Emergence and Widespread Dissemination of OXA-23, -24 and -58 Carbapenemases Among *Acinetobacter* spp. in Asia-Pacific Nations (APAC): Report from the SENTRY Antimicrobial Surveillance Program (2006)

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ABSTRACT

Background:
OXA-type carbapenemases are increasingly found as a resistance (R) determinant in *Acinetobacter* (ACIN) worldwide. OXA-58 has been only reported in Europe, Middle-East and Venezuela. As part of screening carbapenem-resistant (CARB-R) ACIN, the SENTRY Program (APAC) detected OXA-58 encoding genes in 2 additional countries.

Methods:
ACIN from infected patients in 10 countries (41 sites) were susceptibility (S) tested by CLSI broth microdilution method against a wide range of antimicrobials including imipenem (IMP) and meropenem (MERO). Isolates with IMP or MER MIC at > 4 µg/ml were screened for metallo-ß-lactamase (MBL) enzymes (IMP, VIM, SIM, etc.) and OXA-23, -24, -51 (*A. baumannii* [ACB]-assoc.) and -58.

Results:
CARB-R among APAC ACIN was elevated, greatest R in Singapore (SG; 95%), Korea (K; 87%), Taiwan (TA; 63%), Thailand (TH; 59%) and Hong Kong (HK; 50%). OXA-23 was detected in 6 nations (see Table), OXA-24 (3) and OXA-58 (China [C] and TH only). C and TH had the widest variety of enzyme types with 3 TH strains having OXA-23, -24 and -58. MßL-producing ACB isolates were noted: bla_{IMP-4} (Philippines) and bla_{VIM-2} (K). Clonality of isolates was examined via antibiogram patterns and Pulsed-Field Electrophoresis (23 clones, 6 nations, 12 hospitals); most severe in SG.

	Occurrence by nation[a]							
OXA-type	C	HK	I	IN	K	SG	TA	TH
23	16	4	40	0	24	20	0	23
23, 58	11	0	0	0	0	0	0	0
23, 24, 58	0	0	0	0	0	0	0	3
24	0	0	0	1	0	0	2	2
24, 58	0	0	0	0	0	0	0	1
58	2	0	0	0	0	0	0	0
Total	29	4	40	1	24	20	2	29
Sample (%)	182 (16)	8 (50)	131 (31)	67 (2)	46 (52)	21 (95)	29 (7)	49 (59)

a. C = China, HK = Hong Kong; I = India; IN = Indonesia; K = Korea; SG = Singapore; TA = Taiwan; and TH = Thailand. No OXA-producing strains were detected in Australia or the Philippines.

Conclusion:
OXA-23 (83%) alone or with OXA-24 and/or -58 (9%), OXA-24 alone (3%) or with OXA-58 (1%) and OXA-58 alone (1%) have rapidly emerged in APAC and are becoming a global issue that compromises ß-lactam therapy. Only polymyxins (99.1% S) and glycylcyclines (tigecycline; 98.9% S) remained consistently active.

INTRODUCTION

Resistance to the major classes of antimicrobial agents (except polymyxins) has increased substantially among *Acinetobacter* spp. Carbapenems are important therapeutic options for treating infections caused by *Acinetobacter* spp., but they may be hydrolyzed by Ambler class B metallo-ß-lactamases (MßL) as well as class D ß-lactamases.

Five groups of acquired MßL-encoding genes have been identified; however, only bla_{IMP}- and bla_{VIM}-like and more recently bla_{SIM-1} have been reported in *Acinetobacter* spp. IMP and VIM variants have been detected in several eastern countries, including Japan, Hong Kong, South Korea and Australia.

Acquired class D ß-lactamases encoding genes described in *Acinetobacter* spp. have been clustered in three groups, namely, bla_{OXA-23}-, bla_{OXA-24}- and bla_{OXA-58}-types. Additionally, there is a fourth gene cluster (bla_{OXA-51}), which is naturally occurring and chromosomally located in *A. baumannii*. Usually, bla_{OXA-23} and bla_{OXA-58} have been identified on plasmids, while bla_{OXA-24} is usually chromosomal located. The aim of this study was to evaluate the distribution of oxacillinase and MßL genes among *Acinetobacter* spp. isolates recovered from medical centers in the Asia-Pacific (APAC) region.

MATERIALS AND METHODS

Bacterial isolates. During 2006, 41 medical centers located in 10 countries in the APAC region were recruited to participate to the SENTRY Antimicrobial Surveillance Program. Isolates were consecutively collected from bloodstream, respiratory tract, skin and soft tissue, and urinary tract 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 Vitek Systems (bioMérieux; Hazelwood, Missouri, USA), when necessary.

Antimicrobial susceptibility testing. All isolates were susceptibility tested against more than 25 antimicrobials by the broth microdilution procedure described by the Clinical and Laboratory Standards Institute (CLSI; 2006) using validated panels manufactured by TREK Diagnostics (Cleveland, Ohio, USA). Interpretations of susceptibility testing results were performed by CLSI (2007) breakpoint criteria. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were concurrently tested for quality assurance.

Oxacillinase- and MßL-encoding genes detection. Isolates non-susceptible to imipenem or meropenem (MIC, ≥8 µg/ml) were screened using primers able to detect and distinguish alleles encoding three subgroups of acquired oxacillinases (OXA-23-like, OXA-24-like and OXA-58-like) and the intrinsic subgroup of OXA-51-like in a multiplex PCR assay format. MßL screening was performed using generic primers able to detect VIM- and IMP-like, and SPM-1, GIM-1, SIM-1-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/).

