PREVALENCE OF MULTIDRUG RESISTANCE AND MBL GENE DETECTION ON THE UTI ISOLATES OF *Escherichia coli* AND *Klebsiella pneumoniae*

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Abstract

Increase in metalo betalactamase (MBLs) producing microbes in recent years have led to limitations of treatment option. This study aimed to assess the prevalence of MBL producing *E. coli* and *Klebsiella pneumoniae* from UTI isolates. All isolates were evaluated for antibiotic resistance and for the expression of MBL genes by multiplex PCR. Among the various group of antibiotics used in this study, betalactam antibiotics were highly resistance to both genera. Presently, IMP gene was highly predominant on both isolates (43%) and followed by VIM (28.5%). Alarming rate of drug resistance among uropathogens and high rate of MBLs producing isolates was observed. Therefore, if antibiotic resistance needs to be limited, the only way to educate patients and the public is to do so. At the same time, find a new way to control the multidrug resistance.

Index terms - *E.coli, K.pneumoniae*, MBLs, IMP, VIM

I. INTRODUCTION

Antibiotics are drugs used to prevent bacterial infections and to treat them. Several varieties of antibiotics have been used over time for medical purposes. Millions of metric tons of new antibiotic classes have been produced over the past 60 years. Increasing demanded for antibiotics in many sectors have allowed the use of drugs to be cheaper and off-label. In contrast, the enormous and irresponsible use of antibiotics made a significant contribution to the development of resistant strains (Sojib *et al*., 2017). Almost all antibiotics are available on the counter in the developing world and can be purchased without any medical prescription, which is one of the most important factors in causing resistance (Grigoryan *et al*., 2007). Among the antibiotics, β-lactams are the most varied and commonly used agents that account for more than 50% of all systemic antibiotics used. Production of β-lactamases enzymes is the most common cause of bacterial resistance to β-lactam antibiotics. In recent years, the bacterial resistances to β-lactam antibiotics have increased considerably.

Carbapenems are generally used as an alternative to treat severe infections caused by *P. aeruginosa, E. coli and K, pneumoniae*, as these drugs have a good spectrum of activity and are robust for hydrolysis by most β-lactamases, including β-lactamases (ESBL). The use of carbapenems has nevertheless been denatured by the development of strains that produce metallo-β-lactamase (MBL), an enzyme capable of hydrolyzing and inactivating this class of antibiotics. In addition, the spread of MBL-producing clones in remote areas were reported (Wuthi *et al*., 2010). In recent years, MBL genes have spread from *P. aeruginosa* to members of Enterobacteriaceae (Kiska *et al*., 1999).

Currently, no standardized method for MBL detection has been proposed and despite polymerase chain reaction (PCR) being highly accurate and reliable, its accessibility is often limited to reference laboratories. The aim of this study was to detect MBL producing *E. coli* and *K.pneumoniae* from clinical laboratory, and determines antibiograms to guide clinicians in prescribing proper antibiotic and controlling hospital infection.

II. METHODS

2.1 Test pathogens

The clinical isolates of *E.coli and K.pneumoniae* (n=20) were procured from Microtech, Microbiology Laboratory, and Coimbatore and used for the study. All isolates were inoculated onto UTI Chromogenic agar media for confirmation.

2.2 Antibiotic Sensitivity Test

Antibiotic susceptibility screening was done as per the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS). Kirby- Bauer's disc diffusion technique was adapted for antibiogram. The following antibiotics were used such as Gentamycin (G), Cefotaxime (CTX), Imipenem (I), Erythromycin (E), Kanamycin (K), Penicillin (P), Ciproflaxcin (CF), Ampicillin (Amp), Tetracycline (TE), Amoxicilin (A).

