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Carbapenemases-producing Enterobacteriaceae in 2013: Increasing Prevalence of Multiple Carbapenemases in Single Isolates, Expansion of OXA-48-producers and a New KPC-variant

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Abstract

Background: Carbapenemase-producing Enterobacteriaceae have been increasingly reported worldwide. These isolates often can be multidrug resistant (R) and pan-drug R has been described. We updated the occurrence of carbapenemases in the SENTRY Antimicrobial Surveillance Program sampling and describe a new KPC-encoding gene.

Methods: 223 Enterobacteriaceae isolates displaying meropenem or imipenem MIC values ≥2 µg/mL susceptibility tested by CLSI broth microdilution methods were screened for bla_{KPC}, bla_{SME}, bla_{GES}, bla_{NMC-A}, bla_{IMI}, bla_{IMP}, bla_{VIM} and bla_{NDM}. Selected isolates were also screened for bla_{SPM-1}, bla_{GIM-1}, bla_{SIM-1}, bla_{AIM-1}, bla_{KHM-1}, bla_{DIM-1} and bla_{BIC-1}. A new bla_{KPC} and bla_{KPC-2} were cloned using pCR-Blunt II-TOPO as recommended by the manufacturer and colony selection was performed on 50 mg/L of kanamycin. The presence and orientation of inserts was confirmed by PCR/sequencing and MIC testing was performed.

Results: Among 223 isolates, 194 carried carbapenemase genes tested. KPC-2 (8 countries) and KPC-3 (6 countries) were the most common enzymes detected in 61 and 57 isolates, respectively; and were more prevalent in the USA (39 and 48 occurrences, respectively). VIM-1 was also very common and was detected among 25 isolates from 5 European countries. Isolates producing VIM-1 also carried genes encoding KPC-3 (4 isolates from Italy), OXA-244 or OXA-244 and NDM-1 (2 and 1 from Turkey). NDM-1-producing isolates were observed in India (10 isolates; 1 producing OXA-232), China, Philippines, Malaysia, Turkey and Romania (4, 4, 1, 1 and 2 isolates, respectively). Two isolates producing NDM-7 were noted in the Philippines. OXA-48-like was detected among 7 isolates from 6 countries. SME-4 was observed in 3 *S. marcescens* from the USA. Occurrences of GES-18, KPC-4, IMP-1, IMP-4, IMP-26, VIM-23, VIM-32 (USA) and VIM-5 were noted. A new KPC-variant displaying one alteration V29I was detected and when compared to its closest variant KPC-2, displayed similar spectrum for cephalosporins and carbapenems.

Conclusions: Compared to previous data, we noted a larger number of isolates carrying multiple carbapenemase genes and the expansion of OXA-48 that has been previously reported in SENTRY Program mainly from Turkey.

Introduction

Carbapenems are broad spectrum antimicrobial agents recommended for the treatment of severe infections, mainly in geographic regions where multidrug resistance is common. Carbapenems are stable in the presence of various β-lactamase enzymes; however, the emergence and increasing prevalence of acquired carbapenemases jeopardize the use of these agents.

A large number of acquired carbapenemases have been identified and characterized and among these diverse enzymes, KPC-, VIM- and NDM-variants seem to have spread in various continents and bacterial species. Genes encoding carbapenemases are associated with mobile genetic elements that allow rapid dissemination in the clinical setting. Therefore, detection and surveillance of carbapenemase-producing organisms have become matters of major importance for the selection of appropriate therapeutic schemes and the implementation of infection control measures.

In this study, we evaluated 223 Enterobacteriaceae isolates collected worldwide as part of the SENTRY Antimicrobial Surveillance Program. These isolates displayed elevated MIC values for carbapenems (imipenem or meropenem ≥2 µg/mL) and were screened for carbapenemase-encoding genes. Additionally, we performed the preliminary characterization of a new KPC-variant, named KPC-20.

