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Antimicrobial susceptibility studies

Evaluation of a new screen agar plate for detection and presumptive identification of Enterobacteriaceae producing extended-spectrum β-lactamases

Enno Stürenburg[a,*], Ingo Sobottka[a], Rainer Laufs[a], Dietrich Mack[a,b]

[a]*Institut für Infektionsmedizin, Zentrum für Klinisch-Theoretische Medizin, Universitätsklinikum Hamburg-Eppendorf, D-20246 Hamburg, Germany*
[b]*Department of Medical Microbiology and Infectious Diseases, The Clinical School, University of Wales Swansea, Swansea SA2 8PP, United Kingdom*

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Abstract

A new agar screen plate for extended-spectrum β-lactamase (ESBL) detection was evaluated with 50 clinical isolates of ESBL-producing Enterobacteriaceae species: *Enterobacter cloacae* ($n = 10$), *Escherichia coli* ($n = 10$), *Klebsiella oxytoca* ($n = 3$), *Klebsiella pneumoniae* ($n = 25$), and *Proteus mirabilis* ($n = 2$). Fecal samples were artificially inoculated with 2 concentrations (25 and 250 colony forming units [CFU]/plate) of the test strains and then applied to the new agar screen plates. By this approach, the new agar formula detected growth that was suggestive of ESBL activity in 44 of 50 (88%) and 50 of 50 (100%) of ESBL strains with 25 and 250 CFU/plate, respectively. A limitation of the agar screen plates was a lack of some specificity. Among 15 strains with resistant phenotypes other than ESBL (K1 producers of *K. oxytoca*, 6 strains; 9 strains with AmpC phenotype), growth was recorded in 7 (25 CFU/plate) and 11 (250 CFU/plate) of 15 strains. In conclusion, the new agar screen plate is a sensitive and convenient method to directly screen for ESBL organisms in rectal swabs or stool samples, with the potential for incorporation into routine clinical laboratory service.

Keywords: Extended-spectrum β-lactamase detection; Antimicrobial susceptibility testing; Agar screen plate

1. Introduction

Resistance caused by extended-spectrum β-lactamases (ESBLs) has accounted for a significant and ever-increasing proportion of hospital-acquired infections in recent years. Between 1998 and 2001, the incidence of ESBLs in Germany increased approximately 2-fold for both *Escherichia coli* (0.3% versus 0.8%) and *Klebsiella pneumoniae* (4.7% and 8.2%), according to a nationwide multicenter study of the Paul-Ehrlich-Society (Kresken et al, 2003).

Because of their ability to spread rapidly in the hospital environment, these bacteria tend to cause nosocomial outbreaks (Paterson, 2001; Stürenburg & Mack, 2003). Such outbreaks caused by ESBL increase the length of hospital stay (Kim et al, 2002a; Lautenbach et al, 2001), may be responsible for temporary ward closure, and have a high attributable mortality (Du et al, 2002; Kim et al, 2002b; Paterson et al, 2001).

There is convincing evidence that clinical infections by multidrug-resistant organisms such as ESBL represent a minute fraction of the vast population of asymptomatic colonized patients—most of whom are unrecognized—that constitute the institutional reservoir for the spread of these organisms on hands and medical equipment to uncolonized patients (De Champs et al, 1989; Pessoa-Silva et al, 2003). The principal pathogenic reservoir for these bacteria seems to be the gastrointestinal tract of colonized and infected patients (Hobson et al, 1996; Lucet et al, 1996; Pena et al, 1998).

The effectiveness of infection control measures is enhanced by the early detection of patients whose gastrointestinal tracts have been colonized (Lucet et al, 1999). Polymerase chain reaction (PCR) methods that detect the ESBL genes will provide results within a few hours; however, DNA preparation from stool is cumbersome because of high concentrations of PCR inhibitors and test costs are relatively high. With cultural methods, speed and accuracy in identification by colonial morphology are often

* Corresponding author. Tel.: +49-40-42803-3147; fax: +49-40-42803-4881.
E-mail address: e.stuerenburg@uke.uni-hamburg.de (E. Stürenburg).

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difficult because of the presence of a mixed population of commensal bacteria. As primary screening, the American National Committee for Clinical Laboratory Standards (NCCLS) recommends testing growth in Mueller-Hinton broth containing any 2 of the following β-lactam antibiotics: cefpodoxime, 4 μg/mL; ceftazidime, 1 μg/mL; aztreonam, 1 μg/mL; cefotaxime, 1 μg/mL; or ceftriaxone, 1 μg/mL (NCCLS, 2003). However, ESBL screening swabs are commonly plated on selective media, mostly MacConkey agar, and the presence of selective agents such as bile salts may affect the reliability of the test with some strains.

In this study, we report a new ESBL screening test, the ESBL Screen agar (AES Laboratoire, Combourg, France), which is specially designed for the recovery of ESBL from feces or rectal swabs. Using clinical isolates of ESBL-producing bacteria, the microbiological performance of the new agar formula was evaluated for the detection and presumptive identification of ESBL-producing Enterobacteriaceae species.

