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RAPID IDENTIFICATION OF CARBAPENEMASE-PRODUCING *ENTEROBACTERIACEAE*: COMPARISON OF TWO CULTIVATION METHODS

Mario Sarti[2], Maria Valeria Pascuzzi[1], Raffaele Gargiulo[2], Agostino Barozzi[2], Sandra Fregni[2], Anna Mucci[2], Maria Olga Zirilli[2], Ilaria Giovanelli[2], Carla Sabia[1*]

[1]Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy
[2]Provincial Laboratory of Clinical Microbiology, S. Agostino-Estense Hospital, Modena, Italy

Abstract: *We evaluated the performance of chromID CARBA compared with direct plating onto MacConkey agar supplemented with meropenem disk (MCM) for the screening and detection of carbapenemase-producing Enterobacteriaceae (CPE) from rectal swabs. Sensitivity and specificity values were 89.9% and 98.7% for MCM, and 92.4% and 98.8% for chromID CARBA.*

Key Words: carbapenemase-producing *Enterobacteriaceae*, CPE, Sensitivity, Specificity

*Corresponding author: Carla Sabia,
Department of Life Sciences, University of Modena and Reggio Emilia, Via Campi 287, 41125 Modena, Italy
Phone (+39) 059 2055748; Fax (+39) 059 2055483
e-mail: carla.sabia@unimore.it

Introduction

Carbapenems are common as a last-resource antibiotic class for the treatment of infections due to multidrug-resistant *Enterobacteriaceae*. (Carmeli *et al*., 2010). The main mechanism of resistance to carbapenems is represented by the acquisition/expression of new β-lactamase with hydrolytic activity against carbapenems. Most used enzymes are Class A carbapenemase (such as the KPC "*Klebsiella pneumoniae*-carbapenemase"), Class B metallo-β-Lactamase (type VIM, IMP and NDM) and Class D oxacillinases (OXA-48-like) (Luzzaro *et al*., 2012; Nordman *et al*., 2011). The emergence of novel β-lactamases with direct carbapenem-hydrolyzing activity has contributed to increase prevalence of

Carbapenemase-producing *Enterobacteriaceae* (CPE). CPE are particularly problematic due to the frequency with which *Enterobacteriaceae* cause infections (CDC, 2009), the high mortality associated with infections (Gaibani *et al.,* 2011), and the ability of carbapenem resistance to widespread via mobile genetic elements (Cuzon *et al.,* 2010).

An important reservoir and source of the rapid spread of these bacterial strains is the gastro-intestinal tract for this reason in clinical practice it is recommended to perform active surveillance for the detection of carriers of CPE microorganisms by analyzing appropriate specimens such as rectal swabs (Nordman *et al*., 2013; Nordman *et al*., 2012) through a molecular or cultural method. Direct research in molecular biology, thanks to its high sensitivity and specificity, is considered the gold standard for the rapid identification of genes carbapenemases (Schechner *et al*., 2009); however, it has the disadvantage of being expensive and require specialized personnel, so it is not easily practicable in all laboratories. Finally, the molecular method detects only specific genes and does not allow to identify new genes involved in resistance to carbapenems, or you can make a subsequent typing or evaluation of the strain sensitivity to antibiotics.

In recent years, several culture techniques for screening carbapenemase-resistant *Enterobacteriaceae* have been tested: such as MacConkey agar or Tryptic Soy broth containing a 10-μg carbapenem disk, or seeding on commercial chromogenic agar media (Samara *et al*., 2008, Vrioni *et al*, 2011).

The aim of the present study was to evaluate the performance of chromID CARBA (bioMerieux, Florence, Italy) compared with MacConkey agar (bioMerieux, Florence, Italy) plus10 μg meropenem disk for its ability to detect carbapenemase-producing *Enterobacteriaceae* during a rectal swab screening.

A total of thousand (1000) rectal swabs were investigated for screening carbapenemase-resistant *Enterobacteriaceae,* collected from 662 patients hospitalized in departments of internal medicine and long-term care of the Hospitals of USL of Modena where, in the six months preceding the start of the study, it was found a high frequency of CPE isolated.

Every rectal swab was plated onto McConkey agar plus 10 μg meropenem disk (D.I.D., Milano, Italia) (MCM) and onto chromID CARBA. On each medium, evaluation of bacterial growth was made after 24 to 48 h of incubation at 37°C.

Presumptive positive results, with MCM method, were defined as growth of a lactose- fermenting or lactose-non fermenting colonies with a zone of inhibition ≤15 mm by disk of meropenem.

With the MCM method, presumptive CPE colonies were considered those grown as lactose-fermenting or lactose-non fermenting colonies on MacConkey plates at a distance ≤ 15mm from the disk of meropenem. On chromogenic agar plates, presumptive CPE colonies were considered those with a color according to the manufacturer's instructions (green-blue to browny green, pink to burgundy, or brown colonies). Identification and susceptibility testing of the suspect colonies were obtained using the Vitek 2 automated system (bioMerieux, Florence, Italy).

