Journal of Global Antimicrobial Resistance 21 (2020) 86–90

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

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

Rectal screening for carbapenemase-producing Enterobacteriaceae: a proposed workflow

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ARTICLE INFO

Article history:
Received 19 August 2019
Received in revised form 10 October 2019
Accepted 14 October 2019
Available online 19 October 2019

Keywords:
CPE
Carbapenemase
Klebsiella pneumoniae
MALDI-TOF/MS
WASPLab
Rectal screening

ABSTRACT

Objectives: Active screening is a crucial element for the prevention of carbapenemase-producing Enterobacteriaceae (CPE) transmission in healthcare settings. Here we propose a culture-based protocol for rectal swab CPE screening that combines CPE detection with identification of the carbapenemase type.

Methods: The workflow integrates an automatic digital analysis of selective chromogenic media (WASPLab®; Copan), with subsequent rapid tests for the confirmation of carbapenemase production [i.e. detection of *Klebsiella pneumoniae* carbapenemase (KPC)-specific peak by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) or a multiplex immunochromatographic assay identifying the five commonest carbapenemase types]. To evaluate the performance of this protocol in depth, data for 21 162 rectal swabs submitted for CPE screening to the Microbiology Unit of S. Orsola-Malpighi Hospital (Bologna, Italy) were analysed.

Results: Considering its ability to correctly segregate plates with/without Enterobacteriaceae, WASPLab Image Analysis Software showed globally a sensitivity and specificity of 100% and 79.4%, respectively. Of the plates with bacterial growth (n = 901), 693 (76.9%) were found to be positive for CPE by MALDI-TOF/MS (KPC-specific peak for *K. pneumoniae*) or by immunochromatographic assay. Only 2.8% (16/570) of KPC-positive *K. pneumoniae* strains were missed by the specific MALDI-TOF/MS algorithm, being detected by the immunochromatographic assay. The mean turnaround time needed from sample arrival to the final report ranged between 18 and 24 h, representing a significant time saving compared with manual reading.

Conclusion: This workflow proved to be fast and reliable, being particularly suitable for areas endemic for KPC-producing *K. pneumoniae* and for high-throughput laboratories.

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1. Introduction

The global spread of carbapenemase-producing Enterobacteriaceae (CPE) is of great concern to health services worldwide [1,2]. Epidemics of international proportion due to CPE have been described in different countries [3,4]. CPE represent an alarming and dramatic problem for many reasons. First, the morbidity associated with CPE infections is usually high, with a relevant clinical and economic impact [5]. Moreover, therapeutic options for CPE are often limited to a few drugs, thus leading to the emergence and spread of new resistance mechanisms (e.g. polymyxin resistance) [6].

In the last years, several approaches to fight the global burden of CPE have been proposed, among which hospital screening and surveillance protocols as well as strict infection control measures (e.g. hand hygiene, patient isolation, cohort nursing, personal protection equipment, environmental surface decontamination) have been adopted [7,8].

In this context, rectal screening for CPE carriage in high-risk patients represents a common and useful method to limit the spread of CPE [9]. Indeed, several guidance documents suggest performing active surveillance for early detection of colonised patients in order to prevent CPE introduction and transmission [4,10]. Ideally, CPE detection for active screening purposes should have a short turnaround time to ensure timely implementation of infection control measures [7]. Besides the rapid detection of CPE

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http://dx.doi.org/10.1016/j.jgar.2019.10.012
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carriers, identification of the type of carbapenemase is important for surveillance, infection control and treatment purposes [7].

Various laboratory protocols for CPE rectal screening, based both on culture techniques and molecular methods, have been described so far [11–15]. Nevertheless, the optimal workflow in term of sensitivity, specificity and cost:benefit ratio remains unclear and debated [16,17].

In this study, a simple and reliable protocol for rectal CPE screening in an endemic area of Northern Italy was evaluated. This workflow allows CPE detection and identification of carbapenemases by means of a culture-based technique that integrates an automatic digital analysis of chromogenic media (WASPLab®; Copan), followed by rapid confirmation tests [i.e. matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) and/or a multiplex immunochromatographic assay].

