has also been reported that the first characterized MBL from India was VIM-2.\(^9\) A nationwide survey also established the presence of VIM-2, -5, -6 and -11 and the evolution of a new variant, VIM-18, from this country.\(^10\) These studies indicate the dissemination of various MBL genes in the Indian subcontinent.

Although NDM-1 hydrolyses almost all β-lactams efficiently, several of our isolates were found by disc diffusion testing to be susceptible to various carbapenems as well as to piperacillin/tazobactam, which was not discussed in any previously conducted studies. However, MICs of these carbapenems and piperacillin/tazobactam, which were not determined in the present study, could be more informative and would have added more value and clinical significance. Colistin and tigecycline were also found to be active in several cases. In our study it was observed that some of the \(\text{bla}_{\text{NDM}}\)-harbouring isolates were found to be phenotypically susceptible to all three carbapenems (imipenem, meropenem and ertapenem) tested. So, irrespective of antibiograms, all isolates should be routinely screened for molecular detection of the NDM-1 gene in the hospital setting. Thus, the routine susceptibility testing for carbapenems showing false susceptibility may lead to treatment failure.

Recognition of patients at risk and prevention of transmission is urgently needed in our regions. Besides stringent infection control in hospitals and good sanitation in the community, focus on the basis of the evolution of such genes is needed in order to contain their vertical and horizontal spread.

Acknowledgements

We would like to acknowledge the technical staff for their support.

Funding

This study was supported by internal funding.

Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References

9 Toleman MA, Vinodh H, Sekar U et al. \(\text{bla}_{\text{VIM}}\)-harboring integrons isolated in India, Russia, and the United States arise from an ancestral class 1 integron predating the formation of the 3′ conserved sequence. \textit{Antimicrob Agents Chemother} 2007; \textbf{51}: 2636–8.

J Antimicrob Chemother 2011
doi:10.1093/jac/dkr184
Advance Access publication 12 May 2011

Rapid detection of the \(\text{bla}_{\text{NDM}}\)-1 gene by real-time PCR

Danny C. T. Ong1, Tse-Hsien Koh2, Nur Syahidah3, Prabha Krishnan4 and Thean Yen Tan1,*

1 Division of Laboratory Medicine, Changi General Hospital, 2 Simei Street 3, Singapore 529889, Singapore; 2 Department of Pathology, Singapore General Hospital, Outram Road, Singapore 169608, Singapore; 3 Clinical Trials and Research Unit, Changi General Hospital, 2 Simei Street 3, Singapore 529889, Singapore; 4 Department of Laboratory Medicine, Tan Tock Seng Hospital, 11 Jalan Tan Tock Seng, Singapore 308433, Singapore

*Corresponding author. Tel: +65-68504935; Fax: +65-64269507; E-mail: thean_yen_tan@cgh.com.sg

Keywords: β-lactamases, carbapenem resistance, multidrug resistance, laboratory methods, polymerase chain reaction

Sir,
New Delhi metallo-β-lactamase-1 (NDM-1) is a recently reported novel plasmid-borne metallo-β-lactamase that represents an emerging public health threat.\(^3\) To date, the international spread of NDM-1 has already been reported to diverse locations such as the UK, the USA, Japan, Australia and most recently the Middle East.\(^2\) With the increasing need for efficient surveillance and...
isolates were resistant to the four tested carbapenems (MIC ≥ 1 mg/L) from three hospitals, with 89% also demonstrating resistance to ertapenem-resistant (MIC ≥ 4 mg/L) clinical isolates collected from three hospitals, with 89% also demonstrating resistance (MIC ≥ 4 mg/L) to imipenem, meropenem or doripenem. All P. aeruginosa isolates were resistant to the four tested carbapenems (MIC ≥ 64 mg/L). Detection of the blaNDM-1 gene was initially performed by conventional PCR using forward (5′-GAA ACT GTG AGG CTC CAA C-3′) and reverse (5′-GGG CCG TAT GAG TGA TTG C-3′) primers, and all positive PCR results were confirmed by sequencing the obtained amplicon. Study isolates were also screened for extended-spectrum β-lactamase (ESBL) genes (blaTEM and blacTX-M),4,5 metallo-β-lactamase genes (blaIMP, blavIM, blasPM-1, blasIM-1, and blasIM-3),6 the blaKPC gene7 and plasmid-borne ampC genes8,9 Twelve Enterobacteriaceae isolates were positive for the blaNDM-1 gene (eight K. pneumoniae, two Enterobacter cloacae, one E. coli and one P. mirabilis) with the remaining 30 Enterobacteriaceae possessing combinations of ESBL genes (blaOX-M and/or blaOXA-1) (n = 30), blaOXA-like ampC genes (n = 20) and metallo-β-lactamase genes (blaIMP) (n = 4). The five P. aeruginosa isolates were positive for blaVIM.

