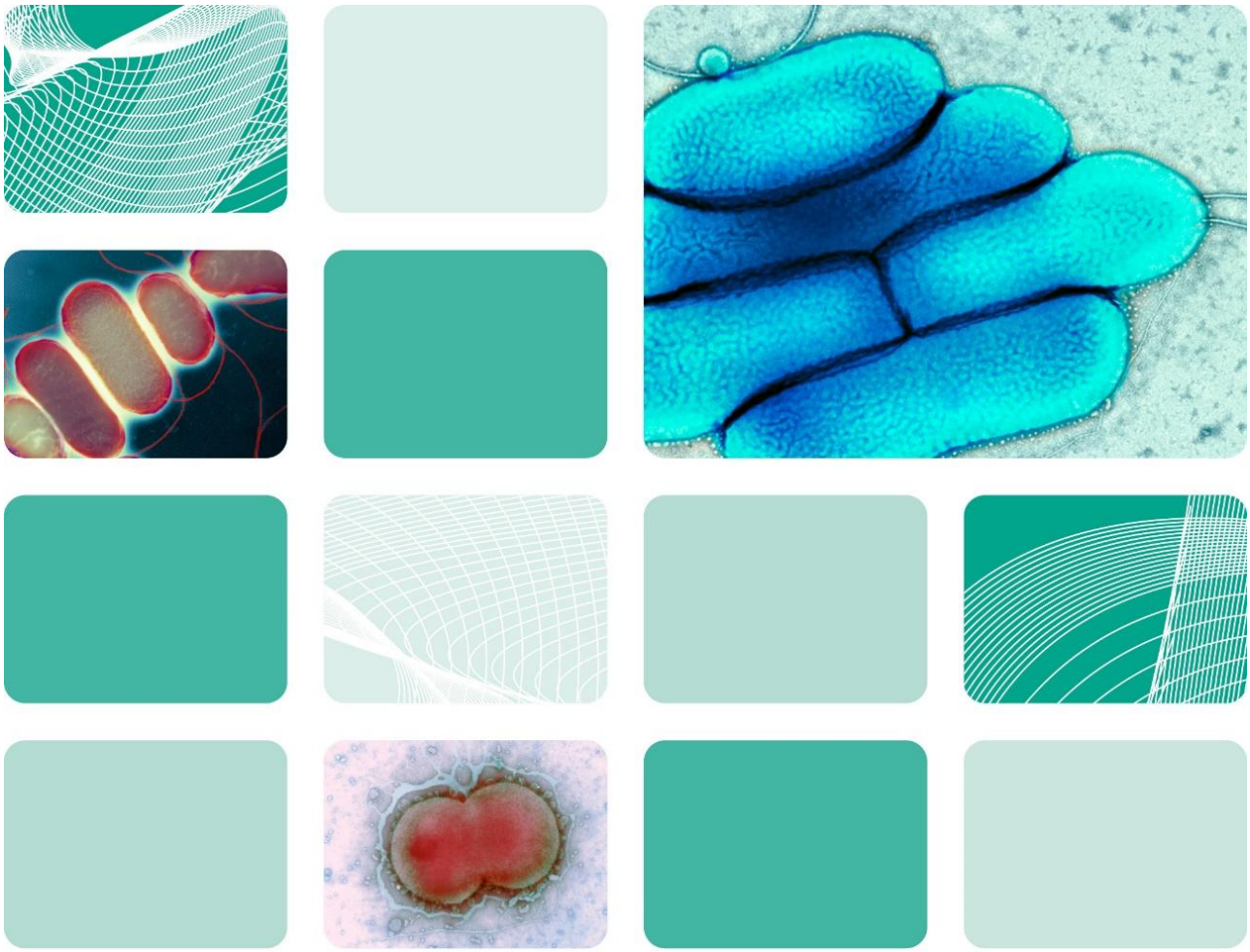
UK Health Security Agency

UK Standards for Microbiology Investigations

Detection of Enterobacteriaceae producing extended spectrum β-lactamases



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Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on the UK SMI website. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee.

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Contents

Amendment table

Each UK SMI document has an individual record of amendments. The amendments are listed on this page. The amendment history is available from standards@ukhsa.gov.uk.

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	7/03.06.26
Issue number discarded	4.1
Insert issue number	4.2
Section(s) involved	**Amendment**
Whole document.	**This is an administrative point change.** **The content of this UK SMI document has not changed.** **The last scientific and clinical review was conducted on 17/08/2016.** Hyperlinks throughout document updated to Royal College of Pathologists website. Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms Partner organisation logos updated. Broken links to devolved administrations replaced. References to NICE accreditation removed. Scope and Purpose replaced with General and Scientific information to align with current UK SMI template. 'Public health responsibilities of diagnostic laboratories' section added.
Amendment table	Incorrect issue number has been corrected.
References	Missing GRADE reference assessment added.

Amendment no/date.	6/17.08.16
Issue no. discarded.	4

Insert issue no.	4.1
Section(s) involved	**Amendment**
References.	GRADE information added to references.

