Detection of ESBL bacteria from clinical specimens: evaluation of a new selective medium

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ABSTRACT

Selective screening media for extended-spectrum β-lactamase (ESBL)-producing bacteria are needed to guide antibiotic therapy and institute appropriate infection control measures. This study evaluates a selective cefpodoxime-incorporated chromogenic agar (CCA) medium for the detection of ESBLs from clinical specimens. The medium was formulated specifically for this study. For all culture-positive urine samples and wound swabs from intensive care unit (ICU) patients, CCA was compared with standard laboratory testing procedures and HPA/BSAC guidance on ESBL detection. The CCA medium was also evaluated for ESBL faecal carriage from patients on ICU and the haematology ward. These patients had no prior evidence of colonisation or infection with ESBL-producing bacteria. All ESBL isolates underwent minimum inhibitory concentration (MIC) testing to cefpodoxime. The Miles and Misra method and the ecometric methods were used to quality control the microbiological performance of the CCA medium, which proved satisfactory. A total of 750 specimens were examined (690 urines, 40 faeces, 20 wound swabs). From urine cultures, 92 suspect colonies were followed up. Eighteen were cefpodoxime-resistant on routine disc testing and all were confirmed subsequently as ESBL-positive. Conventional laboratory methods identified only two urinary ESBLs. Wound cultures revealed two suspect colonies, both of which were ESBL-positive and were also detected by routine methods. Faecal samples produced 10 suspect colonies, six of which were ESBL-positive. All ESBLs had cefpodoxime MICs >10 mg /L (75% were >256 mg/L). Thus, primary conventional culture methods cannot be relied upon to detect suspect ESBL-producing bacteria.

KEY WORDS: Agar.
Chromogenic substrates.
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beta Lactamases.

Introduction

Extended-spectrum β-lactamases (ESBLs) are enzymes that mediate resistance to third-generation cephalosporins (i.e., ceftazidime, cefotaxime and ceftriaxone) and monobactams (e.g., aztreonam) but do not affect cephamycins (e.g., cefoxitin and cefotetan) or carbapenems (e.g., meropenem, imipenem or ertapenem). These enzymes most often are carried on multidrug-resistant plasmids and are capable of being transferred among different species of the family Enterobacteriaceae.[1–3]

Recent surveys confirm a worldwide increase in ESBL-producing Enterobacteriaceae. In the UK, there have been outbreaks in the community setting with CTX-M β-lactamase-producing strains of Enterobacteriaceae, as published by the Health Protection Agency in its report in September 2005. Poland and other European countries have also reported a similar spread of CTX-M-3 ESBL-producing Enterobacteriaceae.[3–5]

Reports from the USA and France have also documented the possibility of their emergence in long-term community care facilities (e.g., nursing homes or rehabilitation units), which may act as reservoirs for the entry of ESBL-producing organisms into acute-care hospitals.[6,7]

The global emergence of ESBL-producing Enterobacteriaceae highlights the need for suitable screening media to aid early identification of ESBL isolates in order that appropriate treatment and implementation of infection control precautions can be introduced rapidly.

Therefore, this study evaluates a new screening medium, which incorporates cefpodoxime as a selective agent in chromogenic agar (CCA medium, Oxoid). Some data are available on the use of antibiotic-incorporated chromogenic and non-chromogenic selective media. This study also aims to add to the limited data and compares CCA with standard laboratory testing procedures and Health Protection Agency/British Society for Antimicrobial Chemotherapy (HPA/BSAC) guidance on ESBL detection.

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Material and methods

Over a six-week study period (February and March 2006), all urine samples from community and hospitalised patients and wound swabs from critical care units at University Hospital Aintree (UHA) and the Walton Neurosurgery Centre were screened for ESBL-producing organisms using the CCA medium. These samples were also processed in parallel using the laboratory's standard operating procedures (SOPs). In addition, CCA was used to evaluate presumptive ESBL producers in faeces from the two

intensive care units (ICUs) and the haematology ward at University Hospital Aintree in patients not known to be colonised or infected with ESBL-producing Enterobacteriaceae.

Following consultation with the Antibiotic Resistance Monitoring Reference Laboratory, HPA, London, it was decided to incorporate 10 mg/L cefpodoxime in the chromogenic agar. This was based on the observation that the vast majority of referred ESBL producers have cefpodoxime minimum inhibitory concentration (MIC) values >10 mg/L. Oxoid specifically formulated the CCA medium for the purpose of this study, including the addition of the antibiotic supplement. The BSAC susceptibility testing methodology was used to perform antibiotic susceptibility tests.

All urine samples submitted to the laboratory were subjected to automated screening and all screen-positive samples were plated on non-selective chromogenic substrate-containing agar (CSA, BD Diagnostics). Antibiotic susceptibility testing (AST) was performed if, after overnight incubation on CSA, there was a pure growth of an isolate with a colony count $>10^8$ colony forming units (cfu)/L, regardless of white blood cell count (WBC), or a colony count of 10^{7-8} cfu/L with a WBC count >20 x10^6/L. Samples not fulfilling these criteria were reported as 'no significant growth' and were not processed further.

