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DETECTION OF *BLA*$_{NDM-1}$ GENE IN *PROVIDENCIA RETTGERI* ISOLATED FROM DIABETIC FOOT ULCERS OF FOUR PATIENTS FROM INDIA

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ABSTRACT

Providencia rettgeri is associated with various infections, including skin infections, diarrhea, bacteremia and urinary tract infections (UTI) andmany hospital acquired infections. Carbapenems are mainstay of treatment fordrug resistant bacterial infections. Enzyme New Delhi Metallo β Lactamase-1encoded by the gene *bla*$_{NDM-1}$ make these bacteria resistant to carbapenems. This gene is located ontransmissible plasmid which may include other antibiotic resistance genes.A total of four strains of *Providencia rettgeri*was isolated from the wound swabs ofpatients with diabetic foot ulcer. They were screened for the production of carbapenemase and confirmed byPolymerase Chain Reaction amplification and sequencing the *bla*$_{NDM-1}$ gene. Presence of this gene can be a potential risk factor in management of diabetic foot infections, which can lead to systemic toxicity and setting of gangrene. To our knowledge this is the first report of*Providencia rettgeri* with *bla*$_{NDM-1}$ isolated from diabetic patients in India.

KEY WORDS:*Providencia rettgeri*, **Diabetic foot ulcer, NDM β Lactamase, *bla*$_{NDM-1}$**

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INTRODUCTION

Providencia rettgeri has been associated with various infections including skin infections, diarrhea, bacteremia and urinary tract infections (UTI). It has been also associated with hospital acquired infections, including catheter related UTI,bacteremia and gastroenteritis.[1,2]Increased use of cephalosporins in serious infections enforce the clinicians to often use the carbapenems as drugs of last resort to treat these infections. Carbapenems is a group of antibiotics that kills the bacteria by inhibiting cell wall synthesis. They are mainstay of treatment for drug resistant bacterial infections. New Delhi Metallo βLactamase-1 is the enzyme which makes the bacteria resistant to β Lactam antibiotics including carbapenems.[3] Synthesis of enzyme NDM-1 is encoded by the gene*bla*$_{NDM-1}$.NDM-1 was first detected in bacteria *Klebsiella pneumoniae* from a Swedish patient of Indian origin in 2008.[4]Subsequently it was also reported from Pakistan, UK, US [5], Canada [6] and Japan.[7] After the report of NDM-1 in *Klebsiella pneumoniae* it was also reported in *E. coli*[8], *Acinetobacter baumannii*[9] and other Enterobacteraceae.[10]NDM-1 has obtained significant importance as this gene is located on transmissible plasmid which may include a number of other antibiotic resistance genes.It may result into spread of resistance among the same or other bacterial pathogen. Hence a case report and other studiesdescribing emergence of NDM-1 are an important warning.Todate there have been only six reports of *Pr. rettgeri* isolates harboring metallo β Lactamase (MBL) encoding genes all over the globe which includes IMP-type MBL producers from Japan[11,12], VIM-type MBL from Korea[13], and NDM- type MBL from Israel[14], Brazil[15] and Nepal[16] and India.[17]Here we report isolation of highly resistant strains of *Providencia rettgeri* from diabetic foot ulcers of four Indian patients which showed presence of *bla*$_{NDM-1}$ gene.

MATERIALS AND METHODS

Bacterial Strains: A total of four strains of *Providencia rettgeri* were isolated from the wound swabs of patients with diabetic foot ulcer. They were identified using standard microbiological procedures.[18]

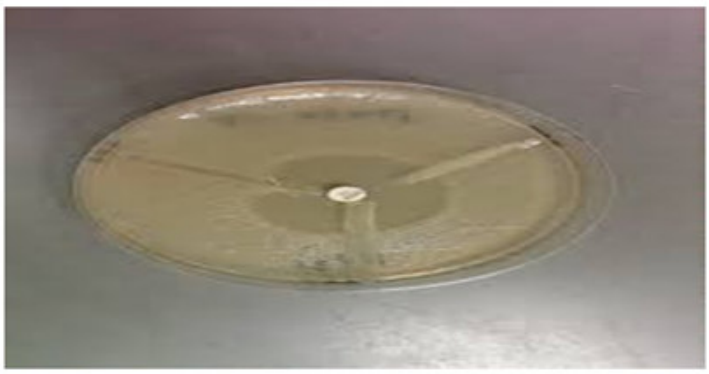
Antimicrobial Susceptibility Testing: All the isolates were subjected to antibiotic sensitivity test by Kirby Baur's Disc Diffusion method and the results were interpreted as per CLSI guidelines.[19]Allthe antibiotic discs were procured from Hi Media, Mumbai, India.*E.coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used for quality control.

Screening and Phenotypic Detection of Carbapenemase Production: All the four isolates were subjectedto Antibiotic Sensitivity test and showed reduced susceptibility to Meropenem and Ertrapenem. These isolates were screened for production of carbapenemaseby Modified Hodg 2test as per CLSIguidelines.[19](Figure:1)

