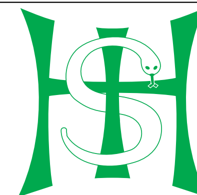
Journal of Hospital Infection 88 (2014) 66–71

Available online at www.sciencedirect.com

Journal of Hospital Infection

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



Routine active surveillance for carbapenemase-producing Enterobacteriaceae from rectal swabs: diagnostic implications of multiplex polymerase chain reaction

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A R T I C L E I N F O

Article history:
Received 16 January 2014
Accepted 27 June 2014
Available online 10 July 2014

Keywords:
Active surveillance
Carbapenemase-producing Enterobacteriaceae
Multiplex polymerase chain reaction
Rectal swabs
Screening

S U M M A R Y

Background: Screening for carriage of carbapenemase-producing Enterobacteriaceae (CPE) is considered an important infection prevention and control strategy. To date, screening has relied primarily on culture although polymerase chain reaction (PCR)-based screening is gaining momentum. Currently there is no gold standard screening method and consequently it is important to consider the implications of different diagnostic strategies used in active surveillance.
Aim: To assess the utility of a multiplex PCR screening strategy, as a component of active surveillance, for detection of CPE in patients admitted to various hospitals.
Methods: A single rectal swab was collected from patients at various hospitals, considered to be at risk of colonization with CPE. Comparison of a modified US Centers for Disease Control and Prevention culture protocol with a PCR-based assay for the detection of the bla_{NDM}, bla_{KPC}, $bla_{OxA\text{-}48\text{-}like}$, bla_{VIM}, bla_{IMP}, and bla_{GES} genes was performed.
Findings: Of the 251 consecutive rectal swabs collected, 30 were PCR positive for one or more carbapenemase genes. Fifteen (50%) were culture positive and CPE only accounted for six isolates. PCR demonstrated excellent sensitivity (100%), specificity (89.8%), and negative predictive value (100%) for detection of CPE, but a positive predictive value of only 46.6% and 16.6% for detection of carbapenemase-producing Gram-negatives and CPE, respectively.
Conclusion: The apparent excellent performance characteristics of PCR for detection of CPE from rectal swabs must be tempered by knowledge of CPE prevalence and be interpreted within a defined epidemiological context. Further comparative research with culture, evaluating the clinical utility of PCR-based assays as a screening tool, is needed.
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Introduction

Carbapenemase-producing Enterobacteriaceae (CPE) are on the increase globally and a major cause for concern. An important strategy advocated by numerous guidelines is to

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http://dx.doi.org/10.1016/j.jhin.2014.06.009
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screen at-risk patients, thus identifying potential carriers who may serve as reservoirs for sustained horizontal transmission.[1–3] Active surveillance is most often initiated in outbreak settings with the aim of detecting unknown reservoirs. This strategy is based on the assumption that colonized patients may perpetuate transmission and that early identification with isolation will curb this. However, the clinical relevance and the subsequent impact of infection prevention and control measures based on active surveillance cultures remain to be determined.[4] To date, the screening of patients for multidrug-resistant Gram-negatives, including CPE, has relied primarily on culture. Various methods of culture have been investigated, yet a universally applicable method remains elusive.

The main drawback to culture from an infection prevention and control (IPC) perspective is the prolonged turnaround time of at least 24–72 h, during which unnecessary IPC measures may be instituted while waiting for a result. Therefore PCR-based assays with their rapid turnaround time and high sensitivity are an attractive alternative. However, the role of PCR-based screening methods has yet to be fully elucidated.[5] The multitude of carbapenemase genes and ubiquitous nature of Enterobacteriaceae may compound the problems associated with PCR-based screening of CPE. In South Africa, multiplex PCR-based screening methods are currently employed, by various laboratories, to assist in the detection of CPE in at-risk patients. Various types of CPE, including NDM-1, KPC and OXA-48-like, have been detected and reported in South Africa.[6–8]

However, this strategy of PCR-based screening for multiple CPE was largely prompted by an outbreak of NDM-1 at a private hospital with a subsequent need for rapid detection of colonized patients. PCR-based screening for CPE has recently garnered interest, although usually as a single or duplex PCR in an established outbreak or high prevalence setting.[9–11] The applicability of a multiplex PCR screening method for surveillance purposes has not been established and the validity of such a method remains to be determined. We sought to assess the applicability of such a strategy in the context of a routine diagnostic laboratory that processes samples from a multitude of different hospitals in the Gauteng region.

