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Laboratory evaluation of *Brilliance*™ CRE Agar for screening carbapenem-resistant Enterobacteriaceae: Performance on a collection of characterised clinical isolates from Greece

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ABSTRACT

The performance of Oxoid *Brilliance*™ CRE Agar (BCRE), a new chromogenic medium designed for screening of carbapenem-resistant Enterobacteriaceae, was evaluated on a collection of clinical isolates of enterobacteria ($n = 175$) and non-fermenters ($n = 55$) with known β-lactam resistance mechanisms and levels of susceptibility to carbapenems. BCRE supported the growth of 100 of 108 enterobacterial isolates that were non-susceptible to at least one carbapenem, whilst excluding 57 of the 67 carbapenem-susceptible isolates. The eight non-susceptible isolates that did not grow on BCRE were carbapenemase-producers with low carbapenem minimum inhibitory concentrations, mostly exhibiting non-susceptibility only to one carbapenem. In total, of 107 carbapenemase-producing enterobacteria that were included in the study, 16 did not grow, with most of them being either susceptible ($n = 8$) or intermediate-susceptible ($n = 5$) to carbapenems. Regarding the 10 carbapenem-susceptible enterobacteria that were not excluded by BCRE, 1 produced a carbapenemase and the rest possessed strong backgrounds of various other β-lactam resistance mechanisms. The medium allowed growth of almost all carbapenem-resistant non-fermenting isolates; nevertheless, non-fermenters were clearly differentiated from Enterobacteriaceae by colony colour and morphology.
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1. Introduction

Nosocomial infections due to carbapenem-resistant Gram-negative bacteria have become one of the most pressing public health problems worldwide. Enzymatic inactivation is the main mechanism of resistance against carbapenems. Clinically important β-lactamases with significant carbapenemase activity belong to molecular classes A (e.g. KPC-type, members of the GES family), B (VIM, IMP, NDM) and D (OXA-23, OXA-48, OXA-58). Acquisition of transmissible carbapenemase genes either by members of the Enterobacteriaceae family or other Gram-negative bacteria encountered in the clinical setting (e.g. *Pseudomonas aeruginosa* and *Acinetobacter baumannii*) can lead to high levels of carbapenem resistance. As these bacteria are usually pan-resistant and therefore difficult to treat with clinically available antibiotics, implementation of infection control measures is probably the only effective response to prevent further spread of carbapenemase-producing strains in healthcare settings. Systematic surveillance cultures and application of screening methodologies play a pivotal role in designing effective control policies [1–4].

Molecular and phenotypic techniques can detect carbapenemase-producing Enterobacteriaceae with high sensitivity and specificity, but their handiness for screening purposes is doubtful as they are expensive and laborious [4,5]. Recently, the industry has introduced selective agar media that can be used for screening of carbapenemase-producing Enterobacteriaceae, mostly by excluding growth of isolates susceptible to carbapenems. A number of the latter media are chromogenic, allowing differentiation of microorganisms to genus or species level [5–15].

In this study, the reliability of a novel chromogenic medium designed to detect carbapenem-resistant enterobacteria was examined. *Brilliance*™ CRE Agar (BCRE) (Thermo Fisher Scientific, Basingstoke, UK), containing a modified carbapenem, was 'challenged' by a collection of characterised clinical isolates of Enterobacteriaceae. A number of *A. baumannii* and *P. aeruginosa* isolates were also assayed to test the chromogenic ability of BCRE

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Table 1
Performance of Oxoid *Brilliance*™ CRE Agar in 175 Enterobacteriaceae by carbapenem susceptibility status.

Performance	CP isolates			CNP isolates			All isolates		
	R/I	S	Total	R/I	S	Total	R/I	S	Total
Growth	90	1	91	10	9	19	100	10	110
No growth	8	8	16	–	49	49	8	57	65
Total	98	9	107	10	58	68	108	67	175

CP, carbapenemase-producing; CNP, carbapenemase-non-producing; R/I, non-susceptible isolates (resistant or intermediate-susceptible to at least one carbapenem); S, isolates susceptible to all four carbapenems tested.

to discriminate carbapenem-resistant enterobacteria from non-fermenters by colony colour and morphology.

2. Materials and methods

2.1. Bacterial isolates

A total of 230 clinical isolates, consisting of Enterobacteriaceae (n = 175) and non-fermenters (n = 55), were included in this study. Isolates were derived from the collections of the Bacteriology Laboratory of the Hellenic Pasteur Institute (Athens, Greece) and the Microbiology Laboratory of the University of Thessaly (Larissa, Greece). Isolates had been collected from different clinical settings and belonged to a variety of clones encountered in Greek hospitals. Previous characterisation of the isolates included determination of β-lactam susceptibility levels as well as characterisation of their β-lactamase content using appropriate methodology [16–18]. Outer membrane protein profiles and the quantity of the produced chromosomal AmpC β-lactamase had also been determined when indicated [19,20]. The enterobacterial sample included 107 carbapenemase-producing isolates [KPC-2 (n = 41), VIM-type (n = 50), KPC-2 + VIM-type (n = 14) and GES-type (n = 2)] that exhibited various levels of susceptibility to carbapenems. The remaining enterobacterial isolates possessed other mechanisms of resistance to β-lactams (n = 37) or were classified as having wild-type β-lactam susceptibility phenotypes (n = 31).

