Scope of the document: This document gives recommendations on laboratory methods of screening for colonisation with ESBL (ESBL $_A$) and plasmid-mediated AmpC (pAmpC/ESBL$_M$) producing *Enterobacterales (EPE).* Indications for screening, the number and interval of screening samples, and the consequences of a positive or negative result are beyond the scope of this document and should be determined by local or national infection control policies. Screening for EPE should always include a rectal swab or fecal sample. Other relevant sites (e.g. urine and wounds) may also be sampled, see national or local guidelines for recommendations.
Note that the term Enterobacterales, including the Enterobacteriaceae family, is used in this document (1).

Background
ESBL and pAmpC are beta-lactamases that hydrolyze penicillins and most cephalosporins without affecting carbapenems. The substrate profile of ESBL enzymes includes penicillins, monobactams, and most extended-spectrum cephalosporins (i.e. 3rd and later generations), except cefamycins. pAmpC enzymes have a similar substrate profile, with the exceptions that they do not hydrolyze 4th generation cephalosporins, but hydrolyze cefamycins (cefoxitin and cefotetan). It should be noted that the level of resistance to extended-spectrum cephalosporins varies depending on the enzyme, co-production of multiple beta-lactamases, and other resistance mechanisms. Thus, the MICs span from intermediate susceptibility to high-level resistance.
ESBL and pAmpC enzymes have been identified in several Gram-negative species. This document focuses on *Enterobacterales* with acquired, usually plasmid-mediated, ESBL and pAmpC genes, referred to as EPE. The main acquired ESBL and pAmpC enzyme families includes CTX-M, SHV- and TEM-variants, and CMY and DHA, respectively.
Non-susceptibility to extended-spectrum cephalosporins in *Enterobacterales* may also be due to non-transferable mechanisms such as production of chromosomal class A beta-lactamases or chromosomal AmpC enzymes, and/or changes in the permeability of the outer membrane of the bacterial cell wall. Clinically important species with chromosomal class A beta-lactamases (conferring resistance to e.g. cefotaxime, but not ceftazidime) include e.g. *Klebsiella oxytoca, Proteus vulgaris,* and *Citrobacter koseri*, whereas species with chromosomal AmpC include e.g. *Escherichia coli, Enterobacter* spp., *C. freundii,* and *Morganella morganii* (*Proteus* spp., *Klebsiella* spp. and *Salmonella* spp. do not have chromosomal AmpC genes). Note that strains with non-transferable mechanisms are often considered less important from an infection control perspective than those with acquired mechanisms.
With respect to clinical infections and infection control, the most important ESBL and/or pAmpC host species are *E. coli, K. pneumoniae*, *P. mirabilis*, and *E. cloacae*. Infections caused by ESBL-producing *E. coli* are often community-acquired, and in Nordic countries, colonisation of healthy people frequently occurs during travel (2). ESBL-producing *K. pneumoniae* are a major cause of hospital-acquired infections and hospital outbreaks all over the world, including in the Nordic countries (3, 4). Outbreaks caused by pAmpC-producing Enterobacterales have also been reported (5, 6).

Laboratory screening strategy
In the current epidemiological situation in the Nordic countries (7-10), culture-based EPE screening is recommended, and should at least cover ESBL-producing *Enterobacterales*. Carriage of pAmpC-producing *bacteria* is considered less common (11), and screening for pAmpC is complicated by chromosomal AmpC production in many species. Each laboratory must therefore decide whether they include screening for pAmpC in their EPE screening strategy. See also the flowchart in Figure 1 below.
For surveillance purposes, detection of EPE by culture-based methods may be confirmed by molecular methods. There is insufficient evidence to rely on direct molecular EPE-screening alone in a non-outbreak situation in the Nordic setting. However, direct molecular screening may be useful when rapid results are required, such as during an outbreak.

Description of the methods
Culture-based screening
Selective culture on commercial antibiotic-containing chromogenic media is currently the most convenient and most used method for EPE screening. Selective culture using in-house antibiotic-containing media or antibiotic discs on non-antibiotic media may also be used.
Several studies have shown that pre-inoculation of the screening sample in enrichment broth (with or without antibiotic supplement) overnight increase sensitivity (12-14). However, specificity may be reduced and thus the workload increased, and it will take an extra day to report results, unless direct culturing is performed in parallel.
When choosing media and methods for EPE screening by culture the following points should be considered:

- The differences between ESBL and pAmpC enzymes and their abilities to hydrolyse 4 th generation cephalosporins and cefamycins makes screening challenging, particularly with regards to finding a single media to detect all types of ESBL and pAmpC enzymes reliably.
- Most commercial EPE media are supplemented with AmpC-inhibitors, thus most strains producing pAmpC will be inhibited and not detected.
- Strains producing chromosomal AmpC enzymes (e.g. *Enterobacter* spp., *Serratia* spp., *C. freundii*, and *M. morganii*) may occasionally grow on ESBL media despite AmpC-inhibitors, and reduce specificity.
- More recently, commercial media without AmpC-inhibitors have become available, enabling detection of both ESBL and pAmpC using one media. However, strains producing chromosomal AmpC enzymes will also grow on combined ESBL/pAmpC media resulting in reduced specificity, increased workload, and possibly delayed reporting of result as confirmation of pAmpC will require molecular confirmation.
- Strains with reduced susceptibility to extended-spectrum cephalosporins due to mechanisms such as production of chromosomal class A beta-lactamases and changes in the permeability of the outer membrane of the bacterial cell wall, may also grow on ESBL and/or pAmpC media.
- EPE strains with low MICs to extended-spectrum cephalosporins may not grow on all media depending on the MIC and the

antibiotic concentration in the media.

