Superbugs causing infections at a tertiary care hospital and the return of pre-antibiotic era!

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ABSTRACT

Aims and Objectives: Increase in the incidence of Escherichia coli and Klebsiella pneumoniae carrying New Delhi metallo beta lactamase-1 (NDM-1) gene are called superbugs is of great concern as presence of blaNDM-1 gene makes E.coli and K.pneumoniae highly resistant to most of currently available antibiotics. This study was planned to observe the burden of blaNDM-1 gene carrying E. coli and K.pneumoniae at a tertiary care hospital in northern India.

Materials and Methods: A total of 1709 E. coli and 327 K. pneumoniae nonrepitive isolates were taken from various clinical samples received in a tertiary care hospital in northern India Lucknow during the period from May 2012 to April 2013. Carbapenemase production was phenotypically detected in all the carbapenem resistant isolates by modified Hodge test. Metallo-β-lactamase production was detected by using meropenem and imipenem discs with and without EDTA and blaNDM-1 gene was detected by polymerase chain reaction.

Results: Over all metallo-β-lactamase production was found in 82% and 88.89% of carbapenem resistant E.coli and K. pneumonia respectively. Out of 366 carbapenem resistant isolates, 102 were found positive for blaNDM-1 gene out of which 89 were E.coli and 13 were K. pneumoniae.

Conclusions: With limited treatment options left for this crisis situation like the pre-antibiotic era; it is an alarm for rational antibiotic usage and intensive education programs.

Key words: New Delhi metallo beta lactamase-1, Escherichia coli, Klebsiella pneumoniae, Metallo-β-lactamase

INTRODUCTION

During the last two decades, appearance of β-lactam resistance specially to 3rd and 4th generation cephalosporin in members of enterobacteriaceae family arose as a serious problem to global health. Currently carbapenem are the therapeutic drug of choice for treating serious infections caused by Escherichia coli and Klebsiella pneumoniae. One of the common mechanism of carbapenem resistance in E.coli and K. pneumonia is production of carbapenemase enzyme. Recently a novel β-lactamase NDM-1 has been reported in multidrug resistant E.coli and K.pneumoniae. NDM-1 gene in Enterobacteriaceae isolates were reported from patients in India, Pakistan, Bangladesh and the UK in 2008–2009. NDM-1 gene is being reported worldwide, however exact burden of NDM-1 gene in world is not known as very few studies has been done on NDM-1 gene and epidemiological data has not been known so far.

It is necessary to detect these strains as soon as possible and suitable infection control interventions should be implemented for all patients infected or colonized with MBL producing bacteria.

MATERIAL AND METHODS

Study isolates

A total of 1709 E.coli and 327 K.pneumoniae isolates were taken from the culture of 20,674 various clinical samples (urine, pus, blood, cerebrospinal fluid, endotracheal aspirate, catheter tips etc) received in the bacteriology laboratory of the Microbiology department during one year period from
May 2012 to April 2013. All the carbapenem resistant isolates included in the study were further identified by following accepted laboratory criteria.4

Antimicrobial susceptibility testing
Antimicrobial susceptibility against isolated *E.coli* and *K.pneumoniae* for various antimicrobial agents using beta-lactam and non-beta lactam antibiotic discs were determined by Kirby-Bauer disc diffusion method and results were interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines.5 Antibiotic discs were procured from Hi-Media, Mumbai, India, except imipenem, which was from BD Diagnostics, Franklin Lakes, NJ, USA. EUCAST guideline 2012 suggest MIC method for colistin drug susceptibility.6 Reference grade powder for imipenem and/or meropenem were obtained from Lac Zene Biosciences, Delhi, India. Minimum inhibitory concentration (MIC) values for imipenem, meropenem and colistin were determined by agar dilution method. Results were interpreted as standard protocol. All the isolates with reduced susceptibility to meropenem and/or imipenem on disc diffusion testing and/or MIC were screened for carbapenemase production. Phenotypic detection for carbapenemase production was done by modified hodge test.7 *K.pneumoniae* ATCC BAA-1705 as positive control and *K. pneumoniae* ATCC BAA-1706 as negative controls were used for MHT.

MBL production was detected in isolates with reduced susceptibility to imipenem and/or meropenem using combined disc diffusion test by imipenem (10 μg) and 0.1 M ethylenediaminetetraacetic acid (EDTA: 292 μg) as described previously by Franklin et al.7 This was complemented by the Modified Hodge test and the double disc synergy test with imipenem (10 μg) alone and imipenem (10 μg)+EDTA (750 μg) for all the isolates resistant to any carbapenem as performed earlier by Lee et al.8

