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Escherichia coli encoding bla_{NDM-5} associated with community-acquired urinary tract infections with unusual MIC creep-like phenomenon against imipenem

Varsha Rani Gajamer[a], Amitabha Bhattacharjee[b], Deepjyoti Paul[b], Chandrayee Deshamukhya[b], Ashish Kr. Singh[a], Nilu Pradhan[a], Hare Krishna Tiwari[a,*]

[a] Department of Microbiology, Sikkim University, Gangtok 737102, Sikkim, India
[b] Department of Microbiology, Assam University, Assam, India

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ABSTRACT

Objectives: Carbapenemase-producing *Escherichia coli* are a major clinical concern. The current study aimed to identify NDM-5-producing *E. coli* associated with community-acquired urinary tract infections (UTIs) co-harbouring extended-spectrum β-lactamases (ESBLs) and showing a phenomenon of imipenem minimum inhibitory concentration (MIC) creep.

Methods: A total of 973 urine samples were collected from females aged between 18–49 years diagnosed with UTI in Northeast India (June 2014–July 2016). Isolates were identified by standard microbiological methods. The presence of bla_{NDM} and ESBL genes was determined by PCR and sequencing. PCR-based replicon typing was performed. Plasmid stability of all β-lactamase-producers and their transformants was analysed by serial passage, and the MIC creep phenomenon was analysed by studying revertants.

Results: Among 34 bla_{NDM-5}-positive *E. coli* isolates, 21 (61.8%) co harboured $bla_{CTX-M-15}$, followed by multiple combinations of genes. This study revealed diverse plasmid types (HI1, I1, FIA + FIB, FIA and Y). The strains showed progressive plasmid loss after 31 passages. Most if the isolates had MICs of 0.5 μg/mL and 1 μg/mL to imipenem, ertapenem and meropenem. However, on studying the MIC creep phenomenon, the MIC was found to be elevated from 0.5 μg/mL to 64 μg/mL and from 1 μg/mL to 128 μg/mL. Analysis of revertants shows that the MIC of most NDM-positive isolates was reduced to 16 μg/mL after the 30th serial passage.

Conclusion: This study observed a unique phenotype of NDM-producers that has not been reported previously. The observed phenomenon poses a global threat as these pathogens may evade phenotypic screening by routine laboratories.

1. Introduction

Carbapenemase-producing Enterobacteriaceae (CPE) causing bacteraemia are of great clinical concern. Carbapenemases are a versatile group of β-lactamases that are characterised by their ability to hydrolyse virtually all β-lactam antibiotics, including cephalosporins and carbapenems, consequently complicating therapy and limiting treatment options [1]. CPE infections are also associated with high mortality rates [2]. Asia is a known epicentre of antimicrobial drug resistance due to extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, with CTX-M being recognised as the most common β-lactamase [3,4]. In the past 10 years, there has been a marked increase in isolation rates of multidrug-resistant Enterobacteriaceae producing ESBLs, AmpC β-lactamases or carbapenemases in community settings [5–7]. The prevalence of ESBL- and carbapenemase-producing Enterobacteriaceae varies remarkably across the Asia-Pacific region [8].

In the last decade, New Delhi metallo-β-lactamase (NDM)- and *Klebsiella pneumoniae* carbapenemase (KPC)-producing Enterobacteriaceae have become endemic on the Indian subcontinent as well as in China [5,7,8]. There are also reports of the occurrence of bla_{NDM-1} and bla_{NDM-5} in a tertiary referral hospital in North India [9]. In the current study, the prevalence of the bla_{NDM} gene among uropathogens isolated from females in Northeast India was

* Corresponding author.
E-mail addresses: hktiwari_2005@rediffmail.com, hktiwari@cus.ac.in (H.K. Tiwari).

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determined. No such studies have been previously undertaken to ascertain the magnitude of carbapenemase-producers in healthcare or community settings in this region. This study was performed to determine the prevalence of carbapenemases, carbapenemase-encoding genes and co-existing ESBL genes among uropathogens. Since carbapenems are not commonly used for the treatment of community-acquired infections in this region, expression of resistance genes was detected by determining minimum inhibitory concentrations (MICs) under carbapenem pressure.

2. Materials and methods

2.1. Collection of bacterial isolates

From June 2014 to July 2016, a total of 973 urine samples were collected from females aged 18–49 years diagnosed with community-acquired UTI in Northeast India. Standard microbiological techniques were used for collection, transportation and processing of the samples [10]. Uropathogens were isolated on cystine–lactose–electrolyte-deficient (CLED) agar, HiChrome UTI agar and MacConkey agar (HiMedia, Mumbai, India) plates by a semiquantitative method [11]. Specimens yielding $\geq 10^5$ CFU/mL were interpreted as significant bacteriuria. All of the isolates were identified on the basis of Gram staining, colony morphology and standard biochemical tests [11], and representative strains were identified using a VITEK®2 Compact instrument (bioMérieux, Marcy-l'Étoile, France).