2. Materials and methods

2.1. Bacterial strains

The bacterial strains selected for this study included 65 oxyimino cephalosporin-resistant isolates of Gram-negative bacteria, most of which were characterized previously (Stürenburg et al, 2003, 2004b). The remaining strains were recent isolates gathered from patients at the University Hospital Hamburg-Eppendorf, Germany, which is affiliated with our institution. Species identification was performed by routine laboratory methods such as the API32E (bioMérieux, Marcy l'Etoile, France). Reference confirmation for ESBL production was made by PCR analysis for β-lactamase genes of the families CTX-M, SHV, and TEM and where applicable, by nucleotide sequencing.

2.2. ESBL-positive strains

Sensitivity was calculated with 50 ESBL-positive strains, which were considered ESBL-positive when a reduction was noted of a least 8-fold in the minimum inhibitory concentrations of either ceftazidime or cefotaxime in the presence of clavulanic acid. In detail, the clinical isolates and their strains consisted of:

- *Enterobacter aerogenes* (n = 3)—CTX-M-1 (n = 2) and SHV-5 (n = 1) ESBLs;
- *Enterobacter cloacae* (n = 3)—SHV-12 (n = 3) ESBLs;
- *Escherichia coli* (n = 11)—SHV-2 (n = 2), SHV-5 (n = 1), SHV-12 (n = 3), TEM-26 (n = 1), TEM-52 (n = 1), TEM-111 (n = 1), CTX-M-1 (n = 1), and CTX-M-23 (n = 1, Stürenburg et al, 2004a);
- *Klebsiella oxytoca* (n = 3)—CTX-M-1 (n = 2) and SHV-12 (n = 1);
- *Klebsiella pneumoniae* (n = 26)—SHV-2 (n = 5), SHV-2a (n = 1), SHV-5 (n = 4), SHV-12 (n = 11), SHV-18 (n = 1), SHV-19 (n = 1), LEN type (ABL: N53S, A201P, and P218A) (n = 1), TEM-47 (n = 1), and TEM-110 (n = 1) and;
- *Proteus mirabilis* (n = 4)—CTX-M-1 (n = 1), CTX-M-15 (n = 1), CTX-M-22 (n = 1), and TEM-92 (n = 1).

2.3. ESBL-negative strains

Specificity was calculated with 15 clinical strains resistant to oxyimino cephalosporins that failed to produce any ESBLs according to the PCR-based technique. Rather, in these strains, the likely type of β-lactamase present was inferred on the basis of the overall susceptibility profile (Livermore, 1995; Livermore et al, 2001). In detail, the non-ESBL strains consisted of 1 strain of *Acinetobacter* spp. (interpretative reading not applicable), 1 strain of *C. freundii*, 2 strains of *Enterobacter cloacae*, 3 strains of *Escherichia coli*, 1 strain of *Morganella morganii* (likely mechanism of resistance: hyperproduction of AmpC chromosomal β-lactamase), 6 strains of *K. oxytoca* isolates (hyperproduction of K1 chromosomal β-lactamase), and 1 strain of *K. pneumoniae* (production of plasmid-mediated AmpC β-lactamase).

2.4. Culture media

The ESBL Screen agar is composed of 2 selective agars for the combined detection of cefotaxime and ceftazidime resistance. In both media, lactose is included as a differential component and an energy source for the organisms capable of its utilization by a fermentation mechanism. The formula per liter of distilled water is the following:

- half-dish 1 (green-blue, DRI+)—resistance to cefotaxime: Drigalski agar (51 g), cefotaxime (1.5 mg);
- half-dish 2 (purple, MC+)—resistance to ceftazidime: MacConkey agar (50 g), ceftazidime (2 mg).

Table 1
Characteristics of Enterobacteriaceae species and nonfermentative Gram-negative bacilli isolated on AES agar screen plates and algorithm for presumptive identification of ESBL activity

Organism	Morphology and/or color (18–24 h incubation)	
	Drigalski side	MacConkey side
Enterobacter spp.	Yellow colonies	Pink-to-red colonies
Escherichia coli	Yellow colonies	Pink-to-red colonies
Klebsiella spp.	Mucoid, yellow colonies	Mucoid, pink-to-red colonies
Other Enterobacteriaceae	Blue colonies	Colorless (transparent) colonies
Pseudomonas spp.	Blue colonies	Colorless (transparent) colonies

Interpretative algorithm
Resistance to cefotaxime and ceftazidime; lactose-positive → high strong presumption of ESBL
Resistance to cefotaxime or ceftazidime; lactose-positive → strong presumption of ESBL
Resistance to cefotaxime and/or to ceftazidime; lactose-negative → presumption of ESBL or multiresistant Gram-negative bacilli
No growth → absence of ESBL

ESBL, Extended Spectrum β-Lactamases.