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

S. A. Abdullahi1*, A. H. Arzai2, I. Yusuf2, S. M. Adamu3, S. Adamu4, Y. A. Koki5, M. A. Rabi’u6 and A. M. Abbas7

1Public Health and Diagnostic Institute, Northwest University Kano, Nigeria.
2Department of Microbiology, Faculty of Sciences, Bayero University, Kano, Nigeria.
3Department of Pathology, National Orthopaedic Hospital Dala, Kano, Nigeria.
4Department of Pharmacy, Infectious Diseases Hospital, Kano, Nigeria.
5Department of Pathology, Murtala Muhammad Specialist Hospital, Kano, Nigeria.
6Jigawa Research Institute, Kazaure, Jigawa, Nigeria.
7Sir Muhammad Sunusi Specialist Hospital, Kano, Nigeria.

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.

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
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 blaNDM-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 Metal β-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, septicemia, 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 (blaNDM-1) codes for NDM-1 [2]. An association with other resistance mechanisms makes majority of Enterobacteriaceae with blanDM-1 gene extensively resistant to antibiotics and susceptible only to colistin and less consistently, tigecycline [1]. Dissemination of the plasmid borne blanDM-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 blaNDM-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 (mgCl₂) 1.5 mM, MgCl₂, 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 pneumoniae* NCTC 13443 was used as the blaNDM-1 positive control. Then, the following conditions were used 94°C for 5 minutes, 94°C for 30 seconds, 43°C for 30 seconds, 72°C for 1 minute, and 72°C 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<sub>NDM-1</sub>* (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* (3.16%) 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<sub>NDM-1</sub>* 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

| 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)**      |
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. pneumonia* 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$_{\text{NDM-1}}$ Gene among Randomly Collected Clinical Bacterial Isolates**

| Bacterial Species | Isolates analyzed | bla$_{\text{NDM-1}}$ 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-1 was 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. pneumonia*. 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. pneumonia* [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    | 7                 | 0(0.0)             |
| swab            | 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.

REFERENCES

1. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balkrishman R. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: A molecular, biological, and epidemiological study. Lancet Infect Dis. 2010;10(9):597-602.

2. Rathinasabapathi P, Hiremath DS, Arunraj R, Parani M. Molecular detection of New Delhi Metallo-Beta-Lactamase-1 (NDM-1) Positive Bacteria from Environmental and Drinking Water Samples by Loop Mediated Isothermal Amplification of blaNDM-1. Indian Journal of Microbiology. 2015;55(4):400–405.

3. Berrazeg MS, Diene M, Medjahed L, Parola P, Drissi, M, Raoult D, Rolain J M. Antibiogram and plasmid profiling of carbapenemase and extended spectrum Beta-lactamase (ESBL) producing Escherichia coli and Klebsiella pneumoniae in Abeckuta, South western, Nigeria. Afr Health Sci. 2013;13(4):1091–1097.

4. Health Protection Agency. Multi-resistant hospital bacteria linked to India and Pakistan: Health Protection Report. 2009; 3(26):3-4.

5. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K. Characterization of blaNDM-1 gene and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. Antimicrob Agents Chemother. 2009;53(12):5046-5054.

6. Centre for disease control and prevention emerging infectious diseases. Response to Detection of New Delhi Metallo-β-Lactamase–Producing Bacteria, Brazil. 2015;21:6.

7. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: 22nd Informational supplement. CLSI Document. 2012;M100-S22.

8. Nordmann Patrice, Laurent Poirel, Amélie Carrér, Mark A. Toleman, Timothy R. Walsh. How to detect NDM-1 Producers J. Clin. Microbiol. 2011;49(2): 718-721.

9. Yusuf I, Arzai AH. First detection of Carbapenemase producing clinical bacteria pathogens in Kano, Nigeria. Biological and Environment Sciences Journal for Tropics. 2011;8(3):163-167.

10. Motayo BO, Akindutu PA, Adeyakinu FA, Okerentugba PO, Nwanze JC, Onoh CG, Innocent-Adiele HC, Okonko IO. Antibiogram and plasmid profiling of carbapenemase and extended spectrum Beta-lactamase (ESBL) producing Escherichia coli and Klebsiella pneumoniae in Abeckuta, South western, Nigeria. Afr Health Sci. 2013;13(4):1091–1097.

11. Yusuf I, Rabiu AT, Haruna M, Abdullahi SA. Carbapenem resistant Enterobacteriaceae (CRE) in Intensive Care Units and Surgical Wards of Hospitals with no history of carbapenem usage in Kano, North West Nigeria. Nigerian Journal of Microbiology. 2015; 27: 1.

12. European Antimicrobial Resistance Surveillance Network. European Centre for Disease Prevention and Control; 2009. Availablefrom: http://ecdc.europa.eu/en/activities/surveillance/EARS-Net/Pages/Database.aspx

13. Oduyebo OO, Falayi OM, Oshun P, Ett AO. Phenotypic determination of carbapenemase producing Enterobacteriaceae isolates from clinical specimens at a tertiary hospital in Lagos, Nigeria. Nigerian Postgraduate Medical Journal. 2016;22(4):223-227.

14. Yusuf I, Yusha’u M, Sherif AA, Getso MI, Yahaya H, Bala JA, Aliyu IA, Haruna M. Detection of metallo beta lactamases among gram negative bacteria isolates from Murtala Muhammed Specialist
Deogratius Okoche, Benon B. Asiimwe, Fred Ashaba Katabazi, Laban Kato, and Christine F. Najjuka. Prevalence and characterization of carbapenem-resistant Enterobacteriaceae isolated from Mulago National Referral Hospital, Uganda. PLoS One. 2015;10(8): e0135745.

Fazeli H, Norouzi-Barough M, Ahadi AAM, Shokri D, Solgi H. Detection of New Delhi Metallo-Beta-Lactamase-1 (NDM-1) in carbapenem-resistant Klebsiella pneumoniae isolated from a university hospital in Iran. Hippokratia. 2015;19(3): 205–209.

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