ORIGINAL RESERCH ARTICLE

MOLECULAR DETECTION OF NEW DELHI METALLO BETA LACTAMASE 1 (NDM-1) PRODUCING BACTERIAL ISOLATES IN KANO- NORTHWESTERN NIGERIA

ABSTRACT

New Delhi Metallo Beta Lactamase 1 (NDM-1) is an enzymewithzinc ions at its active sitethat 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 inKano was investigated. A total of 500 bacterial isolates of *Enterobacteriaeceae*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 (10µg) and imipenem (10µg)by disc diffusion technique. Isolates of 23mm and 21mm for meropenem and imipenem respectively were confirm by modified Hodge test then EDTA Disc Synergy Test using Two meropenem discs, one with MEM (10µg), and other containing 10µl of 0.1M anhydrous EDTA (292µg) for Metallo Beta Lactamase (MBLs) and finallyseventeen MBLs isolates were screened with NDM-1 specific primers by PCRand Four PCR positive products were sequenced for bla_{NDM-1} gene. Of the 500 clinical bacterial isolates screened, 162 (32.4%),43(8.6%) and4(0.8%)were found to produce caberpenemase,MBLsandNDM-1respectively.The highest frequency of NDM-1 producers was found among *Escherichia coli* 3(1.6%)followed by *Klebsiella pnemoniae*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,*Enterobacteriaeceae,*Imipenem, MeropenemNew Delhi Metall B-Lactamase-1, PCR

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 carbapenemase 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 the 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 United Kingdom and recently France. [6]

New Delhi metallo-β-lactamase-1 gene (*bla*$_{NDM-1}$) codes for New Delhi metallo beta lactamase-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 is aimed at detecting the presence of NDM-1 producers in clinical bacterial isolates in kano-Nigeria.

Methodology

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

Modified Hodge test was performed to confirm Caberpenemase production as described by the CLSI guidelines (2012) using Disc diffusion techniques with IPM (10µg) and MEM (10µg).

EDTA disc synergy test was performed as described by the CLSI guidelines (2012)[7] using Disc diffusion techniques with two meropenem discs; one with MEM (10µg) and other

containing 10µl of 0.1M anhydrous EDTA (292µg). A strain producing a diameter of>4mm 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 manufacture's instructions(ThermoFisher Scientific).The DNA was subjected to Polymerase Chain Reaction (PCR)withbla_{NDM-1} gene primersNDM-Fm (5′-GGTTTGGCGATCTGGTTTTC-3′,) and NDM-Rm (5′ CGGAATGGCTCATCACGATC-3′,), as designed by Nordmann*et al*[8]. Using 50µl micro test tubes, 1.5µl of NDM-1 primers each were pipetted and dispensed into the tubes; then 0.2ml of dNTPS each, the cofactor ($mgCl_2$) 1.5mM$_g$ M_gCl_2, 14mM tris-HCl Buffer (PH 8.2) and the Taq polymerase of 1.0µl were added. Finally, 2µl 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, thefollowingconditions were used94^oC for 5 minutes, 94^oC for 30 seconds, 43^oC for 30 seconds, 72^oC for 1 minute, 72^oC for 10 minutes for 35cycles. The amplicons were run on 1.5% agarose gel in TAE buffer at 120volt for 1 hour.Finallythe DNA bands were visualized using UV light box (Gel documentation Unit)

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

Results

Out of 500 clinical bacterial isolates screened, 162 (32.4%) were found to produce caberpenemases. Frequencyof phenotypically detected New Delhi Metallo beta lactamases (MBLs) in this study was found to be43(8.6%)(Table1).

Upon sequencing,fourpositive PCR products showed 100% identity with bla_{NDM-1}(GenBank:KP826710.1 and KJ131191.1 for one *Klebsiella pneumoniea* and three *E. coli*).The overall frequency of New Delhi Metallo beta lactamase 1 (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 pnemoniae* (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)

An illustration of a typical modified Hodge test, a confirmatory test for carbapenemase productionwas shownin plate I. while, typical MBLs and non-MBLs isolates are depicted in plate II. A representative result of Gel electrophoresis showing bla_{NDM-1} genewas given in figure 1.

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. pneumonia	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)**

Table2: 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)**

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)**

Discussion

The prevalence of caberpenemase producing bacterial pathogens (32.4%) was recorded in this study which ishighercompared to that reported byYusuf and Arzai[9]and Motayo*et al*[10]with 14% in Kano, Northwest and9.3% inAbeokuta, Southwest Nigeriarespectively.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 carberpenem 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 countries.Higher prevalence in this study may be attributed toindiscriminate use of antibiotics in the study area.[12]

The prevalence of MBLs was found to be8.6%. The highest producers were*K. pnemoniaae,* followed by *E. coli*, *P. mirabilis*, *Ps. aeruginosa*and*P. vulgaris*.Oduyebo in LagosNigeria [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) recorded24.5% which is higher.[14]The differences in prevalence rates can be due to the differences in sample size and study area.