For the NDM-1 real-time assay, primers and a probe (Table 1) were designed to amplify a 127 bp region based on currently available published sequences of blaNDM-1. GenBank accession numbers AB571289, FN396876, HM853678, HQ171206, HQ259057.1, HQ451074.1, AB614355.1, HQ738352.1, HQ284043.1, HQ284042.1 and HQ256747.1. DNA isolation was performed using the PureLink Genomic DNA Kit (Invitrogen, Carlsbad, CA, USA) from bacterial colonies according to the manufacturer’s instructions. Real-time PCR was performed in 20 μL reaction mixtures containing 800 nM each primer, 200 nM probe, 1× QuantiTect Probe PCR Master Mix (Qiagen, Hilden, Germany) and 5 μL of template DNA using a Rotor-Gene Q (Qiagen, Hilden, Germany). PCR cycling parameters were 95°C for 15 min and 40 cycles at 95°C for 15 s and 58°C for 45 s.

All 12 NDM-1-positive samples were detected by our assay and all 35 NDM-1-negative samples were negative, showing 100% sensitivity and specificity. In order to further elucidate the specificity of the assay, K. pneumoniae ATCC BAA-1705 possessing the blakPC-2 gene was tested by the real-time assay, and this similarly was negative for blakNDM-1.

The linearity and limit of detection of the assay were determined by performing serial 10-fold dilutions from 10 to 10⁶ cfu/mL in triplicate. DNA was isolated from the serial dilutions using 200 μL of culture and eluting in a volume of 200 μL, using the PureLink Genomic DNA Kit according to the manufacturer’s instructions. Our assay was found to correlate well (R² = 0.993) from 10³ to 10⁶ cfu/mL with an efficiency of 91%. The limit of detection at 95% confidence was 10³ cfu/mL (or 25 cfu/reaction).

Recently, Krüttgen et al.9 have also described a similar real-time PCR assay for the detection of blakNDM-1. Their assay provides an alternative method for the rapid screening of blakNDM-1, and has also been validated against clinical isolates of carbapenem-resistant Gram-negative bacilli with well-defined resistance mechanisms.

In conclusion, this real-time assay provided rapid and accurate detection of the blakNDM-1 gene in our tested population, and is a viable method for rapid screening of the NDM-1 gene in carbapenem-resistant Enterobacteriaceae.

**Funding**

This research was supported by internal funding.

**Transparency declarations**

None to declare.

**References**


Cryptococcus and Trichosporon spp. are susceptible in vitro to branched histidine- and lysine-rich peptides (BHKPs)

Patricia E. B. Verwer*, Martin C. Woodle, Teun Boekhout, Irma A. J. M. Bakker-Woudenberg and Wendy W. J. van de Sande

Department of Medical Microbiology & Infectious Diseases, Erasmus MC, University Medical Centre Rotterdam, Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands; Aparna BioSciences Corp., 12111 Parklawn Drive, Suite 125, Rockville, MD 20852, USA; CBS Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

