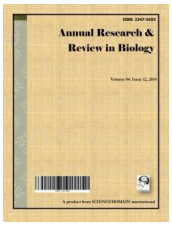

Annual Research & Review in Biology

14(4): 1-6, 2017; Article no.ARRB.33477
ISSN: 2347-565X, NLM ID: 101632869

Molecular Detection of New Delhi Metallo Beta Lactamase 1 (NDM-1) Producing Bacterial Isolates in Kano- Northwestern Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SAA, AHA and IY designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SMA, SA and YAK managed the analyses of the study. Authors MAR and AMA managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2017/33477
Editor(s):
(1) J. David Puett, Department of Biochemistry and Molecular Biology, University of Georgia, Georgia.
(2) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Arun S. Kharat, Dr. Babasaheb Ambedkar Marathwada University, India.
(2) Babatunde Olanrewaju Motayo, Federal Medical Centre, Nigeria.
(3) Payala Vijayalakshmi, Gitam University, India.
(4) Adesoji, A. Timilehin, Federal University Dutsin-Ma, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/19961

Original Research Article

Received 17th April 2017
Accepted 9th June 2017
Published 10th July 2017

ABSTRACT

New Delhi Metallo Beta Lactamase 1 (NDM-1) is an enzyme with zinc ions at its active site that cleaves the amide bond of β-lactam ring and provides resistance against major classes of β-lactam antibiotics. The molecular detection of NDM-1 producing bacterial isolates from tertiary Hospitals in Kano was investigated. A total of 500 bacterial isolates of *Enterobacteriaceae* and *Pseudomonas aeruginosa* from samples of blood, urine, catheter tip were screened for NDM-1 over

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a period of 12 months. The isolates were screened preliminarily for carbapenemases using meropenem (10 µg) and imipenem (10 µg) by disc diffusion technique. Isolates of 23 mm and 21 mm for meropenem and imipenem respectively were confirmed by modified Hodge test then EDTA Disc Synergy Test using two meropenem discs, one with MEM (10 µg), and other containing 10 µl of 0.1 M anhydrous EDTA (292 µg) for Metallo Beta Lactamase (MBLs) and finally seventeen MBLs isolates were screened with NDM-1 specific primers by PCR then four PCR positive products were sequenced for bla_{NDM-1} gene. Of the 500 clinical bacterial isolates screened, 162(32.4%), 43(8.6%) and 4(0.8%) were found to produce carbapenemase, MBLs and NDM-1 respectively. The highest frequency of NDM-1 producers was found among *Escherichia coli* 3(1.6%) followed by *Klebsiella pneumoniae* 1(0.5%). Based on clinical samples, blood (25.0%) was found to have highest prevalence of MBLs followed by catheter tips (21.0%), wound swabs (11.1%) and urine (6.3%). Conclusively, NDM-1 was first detected in Kano, Nigeria.

Keywords: Carbapenemase; Enterobacteriaceae; imipenem; meropenem; New Delhi Metall B-Lactamase-1; PCR.

1. INTRODUCTION

New Delhi Metallo Beta Lactamase-1 (NDM-1) is a newly described Metallo Beta Lactamase (MBLs) that was first identified in 2009 from a single isolates of *Klebsiella pneumoniae* and *Escherichia coli;* both recovered from a patient repatriated to Sweden after treatment in New Delhi hospital, India [1]. NDM-1 is an enzyme that cleaves the amide bond of β-lactam ring and provides resistance against major classes of β-lactam antibiotics [2]. It has zinc ions at its active site that hydrolyses all beta lactam antibiotics excluding aztreonam [1,3].

After the initial report; the Health Protection Agency (HPA) in the United Kingdom (UK) concerned over the rapid increase in the number of cases of human colonization and infection with NDM-1 and other carbapenemases producing *Enterobacteriaceae* in hospitals across the country has raised a national alert in July 2009 [4]. Similarly, to the first case of NDM-1, the majority of patients with NDM-1 positive bacteria in the UK had a history of travel to India or Pakistan where many of them had been hospitalized with various indications including elective surgery and renal dialysis [5]. However, it is presumed that there are other reservoirs of infected patients in the Balkan countries and Middle East. Moreover, NDM-1-producing bacteria have been recovered from many infections such as urinary tract infections, pneumonia, septicaemia, wound and device-associated infections [3]. NDM is reported almost worldwide but did not successfully spread in most countries of Europe except the UK and recently France [6].

New Delhi Metallo-β-lactamase-1 gene (bla_{NDM-1}) codes for NDM-1 [2]. An association with other resistance mechanisms makes majority of *Enterobacteriaceae* with bla_{NDM-1} gene extensively resistant to antibiotics and susceptible only to colistin and less consistently, tigecycline [1]. Dissemination of the plasmid borne bla_{NDM-1} through horizontal gene transfer is a potential threat to the society [2]. Therefore, this research aimed at detecting the presence of NDM-1 producers in clinical bacterial isolates in Kano-Nigeria.

