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Antibiotic Resistance Pattern and Detection of Newly Emerging Resistant Gene New Delhi Metallo-ß-lactamase- 1(blaNDM-1) in *Escherichia coli* and *Klebsiella pneumoniae*

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ABSTRACT

***Escherichia coli* and *Klebsiella pneumoniae* are the normal inhabitants of humans. Most strains of *E. coli* are harmless and benefit their host but some of them may cause a wide variety of intestinal and extra-intestinal diseases, such as diarrhea, urinary tract infections, septicemia, neonatal meningitis and renal complications. Several strains Klebsiella may also cause bacteremia, pneumonia and urinary tract infections. These pathogenic strains are becoming resistant to antibiotic therapy. This antibiotic resistance is posing a major threat to public health and problem in treating various infections. The present study has been designed to evaluate antibiotic resistance pattern and detection of newly emerging gene New Delhi Metallo-ß-Lactamase 1 (blaNDM-1) in *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates. A total of 52 clinical isolates were collected from different hospitals of Karachi in the period of August 2012 - October 2012. Antibiotic sensitivity test was performed by disc diffusion method using different antibiotics. In this study it was found that *E.coli* from diarrheal source and other sources showed highest resistance to Ampicillin (upto 89%) and is highly sensitive to Meropenem and Imipenem (100%). While *Klebsiella pneumoniae* showed highest resistance to Ampicillin (100%) and showed some resistance to Meropenem and Imipenem (13%). In the PCR detection, 04 isolates out of 52 carried the resistant gene blaNDM-1. The presence of this blaNDM-1 gene was significantly higher in *Klebsiella pneumoniae* as compared to *Escherichia coli* strains.**

Keywords: Antibiotic, Disc Diffusion Method, Metallo-ß-Lactamase 1, PCR.

INTRODUCTION

Bacteria from clinical and non-clinical settings are becoming increasingly resistant to conventional antibiotics. The use of the antibiotics was started after some years of Flemings' discovery, who noticed the inhibition of *Staphylococcus species* by *Penicillium spp.* (Sosa *et al*. 2010). Soon after the breakthrough, this was not last for long and proved to be a misplaced belief that available antibiotics would always effectively treat all infections (Sosa *et al*. 2010). Increasing prevalence of resistance has been reported in many pathogens in different regions of the world including developing countries (Byarugaba, 2005). However there are many factors which can influence bacteria to become insensitive to antibiotics; the two main factors are the dissemination of resistance genes and the extensive use of antibiotic. Antibiotic-resistant pathogens are not more virulent than susceptible ones, but the resistant forms are harder to destroy. Bacteria can acquire resistance genes through a few routes. Many inherit the genes from their forerunners. Other, from genetic mutations, which occur readily in bacteria, will spontaneously produce a new resistance trait or will strengthen an existing one (Levy *et al*., 1998). Infections caused by Enterobacteriaceae are treated usually with antibiotics, such as fluoroquinolones, beta-lactams and aminoglycosides. However, they

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are now able to resist these antibiotics and can develop several mechanisms for resistance (Kocsis and Szabó, 2010). By 1983, some strains of Klebsiella found to have extended spectrum ß- lactamases (ESBLs) capable of inactivating extended-spectrum cephalosporins (Pereira *et al*., 2011). Emergence of carbepenamases further complicates the situation as carbapenems were used to treat cephalosporin resistant ESBL-producing organisms (Pereira *et al*., 2011). Different studies indicated that the most common carbapenemase in the US is Klebsiella pnuemoniae cabapenemase (KPC) (Gupta, *et al*., 2011). Recently, a new class B enzyme, New Delhi metallo-ß-lactamase (blaNDM-1) was discovered which confers resistance to all antibiotic except fluoroquinolones and colistin (Yong, *et al*., 2009). Later studies confirmed the prevalence of this resistance factor pretty common in clinical isolates of India, Bangladesh and Pakistan (Kumarasamy *et al*., 2010). Cases have also been reported in other developed countries making it a global threat to human health (Mochon, *et al*., 2011 & Pfeifer, *et al*., 2011). These resistant traits are very serious in nature leaving very few or no remedial options. The aim of this study was to evaluate the resistance pattern of E. coli and K. pneumoniae isolated from clinical samples to different antibiotics. In addition, it was also intended to screen these isolates for the presence of newly emerging New Delhi Metallo-ß-lactamase-1 (blaNDM-1) gene through polymerase chain reaction.

MATERIAL AND METHODS

Study Duration: 03 months (August 2012-October 2012).

