Epidemiologic Detection of Carbapenemase-producing *Klebsiella* spp. by EUCAST or CLSI Clinical Breakpoints

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AMENDED ABSTRACT

Background: Phenotypic detection of carbapenemases generally utilizes reagents/ techniques that are not available in clinical laboratories, limiting clinical utility. CLSI breakpoints for carbapenems are currently under reevaluation and their value for epidemiological screening of carbapenemases production was analyzed and compared to other criteria (CLSI screening and EUCAST breakpoints).

Methods: *Klebsiella* spp. from 2008 surveillance were susceptibility tested (CLSI broth microdilution) against imipenem, meropenem, ertapenem and doripenem. 271 selected strains were tested by PCR for detection of carbapenemase genes encoding KPC, IMP, VIM, NMC-A/IMI, SMEs and OXA-48. Modified Hodge test (MHT) was performed.

Results: All isolates displaying carbapenem MICs ≥1 µg/ml (124 strains) and 147 strains with carbapenem MICs <1 µg/ml (reflecting imipenem MIC distribution) were evaluated. Among 271 *Klebsiella* spp., carbapenemase encoding genes were detected in 62 (39 KPC, 19 VIM and 4 OXA-48). All KPC-producers showed carbapenem MIC ≥4 µg/ml. Imipenem, meropenem and doripenem proposed CLSI breakpoints had high specificity and sensitivity compared to PCR. Ertapenem showed high sensitivity but an elevated number of false-negatives. bla_{OXA-48}-carrying strains caused false-negative results with several carbapenems. Meropenem showed low sensitivity when using EUCAST breakpoints. Across all carbapenems, lowest sensitivity and highest specificity was noted for EUCAST breakpoints and CLSI screening criteria. MHT (120 strains tested) showed 0.95 sensitivity and 1.00 specificity, with only three false-negative (2 OXA-48, 1 VIM).

Conclusions: Proposed carbapenem CLSI breakpoints showed acceptable and balanced sensitivity (0.93-0.98; 0.95) and specificity (0.87-0.98; 0.95) for detection of carbapenemase encoding genes among *Klebsiella* spp. and can be a valuable tool as screening criteria for epidemiological evaluations as well as to predict clinical success.

Abstract Table

Antimicrobial agent	Screening or susceptibility breakpoints	Sensitivity	Specificity	False-positives	False-negative (no. of strains carrying bla_{OXA-48})
Imipenem	≤1 µg/ml[a,b]	0.97	0.98	3	2 (1)
	≤2 µg/ml[c]	0.95	1.00	0	3 (2)
Meropenem	≤1 µg/ml[a,b]	0.93	0.98	2	4 (2)
	≤2 µg/ml[c]	0.85	0.99	1	9 (3)
Doripenem	≤1 µg/ml[b,c]	0.93	0.99	1	4 (3)
Ertapenem	≤0.25 µg/ml[b]	0.98	0.87	27	1 (1)
	≤0.5 µg/ml[c]	0.96	0.90	20	2 (1)
	≤1 µg/ml[a]	0.89	0.96	8	7 (2)

a. CLSI screening concentration criteria for carbapenemase detection.
b. CLSI proposed clinical breakpoint (June, 2009).
c. EUCAST clinical breakpoint (2009).

INTRODUCTION

Carbapenemases are β-lactamase enzymes that have the ability to hydrolyze β-lactam compounds, with significant hydrolysis activity against carbapenems. These enzymes can be divided in metallo-β-lactamases (MβL; Ambler class B) and serine carbapenemases (class A or Bush class 2f) according to the functional requirements and structure of their active site.

The genes encoding most of carbapenemases reside on plasmids or transposons carrying additional genes encoding resistance to other antimicrobial classes. These transferable structures can readily be acquired by Gram-negative pathogens facilitating the dissemination of these potent resistance mechanisms. Additionally, in many cases, these genetic elements confer a multi-drug resistance profile reducing significantly the treatment options for infections caused by carbapenemase-producing isolates.

Several methods for detection of carbapenemases have been proposed. However, these methods usually generate a high number of false-positive results due to hyperproduction of chromosomal cephalosporinases, inhibitory effects of the carbapenemase inhibitor alone, or other artifacts. Moreover, some of these methodologies are not suitable for detection of all carbapenemase enzymes and different tests for detection of serine-carbapenemases and MβLs need to be used.

In this study, we evaluated recently CLSI proposed and EUCAST breakpoints for the epidemiological evaluation of carbapenemase production and these results were compared to molecular methods. Additionally, CLSI screening criteria for detection of carbapenemases and Modified Hodge test were analyzed.

MATERIALS AND METHODS

Bacterial isolates. *Klebsiella* spp. clinical isolates were collected from medical centers located in Europe, North and Latin America from bloodstream, respiratory tract and skin and soft tissue infections according to defined protocols. Isolates belonged to three bacterial species: *K. pneumoniae* (246 strains), *K. oxytoca* (24) and *K. ozaenae* (1). Only clinically significant isolates were included in the study, one per patient episode. Species identification was confirmed by standard biochemical tests and using the 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; 2008) using validated panels (TREK Diagnostics, Cleveland, Ohio, USA). Interpretations of susceptibility testing results were by Clinical Laboratory Standard Institute (CLSI, 2009) criteria. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were concurrently tested for quality assurance; all results were in the published range.

