A new selective broth enrichment automated method for detection of carbapenem-resistant Enterobacteriaceae from rectal swabs

Fabio Arena\textsuperscript{a,b,⁎}, Tommaso Giani\textsuperscript{b,1}, Alberto Antonelli\textsuperscript{b}, Olga Lorenza Colavecchio\textsuperscript{b}, Patrizia Pecile\textsuperscript{c}, Bruno Viaggi\textsuperscript{d}, Riccardo Favilli\textsuperscript{e}, Gian Maria Rossolini\textsuperscript{b,c}

\textsuperscript{a} Department of Medical Biotechnologies, University of Siena, Siena, Italy
\textsuperscript{b} Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy
\textsuperscript{c} Clinical Microbiology and Virology Unit, Florence Careggi University Hospital, Florence, Italy
\textsuperscript{d} Neuro Intensive Care Unit, Florence Careggi University Hospital, Florence, Italy
\textsuperscript{e} Alifax s.r.l., Polverara, Italy

\textbf{ARTICLE INFO}

Keywords: Carriers, Colonization, Laboratory automation, Carbapenemase, Screening, Italy

\textbf{ABSTRACT}

We evaluated the performance of an automated, rapid (TAT 4–8 h), liquid-culture method for carbapenem-resistant Enterobacteriaceae (CRE) detection from rectal swabs, in a setting of KPC-producing \textit{Klebsiella pneumoniae} endemicity. With 600 samples (22 positive for CRE, 3.7%), the system sensitivity and specificity, at 8 h, were 100% and 99.2%, respectively.

The global dissemination of carbapenem-resistant Enterobacteriaceae (CRE) poses a serious threat to healthcare organizations (Rossolini, 2015). CRE infections are associated with high mortality rates due to the limited treatment options (Rossolini, 2015; Gutiérrez-Gutiérrez et al., 2016).

Gut colonization represents the prevalent source for CRE epidemic dissemination in the hospital setting (Lerner et al., 2015; Tacconelli et al., 2014). Infection control strategies mostly rely on screening programs based on rectal swab cultures, contact precautions and cohorting of colonized patients. These precautions must be preventively adopted while waiting for the screening results (Schwaber and Carmeli, 2014; Gagliotti et al., 2014). For this reason short turn-around time (TAT), high sensitivity and specificity, and affordable costs are essential requisites of screening tests for CRE colonization (Viau et al., 2016).

The US CDC reference method consists of a selective broth enrichment step followed by culture on agar medium (CDC. Laboratory Protocol for Detection of Carbapenem-Resistant or Carbapenemase-Producing, 2009) (TAT: 48–72 h). Therefore, different commercially available selective and indicator media and rapid protocols have been adopted allowing CRE detection in a shorter time frame (18–24 h) (Vrioni et al., 2012; Papadimitriou-Oliveris et al., 2014; Giani et al., 2012).

The purpose of this study was to evaluate the performance of a new automated method for the rapid detection of CRE from rectal swabs, which is able to report a putative positivity after 4–8 h of incubation, and definitive negative results after 8 h. The study was carried out in a Italian tertiary care University Hospital, where a surveillance program for CRE is active since 2014, from 1st March to 1st May 2015.

During the study period, rectal swabs for screening (FecalSwabTM system; Copan, Brescia, Italy) were processed in triplicate: 200 μL of the swab liquid medium processed with the SIDECAR® system (Alifax s.r.l. Polverara, Italy); 30 μL with direct culture on a chromogenic selective medium (chromID® CARBA SMART; bioMérieux, Marci Etoile, France) (CHSM); and 200 μL with the reference selective broth enrichment CDC method (BERM) slightly modified (Antonelli et al., 2016).

The SIDECAR system is an integrated system based on light scattering technology (http://www.alifax.com/products/bacteriology-line/show/sidecar). A first module monitors bacterial growth into specific culture broth providing real-time growth curves corresponding to quantitative bacterial cell densities in CFU/mL (maximum loading capability: 60 rectal swabs). The second module is an automated streaking system able to inoculate and store a maximum of 240 Petri dishes.

For the purpose of this study the HB&L CARBAPENEMASE KIT was used (lyophilized antibiotic supplement containing a carbapenem, a Gram-positive bacterial growth inhibitor and an antifungal). The KIT...
composition was optimized using mock specimens inoculated with different bacterial loads of previously characterized CREs (6 K. pneumoniae strains producing different carbapenemases: KPC-type, VIM-1, OXA-48; and a carbapenem non-susceptible porin-deficient strain).

For this evaluation study, 5 μL of each swab liquid medium were automatically plated on MacConkey agar and incubated overnight at 35 ± 2 °C (as a quality control) and 200 μL transferred in the HB&L CARBAPENEMASE KIT growth vial. Growth curves were automatically analyzed during an 8 h period at 5 min time intervals, using a software algorithm developed ad hoc. The software generates real-time results and discriminates between: i) absence of CRE growth; ii) presence of putative CRE growth; iii) sample invalid or turbid (not evaluable). The software, basing on distinctive features of the growth curves, can automatically differentiate between a CRE pattern and growth of a carbapenem-resistant Acinetobacter or Pseudomonas spp.

