Journal of Microbiological Methods 148 (2018) 169–173

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Chromogenic culture media or rapid immunochromatographic test: Which is better for detecting *Klebsiella pneumoniae* that produce OXA-48 and can they be used in blood and urine specimens

Ozlem Genc[a,*], Evrim Aksu[b]

[a] Faculty of Medicine, Department of Medical Microbiology, Dumlupinar University, Kutahya, Turkey
[b] Evliya Celebi Teaching and Research Hospital, Microbiology Laboratory, Dumlupinar University, Kutahya, Turkey

ARTICLE INFO

Keywords:
Klebsiella pneumoniae
OXA-48
Chromogenic culture media
Immunochromatographic test

ABSTRACT

Our goal was to compare a rapid test (OXA-48K-SeT) and four different chromogenic media (CHROMagar KPC, CHROMagar mSuperCARBA, ChromID Carba and ChromID OXA-48) for the detection of OXA-48 producing *Klebsiella pneumoniae* isolates and spiked urine/blood samples with these bacteria. In total 100 *K.pneumoniae* isolates, including 60 OXA-48 positive, 15 other carbapenemase producing, 15 Extended spectrum betalactamases (ESBL) positive and 10 carbapenem sensitive *K.pneumoniae* were included in the study. After all samples were inoculated into all chromogenic media, temocillin discs were placed onto the media. OXA-48K-SeT was studied according to the manufacturer's instructions and the lower detection limit was determined. Sensitivities and specificities of all chromogenic media and rapid test were detected as 100%. All of the OXA-48 producers were found resistant to temocillin on all chromogenic media. The lower detection limit of the rapid assay was determined as 10^6 in both direct bacterial samples and in spiked urine/blood samples. As a result, four chromogenic culture media and OXA-48 K-SeT can be used safely for detection of OXA-48 positive *K.pneumoniae* isolates. Although direct clinical specimens were not used, our study suggests that this media and OXA-48 K-SeT may be used in patient samples like blood and urine. Further studies are needed to assess this suggestion.

1. Introduction

A variety of carbapenemases in *Enterobacteriaceae* have been reported worldwide. Treatment of infections caused by these bacteria, which cause both hospital and community-acquired infections, are very difficult due to multiple antibiotic resistance. Detection of these carbapenemase producers is therefore becoming a major health issue to prevent their spread (Nordmann et al., 2011).

The OXA-48, one of the Ambler class D carbapenemases were first detected in Turkey from a *K.pneumoniae* strain (Carrer et al., 2008). They were then widely reported from the UK, Europe, India and Africa (Nordmann et al., 2011; Jones et al., 2014). Because OXA-48 producing *Enterobacteriaceae* are very common in our country and in many countries, the rapid and proper identification of this carbapenemase type is very important.

While combination disc methods are used for phenotypic confirmation of other classes of carbapenemases, according to EUCAST criteria which is being used as a standard in laboratories in our country since 2016, the only indicator of class D carbapenemases like OXA-48 is high level of temocillin resistance (EUCAST, 2013). Because this is not a confirmation method but an indicator, it is necessary to develop new methods to detect the OXA-48 type carbapenemases. The standard for identification of OXA-48 is based on the use of molecular techniques, mostly Polymerase Chain Reaction (PCR). Although molecular methods which give rapid results for the detection of OXA-48 have been described (Nordmann et al., 2012), they are costly for many laboratories.

Different chromogenic media are available to detect carbapenem resistant bacteria and studies with these media have been reported (Wilkinson et al., 2012; Simner et al., 2015). Brilliance CRE (Oxoid, Basingstoke, UK), Colorex KPC (E&O Laboratories, Bonnybridge, UK), ChromID Carba and ChromID mSuperCarba (by bioMérieux, Paris, France) are some of these media. In these studies the detection capacities of these media were evaluated for direct carbapenemase producing bacteria or for direct/spiked clinical specimens like gaita and blood. However, the number of these studies is very low and studies investigating OXA-48 producing bacteria using chromogenic media are limited (Girlich et al., 2013a; Zarakolu et al., 2015).

A rapid diagnostic test (OXA-48 KSeT, Corisbio, Belgium), which claims to detect OXA-48-producing bacteria rapidly, has been produced. The OXA-48K-SeT test relies on immunological capture of two

* Corresponding author at: Faculty of Medicine, Department of Medical Microbiology, Dumlupinar University, Evliya Celebi Campus, Kutahya 43100, Turkey.
E-mail address: drozlemg@hotmail.com (O. Genc).

https://doi.org/10.1016/j.mimet.2018.04.014
Received 8 March 2018; Received in revised form 12 April 2018; Accepted 17 April 2018
Available online 19 April 2018
0167-7012/

epitopes specific to the OXA-48 variants OXA-48, OXA-181, OXA-204, OXA-232, and OXA-244 using colloidal gold nanoparticles bound to a nitrocellulose membrane within a lateral flow device. The reported sensitivity and specificity were both 100%, with the result obtained in < 10 min (Ote et al., 2015; Pasteran et al., 2016). In addition, gaita and blood samples spiked with carbapenemase producing bacteria were also tested with this rapid test (Nodari et al., 2017; Wareham et al., 2016)

This study was conducted to compare rapid test and chromogenic media for the detection of OXA-48 producing bacteria. In addition we have conducted studies with spiked urine and blood samples to predict the usability of these culture media and the rapid diagnostic testing of direct clinical samples.

