A novel phenotypic detection strategy for class A, B and OXA-48 carbapenemases in Enterobacteriaceae using temocillin

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Introduction

Class A and B carbapenemases in Enterobacteriaceae may be detected using carbapenemase inhibition tests with boronic acid derivatives (BA) and dipicolinic acid (DPA)/EDTA, respectively. However, for OXA-48 (like) carbapenemases, no specific inhibitor is available. Since OXA-48 confers high-level temocillin resistance, a disc diffusion assay using temocillin besides BA and DPA inhibition tests was evaluated for detection of class A, B and OXA-48 carbapenemases.

Methods I

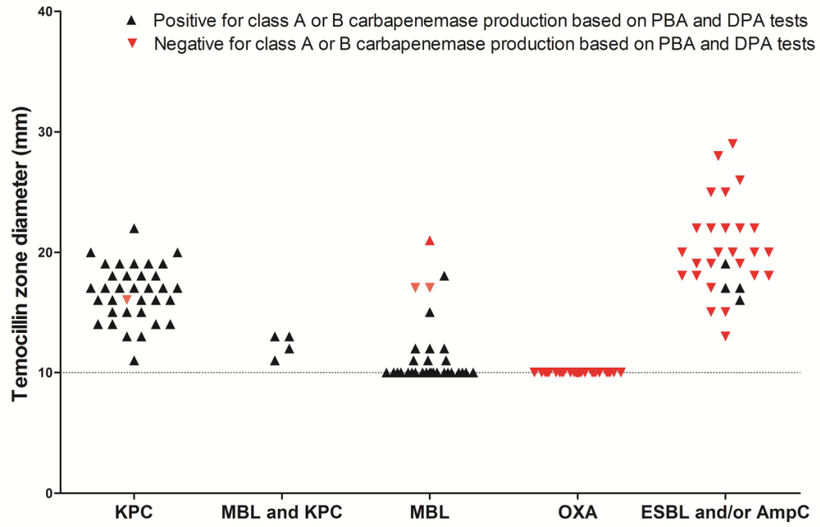
The test collection included 128 well characterized non-repeat Enterobacteriaceae isolates suspected for carbapenemase production, i.e. with meropenem MIC ≥ 0.5 mg/L (83 *K. pneumoniae*, 17 *E. coli*, 23 *Enterobacter* spp., 3 *P. mirabilis*, 2 *S. marcescens*).The isolates produced KPC (n=36), MBL (n=31), KPC plus MBL (n=4), OXA-48 (n=25), OXA-162 (n=2), ESBL (n=19), AmpC (n=10) or ESBL plus AmpC (n=1). PCR and sequencing of beta-lactamase genes was used as reference test. Phenotypic carbapenemase detection was performed with discs (Rosco) containing meropenem (10 ug), temocillin (30 ug), meropenem + phenyl BA (PBA), meropenem + DPA, meropenem + PBA + DPA, and meropenem + cloxacillin (CL).

Methods II

Table 1 shows the strategy for interpretation of carbapenemase inhibition tests. First, to identify KPC and MBL producers, inhibition tests with PBA and/or DPA and CL were evaluated. Second, when no synergy between meropenem and PBA, DPA or both was observed, absence of an inhibition zone (<=10 mm) around the temocillin disc was used to identify OXA carbapenemases.

Results

For identification of class A, B and OXA carbapenemases the sensitivity was 97%, 90% and 100%, respectively. Due to swarming, interpretation of two *P. mirabilis* isolates was false negative. Sensitivity for class B detection in non *Proteus* spp. was 97%. The sensitivity for all classes was 96% (98% in non *Proteus* spp.). None of the 27 OXA producers showed an inhibition zone around the temocillin disc. ESBL and/or AmpC producers had temocillin zone diameters between 13 to 29 mm.



Conclusion

A 30 ug temocillin disc with a zone breakpoint of <=10 mm added to carbapenemase inhibition tests with PBA and DPA, enables sensitive and specific detection and identification of KPC, MBL and OXA-48, the most prevalent carbapenemases in Enterobacteriaceae.

Phenotypic confirmation tests	Class of Carbapenemase			AmpC with reduced permeability	ESBL with reduced permeability
	Class A	Class B	Class D		
Carbapenem +/- BA	+	-	-	+	-
Carbapenem +/- cloxacillin	-	-	-	+	-
Carbapenem +/- DPA	-	+	-	-	-
Temocillin (zone diameter <= 10 mm)	-	+/-	+	-	-

BA= boronic acid; DPA= dipicolinic acid

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