Journal of Hospital Infection xxx (2014) 1–3

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevierhealth.com/journals/jhin

Short report

New screening method to detect carriage of carbapenemase-producing Enterobacteriaceae in patients within 24 hours

N.M. Hanemaaijer [a,b,*,†], R.H.T. Nijhuis [a,†], B.J. Slotboom [a], E.M. Mascini [a], A.A. van Zwet [a]

[a] Department of Medical Microbiology and Medical Immunology, Rijnstate, Velp, The Netherlands
[b] Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, The Netherlands

ARTICLE INFO

Article history:
Received 6 October 2013
Accepted 18 March 2014
Available online xxx

Keywords:
Active surveillance
Carbapenemase-producing Enterobacteriaceae
Carbapenemases
Real-time polymerase chain reaction
Screening

SUMMARY

Rapid identification of patients colonized with carbapenemase-producing Enterobacteriaceae (CPE) is essential to prevent introduction and the spread of CPE in the hospital. This article presents the results of a new screening method to detect patients colonized with CPE within 24 h after hospital admission. From high-risk patients rectal and throat swabs were collected and incubated overnight, after which DNA was isolated and tested for the most prevalent CPE genes (KPC, NDM, OXA-48, VIM and IMP) by a ligation-mediated real-time polymerase chain reaction. In 14 months 454 patients were screened; in six patients CPE were detected (carriage rate 1.3%).

Introduction

Carbapenemase-producing Enterobacteriaceae (CPE) have emerged rapidly and extensively worldwide. Invasive infections with carbapenem-resistant strains are associated with high mortality rates (up to 40–50%), emphasizing the need for fast and accurate detection. In accordance with the Dutch meticillin-resistant *Staphylococcus aureus* (MRSA) policy, known as 'search and destroy', patients suspected of colonization by CPE should be hospitalized in single rooms, applying contact isolation procedures to prevent dissemination of CPE to other patients.

Since there has been documentation of high CPE prevalence in widespread geographical regions, screening for asymptomatic carriers should at least be performed in all patients who have been hospitalized in a foreign hospital, as described in the national guideline 'Multi-resistant micro-organisms' by the Dutch Working Party of Infection Prevention.[1]

The strategy as recommended in the Dutch guideline for the detection of highly resistant micro-organisms is a two-step procedure that consists of a screening step, based on the detection of reduced susceptibility to carbapenems, followed

* Corresponding author. Address: Department of Medical Microbiology, Radboud University Medical Center, P.O. Box 9101, 6500HB Nijmegen, The Netherlands. Tel.: +31 243614356; fax: +31 243540216.
E-mail address: n.hanemaaijer@mmb.umcn.nl (N.M. Hanemaaijer).
† Authors contributed equally to this work.

0195-6701/$ – see front matter
http://dx.doi.org/10.1016/j.jhin.2014.03.003

by phenotypic and genotypic confirmation steps. Screening for gastrointestinal colonization of CPE has been shown to be helpful in reducing the incidence and secondary transmission in the hospital.[2,3] Unfortunately, there is no strong evidence on the best screening strategy to achieve this.[4]

This article presents the results of a new screening method to detect carriage of CPE in patients within 24 h during a 14-month period.

Methods

Screening method

A rectal swab and a throat swab were collected and transported in ∑-Transwab® medium to the laboratory according to the manufacturer's guidelines (MW&E, Corsham, UK). A volume of 100 μL of both the ∑-medium from the rectal swab and of the ∑-medium from the throat swab were incubated together in two different broths each (0.25 μg/mL ertapenem with 50 μg/mL vancomycin and 2 μg/mL ceftazidime with 50 μg/mL vancomycin). After overnight incubation (35 ± 2 °C), 250 μL of each of the two broths were merged and mixed well. Following a wash step and subsequent centrifugation (1 min, 14,000 rpm), the pellet was suspended in 100 μL of the enzyme achromopeptidase and 20 μL internal control (IC). After incubation for 15 min at 37 °C followed by 5 min at 95 °C and centrifugation (1 min, 14,000 rpm), the supernatant was used for detection of the most prevalent carbapenemase genes in Gram-negative bacteria (KPC, NDM, OXA-48, VIM and IMP) using the Check-MDR Carba assay (Check-Points, Wageningen, The Netherlands). The Check-MDR Carba assay was executed according to the manufacturer's guidelines. To each run, a negative isolation control was added to control possible inhibition in each sample. A sample was considered inhibited when the C_T-value of the IC of the corresponding sample was more than two C_T-values higher than the IC of the negative isolation control.

