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Efficacy of an Enrichment Media for Increasing Threshold for Carbapenem-Resistant *Enterobacteriaceae* Screening

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Background: Identification of carbapenem-resistant *Enterobacteriaceae* (CRE) is complex and a major laboratory challenge; clinical cultures may diagnose only some of the CRE carriers among patients, thus it is crucial to perform asymptomatic carriage screening. *Materials and methods:* We compare the efficacy of a rectal sample culture prior to enrichment with BHI (Brain Heart Infusion) Broth and following 18–24 h. All rectal samples were applied on CHROMagar KPC selective growth media and then seeded on MacConkey agar selective growth media with an applied disk of Imipenem antibiotic on top of the media, then inserted into enrichment BHI Broth. After 18–24 h incubation with enrichment media, all samples were applied again on this media. *Results:* From the 2,245 rectal samples, CRE colonies were found in 96 (4.3%). Following enrichment with BHI Broth, CRE colonies were found in 111 (4.9%) CHROMagar KPC plates and 106 (4.7%) MacConkey agar. *Conclusion:* We were able to demonstrate that the number of CRE-positive results increased due to use of additional enrichment with BHI Broth. Therefore, we recommend applying this method of addition of liquid enrichment media as part of a culture protocol routine for CRE screening. J. Clin. Lab. Anal. **30**: 563–566, 2016. © 2015 Wiley Periodicals, Inc.

Key words: Carbapenem-resistant *Enterobacteriaceae*; CHROMagar KPC; MacConkey agar; Brain-Heart Infusion Broth

INTRODUCTION

The term CRE refers to carbapenem-resistant and carbapenemase-producing *Enterobacteriaceae*. CRE are Gram-negative bacteria that have emerged during the past decade, characterized by high levels of resistance to antibiotics (1). The emergence and spread of resistance in *Enterobacteriaceae* are complicating the treatment of serious nosocomial infections and threatening to generate species resistant to a wide range of presently available antibiotic agents. Resistance to carbapenems in the *Enterobacteriaceae* family is generally caused by ability to produce carbapenemases, which are carbapenem-hydrolyzing beta-lactamases. Carbapenemases are classified based on amino acid homology. These enzymes include the class A carbapenemases (KPC (*Klebsiella Pneumoniae Carbapenemase*) types), the class B or metallo-β-lactamases (MBLs; VIM (*Verona integron-encoded metallo-β-lactamase*) and NDM (New Delhi *Metallo-beta-lactamase*) types), and the class D oxacillinases (e.g. (*Metallo-beta-lactamase*), OXA-48-like enzymes) (2). *Escherichia coli* and *Klebsiella* species are types of *Enterobacteriaceae*; these bacteria reside in the human intestine and could become carbapenem resistant (1). Currently, antibiotic treatment of infections caused by CRE includes aminoglycosides, polymyxins, tigecycline, fosfomycin, and temocillin (3). The most significant risk factors for colonization or infection with CRE

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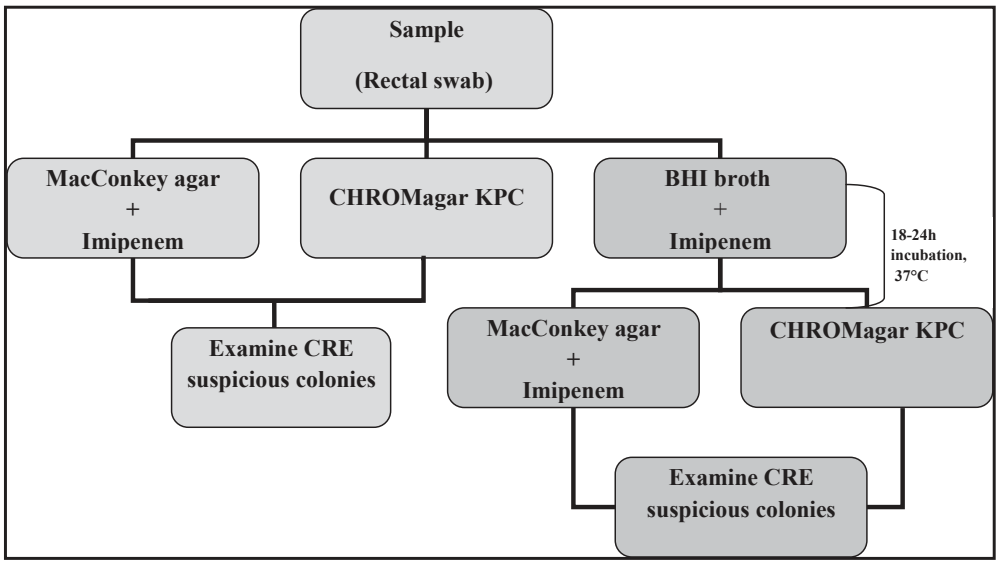

Fig. 1. CRE screening work protocol flowchart.

Gram-negative bacteria among patients are advanced age, decreased functional status, invasive procedures, hospitalization or residence in a long-term care or rehabilitation facility, and recent antibiotic treatment (4, 5).

