Plasmid-mediated AmpC Beta-lactamases

Plasmid-mediated beta-lactamases represent a new threat, since they confer resistance to aminopenicillins, carboxypenicillins, ureidopenicillins, although they are generally susceptible *in vitro* to mecillinam and/or temocillin.

The enzymes provide resistance to third generation cephalosporins and cefoxitin. The enzymes are also active against aztreonam although for some strains the aztreonam MICs are in the susceptible range. Susceptibility to cefepime is little affected (inoculum effect) and the carbapenems are not affected. The enzymes are not affected by beta-lactamase-inhibitors, except for CMY-8 and CMY-9 that are inactivated by tazobactam.
Their expression is generally constitutive, nevertheless inducible plasmid AmpC (ACT-1, DHA-1, DHA-2, CFE-1, CMY-13) have been reported (6).

Plasmid-mediated AmpC beta-lactamases have been found most frequently in species naturally negative for AmpC, such as *K. pneumoniae*, *E. coli*, *K. oxytoca*, Salmonella and *P. mirabilis*. Recently they were also found in *Enterobacter* spp. (2).

The strains with plasmid-mediated AmpC show resistance to cefoxitin (MIC > 16 µg/ml) and ceftazidime (MIC > 32 µg/ml) corresponding to zones of inhibition < 16 mm (McF. 0.5).

Strains with plasmid-mediated AmpC do not show antagonism between cefoxitin and 3rd generation cephalosporins (are not inducible), while inducible plasmid-mediated AmpC (ACT-1, DHA-1, DHA-2, CMY-13) show antagonism between cefoxitin (or imipenem) and third generation cephalosporins.

Isolated that coproduce an ESBL and a plasmid mediated AmpC beta-lactamase may yield a positive confirmatory test for ESBL using cefepime and cefepime+clavulanate (synergism).

Characteristics of AmpC beta-lactamases:

	Chromosonally mediated AmpC (partially derepressed AmpC mutants)	Plasmid-mediated AmpC (derepressed AmpC mutants)	Inducible plasmid-mediated AmpC ACT-1, DHA-1, DHA-2, CFE-1, CMY-13	ESAC in P. aeruginosa (21)
Cefepime+Clavulanate and/or Ceftazidime+Clavula-nate	No synergism	No synergism (except MOX-1, MOX-2)	No synergism	No synergism
Cefoxitin, Imipenem or Amoxycillin+Clavula-nate (7)	Cefoxitin R (zone < 16 mm) **Antagonism** with 3rd gen. cepha.	Cefoxitin R (zone < 16 mm) **No antagonism** with 3rd gen cepha.	Cefoxitin R (zone < 16 mm) **Antagonism** with 3rd gen. cepha.	**No antagonism** Imipenem / 3rd gen cepha.
Ceftazidime Cefepime	S → R S	R (zone < 20 mm) S	S → R S	Ceftazidime R Cefepime I/R
Cloxacillin	Synergism Cloxacillin-cefotaxime	Synergism Cloxacillin+cefotaxi-me Cloxacillin+ceftazidi-me	Synergism Cloxacillin+cefotaxi-me Cloxacillin+ceftazidi-me	Synergism Cloxacillin-cefepime Cloxacillin-Carbapenems
Boronic acid synergy	Cefotaxime-ceftazidime	Cefotaxime-ceftazidime	Cefotaxime-ceftazidime	Carbapenems

Enterobacter spp., *C. freundii, M. morganii, Hafnia alvei, Providencia* spp., Proteus indole positive *and Serratia marcescens*, all produce an inducible chromosomal AmpC beta lactamase, which is not inhibited by clavulanate. There may be seen an antagonism between amoxycillin and clavulanate (smaller zone

with the combination that with amoxycillin alone) due to the presence of the inducible beta-lactamase. All these strains should be reported as resistant to ampicillin/amoxycillin and to amoxycillin+clavulanate (except P. vulgaris).
Using an Amoxycillin+Clavulanate disc (Neo-Sensitabs) better performance is obtained due to the dual action of clavulanic acid: 1) induces expression of inducible plasmid mediated AmpC beta-lactamases (antagonism with 3rd gen. cephalosporins) and 2) permits the detection of an ESBL by enlarging inhibition zones of 3rd gen. cephalosporins (synergism) (7). In the presence of an ESBL + an inducible plasmid-mediated AmpC, both antagonism and synergism can be detected in the same plate (7).