For the purposes of this study, all screen-positive urine samples (1 µL) were plated on one-quarter of a CCA plate. The plates were incubated at 35–37°C aerobically and examined the next day. All suspect colonies on CCA were followed up, regardless of absolute colony count.

Colony colour and morphology on CSA accurately differentiates *Escherichia coli* (rose coloured) from *Klebsiella/Enterobacter* spp. (metallic blue colour) and other Enterobacteriaceae. However, colonial morphology and colour does not distinguish *Klebsiella* spp. from *Enterobacter* spp.[8]

The CCA medium was also evaluated for identification of isolates of *E. coli* and *Klebsiella/Enterobacter* spp. on the basis of colony colour and morphology by plating wild strains of *E. coli, Klebsiella* spp. and *Enterobacter* spp. that were ESBL-positive, together with a control ESBL-positive *Klebsiella pneumoniae* (ATCC 700603). All colonies growing on the CCA were designated 'suspect colonies'.

All presumptive ESBL-producing isolates growing on CCA were plated on cysteine lysine electrolyte-deficient (CLED) agar to confirm a pure growth of the isolate, and AST by BSAC methodology was performed the next day using trimethoprim, nitrofurantoin, amoxicillin, amoxicillin plus clavulanic acid and cefalexin plus a 10 µg cefpodoxime disc for ESBL screening.

Cefpodoxime-resistant isolates (defined as a zone diameter of ≤25 mm) were confirmed for ESBL production using a combination disc method with cefpodoxime, ceftazidime and cefotaxime with/without clavulanic acid.[9] Positive and negative ESBL controls were used for quality control purposes with each batch of isolates tested.

Wound and faecal specimens from patients in ICUs and also faecal specimens from the haematology ward were plated on full CCA plates. These isolates were otherwise processed in exactly the same manner as the urinary isolates.

The Miles and Misra method and ecometric methods were used to quality control microbiological performance of the CCA medium.[10,11] Minimum inhibitory concentration tests of cefpodoxime on Mueller Hinton agar (MHA) were undertaken on all confirmed ESBL-positive isolates, ATCC ESBL-positive and -negative control strains and some cefpodoxime-sensitive 'suspect colonies' that had grown on CCA.

Results

Seven hundred and fifty specimens (690 urines, 20 wound swabs and 40 faecal samples) were examined. One hundred and four specimens yielded 'suspect colonies' (92 urines, two wound swabs and 10 faecal samples) on CCA, of which 27 (26%) were ESBL-positive. Overall, ESBL-positive isolates were obtained from 27/750 (3.6%) specimens after processing on CCA.

Six hundred and ninety urine samples screened on CCA yielded 92 'suspect colonies'. However, only 18/92 (19.5%) were resistant to cefpodoxime. Three isolates susceptible to the cefpodoxime disc but resistant to all other antibiotics used in the primary urine ring were subjected to ESBL confirmatory testing. One isolate confirmed as ESBL-positive was missed by the cefpodoxime disc screening test, while two isolates were ESBL-negative.

Seventeen (90%) ESBL producers were missed during laboratory SOP-based processing. Fifteen (79%) were considered of insignificant growth and not processed further, while two (11%) were missed on routine screening for ESBL.

Of the 20 wound specimens plated on the CCA, only two produced presumptive ESBL-producing isolates that cultured subsequently on CCA. These were also identified by routine laboratory SOP-based processing.

Ten presumptive ESBL isolates were obtained from the 40 faecal specimens plated on CCA. Only one was found to be susceptible on the cefpodoxime disc test. Six were confirmed as ESBL producers, while three were ESBL-negative but resistant to most antibiotics in the primary panel. Overall, only 29 (28%) isolates that grew on CCA were found to be resistant to cefpodoxime. Of these, 27 were confirmed as ESBL producers.

E. coli accounted for 15/27 (55%) of ESBL-positive isolates, while 12/27 (48%) were *Klebsiella/Enterobacter* spp. Twenty (74%) of the ESBL-confirmed isolates had cefpodoxime MICs >256 mg/L, while seven (26%) had MICs of 12–64 mg/L. The MICs for ESBL-positive and ESBL-negative ATCC control strains were 16 mg/L and 0.38 mg/L, respectively.

The ecometric method gave an absolute growth index (AGI) of 95 on CCA for ESBL-positive ATCC 700603 *K. pneumoniae*, while on chromogenic agar without cefpodoxime the AGI was 100. The relative growth index (RGI) of 95% indicates the efficiency of the medium for ESBL-positive isolates. There was no growth of *E. coli* (ATCC 25922) ESBL-negative control (AGI=0) on CCA, giving an RGI of 0%. This indicates that the medium was inhibitory to the *E. coli* ATCC 25922 ESBL-negative control.