Screening for carbapenemase encoding genes. Isolates with reduced susceptibility to imipenem or meropenem (MIC, ≥1 µg/ml) were tested with the Modified Hodge test (MHT) using imipenem and meropenem disks. All isolates were screened for the presence of several carbapenemase-encoding genes using multiplex PCR reactions. Generic primers designed for bla_{IMP}, bla_{VIM}, $bla_{KPC,}$ bla_{SME}, bla_{GES} variants and for bla_{IMI}, bla_{NMC-A}, bla_{OXA-48} were combined in two amplification reactions.

Data analysis. Sensitivity and specificity for carbapenemase detection were calculated for CLSI screening criteria, EUCAST and recently proposed CLSI carbapenem breakpoints were compared with PCR results. MHT results were analyzed for the population with MIC values at ≥1 µg/ml in comparison to PCR.

RESULTS

- During 2008, 1,716 *Klebsiella* spp. were submitted to the SENTRY Antimicrobial Surveillance Program and among those, 124 strains showed elevated MIC values (≥1 µg/ml) for imipenem, meropenem, ertapenem or doripenem. These isolates were tested with MHT and PCR for detection of carbapenemase genes.
- Additionally, 147 strains with MIC values <1 µg/ml for carbapenem compounds were selected according to imipenem MIC distribution. Isolates were recovered from different medical centers and were tested for carbapenemase genes by PCR.
- Among 271 *Klebsiella* spp., carbapenemase encoding genes were observed in 62 (22.9%) strains. Genes encoding KPC-, VIM- and OXA-48-like were detected in 39, 19 and 4 strains, respectively.
- Strains carrying KPC genes displayed MIC results against all carbapenems at ≥4 µg/ml, whereas strains carrying bla_{VIM}- and bla_{OXA-48}-like had MIC values from ≤0.12 to >8 µg/ml for different carbapenem compounds (Figure 1).
- Imipenem showed higher sensitivity (0.95-0.97) and specificity (0.98-1.00) compared to other carbapenems (Figure 1).
- Proposed CLSI breakpoints for imipenem, meropenem and doripenem showed high sensitivity and specificity (Figures 1a, 1b, 1d).
- High sensitivity was obtained with EUCAST (0.96) and proposed CLSI breakpoints (0.98) for ertapenem, but an elevated number of false-negative results was observed (27 strains total; Figure 1c).
- False-negative results were usually due to the presence of bla_{OXA-48}, which is considered to have a weak activity against carbapenem compounds (Figure 1).
- 120 strains were tested by MHT and this method showed 0.95 for sensitivity and 1.00 for specificity, with three false-negative (2 OXA-48, 1 VIM; Figure 2).

Figure 1. MIC distribution of carbapenems and PCR results for carbapenemase genes. Different susceptibility breakpoints are illustrated with distinct lines. False-negative results are underlined and false-positives are in red.

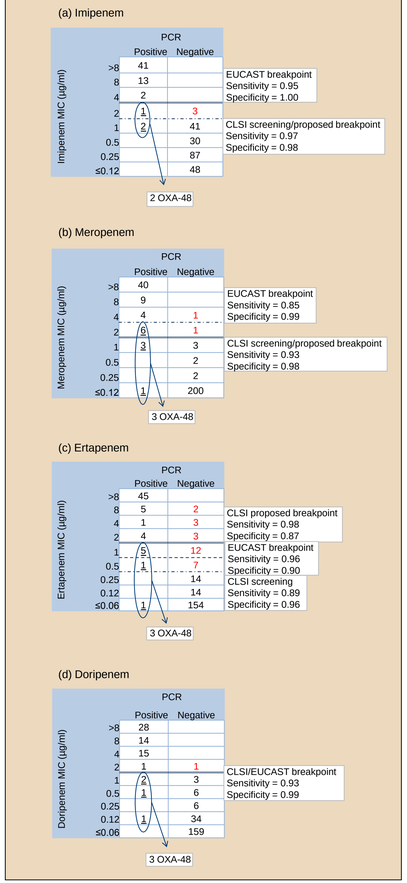

Figure 2. Comparison of PCR results with Modified Hodge test (MHT) performed for 120 *Klebsiella* spp. strains.

MHT	PCR Positive	PCR Negative	
Positive	57	0	Sensitivity = 0.95
Negative	3	61	Specificity = 1.00

CONCLUSIONS

- All KPC-producing *Klebsiella* spp. were detected using the recently proposed international breakpoints for carbapenems (CLSI and EUCAST); however, VIM- and OXA-48-producing strains caused false-negative results with various methods.
- OXA-48-production was detected by MHT in two isolates demonstrating that this carbapenemase can contribute to carbapenem resistance.
- According to our results, MHT is a valuable diagnostic tool for the epidemiological evaluation of carbapenemase-producing *Klebsiella* spp. when used as a confirmatory test.
- Ongoing evaluation of these clinical breakpoints will be required to maintain in vitro test accuracy as these resistance enzymes are disseminated among other Enterobacteriaceae species.

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