2. Materials and methods

2.1. Study setting

The proposed workflow is currently implemented as a routine diagnostic procedure for rectal CPE screening at the Microbiology Unit of S. Orsola-Malpighi University Hospital (Bologna, Italy). This protocol has been adapted to the CPE epidemiological distribution of our geographical area, following regional guidelines (available at: http://assr.regione.emilia-romagna.it/it/servizi/pubblicazioni/rapporti-documenti/indicazioni-pratiche-diagnosi-cpe-2017) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations (http://www.eucast.org). All procedures described below are performed following the manufacturer's instructions; detection of a *Klebsiella pneumoniae* carbapenemase (KPC)-specific peak by MALDI-TOF/MS has been extensively

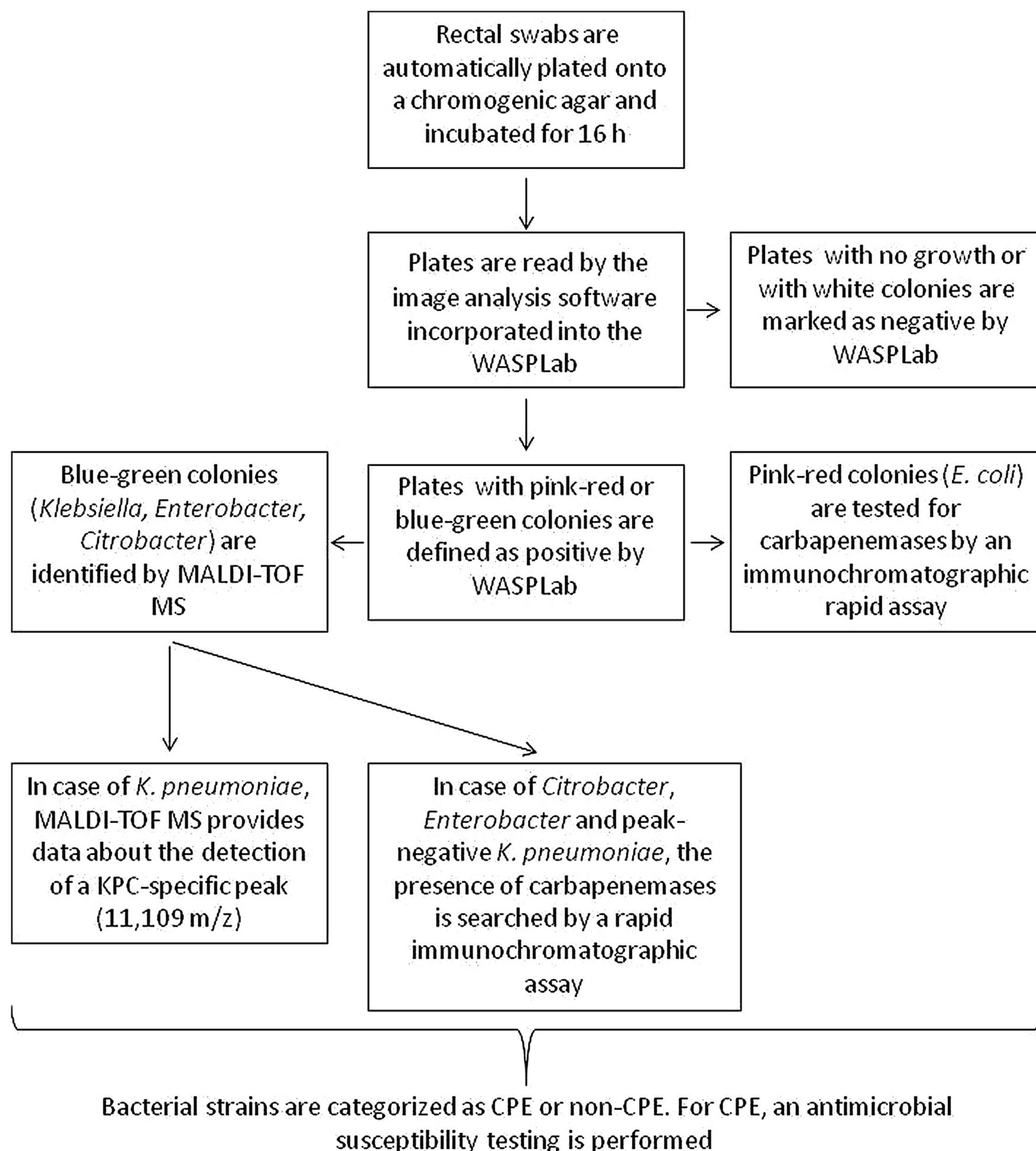

Fig. 1. Workflow for rectal carbapenemase-producing Enterobacteriaceae (CPE) screening.

validated before the introduction in the routine diagnostic workflow, as previously reported [18–20].

To evaluate the performance of the following protocol in depth, data regarding rectal swabs submitted for active CPE screening in a 4-month period (March–June 2019) were collected and analysed.

As suggested by the regional guidelines, rectal swabs are routinely collected from intensive care units, medical wards and surgical wards of the hospital as part of normal CPE screening both in naïve subjects and in the weekly follow-up of colonised patients.

This study was conducted according to the regulations of the Ethical Committee of S. Orsola-Malpighi Hospital as well as the 1964 Helsinki Declaration and its later amendments. All samples remained anonymous throughout the duration of the study.

2.2. Workflow for rectal carbapenemase-producing Enterobacteriaceae screening

The workflow for rectal CPE screening is shown in Fig. 1 and is described in detail below.

(i) Using WASPLab® (Copan, Brescia, Italy) for processing, rectal swabs (eSwab™; Copan) are automatically plated onto a selective chromogenic medium (CHROMagar KPC; Kima Meus, Padua, Italy). This medium contains a carbapenem agent for the direct isolation of Gram-negative bacteria with reduced susceptibility to carbapenems. Moreover, specific chromogens