Guidance to Diagnostic Laboratories

Laboratory Detection & Reporting of Bacteria with Extended-Spectrum b-Lactamases

COMPILED BY

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Minimum recommendations

These guidelines advise on the detection of 'Extended-spectrum b-lactamases' (ESBLs) and are issued at a time when the changing nature and distribution of these important enzymes presents clinicians and diagnostic laboratories with new challenges.

There are several ways to recognise ESBL producers, as outlined in the main body of this document; the strategy below is the simplest way to meet these guidelines

Enterobacteriaceae from infections in hospitalised patients

- Test **both** cefotaxime and ceftazidime on first-line panel, or test cefpodoxime.
- Do ESBL confirmatory tests (below) on isolates found resistant to **ANY** of cefotaxime, ceftazidime or cefpodoxime

Enterobacteriaceae from community patients

- Test cefpodoxime as an indicator on first-line panel (one possible first-line panel for community UTI isolates comprises cefpodoxime, nitrofurantoin, trimethoprim, a fluoroquinolone and **two** out of cephalexin, co-amoxiclav and ampicillin/amoxycillin).
- Do ESBL confirmatory tests (below) on isolates found resistant to cefpodoxime.

Identification to genus/species level is highly desirable for the interpretation of resistance patterns and, as a minimum, should be undertaken on all isolates found resistant to cefotaxime, ceftazidime or cefpodoxime in the above tests.

To confirm ESBL production in isolates found resistant to cefotaxime/ceftazidime or cefpodoxime: use cefpodoxime/clavulanate combination discs or, for *Enterobacter* spp. and *C. freundii*, cefpirome/clavulanate combination discs.

Report ESBL producers resistant to ALL cephalosporins (except cefoxitin).

ESBLs – a changing problem

These guidelines advise on the detection of 'Extended-spectrum b-lactamases' (ESBLs) and are issued at a time when the changing nature and distribution of these enzymes presents clinicians and diagnostic laboratories with new challenges.

The term ESBLs is used to mean acquired class A b-lactamases that hydrolyse and confer resistance to oxyimino- '2nd- and 3rd-generation' cephalosporins, e.g. cefuroxime, cefotaxime, ceftazidime and ceftriaxone.

ESBLs include:

- Cephalosporin-hydrolysing mutants of TEM and SHV - the common plasmid-mediated penicillinases of Enterobacteriaceae. Well over 100 such variants are known (see http://www.lahey.org/studies/inc_webt.asp).
- CTX-M types. These evolved separately, at least some of them via the escape and mutation of chromosomal β-lactamases of *Kluyvera* spp. Over 30 variants are known (1).
- Obscure types, e.g. VEB and PER, not yet of concern in the UK; also OXA (Class D) ESBLs from *Pseudomonas aeruginosa*, in Turkey.

ESBLs are not the sole β-lactamases to confer resistance to 2nd and 3rd generation cephalosporins, but are the most important. They occur mostly in Enterobacteriaceae (e.g. *E. coli, Klebsiella* spp. and *Enterobacter* spp.) and rarely in non-fermenters (e.g. *P. aeruginosa*). They should be distinguished from other important modes of resistance to 2nd and 3rd generation cephalosporins, e.g.:

- Hyperproduced chromosomal AmpC β-lactamases, especially in *Enterobacter* spp.
- Plasmid-mediated AmpC β-lactamases, in *Klebsiella* spp. and *E. coli* (rare)
- Hyperproduced K1 chromosomal β-lactamases in *K. oxytoca* not K. *pneumoniae*)
- Efflux-mediated resistance in *P. aeruginosa*

- Various ill-defined mechanisms in *Acinetobacter* spp.

Guidelines on distinguishing all these resistance mechanisms from strain phenotypes have been updated recently (2;3).

ESBLs are clinically important because:

- They destroy cephalosporins, workhorse hospital antibiotics, given as first-line agents to many severely-ill patients, including those with intra-abdominal infections, community-acquired pneumonias and bacteraemias.

- Delayed recognition and inappropriate treatment of severe infections caused by ESBL producers with cephalosporins has been associated with increased mortality (4-6).

- ESBL-mediated resistance is not always obvious to all cephalosporins *in vitro* (2).