2. METHODOLOGY

A total of 500 clinical bacterial isolates were collected from Microbiology Departments of Aminu Kano Teaching Hospital, Muhammad Abdullahi Wase Specialist Hospital and Murtala Muhammad specialists Hospital, Kano, Nigeria after obtaining an ethical clearance from the respective hospitals' ethical committees. Bacterial isolates were characterized using biochemical tests for *Escherichia coli*, *Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi* and *Salmonella paratyphi* and screened for carbapenemase production according to the procedure described by Clinical and Laboratory Standard Institute guidelines using disc diffusion techniques with imipenem (10 µg) and meropenem (10 µg) obtained from oxoid UK. Any isolate that exhibited resistance or reduced susceptibility of 23 mm and 21 mm for meropenem and imipenem, respectively were subjected to further confirmatory tests [7].

Modified Hodge test was performed to confirm Carbapenemase production as described by the CLSI guidelines using Disc diffusion techniques with IPM (10 µg) and MEM (10 µg).

EDTA disc synergy test was performed as described by the CLSI guidelines [7] using Disc diffusion techniques with two meropenem discs; one with MEM (10 µg) and other containing 10 µl of 0.1 M anhydrous EDTA (292 µg). A strain producing a diameter of >4 mm around the disc with MEM-EDTA and not around the disc with MEM alone was considered phenotypically positive for NDM-1. *Escherichia coli* ATCC 25922 was used as the control strain.

Phenol chloroform method was used for DNA extraction according to manufacturer's instructions (ThermoFisher Scientific).The DNA was subjected to Polymerase Chain Reaction (PCR) with bla_{NDM-1} gene primers NDM-Fm (5′-GGTTTGGCGATCTGGTTTTC-3′,) and NDM-Rm (5′ CGGAATGGCTCATCACGATC-3′,), as designed by Nordmann et al. [8]. Using 50µl micro test tubes, 1.5 µl of NDM-1 primers each were pipetted and dispensed into the tubes; then 0.2 ml of dNTPS each, the cofactor ($mgCl_2$) 1.5 mM_g M_gCl_2, 14 mM tris-HCl Buffer (PH 8.2) and the Taq polymerase of 1.0 µl were added. Finally, 2 µl of the template DNA were also added to the reaction mixture. *Klebsiella pneumoiae* NCTC 13443 was used as the bla_{NDM-1} positive control. Then, the following conditions were used 94℃ for 5 minutes, 94℃ for 30 seconds, 43℃ for 30 seconds, 72℃ for 1 minute, and 72℃ for 10 minutes for 35 cycles. The amplicons were run on 1.5% agarose gel in TAE buffer at 120 volt for 1 hour. The DNA bands were visualized using UV light box (Gel documentation Unit).

Four PCR positive products were sequenced by Sanger sequencing dye termination method using Beckman Coulter Kit and setup according to manufacturer's instructions. Finally the DNA sequence was compared using Basic Local Alignment Search Tool (BLAST).

3. RESULTS

Out of 500 clinical bacterial isolates screened, 162(32.4%) were found to produce carbapenemase. Frequency of phenotypically detected New Delhi Metallo Beta Lactamases (MBLs) in this study was found to be 43(8.6%) (Table 1).

Upon sequencing, four positive PCR products showed 100% identity with bla_{NDM-1} (GenBank: KP826710.1 and KJ131191.1 for one *Klebsiella pneumoniae* and three *E. coli*). The overall frequency of NDM-1 in this study was found to be 0.8%. The highest frequency of NDM-1 producers was found among *Escherichia coli* 3(1.6%) and *Klebsiella pneumoniae* (0.5%) (Table 2)

Based on clinical samples, blood (25.0%) was found to have highest prevalence of MBLs followed by catheter tips (21.0%), wound swabs (11.1%) and urine (6.3%) (Table 3).

A representative result of Gel electrophoresis showing bla_{NDM-1} gene was given in Fig. 1.

4. DISCUSSION

The prevalence of carbapenemase producing bacterial pathogens (32.4%) was recorded in this study which is higher compared to that reported by Yusuf and Arzai [9] and Motayo et al. [10] with 14% in Kano, Northwest and 9.3% in Abeokuta, Southwest Nigeria respectively. However, it is lower to that of Yusuf et al. (34.5%). [11] According to the 2009 data from the European Antimicrobial Resistance Surveillance Network, the rates of carbapenem resistance were: 43.5% in Greece, 17.0% in Cyprus, 1.3% in Italy, 1.2% in Belgium and below 1% in other 23 reporting

Table 1. Prevalence of Carbapenemase and MBLs Producers among Randomly Collected Clinical Bacterial Isolates

Bacterial species	Isolates screened	Carbapenemase producers (%)	MBLs producers (%)
E. coli	187	59(31.6)	16(8.0)
K. pneumoniae	130	48(36.9)	13(10.0)
K. oxytoca	3	1(33.3)	0(0.00)
P. mirabilis	87	20(23.0)	8(9.2)
P. vulgaris	29	8(27.6)	1(3.5)
P. aeruginosa	56	21(37.5)	5(8.9)
S. paratyphi	2	1(50.0)	0(0.00)
S. typhi	6	4(66.7)	0(0.00)
Total	**500**	**162(32.4)**	**43(8.6)**