Culture

In the case of a positive result with the Check-MDR Carba assay, both broths were subcultured on Columbia blood agar and MacConkey agar (both Oxoid, Basingstoke, UK). The species were determined with Matrix-assisted laser desorption–ionization time of flight mass spectrometry (MALDI-TOF MS, Bruker Daltonik GmbH, Bremen, Germany) and antibiotic susceptibility was measured by Vitek2 (bioMérieux, Marcy l'Etoile, France) and confirmed by E-test. Strains were sent to the Dutch National Institute for Public Health and the Environment (RIVM) for identification of the specific class of β-lactamase.

Patient population

After in-house validation, the method was implemented for routine screening on CPE high-risk patients. We defined a high-risk patient as any patient who had been hospitalized in a foreign hospital in the preceding two months. From these patients a throat swab and a rectal swab were collected. The detection of CPE was subsequently performed as described above.

Results

A total of 465 throat and rectal samples from 454 different patients who had been hospitalized abroad were screened between 1st January 2012 and 30th April 2013. Results for the detection of CPE were all available within 24 h. For 384 (85%) of the patients, the country of hospitalization was known. Most patients had been hospitalized in western and southern Europe.

In six of the 454 patients CPE were detected (carriage rate 1.3%). Five patients were colonized with one CPE and one patient with three different isolates harbouring a carbapenemase gene. These patients were screened because of previous hospitalization in southern Europe (Italy, Greece), Africa (Morocco (two), Egypt) or Asia (Jordan). Patient data are recorded in Table I. Detected CPE were identified as five *Klebsiella pneumoniae* (three OXA-48, two KPC), two *Escherichia coli* (both OXA-48) and one *Enterobacter cloacae* (OXA-48). All but one isolate grew in both (ceftazidime and ertapenem) broths. The isolate detected only in the ertapenem broth was identified as *E. coli* (OXA-48) and was extended-spectrum β-lactamase negative.

Discussion

This article describes the routine application of a new screening method to detect carriage of CPE in patients who had been hospitalized abroad.

In the screening method, we used an overnight broth enrichment, followed by real-time polymerase chain reaction for the detection of CPE. Other studies, including the Dutch guidelines, use selective media in the screening step.[5] In this study, the Check-MDR Carba was selected since it was the only commercially available assay able to detect the most prevalent carbapenemase genes (KPC, NDM, OXA-48, VIM and IMP) in one reaction. The choice of this assay was supported by a recent evaluation demonstrating 100% specificity and sensitivity using a wide variety of strains carrying different carbapenemase genes *in vitro*.[6] Despite an excellent performance, a limitation of this assay is the inability to detect the precise type of carbapenemase. Furthermore, as with all molecular assays, novel unidentified carbapenemase genes may not be detected using this assay.

A recent algorithm for carbapenemase detection suggested a three-step method in which an isolate is screened on a special medium, such as the 'Supercarba' medium.[7] Thereafter, the Carba NP test (based on in-vitro hydrolysis of a carbapenem) is performed and subsequently the carbapenemase genes are detected and typed by molecular identification. The results are available within 24 h, which is comparable to our approach. One disadvantage of our screening method is the higher cost associated with molecular diagnostics. However, Tijet *et al.* recently reported an evaluation of the Carba NP test and confirmed its 100% specificity, but the sensitivity and negative predictive value were 72.5% and 69.2% respectively.[8] Mucoid strains and enzymes with low carbapenemase activity (especially bla_{OXA-48}) were associated with false-negative results, which is a serious limitation since OXA-48 is emerging globally and is one of the major clinically relevant carbapenemases. This is underlined by the results of our study, in which the majority of detected isolates were actually OXA-48-producing isolates.

