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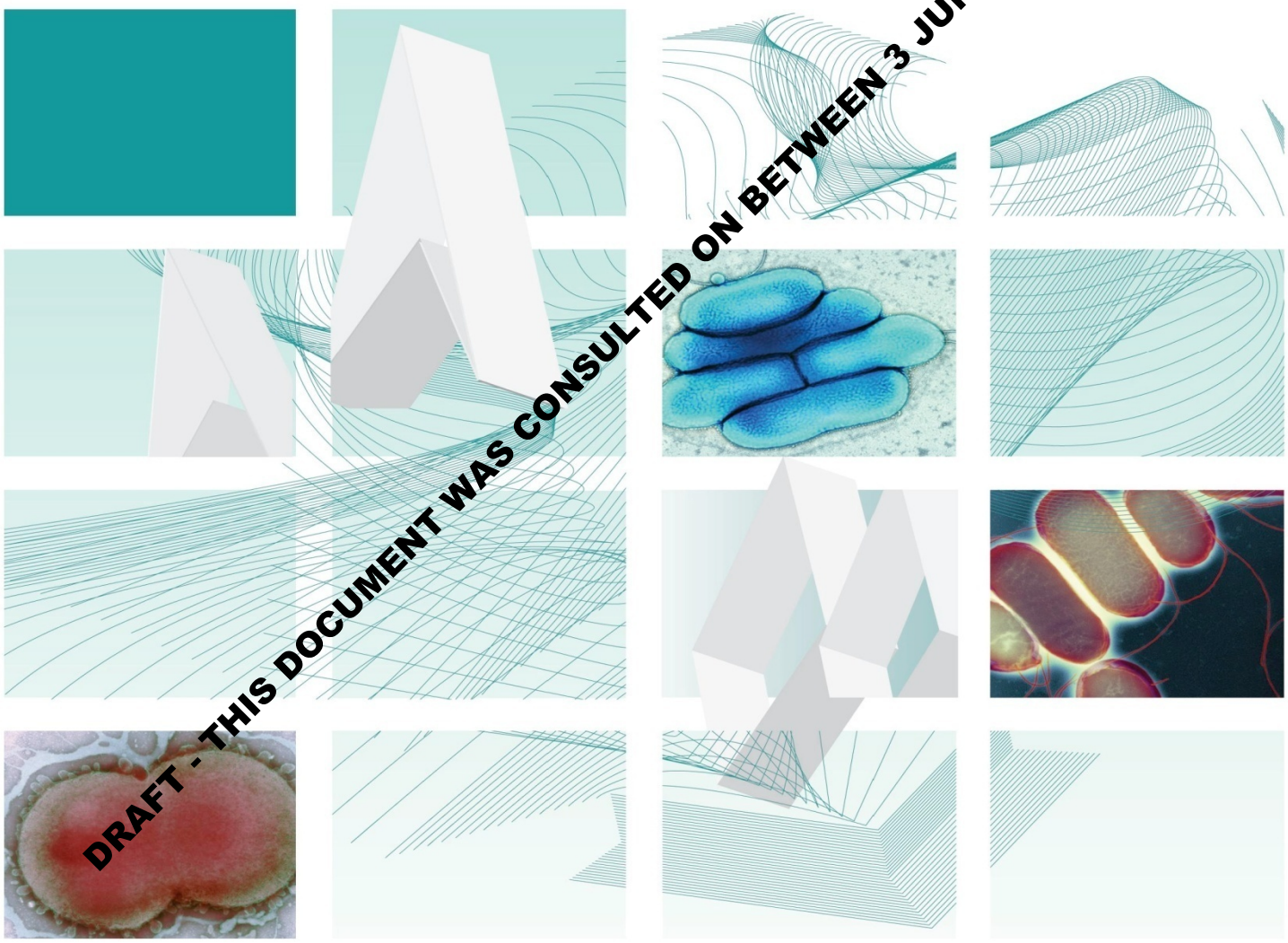
UK Standards for Microbiology Investigations

Detection of Enterobacteriaceae producing Extended Spectrum β-Lactamases



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Amendment table

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

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	5/dd.mm.yy <tab+enter>
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Section(s) involved	

Amendment No/Date.	4/20.09.12
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Section(s) involved	**Amendment**
Whole document.	B59 formerly P2 (previously QSOP51). Document presented in a bacteriology document format.
References.	References reviewed and updated.

UK SMI[#]: scope and purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post-analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs are subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

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The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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Scope of document

Type of specimen

Stool, rectal or peri-rectal swabs, clinical specimens such as blood, wounds or urine

Scope

This 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 BSOP 30.

