

# Quality control assessment of ESBL and antimicrobial susceptibility testing in 5 *Enterobacteriaceae* isolates

**March 2007**

## **PRELIMINARY REPORT**

Culture 1: *Klebsiella pneumoniae*  
Culture 2: *Escherichia coli*  
Culture 3: *Enterobacter aerogenes*  
Culture 4: *Citrobacter freundii*  
Culture 5: *Klebsiella oxytoca*

### **Culture 1: KLEBSIELLA PNEUMONIAE**

<b>ESBL: positive</b>	<u>Reference interpretation</u>
Co-amoxiclav:	I
Piperacillin-tazobactam :	R
Cefuroxime:	R
Cefotaxime:	I/R
Ceftazidime:	R
Cefepime:	I/R

### **Culture 2: ESCHERICHIA COLI**

<b>ESBL: positive</b>	<u>Reference interpretation</u>
Co-amoxiclav:	I
Piperacillin-tazobactam :	S*
Cefuroxime:	R
Cefotaxime:	R
Ceftazidime:	R
Cefepime:	R

\* non interpreted results (no consensus guidelines for beta-lactam/beta-lactam inhibitors associations)

**Culture 3: ENTEROBACTER AEROGENES**

<b>ESBL: Negative</b>	<u>Reference Interpretation</u>
Co-amoxiclav:	R
Piperacillin-tazobactam :	I
Cefuroxime:	R
Cefotaxime:	I/R
Ceftazidime:	R
Cefepime:	S

**Culture 4: CITROBACTER FREUNDII**

<b>ESBL: Positive</b>	<u>Reference Interpretation</u>
Co-amoxiclav:	R
Piperacillin-tazobactam :	I
Cefuroxime:	R
Cefotaxime:	R
Ceftazidime:	R
Cefepime:	S/I

**Culture 5: KLEBSIELLA OXYTOCA**

<b>ESBL: Negative</b>	<u>Reference Interpretation</u>
Co-amoxiclav:	R
Piperacillin-tazobactam :	R
Cefuroxime:	R
Cefotaxime:	I/R
Ceftazidime:	S
Cefepime:	I/R

### **General overview**

This quality control assessment was intentionally designed to evaluate the performance of hospital-based Belgian microbiology laboratories in detection and reporting  $\beta$ -lactam resistance phenotypes in *Enterobacteriaceae* and it focused more specifically on ESBL detection.

Five strains with defined  $\beta$ -lactam resistance mechanisms (three ESBLs-producing isolates, one AmpC overproducing *E. aerogenes* and, one *K. oxytoca* with high level K1/ OXY chromosomal penicillinase) were selected and distributed to 86 laboratories participating to the ESBL multi-centric national survey. Participants were asked to report the clinical susceptibility categorization (S, I, R) according to the guidelines used. Seventy-six (88%) of the laboratories did effectively take part to this quality control assessment and returned their reports.

The antimicrobial susceptibility testing (AST) methods used by the different participants were as follows:

Automates: 29 (38.2%)
Disc diffusion: 22 (29%)
Combination of automates and disc diffusion: 24 (31.6%)
Unknown: 1

Details of the AST methods used are listed hereafter:

VITEK 2: 42	Rosco tablets: 32
PHOENIX: 7	Paper discs: 13 (5 BD, 5 Oxoid, 1 Biorad, 2 unknown)
ATB: 2	
Mini-API: 2	

74 of the participants used the interpretative criteria of the CLSI while criteria of the French Society for Microbiology (CA-FSM) were used by 6 participants.

Methods used for ESBL detection were as follows:

Double disk synergy test (DDST):	23
Double combination discs (DD):	23
DDST + DD:	19
E-test:	4
DDST + E-test:	2
Automates alone	5

Control results were established at one laboratory (UCL Mont-Godinne) by disc diffusion and broth microdilution methods and applying interpretative criteria of the CLSI (M100-S17, January 2007).

ESBLs enzymes were detected phenotypically by DDST, double combination discs (DD) (cefotaxime [30- $\mu$ g], ceftazidime [30- $\mu$ g] and cefepime [30 $\mu$ g] and co-amoxyclav [20/10  $\mu$ g] discs) and by ESBL E- test strips using ceftazidime, cefotaxime and cefepime.

