Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Quick screening and easy detection method of NDM-gene in clinical isolates: A need of the time

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The emerging trend of multidrug resistance is becoming a major threat to community acquired and nosocomial infections, worldwide[1]. The carbapenems are used as last-source drugs because of increasing resistance against beta-lactam groups of antibiotics due to its excessive use to treat wide range of infections[2]. The resistance against carbapenem group of antibiotics has also emerged worldwide for at least one decade and has becoming a major public problem. The latest (metallo beta-lactamase) MBL, named NDM-1 (New Delhi Metallo beta lactamse) has been identified as novel class of carbapenemase found in enterobacteriaceae, first isolated from Swedish patient of Indian origin. He traveled to India and admitted in Ludhiana hospital in Punjab, later he was admitted to the hospital in New Delhi[3,4]. This was the first report of bla_{NDM-1} and the first report of MBL carriage among enterobacteriaceae from UK. NDM-1 carrying isolates confer resistance against all beta-lactamases except colistin and aztreonam[4]. In view of current situation we have developed a quick and easy detection of NDM producers in clinical isolates from site of infection. This method would be effective and economical for hospitals in developing countries where no huge funds are available for using sophisticated infrastructure to identify and detect these resistant markers.

We have screened 100 clinical isolates from Aligarh hospital including (intensive care unit) ICU samples. These isolates were grown on (brain heart infusion) BHI broth and the cells were spread over Imipenem supplemented plates (Figure 1). Only carbapenem resistant strains were found to grow. Of 100, only two isolates (these were later confirmed as *Klebsiella pneumoniae*, data is not needed here) showed growth on the plates containing (IMP) imipenem (5 mg/L). Moreover, all the 100 isolates were grown on Mueller-Hinton agar (BD diagnostics, USA) for an Imipenem/EDTA disc potentiation test to detect production of carbapenemase[5]. The enlargement of inhibition zone was observed as >3.5 mm around the disk in the presence of IMP (10 μg) + EDTA (10 μL of 0.1 M) whereas, no zone was observed around the IMP disk alone. It is the indication of carbapenemase production as being inhibited by EDTA. A single colony was picked from each plate and streaked on Aztreonam (10 mg/L) supplemented plate. No growth was found on these plates. A single colony from IMP-plate was suspended in 100 μL of sterilized water (Figure 1) and was incubated at 95 ℃ for 10 min followed by centrifugation at 8 000 *g* for 5 min. Supernatant was used as template to perform PCR amplification using NDM-F, CATGCCGGGTTTCGGGGCAGTC and NDM-R, GAGCGACTTGGCCTTGCTGTCC primers with the PCR profile, each cycle of denaturation at 95 ℃ for 30 s, annealing at 55 ℃ for 30 s, and extension at 72 ℃ for 1 min for 35 cycles. Both the isolates showed positive for presence of NDM-1. It was further confirmed by sequence analysis. The Etest MBL strip is one of the methods used for detecting metallo-b-lactamase (MBL) producers based on inhibition of MBL activity by EDTA[6] but it is not easily accessible for low income countries hospitals. Nordmann's group in France used automated susceptibility testing by Vitek2 (bioMe ́rieux) for screening NDM-producers followed by molecular characterization[7] and also this technique is not easily available in each hospital. Since efficient prevention of the spread of NDM-1 producers requires a quick and easy screening method that can detect NDM-1 producers as colonizers and thus our method is quick, easy as well as cost effective and can be affordable for any hospital infection

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Foundation project: This work was supported by internal funds of Biotechnology Unit, AMU and DBT grant, BT/PR11453/BID/07/296/2009 to AUK.

control setup. It is of utmost important to develop good laboratories services for surveillance and control measures. This study provides an easy and quick method for the detection of NDM variants among the clinical isolates and hence early prevention of NDM–producers in the community and hospital setting. Moreover, the method is cost effective for low income countries where emerging trends of NDM–1 producers is always high due to poor hygiene conditions.

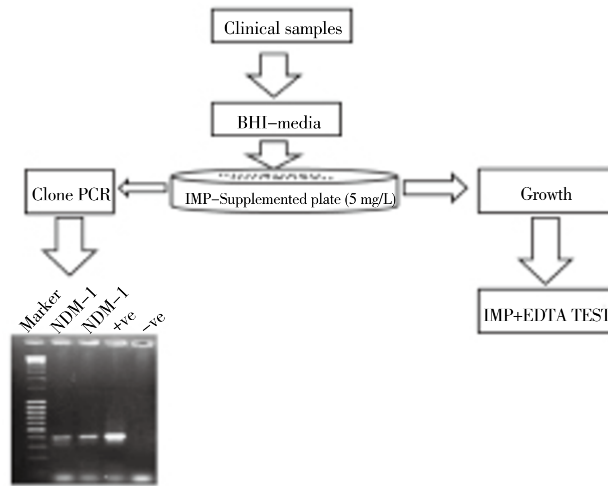

Figure 1. Scheme for easy and quick detection of NDM producers in clinical samples.
Agarose gel showing DNA marker, two NDM–1 producers (*K. pneumoniae*) from clinical isolates, Positive control and negative control. BHI (Brain Heart Infusion), IMP (Imipenem).

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Author acknowledged support of Interdisciplinary Biotechnology from internal funds. TP Mohammad is highly acknowledged for providing isolates.

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