Methods

Clinical specimens

Rectal swabs submitted to our laboratory for screening were received from multiple hospitals within the Gauteng province of South Africa. Screening rectal swabs are submitted from patients considered to be at risk of being colonized with CPE. Although these risk factors may vary between hospitals, there is general agreement among the major hospital groups as to what is considered a risk factor. These include: prior hospitalization within the last three to 12 months; transfer from any other healthcare facility; admission from a long-term care facility; intensive care unit (ICU) admission; use of broad-spectrum antibiotics; invasive device usage; patients on dialysis; and immunocompromised patients. A single dry rayon rectal swab (Copan Diagnostics, Inc., Brescia, Italy) was submitted for analysis, and, upon receipt in the laboratory, was put into 2 mL sterile saline.

PCR-based assay

PCR was performed using the Roche assay developed by TIB MolBiol GmBH (Berlin, Germany) for the detection of six carbapenemase genes. Briefly, 1 mL of the sample-saline solution was processed by the Nuclisens® Easymag instrument (bioMérieux, Marcy l'Etoile, France) to produce a 55 μL eluate, containing isolated nucleic acid from the sample. Five microlitres of the eluate were then added to 15 μL of two separate PCR reaction mixes containing primers and probes for the following carbapenemase genes: (i) bla_{NDM}, $bla_{OXA\text{-}48\text{-}like}$, bla_{KPC}; (ii) bla_{GES}, bla_{IMP}, bla_{VIM}. The sample mixture was then analysed by real-time PCR on a LightCycler 2.0 instrument. A cycle threshold (C_T) value of 36 was considered positive in accordance with the recommendations of the assay developer (TIB MolBiol).

Culture-based assay

A modified CDC protocol for detection of carbapenemase-producing *Klebsiella* spp. and *Escherichia coli* was employed.[12] A 5 mL volume of nutrient broth containing a 10 μg ertapenem disk was inoculated with the remaining 1 mL of rectal swab saline sample, and incubated overnight at 35°C ambient air. The sample was then vortexed and 100 μL subcultured on to a MacConkey agar plate. After 24–48 h incubation, all Gram-negative colonies were identified to species level using matrix-assisted laser desorption–ionization time-of-flight mass spectrometry (bioMérieux) and subjected to the PCR-based assay as described above for determination of the presence of carbapenemase genes: bla_{NDM}, bla_{KPC}, $bla_{OXA\text{-}48}$, bla_{VIM}, bla_{IMP}, bla_{GES}.

Determination of analytical sensitivity and specificity

Control strains

With the exception of *Klebsiella pneumoniae* ATCC® BAA-1705 (KPC) control strains utilized in this study were obtained from clinical isolates of *K. pneumoniae* (OXA-48; NDM-1; GES) and *Enterobacter cloacae* (VIM-1; IMP). These isolates had been independently verified, using PCR techniques, by other private and/or National Institute for Communicable Diseases laboratories. The MIC to ertapenem for each control strain was determined by Etest® (bioMérieux).

PCR and culture

For each control strain, the following procedure was performed. From an initial inoculum of 10^5 cfu/mL, serial 10-fold dilutions were made in normal saline to give a range of 10^5–10^1 cfu/mL. These dilutions were then used for both PCR and culture analytical sensitivity and specificity analysis. A swab (the same one used for collection of rectal screening swab) was inserted into each dilution, rotated and mixed, and then transferred to 2 mL of normal saline in line with the procedure for clinical samples. PCR and culture were then performed on each dilution as described above.

Effect of varied inoculum and MIC on culture results

Six clinical isolates of OXA-48-like *K. pneumoniae* with ertapenem MIC ranging from 2 to 4 μg/mL were prepared in serial 10-fold dilutions of 10^5–10^1 cfu/mL. These were then cultured as described above to determine the limit of

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