Amendment no/date.	5/04.08.16
Issue no. discarded.	3
Insert issue no.	4
Section(s) involved	**Amendment**
Whole document.	B 59 formerly P 2 (previously QSOP 51). Title of document updated. Hyperlinks changed to gov.uk. Document presented in a bacteriology SMI template. The document gives recommendations on the testing of clinical or screening specimens for the detection of Enterobacteriaceae that produce an extended-spectrum β-lactamase (ESBL). Links to different websites/articles updated. Technical limitations/ information section updated.
Page 2.	Updated logos added.
Reporting procedure.	This has been updated with appropriate information.
Appendix.	Flowchart produced for easy guidance.
References.	References reviewed and updated.

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 Scope of document

Type of specimen

Screening specimens include stool, rectal or peri-rectal cultures. Clinical specimens include blood, wounds or urine.

Note: Ideally stool or rectal swabs should be submitted for screening.

This UK SMI describes the examination of clinical or screening specimens for the detection of Enterobacteriaceae that produce an extended-spectrum β-lactamase (ESBL).

This document should not be applied to isolates with carbapenem resistance – these may have ESBLs (or other enzymes) combined with porin loss, or may have acquired carbapenemases or may have both a carbapenemase and an ESBL. Further advice on detection of carbapenem-resistant isolates is provided in UK SMI B 60 - Detection of bacteria with carbapenem-hydrolysing β-lactamases (carbapenemases).

This UK SMI should be used in conjunction with other UK SMIs.

4 Introduction

The term “ESBL” is used in this document to mean acquired class A β-lactamases that hydrolyse and (usually) confer resistance to oxyimino- ‘2nd and 3rd generation’ cephalosporins, eg cefuroxime, cefotaxime, ceftazidime and ceftriaxone, and 4th generation cephalosporins eg cefepime, cefpirome, but not cephamycins (eg cefoxitin) or carbapenems.

ESBLs include:

- cephalosporin-hydrolysing mutants of the TEM and SHV plasmid-mediated penicillinases of Enterobacteriaceae. These were the original ESBLs and over 400 such variants are known (see http://www.ncbi.nlm.nih.gov/projects/pathogens/submit_beta_lactamase).
- CTX-M types. These evolved via the escape of chromosomal β-lactamase genes of *Kluyvera* species to plasmids. Over 170 variants are known, dividing into 5 major groups[1,2]
- minor types, eg VEB, PER and GES[3] – these are rare in Enterobacteriaceae and in the UK

ESBLs are not the only β-lactamases to confer resistance to cephalosporins while sparing carbapenems, but are the most important. Moreover, as plasmid-mediated enzymes, they have great potential for spread. They occur mostly in Enterobacteriaceae (eg *E. coli, Klebsiella* species and *Enterobacter* species). They should be distinguished from other modes of resistance to cephalosporins eg:

- derepressed chromosomal AmpC β-lactamases, especially in *Enterobacter* species
- plasmid-mediated AmpC β-lactamases eg CMY types, in *Klebsiella* species and *E. coli*
- hyperproduced K1 chromosomal β-lactamase in *K. oxytoca*
- advice on distinguishing all resistance mechanisms is available[4,5]

ESBLs are clinically important because they destroy cephalosporins that are used in the treatment of many severely ill patients. Delayed recognition and inappropriate treatment of severe infections caused by ESBL producers with cephalosporins has been associated with increased mortality[6,7].

Until 2000 most ESBLs encountered in the UK were TEM and SHV mutants. They were largely seen in *K. pneumoniae*, including strains causing hospital outbreaks, but did not penetrate *E. coli* or community strains to any major extent. Since 2000, CTX-M ESBLs have proliferated. Unlike earlier types, these are often seen in *E. coli* from the hospital/community interface, eg from urinary infections among elderly out-patients with recent hospitalisation, those who are catheterised, and who have underlying disease [8]. Many patients with infections due to ESBL producers lack recent contact with hospitals; these may be admitted with serious secondary infections, eg bacteraemia where delays in effective therapy increase the risk of death[7].

Similar increases in ESBL prevalence, owing to dissemination of CTX-M enzymes have occurred also in Europe[8], Asia[9,10] and North America[11,12]: whilst CTX-M types have long been prevalent in Argentina[13]. The predominant CTX-M types vary with the country: CTX-M-15 dominates in most of Europe and Asia from India westwards, also North America[12,14]; CTX-M-2 in South America[13] and Israel[15]; CTX-M-14 in the Far East[10] and Spain[2]. The association with *E. coli* and greater community penetration persists irrespective of the particular enzyme. One *E. coli* lineage - Sequence Type (ST) 131- is an especially common ESBL host, especially for CTX-M-15 enzyme, and is disseminated internationally, including in the UK[16].

All cephalosporins except cephamycins (eg cefoxitin and cefotetan) are substrates for ESBLs, but resistance is not always high level, complicating detection and interpretation[4]. Many producers are multi-resistant to non-β-lactam antibiotics including quinolones, aminoglycosides and trimethoprim.

