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BLA-NDM-1 IN CLINICAL ISOLATES OF *Acinetobacter baumannii* FROM NORTH INDIA

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ABSTRACT

The growing incidence of the resistance to carbapenems due to NDM-1 in *Acinetobacter baumannii* is of major concern throughout the world. In this study 200, test cases were studied from October 2015 to January 2017 from six different hospitals of Kanpur. *A.baumannii* were isolated and identified by standard procedure. *A.baumannii* were confirmed genotypically by PCR using OXA 51 primer. All the isolated *A.baumannii* were screened for antimicrobial resistant pattern according to CLSI guideline. The screened isolates were further phenotypically studied for carbapenemase production by the MHT and CDDT. All the screened isolates were also subjected to PCR detection of the blaNDM-1 gene. From 200 patients admitted to ICU of different tertiary care hospitals and total 34 *A.baumannii* was isolated. The prevalence of *A.baumannii* infection in Kanpur region was 10% (20 out of 200 cases) Table 1. The rate of Acinetobacter infection was 9.18 per 1000 ICU days. Antimicrobial resistance pattern of isolated *A.baumannii* was also recorded and it found that only Polymyxins and Tigecycline showed 100% sensitivity. 27 strains were carbapenem resistant. Phenotypically only 18 strains were found to produce carbapenemases by CDDT and 11 strains were MHT positive. Among 34 isolated *A. baumannii*, 11 samples were found positive for bla_{NDM-1}.

KEY WORDS: $bla_{NDM-1,}$ Polymyxins, carbapenem, *A. baumannii*, nosocomial infections

NDM-1 is a novel type of metallo-β-lactamase that hydrolyzes all the β-lactam antibiotics. The gene that encodes for NDM-1 is called blaNDM-1 and is located on a transmissible plasmid and its association with other resistant determinants leads to the extensive drug resistance which is exhibited by a majority of the NDM-1 producing microorganism, leaving only a few therapeutic options (Young et al., 2009 and Kumarawamy et al., 2010)

Therefore, the NDM-1 producing organisms are now being recognized as the world's newest superbugs. In general, the term ''superbugs'' is a colloquial reference to a bacterium that carries resistance genes for many antibiotics (Salcido, 2010).

Acinetobacter baumannii, has emerged as an important opportunistic Gram-negative bacteria in health care institutions globally, as it resists desiccation, is hard to eradicate and has numerous intrinsic and acquired mechanisms of drug resistance. However, the data on the prevalence of NDM-1 producing *A. baumannii* isolates in Indian hospitals is limited. We therefore designed the Present study to evaluate the occurrence of carbapenem resistant A. baumannii and blaNDM-1 in the *A. baumannii* isolates in Kanpur.

MATERIALS AND METHODS

The Bacterial Isolates

A total of 34 non-duplicated A. baumannii isolates were isolated from various clinical samples such as Endotracheal aspirate, Endotracheal tip, sputum, blood, central line tip, urine and urinary catheters at different hospitals of Kanpur (UP). The samples were obtained from ICU admitted patients more than 48 hours between October 2015 and January 2017. The standard microbiological techniques were used for the isolation and the identification of the isolates has been mentioned in our study (Pal et al., 2017). All the isolates were stored in 10% glycerol-supplemented Brain heart infusion broth at -20 ^{0}C. This study was carried out with the consent of the institutional ethics committee.

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Antimicrobial Susceptibility Testing

The Kirby-Bauer disc diffusion method was performed to determine the susceptibilities of the different antibiotics and the results were interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2016). All the antibiotic discs and the media were procured from Hi-media, Mumbai, India. The *E. coli* ATCC 25922 and the Pseudomonas aeruginosa ATCC 27853 strains were used for quality control.

Screening for the Carbapenemase Production

By disc diffusion, all the isolates resistant to imipenem and meropenem (diameter of zones of inhibition, ≤ 21mm) were screened for the production of carbapenemase according to the CLSI guidelines

Phenotypic Detection of the Carbapenemase Production

The phenotypic detection of the carbapenemase production was performed by the Modified Hodge Test (MHT) by using a imipenem disc (10 µg). The detection of metallo-β-lactamase production was also performed by the Combined-Disc Diffusion Test by using two imipenem discs (10 µg), one containing 10µl of 0.1 M EDTA, which were placed 20 mm apart on a Mueller-Hinton agar plate. An increase in the zone diameter of >5 mm around the imipenem-EDTA disc as compared to that of the imipenem disc alone was considered as positive for metallo-β-lactamase production.

Molecular detection of the bla_{NDM-1} genes

DNA was extracted from all the screening positive isolates by using the DNA extraction Kit (Qiagen, Germany) as per the manufacturer's protocol.

