SCREENING FOR EXTENDED-SPECTRUM BETA-LACTAMASE PRODUCTION

INTRODUCTION

The European Antimicrobial Resistance Surveillance System (EARSS) is expanding its scope to include surveillance for antimicrobial resistance in *Escherichia coli* from invasive infections. EARSS considers it desirable to specifically include surveillance for the phenomenon of extended-spectrum β-lactamase (ESBL)-mediated resistance. This document is intended to provide background information for laboratories participating in the programme of *E. coli* surveillance including a suggested approach to surveillance for ESBLs.

BACKGROUND

Production of β-lactamase enzymes is the most common mechanism of resistance to β-lactam antimicrobial agents in Gram-negative bacilli including *E. coli*.

The term extended-spectrum β-lactamase is generally applied to plasmid-encoded β-lactamases that (1) are capable of inactivating extended-spectrum cephalosporins and (2) are inhibited by β-lactamase inhibitors, such as clavulanic acid. These enzymes are generally derived from the common plasmid-encoded enzymes (TEM-1, TEM-2 and SHV-1) that specify resistance to ampicillin. The ability of ESBLs to inactivate a wider range of β-lactam antibiotics is related to one or more mutations in the gene encoding for common TEM and SHV enzymes.

In addition to any plasmid-encoded β-lactamases that may be present, many Enterobacteriaceae also have a chromosomal gene that encodes the production of a β-lactamase enzyme. These chromosomal AmpC β-lactamases are frequently not expressed (particularly in *E. coli*), but if expressed they confer resistance to ampicillin and most cephalosporins and are NOT inhibited by clavulanic acid and related β-lactamase inhibitors.

Following on from the above, phenotypic methods for the detection of ESBLs are based on the measurement of susceptibility to one or more cephalosporin agents both alone and

in the presence of clavulanic acid/a β-lactamase inhibitor. The approach proposed in this document is intended to fulfil the following requirements:

1. It is relatively easy to implement for laboratories not currently screening for ESBL production.
2. The materials are readily available, inexpensive and no specific equipment is required.
3. Interpretation is straightforward.
4. The method is highly sensitive for the detection of ESBL production in *E. coli* (the organism under surveillance) and also in *Klebsiella pneumoniae*, in which ESBLs are probably more common. The use of cefpodoxime is recommended in preference to cefotaxime or ceftazidime (as per NCCLS documents) because cefpodoxime appears to be the most sensitive single substrate for the detection of ESBLS.

PROPOSED METHOD

Ampicillin-susceptible *E. coli*

E. coli categorised as susceptible to ampicillin by any reasonable susceptibility test method are not ESBL producers and no further testing is required.

Ampicillin-resistant *E. coli*

1. Prepare a suspension in saline equal to a 0.5 McFarland standard.
2. Dip a swab into the solution and inoculate the surface of a Mueller Hinton agar plate in three directions to ensure even application of the inoculum. (As for all NCCLS susceptibility testing of *E. coli* Mueller Hinton agar plates with a depth of 3.5 to 4mm are recommended but it is likely that Isosensitest agar or Diagnostic Sensitivity Test agar will give comparable results).
3. Allow the surface of the plate to air-dry.
4. Within 15 minutes of inoculation, apply a 10µg cefpodoxime disc to one half of the plate and a cefpodoxime/clavulanic acid (10µg/1µg) disc to the other half (see figure 1). Discs available from Oxoid.
5. Incubate the plate at 35°C for 18 hours.
6. Measure the diameter of the zone of inhibition of growth around both discs.
7. **INTERPRETATION**: Subtract the diameter of the zone of inhibition around the cefpodoxime disc from the diameter of the zone of inhibition around the cefpodoxime/clavulanic acid disc. If the difference is greater than or equal to 5mm regard the isolates as ESBL-positive.
8. A reduced zone diameter to cefpodoxime with no enhancement by clavulanic acid may be due to the expression of AmpC β-lactamase or some other resistance mechanism.

Laboratories participating in EARSS are invited to submit presumptive ESBL-positive isolates to Dr Dearbhaile Morris, Department of Bacteriology, National University of Ireland, Galway for storage and subsequent further studies. The Department is also happy to receive any cephalosporin-resistant *E. coli* isolates from blood cultures or other *E. coli* with unusual antimicrobial resistance phenotypes.

A great many methods for screening for ESBL production have been proposed. Figure 1 illustrates the recommended NCCLS-style disc diffusion confirmatory test and, for information, figure 2 illustrates the widely used double disc diffusion method for screening for ESBL production.

Figure 1: NCCLS-style disc diffusion confirmatory test for ESBL production using cefpodoxime. The strain tested on this plate is the ESBL-positive control strain *Klebsiella pneumoniae* ATCC 700603. A ≥5mm increase in the zone of inhibition for the cefpodoxime/clavulanic acid-containing disc versus the zone for the disc containing cefpodoxime alone is considered confirmation of ESBL production.

Abbreviations: a, cefpodoxime (10μg); b, cefpodoxime/clavulanic acid (10μg/1μg)

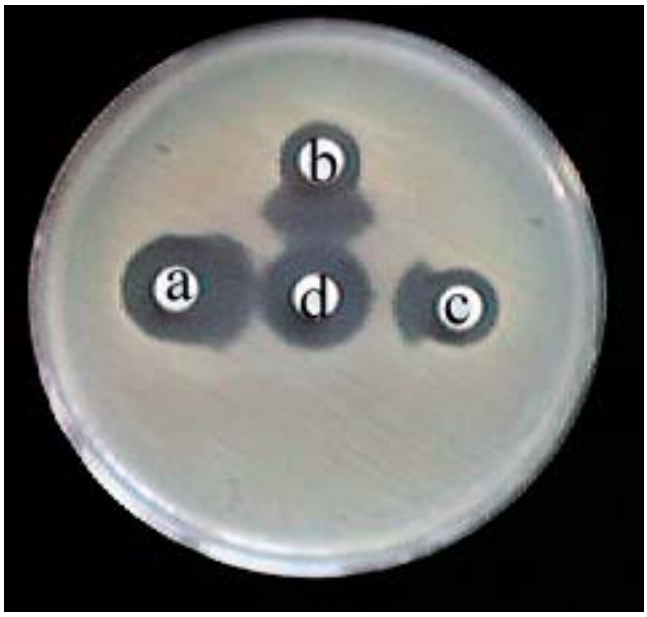

Figure 2: Double-disc diffusion test for the detection of ESBL production in *Klebsiella pneumoniae* ATCC 700603 (ESBL-positive control strain). Enhancement of the zone of inhibition around one or more of the β-lactam-containing discs towards the clavulanic acid-containing disc is indicative of ESBL production. Precise placement of discs is important (a distance of 15mm between the discs is recommended) and interpretation is subjective.

Abbreviations: a, ceftriaxone (30μg); b, aztreonam (30μg); c, cefpodoxime (10μg); d = amoxycillin/clavulanic acid (20μg/10μg)

For comments or discussion of this document or to obtain an isolate for use as control strain please contact Professor Martin Cormican at martin.cormican@bsi.ie