4.1 Laboratory detection: screening and confirmation

ESBLs may be detected fortuitously during the processing of clinical samples. Alternatively, they may be detected during targeted screening of faecal samples.

How to recognise ESBL Producers

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 hospitalised patients

- test both cefotaxime and ceftazidime on the first-line panel, or test cefpodoxime. Unless cefpodoxime is tested, it is required that both cefotaxime (or ceftriaxone) and ceftazidime are used as indicator cephalosporins, as there may be large differences in MICs of cefotaxime (or ceftriaxone) and ceftazidime for different ESBL-producing isolates. Cefpodoxime is the most sensitive individual indicator cephalosporin for detection of ESBL production. Cefpodoxime may be used for screening, but not for confirmation testing as it is less specific than the combination of cefotaxime (or ceftriaxone) and ceftazidime
- perform 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
- perform ESBL confirmatory tests (below) on isolates found resistant to cefpodoxime

Note: 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.

To confirm ESBL production in isolates found resistant to cefotaxime/ceftazidime or cefpodoxime

Use cefpodoxime/clavulanate combination discs for all Enterobacteriaceae except *Enterobacter* species and *Citrobacter freundii,* where cefpirome/clavulanate or cefepime/clavulanate combination discs are used.

Note:

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

The basic strategy to detect ESBL producers, outlined above, is to use an indicator cephalosporin to screen for likely producers, then to seek cephalosporin/clavulanate synergy, which distinguishes ESBL producers from strains that hyperproduce either AmpC or K1 enzymes[4].

Screening

The ideal indicator cephalosporin is one to which all ESBLs confer resistance, even when production is scanty[4]. Choice is predicated by the following general traits:

- TEM and SHV ESBLs – obvious resistance to ceftazidime, variable to cefotaxime
- CTX-M ESBLs – obvious resistance to cefotaxime, variable to ceftazidime
- all ESBLs – resistance to cefpodoxime, however, low-level cefpodoxime resistance is common in isolates with no ESBL or other substantive mechanism[17]

- cefuroxime, cephalexin and cephradine are unreliable indicators for ESBL production and are not recommended.

Selective culture media

Chromogenic media have been developed for detection of ESBL-producers in faecal screening whereas for routine diagnostic testing using EUCAST disc diffusion method, the screening cut off values could be employed for detecting ESBLs (see EUCAST detection of resistance mechanisms) as referred to in section 4.7 of this document[18-20]. Although chromogenic agar use may have its limitation, such as being likely to be less specific, particularly in areas where ESBL producers are commonplace, they are still the preferred option[19-24]. Some commercial products include selective antimicrobial agents incorporated into the medium. Others will require placement of indicator cephalosporin discs.

Clinical specimens can also be screened using MacConkey or CLED agar with an antibiotic disc. Although not a well validated or tried method, if used by laboratories, they should ensure that this has been validated and verified locally.

For further information on the different screening methods, see section 4.7.

Confirmatory tests for ESBLs: inhibitor-based tests

Enterobacteriaceae isolates resistant to any indicator cephalosporin but susceptible to carbapenems in the screening tests above should be subjected to confirmatory tests. These depend on demonstrating synergy between clavulanate and the indicator cephalosporin(s) to which the isolate was found resistant. Three methods can be used:

- **Double disc synergy tests**

  A plate is inoculated with the test organism as for a routine susceptibility test. Discs containing cefotaxime and ceftazidime 30µg (or cefpodoxime 10µg) are applied either side of one with co-amoxiclav 20+10µg; and are placed 20mm away (centre to centre) from it. This distance is optimal for cephalosporin 30µg discs[4]. However, it has been suggested that the sensitivity of this test can be increased by reducing the distance between the discs to 15mm or expanding to 30mm for strains with very high or low levels of resistance respectively[25].

  ESBL production is inferred when the zone of either cephalosporin is expanded by the clavulanate. The method is inexpensive, but the optimal disc separation varies with the strain and some producers may be missed. It is therefore not recommended.

Figure 1: Detection of ESBL production using the double disc method.
The disc on the left is cefotaxime (30µg): the disc in the centre is co-amoxiclav (20+10 µg): the disc on the right is ceftazidime (30µg). Note the expansion of the zones around the cefotaxime and ceftazidime discs adjacent to the co-amoxiclav (courtesy of Jenny Andrews of the Sandwell and West Birmingham NHS Trust[26]).

- **Combination disc tests[27,28]**

  These compare the zones of cephalosporin discs to those of the same cephalosporin plus clavulanate. These are commercially available. According to the supplier, either the difference in zone diameters, or the ratio of diameters, is compared, with zone diameter increases of ≥5mm or ≥ 50 % in the presence of the clavulanate implying ESBL production[28,29]. These tests are inexpensive and do not require critical disc spacing, but care should be taken regarding controls (see below) especially if the discs are from different batches.

- **Gradient ESBL strips**

  These have a cephalosporin gradient at one end and a cephalosporin plus 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 compared with cephalosporin + clavulanate MIC is ≥8. These are accurate and precise, but more expensive than combination discs. The test should be used for confirmation of ESBL production only and is not reliable for determination of the MIC.

