# Journal Pre-proof

Rectal screening of carbapenemase-producing *Enterobacteriaceae*: a proposed workflow

Claudio Foschi, Paolo Gaibani, Donatella Lombardo, Maria Carla Re, Simone Ambretti

PII: S2213-7165(19)30266-8

DOI: https://doi.org/10.1016/j.jgar.2019.10.012

Reference: JGAR 1069

To appear in: *Journal of Global Antimicrobial Resistance*

Received Date: 19 August 2019

Revised Date: 10 October 2019

Accepted Date: 14 October 2019

Please cite this article as: Foschi C, Gaibani P, Lombardo D, Re MC, Ambretti S, Rectal screening of carbapenemase-producing *Enterobacteriaceae*: a proposed workflow, *Journal of Global Antimicrobial Resistance* (2019), doi: https://doi.org/10.1016/j.jgar.2019.10.012

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2019 Published by Elsevier.

Journal Pre-proof

**Rectal screening of carbapenemase-producing *Enterobacteriaceae*: a proposed workflow**

Claudio Foschi[a,b*], Paolo Gaibani[b], Donatella Lombardo[b], Maria Carla Re[a,b], Simone Ambretti[b]

[a]Microbiology, DIMES, University of Bologna, via Massarenti 9, Bologna, Italy

[b]Microbiology Unit, S.Orsola-Malpighi Hospital, Via Massarenti n 9, Bologna, Italy

*Corresponding author:

Claudio Foschi, Microbiology, DIMES, University of Bologna,

St. Orsola Hospital, Via Massarenti, 9, 40138 Bologna, Italy.

Tel: +39 051 2144513; Fax: +39 051 307397; email: claudio.foschi2@unibo.it

Journal Pre-proof

**HIGHLIGHTS**

- We propose a culture-based protocol that combines rectal CPE detection and carbapenamase identification
- The workflow integrates an automatic digital analysis of chromogenic media (WASPLab), with rapid tests for carbapenemase production (MALDI-TOF MS; immunochromatographic assay)
- Considering its ability to correctly segregate plates with/without *Enterobacteriaceae,* WASPLab showed a sensitivity and a specificity of 100% and 79.4%,

Journal Pre-proof

- The workflow proved to be fast and reliable, being particularly suitable for KPC-*K. pneumoniae* endemic areas


## ABSTRACT

**Objectives:** Active screening is a crucial element for the prevention of carbapenemase-producing *Enterobacteriaceae* (CPE) transmission in healthcare settings. Here, we proposed a culture-based protocol for rectal swab CPE screening that combines the detection of CPE and the identification of carbapenamase 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* KPC-specific peak by MALDI-TOF mass spectrometry; a multiplex immunochromatographic assay identifying the five commonest carbapenemase types). To in-depth evaluate the performance of this protocol, data about 21 162 rectal swabs submitted for CPE screening at the Microbiology of S. Orsola-Malpighi Hospital in Bologna were analyzed.

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

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


Journal Pre-proof

Journal Pre-proof

**KEYWORDS:** CPE; carbapenemases; *Klebsiella pneumoniae*; MALDI-TOF MS; WASPLab; rectal screening

## 1. INTRODUCTION

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

In the last years, several approaches to fight the global burden of CPE have been proposed. Among all, screening and surveillance hospital 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, the rectal screening for CPE carriage in high-risk patients represents a common and useful method to limit CPE spread (9). Indeed, several guidance documents suggest performing active surveillance for early detection of colonized patients, to prevent CPE introduction and transmission (4, 10). Ideally, CPE detection for active screening purposes should have a short turn-around time (TAT), to ensure timely implementation of infection control measures (7). Besides the rapid detection of CPE carriers, the identification of the type of carbapenemase is important for surveillance, infection control and treatment purposes (7).

Various laboratory protocols for CPE rectal screening, both based on culture techniques and on 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).