CRE bacterial identification is complex and a major laboratory challenge; clinical cultures may diagnose only some of the CRE carriers among patients. For this reason, it is crucial to perform asymptomatic carriage screening (rectal swab or stool culture). This allows rapid detection of carriers among suspected patients and consequent early contact isolation in order to prevent transmission between CRE carriers and other patients at risk. In recent years, different selective growth media targeted for CRE screening have been developed, such as Brilliance CRE *Agar* and CHROM KPC *agar*. These growth media demonstrated efficacy in 95–98% of studies and enabled preliminary bacteria identification by colony color (6, 7). Also, the molecular biology platform offers technology that enables rapid and reliable CRE detection directly from the sample. In comparison to culture technique, this technology has high sensitivity and specificity for detection of an enzyme that enables the resistance ability in CRE (8, 9). Molecular biology methods are often very expensive and therefore not yet in routine use in microbiology laboratories. The main problem regarding use of a culture technique for CRE bacteria screening is the small quantity of bacteria on a swab or low-quality sample (especially with rectal cultures). One proposed solution could be increasing the sensitivity through enrichment of the original sample with liquid enrichment media.

Our primary goal is to compare the efficacy of a rectal sample culture technique on two different selective growth media targeted for CRE screening prior enrichment and after enrichment with Brain-Heart Infusion Broth with Imipenem.

MATERIALS AND METHODS

Specimen Collection

Between January 2013 and July 2014, 2,245 rectal samples were obtained in Poriya Medical Center in the north of Israel. All specimens were collected with *Amies* Media *Swabs* (Copan, Italia) *by nurses and* sent to the Laboratory of Clinical Microbiology within a maximum of 12 h from sample collection.

Culture

Each sample was seeded on two selective growth media; first on CHROMagar KPC (Hy-Laboratories Ltd., Israel) and then on MacConkey agar (BD Diagnostics, Sparks, MD), and afterwards transferred into enrichment media (Brain-Heart Infusion Broth, BD Diagnostics) containing a 10 μg Imipenem disk (BD Diagnostics). Following seeding on MacConkey agar, a 10 μg Imipenem disk was added on top of the densest seeded area. All the growth media were incubated for 18–24 h at 37°C. At the end of the incubation period, plates were examined for presence of CRE suspicious colonies. For example, on CHROMagar KPC, presence of blue or pink colored colonies meant suspicion of *Klebsiella* or *E. coli,* respectively. MacConkey agar plates were examined for the presence of CRE suspicious colonies within the inhibition zone around the

TABLE 1. Comparison of CRE Screening Prior to Brain-Heart Infusion Broth Enrichment and Following Enrichment With Brain-Heart Infusion Broth Media

Method/growth media	Routine CRE screening protocol (no. of CRE-positive samples)	Routine CRE screening protocol + addition of enrichment media BHI (no. of CRE-positive samples)
MacConkey agar + Imipenem	96	106
CHROMagar KPC	96	111

Imipenem disk. At the end of the 18–24 h incubation period, 25 μl of bacterial suspension from Brain-Heart Infusion Broth enrichment media was seeded first onto CHROMagar KPC and then on MacConkey agar, followed by addition of an Imipenem disk. All the growth media were incubated again for 18–24 h at 37°C and then examined for presence of CRE suspicious colonies (Fig. 1). Each suspected CRE bacterial growth was characterized by the VITEK 2 system (bioMérieux, Durham, NC) and by modified *Hodge Test* (MHT) (10). PCR (Polymerase Chain Reaction) method was used to confirm presence or absence of carbapenemase genes (KPC, MBLs, VIM, NDM, and OXA48) in isolates that were screened as CRE positive.

RESULTS

From the total 2,245 rectal samples, CRE isolates were found in 96 (4.3%) CHROMagar KPC and MacConkey agar plates (with Imipenem disk added). Following the enrichment with Brain-Heart Infusion Broth that also contained an Imipenem disk, CRE isolates were found in 111 (4.9%) CHROMagar KPC plates and 106 (4.7%) MacConkey agar plates (Table 1). The total 111 CRE-positive bacteria were distributed as follows: 78 *K. pneumonia* (70.3%), 27 *E. coli* (24.3%), five *Enterobacter cloacae* (4.5%), and one *Providencia rettgeri* (0.9%).

DISCUSSION

Screening for antibiotic-resistant bacteria is certainly one of the major, complex, and significant challenges that a microbiology laboratory struggles with. Screening for CRE bacteria is perhaps more important than others, given the significant mortality rate caused by these bacteria (11). The ability to provide fast, accurate, and reliable results is probably one of the main issues concerning screening for antibiotic-resistant bacteria. Rapid detection of CRE carriers enables early contact isolation in order to prevent transmission between CRE carriers and other patients at risk. Therefore a CRE detection method has to have high sensitivity and specificity, and be as rapid as possible. In this work, we have investigated the efficacy of enrichment media in liquid broth in accordance with a culture protocol for CRE carrier screening. In our current work, we were able to demonstrate that the number of CRE-positive results has increased due to additional enrichment with Brain-Heart Infusion Broth with the addition of an Imipenem disk; this is particularly significant for the *E. coli* bacterium. However, applying the culture-enrichment technique will extend the process for at least 24 h. This means that receiving results for negative CRE screening will also be delayed for 24 h, an issue that could create a heavy burden on hospitals where the availability of isolation rooms for CRE suspected patients is already limited. Another interesting fact found in our current work was the equal efficacy of results for MacConkey agar with Imipenem disk added and for CHROMagar KPC. Nevertheless, the main disadvantage of working with MacConkey agar is the need to perform a complete identification routine for bacterial colonies that grow in the antibiotic inhibition zone; this may delay the final result except in laboratories with available molecular biology technology or mass spectrometry. These are becoming more available in microbiology laboratories and enable rapid bacterial identification (12, 13).

In summary, we found a cost-effective, simple, and accessible technique for optimization of the known CRE screening routine. Despite the process prolongation, we recommend applying this method of addition of liquid enrichment media as part of a culture protocol routine for CRE screening with an intention to not misidentify CRE carriers among suspected patients due to the small quantity of bacteria in the original swab sample.

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