- **Automated systems**

  There are many commercially available systems for ESBL detection. Although some authors report false positives, automated or semi-automated systems generally can be used to detect ESBLs[30-33]. Some cards and panels include cephalosporin-clavulanate synergy tests; others infer ESBL production from overall antibiograms. Care should be taken to ensure that control strains (see below) give the appropriate result with the card or panel used, as problems have arisen with particular card types[34].

Confirmatory tests for ESBLs: rapid methods

Molecular tests: PCR has been successfully utilized for the detection of ESBL genes directly from clinical or screening samples[35]. Obvious advantages include a greater speed of detection and potentially a higher sensitivity than that offered by culture[19]. Disadvantages include a higher cost for processing samples and the need for specialised equipment and/or expertise and so might be considered expensive in some settings.

Gene sequencing and DNA microarray-based method have also been recommended for the genotypic confirmation of the presence of the ESBL genes[36,37]. Test results are usually obtained within 24hrs, however, molecular methods may not detect sporadically occurring ESBL genes or new ESBL genes[25].

Matrix-Assisted Laser Desorption/Ionisation - Time of Flight (MALDI-TOF): This is increasingly available to diagnostic laboratories; and has definite potential to discriminate antibiotic-resistant strains due to ESBL and carbapenemase production from non-producing strains, but this performance is not yet sufficiently reliable for routine microbiological diagnostics[38]. However, MALDI-TOF has been shown to be a rapid and efficient method for the early detection of ESBL-producing Enterobacteriaceae from clinical samples such as positive blood cultures thus allowing early administration of an appropriate antibiotic therapy[39].

This assay has also been noted to be much faster than the methods used routinely in clinical practice. It has the potential to provide an answer on day 1 if used with a clinical specimen or on day 2 if used on colonies. This option is not commercially available at the time. The overall expected time from the protein extraction to the spectrum acquisition and analysis is <2hr. Another additional advantage is its relatively low cost[39].

4.2 Controls for ESBL tests

Quality Control of the cephalosporin discs used in the routine primary screening should follow standard EUCAST/BSAC or CLSI recommendations.

Positive controls should be used to ensure the performance of ESBL confirmatory tests. Three ESBL-positive *E. coli* strains suitable for purpose are available from the NCTC (www.phe-culturecollections.org.uk/media/63614/m01520130827v4_antimicrobresmech-a4.pdf). They are as follows:

- CTX-M-15 (cefotaximase, less active against ceftazidime) NCTC 13353
- TEM-3 (broad-spectrum ESBL) NCTC 13351
- TEM-10 (ceftazidimase, less active against cefotaxime) NCTC 13352

Alternatively, some strains may be obtained commercially from other suppliers.

Table 2 showing ESBL control strains available from the NCTC

2. Extended-Spectrum β-Lactamases (ESBL):

2.1 TEM β-lactamases

Organism	NCTC® Strain Reference	Characteristics
Escherichia coli	NCTC 13351	TEM-3 ESBL – Transconjugant of strain isolated in Clermont Ferrand in 1985
Escherichia coli	NCTC 13352	TEM-10 ESBL – Transconjugant of original TEM-10 producer isolated in Chicago in 1988

2.2 SHV β-lactamases

Organism	NCTC® Strain Reference	Characteristics
Klebsiella pneumoniae	NCTC 13368	SHV-18 (ATCC 700603)

2.3 CTX-M β-lactamases

Organism	NCTC® Strain Reference	Characteristics
Escherichia coli	NCTC 13353	Strain EO 487. CTX-M-15 ESBL producer. Control strain for group 1 bla_{CTX-M} multiplex PCR assays
Escherichia coli	NCTC 13441	Strain EO 499. CTX-M-15 ESBL producer – Uropathogenic strain O25:H4 sequence type (ST) 131. Clinical isolate harbouring sequenced plasmid pEK499 (see NCTC 13400); Control strain for group 1 bla_{CTX-M} multiplex PCR assays
Escherichia coli	NCTC 13400	Strain Tr499 = DH5-α derivative. Source of pEK499 (fully sequenced plasmid GenBank Accession No EU935739) encoding CTX-M-15 enzyme. Fusion of type FII and FIA replicons, and harbours 10 antibiotic resistance

The CLSI recommends *K. pneumoniae* ATCC 700603 as a single ESBL-producing QC control. This strain may be sourced from the ATCC.

Either *E. coli* NCTC 10418 or ATCC 25922 should also be used as a negative control in ESBL confirmation tests. Negative controls are especially important when cephalosporin and cephalosporin plus clavulanate combination discs are from different batches, which may vary in retained potency. Zones of the cephalosporin and cephalosporin and clavulanate discs for ESBL-negative *E. coli* should be equal or within 2mm. Any greater difference implies malfunction or deterioration.

4.3 Detecting ESBLs in AmpC-Inducible species

ESBLs are harder to detect in species of *Enterobacteriaceae* with inducible, chromosomal AmpC enzymes (eg *Enterobacter*, *Citrobacter freundii*, *Morganella morganii*, *Providencia* and *Serratia*) than in *E. coli* and *Klebsiella* because AmpC activity induced by the clavulanate may attack the indicator cephalosporin, masking any synergy arising from inhibition of the ESBL.