AmpC cephalosporinases were assessed by DDST and DD as above in the presence of cloxacillin (250 µg/ml) containing Mueller-Hinton agar and by a disc synergy test between ceftazidime and cloxacillin 500-µg tablets (Rosco NeoSensitabs).

ESBL enzymes were characterized by their isoelectric point determined by IEF and by PCR for *bla* genes of TEM, SHV, and CTX-M families using specific primers designed in house. Identification of TEM, SHV and CTX-M types and subgroups was performed using DNA sequencing of full-length ESBL gene PCR products.

### **Culture 1**

*Klebsiella pneumoniae* (UCL-MG #N6102902) was an ESBL producing strain (TEM-24 β-lactamase) characterized by a higher level of resistance to ceftazidime (> 256 µg/ml) than to cefotaxime (2-4 µg/ml) and it was correctly identified by 99% of the laboratories (75 of 76 participants). 74 participants provided a correct identification; the isolate was incorrectly identified (to the species level) as *K. oxytoca* by 2 laboratories.

<b>Antibiotic</b>	<b>Reference results</b>	<b>Total Nr Labs</b>	<b>S</b>	<b>I</b>	<b>R</b>
Co-amoxyclav	<b>I</b>	74	11	30	33
Piperacillin/tazobactam	<b>R</b>	72	18	16	38
Cefuroxime	<b>R</b>	74	-	4	70
Cefotaxime	<b>I/R</b>	67	5	10	52
Ceftazidime	<b>R</b>	76	-	-	76
Cefepime	<b>I/R</b>	74	8	20	46

NB: Reported results refer to interpreted results

False susceptible results were reported by 15% of the labs for co-amoxyclav and by 25% of them for piperacillin-tazobactam. Most of the very major errors to these two antimicrobial agents were reported by laboratories using Rosco Neosensitabs tablets for co-amoxyclav and by those using VITEK 2 automated systems for piperacillin/tazobactam.

Noteworthy also, some participants incorrectly reported this strain as susceptible to cefotaxime or to cefepime despite they had correctly reported the isolate as ESBL-producer. Obviously this isolate had an MIC of 2-4 µg/ml to cefotaxime and of to 2 µg/ml to cefepime but as an ESBL-producer it should have been interpreted as non-susceptible (resistant or intermediate) to all extended-spectrum cephalosporins by CLSI or CA-SFM guidelines.

## **Culture 2**

*Escherichia coli* (UCL-MG #N6042271) produces the CTX-M-1 ESBL enzyme which makes it particularly suitable to hydrolyze cefotaxime. Consequently, it is characterized by a higher level of resistance to cefotaxime (> 256 µg/ml) than to ceftazidime (8 µg/ml). Cefepime was also slightly affected by this enzyme (32 µg/ml). On the other hand it was susceptible to piperacillin-tazobactam (8 µg/ml).

All 76 laboratories correctly reported this *E. coli* isolate as an ESBL-producing strain although three laboratories did not identify the isolate to the species level (2 participants misidentified the strain as *K. oxytoca*, and one as *Salmonella arizonae*).

The overall concordance of results with the reference value was good for all tested agents but piperacillin-tazobactam. This low rate of agreement was not based on discordances of results based on zone diameters or MIC but on the fact that a majority of the participants reported it as resistant or intermediate after applying interpretative readings or expert rules despite the fact that CLSI does not provide specific interpretative guidelines for beta-lactam/inhibitor associations against ESBL-producing isolates.

It may however appear justified to report susceptibility to piperacillin/tazobactam with caution owing to the lack of clinical data substantiating the effectiveness of this drug for the treatment of infections caused by ESBL-producing isolates.

Four participants incorrectly reported this strain as susceptible to cefepime despite detection of ESBL.

