Background: Phenotypic detection of carbapenemase-producing K. pneumoniae generally utilizes nucleic acid techniques that are not available in clinical laboratories, limiting clinical utility. CLSI breakpoints for carbapenemase production are currently undergoing reevaluation 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 isolates were selected for PCR for detection of carbapenemase genes encoding KPC, IMP, VIM, NDM-1, AHC 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., carbapenem encoding genes were detected in 62 (39 K. pneumoniae, 19 VIM and 4 OXA-48). All KPC-producing isolates showed carbapenem MIC >4 µg/ml. Imipenem, meropenem and doripenem detected OXA-48 with high specificity and sensitivity compared to PCR. Ertapenem showed high sensitivity but an elevated number of false-negatives. False-negative results with various methods, several carbapenemases were detected by PCR. meropenem disk showed low sensitivity and specificity compared to PCR. Ertapenem showed high sensitivity but low specificity and low numbers of false-negatives. Carbapenemase genes were detected in 100% of the carbapenem MIC ≥1 µg/ml isolates tested. Using MHT, all carbapenemase positive isolates were determined to be positive. Carbapenemase-producing isolates were selected for further analysis.

Conclusions: Sensitivity and specificity of MHT for detection of carbapenemase-producing K. pneumoniae were very high. OXA-48 and NDM-1 enzymes can be detected by MHT. False-negative results were very low.

INTRODUCTION

Carbapenemase genes are β-lactamases enzymes that have the ability to hydrolyze β-lactam antibiotics, with significant hydrolytic activity against carbapenems. These enzymes can be found on plasmids (class A, B and C) or as chromosomal elements (class B, C and D) 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.

Carbapenemase are β-lactamases enzymes that have the ability to hydrolyze β-lactam antibiotics, with significant hydrolytic activity against carbapenems. These enzymes can be found on plasmids (class A, B and C) or as chromosomal elements (class B, C and D) according to the functional requirements and structure of their active site.

Several methods for detection of carbapenemases have been proposed. High sensitivity and specificity usually generate a high number of false-positive results due to high-hydrolysis of chromosomal carbapenemases and inhibitory effects of the carbapenemase inhibitor alone, or other β-lactams. Moreover, some of these methodologies are not suitable for detection of all carbapenemase enzymes and different detection of serine-carbapenemases and MBLs need to be used.

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

BACTERIAL ISOLATES: Klebsiella spp. clinical isolates were collected from different 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 different species: K. pneumoniae (248 strains), K. oxytoca (24) and K. aerogenes (1). Only clinical isolates with carbapenem MIC ≥1 µg/ml were included in the study, one per patient episode. Species identification was confirmed by standard biochemical tests and using the Vitek systems (bioMerieux, Hazelwood, Missouri, USA), where necessary.

Susceptibility testing: All isolates were susceptibility tested against meropenem, imipenem, ertapenem and doripenem. Modified Hodge test was performed for detection of carbapenemase genes.

During 2008, 1,716 Klebsiella spp. were submitted to the LINDT (International 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 by CLSI recommended PCR for detection of carbapenemase genes.

Additionally, 147 strains with MIC values <1 µg/ml for carbapenem compounds were selected for PCR testing, according to imipenem MIC distribution. Isolates were recovered from different medical centers and were tested for carbapenemase genes by PCR; CLSI proposed clinical breakpoint (June, 2009).

Ertapenem showed high sensitivity (0.95-0.97) and specificity (1.00 and 0.87) compared to PCR. Ertapenem showed high sensitivity and specificity (0.95 and 1.00) compared to MHT. These results showed that this method could contribute to carbapenem resistance.

During 2009, 980 isolates were evaluated by both PCR and MHT. All isolates displaying carbapenem MICs ≥1 µg/ml in comparison to PCR were tested against more than 25 antimicrobials by broth microdilution. Data analysis was performed for carbapenem compounds were selected for analysis according to imipenem MIC distribution. Isolates were recovered from different medical centers and were tested for carbapenemase genes by PCR; CLSI proposed clinical breakpoint (June, 2009).

All KPC-producing Klebsiella spp. were detected using the recently proposed international breakpoints (CLSI and EUCAST), however, VIM- and OXA-48-producing strains caused false-negative results with various methods. OXA-48 was detected by MHT in few isolates demonstrating that this method could contribute to carbapenem resistance.

According to our results, MHT is a valuable diagnostic tool for the phenotypic identification 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. These resistance enzymes are disseminated among other Enterobacteriaceae species.

REFERENCES