2.3 Isolation of MBLs producing isolates by Multiplex PCR

DNA template preparation was performed according to Sadasivam and Manickam, 2008 procedure. The following reaction mixture was used for the amplification of MBLs genes (IMP and VMP). Each PCR reaction mixture (25µl) contained 2µl of template DNA (plasmid DNA), 10 µl of 10 X PCR mix, 0.5 µl of (0.5 µM) each of the primers and 12 µl of molecular grade water. The PCR program was performed in a Thermal Cycler and it consisted of an initial incubation of 10 min at 37°C and an initial denaturation step at 94°C for 5 min, followed by 30 cycles of DNA denaturation at 94°C for 1 min, primer annealing at

54°C for 1 min, and primer extension at 72°C for 1.5 min. Following PCR, aliquots (20 µl) of the reaction mixtures were analyzed by electrophoresis on a 1.5% Agarose gel, containing ethidium bromide (0.2 mg/ml), in the presence of an appropriate DNA molecular weight marker. Then observe the amplification bands under UV Transilluminater and detection of resistance genes with the use of marker (Essa and Afifi, 2007).

For MBL-IMP gene: IMP-A (5`GAAGGCGTTTATGTTCATAC-3`) and IMP-B (5`-GTACGTTTCAAGAGTGATGC-3`), which give an amplified product of 587-bp.

For MBL-VIM gene: VIM2004A (5`-GTT TGG TCGCAT ATC GCA AC-3`) and VIM2004B (5`-AAT GCG CAG CAC CAG GATAG-3`), which give an amplified product of 382-bp.

III. RESULTS AND DISCUSSION

In the present study, UTI isolates were procured from clinical laboratory and which were reconfirmed by morphological characterization on chromogenic media. The pink colour colony and mucoid blue colony on Chromogenic media which was known as *E.coli and K.pneumoniae*. Among the 20 isolates, 9 of were confirmed as *E.coli* and 5 of were *K.pneumoniae.*

Antimicrobial resistance has now been recognized as a major public health and patient care problem. It is more disturbing for developing countries. In the present study, Ampicillin showed 100% resistance and followed by Erythromycin (90%) Ampicillin (89%). In this study, *E. coli* were highly resistant to Erythromycin, Penicillin and amoxicillin (100%) and least resistant to Kanamycin (22.2%) (Fig.1). High resistance of *E. coli* to antimicrobial agents tested was observed in this study which is similar to what was observed by previous report in Tamilnadu (Manikandan and Amsath, 2014). Resistance to amoxicillin and ampicillin observed in this study was similar to previous in Chennai, India (Roshene *et al*., 2015).

In case of *K.pneumoniae,* a hundred percentages of isolates were resistance to amoxicillin and ampicillin. Similar result was shown in different studies (Pooja *et al*., 2017; Chander and Shrestha, 2013; Mekki *et al*., 2010). More than 60% of *K. pneumoniae* isolates were resistant to number of antibiotics including Imipenem, Cefotaxime, Erythromycin, Tetracycline and Ciproflaxcin. According to previous study of Pooja *et al* (2017), Imipenem resistance was high in present study.

The high level of resistance seen to most of the betalactam antibiotics, which phenomenon become took place by means of out of control and irrelevant use of those marketers in hospitals. This is promoted by the lack of an antibiotic policy and the availability of antibiotics sold over the counter in India. The high rate of antimicrobial resistance has major therapeutic implications insofar as 71.1% of our *E. coli* population was noted to be multidrug resistant. In the present study, compared to betalactamase antibiotics, non betalactamase antibiotic of Kanamycin was most effective drug against both *E.coli* and *K. pneumoniae*.

The presence of MBL-producing bacteria in a health center is commonly associated with a therapeutic problem; in addition to a serious situation for infection control management. Clinical laboratories must be able to distinguish MBL-producing isolates from those with other mechanisms responsible for betalactam resistance as it is considered to be the most worrisome resistance mechanism. In the present study, multiplex PCR method was used for the determination of MBL producing isolates. The 14 isolates of *E.coli* and *K.pneumoniae* were utilized for the PCR analysis. Among the 2 genes, IMP gene was highly predominant on both isolates (43%) and followed by VIM (28.5%) (Plate 1). Similar result was obtained from Essa and Afifi, (2007) studies. These MBLs genes were obtained from both Imipenem resistance and sensitive isolates, but mostly obtained from resistance isolates, which corroborates with other report where *K. pneumoniae* and *E. coli* were found to be carbapenem sensitive but positive for MBL genes (Debata *et al*., 2013).