allow the development of colorimetric changes in bacterial colonies on the basis of the species: *Escherichia coli* colonies appear dark pink to reddish; colonies of *Klebsiella* spp., *Citrobacter* spp. and *Enterobacter* spp. are metallic blue; and *Pseudomonas* spp. and *Acinetobacter* spp. appear translucent or opaque cream-colored.

Inoculated plates are moved by a conveyor belt to a digital imager, where an image is obtained at time point 0, and are then moved into the WASPLab incubator, where the plates are incubated at 35 °C in an aerobic atmosphere for 16 h.

(ii) As described elsewhere [21–23], the WASPLab contains a digital imager to automatically take images of plates at programmable time points throughout incubation. In our workflow, a plate image is taken at 16 h post-inoculation, defined as the final incubation time on WASPLab for 100% detection sensitivity for CPE [24]. Plates are automatically screened by the Chromogenic Detection Module image analysis software incorporated into WASPLab. This software analyses the plates to identify differences in growth and colony colour and is programmed to correspond specifically to the medium type used by the laboratory. By means of an internal algorithm, the software automatically separates 'negative' from 'non-negative' plates: in our protocol, plates with no bacterial growth or with white/cream colonies are marked as negative for CPE, whereas plates with pink-red or green-blue colonies are defined as positive.

(iii) All of the plates segregated as negative by the WASPLab are quickly checked (30 plates at a time on WASPLab monitor) to confirm the absence of potentially CPE, whereas plates marked as positive are read to evaluate the presence and type of bacterial colonies.

(iv) In the case of blue/green colonies (i.e. *Klebsiella* spp., *Citrobacter* spp. and *Enterobacter* spp.), bacterial species identification is achieved by MALDI-TOF/MS using a Bruker microflex™ instrument (Bruker Daltonik GmbH, Bremen, Germany). In the case of *K. pneumoniae* strains only, along with species identification, MALDI provides data about the detection of a KPC-specific peak (11 109 m/z) by a dedicated algorithm integrated into the MALDI Biotyper system [18–20].