Sample Size: A total of 52 isolates belong to *Escherichia coli* and *Klebsiella pneumoniae spp.* were collected from different laboratories of Karachi. These bacterial strains were isolated from diarrhea, sputum, urine and nasal swab. *Purification* and

Identification of Isolates: All isolates were initially processed to purify and confirm for their species identification. Briefly, bacterial isolates were streaked on Eosin Methylene Blue (EMB) agar plates for isolated colonies and incubated for 24hrs at 37°C. All pure cultures were identified on the basis of their morphological, cultural and biochemical characteristics.

Antimicrobial Susceptibility Testing: Kirby-Bauer disk diffusion method as described earlier in many studies was used to determine the antibiotic resistance of the organisms (Lalitha,2003). Briefly, bacterial lawns were made on pre-incubated agar plates and antibiotic discs were placed aseptically. After incubation, zone of inhibitions were recorded and results were interpreted using clinical and laboratory standard institute, guidelines (CLSI, 2011).

Polymerase Chain Reaction (PCR) for the Detection of blaNDM-1: Polymerase chain reaction (PCR) was used to enumerate blaNDM-1 resistant gene. The primer sequences and conditions as reported earlier were used in this study (Kruttgen, 2011). Briefly, initial denaturation was done at 95°C for 5 minutes followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 40 seconds, and extension at 72°C for 50 seconds. The thermal cycling was terminated with a final extension of 72°C for 5 minutes. These primers amplified an internal sequence of 206 base pairs of blaNDM-1 gene. Agarose gel electrophoresis and ethidium bromide staining was performed to analyse PCR products. The sequences of the forward and reverse primers were: NDM-Fm: 5'- CTTCCAACGGTTT GATCGT C-3', NDM-Rm: 5'-ATTGGCATAAGT CGCAA TCC-3'.

RESULTS

A total of 52 isolates were collected from different laboratories which belong to *E. coli* and *Klebsiella pneumoniae spp.* Antibiotic susceptibility testing was performed for all the isolates. A total of 7 antibiotics were used. All the strains showed high resistance to Ampicillin. *E.coli* from diarrheal sources and other sources showed resistance to Ampicillin as 82% and 89% respectively, and highly sensitive to Imipenem and Meropenem. While *Klebsiella*

strains showed 100% resistance to Ampicillin and 13% resistance to Imipenem and Meropenem. Resistances to other antibiotics are shown in Table: 1 and Figure 1.

Table I: Antibiotic Resistance Pattern of *E. coli* and *K. pneumoniae*

ISOLATES	AMP %	TE%	CIP%	LEVO%	C %	IPM%	MEM%
E.coli from diarrheal source	83 (25)	67(20)	30(09)	30(09)	17(05)	0	0
E.coli from different sources	88(08)	67(06)	44(04)	22(02)	22(02)	0	0
K. pneumoniae from different sources	100(15)	67(10)	7(01)	7(01)	20(03)	13(02)	13(02)

Key: AMP=Ampicillin, TE=Tetracycline, CIP=Ciprofloxacin, LEVO=levoflaxacin,
C=Chloramphenicol, IPM=Imipenem, MEM=Meropenem

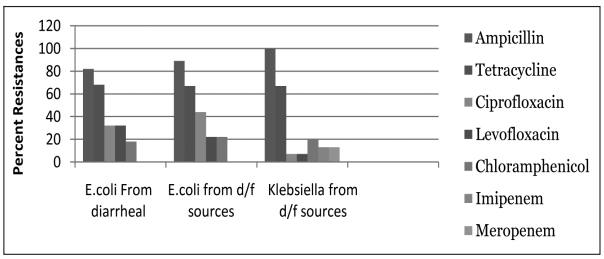

Figure 1: Graphical representation of Table 1.0

PCR DETECTION OF blaNDM-1 GENE: All the 52 isolates were subjected to PCR using primers for blaNDM-1 gene. These primers amplified an internal sequence of 206 base pairs of blaNDM-1 gene. Four isolates out of fifty two were positive and showed bands on the gel (Figure 2 & 3). Among the 04 positive strains, 03 strains were of *K. pneumonia*e and only 01 strain was of *E. coli*.

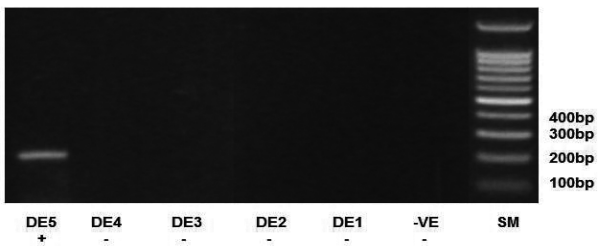

Figure 2: Agarose Gel electrophoresis of *E. coli* from Diarrheal samples. SM: 100bp DNA size marker.