2. Material and methods

2.1. Preparation of inoculums and spiked urine/blood samples

A total of 100 isolates of *K.pneumoniae*, including 60 OXA-48 positive and 15 other carbapenemase producing (with PCR confirmed in a previous study) *K.pneumoniae* (10 NDM 4 VIM, 1 IMP), carbapenem susceptible 15 Extended spectrum betalactamases (ESBL) positive and 10 carbapenem susceptible *K.pneumoniae* were included in the study (Genc et al., 2016).

First, each isolate was subcultured on blood agar medium (RTA, Nazar Tıp, Turkey) and incubated for 24 h at 37 °C. A 1 McFarland bacterial inoculum of each bacterium was prepared and these suspensions were diluted with % 0.9 NaCl to obtain a 0.5 McFarland bacterial suspension. One-microliter aliquots of these suspensions were delivered onto the chromogenic media (Wilkinson et al., 2012). Each isolate was inoculated twice on each chromogenic medium, without and with 30 µg temocillin discs (Bioanalyse, Turkey) to investigate temocillin sensitivity (Fig. 1). After incubation for 24 h at 37 °C, all media were evaluated.

Bacteria-free urine and blood samples that were sent routinely to our laboratory were used for obtaining spiked samples. 0.5 ml of each prepared 1 McFarland bacterial suspension was mixed with the same amount of blood/urine for the preparation of spiked samples. One-microliter aliquots of the 0.5 McFarland spiked urine/blood samples were inoculated to all chromogenic media. Each isolate was inoculated twice on each chromogenic medium, without and with 30 µg temocillin discs to investigate temosilin sensitivity (Fig. 1). After incubation for 24 h at 37 °C, all media were evaluated.

In the study, *K.pneumoniae* ATCC 2146 (NDM positive), *K.pneumoniae* ATCC 1705 (KPC positive) and *K.pneumoniae* NCTC 13442 (OXA-48 positive) were used as controls.

2.2. Chromogenic media

Four different chromogenic media were from two different companies used to detect carbapenemase producing bacteria. ChromID Carba and ChromID OXA-48 (was produced to detect OXA-48) were provided by bioMérieux, La Balme-les-Grottes, France. CHROMagar KPC and CHROMagar mSuperCARBA were provided by CHROMagar, Paris, France. Carbapenem resistant *K.pneumoniae* forms metallic blue colonies on CHROMagar KPC and CHROMagar mSuperCARBA media. These bacteria are growing as green colonies on ChromID Carba. Only OXA-48 producing bacteria are growing on ChromID OXA-48 according to manufacturer's instructions. OXA-48 producing *K.pneumoniae* forms green colonies on ChromID OXA-48.

2.3. Rapid diagnostic test for OXA-48

OXA-48 presence was investigated with OXA-48 K-SeT (Coris, Belgium) in bacterial suspension and in spiked samples. This test is produced to perform directly from bacterial colonies. Three to five colonies of bacteria were suspended in 10 drops of buffer (supplied in the OXA-48K-SeT kit) and 3 drops of this suspension was applied to the sample well as recommended by the manufacturer. The test was evaluated within 15 min (Fig. 2).

In order to study spiked urine and blood samples by OXA-48K-SeT; 0.5 ml of the spiked samples prepared as 0.5 McFarland were mixed with 10 drops of buffer and studied as described (Fig. 2).

To determine the lower detection limit of OXA-48K-SeT assay, a serial ten-fold dilution of 0.5 McFarland inoculum of *K.pneumoniae* NCTC 13442 and three different randomly selected OXA-48 positive *K.pneumoniae* were used. Each dilution was plated for viable colonies counting (Nodari et al., 2017). The number of viable colonies ranged from 10^2 to 10^8 CFU/ml. First, 10 drops of buffer was mixed with 0.5 ml of these dilutions and the test was run. Secondly, the bacterial pellet was obtained after centrifugation of all 0.5 ml serial dilutions and 10 drops of buffer were added to the pellet (Wareham et al., 2016). As previously described, spiked urine and blood samples were prepared from these four bacteria. This method was repeated with these spiked samples for determining the lower detection limit of OXA-48K-SeT assay (Fig. 3).

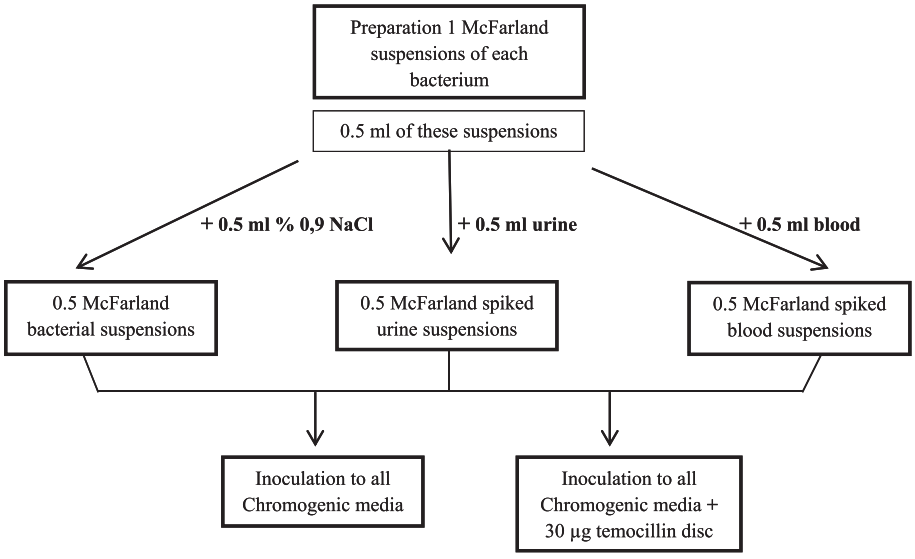

Fig. 1. Method of inoculation to chromogenic media.