For strains belonging to other species (i.e. *Citrobacter*, *Enterobacter*) and for *K. pneumoniae* strains negative for the KPC-specific peak, a multiplex immunochromatographic assay (NG-Test CARBA 5; NG Biotech, Guipry, France) is performed [25,26]. This test allows rapid (~15 min) detection of the five commonest carbapenemases enzymes (i.e. KPC, IMP, VIM, NDM and OXA-48-like) directly on bacterial colonies.

(v) Pink-reddish colonies (i.e. *E. coli*) immediately undergo immunochromatographic assay for carbapenemase detection (NG-Test CARBA 5), with no MALDI-TOF/MS processing.

(vi) Finally, on the basis of MALDI-TOF/MS and/or NG-Test CARBA 5 results, bacterial strains are categorised as CPE or non-CPE. For CPE, species identification and the type of carbapenemase are reported and antimicrobial susceptibility testing is also performed.

3. Results

During the study period, a total of 21 162 rectal swabs were submitted to the Microbiology Unit for CPE screening. Using WASPLab Image Analysis Software, 16 088 plates (76.0%) were correctly segregated as negative for Enterobacteriaceae, with no false-negative results. Conversely, automatic reading marked 5074 plates (24.0%) as potentially positive, but only 901 (17.8%) of them showed colonies suggestive for Enterobacteriaceae. In the remaining cases (4173 plates; 19.7% of the total), no bacterial growth was found; the presence of abundant faecal material and other interfering substances led to the creation of pink/green halos on the plates, wrongly considered as bacterial colonies by the image software. Considering its ability to correctly segregate plates with/without Enterobacteriaceae, WASPLab showed globally a sensitivity and specificity for Enterobacteriaceae detection of 100% and 79.4%, respectively. Of the 901 plates with a bacterial growth, 693 (76.9%) were found to be positive for CPE by MALDI-TOF/MS (KPC-specific peak for *K. pneumoniae*) or by the immunochromatographic assay (total prevalence rate of CPE, 3.3%). In contrast, detection of carbapenemase was negative in 208 samples (23.1%) despite the presence of suggestive bacterial colonies. Overall, the chromogenic medium (CHROMagar KPC) showed a positive predictive value (PPV) for CPE of 76.9%.

Strains negative for carbapenemase production but grown on the selective chromogenic medium (i.e. potentially carbapenem-resistant) were represented mainly by *Klebsiella* spp. (46.7%) and *Enterobacter* spp. (30%).

In Table 1, CPE are stratified by bacterial species and type of carbapenemase detected. KPC was the most common enzyme in

Table 1
Carbapenemase-producing Enterobacteriaceae (n = 693) stratified by bacterial species and type of carbapenemase[a].

Species	KPC (n = 583; 84.1%)	NDM (n = 48; 6.9%)	VIM (n = 29; 4.2%)	OXA-48 (n = 21; 3.0%)	Double mechanism (n = 12; 1.7%)[b]
Klebsiella pneumoniae (n = 626; 90.3%)	570 (97.8%)	26 (54.2%)	10 (34.5%)	11 (52.4%)	9 (75.0%)
Escherichia coli (n = 46; 6.6%)	9 (1.5%)	22 (45.8%)	4 (13.8%)	8 (38.1%)	3 (25.0%)
Enterobacter spp. (n = 9; 1.3%)[c]	1 (0.2%)	0 (0.0%)	7 (24.1%)	1 (4.8%)	0 (0.0%)
Klebsiella oxytoca (n = 6; 0.9%)	1 (0.2%)	0 (0.0%)	4 (13.8%)	1 (4.8%)	0 (0.0%)
Citrobacter freundii (n = 5; 0.7%)	1 (0.2%)	0 (0.0%)	4 (13.8%)	0 (0.0%)	0 (0.0%)
Raoultella ornithinolytica (n = 1; 0.1%)	1 (0.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