In all presumptive CPE isolates, carbapenemases were detected using the modified Hodge test (Lee *et al*., 2001), combining disk test with IPM and IPM plus phenylboronic acid (PBA) and ethylenediaminetetraacetic acid (EDTA) as inhibitors of KPC or MBLs, respectively (Tsakris *et al*., 2009). The presence of genes bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{NDM} and bla_{OXA} was confirmed by PCR using primers previously described (Giani *et al*., 2009; Lauretti *et al*., 1999; Pagani *et al*., 2005; Roy *et al*., 2011; Dallenne *et al*., 2010).

For both cultural methods the following parameters at 24 hours and 48 hours, have been calculated: sensitivity (Se); specificity (Sp); positive predictive value (PPV); negative predictive, overall accuracy. True-positive strains were defined as all presumptive CPE growing on the media and genotypically confirmed to be CPE positive. False-positive strains were defined to be all presumptive CPE growing on the media that were genotypically confirmed to be CPE negative. No growth by all screening methods was characterized as a true-negative result. No recovery of a genotypically confirmed CPE-positive strain using a particular screening method was characterized as a false-negative result for this specific screening method. Differences in sensitivity and specificity among the various screening methods were analyzed using the chi-square test. Statistical significance was taken $P<0.05$.

CPE were isolated from 79 of the 1000 rectal swabs analyzed (7.9%), these samples were collected from 53 (8%) of 662 patients. Table 1 contains a list of the different isolations CPE characterized. Of particular interest was the finding, from 4 samples collected at different times on the same patient, of two distinct species of CPE simultaneously present, as well as that of a strain of *K. pneumoniae*, also in this case

isolated on four occasions by the same patient, who possessed both the gene *bla*$_{KPC}$ and *bla*$_{VIM}$. All strains were negative for bla$_{NDM}$ and bla$_{OXA}$ genes.

The recognition of suspicious colonies CPE on the chromID CARBA turned out to be rather easy as their differentiation for microbial species as a function of different color developed.

By use of the method MCM was identified a colonization by CPE in 71 cases after reading at 24 hours and in 2 cases after further reading at 48 hours, while with the chromID CARBA were recognized 73 colonizations to the first reading and 4 other colonizations in the second day. In parallel the number of false positivity was observed slightly higher at 48 hours rather than 24 hours, being increased from 12 to 13 cases for MCM and 11 to 14 cases for CARBA.

In order to evaluate the performance of the two cultural methods were calculated values of sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV) and overall accuracy, for the readings at 24 and 48 hours (Table 2). The sensitivity and specificity of screening to 24 hours (considering as true positive and true negative samples for which the two techniques have given concordant results) were respectively 89.9% and 98.7% for MCM, 92.4% and 98.8% using chromID CARBA.

The performance values remained high for both methods (after 24 and 48 hours of incubation), results with slightly higher for the method chromID CARBA, compared to the method MCM for all the parameters considered.

The chi-square test does not has given statistically significant differences, either by comparing the results of different cultural methods, either by comparing the results obtained by reading to 24 hours rather than 48 hours.

There is an alarming spread of CPE, in some cases responsible for serious systemic infections difficult to treat therapeutically and burdened by a high mortality rate. The main problem is on the adoption of measures to monitor and control this phenomenon that are not always efficient. Such measures to be effective they must be based on timely and accurate detection of patients who already have established a state of intestinal colonization. Culture test screening and monitoring of colonization by CPE must be characterized by high sensitivity (good recognition ability of patients colonized) and adequate specificity (content recourse to methods of confirming phenotypic or genotypic).

The method with chromogenic medium chromID CARBA has demonstrated, especially after 48 hours of incubation, a greater sensitivity than the method based on

the use of the method MCM, while maintaining a good specificity; these differences are still found in our cases statistically significant.

The use of this medium can on one side be easier to read and easy to use but on the other hand be burdened with higher costs, the decision whether to use an alternative method MCM should be taken also in function of the feedback of technical-organizational and economic impact.

Conflict of interest

All authors declare to have no conflict of interest

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Appendix A:

Table 1: List of isolates characterized as Enterobacteriaceae producing carbapenemases (CPE)

CPE	Number of Swabs	Number of patients
K. pneumoniae KPC	59	41
K. pneumoniae IMP	1	1
K. pneumoniae KPC + VIM	4	1
E. coli KPC	1	1
E. coli VIM	1	1
E. coli IMP	2	1
E. cloacae VIM	3	2
C. freundii VIM	3	3
P. mirabilis VIM	1	1
K. pneumoniae KPC + *P. mirabilis* VIM	4	1

Table 2: Performance for the two methods at 24 and 48 hours

Method	Se* (%)	Sp** (%)	VPP*** (%)	VPN**** (%)	Accuracy (%)
MCM 24 h	89,9	98,7	85,5	99,1	98
chromID CARBA 24h	92,4	98,8	86,9	99,3	98,3
MCM 48 h	92,4	98,6	84,9	99,3	98,1
chromID CARBA 48h	97,5	98,5	84,6	99,8	98,4

MCM; Mc Conkey agar plus 10 μg meropenem disk

Sensibility (Se*); Specificity (Sp**); Positive predictive value (VPP***); Negative predictive value (VPN****)