- if ESBL tests are to be done on AmpC-inducible species it is best to use an AmpC-stable cephalosporin (ie cefepime or cefpirome) in the clavulanate synergy tests[40]. Cefepime-clavulanate gradient strips or combination discs and cefpirome-clavulanate combination discs are available. Once again, a >8-fold MIC reduction or >5mm zone expansion indicates a positive ESBL result
- cephalosporins are in any case not recommended as therapy for infections due to AmpC-inducible species, owing to the risk of selecting AmpC-derepressed mutants, with consequent failure[41]
- ESBL tests have poor sensitivity (but good specificity) for *Enterobacter* species even if using cefepime or cefpirome, especially if AmpC is concurrently hyperproduced. Some producers are only revealed by molecular testing

4.4 Distinguishing ESBLs from carbapenemases

The presence of ESBLs may also be masked by carbapenemases such as MBLs or KPCs (but not OXA-48-like enzymes) and/or severe permeability defects (refer to UK SMI B 60 -Screening and detection of bacteria with carbapenem-hydrolysing β-lactamases (carbapenemases)). The epidemiological importance of ESBLs in these contexts could be questioned, since the carbapenemase has greater public health importance, but if detection is still considered relevant it is recommended that molecular methods for ESBL detection are used.

4.5 Distinguishing ESBLs from K1 enzyme

Around 10-20% of *K. oxytoca* isolates hyperproduce their class A "K1"chromosomal β-lactamase. These are resistant to cefpodoxime, aztreonam and piperacillin-tazobactam, but not ceftazidime[5].

- they may give weak positive clavulanate synergy tests with cefotaxime or cefepime (not ceftazidime), leading to confusion with ESBL producers[42]. K1 hyperproduction should be suspected if a *Klebsiella* isolate is indole-positive and has high-level resistance to piperacillin/tazobactam, cefuroxime and aztreonam - but only borderline resistance or susceptibility to cefotaxime and full susceptibility to ceftazidime.

5 Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective media in screening procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen containers[43,44]

UK SMIs use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".

Quality control

The discs that are used should be quality control tested using disc diffusion methods and quality control strains as described in the BSAC or EUCAST or CLSI guideline documents. Follow guidelines for frequency of disc quality control testing and corrective action if results are out of range.

Chromogenic media

Chromogenic media are affected by light and plates should be stored in the dark and not left in the light before or after inoculation. Incubation times for chromogenic media should be as recommended by the manufacturers.

6 Safety considerations[43-59]

6.1 Specimen collection, transport and storage[43-48]

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Collect swabs into appropriate transport medium and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

6.2 Specimen processing[43-59]

Containment Level 2 pathogens.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[51].

Refer to current guidance on the safe handling of all organisms documented in this UK SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

7 Specimen collection

7.1 Type of specimens

Screening specimens include stool, rectal or peri-rectal cultures. Clinical specimens include blood, wounds or urine.

7.2 Optimal time and method of collection[60]

For safety considerations refer to Section 6.1.

Collect specimens before starting antimicrobial therapy where possible[60].

Unless otherwise stated, swabs for bacterial culture should be placed in appropriate transport medium[61-65]

Collect specimens into appropriate CE marked leak proof containers and place in sealed plastic bags.

7.3 Adequate quantity and appropriate number of specimens[60]

There should be visible faecal material on the rectal or peri-rectal swabs taken.

Numbers and frequency of specimen collection are dependent on the clinical condition of patient or for screening specimens, on local policies and practices.

8 Specimen transport and storage[43,44]

8.1 Optimal transport and storage conditions

For safety considerations refer to Section 6.1.

Specimens should be transported and processed as soon as possible[60].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[60].

9 Specimen processing/procedure[43,44]

9.1 Test selection

N/A

9.2 Appearance

N/A

9.3 Sample preparation

For safety considerations refer to Section 6.2.

9.4 Microscopy

9.4.1 Standard

N/A

9.4.2 Supplementary / preparation of smears

N/A

9.5 Culture and investigation

Direct culture

Inoculate culture media with swab or other sample (refer to UK SMI Q 5 – Inoculation of culture media in bacteriology).

Enrichment culture

Remove the cap aseptically from the container and place the swab(s) in the broth, break off (or cut) the swab-stick(s) and replace the cap.