Molecular detection of *bla*~NDM-1~ gene-
Template DNA from meropenem and/or imipenem resistant strain was prepared from freshly cultured by re-suspending 3-5 colonies in 100 μL of molecular grade water, and then by heating at 95°C for 10 minutes. The bacterial lysate was centrifuged at 10,000 rpm for 2 min, the supernatant was transferred to new eppendorf tube and used as DNA template for PCR amplification. *K.pneumoniae* ATCC BAA-2146 procured from Eurofins Genomics, MWG Biotech AG primer microbiologies KWIK STIC was used as positive control for NDM-1 gene. The resistance gene was amplified by polymerase chain reaction (PCR) by using previously published primers NDM-Fm 5’GGTTTGGGCATCCTGTTTTC-3’ and NDM-Rm-5’CGGATGCTCATCAAGT-3’.9-11 specific for *bla*~NDM-1~ in selected meropenem and/or imipenem resistant strains.12 The Amplified product was analysed on 2% (w/v) agarose gel containing (0.5 μl/ml) of ethidium bromide along with molecular weight marker (100 bp) electrophoretically. Constant current (80V) was maintained for 2 hr and analysed by 264 nm wavelength UV transilluminator and gel doc (alphalmager3400HP) was used for documentation. A sharp band 621 bp of amplified DNA visualized, considered to be positive (Figure 1).

RESULTS
Out of 1709 *E.coli* and 327 *K. pneumoniae* isolates, 330 *E.coli* and 36 *K.pneumoniae* isolates were resistant to carbapenem. The patients suffering from these carbapenem resistant isolates having various risk factors [Table 1]. Percentage of *bla*~NDM-1~ positive isolates among carbapenem resistant *E.coli* and *K.pneumoniae* were 26.97%(89/330) & 36.11%(13/36) respectively. Majority of patients having infection with *bla*~NDM-1~ gene containing isolates were in 15-60 years of age group. Majority of the *bla*~NDM-1~ gene positive isolates were obtained from ICU and wards. All *bla*~NDM-1~ producing *E.coli* and *K. pneumoniae* were susceptible to colistin on MIC however only 53.9% *E.coli* and 61.5% *K. pneumoniae* were susceptible to tigecycline. For other antimicrobials, *bla*~NDM-1~ positive isolates showed variable susceptibility pattern [Table 2]. Out of total 89 *bla*~NDM-1~ positive *E.coli*, only 1 isolate was found moderately sensitive to meropenem on MIC, however it was resistant to imipenem. All isolates of *K. pneumoniae* with *bla*~NDM-1~ positive were resistant to both meropenem and imipenem by MIC method. Range of MIC value for meropenem, imipenem and colistin were 2-16 μg/ml, 4-16 μg/ml and 0.5-1 μg/ml respectively for *E.coli* and 4-16 μg/ml, 4-8 μg/ml and 0.5-1 μg/ml respectively for *K.pneumoniae*.

DISCUSSION
Enterobacteriaceae members are common causes of both health care and community acquired infections, raising the possibility of spread of resistant organisms

![Figure 1: Representative gel picture showing amplification of *bla*~NDM-1~ gene (621bp). Lane M: 100 bp DNA ladder; Lanes 1 and 2: Positive control Klebsiella pneumoniae ATCC BAA-2146; Lane 3: Negative control; Lane 4-9: Clinical isolates](image-url)
into the community. Our study gives an initial account on the presence of \textit{blaNDM-1} gene in a tertiary care hospital in northern India. Amongst all carbapenem resistant study isolates, \textit{blaNDM-1} gene was present in 89 isolates of \textit{E. coli} (26.97%) and 13 \textit{K. pneumoniae}. In a study done by Kumarasamy et al., in Chennai (India), \textit{blaNDM-1} gene was found in 25.33% \textit{E. coli} and 23.33% isolates of \textit{K. pneumoniae} in carbapenem resistant isolates. Over all \textit{blaNDM-1} gene was found in 5% of isolated \textit{E. coli} and 23.33% isolates of \textit{K. pneumoniae} in carbapenem resistant isolates. All NDM-1-producing isolates of \textit{E. coli} and \textit{K. pneumonia} were susceptible to colistin on MIC while in a study done by Kumarasamy et al., colistin showed variable sensitivity pattern to NDM-1 gene isolates. Reason may be different geographical distribution. Susceptible to tigecycline which are almost consistent with a study done by Seema et al., 2011 in which tigecycline susceptibility was present in 46.2% and Kumarasamy et al., 2010 in which it was 64% and 67%. In our study some of the \textit{blaNDM-1} harbouring isolates were found phenotypically susceptible to one or more carbapenems tested.

So all the carbapenem disc should be used for drug susceptibility with, and if possible molecular analysis should be done in hospital setting because false susceptibility testing may lead to treatment failure.

### CONCLUSION

Present study shows higher rate of resistance to commonly used carbapenems in a tertiary care set up in northern India which may be due to irrational use of antibiotics and responsible genes can transfer from one bacteria to other. It is necessary to detect these strains as soon as possible and suitable infection control interventions should be implemented for all patients infected or colonized with MBL producing bacteria.

### REFERENCES

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All the authors were equally involved in drafting and finalizing the manuscript. RK - Concept and design of study, statistically data analysis, supervised research work, manuscript preparation and editing, finalised manuscript; SR - Concept, collected data and literature search, did experimental work, helped in data analysis, helped in manuscript preparation and editing; JA - Concept of study, helped in molecular work, critical revision and editing of manuscript; MS - Concept of study, helped in molecular work, manuscript editing and review.

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