Molecular Detection of NDM Gene

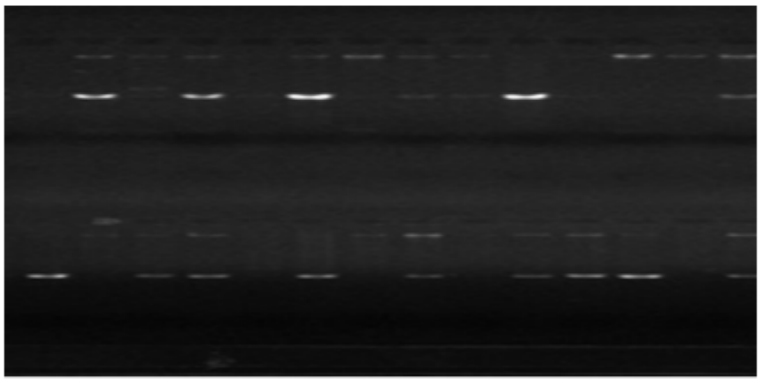
The presence of MetalloβLactamase enzymes were further confirmed by Polymerase Chain Reaction (PCR) amplification and sequencing the *bla*$_{NDM-1}$gene from all four of the *Providencia rettgeri* isolates.Detection of NDM-1 in Gram-Negative Clinical Isolates[20]Glycerol stocks were prepared from the isolates; DNA was extracted from the isolates using PureLink® Pro 96 Genomic DNA Purification Kit (Invitrogen, USA). PCR amplification was done using NDM-1 forward (5'GGTTTGGCGATCTGGTTTTC-3') and NDM-1 reverse primers (3'CGGAATGGCTCATCACGATC-5') Product Size being 621bp (Annealing temperature 52 degree Celsius) PCR was performed for 35 cycles using a GeneAmp® PCR System 9700 Thermal Cycler (Applied BiosystemsInc, USA). The amplification was confirmed on 1% agarose gel by gel electrophoresis. A 1kb+ ladder was loaded along with the PCR products. Original plasmid of *K. pneumoniae* was used as a positive control.The bands at approximately 620bp were considered to be NDM-1 positive. The PCR products were then purified by PEG-NaCl, and sequenced using the above primers by Sanger sequencing on 3730XL DNA Analyzer, BDT version 3.1. (Figure 2).

Figure 1
Detection of Carbapenemase by Modified Hodge Test



MHT Showing Positive Result by Indentation of Lawn Growth Near Test Strain

Figure 2
Detection of blaNDM-1 by Polymerase Chain Reaction



Agarose Gel Showing PCR Amplified Product of bla_{NDM-1} Gene

RESULTS

Three out of four isolates were resistant to carbapenems (Imipenem and Meropenem) whereas the fourth showed intermediate susceptibility to Imipenem. All the isolates were resistant to Cephalosporins (Cefaparazone, Cefuroxime, Cefepime and Ceftazidime) and Aminoglycosides(Gentamicin and Amikacin). They were sensitive to Tigecycline and Colistin. All the isolates gave Modified Hodge Test positive and also revealed the presence of bla_{NDM-1}on PCR.

Table1
Antimicrobial Resistance Profiles of the FourPr.rettgeri Isolates

Antibiotic Isolate No.	CEF	CXM	CPM	CTZ	GM	AK	IPM	MRP	CL	TG
1	R	R	R	R	R	R	R	R	S	S
2	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**S**
3	R	R	R	R	R	R	R	R	S	S
4	R	R	R	R	R	R	S	R	S	S

(CEF-Cefaparazone,CXM- Cefuroxime, CPM-Cefepime, CTZ-Ceftazidime, GM- Gentamicin, AK-Amikasin, IPM-Imipenem, MRP-Meropenem, CL-Colistin, TG-Tigecycline)

DISCUSSION

Introduction of third generation cephalosporins was considered a boon in the treatment of infectious bacterial diseases. However, their increaseduse has resulted into emergence of resistant Enterobacteraceae possessing ESBLs. NDM β Lactamase has attracted significant attention asthe gene encoding MBL is located on a very mobile genetic element and it spreads in a very complex and unpredictable manner. Hence there are chances of finding this gene or gene cassette in other gram negative bacteria harbored by the same patient. A multinational study published by The Lancet of Infectious diseases reported 37 cases in UK, 44 in Chennai, 26 in Haryana and 73 other sites in India and Pakistan.[3] NDM-1 resistance can spread globally because of increased levels of travelers.However, there are very few reports (6 till date) of detection of *bla*$_{NDM-1}$ gene in *Providencia rettgeri.* This is probably the first report of *bla*$_{NDM-1}$ gene in *Providencia rettgeri* from Indian patients with diabetic foot ulcers. *Providencia rettgeri* has been also reported to be isolated from diabetic foot ulcer by Jain et al. from Ahmedabad, India.[21] Diabetic foot infections are poly-microbial in nature. Prevalence of isolates increase with the increase in the Wagner's scale. Isolation of a highly resistant organism like the one harboring *bla*$_{NDM\ -1}$ can lead to devastating complications in such cases. The presence of*bla*$_{NDM-1}$ gene and high level resistance observed in the present study may be due to wide spread use of broad spectrum antibiotics leading to survival advantage to the bacteria.Global spread of NDM-1 mediated antibiotic resistance has also been reported by Johnson and Woodford.[22]In this respect the authors agree with Senthil Kumar et al. who have stressed the need for development of a newer antibiotic to treat the critical infections by drug resistant bacteria.[23]Presence of resistant genes like *bla*$_{NDM-1}$ leads to multidrug resistance which can be a potential risk factor in management of diabetic foot infections and can lead to complications like systemic toxicity and setting of gangrene.

CONCLUSION

To our knowledgethis is the first report describing *Providencia rettgeri*strains harboring*bla*$_{NDM-1}$ in diabetic patients from India. They were highly resistant to β lactams and aminoglycosides. Presence of *bla*$_{NDM-1}$ gene with other resistance determinants on transmissible plasmid constitutes a significant threat to global healthcare. Hence detection and reporting of NDM-1 producing bacteria with any reduced susceptibility to carbapenems should be considered as high alert as it underlines the need for adopting effective measures to control such type of infection by prudent usage of antibiotics and strict implementation of infection control policy.

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