9.5.1 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Clinical samples submitted for diagnostic culture and susceptibility testing: Any condition + detection of ESBL-producing Enterobacteriaceae	Any sample	Process as requested in accordance with the relevant SOPS. Include the indicator antimicrobials: screening indicator drugs as per standardised method used and if using EUCAST, refer to Table 3 for the EUCAST cut off values on any Enterobacteriaceae isolated	35-37	Aerobic	18-24hr	≥18hr	ESBL producing Enterobacteriaceae
Screening: Screening test for ESBL-producing Enterobacteriaceae	Screening specimens – Stool, Rectal or Peri-rectal swabs	Chromogenic agar using 30µg cefotaxime and ceftazidime 30µg (or 10µg cefpodoxime only)[66] OR alternatively, MacConkey[67]/ CLED agar + 30µg cefotaxime and 30µg ceftazidime (or 10µg cefpodoxime only)	35-37	Aerobic	18-24hr	≥18hr	ESBL producing Enterobacteriaceae most especially *Klebsiella* species *Escherichia coli*
Other organisms for consideration – ESBLs are harder to detect in species of Enterobacteriaceae with inducible, chromosomal AmpC enzymes (eg *Enterobacter*, *Citrobacter freundii*, *Morganella morganii*, *Providencia* and *Serratia*) but some confirmatory tests (cefpirome/clavulanate or cefepime/clavulanate) can be used for identification of these. Refer to the Introduction.							

9.6 Identification

Refer to individual UK SMIs for organism identification.

9.6.1 Minimum level of identification in the laboratory

Klebsiella species *Escherichia* species *Enterobacter* species *Citrobacter* species	species level UK SMI ID 16 - Identification of Enterobacteriaceae
Pseudomonas species *Acinetobacter* species *Stenotrophomonas maltophilia*	species level UK SMI ID 17 - Identification of Pseudomonas species and other non-glucose fermenters **Note:** The methods described herein are not suitable for detecting ESBLs in *Acinetobacter* species, which are often susceptible to clavulanic acid and so may yield a false ESBL-positive result. Ceftazidime-clavulanate synergy may be used to indicate ESBL production (usually VEB or PER enzymes) in isolates of *Pseudomonas* species, but this is uncommon in the genus and should not be routinely sought.

Organisms may be further identified if this is clinically or epidemiologically indicated.

9.7 Antimicrobial susceptibility testing

Testing cultured bacterial isolates

The recommended methods for detecting Enterobacteriaceae for ESBL production in routine samples are broth dilution, agar dilution, disc diffusion or an automated system.

The indicator drugs should be included in primary susceptibility testing done eg by the method of the British Society for Antimicrobial Chemotherapy (http://bsac.org.uk/susceptibility/methodologylatestversion/)[68,69]. Refer to the European Committee on Antimicrobial Susceptibility Testing (EUCAST)/ British Society for Antimicrobial Chemotherapy (BSAC), or Clinical and Laboratory Standards Institute (CLSI) guidelines. Species identification is highly desirable to allow proper interpretation of results. BSAC recommended breakpoints for the cephalosporins advocated are updated annually and should be sought from the link above.

Table 3: ESBL detection criteria for Enterobacteriaceae[25]

Method	Antibiotic	Perform ESBL-testing if
Broth or agar dilution[1]	Cefotaxime/ceftriaxone AND Ceftazidime	MIC >1 mg/L for either agent
	Cefpodoxime	MIC >1 mg/L
Disc diffusion[1]	Cefotaxime (5µg) / Ceftriaxone (30µg) AND Ceftazidime (10µg)	Inhibition zone < 21 mm Inhibition zone < 23 mm Inhibition zone < 22 mm
	Cefpodoxime (10µg)	Inhibition zone < 21 mm

[1] With all methods either test cefotaxime or ceftriaxone AND ceftazidime OR cefpodoxime can be tested alone.

Note: It should be noted that the inhibition zone sizes in Table 3 apply only when the standardised methodology (EUCAST/ BSAC or CLSI) is used and not on MacConkey/CLED agar plates.

Direct testing of clinical or screening samples with indicator discs

In clinical or screening samples inoculated directly on agar plates with cephalosporin indicator discs, any isolates of presumptive Enterobacteriaceae with a zone size of within 20mm should be identified and submitted for formal susceptibility testing in accordance with EUCAST/ BSAC, or CLSI methodology[25,68].

Confirmatory Tests for ESBLs: inhibitor-based tests

Enterobacteriaceae isolates resistant to any indicator cephalosporin, but susceptible to all carbapenems in the screening tests above, should be subjected to confirmatory tests. These depend on demonstrating synergy between clavulanate and the indicator cephalosporin(s) to which the isolate was found resistant.

Table 4: ESBL confirmation methods for Enterobacteriaceae that are positive in the ESBL screening[25]

Method	Antimicrobial agent (disc content)	ESBL confirmation is positive if
ESBL gradient test	Cefotaxime +/- clavulanic acid	MIC ratio ≥ 8 or deformed ellipse Present
	Ceftazidime +/- clavulanic acid	MIC ratio ≥ 8 or deformed ellipse Present
Combination disc diffusion test	Cefotaxime (30 µg) +/- clavulanic acid (10 µg)	≥ 5 mm increase in inhibition zone
	Ceftazidime (30 µg) +/- clavulanic acid (10 µg)	≥ 5 mm increase in inhibition zone
Double disc synergy test	Cefotaxime, ceftazidime and cefepime	Expansion of indicator cephalosporin inhibition zone towards amoxicillin-clavulanic acid disc
Other organisms for consideration – ESBLs are harder to detect in species of Enterobacteriaceae with inducible, chromosomal AmpC enzymes (eg *Enterobacter*, *Citrobacter freundii*, *Morganella morganii*, *Providencia* and *Serratia*) but some confirmatory tests (cefpirome/clavulanate or cefepime/clavulanate) can be used for identification of these. Refer to the Introduction.		