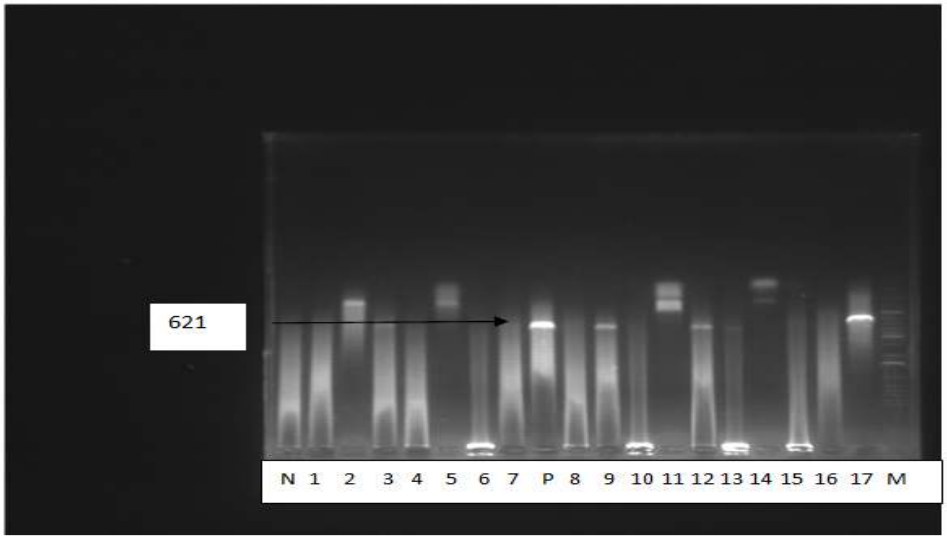

Fig. 1. Gel electrophoresis of bla_{NDM-1} gene

Key: Lane M is molecular maker graduated on hundred base pair (100 bp)
Lane N negative control, Lane P positive control,
Lane 3, 9, 12, and 13 are positive bands (Klebsiella pneumoniae and three E. coli respectively).
Lane 1, 2, 4, 5, 6, 7, 8, 10, 11, 14, 15, 16 and 17 are negative bands

countries. Higher prevalence in this study may be attributed to indiscriminate use of antibiotics in the study area [12].

The prevalence of MBLs was found to be 8.6%. The highest producers were *K. pneumoniae* followed by *E. coli*, *P. mirabilis*, *Ps. aeruginosa* and *P. vulgaris*. Oduyebo in Lagos Nigeria [13] reported slightly lower (8.5%) than this. However, the first report of MBLs detection among clinical bacterial isolates in Kano and Kaduna (Northwestern Nigeria) recorded 24.5% which is higher [14]. The differences in prevalence rates may be due to the differences in sample size and study area.

Table 2. Prevalence of bla_{NDM-1} Gene among Randomly Collected Clinical Bacterial Isolates

Bacterial Species	Isolates analyzed	Bla_{NDM-1} gene (%)
E. coli	187	3(1.6)
K. pneumonia	130	1(0.5)
K. oxytoca	3	0(0.0)
P. mirabilis	87	0(0.0)
P. vulgaris	29	0(0.0)
Ps. aeruginosa	56	0(0.0)
S. paratyphi	2	0(0.0)
S. typhi	6	0(0.0)
Total	**500**	**4(0.8)**

In this study, NDM-1was detected in Kano with the prevalence of 0.8% which is lower than the findings of Deogratius *et al.*in Uganda (2.6%) and Fazeli et al. in Iran (12.2 %) which could be due to variation in sample size and study area [15, 16]. The highest prevalence of NDM-1 producers was found among *E. coli* followed by *K. pneumoniae.* This correlated with the work of Kumarasamy et al. in India, Pakistan, and UK who reported highest prevalence of NDM-1 among *E. coli* and *K. pneumoniae* [1].

Table 3. Distribution of MBLs Producing Clinical Bacterial Isolates among Clinical Samples

Clinical samples	Isolates screened	MBLs producers (%)
Blood	36	9(25.0)
Catheter tip	19	4(21.0)
Ear swab	20	0(0.0)
High virginal swab	7	0(0.0)
Sputum	10	0(0.0)
Stool	5	0(0.0)
Urine	301	19(6.3)
Wound swab	99	11(11.1)
Total	**500**	**43(8.6)**

Blood samples were found to have the highest prevalence of MBLs (5.6%) which may be attributed to factors like improper use of syringes

or needles, inadequate disinfection of skin of prolonged hospital stayed patients during phlebotomy or transfusion and poor hand washing technique among health practitioners.

5. CONCLUSION

A novel carbapenemase, New Delhi Metallo Beta Lactamase 1(NDM-1) was first detected among clinical bacterial isolates in Kano, Northwestern Nigeria. Prevalence of NDM-1 producers is highest among blood samples. Therefore its identification in clinical bacterial infections will be suspected on any decreased susceptibility to carbapenem in *Enterobacteriaceae.*

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/19961