This SMI should be used in conjunction with other SMIs.

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, but not cephamycins or carbapenems eg cefoxitin.

ESBLs include:

- cephalosporin-hydrolysing mutants of the TEM and SHV plasmid-mediated penicillinases of Enterobacteriaceae. These were the original ESBLs and over 200 such variants are known (see http://www.lahey.org/studies).
- CTX-M types. These evolved via the escape of chromosomal β-lactamase genes of *Kluyvera* species to plasmids. Over 100 variants are known, dividing into 5 major groups[1,2].
- minor types, eg VEB and PER [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:

- depressed 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.*
- metallo (IMP, VIM, NDM) and non-metallo (KPC and OXA-48) carbapenemases.

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.

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. 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.
- Pperform 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.

Laboratory detection: screening and confirmation

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 predicted 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

Clinical specimens are screened using MacConkey or CLED agar with the antibiotic disc. Chromogenic media have also been developed for detection of ESBL-producers[18-20], but these are likely to be less specific, particularly in areas where ESBL producers are commonplace and no advantage has yet been demonstrated in trials with clinical samples[19-24]. Some products include selective antimicrobial agents incorporated into the medium. Others will require placement of indicator cephalosporin discs.

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 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 cheap, 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 (30 µg). Note the expansion of the zones around the cefotaxime and ceftazidime discs adjacent to the co-amoxiclav (courtesy of Jenny Andrews).

- **Combination disc tests[26,27]**

  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 [27] or ≥ 50 %[28] in the presence of the clavulanate implying ESBL production. These tests are cheap and do not require critical disc spacing, but care should be taken regarding controls (see below) especially if the discs are from different batches.

- **Etest 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[29,30], automated or semi-automated systems generally can be used to detect ESBLs[31,32]. 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[33].

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[34]. 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[35,36]. 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 in 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[37]. 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[38].

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. Neither option is currently 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[38].

Controls for ESBL tests

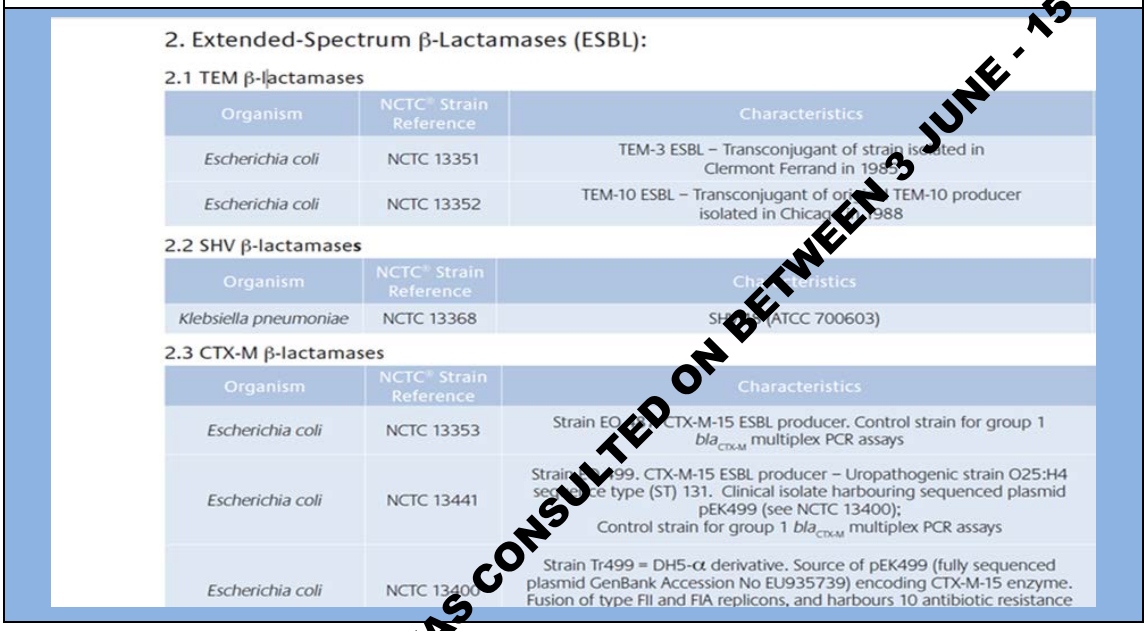
Quality Control of the cephalosporin discs used in the primary screening should follow standard BSAC, EUCAST or CLSI recommendations.

