

INTRODUCTION

Plasmid mediated AmpC type β -lactamases are commonly isolated from cephalosporin-resistant Enterobacteriaceae and can cause hospital outbreaks.

The aim of this study was to test a fast detection assay (eazyplex® SuperBug AmpC) for detection of the most common plasmid mediated AmpC (pAmpC) β -lactamase genes in Enterobacteriaceae (ACC, CMY-II group, DHA and MOX).

MATERIALS AND METHODS

The eazyplex® SuperBug AmpC assay (Amplex Diagnostics) is based on loop-mediated isothermal amplification (LAMP) and real-time detection of four different target genes coding for AmpC type β -lactamases (Table 1).

A collection of 35 Enterobacteriaceae isolates previously confirmed by molecular methods (in-house PCR and Check-Points microarray kit, Check-MDR 103XL) were used in the evaluation (Table 2). Of the 35 isolates tested, 9 were reference strains from Culture Collection, University of Göteborg (CCUG), Sweden (7 pAmpC positive and 2 negative), 11 were clinical isolates from our culture collection and 14 were consecutive clinical isolates.

The assay was performed according to the manufacturer's instructions on Genie II (Amplex Diagnostics).

CONCLUSIONS

The eazyplex® SuperBug AmpC assay (Amplex Diagnostics) obtained 100% sensitivity and specificity for the target genes ACC, CMY-II and DHA for the Enterobacteriaceae isolates.

The assay can also detect some of the MOX-genes. However, the only tested MOX-positive isolate obtained from the Culture Collection, University of Göteborg, Sweden (CCUG), was not detected by the assay.

Table 1: AmpC β -lactamase genes included in the assay

Group	Specificity
ACC	ACC 1-6
CMY-II	CMY-II group
DHA	DHA 1-24
MOX	MOX 1-4, 8, 10,11 and CMY-II 1, 8-11, 19



A small part of a single bacterial colony is suspended in 500 μ l RALF buffer and incubated at 99°C for 2 min.

25 μ l of the RALF suspension is transferred to each single tube of the test strip.



LAMP reaction and detection is monitored in real time by the GENIE II® instrument.

RESULTS

The eazyplex® SuperBug AmpC assay (Amplex Diagnostics) identified all target genes correctly with the exception of one MOX-positive E. coli isolate (CCUG 58538).

Thus, a sensitivity of 100% for the target genes ACC, CMY-II and DHA were obtained.

No false-positive results were observed resulting in a specificity of 100% for ACC, CMY-II and DHA.

Table 2: Results from Amplex eazyplex® SuperBug AmpC compared to reference testing

	In-house PCR/Check-points microarray			
	positive			negative
	ACC	DHA	CMY-II	MOX
eazyplex® positive ACC	2	0	0	0
eazyplex® positive DHA	0	12	0	0
eazyplex® positive CMY-II	0	0	9	0
eazyplex® positive MOX	0	0	0	0
eazyplex® negative				12

REFERENCES

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- Bush, K and Jacoby, GA. Updated functional classification of beta-lactamases. Antimicrob Agents Chemother. 2010; 54(3): 969-76.