Journal Pre-proof

In this study, we evaluated a simple and reliable protocol for rectal CPE screening in an endemic area of the Northern Italy. This workflow allows CPE detection  and the identification of carbapenamases, by means of a culture-based technique that integrates an automatic digital analysis of chromogenic media (WASPLab, Copan), followed by rapid confirmation tests (MALDI-TOF mass spectrometry and/or a multiplex immunochromatographic assay).

## 2. MATERIALS AND METHODS

**2.1. Study setting.** The proposed workflow is currently implemented as routine diagnostic procedure for rectal CPE screening at the Microbiology Unit of S. Orsola-Malpighi University Hospital of Bologna, Italy. This protocol has been adapted to 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 EUCAST recommendations (www.eucast.org). All procedures described below are performed following manufacturer's instructions; the detection of a KPC-specific peak by MALDI-TOF MS has been extensively validated before the introduction in the routine diagnostic workflow, as previously reported (18-20).

To in-depth evaluate the performance of the following protocol, data about the rectal swabs submitted for active CPE screening in a four-months period (March-June 2019) were collected and analyzed.

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

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

Journal Pre-proof

**2.2. Rectal CPE screening workflow.** The workflow is shown in Figure 1 and it is described in detail below.

(i) Using WASPLab (Copan) for processing, rectal swabs (E-Swab, Copan, Brescia Italy) are automatically plated onto a selective chromogenic agar (CHROMagar KPC; Kima Meus, Italy). This medium contains a carbapenem agent for the direct isolation of Gram-negative bacteria harboring 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, whereas *Pseudomonas* and *Acinetobacter* species 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 then moved into the WASPLab incubator, where the plates are incubated at 35°C in 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 a 100%-detection sensitivity for CPE (24). Plates are automatically screened by the Chromogenic Detection Module (CDM) image analysis software incorporated into the WASPLab. This software analyses the plates to identify differences in growth and colony colour and it 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 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.

Journal Pre-proof

(iv) In case of blue/green colonies (i.e. *Klebsiella* spp., *Citrobacter* spp. and *Enterobacter* spp.), bacterial species identification is achieved by MALDI-TOF mass spectrometry (MS), using a Bruker Microflex instrument (Bruker Daltonics, Bremen, Germany). Only in case of *K. pneumoniae* strains, along with the 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, France) is performed (25, 26). This test allows the rapid (about 15 minutes) 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 the immunochromatographic assay for carbapenamase 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 categorized as CPE or non-CPE. For CPE, the species identification and the type of carbapenemase are reported and an antimicrobial susceptibility testing is performed, as well.

## 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, a total of 16 088 (76.1%) plates were correctly segregated as negative for *Enterobacteriaceae*, with no false-negative results. Conversely, the automatic reading marked 5 074 plates as potentially positive (23.9%), but only 901 (17.7%) of them showed colonies suggestive for *Enterobacteriaceae*. In the remaining cases (4 173 plates; 19.6% 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

Journal Pre-proof

plates with/without *Enterobacteriaceae*, WASPLab showed globally a sensitivity and a specificity for *Enterobacteriaceae* detection of 100% and 79.4%, respectively. Out of the 901 plates with a bacterial growth, 693 (76.9%) were found positive for CPE by MALDI-TOF MS (specific KPC-peak for *K. pneumoniae*) or by the immunochromatographic assay (total prevalence rate of CPE found: 3.3%). On the contrary, the 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 carbapenamase production but grown on the selective chromogenic medium (i.e. potentially carbapenem-resistant) were mainly represented mainly by *Klebsiella* spp. (46.7%) and *Enterobacter* spp. (30%).

In Table 1, CPE are stratified by the bacterial species and by the type of carbapenamase detected. KPC was the most common enzyme in 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, whereas in 12 samples (12/693; 1.7%) the contemporary production of two different carbapenemases was detected. In this latter group, the most frequent double mechanisms were represented by KPC plus NDM (6/12) and by OXA-48 plus 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* accounted each for about 3.5% of all CPE. 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%) and *E. coli* (38%). 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 characterized by excellent performances in term of TAT and ease of use. Indeed, the time needed for MALDI-TOF and immunochromatographic analyses is very short (less than 30 minutes) and the whole protocol is simple with reduced hands-on time, being

Journal Pre-proof

particularly suitable for a high-throughput laboratory. Globally, the mean TAT needed from the 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 to 24 hours. Moreover, considering a high number of samples per day (400-500 rectal samples), the automatic segregation of the plates leads to a time saving of 2-4 hours compared to a 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, by using an automatic digital analysis of chromogenic media (WASPLab) and rapid confirmatory tests (MALDI-TOF MS and an immunochromatographic assay).

