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Can combination disks with EUCAST potencies for cefotaxime and ceftazidime be used for confirmation of ESBL in Enterobacteriaceae?

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Introduction

The prevalence of Extended-Spectrum β-Lactamase (ESBL)-producing Enterobacteriaceae is continuously increasing worldwide. Disk diffusion with cephalosporin disks with and without clavulanic acid (CLAV) can be used for phenotypic confirmation of ESBL. For this purpose, EUCAST and CLSI recommend the use of cefotaxime (CTX) and ceftazidime (CAZ) 30 µg disks ± CLAV.

Objective

The objective of this study was to investigate if combination disks with EUCAST disk potencies, cefotaxime 5 µg and ceftazidime 10 µg, can be used for phenotypic confirmation of ESBL in Enterobacteriaceae.

Methods

A total of 45 *Escherichia coli* and 5 *Klebsiella pneumoniae* were tested blindly according to EUCAST disk diffusion methodology at three laboratories using Oxoid (Thermo Fisher Scientific) Mueller-Hinton agar. The presence or absence of beta-lactam resistance genes were identified with amplicon-based next-generation sequencing (Ion Torrent), see **Table 1**.

Two sets of combination disks were evaluated: CTX 5 µg ± CLAV 10 µg and CAZ 10 µg ± CLAV10 µg from MAST and Liofilchem. Combination disks with CTX 30 µg ± CLAV 10 µg and CAZ 30 µg ± CLAV 10 µg from MAST were used as control. Isolates were considered ESBL positive if, for one or both agents, the increase in zone diameter in the presence of clavulanic acid was ≥ 5 mm compared with the cephalosporin alone.

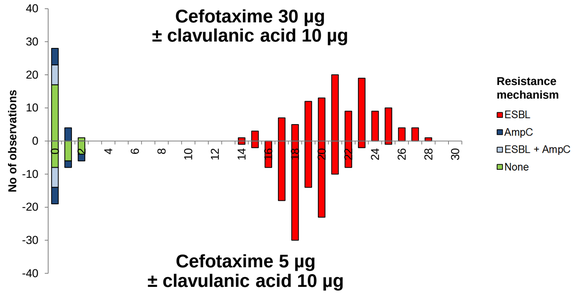

Figure 1. Distributions showing the increase in zone diameter (mm) caused by the addition of clavulanic acid to cefotaxime 30 µg and 5 µg for 50 Enterobacteriaceae vs. resistance mechanism.

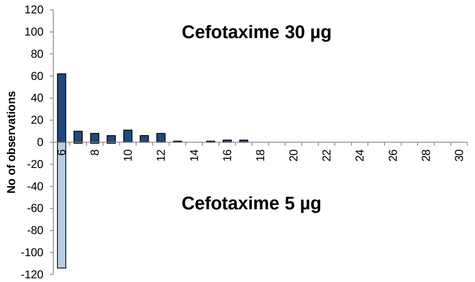

Figure 2. Inhibition zone diameter (mm) distributions for cefotaxime 30 µg and 5 µg with 39 ESBL-producing Enterobacteriaceae tested in triplicate.

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Results

The sensitivity and specificity were 100 % for all combination disks tested at the three laboratories. All isolates with ESBL alone (39/50) were found to be ESBL positive and, as expected, isolates with both ESBL and AmpC (2/50) could not be detected with any of the combination disks.

The increase in zone diameter produced by clavulanic acid was similar for disks with different potencies, for cefotaxime ranging from 0-2 mm for ESBL-negative isolates and 14-28 mm for ESBL-positive isolates (**Figure 1**). For the ESBL-positive isolates, 98 % of readings showed no zone (6 mm) for CTX 5 µg, as compared with 53 % for the CTX 30-µg disk (**Figure 2**).

Conclusions

In this multicenter study, combination disks for confirmation of ESBL in Enterobacteriaceae, containing CTX 5 µg and CAZ 10 µg with and without clavulanic acid 10 µg exhibited the same sensitivity and specificity as disks containing CTX 30 µg and CAZ 30 µg. Using the same disk potencies for primary susceptibility testing and the confirmation of ESBL would be an advantage to clinical laboratories. The design of the study meets the requirement for EUCAST to recommend the method as an alternative confirmation method.

Table 1. Characteristics of the study isolates (50 Enterobacteriaceae) as detected by next-generation sequencing.

Resistance mechanism	None	ESBL			AmpC		ESBL + AmpC
Resistance genes	-	CTX-M group 1	CTX-M group 9	CTX-M groups 1+9	CIT	DHA	CTX-M group 1 + CIT
E. coli	6	10	23	1	2	1	2
K. pneumoniae	0	5	0	0	0	0	0

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