Methods

Bacterial isolates: A total of 18,409 Enterobacteriaceae isolates were collected from 172 hospitals participating in the SENTRY Antimicrobial Surveillance Program during 2013. These isolates were recovered from bloodstream (3909 of isolates), intra-abdominal (282), skin/soft tissue (4832), urinary tract (4299) infections, pneumonia in hospitalized patients (3692) and other sites (1736). Only clinically significant isolates were included in the study, one per patient episode according to defined protocols. Species identification was confirmed by standard biochemical tests and using the MALDI Biotyper (Bruker Daltonics, Billerica, Massachusetts, USA) according to the manufacturer instructions, where necessary.

Susceptibility testing: All isolates were susceptibility tested using broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI) guidelines using validated panels. Categorical interpretations for all antimicrobials were those found in the CLSI document M100-S24 and the EUCAST website, and quality control (QC) was performed using *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. All QC results were within specified ranges as published in CLSI documents.

Screening for carbapenemase encoding genes: Isolates with reduced susceptibility to imipenem and/or meropenem (MIC, ≥2 µg/mL) were screened for the presence of bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{KPC}, bla_{SME}, bla_{GES}, bla_{IMI}, bla_{NMC-A} and bla_{OXA-48} variants by multiplex PCR reactions. Amplicons were sequenced on both strands. Results were analyzed using Lasergene® software (DNAStar, Madison, Wisconsin) and compared to available sequences through the internet using BLAST (http://www.ncbi.nlm.nih.gov/blast/). Isolates displaying negative results to the genes described above were screened for the presence of bla_{SPM-1}, bla_{GIM-1}, bla_{SIM-1}, bla_{AIM-1}, bla_{KHM-1}, bla_{DIM-1} and bla_{BIC-1}.

Characterization of KPC-20-encoding genes: The gene encoding KPC-20 was cloned using the cloning vector pCR-Blunt II-TOPO (Zero Blunt TOPO PCR Cloning kit; Life Technologies) as recommended by the manufacturer and colony selection was performed on plates containing 50 µg/mL of kanamycin and 0.5 µg/mL of ceftazidime. The presence and orientation of inserts was confirmed by PCR and sequencing. MIC testing was performed as described above.

Results

- Overall, 223 (1.2%) Enterobacteriaceae isolates displayed elevated carbapenem MIC values belonging to the following bacterial species: *Citrobacter freundii* (7), *Enterobacter aerogenes* (11), *Enterobacter cloacae* (34), *E. coli* (16), *Klebsiella oxytoca* (4), *Klebsiella pneumoniae* (133), *Proteus mirabilis* (6), *Providencia stuartii* (2) and *Serratia marcescens* (10).
- Carbapenem non-susceptible isolates were detected in Asia-W. Pacific (2.4% of overall isolates in the region; 40/1694), Latin America (1.7%; 24/1448), North America (1.1%; 103/9263) and Europe (0.9%; 56/6004). These isolates were recovered from bloodstream (28 isolates), intra-abdominal (7), respiratory tract (hospitalized patients; 75), skin/soft tissue (39), urinary tract (46) and other sites (22).
- KPC-encoding genes were detected among the120 isolates (Table 1) and KPC-2 and KPC-3 were the most common genes being detected among 61 and 57 isolates, respectively. KPC-4 and a new KPC-variant (KPC-20) were detected among one isolate each (*E. cloacae* and *E. coli*, respectively) from the USA.
- The majority of KPC-producing isolates were detected in the USA (38 KPC-2-, 48 KPC-3-, one of each KPC-4 and KPC-20), but the genes encoding KPC-2 and KPC-3 were also detected in another seven and five countries, respectively, including several Latin American countries (KPC-2 in five countries and KPC-3 in Colombia).

Table 1. Distribution by country and bacterial species of 120 KPC-producing strains detected during 2013 in hospitals participating on the SENTRY Antimicrobial Surveillance Program.