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

Our results agree with previous studies about the use of WASPLab for automated scoring of chromogenic media for the detection of methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci (21-22). Indeed, the major finding was a 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 report about the digital plate reading of a chromogenic medium for Gram-negative rods: the excellent ability in categorizing 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 about 20% of cases, grown bacteria were negative for carbapenamase production at the confirmatory tests. Indeed, other mechanisms,

Journal Pre-proof

different from carbapenemase, can be responsible of carbapenem resistance. In *Enterobacteriaceae*, the presence of extended-spectrum β-lactamases (ESBL) or AmpC enzymes plus porin loss can lead to carbapenem resistance and to the 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 the detection of *K. pneumoniae* KPC-specific peak; (ii) the 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 been previously shown that single-peak MALDI-TOF detection assay predicts KPC production with high accuracy in *K. pneumoniae*, with overall PPV and NPV of 98.7% and 96.8%, respectively (19, 20). Here, we confirmed the excellent sensitivity of MALDI-TOF for KPC detection, with less than 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 the presence of KPC-*K. pneumoniae* is highly endemic, the use of MALDI-TOF is of particular diagnostic utility, considering the reduced TAT and the extreme ease-of-use. Moreover, MALDI-TOF identification of KPC-*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 it provides a final result in less than 15 minutes (25). Moreover, unlike MALDI-TOF, the immunochromatographic test allows the detection of strains producing more than one carbapenemase at the same time. Even though this information is not fundamental for patient's management in term of infection control measures, it can be useful for epidemiological and surveillance purposes, as well as for adequate treatment in case of CPE infections.

Journal Pre-proof

The lack of data about double mechanisms in 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, through the antimicrobial susceptibility testing, it's possible to eventually recover *K. pneumoniae* positive for both KPC and a metallo-beta-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 beta-lactamase (i.e. GES, NmcA, IMI and SME etc). Indeed, these enzymes are uncommonly worldwide and their distribution is mainly restricted to 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 [carbaNP] 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 carbapenamases with low-level hydrolytic activity toward carbapenems (i.e. OXA-48). However, the percentage of OXA-48 positive strains found during the study period (i.e. 3%) is in line with other national epidemiological reports (33). Thus, even though 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 proposed a CPE screening workflow characterized by a high ease of use and a low TAT, that combines different reliable technologies and that improves process traceability.

Journal Pre-proof

This protocol allows to obtain both CPE detection and carbapenemase identification and it is particularly suitable for KPC-*K. pneumoniae* endemic areas.

Further studies are needed to better evaluate the potential clinical impact of this protocol on patients' management.

## DECLARATIONS

**Funding:** This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Competing Interests:** This study was conducted in the absence of any commercial or financial relationships that could be construed as potential conflict of interest. All the authors declare the absence of any dual or conflicting interest.

**Ethical Approval:** This study was conducted according to the regulations of the S. Orsola-Malpighi University Hospital Ethical Committee and to the 1964 Helsinki declaration and its later amendments. All the samples were kept anonymous throughout the duration of the study.

## AUTHOR'S CONTRIBUTION

SA, PG, CF and MCR conceived and designed the study. CF and DL analysed the data. CF, PG and SA wrote the paper. All authors read, reviewed and approved the final version of the manuscript.

## ACKNOWLEDGEMENTS

We would like to thank all the technical staff and residents of the Microbiology Unit for their excellent assistance.

Journal Pre-proof

Journal Pre-proof

**REFERENCES**

1- Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. Carbapenemases in *Klebsiella pneumoniae* and other *Enterobacteriaceae*: an evolving crisis of global dimensions. Clin Microbiol Rev 2012;25:682-707. https://doi.org/10.1128/CMR.05035-11.