[a] No case of IMP was observed.
[b] KPC + NDM (n = 6), NDM + OXA (n = 5) and VIM + OXA (n = 1).
[c] Including *Enterobacter asburiae*, *Enterobacter cloacae* and *Enterobacter aerogenes*.

our setting (583/693; 84.1%), followed by NDM (48/693; 6.9%), VIM (29/693; 4.2%) and OXA-48 (21/693; 3.0%); no case of IMP was observed, Simultaneous production of two different carbapenemases was detected in 12 samples (1.7%). In this latter group, the most frequent double resistance mechanisms were represented by KPC + NDM (6/12) and OXA-48 + NDM (5/12).

Overall, KPC-producing *K. pneumoniae* represented the vast majority of all CPE strains (>80%), whereas NDM-positive *K. pneumoniae* and *E. coli* each accounted for ~3.5%. In *Citrobacter* spp. and *Enterobacter* spp. strains, VIM was the most common carbapenemase detected. Finally, OXA-48 enzymes were found mainly in *K. pneumoniae* (52.4%) and *E. coli* (38.1%).

It is worth underlining that only 2.8% (16/570) of KPC-positive *K. pneumoniae* strains were missed by the specific MALDI-TOF/MS algorithm (KPC-specific peak), being detected by the immunochromatographic assay.

The proposed workflow was characterised by excellent performances in term of turnaround time (TAT) and ease of use. Indeed, the time needed for MALDI-TOF and immunochromatographic analysis is very short (<30 min) and the whole protocol is simple with reduced hands-on time, being particularly suitable for a high-throughput laboratory. Globally, the mean TAT needed from sample arrival to the final report (i.e. positive or negative for CPE; species identification and type of carbapenemase for positive samples) ranges between 18 and 24 h. Moreover, considering a high number of samples per day (400–500 rectal samples), automatic segregation of the plates leads to a time saving of 2–4 h compared with manual reading.

4. Discussion

Active rectal screening is a crucial element to prevent CPE transmission in healthcare settings [7]. Here we proposed a simple and reliable workflow for rectal CPE screening using an automatic digital analysis of chromogenic media (WASPLab®) and rapid confirmatory tests (MALDI-TOF/MS and an immunochromatographic assay).

First, we found that WASPLab Image Analysis Software is particularly accurate at identifying negative CPE plates with outstanding sensitivity (100%). On the other hand, it was observed that agar plates can be falsely called positive by the WASPLab software (specificity of ~80%) because of the presence of colorimetric pigmentation due to residual interfering substances.

The current results are in agreement with previous studies about the use of WASPLab for automated scoring of chromogenic media for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci [21,22]. Indeed, the major finding was 100% sensitivity for the detection of 'non-negative' specimens, with a lower specificity (89–90%) [21,22]. To the best of our knowledge, this is one the first reports about digital plate reading of a chromogenic medium for Gram-negative rods: the excellent ability in categorising negative plates, in conjunction with the high automation and greatly reduced labour costs, makes WASPLab an excellent choice for CPE screening in high-throughput laboratories.

Second, when evaluating the performance of the chromogenic medium, a good PPV for the detection of CPE was found. It is not surprising that in ~20% of cases, grown bacteria were negative for carbapenemase production by the confirmatory tests. Indeed, other mechanisms, different from carbapenemase, can be responsible of carbapenem resistance. In Enterobacteriaceae, the presence of extended-spectrum β-lactamases (ESBLs) or AmpC β-lactamases plus porin loss can lead to carbapenem resistance and subsequent growth on selective media [27].

After WASPLab analysis, except for *E. coli*, we suggest a two-step protocol to confirm carbapenemase production: (i) MALDI-TOF/MS species identification combined with detection of a KPC-specific peak; (ii) use of a multiplex immunochromatographic test for *K. pneumoniae* negative for KPC-associated peak and for all the remaining bacterial species (e.g. *Enterobacter* spp., *Citrobacter* spp.).

