A Laboratory Evaluation of Chromogenic Screening Media for the Detection of Extended-Spectrum Beta-Lactamase-Producing Bacteria

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Overview

Purpose: To examine the performance of Thermo Scientific™ Oxoid™ *Brilliance*™ ESBL Agar (Thermo Fisher Scientific) for the detection of ESBL-producing bacteria in comparison with chromID® ESBL (bioMérieux).

Methods: Eighty pure culture isolates, comprising 23 ESBLs, 13 chromosomal AmpC and 44 other non-ESBL producing organisms (including Gram-negative and Gram-positive isolates) were prepared as 0.5 McFarland suspensions. Each ESBL was serially diluted and spread over the surface of the medium. Non-ESBL organisms were inoculated directly from the 0.5 McFarland suspensions with a 10µL loop, using the diminishing sweep technique. All plates were incubated aerobically at 37°C for 24 hours.

Results: Both media examined in this study achieved high sensitivity and specificity within 24 hours and are suitable for the detection of most of the commonly encountered ESBL types. *Brilliance* ESBL Agar provided marginally better sensitivity and specificity in this study.

Introduction

Since the 1980s, the increasing prevalence of plasmid-encoded extended-spectrum beta-lactamases (ESBLs) has been of major concern[1,2]. Treatment options for infections caused by bacteria possessing such plasmids are limited due to their resistance to beta-lactams, monobactams and cephalosporins. *In vivo* resistance to aminoglycosides, fluoroquinolones and trimethoprim-sulfamethoxazole has also been widely reported, leaving carbapenems as the currently preferred therapeutic option[2].

The EU-funded Mastering Hospital Antimicrobial Resistance (MOSAR) project is currently conducting a large, multi-site trial to determine the prevalence of ESBL producing bacteria across Europe. The MOSAR project has recognized the benefits of chromogenic media for screening and has selected *Brilliance*™ ESBL Agar for their study. Routine screening for ESBL-producing Enterobacteriaceae using culture on chromogenic media is becoming more widely adopted.

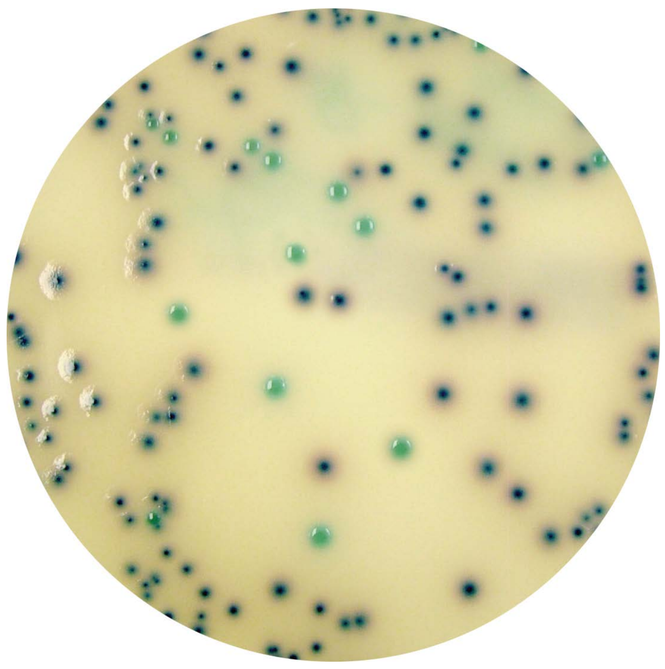
The aim of this study was to examine the performance of *Brilliance* ESBL Agar, a new chromogenic screening medium for the detection of ESBL-producing bacteria, in comparison with chromID ESBL.

Methods

Eighty pure culture isolates, comprising 23 ESBLs (*E. coli, K. pneumoniae, E. cloacae* and *Ps. aeruginosa*, harbouring CTX-M, TEM, SHV, or PER), 13 chromosomal AmpC (*E. coli, Enterobacter* spp., *Klebsiella* spp., *Citrobacter* spp., *S. marcescens*, *A. baumanii* and *Ps. aeruginosa*, including inducible and derepressed variants) and 44 other non-ESBL producing organisms (including Gram-negative and Gram-positive isolates) were prepared as 0.5 McFarland suspensions.

Each ESBL isolate was serially diluted to provide an inoculum of 10 to 100 cfu from a 50mL volume, which was spread over the surface of each medium. Non-ESBL organisms were inoculated directly from the 0.5 McFarland suspensions with a 10µL loop, using the diminishing sweep technique. All plates were incubated aerobically at 37°C for 24 hours.

FIGURE 1. Typical morphology of colonies of ESBL-producing *E. coli* (blue) and *K. pneumoniae* (green) on *Brilliance* ESBL Agar, following aerobic incubation at 37°C for 24 hours.



Results

Both media obtained sensitivity and specificity of >90% in this study (Table 1). Two *E. coli* ESBL isolates (SHV-2 and TEM-3) were not detected by chromID ESBL and one *Ps. aeruginosa* (PER-1) was not detected by *Brilliance* ESBL Agar. With regard to false positives, both media allowed growth of *Ps. aeruginosa* possessing OXA-10 and a *Klebsiella* spp. with derepressed chromosomal AmpC, though growth of this isolate was more prolific on the chromID medium. *Brilliance* ESBL Agar also allowed growth of two *Ps. aeruginosa* isolates with inducible AmpC, while chromID ESBL allowed growth of an *A. baumanii* isolate with inducible AmpC, as well as isolates of *Ps. putida* and *B. cepacia.*

TABLE 1. Sensitivity and specificity of *Brilliance* ESBL Agar and chromID ESBL following aerobic incubation at 37°C for 24 hours.

n=80	Sensitivity	Specificity
Brilliance ESBL Agar	95.7% (22/23)	93.0% (53/57)
chromID ESBL	91.3% (21/23)	91.2% (52/57)

Each medium also enabled differentiation of *E. coli* and coliforms from other Gram-negative bacteria due to enzymic cleavage of specific chromogenic substrates. chromID ESBL typically produces pink or burgundy colonies for *E. coli* and green colonies for coliforms, while *Brilliance* ESBL Agar typically produces blue colonies for *E. coli* and green colonies for coliforms (Figure 1).

Conclusion

Previous studies have demonstrated the advantages of chromogenic screening media over traditional culture media for the presumptive identification of ESBL-producing Enterobacteriaceae[3,4]. Chromogenic screening media can provide earlier presumptive identification than traditional culture-based methods, thereby reducing time to reporting and enabling infection control teams to implement timely control measures.

Both media examined in this study achieved high sensitivity and specificity within 24 hours and are suitable for the detection of most of the commonly encountered ESBL types. *Brilliance* ESBL Agar provided marginally better sensitivity and specificity in this study. Further studies using routine clinical screening samples are currently being undertaken.

References

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