2- European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2014. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm: ECDC; 2015.

3- Giani T, Pini B, Arena F, Conte V, Bracco S, Migliavacca R; AMCLI-CRE Survey Participants, Pantosti A, Pagani L, Luzzaro F, Rossolini GM. Epidemic diffusion of KPC carbapenemase-producing *Klebsiella pneumoniae* in Italy: results of the first countrywide survey, 15 May to 30 June 2011. Euro Surveill 2013;18:pii=20489.

4- Grundmann H, Livermore DM, Giske CG, Canton R, Rossolini GM, Campos J, et al.; CNSE Working Group. Carbapenem-non-susceptible *Enterobacteriaceae* in Europe: conclusions from a meeting of national experts. Euro Surveill 2010;15:pii=19711. https://doi.org/10.2807/ese.15.46.19711-en.

5- Daikos GL, Tsaousi S, Tzouvelekis LS, Anyfantis I, Psichogiou M, Argyropoulou A, et al. Carbapenemase-producing *Klebsiella pneumoniae* bloodstream infections: lowering mortality by antibiotic combination schemes and the role of carbapenems. Antimicrob Agents Chemother 2014;58:2322-2328. https://doi.org/10.1128/AAC.02166-13.

6- Monaco M, Giani T, Raffone M, Arena F, Garcia-Fernandez A, Pollini S; Network EuSCAPE-Italy, Grundmann H, Pantosti A, Rossolini GM. Colistin resistance superimposed to endemic carbapenem-resistant *Klebsiella pneumoniae*: a rapidly evolving problem in Italy, November 2013 to April 2014. Euro Surveill 2014;19:pii=20939. https://doi.org/10.2807/1560-7917.es2014.19.42.20939.

Journal Pre-proof

7- Akova M, Daikos GL, Tzouvelekis L, Carmeli Y. Interventional strategies and current clinical experience with carbapenemase-producing Gram-negative bacteria. Clin Microbiol Infect 2012;18: 439-448. https://doi.org/10.1111/j.1469-0691.2012.03823.x.

8- De Rosa FG, Corcione S, Cavallo R, Di Perri G, Bassetti M. Critical issues for *Klebsiella pneumoniae* KPC-carbapenemase producing *K. pneumoniae* infections: a critical agenda. Future Microbiol 2015;10:283-294. https://doi.org/10.2217/fmb.14.121.

9- European Centre for Disease Prevention and Control. Risk assessment on the spread of carbapenemase-producing *Enterobacteriaceae* (CPE) through patient transfer between healthcare facilities, with special emphasis on cross-border transfer. Stockholm: ECDC, 2011.

10- Centers for Disease Control and Prevention (CDC). Guidance for control of infections with carbapenem-resistant or carbapenemase-producing *Enterobacteriaceae* in acute care facilities. MMWR Morb Mortal Wkly Rep 2009;58:256-260.

11- Antonelli A, Arena F, Giani T, Colavecchio OL, Valeva SV, Paule S, et al. Performance of the BD MAX™ instrument with Check-Direct CPE real-time PCR for the detection of carbapenemase genes from rectal swabs, in a setting with endemic dissemination of carbapenemase-producing *Enterobacteriaceae*. Diagn Microbiol Infect Dis 2016;86:30-34. https://
doi.org/10.1016/j.diagmicrobio.2016.06.002

12- Shorten RJ, Ashcroft P, Dodgson AR. Rectal swab screening for the detection of carriage of carbapenemase-producing Enterobacteriaceae. J Hosp Infect 2016;94:131-132. https://doi.org/10.1016/j.jhin.2016.07.009.

13- Tato M, Ruiz-Garbajosa P, Traczewski M, Dodgson A, McEwan A, Humphries R, et al. Multisite Evaluation of Cepheid Xpert Carba-R Assay for Detection of Carbapenemase-Producing Organisms in Rectal Swabs. J Clin Microbiol 2016;54:1814-1819. https://doi.org/10.1128/JCM.00341-16.