Differentiation of AmpC beta-lactamases in E. coli

Mirelis et al (9) and Aragon (15) found a simple phenotypic method for the differentiation between plasmid-mediated and chromosomal AmpC-ß-lactamases in E. coli and P. mirabilis using Cloxacillin Diatabs and by visual examination of the antibiogram plates. The presence of scattered colonies located near the edge of the zone of inhibition of cefoxitin, cefotaxime, ceftazidime and aztreonam indicated the presence of plasmid-mediated AmpC beta-lactamases.
Cloxacillin or Boronic acid alone do not distinguish between chromosomal or plasmidic AmpC beta-lactamases.

E. coli (AMC I/R, Cefotaxime I/R, Ceftazidime I/R Cefoxitin: most I/R)

	Plasmid AmpC	**Inducible plasmid AmpC**	**Chromosomal AmpC hyperprod.**	**Chromosomal ESAC (14, 18)**
Cloxacillin	Synergy with ceftazidime or cefotaxime	Synergy with ceftazidime or cefotaxime	Synergy with ceftazidime or cefotaxime	Synergy
Boronic acid	Synergy with Ceftazidime and/or Cefotaxime	Synergy with Ceftazidime and/or Cefotaxime	Synergy with Ceftazidime. and/or Cefotaxime	Synergy
Cefoxitin Imipenem	No antagonism with 3rd gen. cephalosporins	Antagonism with 3rd gen. cephalosporins	No antagonism	No antagonism
Antibiogram	Scattered colonies (resistant mutants) near the edge of the zone of cefoxitin, cefotaxime, ceftazidime and aztreonam	Scattered colonies	Well defined edge of zone	
Cefepime MIC µg/ml	Cefepime MIC ≤1 µg/ml	Cefepime MIC ≤1 µg/ml	Cefepime MIC ≤1 µg/ml	Cefepime MIC 1-8 µg/ml (zone<26mm) Ceftazidime R Cefoxitin R

- ESAC=extended spectrum AmpC (14)
- The same procedure will be appropriate for K. pneumoniae and P. mirabilis strains.
- Chromosomal AmpC's of E.coli are not inducible.

Plasmid-mediated AmpC beta-lactamases MIC µg/ml

Beta-lactamases	FOX	CAZ	AZT	FEP	IMP	MRP	Microorganisms
AAC-1	4-8	≥32	1	0.25	0.125-0.8	0.03	E. coli, K. pneumoniae, P. mirabilis, Salmonella, C. freundii
ACT-1	> 256	4 - > 128	4 → > 128	≤ 0.06-8	1	·	E. coli, K. pneumoniae, E. cloacae (inducible)
BIL-1 (CMY-2)	R	> 16	4-16	1	0.5	0.06	E. coli
CMY-1	256	4-128	32	0.25-4	≤ 0.5	0.06	K. pneumoniae
CMY-2	32-256	32-128	16-64	0.5-4	≤ 0.5	0.06	E. coli, Salmonella, K. pneumoniae
CMY-3	128	64	32-256	1	0.25	0.03	P. mirabilis
CMY-4	8 - > 256	8-256	0.5-32	0.06-4	0.25	0.125	E. coli, Salmonella, P. mirabilis
CMY-5	R	256	64	-	0.5-1	-	K. oxytoca
CMY-6	256	256	64	0.5	0.25	0.06	E. coli
CMY-7	R	> 32	·	I	0.25	< 2	E. coli, Salmonella
CMY-8	> 256	32-64	·	·	0.25-0.5	-	K. pneumoniae
CMY-9	> 128	128	8	0.25	0.5	0.06	E. coli
CMY-10	> 128	8-64	4-128	0.12-0.5	0.25-0.5	≤ 0.125	E. coli, E. aerogenes, K. pneumoniae
CMY-11	> 256	256	128	·	·	·	E. coli
CMY-12	256	128	8-32	16	0.25-4	0.5	P. mirabilis
CMY-13	512	256	64	1	0.25	≤ 0.03	E. coli (inducible)
CMY-14	128	128	16-32	0.5-32	0.25-2	0.06	P. mirabilis
CMY-15	512	128	8-32	0.25-8	0.25-16	4	P. mirabilis
CMY-16	≥ 32	≥ 32	1	2	2	0.05	P. mirabilis / Synergy TAZO-FEP
CMY-19	≥ 128	> 128	16	4	0.25	≤ 0.06	K. pneumoniae (8)
CMY-20	≥ 128	> 128	>64	4	-	1	E. coli
CMY-21	> 64	64	32	0.5	0.25	0.06	E. coli
CMY-29	≥ 256	128	·	1	0.5	0.12	E.coli
CMY-30	128	32	128	5	0.5	0.12	E.coli
ESAC (14)				1-8			E.coli
ESAC (18)	> 256	> 256	≥32	32			E.coli
CFE-1	R	64	8	0.25	0.25	·	E. coli (inducible)
DHA-1	128-512	8-64	1-16	≤ 0.125-2	≤ 0.125 - 0.5	·	E. coli, K. pneumoniae, Salmonella, P. mirabilis (inducible)
DHA-2	16	8	2	0.03	0.25	·	K. pneumoniae (inducible)
FOX-1	128	8	1	1	0.25	≤ 0.03	E. coli, K. pneumoniae
FOX-2	256	32	2	0.13	0.5	0.03	E. coli
FOX-3	64	16	1	≤ 0.06	0.12	-	E. coli, K. oxytoca
FOX-4	> 512	> 128	64	2	0.5	0.12	E. coli
FOX-5	512	128	8-16	0.5	0.5	-	K. pneumoniae, E. coli
FOX-7	-	-	-	-	-	-	E. coli, K. pneumoniae, E. cloacae
LAT-1	64-256	> 128	64	1	0.25-2	0.06	K. pneumoniae
LAT-2 (CMY-2)	256	> 256	64-256	-	-	-	E. coli, K. pneumoniae, E. aerogenes
LAT-3 (CMY-6)	256	128	64	0.5	0.25	0.06	E. coli
LAT-4 (CMY-1)	64-256	8-256	8-128	0.125-1	0.25	0.125	E. coli
MIR-1	≥ 256	128	128	1	1	0.125	E. cloacae, K. pneumoniae
MOX-1	R	16 → R	16	-	0.5	-	K. pneumoniae,
MOX-2	≥ 128	4-256	·	0.25-4	≤ 0.125	-	K. pneumoniae