Carbapenemase (s) Country (no. of strains)	No. of isolates by species producing carbapenemase(s):						
	CF	EA	EC	ECL	KOX	KPN	SM
KPC-2 (60)	1	7	3	6		41	2
Argentina (3)			1	1		1	
Brazil (3)				2		1	
China (6)		2				4	
Colombia (2)		1					1
Ecuador (1)						1	
Greece (1)						1	
USA (38)	1	1	2	2		31	1
Venezuela (6)		3		1		2	
KPC-2, VIM-4 (1)						1	
USA (1)						1	
KPC-3 (53)	1	1	2	5	2	41	1
Colombia (1)						1	
Germany (1)						1	
Israel (1)						1	
Italy (1)						1	
Turkey (1)						1	
USA (48)	1	1	2	5	2	36	1
KPC-3, VIM-1 (4)						4	
Italy (4)						4	
KPC-4 (1)				1			
USA (1)				1			
KPC-20 (1)			1				
USA (1)			1				

Abbreviations: CF - *C. freundii;* EA - *E. aerogenes;* EC - *E. coli;* ECL - *E. cloacae;* KOX - *K. oxytoca;* KPN - *K. pneumoniae;* SM - *S. marcescens*.

- Among non-KPC-carbapenemases, NDM-1 and VIM-1 were the most prevalent carbapenemases and were detected in 24 isolates each, alone or with other carbapenemases (including KPC, as described above). NDM-1-producing isolates were more common in India (10 occurrences), but were also observed in China (4), Philippines (4), Romania (2), Turkey and Malaysia (one each; Table 2).
- OXA-48-like enzymes were observed among 15 isolates and variants included: OXA-48 (7 occurrences), OXA-232 (5) and OXA-224 (3). Isolates producing these enzymes were noted in eight countries (India, Turkey, Ireland, Romania, Russia, Spain, Ukraine and USA; Table 2).
- Two other NDM-variants were detected: NDM-5 in two isolates (China and India; Table 2) and NDM-7 in three isolates (one in India and two in the Philippines).
- Multiple carbapenemase encoding genes were observed among 11 isolates (Tables 1 and 2). Four isolates from Italy harbored bla_{VIM-1} in addition to bla_{KPC-3} and one isolate from New York, USA carried genes encoding VIM-4 and KPC-2 (Table 1). Three isolates from India carried bla_{NDM-1} and genes encoding NDM-1 and OXA-232 and one from Turkey carried bla_{NDM-1}, $bla_{OXA-224}$ and bla_{VIM-1}. Another isolate from Turkey carried only $bla_{OXA-224}$ and bla_{VIM-1} (Table 2).
- Nine other carbapenemase-encoding genes were noted among 1-3 isolates (Table 2) and 29 isolates were negative for all carbapenemase genes tested. Only five of these carbapenemase-negative isolates had imipenem and meropenem MIC values >2 µg/mL.
- KPC-20 displayed one aminoacid alteration (V29I; Figure 1) when compared to KPC-2, its closest variant (99.7% homology). When these two genes were expressed in the same *E. coli* background, MIC values for carbapenems and cephalosporins were very similar (Table 3).

Table 3. Susceptibility results for clinical and recombinant isolates carrying genes encoding KPC-20 and KPC-2 compared.

Antimicrobial agent	Isolate (MIC µg/mL)[a]			
	E. coli clinical isolate carrying bla_{KPC-20}	*E. coli* TO10 pCR Blunt-II TOPO (bla_{KPC-20})	*E. coli* TO10 pCR Blunt-II TOPO (bla_{KPC-2})	*E. coli* TO10 recipient
Imipenem	4	1	1	0.25
Meropenem	4	0.06	0.12	≤0.06
Doripenem	2	≤0.12	≤0.12	≤0.06
Cefoxitin	>16	8	16	4
Ceftriaxone	>8	1	1	0.12
Ceftazidime	32	1	1	0.25
Cefepime	16	≤0.5	≤0.5	≤0.5
Aztreonam	>16	4	4	≤0.12
Ampicillin	>8	>8	>8	4
Ampicillin/sulbactam	>32	32	32	4
Amoxicillin/clavulanate	>8	>8	>8	8
Piperacillin/tazobactam	8	8	8	2
Amikacin	16	NT	NT	NT
Tobramycin	>16	NT	NT	NT
Ciprofloxacin	>4	NT	NT	NT
Polymyxin B	0.5	NT	NT	NT

a. NT=Not tested.