In this study,NDM-1was detected in Kano with the prevalence of 0.8%which is lower than the findings ofDeogratius*et al*.in Uganda(2.6%)andFazeli*et al*in Iran (12.2 %) which could be due to variation in sample sizeand study area.[15, 16] The highest prevalence of NDM-1 producers was found among *E. coli* followed by *K. pnemoniaae.* 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]

Blood samples were found to have the highest prevalence of MBLs (5.6%). This 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.

Conclusion

A novel carberpenemase, 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 identification of NDM-1 producers in clinical bacterial infections will be suspected on any decreased susceptibility to caberpenem in *Enterobacteriaceae.*

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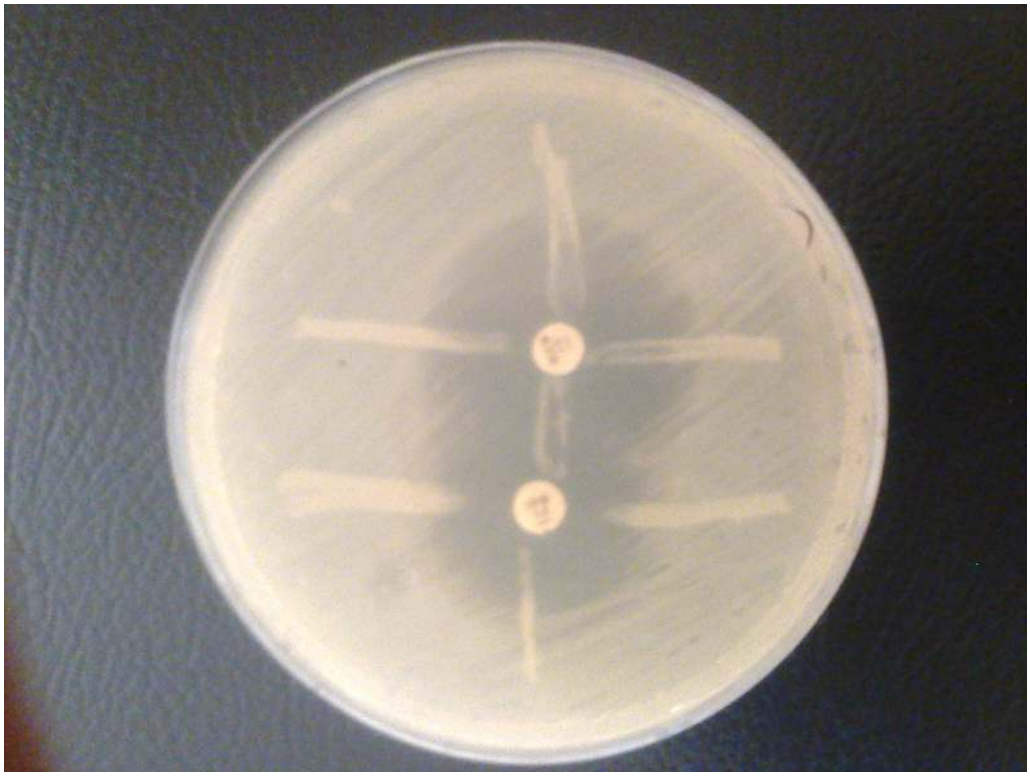