Gupta et al. (3) describes isolation of multiresistant Salmonella, with plasmid-mediated AmpC beta-lactamase, from cattle and humans in the USA.

Three cases of invasive infections caused by Salmonella enterica serotype cholerasuis found in Taiwan (5). The strains were resistant to ciprofloxacin (mutations gyrA and ParC) and to ceftriaxone (presence of plasmid-mediated CMY-2 beta-lactamase).
Recent studies (4) show that the outcome of cephalosporin treatment in serious infections due to AmpC beta-lactamase producing K. pneumonia isolates was poor. A standard test for detection of plasmid-mediated AmpC beta-lactamases is needed. Emergence of cefepime-hydrolyzing CMY-19 in Japan (8).

Detection of Plasmid-mediated-AmpC beta-lactamases

The capability to detect AmpC is important to improve the clinical management of infections and provide sound epidemiological data. Reduced susceptibility to cefoxitin in the Enterobacteriaceae may be an indicator of AmpC activity, but it should be confirmed by other tests. Laboratories should be able to recognize AmpC derepressed strains and those with plasmid AmpC. Guidelines from the CLSI are not yet available for detection of bacteria with AmpC beta-lactamases.

Screening

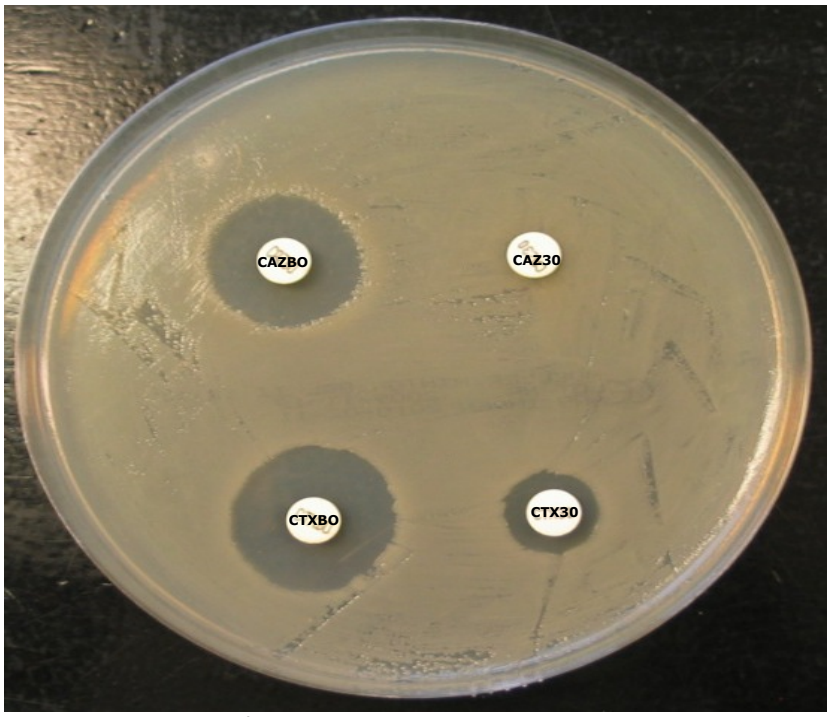
Derepressed/plasmid AmpC should be suspected when we see:

- Resistance to 3rd generation cephalosporins – NOT Cefepime.
- Resistance to Cefoxitin (inhibition zone < 16 mm).
- No cephalosporin / Clav. synergism.
- I / R to Amoxycillin + Clav.
- AmpC derepressed Serratia are S to ceftazidime.
- Providencia, Morganella and Serratia inducible & derepressed may appear S /I to cefoxitin.
- Strains producing AAC-1 beta-lactamase are susceptible to cefoxitin

Confirmation

1.Combined disk test. AmpC Confirm ID kit

Apply Cefotaxime, Cefotaxime+Boronic, Ceftazidime, Ceftazidime+Boronic, Cefotaxime+Cloxacillin and Ceftazidime+ Cloxacillin in an inoculated plate.



E.coli ATCC FN9414 AmpC positive

Interpretation

A Cefotaxime+Cloxacillin inhibition zone ≥ 5 mm than Cefotaxime alone and/or a Ceftazidime+Cloxacillin zone ≥ 5 mm than Ceftazidime alone indicates the presence of an AmpC.

A Cefotaxime+Boronic inhibition zone ≥5 mm than Cefotaxime alone and/or a Ceftazidime+Boronic inhibition zone ≥5 mm than Ceftazidime alone indicates the presence of an AmpC.

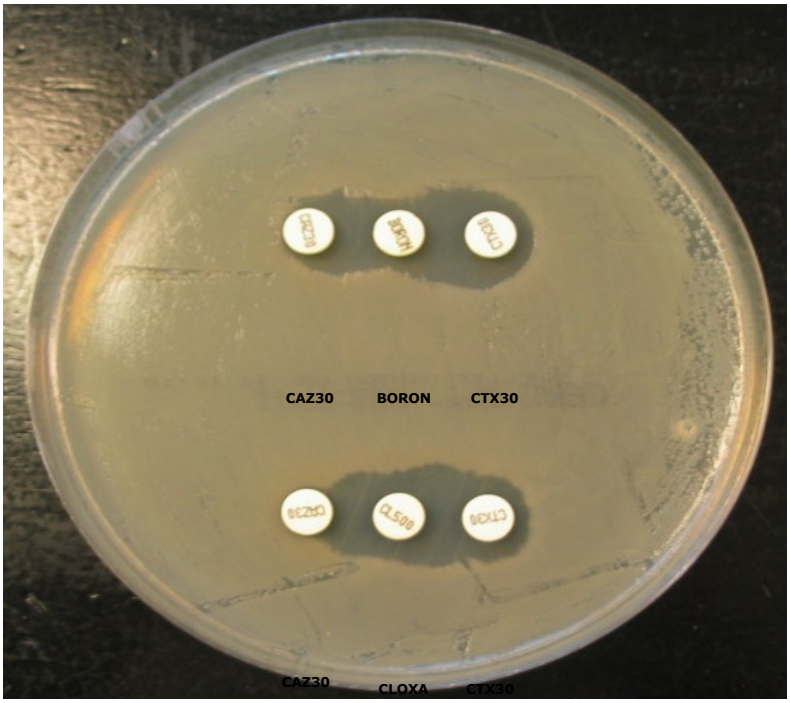
Inhibition zone ≥ 5 mm than Ceftazidime alone, indicates the presence of AmpC.

2. Double disk synergy test

Apply one Cefotaxime (CTX) and one Ceftazidime (CAZ) Neo-Sensitabs on an inoculated MH agar plate. In between apply one Boronic Acid Diatabs (BOR) at a distance of approx. 10 mm (edge to edge). If the strain is totally resistant to the Cefalosporins combination, the distance should be reduced to 5 mm.

