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Letter to Editor

Microbiology Section

# Universal Presence of *bla*$_{NDM-1}$ Gene in Carbapenem-Resistant Gram-Negative Bacilli in an Indian Hospital in 2015

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Dear Editor,

Carbapenem-resistant Gram-Negative Bacilli (GNB) which is simultaneously resistant to most other antimicrobials is now found in many hospitals worldwide [1]. Resistant strains are associated with high mortality; therefore, it is important to investigate resistance mechanisms to guide efforts to combat them.

The study started with all GNB strains (n=1544) isolated from routine clinical specimens received at the diagnostic laboratory of our tertiary-care hospital during the period January 2015 to June 2015. Out of these strains, 194 were found to be carbapenem-resistant by the Kirby-Bauer disc diffusion method, interpreted according to Clinical Laboratory Standard Institute (CLSI) guidelines [2], were included in the study. Subsequently, six isolates of *Elizabethkingia meningoseptica* and four of *Stenotrophomonas maltophilia* were excluded because of intrinsic carbapenem-resistance. Carbapenem Minimum Inhibitory Concentrations (MIC) of the remaining strains (n=184) were determined using E-test strips (bioMérieux, India) on Muller Hinton II Agar (Becton Dickinson, USA) [3]; MIC values were interpreted according to CLSI guidelines [2]. Mean carbapenem MICs of resistant isolates were above 25 µg/mL for all organisms/carbapenem combinations studied. Isolates were identified with standard biochemical methods [4], supplemented with Vitek 2 GNID panels if needed. Resistant strains were comprised of 104 isolates of *Acinetobacter calcoaceticus-baumannii* Complex (ACBC), 49 of family Enterobacteriaceae, and 31 of *Pseudomonas aeruginosa*. Among Carbapenem-Resistant Enterobacteriaceae (CRE), *Klebsiella pneumoniae* (17) and *Escherichia coli* (15) were the most common species, followed by *Enterobacter cloacae* (7), *Enterobacter aerogenes* (5), *Citrobacter freundii* (4), and *Citrobacter koseri* (1).

PCR was performed for *bla*$_{NDM-1}$, *bla*$_{VIM}$, *bla*$_{KPC}$ and *bla*$_{OXA-48}$ carbapenemase genes with positive and negative controls in each run [5-7]. Phenotypic tests for carbapenemases were also used; these included Modified Hodge Test (MHT), Carba NP Test (CNPT), Blue Carba test (BCT), and Carba Acineto NP test (CANPT). MHT and CNPT were performed according to CLSI protocols [2], while BCT and CANPT were performed according to protocols in publications reporting these tests for the first time [8,9]. No phenotypic assay for carbapenemase detection had sensitivity above 90% in our hands when compared with PCR.

All GNB isolates with acquired carbapenem resistance carried the *bla*$_{NDM-1}$ gene [Table/Fig-1]. In addition, the *bla*$_{VIM}$ gene was detected in 24 isolates, which included *P. aeruginosa* (n=20), *Acinetobacter calcoaceticus-baumannii complex* (n=3) and *Enterobacter cloacae* (n=1). The *bla*$_{OXA-48}$ was detected only in *K. pneumoniae* (n=8). No isolate carried the *bla*$_{KPC}$ gene.

Studies on New Delhi Metallo-beta-lactamase 1 (NDM-1) in Southern Asia, starting with the seminal article by Kumarasamy KK et al. in 2010, are too numerous to quote [10]. The prevalence of *bla*$_{NDM-1}$ gene in India has increased steadily since then, and a PubMed search revealed an article from 2012 reporting its presence in all (n=17) carbapenem-resistant isolates of *K. pneumoniae* in Guwahati, Assam, India [11]. Another study from 2014 reported the presence of *bla*$_{NDM-1}$ gene in all [12] carbapenem-resistant isolates in Sharjah, UAE, where many patients travel frequently to Southern Asia [13]. However, ours is the first to report the universal presence of the *bla*$_{NDM-1}$ gene in such a large number (n=184) of carbapenem-resistant isolates. Ours is also the first to report the high incidence (12.56%) of carbapenem-resistance in clinical isolates of GNB, and the presence of *bla*$_{NDM-1}$, from our mountainous state in Northern India.

| | | *Acinetobacter* | *P. aeruginosa* | Enterobacteriaceae |
|---|---|---|---|---|
| Total strains 184 | | | | |
| Number of strains carrying the gene concerned | *NDM-1* | 104 | 31 | 49 |
| | *VIM* | 03 | 20 | 01 |
| | *OXA-48* | 0 | 0 | 08 |
| | *KPC* | 0 | 0 | 0 |

**[Table/Fig-1]:** Carbapenemase genes in carbapenem-resistant Gram-negative bacilli. (Total strains 184)

The universal presence of *bla*$_{NDM-1}$ in our carbapenem-resistant isolates, along with similar or identical findings in other places in Asia, is worrisome because Ambler Class B metallo-beta-lactamases are not inhibited by the newly developed beta-lactamase inhibitors, avibactam and relebactam, which target serine carbapenemases of Ambler Class A and C only. This emphasizes the need to develop inhibitors of Ambler Class B carbapenemases. Fortunately, cyclobutanone and bisthiazolidine derivatives have displayed promising activity against metallo-beta-lactamases, and it is hoped that structural modifications will improve their activity to clinically significant levels in the near future [12,14].

To conclude, it is important to monitor the nature of carbapenemases to provide impetus to the development of newer inhibitors, and also guide their subsequent use, especially on an empiric basis. Since it is neither feasible nor economical to do this on all carbapenem-resistant isolates, nationally coordinated surveys must be done periodically with significant numbers of geographically representative isolates to maintain an up-to-date picture of resistance mechanisms in different parts of the country.

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