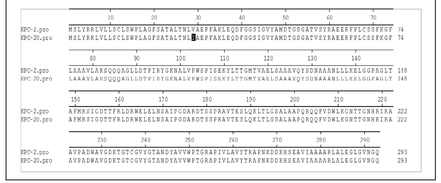
Table 2. Distribution of non-KPC carbapenemases by country and bacterial species detected during 2013 in hospitals participating in the SENTRY Antimicrobial Surveillance Program.

Country Carbapenemase(s) (no. of strains)	No. of isolates by species producing carbapenemase(s):								
	CF	EA	EC	ECL	KOX	KPN	PM	PST	SM
Belgium (1)	1								
GES-18 (1)	1								
China (10)		1	3	1	2	3			
IMP-1 (1)					1				
IMP-26 (3)				1	1	1			
IMP-4 (1)						1			
NDM-1 (4)		1	2			1			
NDM-5 (1)			1						
Croatia (2)				2					
VIM-1 (2)				2					
Greece (10)				6		1	1	2	
VIM-1 (10)				6		1	1	2	
India (15)			4			7	4		
NDM-1 (9)			1			4	4		
NDM-1, OXA-232 (3)			1			2			
NDM-5 (1)			1						
NDM-7 (1)			1						
OXA-232 (1)						1			
Ireland (1)						1			
OXA-48 (1)						1			
Italy (4)			4						
VIM-1 (4)			4						
Malaysia (1)						1			
NDM-1 (1)						1			
Mexico (2)			1			1			
GES-like[a] (1)						1			
VIM-23 (1)			1						
Philippines (6)						6			
NDM-1 (4)						4			
NDM-7 (1)						2			
Poland (3)	1		2						
VIM-1 (1)	1								
VIM-4 (2)			2						
Romania (3)			1			1			1
NDM-1 (2)			1						1
OXA-48 (1)						1			
Russia (1)						1			
OXA-48 (1)						1			
Spain (1)						1			
OXA-48 (1)						1			
Turkey (8)			2			6			
IMP-1 (1)						1			
NDM-1, OXA-244, VIM-1 (1)						1			
OXA-244, VIM-1 (2)						2			
OXA-48 (2)						2			
VIM-1 (1)			1						
VIM-5 (1)			1						
Ukraine (1)						1			
OXA-48 (1)						1			
USA (5)		1				1			3
OXA-232 (1)						1			
SME-4 (3)									3
VIM-32 (1)	1								

Abbreviations: CF - *C. freundii;* EA - *E. aerogenes;* EC - *E. coli;* ECL - *E. cloacae;* KOX - *K. oxytoca;* KPN - *K. pneumoniae;* PM - *P. mirabilis;* PST - *P. stuartii;* SM - *S. marcescens*.

a. Multiple peaks in sequencing were noted, but at least one carbapenemase-encoding gene was identified. Further investigations of this isolate are being performed.

Figure 1. Alignment of the amino acid sequences of KPC-20 detected in an *E. coli* isolate from a New York City hospital compared to its closest variant KPC-2.



Conclusions

- An increasing diversity of carbapenemase encoding genes was noted in 2013 when compared to previous years of our surveillance for these genes. OXA-48-, NDM-variants and KPC-2 and KPC-3 seem to be spreading in various countries with endemic occurrences in Turkey, India and USA, respectively.
- A new KPC-encoding gene was identified displaying one amino acid alteration compared to KPC-2. This new gene was detected in an *E. coli* isolate in a New York City hospital in which KPC-producing isolates are very prevalent.
- Carbapenemase-producing strains are a serious concern for the use of carbapenems for treatment of severe infections in areas where these organisms are prevalent. Although the overall prevalence of carbapenemase-encoding genes is low, the limited therapeutic choices in clinical use and in the antimicrobial pipeline specially for metallo-β-lactamase-producing isolates might jeopardize the care for patients with infections due to these organisms.

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