- Many ESBL producers are multi-resistant to non-β-lactam antibiotics such as quinolones, aminoglycosides and trimethoprim, narrowing treatment options.

- Some producer strains achieve outbreak status, spreading among patients and locales, perhaps owing to particular pathogenicity traits.

New guidance on ESBLs is needed because:

- Until 2001/2 most ESBL producers in the UK were *Klebsiella* spp. with TEM and SHV mutants. They were largely from specialist units, where multi-resistance is anticipated.

- Since 2000, CTX-M ESBLs have emerged in the UK. They are often in *E. coli* from the hospital/community interface, for instance in urinary infections among out-patients with recent hospitalisation, who are catheterised, and who have underlying disease. Some patients with such infections do not appear to have had contact with hospitals. They may be admitted with serious secondary infections, such as bacteraemia, and be treated inappropriately due to delayed recognition that the organism is an ESBL producer, with serious sequelae. High mortality has been observed in these cases.

- ***CTX-M β-lactamase-producing E. coli have been sent to the Antibiotic Resistance Monitoring & Reference Laboratory (ARMRL) from over 60 UK diagnostic labs. About one quarter of all the isolates received belong to one***

major outbreak E. coli strain, with CTX-M-15 enzyme and with a distinctive DNA profile. This strain has been reported from 6 centres and is dominant in 2. At least 4 other outbreak strains occur, also with CTX-M-15; in addition, this enzyme has been found in many sporadic E. coli strains (7;8). A few isolates have other CTX-M-types, principally CTX-M-3, -9 or –14. CTX-M enzymes are also occurring in Klebsiella spp.; a general increase is indicated by the BSAC Bacteraemia Surveillance and we are aware of ongoing outbreaks at 2 hospitals in the West Midlands, one with a CTX-M-15 producer, the other with a CTX-M-26 producer(9).

- Similar shifts to increased ESBL prevalence, dissemination and towards CTX-M types are occurring widely in Europe (10-13), Asia (14) and Canada (15): CTX-M types have long been dominant in Argentina (16). The predominant CTX-M types do, however vary with the country: CTX-M-15 in the UK, Canada and Russia, CTX-M-2 in Argentina and Israel; CTX-M-14 in parts of China and CTX-M-9 and –12 in Spain.

These changes mean that ESBLs must be sought more widely than previously. Moreover CTX-M enzymes present different detection challenges to the TEM and SHV ESBLs, which, themselves, remain scattered and, probably, under-detected.

Laboratory detection: Screening then Confirmation

The basic strategy to detect ESBL producers is to **use an indicator cephalosporin to screen** for likely producers, then to **seek cephalosporin/clavulanate synergy**, which distinguishes ESBL producers from, for example, strains that hyperproducer AmpC or K1 enzymes.

SCREENING: CHOICE OF INDICATOR CEPHALOSPORIN

The ideal indicator cephalosporin is one to which all ESBLs confer resistance, even when their production is scanty. Choice is predicated by the following general traits:

TEM & SHV ESBLs – obvious resistance to ceftazidime, variable to cefotaxime

CTX-M ESBLs – obvious resistance to cefotaxime: variable to ceftazidime

All ESBLs – obvious resistance to cefpodoxime

Cefuroxime, cephalexin and cephradine (see below) are unreliable indicators

It follows that the logical indicator is either cefpodoxime or BOTH of cefotaxime and ceftazidime.

An alternative strategy has been proposed for community urines: testing cephalexin or cephradine as the indicator drug, then doing confirmatory ESBL tests on all isolates that are found resistant (these include e.g. all Enterobacter spp. and some hyperproducers of classical TEM, as well as the ESBL producers). This is* NOT *recommended, as some CTX-M-15 producers, principally those belonging to the major UK outbreak strain, appear borderline susceptible (submitted).

WHICH SPECIMENS & ISOLATES TO SCREEN

The spread of CTX-M enzymes into out-patient/community *E. coli* means that the indicator cephalosporin(s) should be tested first-line against **ALL Enterobacteriaceae** or, if direct sensitivities are done, on all clinical specimens likely to harbour producers.