<b>Antibiotic</b>	<b>Reference Results</b>	<b>Total Nr Labs</b>	<b>S</b>	<b>I</b>	<b>R</b>
Co-amoxyclav	<b>I</b>	74	7	37	30
Piperacillin/tazobactam	<b>S*</b>	72	32	15	25
Cefuroxime	<b>R</b>	73	-	-	73
Cefotaxime	<b>R</b>	66	-	1	65
Ceftazidime	<b>R</b>	76	-	12	64
Cefepime	<b>R</b>	73	4	18	51

\* piperacillin-tazobactam MIC is 8 µg/ml. The fact that CLSI does not provide interpretative guidelines for beta-lactam/inhibitor associations against ESBL-producing isolates most probably explains the low concordance of results observed for this drug

## **Culture 3**

*Enterobacter aerogenes* (UCL-MG #N6102780) is a partially derepressed AmpC producing isolate displaying a moderate level of resistance to ceftazidime (32-64 µg/ml), to cefotaxime (16 µg/ml) and to piperacillin-tazobactam (32 µg/ml) but it remains susceptible to cefepime (0.25-0.5 µg/ml). Besides the derepressed chromosomal AmpC cephalosporinase, this isolate co-produced a TEM-1 narrow-spectrum β-lactamase but it had no ESBL.

The majority of the participants (69 of 76 [91%]) correctly identified this isolate as a non ESBL-producer. Two participants reported the strain as ESBL-positive and five as “not determinable” because of discrepant results between the different detection tests they were using. Of note, six out of the seven

laboratories reporting the isolate as ESBL-positive were using automatic systems (VITEK 2) alone or in association with an disc based test method. It should be emphasized here again that no guidelines are currently available for detecting ESBL enzymes in AmpC inducible species and also that the current algorithms of the automated systems are not well adapted nor validated to distinguish ESBLs from overproduced AmpC in *Enterobacter* spp. isolates.

Antibiotic	Reference Results	Total Nr Labs	S	I	R
Co-amoxyclov	R	74	1	3	70
Piperacillin/tazobactam	I	72	31	20	21
Cefuroxime	R	73	1	2	70
Cefotaxime	I/R	67	28	22	17
Ceftazidime	R	75	21	16	38
Cefepime	S	73	62	8	3

A false susceptibility to piperacillin-tazobactam (43%) cefotaxime (41%) and to ceftazidime (28%) was found (as crude result based on zone diameter and/or MIC value) in a large number of laboratories whatever the testing method they used, but it was particularly frequently recorded by VITEK2 users both for piperacillin-tazobactam (38 out of 42 participants) and for cefotaxime (40 out of 42 participants). It is known that AmpC-mediated resistance may be heterogeneously expressed (only 1 out of  $10^8$  CFU would overproduce AmpC) and that detection of such resistance mechanism is challenging for rapid automated systems which monitor *in vitro* bacterial growth kinetics during only the first few hours (4 to 8 hrs).

Again, CLSI does not provide specific interpretative guidelines for chromosomal AmpC-producing *Enterobacteriaceae* (e.g. *Enterobacter* species, *Citrobacter freundii*, *Morganella morganii*, *Providencia* species and *Serratia* species). However, clinical failure associated with the emergence of resistance to third-generation cephalosporins has been well documented during therapy with these agents so that a susceptible result should probably not be reported to clinicians.

On the contrary, 11 participants correctly assessed this isolate as susceptible to cefepime by MIC or zone size results, but reported an 'intermediate' or 'resistant' interpretative results. It should be here reminded that 4<sup>th</sup> generation cephalosporins (cefepime, ceftipime) usually retain excellent activity against AmpC derepressed *Enterobacteriaceae* isolates and have been shown to be clinically effective against these organisms.

#### **Culture 4**

***Citrobacter freundii*** (UCL-MG #N6010290) is an AmpC overproducing clinical isolate which co-produces two different ESBL enzymes: SHV-12 like and CTX-M-9.

This strain displays high level resistance to both cefotaxime (256 µg/ml) and ceftazidime (256 µg/ml) and it shows intermediate susceptibility to piperacillin-tazobactam (16-32 µg/ml). Based on actual CLSI breakpoints, it is categorized as borderline susceptible to cefepime (8 µg/ml).

This organism was correctly identified as ESBL-producer by only 31 (41%) of the participants while 37 (49%) failed to identify an ESBL; Further, 8 laboratories reported this isolate as “not determinable” or as “possible ESBL-producer”.