Journal Pre-proof

14- Arena F, Giani T, Antonelli A, Colavecchio OL, Pecile P, Viaggi B, et al. A new selective broth enrichment automated method for detection of carbapenem-resistant *Enterobacteriaceae* from rectal swabs. J Microbiol Methods 2018;147:66-68. https://doi.org/ 10.1016/j.mimet.2018.03.002.

15- O'Connor C, Kiernan MG, Finnegan C, O'Hara M, Power L, O'Connell NH, et al. An optimized work-flow to reduce time-to-detection of carbapenemase-producing *Enterobacteriaceae* (CPE) using direct testing from rectal swabs. Bioengineered 2017;8:217-224. https://doi.org/10.1080/21655979.2016.1222335.

16- Ciesielczuk H, Phee LM, Dolphin H, Wilks M, Cherian BP, Wareham DW. Optimal detection of carbapenemase-producing *Enterobacteriaceae* from rectal samples: a role for enrichment? J Hosp Infect 2018;98:270-274. https://doi.org/10.1016/j.jhin.2017.10.012.

17- Simner PJ, Martin I, Opene B, Tamma PD, Carroll KC, Milstone AM. Evaluation of Multiple Methods for Detection of Gastrointestinal Colonization of Carbapenem-Resistant Organisms from Rectal Swabs. J Clin Microbiol 2016;54:1664-1667. https://doi.org/10.1128/JCM.00548-16.

18- Cordovana M, Kostrzewa M, Glandorf J, Bienia M, Ambretti S, Pranada AB. A Full MALDI-Based Approach to Detect Plasmid-Encoded KPC-Producing *Klebsiella pneumoniae*. Front Microbiol 2018;9:2854. https://doi.org/10.3389/fmicb.2018.02854.

19- Gaibani P, Galea A, Fagioni M, Ambretti S, Sambri V, Landini MP. Evaluation of Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry for Identification of KPC-Producing *Klebsiella pneumoniae*. J Clin Microbiol 2016;54:2609-2613. https://doi.org/10.1128/JCM.01242-16.

20- Gaibani P, Ambretti S, Tamburini MV, Vecchio Nepita E, Re MC. Clinical application of Bruker Biotyper MALDI-TOF/MS system for real-time identification of KPC production in *Klebsiella pneumoniae* clinical isolates. J Glob Antimicrob Resist 2018;12:169-170. https://doi.org/10.1016/j.jgar.2018.01.016.

Journal Pre-proof

Journal Pre-proof

21- Faron ML, Buchan BW, Vismara C, Lacchini C, Bielli A, Gesu G, et al. Automated Scoring of Chromogenic Media for Detection of Methicillin-Resistant *Staphylococcus aureus* by Use of WASPLab Image Analysis Software. J Clin Microbiol 2016;54:620-624. https://doi.org/doi: 10.1128/JCM.02778-15.

22- Faron ML, Buchan BW, Coon C, Liebregts T, van Bree A, Jansz AR, et al. Automatic Digital Analysis of Chromogenic Media for Vancomycin-Resistant-*Enterococcus* Screens Using Copan WASPLab. J Clin Microbiol 2016;54:2464-2469. https://doi.org/10.1128/JCM.01040-16

23- Kirn TJ. Automatic Digital Plate Reading for Surveillance Cultures. J Clin Microbiol 2016;54:2424-2426. https://doi.org/10.1128/JCM.01279-16.

24- Cherkaoui A, Renzi G, Vuilleumier N, Schrenzel J. Copan WASPLab automation significantly reduces incubation times and allows earlier culture readings. Clin Microbiol Infect 2019; pii: S1198-743X(19)30152-1. https://doi.org/10.1016/j.cmi.2019.04.001.

25- Hopkins KL, Meunier D, Naas T, Volland H, Woodford N. Evaluation of the NG-Test CARBA 5 multiplex immunochromatographic assay for the detection of KPC, OXA-48-like, NDM, VIM and IMP carbapenemases. J Antimicrob Chemother 2018;73:3523-3526. https://doi.org/10.1093/jac/dky342.