PCR reaction

PCR was carried out in 20 µl reaction volume with 10µl master mix (Takara), 5µl nuclease free water, 1 µl forward and reverse primer [NDM-Fm (5′-CTGAGCACCGCATTAGCC-3′) and NDM-Rm (5′- GGCCGTATGAGTGATTGC-3′) (GCC biotech, Calcutta)] each and 3µl DNA template. Conditions for PCR were as below, initial denaturation 94 °C for 5 min, number of cycles were 34 in each cycle denaturation was set at 94 °C for 30 sec, annealing at 50 °C for 30 sec and then 72°C for 1 min for extension, followed by a final extension at 72°C for 5 min. After amplification, amplified DNA was identified by 1% agarose gel electrophoresis. Loading and electrophoresis of amplified PCR product and visualization of the band on agarose gel were performed as mentioned before.

The PCR product of the blaNDM-1 gene was sent for sequencing to Chromus biotech Pvt. Ltd., Bangalore, India.

RESULTS

In this study 200, test cases were studied from October 2015 to January 2017 from six different hospitals of Kanpur. From 200 patients admitted to different ICU, total 315 samples were collected and microbiologically processed.

In this study total of 200 patients were observed for nosocomial infections, admitted to ICU of different tertiary care hospitals and total 34 *Acinetobacter baumannii* was isolated. These 34 isolated A. baumannii also gave the positive result in PCR with OXA 51 primer.

On the basis of microbiological and clinical correlation among 34 isolated *A.baumannii*, 20 were contributed to the infection. Hence, the prevalence of *A.baumannii* infection in Kanpur region was 10% (20 out of 200 cases) Table 1. The rate of Acinetobacter infection was 9.18 per 1000 ICU days. Antimicrobial resistance pattern of isolated *A.baumannii* were shown in table 2. Only Polymyxins and Tigecycline showed 100% sensitivity. 27 strains were carbapenem resistant. Phenotypically only 18 strains were found to produce carbapenemases by CCDT [Table 3]. While only 11 strains were MHT positive. [Table 3] Sensitivity and specificity of tests have mentioned in table 4. After the analysis of phenotypically all the isolated *A .baumannii* strains were subjected to bla $_{NDM}$ detection. Among 34 isolated *A. baumannii*, 11 samples were found positive for bla_{NDM-1} [Fig 1].

Table 1: Distribution of A. baumannii associated with infection and colonization

Infection/colonizer	No. of *A. baumannii*
Associated with infection	20
Associated with colonizer	14

Table 2: Antibiotic resistant pattern of *A. baumannii*

S.N.	Antibiotics	Antibiotics	No. of Strains	Percent
1.	Piperacillin(100µg)	PI	34	100.0
2.	Ampicillin/Sulbactum(10/10µg)	A/S	34	100.0
3.	Ticarcillin(75µg)	TC	34	100.0
4.	Piperacillin/Tazobactum(100/10µg)	PIT	28	82.4
5.	Cefotaxime(30µg)	CTX	34	100.0
6.	Ceftriaxone(30µg)	CTR	34	100.0
7.	Ceftazidime(30µg)	CAZ	34	100.0
8.	Cefepime(30µg)	CPM	34	100.0
9.	Cefepime/Tazobactum(30/10 µg)	CPT	33	97.1
10.	Cefoperazone/Sulbactam	CFS	30	88.2
11.	Aztreonem	AT	34	100
12.	Amikacin(30µg)	AK	27	79.4
13.	Gentamycin(10µg)	GEN	29	85.3
14.	Tobramycin(10µg)	TOB	33	97.1
15.	Netilimicin	NET	32	94.1
16.	Ciprofloxacin(5µg)	CIP	30	88.2
17.	Ofloxacin(1 µg)	OF	29	85.3
18.	Levofloxacin(5µg)	LE	28	82.4
19.	Tetracycline(30µg)	TE	29	85.3
20.	Cotrimoxazole(1.25/23.75µg)	COT	32	94.1
21.	Imipenem(10µg)	IMP	27	79.4
22.	Meropenem(10µg)	MRP	27	79.4
23.	Polymyxin B	PB	0	0.0
24.	Tigecycline	TGC	0	0.0

Table 3: Different methods of detection of Carbapenem susceptibility

	Screening test	MHT	CDDT	Genotypically
Carbapenem resistance	27	11	18	23

Table 4: Diagnostic test evaluation; Sensitivity and specificity of MHT and CDDT

	MHT	CDDT
Sensitivity	47.83%	78.26%
Specificity	100%	100%
Positive predictive value	100%	100%
Negative predictive value	47.83%	68.75%