Using the Miles and Misra method, the ESBL-positive control (*K. pneumoniae* ATCC 700603) exhibited confluent growth at a dilution of 10^{-5} on the CCA. However, at a dilution of 10^{-6} the mean of the six counts was 18. Similarly, the mean was 19 colonies for chromogenic agar without the cefpodoxime at a dilution of 10^{-6}. The ESBL-negative control

(*E. coli* ATCC25922) had a mean colony count of six on CCA at a dilution of 10^{-1}. In comparison, the mean colony count of six on chromogenic medium without cefpodoxime at a dilution of 10^{-6} was 15.

Discussion

Infections due to ESBL-producing organisms are no longer confined to hospitals as increasing global prevalence of these organisms is being reported in the community.[12–14] In view of this, a guidance note was issued by the Standards Unit, Evaluations and Standards Laboratory, Centre for Infections, in 2006, (QSOP 51, HPA) for the laboratory detection and reporting of bacteria with ESBLs. This stated that the spread of CTX-M enzymes into out-patient/community *E. coli* means that the indicator cephalosporin(s) should be a first-line test against all Enterobacteriaceae, or, if direct sensitivities are performed, on all clinical specimens likely to harbour ESBL producers.

The guidance, though very useful and helpful, is an unrealistic approach to diagnostic microbiology. Most laboratories do not screen all Enterobacteriaceae for ESBLs as it can prove to be quite labour-intensive; therefore, the true prevalence of ESBLs may be under-diagnosed.

This highlights the need for a selective medium, as is the case for methicillin-resistant *Staphylococcus aureus* (MRSA), which could be used reliably to screen for ESBLs directly from clinical specimens, thus improving primary isolation. This could in turn serve as a surveillance tool for patients from high-risk areas such as ICUs and haematology wards.

A recent newsletter issued by the Antibiotic Resistance Monitoring Reference Laboratory (HPA) has hinted that Mast Diagnostics plans to launch a colorimetric test, using a chromogenic cephalosporin in combination with ESBL inhibitors, and stresses the potential value of these tests for the identification of ESBL producers 24 hours after specimens are obtained from the patient. Currently, as far as is known, two commercial selective media – BLSE agar incorporating cefotaxime and ceftazidime (AES Chemunex), and ESBL–Bx (bioMérieux, Marcy I'Etoile), which was introduced late last year and uses cefpodoxime as the selective agent in chromogenic agar – are available for the presumptive isolation of ESBLs.

Relatively few studies have evaluated the role of selective media for screening ESBLs from clinical specimens.[15–17] These have used cefotaxime, ceftazidime and cefpodoxime as selective agents incorporated in either MacConkey agar or chromogenic agar. Glupczynski *et al*.[17] compared MacConkey agar supplemented with ceftazidime with ESBL–Bx (bioMérieux) and found that ESBL–Bx had an advantage in terms of its chromogenic character, its sensitivity (97.7%) and specificity (89%), compared to ceftazidime-incorporated agar. This agar reduced by 27% the need for unnecessary identification and confirmation of ESBLs, as all colourless colonies growing on the medium could be disregarded.

The CCA medium used in the present study performed well with faecal and wound specimens, although the numbers were small and so it is difficult to draw firm conclusions. Of the 10 presumptive ESBLs obtained from faeces, six were confirmed as ESBL-producing isolates. Three, all *Enterobacter/Klebsiella* spp., were resistant to almost all first-line antibiotics, indicating other mechanisms of resistance.

The quality control methods showed that the medium inhibited the growth of the ESBL-negative control strain (*E. coli* ATCC 25922) while supporting the growth of the ESBL-positive control strain (*K. pneumoniae* ATCC 700603). The result of the Miles and Misra test suggests that the medium may be affected by the strength of the inoculum. Non-ESBL-producing Enterobacteriaceae can grow on the medium if the colony count exceeds 10^6 or 10^7 cfu/mL.

The initial inoculum may be quite high and may therefore, despite sensitivity to cefpodoxime, overwhelm the antibiotic incorporated in the medium. This phenomenon could have resulted in the large number of 'suspect colonies' obtained from the urine samples. Thus, inoculum dependence raises doubts about the use of this medium as a screening tool for urinary specimens.

The CCA medium may have a role in screening for ESBL producers in wound and faecal specimens from high-risk areas or areas where broad-spectrum antibiotics are often used (e.g., critical care units, haematology wards and renal units). Identification of presumptive ESBL producers in 24 hours can have a substantial impact on the choice of empirical antibiotic therapy for critically ill patients, in whom a 42% higher mortality has been reported when ESBL-associated bacteraemia was treated with inappropriate antimicrobial therapy.[18] Duration of hospital stay and costs may be higher in patients infected with ESBL-producing organisms than with non-ESBL-producing organisms of the same species.[18]

Clearly, the CCA medium needs to be evaluated further, as the sample size in this initial study is small and firm conclusions cannot be drawn. On the other hand, the results obtained with urine samples have uncovered shortcomings that need to be addressed before CCA can be adopted routinely as a screening method. □

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