26- Takissian J, Bonnin RA, Naas T, Dortet L. NG-Test Carba 5 for Rapid Detection of Carbapenemase-Producing Enterobacterales from Positive Blood Cultures. Antimicrob Agents Chemother 2019;63:pii:e00011-19. https://doi.org/10.1128/AAC.00011-19.

27- Paterson DL. Resistance in gram-negative bacteria: *Enterobacteriaceae*. Am J Infect Control 2006;34:S20-S28. https://doi.org/10.1016/j.ajic.2006.05.238

28- Youn JH, Drake SK, Weingarten RA, Frank KM, Dekker JP, Lau AF. Clinical Performance of a Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry Method for

Journal Pre-proof

Detection of Certain blaKPC-Containing Plasmids. J Clin Microbiol 2016;54:35-42. https://doi.org/10.1128/JCM.01643-15

29- Iacchini S, Sabbatucci M, Gagliotti C, Rossolini GM, Moro ML, Iannazzo S, et al. Bloodstream infections due to carbapenemase-producing *Enterobacteriaceae* in Italy: results from nationwide surveillance, 2014 to 2017. Euro Surveill 2019;24(5). https://doi.org/10.2807/1560-7917.ES.2019.24.5.1800159.

30- Diene SM, Rolain JM. Carbapenemase genes and genetic platforms in Gram-negative bacilli: *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter* species. Clin Microbiol Infect 2014;20:831-838. https://doi.org/10.1111/1469-0691.12655.

31- Doyle D, Peirano G, Lascols C, Lloyd T, Church DL, Pitout JD. Laboratory detection of *Enterobacteriaceae* that produce carbapenemases. J Clin Microbiol 2012;50:3877-3880. https://doi.org/10.1128/JCM.02117-12.

32- Dortet L, Poirel L, Nordmann P. Rapid identification of carbapenemase types in *Enterobacteriaceae* and *Pseudomonas* spp. by using a biochemical test. Antimicrob Agents Chemother 2012;56:6437-6440. https://doi.org/10.1128/AAC.01395-12.

33- Barbarini D, Russello G, Brovarone F, Capatti C, Colla R, Perilli M, et al. Evaluation of carbapenem-resistant *Enterobacteriaceae* in an Italian setting: report from the trench. Infect Genet Evol 2015;30:8-14. https://doi.org/10.1016/j.meegid.2014.11.025.