The proportion of correct answers was not statistically different among participants who used automated systems (10/29 [34%] ESBL-positive), disc method (11/22 [50%]) or a combination of automated and disc method (10/24 [42%]). Likewise, no difference in the proportion of accurate ESBL detection was found when comparing the number and combination of methods used (DD, DDST or E-test) nor when looking at the number of antibiotic substrates used. However, the inclusion of cefepime as substrate indicator was associated with a higher rate of ESBL detection (19/36 [53%] vs 1/10 [10%] when cefepime was not used;  $p=0.01$ ).

This observation underscores the importance of the choice of appropriate indicator substrates for ESBL detection in selected species, especially among AmpC-producing isolates. Indeed, several studies have shown that 4<sup>th</sup> generation cephalosporins (cefpirome or cefepime) are better substrates for ESBL detection in AmpC-producing organisms because of their enhanced stability to these enzymes in comparison to other extended-spectrum cephalosporins.

Almost 60% of the participants recorded this isolate as cefepime-susceptible (which is indeed correct according to current CLSI breakpoints). Yet, 25 of the 31 participants who detected the ESBL applied an interpretative reading and reported this organism as intermediate or resistant to cefepime (9 and 16 participants, respectively), while six others would have reported it as susceptible to the clinician.

Once again, CLSI does not provide specific interpretative guidelines for ESBL reporting in *Citrobacter freundii* nor in any other AmpC-producing species.

Data concerning the clinical outcome of infections caused by ESBL-and AmpC co-producing isolates (such as *Citrobacter freundii* or *Enterobacter* spp.) in patients treated with cefepime are scarce so that this agent should probably not be administered as first line therapy (carbapenems should usually be preferred instead in this setting) but cefepime could be considered in individual setting taking into account the sites of infection and the MIC value of the pathogen (PK/PD data suggest that at conventional daily dosage of 2 g bid (6 g/d), cefepime would be anticipated to be clinically effective for MIC value up to 8 µg/ml).

Antibiotic	Reference Results	Total Nr Labs	S	I	R
Co-amoxyclav	R	74	-	-	74
Piperacillin/tazobactam	I	73	7	22	44
Cefuroxime	R	73	-	-	73
Cefotaxime	R	68	2	3	63
Ceftazidime	R	76	-	-	76
Cefepime	S/I*	74	43	13	18

\* isolate had a MIC of 8 µg/ml for cefepime (borderline susceptible by CLSI criteria); CLSI does not give interpretative guidelines for-AmpC producing species

### **Culture 5**

*Klebsiella oxytoca* (MG #N6050217) is a K1/ OXY- chromosomal  $\beta$ -lactamase hyperproducer and no ESBL was present in this isolate. It was characterized by a high level resistance to cefuroxime and to piperacillin-tazobactam (> 256  $\mu\text{g/ml}$ ) and it showed moderate resistance to cefotaxime (16  $\mu\text{g/ml}$ ) and to cefepime (16  $\mu\text{g/ml}$ ) but not to ceftazidime to which it was susceptible (2  $\mu\text{g/ml}$ ). This organism was correctly identified as non ESBL-producer by 68 participants (89%). Six laboratories (8%) misidentified the isolate as ESBL-producing and two others reported the ESBL status as “not determinable” or “possible ESBL”. Errors occurred both in participants using automated systems, disc tests or a combination of manual and automated methods.

<b>Antibiotic</b>	<b>Reference Results</b>	<b>Total Nr Labs</b>	<b>S</b>	<b>I</b>	<b>R</b>
Co-amoxyclov	<b>R</b>	75	-	-	75
Piperacillin/tazobactam	<b>R</b>	73	-	-	73
Cefuroxime	<b>R</b>	74	-	-	74
Cefotaxime	<b>I/R</b>	68	24	18	26
Ceftazidime	<b>S</b>	76	64	3	9
Cefepime	<b>I/R</b>	74	25	22	27