PlateI: Zone inhibition of Positive modified Hodge test

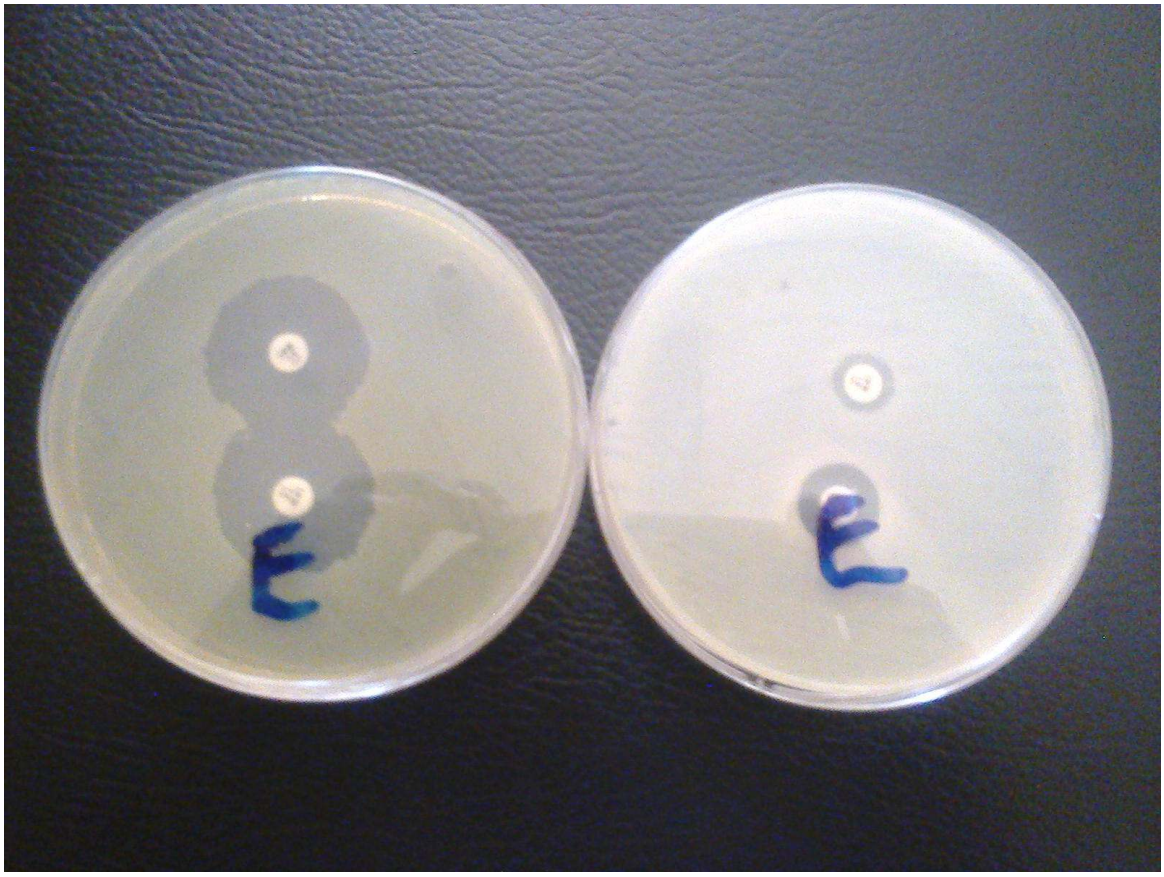

Plate II: Zone of inhibition of MBL (right) and non MBL producers (left)

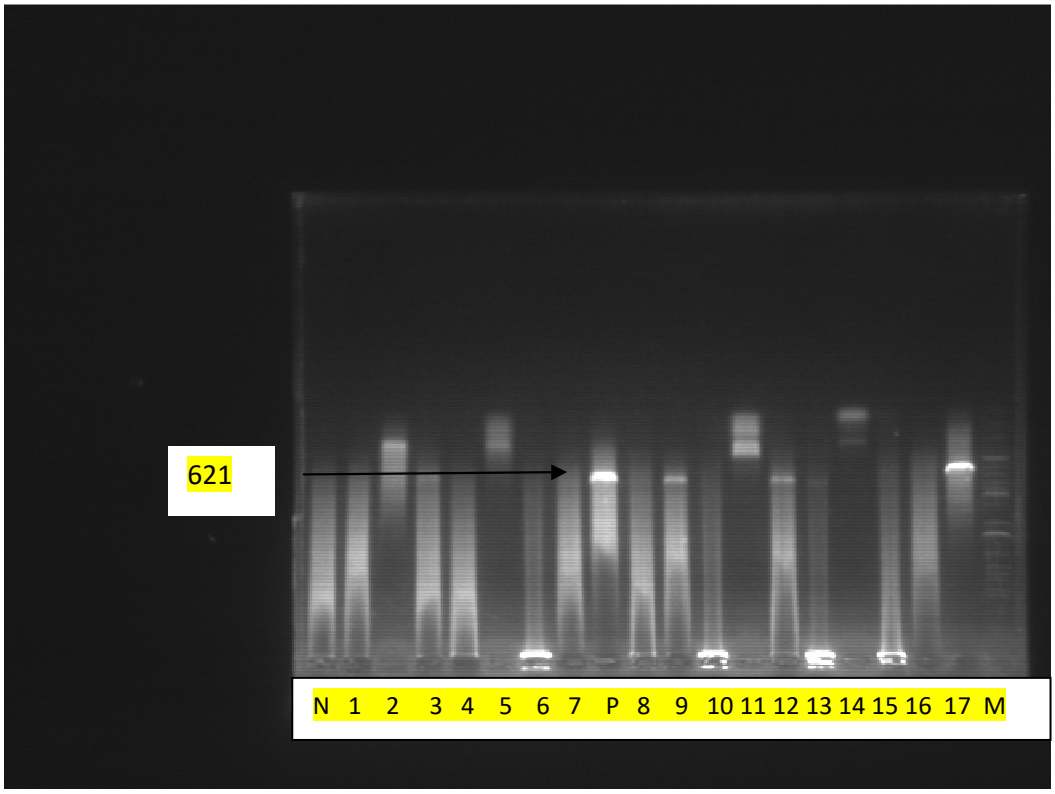

Figure 1: Gel electrophoresis of bla_{NDM-1} gene

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