2.2. Molecular detection of the bla_{NDM} gene

The presence of bla_{NDM} was determined by PCR using the primers NDM-F (5′-GGGCAGTCGCTTCCAACGGT-3′) and NDM-R (5′-GTAGTGCTCAGTGTCGGCAT-3′) [9]. PCR was performed under the following conditions: initial denaturation at 94 °C for 10 min; 30 cycles of denaturation at 94 °C for 40 s, annealing at 55 °C for 40 s and extension at 72 °C for 1 min; and a final elongation step at 72 °C for 7 min. Amplified products were purified using a MinElute® PCR Purification Kit (QIAGEN, Hilden, Germany) and were sequenced to confirm the bla_{NDM} variant.

2.3. Plasmid preparation and transformation

NDM-positive bacterial isolates were grown overnight in Luria–Bertani (LB) broth (HiMedia) containing 0.25 μg/mL imipenem. Plasmid DNA was extracted from the overnight cultures using a QIAprep Spin Miniprep Kit (QIAGEN) and the extracted plasmids were then subjected to transformation by the heat-shock method using *Escherichia coli* JM107 as the recipient strain. Transformants were selected on LB agar supplemented with 2 μg/mL imipenem and were further confirmed both by phenotypic and PCR analysis.

2.4. PCR-based replicon typing (PBRT)

PBRT was carried out targeting 18 different replicon types, including FIA, FIB, FIC, HI1, HI2, I1/Iγ, L/M, N, P, W, T, A/C, K, B/O, X, Y, F and FIIA, targeted in five multiplex and three simplex PCRs [12].

2.5. Plasmid stability testing

Plasmid stability of all β-lactamase-producers as well as their transformants was analysed by serial passage for 110 consecutive days at 1:1000 dilutions in LB broth without antimicrobial pressure [13]. After each passage, PCR was carried out to detect the presence of *bla* genes in the isolates.

2.6. Detection of co-existence of extended-spectrum β-lactamase genes

PCR was performed to determine the presence of ESBL genes. The genes were detected by multiplex PCR targeting bla_{TEM}, bla_{PER}, bla_{OXA-2}, bla_{SHV}, bla_{CTX-M}, bla_{VEB} and bla_{GES}. PCR was performed under the following conditions: initial denaturation at 94 °C for 5 min; 33 cycles at 94 °C for 35 s, 51 °C for 1 min and 72 °C for 1 min; and final extension at 72 °C for 7 min. Amplified products were further sequenced to confirm the co-existence of ESBL genes [14].

2.7. Sanger sequencing

Amplified products were selected for sequencing to identify the bla_{NDM} variant. DNA was sequenced at SciGenom Labs (Cochin, India) using the dideoxynucleotide chain termination method. The ABI sequence files were assembled and contigs were prepared using Codon Code Aligner software v.7.0.1.

Nucleotide sequence similarity searches were performed using the National Center for Biotechnology Information (NCBI) BLAST (Basic Local Alignment Search Tool) server on GenBank database release 138.0 12.

2.8. Antimicrobial susceptibility testing

The antimicrobial susceptibility pattern of the isolates was determined by the Kirby–Bauer disk diffusion method against ampicillin (10 μg), gentamicin (120 μg), piperacillin/tazobactam (TZP) (100/10 μg), netilmicin (30 μg), norfloxacin (10 μg), cefalotin (30 μg), cefoxitin (30 μg), cefuroxime (30 μg), ciprofloxacin (30 μg), ceftazidime (30 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), nitrofurantoin (300 μg) and imipenem (10 μg) on Muller–Hinton agar (HiMedia) plates. *E. coli* ATCC 25922 was used as a control and the results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [15].

2.9. Minimum inhibitory concentration determination of NDM-positive isolates

MICs of isolates harbouring the bla_{NDM} gene were determined by the agar dilution method against imipenem (Lupin India, Mumbai, India), ertapenem (INVANZ®; MSD, India) and meropenem (Meronem®; AstraZeneca, Bangalore, India). MIC interpretive standards for the susceptibility categories were categorised according to the breakpoints recommended by the CLSI [15]. The concentrations of drugs analysed were 0.5–256 μg/mL. *E. coli* ATCC 25922 was inoculated on each plate as a growth control.

2.10. Observation of MIC creep

NDM-positive isolates having an MIC of ≤4 μg/mL were subjected to serial passage in LB broth with a gradually increasing concentration of imipenem from 0.25 μg/mL to 8 μg/mL for six consecutive days.

2.11. Analysis of revertants

The isolates tested for MIC creep with an elevated MIC against imipenem were subjected to serial passage in LB broth at 1:1000 dilutions in the absence of imipenem for 30 consecutive days [16]. The MIC of each strain was subsequently determined on the 10th, 20th and 30th day of passage.