Concordance of results between the participating laboratories and the reference lab almost reached 100% for co-amoxyclov, piperacillin-tazobactam and cefuroxime. Owing to the moderate level of resistance it comes as no surprise that lower concordance were found for cefotaxime and cefepime. Again, most false susceptible results were observed in laboratories using automated systems; indeed out of 42 VITEK 2 users, 37 and 29, respectively reported this isolate as susceptible to cefotaxime (8  $\mu\text{g/ml}$ ) and to cefepime (4-8  $\mu\text{g/ml}$ ). Twelve participants (including all 6 who categorized the strain as ESBL-positive) reported this strains as resistant or intermediate to ceftazidime. CLSI currently does not provide any recommendations nor interpretative guidelines for reporting the susceptibility to extended cephalosporins among K1-OXY hyperproducing *K. oxytoca* isolates. Overproduction of K1-OXY penicillinase is present in about 25% of *K. oxytoca* isolates and is due to punctual mutations in the promoter leading lead to increased transcription of the *bla<sub>OXY</sub>* gene. High-level resistance to cefuroxime, aztreonam, and to piperacillin-tazobactam is typical for this mechanism; it confers moderate resistance to ceftriaxone, cefotaxime and to cefepime while ceftazidime remains unaffected. Since this enzyme belongs to Ambler class A penicillinase, it is susceptible to inhibition by clavulanate. It should be reemphasized that the occurrence of a synergy between clavulanate and cefotaxime or cefepime is characteristic for OXY  $\beta$ -lactamases in *K. oxytoca* and that it does not imply the presence of an ESBL in this organism. On the other hand, synergy between clavulanate and ceftazidime represents the single most important phenotypic marker for ESBL detection in this species.

## **Conclusions**

This external quality assessment showed that the performance standards of laboratories for ESBL detection was globally satisfactory but that it was obviously variable depending on the isolates and that it was significantly lower in organisms in which several resistance mechanisms were superimposed (40% accurate detection in one AmpC overproducing *Citrobacter freundii*). Likewise, *Enterobacteriaceae* isolates with a resistance mechanism other than ESBL (one K-1 OXY- $\beta$  lactamase overproducing *K. oxytoca* and one AmpC *E. aerogenes* hyperproducer) were incorrectly assigned as ESBL-producers by about 10% of the participants. Interestingly, no difference in performance was found in this external QC assessment between laboratories who used automated or those employing manual methods. As a rule, the majority of the laboratories even those using automated systems currently use a combination of different methods including disc-based synergy tests.

No relation could be established between accuracy of ESBL detection and the number of indicator substrates used, though it should be underscored that the majority of the participants did use at least two or three cephalosporin indicators for ESBL detection. Almost three-quarter of the participants (56 of 76) employed cefepime as a marker of ESBL detection in AmpC producing species isolates (which is not yet endorsed by CLSI) and this was associated with a better performance for detection of ESBL in the *C. freundii* isolate.

One important issue raised by this survey was that despite accurate detection of the involved resistance mechanisms, category reports were still often at variance with CLSI interpretative guidelines. In particular, false susceptible results to cefotaxime and to cefepime were reported by 5 to 11% of the participants for *E. coli* and for *K. pneumoniae* (despite ESBL detection). Likewise false susceptible results to third generation cephalosporins (cefotaxime, ceftazidime) were reported by a substantial number of participant in one AmpC derepressed *Enterobacter aerogenes* isolate. While discordances with the reference results for some antibiotics could occasionally be attributed to a lack of detection of low-level resistance, they most often occurred because of lack of guidelines (for instance; report for piperacillin-tazobactam in ESBL-positive organisms; reports for extended spectrum cephalosporins in AmpC producing isolates....).

In summary, this survey allowed to determine the baseline performance of Belgian clinical microbiology laboratories in detecting and reporting ESBL and  $\beta$ -lactam resistance phenotypes in *Enterobacteriaceae* isolates. Globally, results of this study were satisfactory and on the average better than those of similar recent surveys performed in other European countries and in the USA. Nevertheless several problems were identified (lack of identification of ESBL in organisms with superimposed resistance mechanisms, discordances with current CLSI guidelines in interpretative reports) and suggest that there is substantial room for improvement. Further studies to fulfil these goals will be planned in the future (a second external quality control assessment is foreseen during the first trimester of 2008).

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