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Carbapenemase Occurrences among *Escherichia coli* and *Klebsiella* spp. from Europe and the Americas (2007 – 2009)

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SENTRY ANTIMICROBIAL SURVEILLANCE

Abstract

Background: Occurrence rates of carbapenemase (CARBase)-producing *Klebsiella* spp. (KSP) and *E. coli* (EC) collected from surveillance programs in USA, European (EU) and Latin American (LA) hospitals were evaluated for a 3-year period.

Methods: 14,243 KSP and EC were susceptibility (S) tested by CLSI methods (M07-A8 and M100-S20-U). Strains that met the CLSI screening criteria for CARBase production (imipenem and/or meropenem MIC, ≥2 µg/mL), current CLSI breakpoint for non-S, were evaluated. Selected strains were subjected to Modified Hodge Test (MHT) and PCR (KPC, IMP, VIM, SME, IMI, NMC-A, GES, OXA-48). Amplicons were sequenced. Annual trend rates were determined by χ^2; *P* values <0.05 were considered statistically significant.

Results: 325 (2.3%) strains met the screening criteria (carbapenem-non-S, CARB-NS). An increase in CARB-NS was noted in all regions, reflecting in an overall increased rate of 1.8 – 1.9% in the first two years, to 3.4% in 2009 (*P*<0.001). 219 (1.5%) strains were CARBase producers by MHT and PCR. KPC-2 and -3 were increasingly detected in USA (from 1.7 – 2.1% [2007 and 2008] to 3.4% [2009]) and LA (all KPC-2; Brazil and Argentina; from 0.1% [2007] to 2.4% [2009]). OXA-48 strains were detected in Turkey (all years) and Argentina (2007 and 2008). Several MβL (17 VIM-like) strains were noted in Greek centers in 2008, which did not participate in 2007 or 2009, otherwise MβL rates were stable (*P* =0.9314). MβL strains were not observed in USA.

	Number (%) by year				
Phenotype/Genotype	2007	2008	2009	Total	OR (95 CI %)[a]
Carbapenem-non-S	97(1.8)	98(1.9)	130(3.4)	325(2.3)	0.52 (0.39-0.68)
CARBase producer (MHT + PCR)	62(1.2)	67(1.3)	90(2.4)	219(1.5)	0.48 (0.34-0.67)
KPC-like	50(0.9)	39(0.8)	68(1.8)	157(1.1)	0.51 (0.35-0.75)
OXA-48	3(<0.1)	8(0.2)	17(0.4)	28(0.2)	0.12(0.03-0.45)
MBL	6(0.1)	20(0.4)[b]	4(0.1)	30(0.2)	1.06 (0.23-4.45)
Total	5,351	5,121	3,771	14,243	–

a. *P* values were calculated by χ^2 for trend test; all *P* values were <0.0001, except for MBL (*P* =0.9314). OR and respective 95% CI refer to comparisons between the years of 2007 and 2009.
b. Vast majority (85.0%) of MBL strains originated from two Greek medical centers, not part of the surveillance program in 2007 and 2009.

Conclusions: Rates of KPC-producing strains continue to increase in USA and has emerged in LA, while a stable occurrence was noted in EU. Rates of MβL producers were stable and limited to a few EU sites (Spain, Italy and Greece), where CARBase strains are endemic/epidemic.

Introduction

A large number of acquired carbapenemases have been identified during the past few years among Gram-negative pathogens, including Enterobacteriaceae. These diverse enzymes belonging to either molecular class B (metallo-β-lactamases) or molecular classes A and D (serine carbapenemases and oxacillinases), have emerged globally and represent serious public health challenges, compromising therapeutic choices and complicating patient management.

The genes encoding carbapenemases are associated with mobile genetic elements that allow 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 the rates of carbapenem resistance and carbapenemase production among *Escherichia coli* and *Klebsiella* spp. strains from United States (USA), European and Latin American hospitals collected during the 2007-2009 period and evaluated as part of the SENTRY Antimicrobial Surveillance Program.