Corresponding author. Tel: +3110-7031975; Fax: +3110-7033875; E-mail: p.verwer@erasmusmc.nl

Keywords: susceptibility testing, peptides, antifungal, fungi

Sir,

With the increase in immunocompromised hosts during recent decades, fungal infections, which are difficult to treat, develop more frequently. As a result, there is an ongoing need for new antifungal agents. A group of potential antifungal agents are branched histidine- and lysine-rich peptides (BHKPs), originally developed as gene delivery agents (Figure 1). It is thought that the mechanism of action of these BHKPs is similar to that of histatins, a group of naturally occurring peptides with antimicrobial activity. Histatin-5 is considered its most potent representative. In a search for new antifungal agents, the susceptibility of a panel of fungi and yeasts to three BHKPs was tested.

This panel included 9 Aspergillus strains, 5 Candida strains, 21 Cryptococcus strains and 8 Trichosporon strains (Table 1). MICs were determined for the BHKPs H2K4b, H3K4b(H) and H3K4b(G) (Aparna BioSciences Corp., Rockville, MD, USA), histatin-5 (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and amphotericin B, caspofungin and voriconazole as reference agents. All strains were tested according to the broth microdilution methods described by the CLSI. Differences in MICs were analysed by two-tailed Mann–Whitney tests (GraphPad Prism, San Diego, CA, USA).

As can be seen in Table 1, for all strains MICs of amphotericin B, voriconazole and caspofungin were in agreement with previously described MICs. Aspergillus and Candida spp. had high MICs of histatin-5 (MICs >16 mg/L) and of the three new peptides H2K4b, H3K4b(H) and H3K4b(G) (MICs >128 mg/L), two A. niger strains excepted. The MICs for Candida spp. obtained in our study were higher than reported by Zhu et al., which could be explained by differences in media: yeast–maltose medium versus RPMI-1640. The median MICs for A. niger were 16 mg/L of H2K4b and >128 mg/L of H3K4b(H) and H3K4b(G). This fungus apparently had low MICs of H2K4b only. Microscopic observations showed that exposure to a sub-MIC concentration of H2K4b (128 mg/L) did not result in morphological changes of hyphae in A. fumigatus ATCC 204305 (data not shown).

Cryptococcus and Trichosporon spp. had lower MICs of the BHKPs than Aspergillus and Candida spp. Cryptococcus gattii was inhibited significantly more efficiently by BHKPs (median MICs of 4, 4 and 4 mg/L of H2K4b, H3K4b(H) and H3K4b(G), respectively) than Cryptococcus neoformans (median MICs of 8, 16 and 16 mg/L of H2K4b, H3K4b(H) and H3K4b(G), respectively) (P = 0.003, P = 0.004 and P = 0.035, respectively). Neither the presence of a capsule (microscopic observation using Indian ink) nor addition of 5% fetal calf serum (not heat-inactivated) influenced susceptibility (data not shown).

Like in Cryptococcus spp., Trichosporon spp. were most efficiently inhibited by H2K4b. Histatin-5 did not inhibit growth of Cryptococcus or Trichosporon spp.

In our study, we found differences in susceptibility to BHKPs for the four fungal species. Aspergillus and Candida belong to the Ascomycota, whereas both Cryptococcus and Trichosporon belong to the Basidiomycota. Ascomycota and Basidiomycota differ in cell wall composition. For instance, Cryptococcus and Trichosporon spp. both express glucuronoxylomannan (GXM) on their cell walls, whereas Aspergillus and Candida spp. express Glucan 1,3 β-glucan and Mannoproteins.

Figure 1. Schematic structure of BHKPs. The peptides consist of terminal branches originating from a lysine core. This lysine core consists of three lysines. R represents KHKHHKHHKHHKHKHHKHK for H2K4b, KHHHKHHKHHKHHKHHKHHHKH for H3K4b(H) and KHHHKHHKHHKHHKHHKHHHK for H3K4b(G).

Advance Access publication 4 May 2011