Journal Pre-proof

**FIGURE LEGENDS**

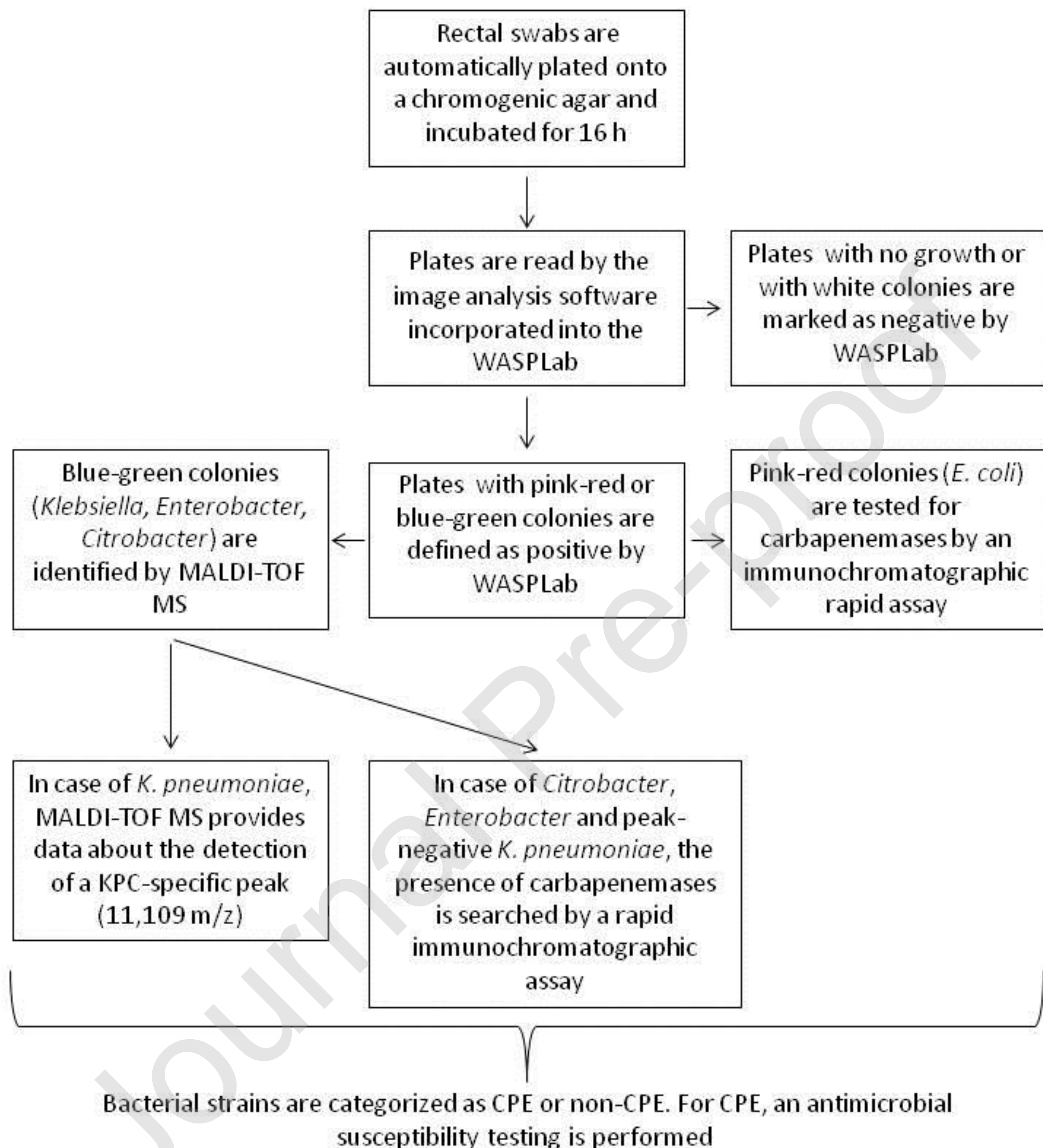


**Figure 1. Workflow for rectal CPE screening.**

Journal Pre-proof

**TABLES**

**Table 1. CPE stratified by the bacterial species and type of carbepenemase.**

| **CPE** (n=693) | **KPC** (n=583; 84.1%) | **NDM** (n=48; 6.9%) | **VIM** (n=29; 4.1%) | **IMP** (n=0; 0.0%) | **OXA-48** (n=21; 3.0%) | **Double mechanism**[#] (n=12; 1.7%) |
|---|---|---|---|---|---|---|
| *Klebsiella pneumoniae* (n=626; 90.3%) | 570 (97.7%) | 26 (54.1%) | 10 (34.5%) | 0 (0.0%) | 11 (52.3%) | 9 (75.0%) |
| *Escherichia coli* (n=46; 6.6%) | 9 (1.5%) | 22 (45.9%) | 4 (13.8%) | 0 (0.0%) | 8 (38.1%) | 3 (25.0%) |
| *Enterobacter spp.** (n=9; 1.3%) | 1 (0.2%) | 0 (0.0%) | 7 (24.1%) | 0 (0.0%) | 1 (4.8%) | 0 (0.0%) |
| *Klebsiella oxytoca* (n=6; 0.8%) | 1 (0.2%) | 0 (0.0%) | 4 (13.8%) | 0 (0.0%) | 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%) | 0 (0.0%) |
| *Roultella ornithinolytica* (n=1; 0.1%) | 1 (0.2%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |

[*] including *E. asburiae*, *E. cloacae* and *E. aerogenes*

[#] 6 cases KPC+NDM; 5 cases NDM+OXA; 1 case VIM+OXA

Journal Pre-proof