EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

EUCAST technical guidance on the use of the combination disk test (CDT) for confirmation of ESBL in Enterobacterales

New disk potencies for combination disks containing cefotaxime and ceftazidime without and with clavulanic acid

The prevalence of Extended-Spectrum Beta-Lactamase (ESBL)-producing Enterobacterales is increasing worldwide. Disk diffusion with cephalosporin disks without and with clavulanic acid are used for phenotypic confirmation of ESBL production in Enterobacterales.

The EUCAST standard disk potencies for antimicrobial susceptibility testing (AST) of cefotaxime and ceftazidime, are 5 and 10 µg, respectively. However, for ESBL confirmation, EUCAST (as CLSI) has hitherto recommended the use of cefotaxime 30 µg and ceftazidime 30 µg disks without and with clavulanic acid (CLAV) 10 µg. EUCAST now recommends the use of combination disks with the same cephalosporin disk content (cefotaxime 5 µg and ceftazidime 10 µg) for standard disk diffusion and ESBL confirmation.

EUCAST has, in a multicenter study (**Appendix 1**), compared the performance of previously recommended disks and the proposed new disks. Combination disks containing cefotaxime 5 µg ± CLAV 10 µg and ceftazidime 10 µg ± CLAV 10 µg showed the same sensitivity and specificity as disks containing cefotaxime 30 µg and ceftazidime 30 µg.

Interpretive criteria

The test is positive for ESBL production if, for one or both agents, the increase in zone diameter in the presence of clavulanic acid is ≥ 5 mm compared with the cephalosporin alone.

Quality Control of disks for confirmation of ESBL

EUCAST recommend quality control testing of the ESBL confirmation disks with both *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603. EUCAST QC Tables list target values and ranges for cefotaxime 5 µg and ceftazidime 10 µg. For the combination disks, testing with *E. coli* ATCC 25922, should yield inhibition zone diameters within ± 1 mm as compared with the cephalosporin alone. When testing the combination disks with *K. pneumoniae* ATCC 700603, the following criteria should be met:

***K. pneumoniae* ATCC 700603**

Disk	Criteria*
Cefotaxime 5 µg + clavulanic acid 10 µg	≥3 mm increase in zone diameter with cefotaxime + clavulanic acid compared with cefotaxime alone.
Ceftazidime 10 µg + clavulanic acid 10 µg	≥5 mm increase in zone diameter with ceftazidime + clavulanic acid compared with ceftazidime alone.

*Criteria identical to combination disks containing cefotaxime 30 µg ± CLAV 10 µg and ceftazidime 30 µg ± CLAV 10 µg

Recommendation

EUCAST recommends laboratories to introduce combination disks containing cefotaxime 5 µg and ceftazidime 10 µg without and with clavulanic acid 10 µg for routine phenotypic confirmation of ESBL production in Enterobacterales. EUCAST tasks manufacturers of disks for diagnostic use to make available the cefotaxime 5 µg ± CLAV 10 µg and the ceftazidime 10 µg ± CLAV 10 µg disks AND to ascertain that the biological activity of cefotaxime and ceftazidime in disks without and with clavulanic acid is identical. For this purpose, disks without and with clavulanic acid must be produced in parallel and sold as a diagnostic kit.

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Appendix 1

Multicenter study on combination disks for confirmation of ESBL in Enterobacterales

Objective

The aim of this study was to investigate if combination disks with cefotaxime (CTX) 5 µg and ceftazidime (CAZ) 10 µg without and with clavulanic acid (CLAV) 10 µg, give the same results as disks containing CTX 30 µg and CAZ 30 µg, for the confirmation of ESBL in Enterobacterales.

Method

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. Three sets of combination disks were evaluated; CTX 5 µg ± CLAV 10 µg and CAZ 10 µg ± CLAV10 µg produced by MAST and Liofilchem were compared to CTX 30 µg ± CLAV 10 µg and CAZ 30 µg ± CLAV 10 µg from MAST. 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. The presence or absence of beta-lactam resistance genes were identified with amplicon-based next-generation sequencing (Ion Torrent), see **Table 1**.

Table 1. Characteristics of the study isolates (50 Enterobacterales), 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

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 containing CTX 30 µg and CAZ 30 µg as with disks containing CTX 5 µg and CAZ 10 µg (**Table 2**).

Table 2. Increase in zone diameter caused by the addition of clavulanic acid (mm)

Disk	Disk manufacturer	Negative results	Positive results
Cefotaxime 30 µg ± clavulanic acid 10 µg	Mast	0 - 2	14 - 28
Ceftazidime 30 µg ± clavulanic acid 10 µg	Mast	0 - 4	4 - 22
Cefotaxime 5 µg ± clavulanic acid 10 µg	Mast	0 - 2	16 - 26
Ceftazidime 10 µg ± clavulanic acid 10 µg	Mast	0 - 4	4 - 23
Cefotaxime 5 µg ± clavulanic acid 10 µg	Liofilchem	0 - 2	14 - 25
Ceftazidime 10 µg ± clavulanic acid 10 µg	Liofilchem	0 - 2	3 - 21

Conclusion

The study showed that combination disks for confirmation of ESBL production in Enterobacterales, containing CTX 5 µg and CAZ 10 µg without and with clavulanic acid 10 µg exhibited the same sensitivity and specificity as disks containing CTX 30 µg and CAZ 30 µg. In the laboratory, it is an advantage to use the same disk potencies for standard susceptibility testing as for the confirmation of ESBL.