Please cite this article in press as: Hanemaaijer NM, et al., New screening method to detect carriage of carbapenemase-producing Enterobacteriaceae in patients within 24 hours, Journal of Hospital Infection (2014), http://dx.doi.org/10.1016/j.jhin.2014.03.003

Table I
Epidemiological data for detected carbapenemase-producing Enterobacteriaceae isolates

Patients: sex, age, country	Date	Isolate	Carbapenemase	MIC meropenem (μg/mL)	Patient information
Male, 60 years, The Netherlands	09/08/2012	*Klebsiella pneumoniae*	KPC	>32	Hospitalization for two weeks including one week ICU in Italy after an out-of-hospital reanimation.
Female, 61 years, Greece	05/11/2012	*K. pneumoniae*	KPC	>32	Hospitalization for 3.5 months including ICU in Greece because of respiratory failure due to an exacerbation of COPD. Before hospitalization already living in Greece for several years.
Male, 85 years, Morocco	27/12/2012	*K. pneumoniae*	OXA-48	12	Hospitalization including ICU in Morocco because of gastrointestinal bleeding. Before hospitalization already living in Morocco for several years.
Female, 79 years, Morocco	14/01/2013	*Enterobacter cloacae*	OXA-48	6	Hospitalization for 2–3 months in Morocco because of a CVA and a myocardial infarction. Before hospitalization already living in Morocco for several years.
Male, 58 years, The Netherlands	21/01/2013	*K. pneumoniae*	OXA-48	16	Hospitalization for 2.5 weeks in Jordan because of pneumonia.
Male, 53 years, The Netherlands	20/03/2013	*K. pneumoniae*, *Escherichia coli* (2×)	OXA-48	1.5, 2, 0.75, respectively	Hospitalization including surgery in Egypt for an acute herniated disc.

MIC, minimum inhibitory concentration; ICU, intensive care unit; COPD, chronic obstructive pulmonary disease; CVA, cerebrovascular accident.

In conclusion, this study reports the results of a new screening method to identify CPE in patients within 24 h. Since early identification and isolation of carriers are key components of an effective infection control strategy in hospitals, this method may be a major improvement in the challenge to reduce the spread of CPE.

Conflict of interest statement
A.A. van Zwet is an advisor for Check-Points.

Funding sources
None.

References

1. Dutch Working Party on Infection Prevention. National guideline 'multi-resistant micro-organisms'; 2012. Available at: http://wip.nl.
2. NVMM Guideline Laboratory detection of highly resistant microorganisms, version 2.0; 2012. Available at: http://www.nvmm.nl.
3. Poulou A, Voulgari E, Vrioni G, *et al*. Imported *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* clones in a Greek hospital: impact of infection control measures for restraining their dissemination. *J Clin Microbiol* 2012;**50**:2618–2623.
4. Nordmann P, Gniadkowski M, Giske CG, *et al*. Identification and screening of carbapenemase-producing Enterobacteriaceae. *Clin Microbiol Infect* 2012;**18**:432–438.
5. Wilkinson KM, Winstanley TG, Lanyon C, *et al*. Comparison of four chromogenic culture media for carbapenemase-producing Enterobacteriaceae. *J Clin Microbiol* 2012;**50**:3102–3104.
6. Cuzon G, Naas T, Bogaerts P, Glupczynski Y, Nordmann P. Probe ligation and real-time detection of KPC, OXA-48, VIM, IMP, and NDM carbapenemase genes. *Diagn Microbiol Infect Dis* 2013;**76**:502–505.
7. Nordmann P, Poirel L. Strategies for identification of carbapenemase-producing Enterobacteriaceae. *J Antimicrob Chemother* 2013;**68**:487–489.
8. Tijet N, Boyd D, Patel SN, Mulvey MR, Melano RG. Evaluation of the Carba NP test for rapid detection of carbapenemase-producing Enterobacteriaceae and *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2013;**57**:4578–4580.

Please cite this article in press as: Hanemaaijer NM, et al., New screening method to detect carriage of carbapenemase-producing Enterobacteriaceae in patients within 24 hours, Journal of Hospital Infection (2014), http://dx.doi.org/10.1016/j.jhin.2014.03.003