Molecular typing. Oxacillinase-producing isolates were typed using Pulsed-Field Gel Electrophoresis (PFGE). Briefly, genomic DNA was immobilized in agarose blocks and digested with the restriction enzyme SmaI (Life Technologies, Gaithersburg, Maryland). Electrophoresis was performed on the CHEF-DR III (BioRad, Richmond, California), under the following conditions: 0.5 x TBE, 1 % agarose, 13°C , 200V, for 23-24 h with the switch time ramped from 5 to 60-90 seconds. Gels were stained with ethidium bromide, destained in distilled water, and photographed under UV light. Isolates with identical profiles were assigned to the same type. Isolates that differed by one to three bands were assigned as subtypes. Strains were considered unique by PFGE if they differed by more than six bands.

RESULTS

- Among 543 *Acinetobacter* spp. isolates recovered in the APAC region in 2006, polymyxins (99.1% susceptibility) and glycylcyclines (tigecycline; 98.9% susceptibility) were the most active antimicrobial agents, followed by imipenem and meropenem (59.6% and 58.5% susceptibility, respectively) (Table 1).
- A total of 229 (42.2%) were non-susceptible (MIC, ≥8 µg/ml) to imipenem or meropenem, with highest rates in Singapore (95.2%), Korea (87.0%), Taiwan (62.5%), Thailand (59.2%) and Hong Kong (50.0%).
- Among the non-susceptible isolates, oxacillinase production was detected in 65.1% (149 isolates) of the isolates and MßLs was found in only 0.9% (two isolates).
- bla_{OXA-23} was detected in 23.4% of the isolates, which were distributed among six nations (Figure 1); while bla_{OXA-24} and bla_{OXA-58} were detected in 0.9% and 0.4% of the isolates, respectively, only noted in China and Thailand.
- The widest variety of oxacillinase enzyme types was observed in Thailand, where three *Acinetobacter* spp. isolates (one clone) harbored the bla_{OXA-23}, bla_{OXA-24} and bla_{OXA-58} (Figure 1).
- MßL-encoding genes were detected in one isolate recovered from the Philippines (bla_{IMP-4}) and one isolate recovered from Korea (bla_{VIM-2}) (Figure 1).
- In general, PFGE analysis showed clonal dissemination of *Acinetobacter* spp. isolates carrying oxacillinase genes within medical centers (Table 2).

Table 1. Activity of antimicrobial agents tested against *Acinetobacter* spp. isolates recovered from APAC region during 2006.

Antimicrobial agent	MIC_{50}	MIC_{90}	Range	% susceptible/resistant[a]
Acinetobacter spp.[b] (543)				
Ceftazidime	>16	>16	≤1->16	37.5 / 61.0
Cefepime	>16	>16	≤0.25->16	37.3 / 55.8
Ampicillin/sulbactam	>16	>16	≤2->16	42.5 / 51.6
Imipenem	2	>8	≤0.12->8	59.6 / 37.4
Meropenem	2	>8	≤0.12->8	58.5 / 39.4
Levofloxacin	>4	>4	≤0.5->4	38.3 / 50.6
Amikacin	>32	>32	≤4->32	44.4 / 53.8
Gentamicin	>8	>8	≤2->8	36.9 / 63.9
Tigecycline	0.5	1	≤0.03->4	98.8 / 1.2
Polymyxin B	≤0.5	1	≤0.5->4	99.1 / 1.0

a. According to CLSI M100-S17.
b. Includes 392 non-oxacillinase- and MßL-producing isolates, 149 oxacillinase-producing isolates and 2 MßL-producing isolates.

Table 2. Clonality of isolates examined via antibiogram patterns and PFGE using SmaI.

Enzyme	No. of strains	No. of clones[a]	Medical center	Country
OXA-23	4	1	204	Hong Kong
	20	5	217	Singapore
	6	3	218	China
	15	4	224	Korea
	9	1	225	Korea
	15	4	226	Thailand
	8	3	227	Thailand
	9	1	231	China
	1	-	238	China
	7	2	243	India
	3	-	246	India
	23	4	248	India
	4	2	252	India
OXA-24	5	4	221 / 226 / 242	Taiwan/ Thailand/ Indonesia
OXA-58	2	1	232 / 234	China
OXA-23 and -58	11	2	231	China
OXA-24 and -58	1	-	226	Thailand
OXA-23, -24 and -58	3	1	226	Thailand

a. Indicates the number of clones carrying oxacillinase-encoding genes within each participating medical center.

Figure 1. Distribution of acquired MßL- and oxacillinase-encoding genes in *Acinetobacter* spp. recovered from medical centers in the APAC region. Indicated countries represent the location of the participating medical centers and the MßL- or oxacillinase-encoding genes, followed by the number of isolates.

CONCLUSIONS

- High rates of carbapenem-resistance in *Acinetobacter* spp. isolates were found in the APAC region (42.2%).
- Carbapenem resistance was highly associated with the presence of oxacillinase enzymes and not MßL production. Only one isolate harboring bla_{OXA-58} showed a susceptible phenotype towards carbapenems ($P < 0.001$).
- OXA-23-encoding gene is highly disseminated in *Acinetobacter* spp. isolates from the APAC region.
- OXA-24- and OXA-58-encoding genes have rapidly emerged in this region and bla_{OXA-58} is as prevalent as bla_{OXA-23} in China.
- Several *Acinetobacter* spp. isolates harbored more than one acquired OXA-encoding gene, characteristics not previously observed, illustrating the capability for dissemination of these resistance determinants.
- MßL-encoding genes were not highly prevalent among carbapenem-resistant *Acinetobacter* spp. Additionally, SIM-1-encoding gene does not seem to have spread in Seoul, Korea, where it was first described.
- Oxacillinase-producing *Acinetobacter* spp. are becoming a global persistent problem that compromises all ß-lactam therapy. Only polymyxins and tigecycline remained consistently active.

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