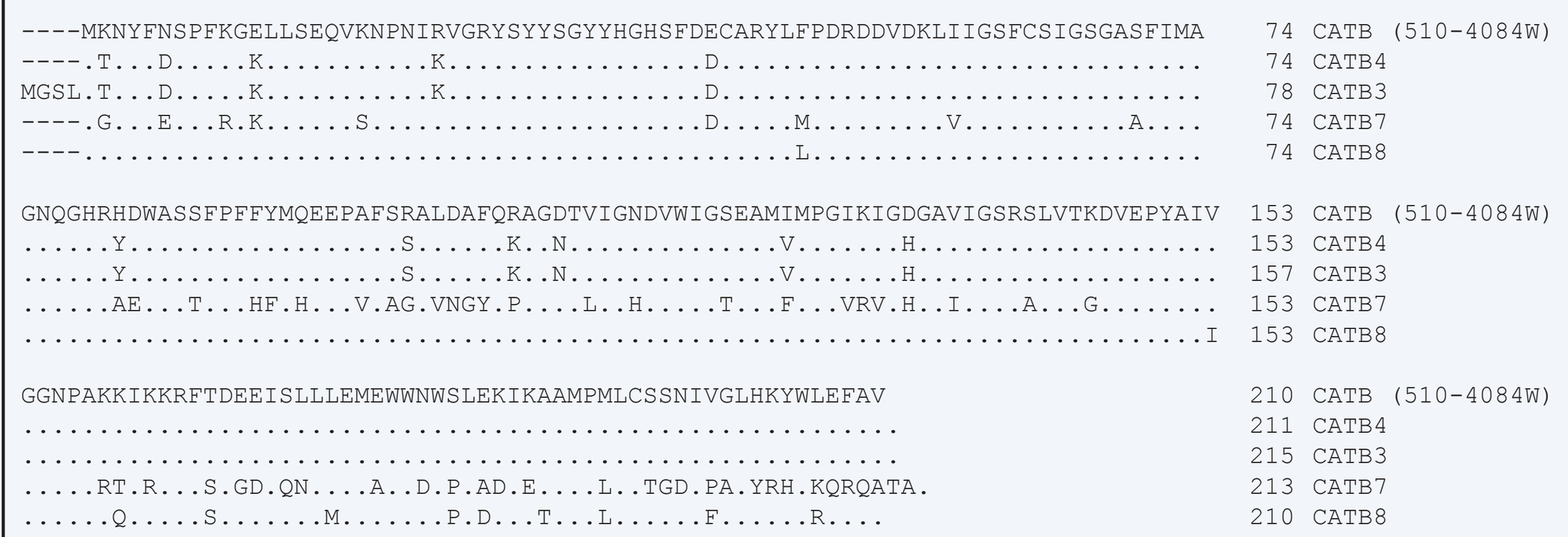

Figure 2B

Percent Identity	1	2	3	4	5		
1		94.9	93.1	71.4	94.9	1	CATB (510-4084W)
2	5.4		97.7	71.6	89.9	2	CATB4
3	5.4	0.0		69.7	88.0	3	CATB3
4	37.5	36.7	36.7		71.4	4	CATB7
5	5.4	11.3	11.3	37.5		5	CATB8
Divergence	1	2	3	4	5		

CONCLUSIONS

- This study describes the detection of an *A. lwoffii* clinical isolate with a multiresistance plasmid DNA harboring several resistance determinants, including two carbapenamase genes and a new chloramphenicol acetyltransferase gene.
- Co-production of carbapenemases has been previously described in *A. junii* and *A. baumannii* from Australia and Singapore (IMP-4 and OXA-58), and *A. baumannii* from Greece (VIM-1/4 and OXA-58); however, these isolates showed carbapenem-resistance phenotype.
- Although the *A. lwoffii* 510-4084W isolate carried both carbapenemase genes, carbapenem resistance was not obvious, which limits detection and facilitates spread of these genes.
- This report emphasizes the ability of *Acinetobacter* spp. to acquire foreign DNA present in the nosocomial environment, including resistance genes.

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