HOW TO SCREEN WITH THE INDICATOR

The indicator drugs should be included in primary susceptibility testing done e.g. by the method of the British Society for Antimicrobial Chemotherapy(17). The indicators also work in Stokes' comparative disc method, though this is no longer recommended, owing to its poor standardisation. **Species identification is highly desirable to allow proper interpretation of results. BSAC recommended breakpoints for the cephalosporins advocated are:**

Antibiotic & disc content	**Zone breakpoints (mm**		**MIC (mg/L)**	
	R, ≤	S, ≥	R, >	S, ≤
Cefotaxime, 30 μg	29	30	1	1
Ceftazidime, 30 μg *E. coli* & *Kleb*	21	22	2	2
Ceftazidime, 30 μg , other species	27	28	2	2
Cefpodoxime, 10 μg	25	26	1	1

If NCCLS methodology is followed, attention should be paid to the Standards' low breakpoints for ESBL detection, not only their (much higher) therapeutic breakpoints. Automated systems -e.g. Vitek and Phoenix- incorporate ESBL detection tests or strategies and are an alternative to the present recommendations (18;19).

Confirmatory tests for ESBLs

Enterobacteriaceae isolates resistant to any indicator cephalosporin in the screening tests outlined above should be subjected to confirmatory tests. Confirmation of ESBL production depends on demonstrating synergy between clavulanate and those indicator cephalosporin(s) to which the isolate was initially found resistant. Three methods can be used:

(i) ***Double disc tests.*** A plate is inoculated as for a routine susceptibility test. Discs containing cefotaxime and ceftazidime 30 µg (or cefpodoxime 10 µg) are applied either side of one with co-amoxiclav 20+10 µg; and *c*. 20-30 mm away from it.

ESBL production is inferred when the zone of *either* cephalosporin is expanded by the clavulanate. The method is cheap, but the optimal disc separation varies with the strain and some producers may be missed. **We therefore no longer advocate this method for routine use**.

(ii) ***Combination disc methods***. (Oxoid or Becton Dickinson 'Combination Discs' and Mast 'MAST DD'). These compare the zones of cephalosporin discs to those of the same cephalosporin plus clavulanate. According to the supplier, either the difference in zone diameters, (Oxoid) or the ratio of diameters, is compared (Mast and BD) with zone diameter increases of $\geq$5 mm (20) or $\geq$50% (21) in the presence of the clavulanate implying ESBL production. **These tests are cheap and do not require critical disc spacing.**

(iii) ***Etest ESBL strips*** **(AB Biodisk, Solna, Sweden; Bio-Stat, Stockport, UK).** These have a cephalosporin gradient at one end and a cephalosporin + clavulanate gradient at the other. Users should follow the manufacturer's instructions, including for a heavier inoculum than in BSAC disc tests. ESBL production is inferred if the MIC ratio for cephalosporin alone: cephalosporin + clavulanate MIC is $\geq$8. **These are accurate and precise, but more expensive than combination discs.**

Pitfalls and problems for ESBL tests

Species with inducible AmpC b-lactamases: ESBLs are harder to detect in those Enterobacteriaceae with inducible AmpC chromosomal enzymes (e.g. *Enterobacter* spp., *Citrobacter freundii, Morganella morganii, Providencia* spp. and *Serratia* spp.). The AmpC enzymes may be induced by clavulanate (which inhibits them poorly) and may then attack the cephalosporin, masking synergy arising from inhibition of the ESBL.

- If ESBL tests are to be done on *Enterobacter* spp. (**10-20% of cephalosporin resistance in enterobacters *is* due to ESBLs, not derepressed AmpC**) it is best to use an AmpC-stable cephalosporin (i.e. cefepime or cefpirome) in the clavulanate synergy tests (though **NOT** as the first indicator compound). **Cefepime/clavulanate Etests (AB Biodisk) and cefpirome/clavulanate combination discs (Oxoid) are available, and should be used with these genera.**

- Cephalosporin therapy of *Enterobacter* and *C. freundii* infections is anyway not recommended, owing to the risk of selecting AmpC-derepressed mutants; and clinicians should be steered away from the use of these agents (22;23).

K. oxytoca: 10-20% of *K. oxytoca* isolates hyperproduce their class A "K1"chromosomal β-lactamase. These are resistant to cefpodoxime and (often) cefotaxime but not to ceftazidime.