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 1988
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 EC958. CTX-M-15 ESBL producer. Control strain for group 1 bla_{CTX-M} multiplex PCR assays
Escherichia coli	NCTC 13441	Strain Tr499. 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 12418 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 retained potency. Zones of the cephalosporin and cephalosporin and clavulanate discs for ESBL-negative *E. coli* should be equal or within 2 mm. Any greater difference implies malfunction or deterioration.

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[39]. Cefepime-clavulanate Etests 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[40].
- 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.

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. 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.

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[41]. 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.

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[42,43]

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.

1 Safety considerations[42-58]

1.1 Specimen collection, transport and storage[42-47]

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.

1.2 Specimen processing[42-58]

Containment Level 2 pathogens.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[50].

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

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

2 Specimen collection

2.1 Type of specimens

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

2.2 Optimal time and method of collection[59]

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[59].

Unless otherwise stated, swabs for bacterial culture should be placed in appropriate transport medium[60-64].

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

2.3 Adequate quantity and appropriate number of specimens[59]

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

3 Specimen transport and storage[42,43]

3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[59].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[59].

4 Specimen processing/procedure[42,43]

4.1 Test selection

N/A

4.2 Appearance

N/A

4.3 Sample preparation

For safety considerations refer to Section 1.2.

4.4 Microscopy

4.4.1 Standard

N/A

4.4.2 Supplementary / preparation of smears

N/A

4.5 Culture and investigation

Direct culture

Inoculate culture media with swab or other sample (refer to 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.

4.5.1 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Clinical samples: Any condition + detection of ESBL-producing Enterobacteriaceae	Any sample	Process as requested in accordance with the relevant SOPS. Include the indicator antimicrobials: 30µg cefotaxime and 30µg ceftazidime (or 10µg cefpodoxime only)	35-37	Aerobic	18-24hr	≥18hr	ESBL producing Enterobacteriaceae
Screening: Screening test for ESBL-producing Enterobacteriaceae	Screening specimens – Stool, Rectal or Peri-rectal swabs	MacConkey agar [65] or CLED agar + 30µg cefotaxime and 30µg ceftazidime (or 10µg cefpodoxime only) OR Chromogenic agar using 30µg cefotaxime and ceftazidime 30µg (or 10µg cefpodoxime only) [66] as necessary	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.							

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Klebsiella species *Escherichia* species *Enterobacter* species *Citrobacter* species	species level ID 16 -Identification of enterobacteriaceae
Pseudomonas species *Acinetobacter* species	species level ID 17 -Identification of *Pseudomonas* species and other non-

Stenotrophomonas maltophilia	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.

4.7 Antimicrobial susceptibility testing

Screen with indicator

The recommended methods for screening Enterobacteriaceae for ESBL production are broth dilution, agar dilution, disc diffusion or an automated system. 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.

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/)[67,68]. Refer to British Society for Antimicrobial Chemotherapy (BSAC), the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 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 screening methods for Enterobacteriaceae [25]

Method	Antibiotic	Perform ESBL-testing if
Broth or agar dilution	Cefotaxime/ceftriaxone AND Ceftazidime	MIC >1 mg/L for either agent
	Cefpodoxime	MIC >1 mg/L
Disc diffusion [1]	Cefotaxime (5µg) **OR** Ceftriaxone (30µg) AND Ceftazidime (10µg)	Inhibition zone < 21 mm Inhibition zone < 23 mm Inhibition zone < 22 mm
	Cefpdoxime (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 (BSAC, EUCAST or CLSI) is used and not on MaConkey/CLED agar plates.

Screening of samples with indicator discs

In clinical or screening samples inoculated on MacConkey or CLED agar 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 BSAC, EUCAST or CLSI methodology.

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.		

4.8 Referral for outbreak investigations

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

- representatives 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)

4.9 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 Reference Unit (AMRHAI)
Bacteriology Reference Department
Microbiology Services Division
Public Health England
61 Colindale Avenue
London
NW9 5EQ

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

Telephone: +44 (0) 208 3276511/ 7877

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

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/rmlab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting procedure

5.1 Microscopy

N/A

5.2 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

5.2.1 Culture reporting time

Clinically urgent culture results to be telephoned or sent electronically when available. Written report within 72hr stating, if appropriate, that a further report will be issued.

5.3 Antimicrobial susceptibility testing

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

5.3.1 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*[69]. 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[36] 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.

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 cephalosporin with co-amoxiclav should be effective in principle, but have not been formally evaluated and may be antagonistic *vs. Enterobacter* species[70].