Carbapenemase-resistance isolates particularly MBLs producing strains represents a major hazard to human health due to fail in most antibiotic treatment. In recent years, the detection of that enzyme among gram-negative isolates has been observed from developing countries; however, that report in India is not sufficient and need to be more investigation. Therefore, if antibiotic resistance needs to be limited, the only way to educate patients and the public is to do so. At the same time, find a new way to control the multidrug resistance isolates.

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Fig.1

Antibiotic resistance patterns of UTI isolates

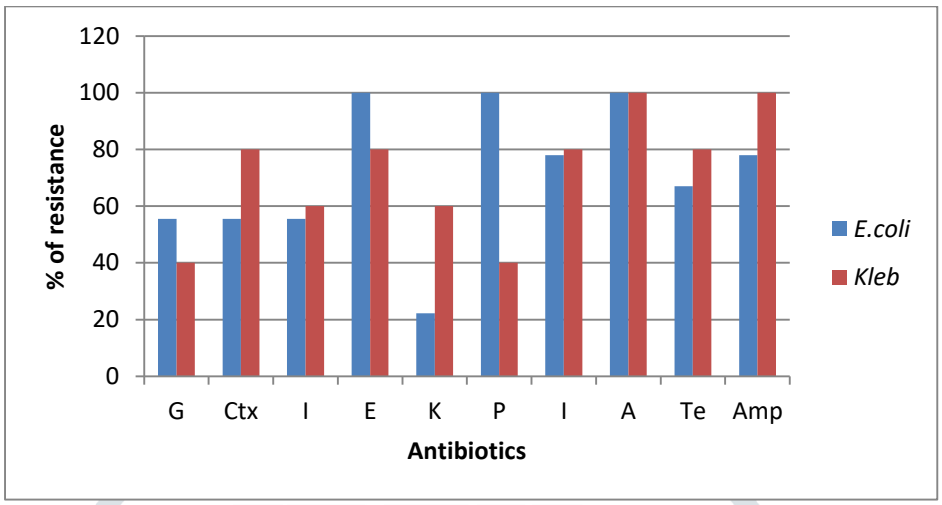
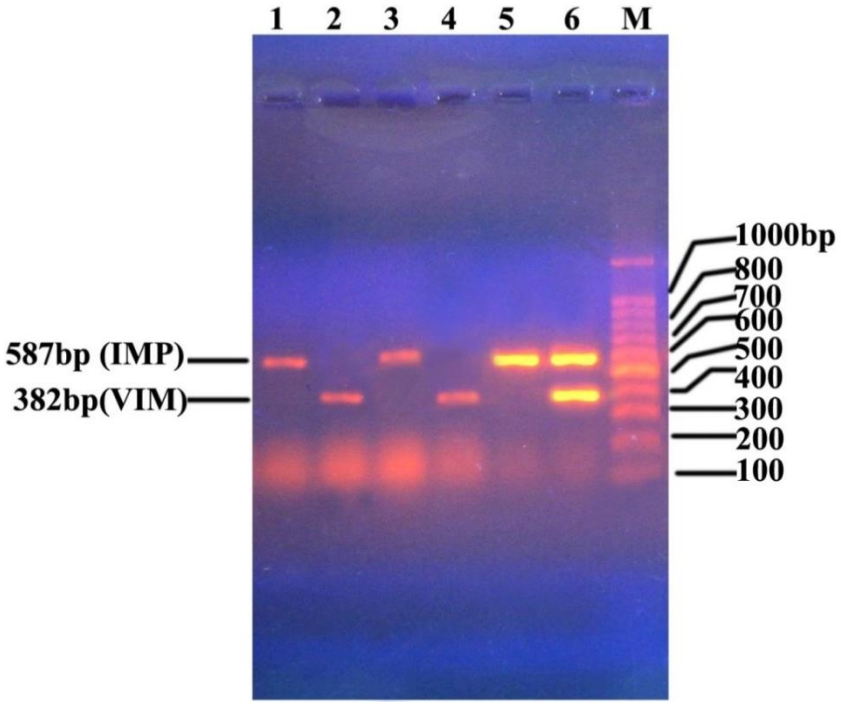


Plate 1

Amplification of MBLs genes



Lane 1 to 3- *E.coli*, Lane 4 to 6 –*K.pneumoniae*, Lane M- 100bp DNA marker