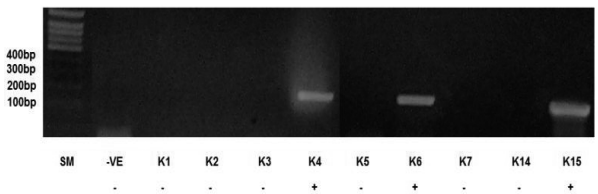

Figure 3: Agarose gel electrophoresis *K. pneumoniae.* SM: 100bp DNA size marker

DISCUSSION

Escherichia coli and *Klebsiella pneumoniae* are the normal inhabitants of humans, but sometimes they become opportunistic pathogen and cause infections. These bacterial infections are often treated with antibiotics. Now days, many bacterial strains are showing to have resistance against the drugs. This antibiotic resistance is posing a major threat to public health and in treating various infections. The present study has been designed to observe the sensitivity pattern through disc diffusion method and to detect the resistant gene blaNDM-1 in *Escherichia coli* & *Klebsiella pneumoniae* through PCR assay.

A total of 52 isolates belong to *E. coli* and *K. pneumoniae* was included in this study. These isolates were collected from different laboratories of Karachi in the period of August 2012- October 2012. All the isolates were subcultured and identified on the basis of morphological, cultural and biochemical properties.

Antibiotic susceptibility test was performed for the detection of resistance pattern in *Escherechia coli* and *Klebsiella pneumoniae*. Results showed that *E. coli* from diarrheal specimens showed 33% resistance, *E. coli* from various sources showed 35% resistance and Klebsiella from different sources showed 32% resistance. All the strains showed high resistance to Ampicillin, i.e *E. coli* strains showed (82%-89%) resistance, while Klebsiella strains showed (100%) resistance. This resistance pattern is similar to the previous study (Oplustil *et al*., 2001). The percentage of resistance among outpatients to ampicillin was 11.3% and, to ampicillin alone, the resistance was 94.5%. A similar profile was observed for inpatients, where we had 33.7% resistance to ampicillin /sulbactam, and 97% to ampicillin alone.

All the *E. coli* and *K. pneumoniae* strains showed highest susceptibility to imipenem and meropenem i.e. 100% and 87% respectively. This pattern is similar to the study (Alzahrani *et al*., 2005). ESBL-producing *E. coli* isolates showed highest susceptibilty to meropenem (95.8%) followed by amikacin (93.7%), and imipenem (91.7%). ESBL-

producing *K. pneumoniae* showed highest susceptibility to meropenem (94.4%) followed by gentamicin & piperacillin/tazocin (88.9%), and amikacin, ciprofloxacin & levofloxacin (83.3%).

Polymerase chain reaction (PCR) of all the 52 isolates was done and it was found that 04 isolates out of 52 carried the resistant gene blaNDM-1. The sample size was not so large, but still we were able to have some positive isolates carrying this resistant gene.

The comparison of antibiotic susceptibility testing and PCR showed some contrast in the results. Three *K. pneumoniae* and one *E. coli* were carrying blaNDM-1, but phenotypically they were sensitive to carbepenems. Possible explanation of these types of results might be the presence of any point mutation or inclusion of insertion sequence which inhibit the expression of respective protein and thus no resistance in antibiotic susceptibility test. So with this study, it can be concluded that blaNDM-1 gene is prevalent in local clinical isolates which results in resistance to variety of antibiotics including carbepenems.

REFERENCES

Alzahrani A. J. *et al*. 2005. Susceptibility Patterns of Extended Spectrum ß- Lactamase (ESBL)-producing Escherichia coli and Klebsiella pneumoniae isolated in a teaching hospital. Pakistan J. Med. Res.Vol. 44, No.2.

Byarugaba D. K. *et al*. 2005. Antimicrobial resistance and its containment in developing countries. In Antibiotic Policies: Theory and Practice, New York: Springer. Ed. I. pp 617–646.

Clinical and Laboratory Standards Institute: Performance Standards for Antimicrobial Susceptibility Testing; Tweny-first Informational Supplement. CLSI document M 100-S 21 (ISBN 1-56238-742-1). Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2011.

Gupta N, M. Brandi *et al*. 2011.Carbapenem-ResistantEnterobacteriaceae Epidemiology and Prevention, Clinical Infectious Diseases;53(1):60–67)