Journal of Hospital Infection 96 (2017) 123–128

Available online at www.sciencedirect.com

Journal of Hospital Infection

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

Detection of colonization by carbapenem-resistant organisms by real-time polymerase chain reaction from rectal swabs in patients with chronic renal disease

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ARTICLE INFO

Article history:
Received 24 January 2017
Accepted 20 March 2017
Available online 24 March 2017

Keywords:
Rectal swabs
Surveillance
Real-time PCR

SUMMARY

Background: Carbapenem-resistant organism (CRO) colonization is a serious problem that increases the risk of infection and contributes to dissemination of antimicrobial resistance in healthcare-associated environments. The risk of acquisition and dissemination of CRO is high in chronic renal failure patients and the surveillance culture is recommended as a component of infection control programmes.

Aim: To assess colonization by CRO, comparing phenotypic and molecular-based methods of diagnostics, in rectal swabs in a large population of chronic renal failure patients.

Methods: A total of 1092 rectal swabs (ESwab™) were collected at two different times from 546 chronic kidney disease (CKD) patients from a specialized tertiary care university centre. They were divided into three groups: conservative treatment ($N = 129$), dialysis ($N = 217$), and transplanted patients ($N = 200$). A chromogenic (CHROMagar™) KPC agar and the multiplex real-time polymerase chain reaction (qPCR) targeting carbapenemase-encoding genes were tested as phenotypic and molecular screening for carbapenemase production. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and conventional PCR were also performed on the isolates grown on chromogenic agar.

Findings: Among the 1092 samples, 150 (13.7%) were identified as CRO producers according to chromogenic agar. Only 26 (2.4%) were confirmed as KPC by conventional PCR. According to qPCR direct from swab, 31 (2.8%) were positive for KPC, 39 (3.6%) for GES, and three (0.3%) for SPM with kappa index of 0.256.

Conclusion: The qPCR technique provides faster results when compared to culture method and enables rapid implementation of control measures and interventions to reduce the spread of CRO in healthcare settings, especially among CKD patients.

Introduction

The global spread of carbapenem-resistant organisms (CRO) is a critical public health issue, especially in immunocompromised individuals such as chronic kidney disease (CKD)

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http://dx.doi.org/10.1016/j.jhin.2017.03.021
0195-6701/

patients. CRO colonization and infection in CKD individuals are increasingly reported, and infection has become a major cause of morbidity in all stages of the disease which may directly contribute to mortality [1].

The treatment of these micro-organisms is difficult due to the production of enzymes responsible for conferring resistance to carbapenems. Resistance to carbapenems is caused by the production of carbapenem-hydrolysing enzymes (serine carbapenemases and metallo-β-lactamases), or by a combination of membrane impermeability with the production of extended-spectrum β-lactamases (ESBLs), AmpC or overexpression of efflux pump and penicillin-binding protein alterations. Carbapenem-hydrolysing enzymes are the most widespread mechanisms since some genes encoding carbapenem resistance, such as bla_{NDM}, bla_{OXA-48}, bla_{IMP}, bla_{VIM}, bla_{SPM} and bla_{KPC}, are carried out by mobile DNA elements [2].

Gastrointestinal microbiota is the primary source of these CROs and may play a key role in the spread of antibiotic resistance and healthcare environments. *Klebsiella* species and *Escherichia coli* are examples of healthy human gut bacteria that may become resistant to antimicrobials. To control their spread, proper surveillance and infection control programmes must be established to identify the colonization of the gastrointestinal tract by these resistant organisms [3].

The prevention of the CRO transmission within healthcare facilities requires accurate and rapid laboratory detection methods, aiding the infection control precautions to be quickly implemented [4]. There are some current methods described for surveillance directly from rectal swabs, and they include broth enrichment, direct selective culture, chromogenic media and molecular detection of carbapenemase genes [4,5].

Thus, the aim of the present study was to assess the CRO colonization, comparing the culture and molecular real-time polymerase chain reaction (qPCR) methods applied to rectal swabs in a large population of chronic renal disease (CKD) patients.