Non-fermenting isolates included 28 *A. baumannii* and 27 *P. aeruginosa*. All *A. baumannii* isolates produced carbapenemases [OXA-58 (n = 18), OXA-58 + VIM-1 (n = 8) and OXA-23 (n = 2)] and, except for 1 isolate that exhibited minimum inhibitory concentrations (MICs) in the intermediate range for imipenem and meropenem and was susceptible to doripenem, all were highly resistant to carbapenems. Of the *P. aeruginosa* isolates, 23 were highly resistant to carbapenems and produced VIM-type metallo-enzymes [VIM-2 (n = 18) and VIM-4 (n = 5)], and 4 exhibited wild-type β-lactam susceptibility phenotypes.

2.2. Classification of the isolates into carbapenem susceptibility categories

Susceptibility levels of the isolates to imipenem, meropenem, ertapenem and doripenem were determined by Etest (bioMérieux, La Balme-les-Grottes, France) according to the manufacturer's instructions. For susceptibility categorisation of the isolates, European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints were used (http://www.eucast.org). Each isolate was further classified as carbapenem-non-susceptible if exhibiting an MIC in the resistant or intermediate ranges for at least one of the carbapenems tested.

2.3. Culture conditions and interpretation

Bacterial suspensions were prepared from colonies grown on Oxoid Columbia Agar (Thermo Fisher Scientific) and were suspended in saline solution (0.9%, w/v) to a final density of ca. 10^8 CFU/mL estimated using a DensiCheck turbidimeter (bioMérieux, Marcy l'Étoile, France). The initial suspension was further diluted by serial 10-fold dilutions. BCRE was inoculated with 0.1 mL of the 10^1, 10^2 and 10^4 CFU/mL suspensions of each isolate. Results were recorded after incubation for 18 h at 36 °C. Colonies grown on each plate were counted and their colour and morphology were recorded. In cases of unexpected or inconsistent results, species identification, carbapenem susceptibility testing and plating of the respective isolates on BCRE were repeated twice.

3. Results

3.1. Performance of the Brilliance™ CRE Agar in Enterobacteriaceae

Of the 175 enterobacterial isolates, 108 were categorised as non-susceptible and 67 as susceptible to carbapenems. Of the non-susceptible isolates, 100 (92.6%) grew on BCRE, whilst growth was inhibited for 57 (85.1%) of the susceptible isolates (Table 1). Furthermore, 91 of those that grew (90 non-susceptible and 1 susceptible to carbapenems) were carbapenemase-producers, accounting for 85% of the total 107 carbapenemase-producing Enterobacteriaceae that were included in the study. Detailed results from testing the performance of BCRE as related to bacterial species, carbapenem susceptibility status and background mechanisms of β-lactam susceptibility of the isolates are presented in Table 2.

3.1.1. Klebsiella pneumoniae

Of the 112 isolates of *K. pneumoniae* tested, BCRE allowed growth of 82 (98.8%) of 83 non-susceptible isolates and excluded 23 (79.3%) of 29 susceptible isolates to carbapenems (Table 2). The sole carbapenem-non-susceptible isolate that failed to grow on the medium was a KPC-2 producer with MICs of imipenem, meropenem, and doripenem in the intermediate susceptibility range and an ertapenem MIC of 6 mg/L (Table 3). BCRE detected the majority (96.3%) of the carbapenemase-producing *K. pneumoniae* isolates (78/81 isolates), including 1 of 3 carbapenemase-producing isolates that were susceptible to carbapenems. Ten non-carbapenemase-producing *K. pneumoniae* also grew on BCRE. All of the latter possessed multiple mechanisms of resistance to the newer β-lactams, but only five of them [porin-deficient extended-spectrum β-lactamase (ESBL)-producers] had been categorised as carbapenem-non-susceptible, mostly due to elevated ertapenem MICs (6–128 mg/L).

K. pneumoniae isolates grown on BCRE developed steel-blue, smooth colonies varying in size; 34 (38.6%) of them were recovered even from 10^1 CFU/mL suspensions, whilst for the remainder the lower limit of detection was raised to 10^2 or 10^4 CFU/mL (51.1% and 10.2% of the isolates, respectively). Thus, ca. 90% of the *K. pneumoniae* isolates that grew on BCRE could be detected from low inocula ($<10^3$ CFU/mL).

3.1.2. Escherichia coli

Of the 28 *E. coli* isolates tested (12 non-susceptible and 16 susceptible to carbapenems), BCRE selected 9 (75%) of the

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