Methods

Bacterial isolates: A total of 14,243 *E. coli* and *K. pneumoniae* isolates were collected from medical centers located in the USA, Europe and Latin America from 2007 to 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 Vitek Systems (bioMérieux; Hazelwood, Missouri, USA), where necessary.

Susceptibility testing: All isolates were susceptibility tested against more than 25 antimicrobials by broth microdilution procedure described by the Clinical and Laboratory Standards Institute (CLSI; 2009) using validated panels manufactured by TREK Diagnostics (Cleveland, Ohio, USA). Interpretations of susceptibility testing results were by M100-S20-U document (CLSI, 2010). *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were concurrently tested for quality assurance; all results were in the CLSI published range.

Screening for carbapenemase encoding genes: All isolates with reduced susceptibility to imipenem or meropenem (MIC, ≥2 µg/ml) were tested with the Modified Hodge test (MHT) using imipenem and meropenem disks. Isolates were screened for the presence of bla_{IMP}, bla_{VIM}, bla_{KPC}, bla_{SME}, bla_{GES} variants and for bla_{IMI}, bla_{NMC-A}, bla_{OXA-48} by multiplex PCR reactions. Amplicons were sequenced on both strands. Results were analyzed using Lasergene® software (DNAStar, Madison, Wisconsin, USA) and compared to available sequences through the internet using BLAST (http://www.ncbi.nlm.nih.gov/blast/).

Statistical analysis: Carbapenem resistance and carbapenemase production rates among different groups were calculated by χ^2 test using the Epi Info™ Version 3.4.1 software package (Centers for Disease Control and Prevention, Atlanta, Georgia, USA). *P* values <0.05 were considered to be significant.

Results

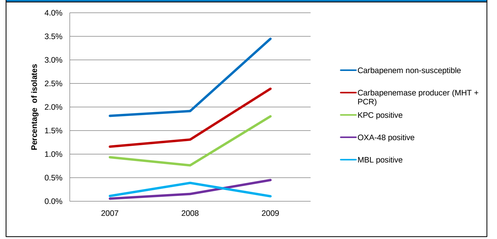
- Among 14,243 *E. coli* and *Klebsiella* spp. strains collected from 2007 through 2009, 325 (2.3%) were non-susceptible to imipenem and/or meropenem according to the new CLSI breakpoints (M100-S20-U).
- Carbapenem non-susceptible strains significantly increased over time in all geographic regions (Table 1). In the first two years, carbapenem non-susceptibility rates for all regions combined were 1.8 and 1.9% increasing to 3.4% in 2009 (*P*<0.001; OR [95 CI %], 0.52 [0.39-0.68]; Figure 1).
- Carbapenemase production, detected by MHT and PCR, was observed among 219 (1.5%) strains: 62 (1.1%) identified in 2007, 67 (1.3%) in 2008 and 90 (2.4%) in 2009 (Table 1). These numbers show a statistically significant escalating trend (*P*<0.001; OR [95 CI %], 0.48 [0.34-0.67]).
- Genes encoding OXA-48 (28 strains) and IMP-type enzymes (2), were detected in Europe and Latin America, VIM-type enzymes (28) were observed only in Europe, and KPCs (157) were detected in all regions, with higher prevalence in the USA and Israel.
- In USA hospitals, KPC-production was 1.7% in 2007, increasing to 2.1% in 2008 and 3.4% in 2009, whereas in Europe, KPC production was stable over the monitored years (9-10 strains per year).
- One KPC-producer was detected in Latin America (Argentina) in 2007-2008. However, in 2009, a total of ten KPC-2-producing strains were detected in four hospital located in Brazil and Argentina.
- OXA-48-producing strains were detected in Turkey and in two Argentinean hospitals in 2007 and 2008. The number of OXA-48-producing strains in Turkish hospitals increased from two and six strains in 2007 and 2008, respectively, to 17 strains in 2009.
- Metallo-β-lactamase (MβL)-production rates were stable throughout the three recent years surveyed (*P* =0.9314, OR [95 CI %], 0.52 [0.39-0.68]). The increase in MβL production observed in 2008 (Figure 1) was due to the detection of 17 VIM-like strains in Greek hospitals; however, institutions from this country were not sampled in 2007 or 2009.
- Strains producing MβLs were mainly noted in Europe. VIM enzymes (VIM-1 and -2) were detected in Spain, Italy, Turkey and Greece (2008 only).
- Only one MβL-producer was detected in Latin America: an IMP-18-producing *K. oxytoca* from Mexico. MβL-producing strains were not observed in the USA during this study period.