9.8 Referral for outbreak investigations

In England, the AMRHAI Reference Unit at UKHSA Colindale does not seek to confirm all ESBL producers, but the following should be submitted:

- representative isolates from major outbreaks
- representative isolates from unusual settings, eg neonatal units, especially if multiple cases occur
- isolates giving concerns based on a patient's history (contact laboratory to discuss)

9.9 Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit
Bacteriology Reference Department
National Infections Service
UK Health Security Agency
61 Colindale Avenue
London
NW9 5EQ

https://www.gov.uk/amrhai-reference-unit-reference-and-diagnostic-services

Telephone: +44 (0) 208 3276511/ 7877

Contact UKHSA's main switchboard: Tel. +44 (0) 20 8200 4400

England

Wales

Scotland

Northern Ireland

10 Reporting procedure

10.1 Microscopy

N/A

10.1.1 Microscopy reporting time

N/A

10.2 Culture

Screening samples

Negatives

"ESBL-producing Enterobacteriaceae not isolated"

Positives

"ESBL-producing Enterobacteriaceae (insert genus and species identification) isolated" eg ESBL-producing *Klebsiella pneumoniae* isolated

10.2.1 Culture reporting time

Interim or preliminary results should be issued on detection of potentially clinically significant isolates as soon as growth is detected, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

Final written or computer generated reports should follow preliminary and verbal reports as soon as possible.

10.3 Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated (noting the caveats below). Prudent use of antimicrobials according to local and national protocols is recommended.

10.3.1 Cephalosporins

There is a division of opinion about the reporting of cephalosporin susceptibility for ESBL producers. For several years it was considered, by BSAC/EUCAST and CLSI and based on clinical experience that all ESBL producers should be reported as resistant to all cephalosporins and aztreonam, irrespective of susceptibility test results.

Latterly, EUCAST and CLSI have taken the contrary view, arguing that, with the low breakpoints now adopted by both organisations, cephalosporin susceptibility results can be taken at face value, and that cephalosporins can be used as therapy so long as ESBL producers appear susceptible *in vitro*[70]. This view is based upon pharmacodynamic analysis, animal studies and on several reports of positive treatment outcomes when MICs were 1-2mg/L.

However, this revised view is challenged on the grounds (i) that the evidence of predictable clinical success for cephalosporins against low-MIC ESBL producers is far from overwhelming, with cephalosporin failures also reported *vs.* low-MIC ESBL-

positive strains, and (ii) 'susceptible' MIC and zone test results for ESBL producers often have poor reproducibility[37].

In the face of this disagreement, the best advice is to apply utmost caution if cephalosporins are to be used in severe infections due to ESBL producers.

It should also be added that the great majority of ESBL producers in the UK are clearly resistant to all oxyimino-cephalosporins at BSAC-EUCAST breakpoints and that this debate relates only to a minority of isolates (this situation is different in countries where producers of CTX-M-2 and -14 dominate, as MICs of ceftazidime for these often are 2-4mg/L).

Combinations of a cephalosporin with co-amoxiclav should be effective in principle, but have not been formally evaluated and may be antagonistic against some ESBL-negative *Enterobacter* species[71].

10.3.2 Penicillins and penicillin-inhibitor combinations

Organisms with ESBLs are resistant to all parenteral penicillins except temocillin, which is stable and generally active. Mecillinam may appear active *in vitro*, but its efficacy remains unproven, with anecdotal reports of failures as well as one positive case series[72,73].

Susceptibility to β-lactamase inhibitor combinations varies with the isolate. ESBLs are inhibited by tazobactam and clavulanate but many isolates with CTX-M-15 (the commonest ESBL in the UK) also have OXA-1, an inhibitor-resistant penicillinase, conferring resistance.

A recent analysis showed that inhibitor combinations can be used against ESBL producers when these appear susceptible *in vitro*[74].

10.3.3 Carbapenems

Carbapenems (imipenem, ertapenem, meropenem and doripenem) are stable to ESBLs and remain active against ESBL producers unless the organism

- also loses porins, reducing permeability - a mechanism that particularly compromises ertapenem or
- acquires DNA encoding a carbapenemase[75]. For further information, refer to UK SMI B 60 – Detection of bacteria with carbapenem-hydrolysing β-lactamases (carbapenemases)

11 Notification to UKHSA [76,77,] or equivalent in the devolved administrations[78-81]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify UK Health Security Agency (UKHSA) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local UKHSA Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to UKHSA. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to UKHSA and many UKHSA Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under ‘Notification Duties of Registered Medical Practitioners’: it is not noted under ‘Notification Duties of Diagnostic Laboratories’.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[78,79], Wales[80] and Northern Ireland[81].