It has previously been shown that single-peak MALDI-TOF detection assay predicts KPC production with high accuracy in *K. pneumoniae*, with an overall PPV and negative predictive value (NPV) of 98.7% and 96.8%, respectively [19,20]. Here we confirmed the excellent sensitivity of MALDI-TOF for KPC detection, with <3% of KPC-positive *K. pneumoniae* missed by MALDI-TOF and detected only by the immunochromatographic test. In this context, it should be remembered that the gene encoding the 11 109-Da protein is lacking in some plasmids carrying the bla_{KPC} gene, leading to the possibility of false-negative results [28].

In our setting where KPC-producing *K. pneumoniae* is highly endemic, use of MALDI-TOF is of particular diagnostic utility considering the reduced TAT and extreme ease of use. Moreover, MALDI-TOF identification of KPC-positive *K. pneumoniae* saves the use of the immunochromatographic test with a significant cost reduction and a better cost:benefit ratio.

However, when necessary, the multiplex immunochromatographic test is easy to perform, with little hands-on time, and provides a final result in <15 min [25]. Moreover, unlike MALDI-TOF, the immunochromatographic test allows the detection of strains producing more than one carbapenemase at the same time. Although this information is not fundamental for patient management in term of infection control measures, it can be useful for epidemiological and surveillance purposes as well as for adequate treatment in the case of CPE infections.

The lack of data about double resistance mechanisms in the case of KPC-producing *K. pneumoniae* detected by MALDI-TOF could be a significant limitation of our protocol. However, CPE harbouring more than one carbapenemase gene are still very rare in Italy. Recent national surveillance data show a rate of 'double mechanism' strains of 1.3% in CPE bloodstream infections [29]. Moreover, by antimicrobial susceptibility testing, it is possible to eventually recover *K. pneumoniae* positive for both KPC and a metallo-β-lactamase (e.g. NDM, VIM) by checking strains showing resistance to ceftazidime/avibactam.

The main limitation of the proposed workflow is the inability to detect CPE strains harbouring rare types of carbapenemase belonging to class A or class B β-lactamase (i.e. GES, NmcA, IMI and SME, etc.). Indeed, these enzymes are uncommon worldwide and their distribution is mainly restricted to a few Gram-negative species [30]. Therefore, considering the marginal role of these carbapenemases in the Italian epidemiology, the proposed protocol may be well adapted to laboratories with a CPE distribution similar to our country.

At the same time, our workflow could be easily integrated with different phenotypic tests [i.e. biochemical colorimetric assays (Carba NP) or combination disk test methods] able to detect any carbapenemase activity [31,32].

A second limitation lies in the possibility to miss, by using CHROMagar KPC medium, strains harbouring carbapenemases with low-level hydrolytic activity towards carbapenems (i.e. OXA-48). However, the percentage of OXA-48-positive strains found during the study period (3.0%) is in line with other national epidemiological reports [33]. Thus, although additional in-depth evaluations of its performance against carbapenemases are needed, CHROMagar KPC medium could be suitable to support the growth of OXA-48-positive strains.

In conclusion, in view of laboratory automation, we propose a CPE screening workflow characterised by a high ease of use and a low TAT that combines different reliable technologies and improves process traceability.

This protocol allows to achieve both CPE detection and carbapenemase identification and is particularly suitable for areas endemic for KPC-positive *K. pneumoniae*. Further studies are needed to better evaluate the potential clinical impact of this protocol on patient management.

Acknowledgement

The authors would like to thank all of the technical staff and residents of the Microbiology Unit of S. Orsola-Malpighi Hospital (Bologna, Italy) for their excellent assistance.

Funding: None.

Competing interests: None declared.

Ethical approval: This study was conducted according to the regulations of the Ethical Committee of S. Orsola-Malpighi University Hospital and the 1964 Helsinki Declaration and its later amendments. All samples remained anonymous throughout the duration of the study.

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