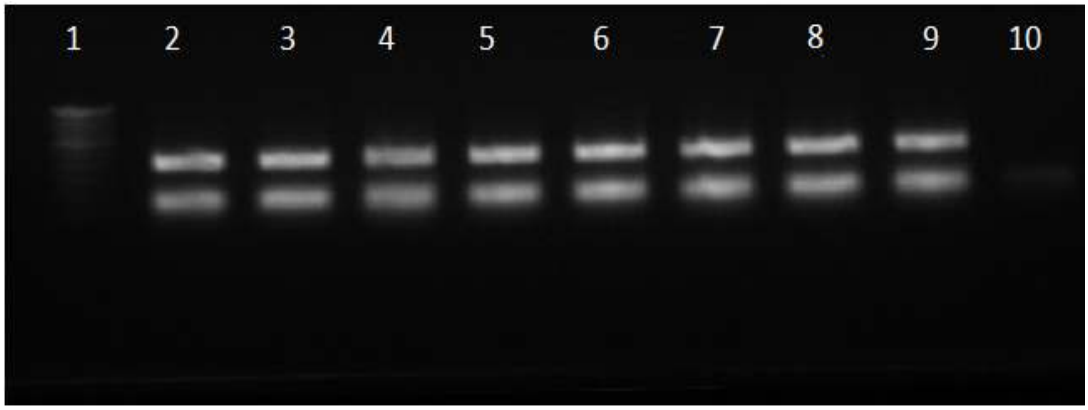

Fig. 1: The photographs show positive confirmation of $bla_{NDM\ 1}$ gene from lane 2 to 9 were found positive samples, lane 10 shows negative result and lane 1 associated with 100bp ladder

DISCUSSION

Non fermenting Gram-negative bacteria *Acinetobacter baumannii*, widely associated with long term care facilities. In this study 200, test cases were studied from six different hospitals of Kanpur and 315 samples were collected and microbiologically processed.

In this study total of 200 patients were observed for nosocomial infections, admitted to ICU of different tertiary care hospitals and total 34 *Acinetobacter baumannii* was isolated. Species A. baumannii was confirmed by presence of $bla_{OXA\ 51}$ gene. On the basis of microbiological and clinical correlation among 34 isolated *A. baumannii*, only 20 were contributed to the infection. The rate of Acinetobacter infection was 9.18 per 1000 ICU days.

Omer et al reported approx similar result, 9.5% of Acinetobacter infection in their study (Omer et al., 2015). There were some another studies, in Rajasthan 83.2% of *A. baumannii* was reported as the most common nosocomial pathogen especially from ICUs (Sharma et al., 2013). Similarly, in Pune, Acinetobacter was the most common isolates from ICU (Patwardhan et al., 2008). The differences among the studies can be attributed to differences in study populations and locality, availability of resources, overcrowding or shortage of nurseries, antibiotic use and the difference in surveillance methods for detection of nosocomial infections.

Here only polymyxins and tigecycline was found to be 100% sensitive other studies showed also that these are the most effective drugs (Urban et al., 2003 and Jaggi et al., 2012). This study brings up an important aspect of increasing resistance in *A.baumannii* towards carbapenems and harbor bla_{NDM-1}.

The increasing reports on the NDM-1 producing bacteria are a major concern worldwide. Although, NDM-1 seems to be endemic in the Indian subcontinent, other studies have suggested that the Balkan countries may act as another reservoir of the NDM-1 producers (Kumarasamy et al., 2010 and Lascols et al., 2009)

Here, we are reporting the presence of NDM-1 producing A.baumannii isolates in various clinical samples from a tertiary care hospital. The overall prevalence of the blaNDM-1 possessing A. baumannii isolates in Kanpur was found to be 5.5% (11/200).

Screening for the drug susceptibility reported 27 strains were carbapenem resistant. Phenotypically only 18 strains were found to produce carbapenemases by CDDT, while only 11 strains were MHT positive.

MHT has been non specific for the detection of metallo-beta-lactamases (Thomson et al., 2010) in the present study MHT detected 6 of the 11 NDM-1 positive isolates. The CDDT detected 9 of the 11 isolates that were positive for the bla_{NDM-1}gene. Therefore only molecular tools were capable of detecting NDM in *A. baumannii*, and it is likely that

carbapenem resistance is the result of decreased production of outer membrane porins, together with low-level expression of NDM (Naas et al., 2009 and Poirel et al., 2006).

In this study, samples were collected from ICU. Among the 11 bla_{NDM-1} positive *A. baumannii* isolates, a majority of the infected patients were died (5/11).

Bonnin et al. recently suggested that *A. baumannii* not only might accept resistance genes but also could act as a gene donor, spreading resistance genes to other bacteria, including Enterobacteriaceae (Bonnin et al., 2014). This possibility emphasizes our concerns about dissemination of these genes in our country as in many other countries where NDM-1-producing *A. baumannii* have been isolated, making these findings a global public health matter, as suggested by Johnson and Woodford (Bonnin et al., 2014).

CONCLUSION

Carbapenem Resistant *A. baumannii* was prevalent in our Kanpur region. Carbapenemase genes bla_{NDM} in clinical isolates of *A. baumannii* is a worrying trend and it underlines the need for a stringent infection control practices along with antimicrobial stewardship to curb these *Acinetobacter* species.

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