ORIGINAL ARTICLE

Utility of CHROMagar mSuperCARBA for surveillance cultures of carbapenemase-producing *Enterobacteriaceae*

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Abstract

Culture of carbapenemase-producing *Enterobacteriaceae* (CPE) as part of active surveillance is one of the most useful strategies for successful infection control programmes. Our objective was to compare the recently introduced CHROMagar mSuperCARBA agar for CPE detection in surveillance cultures from perineal swabs with the US Centers for Disease Control and Prevention method. Our results showed that this agar is a useful and affordable alternative (sensitivity 93.05%, specificity 96.21%, diagnostic accuracy 95.2%) to detect CPE in hospital settings.

Keywords: Carbapenemases, chromogenic agar, *Enterobacteriaceae*, performance, surveillance cultures
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Introduction

Carbapenemase-producing *Enterobacteriaceae* (CPE) were detected about 20 years ago in the United States and are currently a global epidemic [1]. The infections caused by these multidrug-resistant microorganisms have high rates of morbidity and close to 50% mortality, mostly due to the few therapeutic options available, such as tigecycline, polymyxins, fosfomycin and aminoglycosides [2,3].

More than 2000 genetic variants of carbapenemases have been described. The predominant enzymes are as follows: *Klebsiella pneumoniae* carbapenemase (KPC; serin carbapenemase, Ambler group A), New Delhi metallo-β-lactamase (NDM; metallo-β-lactamase, Ambler group B) and OXA-48 (oxacillinase, Ambler group D). KPC carbapenemase predominates in endemic countries such as Greece, Italy and Israel, with incidences exceeding 30% [4], while Turkey and other Mediterranean countries report the OXA-48 variant as predominant [5]. NDM carbapenemase predominates in countries such as India, where it has a prevalence of 50% [6]. Regarding Latin America, KPC carbapenemase is considered endemic in some countries such as Colombia, Argentina and Brazil, while Chile only reports outbreaks caused by this microorganism. NDM variant predominates in countries such as Guatemala. However, the latter variant and OXA-48 carbapenemase are usually reported with less frequency in Latin America [7].

The US Centers for Disease Control and Prevention (CDC) and the European Society of Clinical Microbiology and Infectious Diseases recommend early detection to prevent the spread of CPE [8–10]. The early identification of these microorganisms is frequently carried out through rectal swab cultures. Given the fact that sensitive and specific methodologies with good turnaround times are required, several investigators have supported the use of chromogenic agars for identification [10]. The sensitivity of these agars varies according to the type of carbapenemase studied and the brand.

The CDC recommends the use of Landman's protocol for the detection of intestinal colonization of carbapenemase-producing *Enterobacteriaceae* [11]. The CHROMagar mSuperCARBA agar (CHROMagar) was marketed in 2016 as an

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appropriate alternative for the detection of KPC, NDM and OXA-48 like carbapenemases in *Enterobacteriaceae*. This chromogenic agar is based on a previous culture medium designed by Nordmann et al. in 2012 [32], which had shown the best performance for CPE detection in many studies [10,12,13]. However, to date, there is little scientific literature demonstrating its usefulness in surveillance cultures with patient samples.

Our objective was to compare the performance of CHROMagar mSuperCARBA agar with the method recommended by the CDC for detection of intestinal colonization by carbapenemase-producing *Enterobacteriaceae*.

Materials and methods

We carried out a prospective and multicentre study from February to April 2016. We included seven adult intensive care units from Guayaquil (Ecuador). Informed consent was obtained from each patient or their relatives for the sampling.

Patient selection

Perineal swabs were collected weekly in each patient who had more than 48 hours of hospitalization.

Microbiologic surveillance cultures

Amies media was used to transport samples to the laboratories. All the swabs were processed by the CDC method as previously described [11]. The samples were suspended in 5 mL of trypticase soy broth (Oxoid), and subsequently a 10 μg ertapenem (Oxoid) disc was added. The broth was incubated in air atmosphere for 24 hours at 35°C. After incubation, 100 μL of suspension was inoculated on MacConkey II agar (Becton Dickinson) [11]. It was incubated for 24 hours at 35°C in air atmosphere. The CHROMagar mSuperCARBA agar (SC) (CHROMagar) processing was performed in the bacteriology laboratory of the National Institute of Public Health Research 'Dr Leopoldo Izquieta Pérez.' The swab was plated in the agar prepared according to the manufacturer's instructions (≤72 hours of preparation) and incubated for 24 hours at 35°C in air atmosphere.

Bacterial identification and antimicrobial susceptibility

For the CDC method, we considered presumptive CPE colonies to be those growing as lactose positive, following CDC recommendations. Additionally, we also considered lactose-negative colonies (Hardy Diagnostics). In the SC method, according to the manufacturer's instructions, red colonies were considered *Escherichia coli,* metallic blue the remainder of the coliforms and colorless colonies other Gram-negative bacteria. All presumptive CPE colonies cultured in the two agars were identified by the API 20E system (bioMérieux). We performed antimicrobial susceptibility testing by the disc diffusion method [14]. Imipenem (IMP) 10 μg and meropenem 10 μg were used. Carbapenem-resistant *Enterobacteriaceae* (CRE) and nonresistant strains were defined according to Clinical Laboratory Standards Institute breakpoints. CRE strains were those with zone diameters ≤22 mm, and those with no resistance to carbapenem had zone inhibition diameters ≥23 mm [14].

Phenotypic detection of carbapenem resistance mechanisms

We tested all CRE with the modified Hodge test (MHT) [14]. Combined-disc tests of meropenem with and without phenylboronic acid (PBA) (Liofilchem) to detect serin carbapenemases and imipenem with and without EDTA (Liofilchem) to detect metallobetalactamase was performed as described elsewhere [4,15]. Carbapenemase production was confirmed if MHT was positive and the combined-disc test with PBA or EDTA was positive. CRE with a negative or undetermined MHT and/or a combined-disc–negative test were also tested with the carbapenem inactivation method [16] and MHT with Müller-Hinton agar (Becton Dickinson) supplemented with 250 mg/mL cloxacillin sodium salt (MHT-C) (MilliporeSigma) and meropenem disc (10 μg) according to previously described protocols [17]. We defined isolates with negative carbapenem-inactivation method and MHT-C as CRE–non-producing carbapenemase (CRE non-PC). These isolates were also studied for extended-spectrum β-lactamase (ESBL) and AmpC production. An ESBL phenotype was defined in *Enterobacteriaceae* with a synergy effect observed among cefepime (30 μg), cefotaxime (30 μg) and ceftazidime (30 μg) discs and the amoxicillin/clavulanic acid disc (20 μg/10 μg), which was placed at 15 mm from centre to centre of ceftazidime, cefepime and cefotaxime [14]. An AmpC phenotype was considered in CRE non-PC isolates, with a synergistic effect observed with the double-disc method with ertapenem (10 μg) and phenylboronic acid (300 μg) (PBA) discs (Kirby-Bauer method with ertapenem and PBA discs placed 15 mm from centre to centre in MHT-C) [14].

Escherichia coli ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603 and *K. pneumoniae* ATCC BAA-1705 were used as quality control strains for the described tests.

Molecular detection of carbapenemases

All CRE were subcultured on trypticase soy agar (Oxoid) for 24 hours at 35°C in air atmosphere. DNA was extracted using the Wizard Genomic DNA purification kit (Promega) following the manufacturer's recommendations. We assessed the DNA quality by analysing the ratio of the absorbance at 260 nm/