After 8 h monitoring, 5 μL of samples (including those signaled as invalid) were automatically streaked on a chromID® CARBA SMART (bioMérieux, Marcy l’Etoile, France).

The nature of suspect CRE strains grown in selective media was confirmed separately by MALDI-TOF identification (Vitek MS, bioMérieux) and detection of carbapenemases by real-time PCR (Antonelli et al., 2016) (Fig. 1). The K. pneumoniae strains KKBO-1, MR1106, 02C05 and KP7001 were used to determine the SIDECAR Limit of Detection (LOD) for blaKPC, blaVIM, blaOXA-48, and blaNDM targets, respectively (Antonelli et al., 2016). A suspension of 1 × 10^8 CFU/mL in normal saline of each strain was spiked in serial dilutions of a pool of negative rectal swabs samples (previously tested negative for CRE by CHSM and BERM). The CFU/mL count, was measured by direct plating 100 μL of the serial dilutions of each sample on chromID® CARBA SMART plates.

A total of 600 consecutive surveillance rectal swabs were analyzed. Using the reference method (BERM), 22 samples (3.7%) were positive for CRE (all KPC-producing K. pneumoniae; in 4 cases together with a KPC-producing E. coli).

No discrepancies were observed between results obtained with CHSM and BERM. The meropenem MICs range (MICs determined by reference broth microdilution method) were 8 - > 128 μg/mL for the K. pneumoniae and 1–16 μg/mL for the E. coli isolates.

The SIDECAR test was invalid with 71 samples (11.8%). Four of these invalid samples were positive for CRE by the BERM. In the majority of cases the sample was considered not evaluable due to the excessive turbidity of the medium after the inoculum in the HB&L CARBAPENEMASE KIT broth.

Sensitivity of the SIDECAR method increased progressively over the time reaching 100% after 8 h monitoring (NPV > 97%). Specificity was invariably above 99% (Table 1).

Six (33.3%) and twelve (66.6%) of the 18 evaluable positive samples were already correctly reported by SIDECAR at 4 and 6.5 h of incubation, respectively. After overnight incubation with SIDECAR all the four invalid samples positive for CRE with BERM were recovered.

The SIDECAR system LOD with mock specimens differed depending on the resistance mechanism. However the sensitivity of the system was good in all cases with LOD ranging from approx. 10–200 CFU/mL. The lower LOD was documented with the KPC-producing reference strain and the highest with the VIM-producing strain (Table 2).

The prompt recognition of carriers is of paramount importance to limit CRE dissemination in the hospital setting (Tacconelli et al., 2014). Furthermore, since patients with unknown colonization status are generally preventively placed on (functional) isolation, which is withdrawn in case of negative results, a rapid knowledge of screening results is expected to reduce days of unnecessary isolation.

Overall, the SIDECAR-based method resulted to be a rapid and sensitive method for the detection of intestinal colonization by CRE. Interestingly, compared to the reference method, the system was able to report putative CRE presence with significant advance (after only 4 and 6.5 h of incubation in some cases). Results obtained at 8 h were overall...
comparable to those obtained with reference method after 48 h. We can conclude that the SIDECAR system represents a fast culture-based alternative to BERM and CHSM, with TATs slightly longer than those of molecular methods. Comparing to molecular methods, the SIDECAR system has the advantages of more affordable costs and direct availability of the colonizing strain, without further sub-culture steps (Antonelli et al., 2016).

Among limitations of the system we should mention that SIDECAR is unable to distinguish between carbapenemase-producing Enterobacteriaceae (the subgroup of CRE considered epidemiologically more relevant) and non-carbapenemase producing CREs. Although a relatively high percentage of turbid or invalid samples was found, all the samples not evaluable at 4–8 h were recovered after the overnight culture step on solid medium automatically performed by the instrument. Slight modifications to the protocol are under evaluation to reduce the percentage of not evaluable cases.

In this study it was impossible to evaluate the performance with strains producing carbapenemases other than KPC, which could be considered a limitation of the study. However, in a preliminary evaluation, the system correctly detected two samples positive for blaOXA-48 and blaVIM-type (co-present with blaKPC) carbapenemases, respectively (European Congress of Clinical Microbiology and Infectious Diseases, 25–28 April 2015, Copenhagen. Abstract: P0152). Further evaluation steps will be needed in settings with presence of OXA-48, VIM-type and/or NDM carbapenemases.

Compared to other existing cultural methods for the detection of CRE intestinal colonization, the SIDECAR system represents an attractive and sustainable alternative due to its ability to process surveillance rectal swabs automatically and to detect positivity or confirm negativity for CRE carriage within a shorter timeframe. Considering that in settings with CRE endemic dissemination there is an increasing need to perform rectal swabs, the SIDECAR high level of automation represents a significant advantage for the Clinical Microbiology Laboratory and for infection control programs.

References