5.3.2 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.

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* [71].

5.3.3 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 [72]. For further information, refer to B 60: Screening and detection of bacteria with Ccarbapenem-hydrolysing β-lactamases (carbapenemases)

6 Notification to PHE[73,74] or equivalent in the devolved administrations[75-78]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) 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 PHE 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.

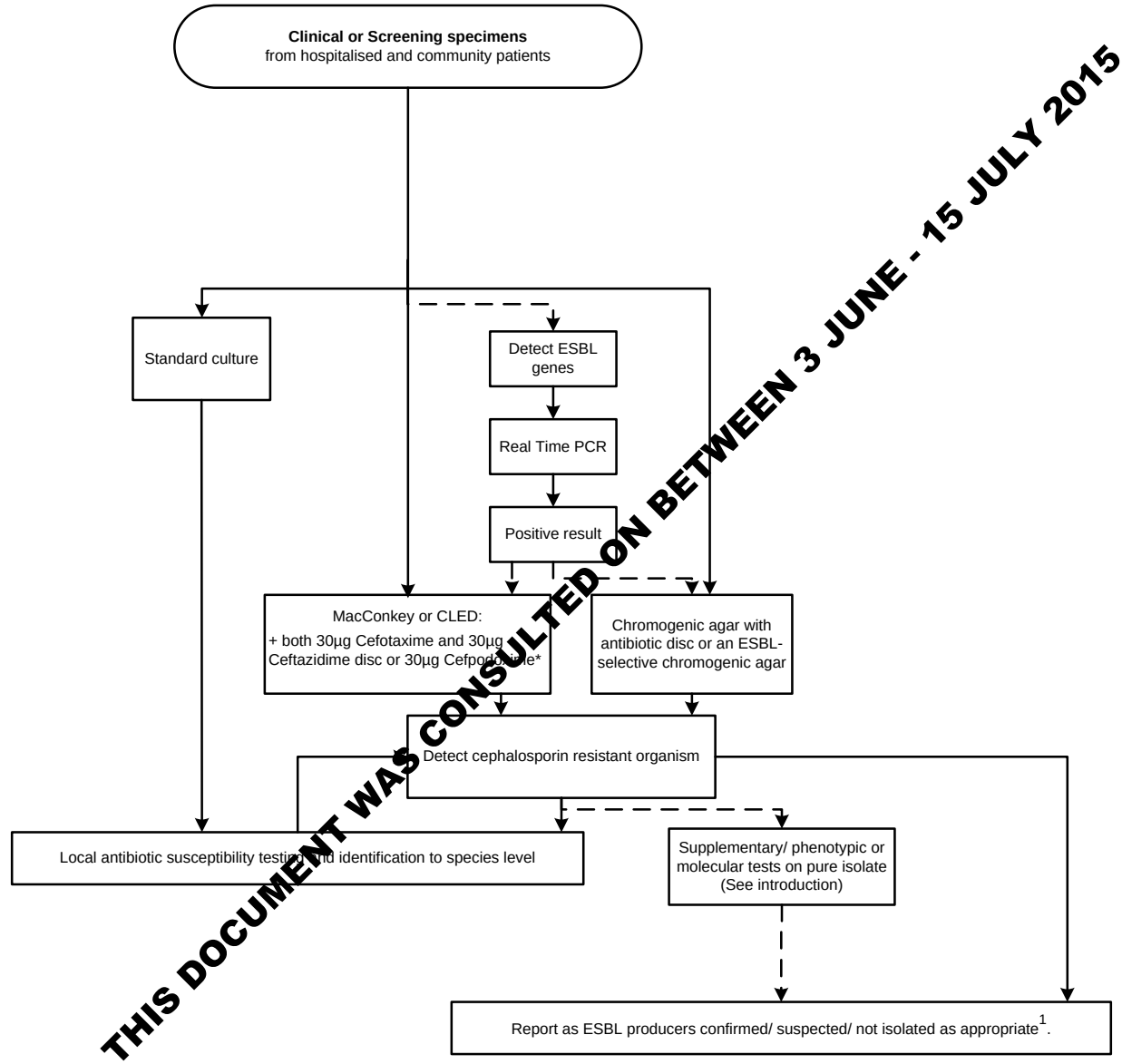
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local 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[75,76], Wales[77] and Northern Ireland[78].

Appendix: Flowchart for the screening and detection of ESBLs



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

Note: The branch with the dotted lines in this flowchart is optional but useful for diagnostic laboratories that have advanced into using molecular methods.

The flowchart is for guidance only.

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