- They may give positive clavulanate synergy tests with cefotaxime or cefepime (never ceftazidime), so that producers are confused with ESBL producers. K1 hyperproduction resistance should be suspected if a *Klebsiella* isolate is indole-positive and has high-level resistance (growth up to the disc?) to piperacillin/tazobactam and cefuroxime, but has borderline susceptibility to cefotaxime and full susceptibility to ceftazidime.

Acinetobacter spp., P. aeruginosa & Stenotrophomonas maltophilia: ESBL tests were **NOT** developed for these species and should **NOT** be used for them. False positive results with *Acinetobacter* are common owing to inherent susceptibility to clavulanate, whilst *S. maltophilia* may give positive results *via* inhibition of its chromosomal L-2 β-lactamase. ESBLs may occur in these genera (e.g. VEB-1 in

Acinetobacter spp. in France), but are not the common cause of cephalosporin resistance in them, and should not be routinely sought.

Enzymes with marginal ESBL activity, those expressed weakly, and those produced alongside other enzymes (e.g. derepressed AmpC) are the hardest to detect. The methods outlined here will never be so precise as the best molecular analysis, but will detect most producers.

Reporting for ESBL producers

b**-Lactams:** Organisms inferred to have ESBLs should be reported resistant to ALL penicillins (except temocillin), cephalosporins (except cefoxitin), and to aztreonam, irrespective of routine susceptibility results. ***Treatment failures and death have occurred when cephalosporins were used against ESBL producers that appeared susceptible in vitro*** (4-6). **The cephalosporin advice includes the fourth-generation compounds, cefepime and cefpirome- which were developed for stability to AmpC enzymes, not ESBLs.**

- Carbapenems (imipenem, meropenem and ertapenem) are consistently active and are the treatment of choice in severe infections due to ESBL producers.
- Susceptibilities of ESBL producers to β-lactamase inhibitor combinations vary with the isolate and its amount of enzyme, but should be accepted at face value.
- Mecillinam often appears active *in vitro*, but its efficacy against ESBL producers remains unproven- we are cautious of advocating its use in severe infection, but further study is needed.
- Combinations of a cephalosporin with co-amoxiclav should work in principle, but have not been formally evaluated in treatment, and may be antagonistic *vs. Enterobacter* spp.; we are therefore cautious of advocating clinical use.

Non-b**-lactams:** Many ESBL producers, including community isolates with CTX-M enzymes are multi-resistant to fluoroquinolones and aminoglycosides, but susceptibilities vary, and these are good options where a strain is susceptible.. **The predominant CTX-M-15 producing *E. coli* strains disseminating in the UK are resistant to fluoroquinolones, trimethoprim, co-trimoxazole, tetracyclines and amikacin; gentamicin resistance is variable and is absent from the predominant strain.**

Oral therapies: Among oral agents suitable for community use in UTI, nitrofurantoin and fosfomycin (not readily available in the UK) are active *vs.* many ESBL producers including most of the present CTX-M-15 producing *E. coli*. See above comments on mecillinam and on clavulanate combinations.

Empirical treatment: Empirical treatment strategies may need to be re-thought in settings where ESBLs producers are prevalent and/or where there is a significant perceived risk (e.g. for a patient with a history of UTI, admitted from the community with an overspill bacteraemia). **It may be appropriate to use a carbapenem until the infection has been proved NOT to involve an ESBL producer, then to step down to a narrower-spectrum antibiotic. Ertapenem may, arguably, be the preferred agent, as applying the least selection pressure for resistance in colonising non-fermentative bacteria.**

Which ESBL producers to send to ARMRL?

ARMRL does not seek every ESBL producer, least of all every nosocomial *Klebsiella* sp. isolate believed to harbour an ESBL. We do, however, seek:

- Representatives from major outbreaks
- ESBL-positive *E. coli* from any laboratory where these have not been encountered previously (we will then advise on which further isolates to send).
- ANY suspected producers from a patient without recent hospital contact.

If in doubt, please phone to ask 020-8327-7223 (David Livermore) or 020-8327-7255 (Neil Woodford). Even where we do not seek the isolates themselves we are always happy to provide advice.

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