Table 1. Distribution of carbapenem-non-susceptible and carbapenemase-producing *E. coli* and *Klebsiella* spp. strains in the USA, Europe and Latin America during 2007-2009 (SENTRY Program).

	Geographic Region (no. of strains [%])			
Year/Phenotype or genotype	Overall	USA	Europe	Latin America
2007 (no. of strains)	(5,351)	(1,912)	(2,570)	(869)
Carbapenem-non-susceptible	97 (1.8)	46 (2.4)	26 (1.0)	25 (2.9)
Carbapenemase-producer[a,b]	62 (63.9)	43 (93.5)	17 (65.4)	2 (8.0)
KPC-positive[c]	50 (80.6)	40 (93.0)	9 (52.9)	1 (50.0)
OXA-48-positive[c]	3 (4.8)	0 (0.0)	2 (11.8)	1 (50.0)
MβL-positive[c]	6 (9.7)	0 (0.0)	6 (35.3)	0 (0.0)
2008 (no. of strains)	(5,121)	(1,793)	(2,495)	(833)
Carbapenem-non-susceptible	98 (1.9)	36 (2.0)	45 (1.8)	17 (2.0)
Carbapenemase-producer[a,b]	67 (51.0)	30 (83.3)	35 (77.8)	2 (11.8)
KPC-positive[c]	39 (78.0)	30 (100.0)	9 (25.7)	0 (0.0)
OXA-48-positive[c]	8 (16.0)	0 (0.0)	6 (17.1)	2 (100.0)
MβL-positive[c]	20 (6.0)	0 (0.0)	20 (57.1)	0 (0.0)
2009 (no. of strains)	(3,711)	(1,414)	(1,942)	(415)
Carbapenem-non-susceptible	130 (3.4)	55 (3.9)	51 (2.6)	24 (5.8)
Carbapenemase-producer[a,b]	90 (69.2)	48 (87.3)	31 (60.8)	11 (45.8)
KPC-positive[c]	68 (75.6)	48 (100.0)	10 (32.3)	10 (90.9)
OXA-48-positive[c]	17 (18.9)	0 (0.0)	17 (54.8)	0 (0.0)
MβL-positive[c]	4 (4.4)	0 (0.0)	3 (9.7)	1 (9.1)

a. Carbapenemase-producing strains were defined as positive by MHT and PCR-positive for carbapenemase-encoding genes.
b. Rates were calculated based on the number of carbapenem-non-susceptible isolates.
c. Rates were calculated based on the number of carbapenemase-producers.

Figure 1. Trends of carbapenem-non-susceptibility and carbapenemase production among *E. coli* and *Klebsiella* spp. isolates collected from USA, European and Latin American medical sites (SENTRY Program, 2007-2009).



Conclusions

- Carbapenem resistance rates and carbapenemase production were increasingly observed, whereas MβL rates were stable over the study period or associated with local epidemic, clonal occurrence. The dissemination of KPC-producing strains in all geographic regions and OXA-48 in Turkish hospitals contributed directly to these escalating resistance trends.
- KPC-encoding genes are efficiently disseminating worldwide. bla_{KPC} is associated with a transposon structure (Tn*4401*) that is likely responsible for the successful spread of these resistance determinants.
- Carbapenemase production cannot be inferred from the antimicrobial resistance profile, thus, the dissemination of these enzymes must be closely monitored (molecular and/or phenotypic tests [MHT]) and strategies should be devised for implementation of effective surveillance initiatives. Some guidelines are available from the Centers for Disease Control (CDC, USA) and European Centre for Disease Control (ECDC).

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