Methods

From September 2013 to February 2015, a total of 546 CKD patients admitted or supported by a tertiary centre specializing in chronic renal failure care at São Paulo, Brazil, were included in the study. These patients were divided into three groups: conservative treatment ($N = 129$), patients under dialysis ($N = 217$), and kidney transplant ($N = 200$). The CKD patients had a rectal swab monitoring collection held at two different times. The first collection was performed at the admission of the patient in the study, followed by a collection from seven to 14 days after the first collection so that the study included a total of 1092 samples.

Rectal samples were collected using an ESwab™ flocculated nylon liquid medium (Copan Diagnostics, Inc., Corona, CA, USA) and then transported to the Special Laboratory of Clinical Microbiology with the aim of isolating and identifying carbapenem-resistant strains. The ESwabs were sown in a culture medium specific for this purpose, called chromogenic agar KPC. Samples grown in the culture medium had the colonies seeded on to MacConkey agar for isolation and subsequent identification of the species by MALDI-TOF and research of resistance genes by conventional PCR.

At the same time that the culture of these 1092 ESwabs surveillance was performed, the real-time PCR of these samples was also performed directly from the liquid of the rectal ESwab, without previous enrichment, because the research aimed to minimize the time to detect resistance genes. The genes investigated in the study were: bla_{KPC}, bla_{NDM}, bla_{OXA-48}, bla_{GES}, bla_{VIM} and bla_{SPM}.

The project was approved by the Research Ethics Committee of UNIFESP, CEP No. 1630/11, and the patients were included in the study after signing the informed consent.

Phenotypic method detection

The chromogenic KPC agar (Probac Brazil, São Paulo, SP, Brazil) was used as the culture method for the detection of CRO. The plates were incubated overnight at ±37°C and then examined for growth (*Klebsiella*, *Enterobacter*, and *Citrobacter* spp., metallic blue colonies; *Escherichia coli*, dark pink or red colonies; *P. aeruginosa*, translucent colonies).

Each individual isolate suspected of producing CRO was subcultured on MacConkey agar for identification and carbapenemase detection by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany). In addition, we performed the screening for carbapenemase genes detection (bla_{KPC}, bla_{NDM}, bla_{GES}, bla_{VIM}, bla_{OXA-48} and bla_{SPM}) by convencional PCR.

Detection of carbapenemase activity by MALDI-TOF MS

A fresh ertapenem solution (0.5 g/L) was prepared using Tris–HCl 20 mM pH adjusted (pH 6.8). A 500 mL aliquot of ertapenem (ETP: 0.25 mg/mL; Merck Sharp & Dohme, NJ, USA) solution was added to the bacterial pellet tube, gently mixed and incubated at 37°C for 2 and 4 h. The tubes were then centrifuged at 13,000 rpm for 2 min and 1 mL of the supernatant was spotted on to a stainless steel MALDI target plate. One microlitre of α-cyano-4-hydroxycinnamic acid matrix solution was placed on each spot and allowed to dry at room temperature. A mass spectrum was obtained using a Bruker Daltonics Microflex LT instrument, operating according to the manufacturer's instructions as recommended. Carbapenem hydrolysis was considered positive if the corresponding ertapenem intact molecule mass peak (475 m/z and its monosodium salt, 497 m/z) completely disappeared. In each assay, a fresh inoculum of *K. pneumoniae* ATCC BAA 1705 and *K. pneumoniae* ATCC BAA 1706 was tested as the positive and negative controls, respectively, as well as a tube containing only the ertapenem solution [6].

Detection of carbapenem resistance genes

The isolates obtained on chromogenic agar from the 1092 surveillance rectal swabs were screened for the presence of the six most prevalent carbapenemase genes described. The rectal Eswabs and the respective colonies phenotypically tested had their DNA extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

Conventional PCR was performed directly from colonies that grew in chromogenic media for single detection of the genes: bla_{KPC}, bla_{NDM}, bla_{GES}, bla_{VIM} bla_{OXA-48} and bla_{SPM}.