Evaluation of a new method for detecting KPC-producing bacteria based on the combination of a screening medium and a gradient diffusion system

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INTRODUCTION

Carbapenemes are commonly used to treat infections caused by multidrug-resistant Enterobacteriaceae. An increasing mechanism of carbapenem resistance is the Klebsiella pneumoniae carbapenemase (KPC).
The KPC ß-lactamase occurs most commonly in *K. pneumoniae,* but it has also been reported sporadically in other species of *Enterobacteriaceae* (*Klebsiella oxytoca, Enterobacter* spp., *Escherichia coli, Salmonella spp., Citrobacter freundii* and *Serratia* spp.) and *Pseudomonas aeruginosa* .
The KPC enzyme confers resistance to all β-lactam agents including penicillins, cephalosporins, monobactams and most β-lactam/β-lactamase inhibitors.
KPC hydrolyzes the carbapenems (ertapenem, imipenem and meropenem) reducing their activity and poses a considerable threat to clinical patient care and public health.

OBJECTIVES

Laboratory identification of KPC-producing clinical isolates will be essential for limiting the spread of strains possessing this resistance mechanism.
We evaluated a new method for the detection of potential KPC producers using first Chromatic CRE for the screening and then MTS Ertapenem/Ertapenem+Boronic acid and Meropenem/Meropenem+Boronic acid for confirmation.

METHODS

Bacterial isolates: 91 bacterial isolates with known resistance mechanisms (acquired *ampC*, MBL, KPC and ESBL producing *Enterobacteriaceae*) determined by molecular methods were included in the study.
All the investigated isolates were frozen at -20°C and subcultured on Mueller Hinton Agar plates for two consecutive days prior using to ensure the purity of the culture.

Screening medium

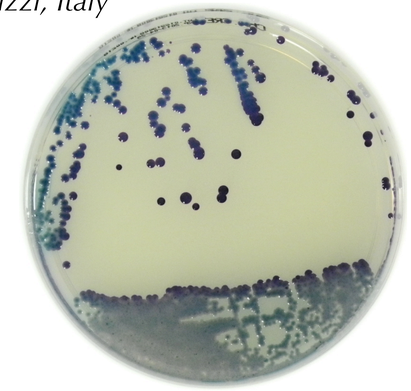
Chromatic CRE (Liofilchem®, Italy) is used as a chromogenic medium designed to detect carbapenem-resistant *Enterobacteriaceae*.
All plates are incubated aerobically at 36+/-1°C, read after 18-24 h and interpreted according to manufacturers' instructions.

Gradient diffusion system

MTS Ertapenem/Ertapenem+Boronic acid (Liofilchem®, Italy) is a double-sided gradient strip of Ertapenem 8-0.125 mg/L and Ertapenem 2-0.032 plus a constant level of Boronic acid.
MTS Meropenem/Meropenem+Boronic acid (Liofilchem®, Italy) is a double-sided gradient strip of Meropenem 8-0.125 mg/L and Meropenem 2-0.032 plus a constant level of Boronic acid.
All strips are inoculated on Mueller Hinton Agar (Liofilchem®, Italy). Interpretation of results was performed after 24 hours incubation.
All strains were tested on Chromatic CRE medium and with MTS Ertapenem/Ertapenem+Boronic acid and Meropenem/Meropenem+Boronic acid on Mueller Hinton Agar. Sensitivity and specificity were calculated.

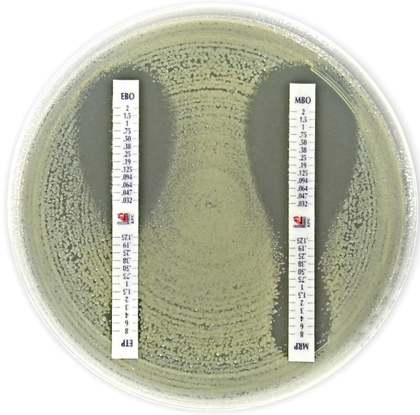
RESULTS

KPC-producing *K. pneumoniae* grew uninhibited on Chromatic CRE and yielded green or blue colonies (100% sensitivity).
Poor growth/no growth/atypical colony color was evident with KPC-non producing Enterobacteriaceae (77% specificity).
MTS Ertapenem/Ertapenem+Boronic acid and MTS Meropenem/Meropenem+Boronic acid were evaluated with KPC positive and negative strains.
Both tests show 100% sensitivity and 89.3% specificity for MTS Ertapenem/Ertapenem+Boronic acid and 95.6 % specificity for MTS Meropenem/ Meropenem+Boronic acid, respectively.



growth on Liofilchem® Chromatic CRE

KPC-positive
Klebsiella pneumoniae



Liofilchem® MIC Test Strip
Ertapenem/Ertapenem+Boronic acid
Meropenem/Meropenem+Boronic acid

CONCLUSION

The study demonstrates the efficiency of Chromatic CRE intended as a screening step of KPC producing strains, followed by MTS Ertapenem/Ertapenem+Boronic acid and MTS Meropenem/Meropenem+Boronic acid as subsequent confirmatory steps; this combination allows the rapid detection of carbapenem-resistant organisms and the immediate implementation of infection control measures avoiding the spread of carbapenem resistance.

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