- Be aware that most carbapenemase producing Enterobacteriales (CPE), except those producing OXA-48-like enzymes only, will grow on EPE-media as well. However, selective CPE media are recommended for detection of CPE. See also the NordicAST methods document for CPE-screening.

When using selective media, growth of enteric bacteria on simultaneously inoculated non-selective agar may help validate a negative screening result, i.e. no growth of enteric bacteria may suggest the absence of fecal material and therefore an invalid sample.

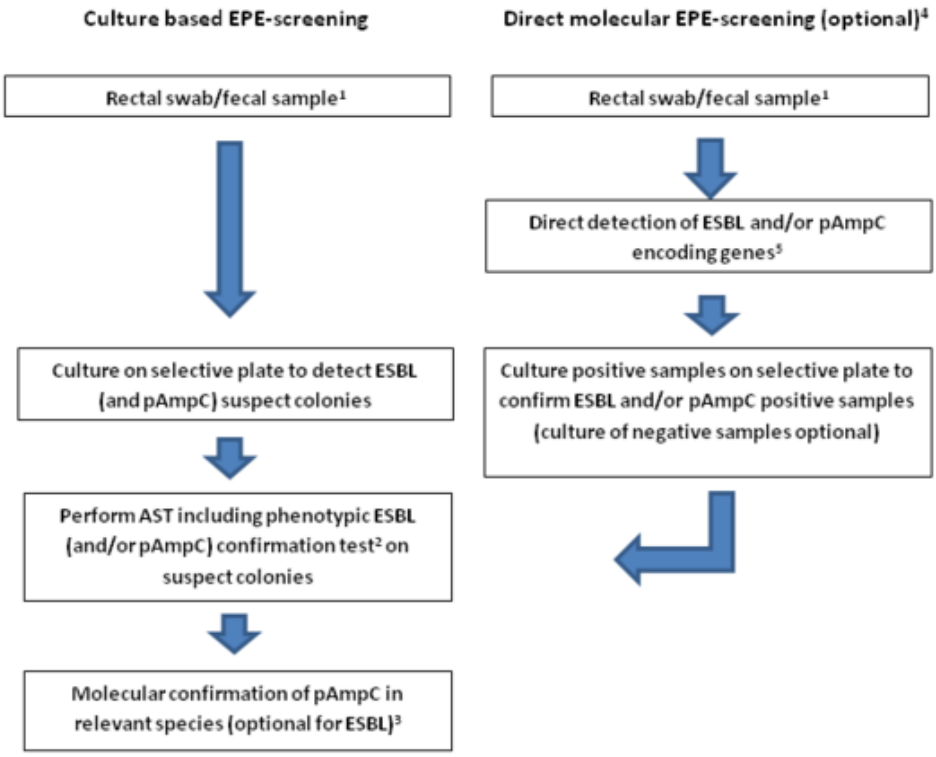
Confirmatory tests on suspected EPE colonies

Species identification and antimicrobial susceptibility testing (by disc diffusion or an MIC method) should always be performed to confirm suspected EPE colonies. Decreased susceptibility to extended-spectrum cephalosporins should be confirmed, and a phenotypic confirmation test, i.e. clavulanic acid and cloxacillin synergy, for ESBL and pAmpC (in species lacking chromosomal AmpC), respectively, should be performed. Note that an AmpC phenotype may either be due to pAmpC or chromosomal AmpC production, but phenotypical tests cannot differentiate between these mechanisms. Thus, to confirm the presence of pAmpC, a molecular test is required in species which also have chromosomal AmpC (15). The presence of ESBL encoding genes may also be confirmed by molecular analysis, however this is not required in routine clinical work as the phenotypic confirmation test is considered highly accurate.
See also the NordicAST methods document for detection of ESBL-A/M/CARBA in Enterobacteriales and the EUCAST guidelines for detection of resistance mechanisms.

Direct molecular screening

Molecular methods can also be used to detect EPE directly in screening samples (16). Molecular tests have a shorter turnaround time and are useful where rapid results are required and the target gene is known, such as in an outbreak setting.
If direct molecular screening is used in the routine setting, the test should cover the clinically most important ESBL encoding genes *bla*$_{CTX-M}$ group 1 and 9, and the pAmpC genes *bla*$_{CMY}$ and *bla*$_{DHA}$ might also be included. The most important limitations of direct molecular tests for routine screening are that they will not identify rare or new ESBL-genes, or ESBL-genes not included in the assay, neither do they provide information on the host species. Thus, they should always be used in combination with culture-based methods and the final decision of carriage status should be based on isolation of an EPE strain.

Figure 1: Overview of recommended EPE screening strategy in the Nordic setting.



1 EPE-screening must include a rectal swab or fecal sample. Other relevant sites may also be sampled.
2 According to EUCAST/NordicAST reccomendations.
3 The molecular assay should include the pAmpC genes blaCMY and blaDHA (and the ESBL genes blaCTX-M group-1 and -9).
4 Direct molecular screening, as a supplement to culture based screening, may be useful when rapid results are required, such as during an outbreak.
5 A direct molecular assay should include the pAmpC genes blaCMY and blaDHA, and the ESBL genes blaCTX-M group-1 and -9, and/or the relevant target gene in an outbreak situation.

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Changes

Version	Changes
2018 -	New document