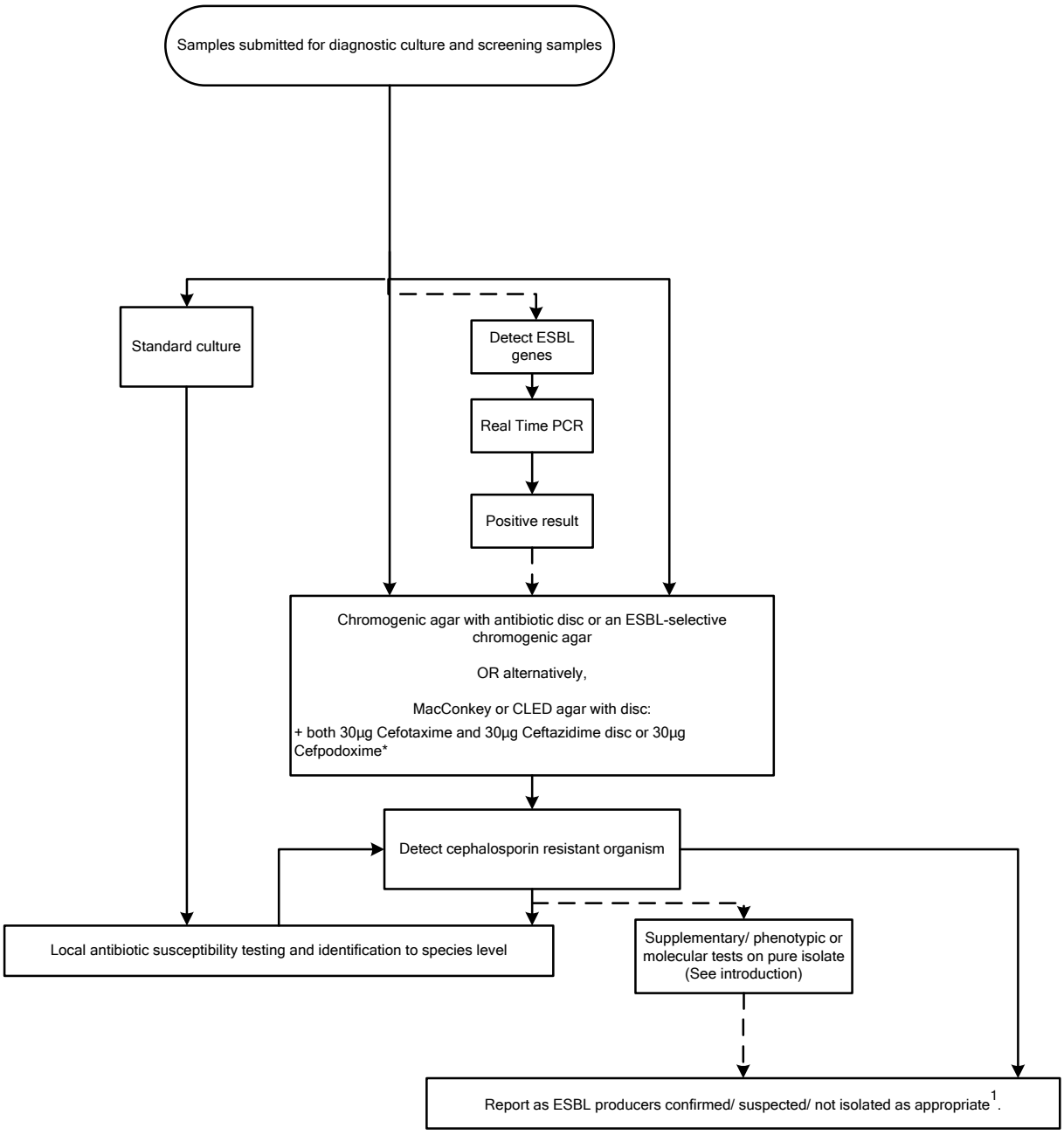
12 Public health responsibilities of diagnostic laboratories

Diagnostic laboratories have public health responsibility as part of their duties. Amongst these are additional local testing, or referral to further characterise the organism as required, primarily for public health purposes e.g. routine cryptosporidium detection; serotyping or microbial subtyping; and a duty to refer appropriate specimens and isolates of public health importance to a reference laboratory.

Diagnostic laboratory outputs inform public health intervention, and surveillance data is required to develop policy and guidance forming an essential component of healthcare. It is recognised that additional testing and referral of samples may entail some costs that has to be borne by the laboratory but in certain jurisdictions these costs are covered centrally.

Diagnostic laboratories should be mindful of the impact of laboratory investigations on public health and consider requests from the reference laboratories for specimen referral or enhanced information.

Algorithm: Flowchart for the screening and detection of ESBLs[25]



[1] If concerned about a result based on a patient's history, send to the UKHSA reference laboratory for further testing.

Note: The branch with the dotted lines in this flowchart is optional but useful for diagnostic laboratories that have molecular methods available locally. For more information, see link: http://www.eucast.org/resistance_mechanisms/.

The flowchart is for guidance only.

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An explanation of the reference assessment used is available in the scientific information section on the UK SMI website.

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