Apply one Ceftazidime (CAZ) and one Cefotaxime (CTX) Neo-Sensitabs. In between at a distance of 5-10 mm edge to edge, apply one Cloxacillin Neo-Sensitabs.

Instead of Cefotaxime and Ceftazidime their combinations with clavulanate may be used for the synergy test (19).



E.coli ATCC FN9414 AmpC positive

Interpretation

A keyhole or ghost zone (synergism) between Boronic Acid and any of Cefotaxime or Ceftazidime indicates the presence of an AmpC beta-lactamase.

A keyhole or ghost zone between Cloxacillin and Ceftazidime and/or Cefotaxime indicates the presence of an AmpC beta-lactamase.

Plasmid mediated AmpC differ from chromosomal AmpC in being uninducible (few exceptions).Strains producing inducible plasmid AmpC beta-lactamases (ACT-1, DHA-1, DHA-2, CFE-1, CMY-13) will show antagonism (distorted zone) between Cefoxitin or Imipenem and 3rd generation cephalosporins.

Strains of Klebsiella spp, Salmonella spp and P. mirabilis showing synergism with Boronic Acid and/or Cloxacillin possess presumptively plasmid mediated AmpC beta-lactamases.

The method cannot distinguish between chromosomal and plasmid mediated AmpC beta-lactamases in E. coli, but the test is useful to select strains for further analysis. Plasmid mediated are often multiresistant and may show scattered colonies near the edge of the zone of third gen. cephalosporins and aztreonam disks.

Inducible phenotype

The inducible phenotype is identified by a tablet approximation test, using Imipenem or Cefoxitin against 3rd generation cephalosporins (distance 15 mm from edge to edge).

Distorted zones indicate the presence of an inducible AmpC beta-lactamase.

Treatment with 3rd generation cephalosporins should be avoided in severe Enterobacter, C. freundii, Serratia and Morganella infections except in UTI, because of risk for selection of cephalosporin-resistance during therapy.

AmpC + ESBL. ESBL +AmpC Confirm ID kit

Screening criterion for ESBL presence among AmpC-producing Enterobacter, C. freundii and Serratia marcescens is Cefepime MIC > 1 ug/ml (inhibition zone< 26 mm).

High level expression of AmpC may prevent recognition of an ESBL. Use of Cefepime is more reliable to detect these strains because high AmpC production has little effect on cefepime activity.

Combined disk test (ESBL+AmpC)

Apply one of each:

A) Cefotaxime (CTX 30)
B) Cefotaxime+Clavulanate (CTX+C)
C) Cefotaxime+Cloxacillin (CTXCX)
D) Cefotaxime+Clavulanate+Cloxacillin (CTXCX) on the inoculated MH plate.

Interpretation

		Cefotaxime CTX30	**Cefotaxime+Clav. CTX+C**	**Cefotaxime+Cloxa. CTXCX**
ESBL	CTX+C or CTXCC	≥ 5 mm -	- <4 mm	- ≥ 5 mm
AmpC	CTXCX or CTXCC	≥ 5 mm -	- ≥ 5 mm	- <4 mm
ESBL+AmpC	CTX+C and CTXCC	<4 mm -	- ≥ 5 mm	- ≥ 5 mm

Neither ESBL or AmpC: All zones within 2 mm of each other CTXCC= Cefotaxime+Clavulanate+Cloxacillin.

Detection of ESAC in P.aeruginosa (10)

ESAC in P. aeruginosa constitutes a favorable background for the selection of carbapenem-resistant strains. P. aeruginosa isolates being I/R to Imipenem and Ceftazidime should be tested for ESAC.

Place 1 Cloxacillin Diatabs between Imipenem and Cefepime Neo-Sensitabs (distance 8 mm from edge to edge). Synergy between Cloxacillin and Imipenem and/or Cefepime indicates the presence of and ESAC (extended spectrum AmpC)

Please note:
KPC and other class A carbapenemases (Sme, IMI, GES, NmcA) may give a positive synergy test with Boronic acid. They do not show synergism when using Cloxacillin (differentiation from AmpC).
K. oxytoca hyperproducing K-1 enzyme may give a positive synergy test with Boronic acid, but they show susceptibility to Ceftazidime and are therefore easily detected.

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