INTRODUCTION

- Urinary Tract Infections (UTI) are one of the most commonly occurring infections in the world affecting people of all age groups and majority of UTIs are caused by the bacterium E. coli.
- It’s a major public health problem with growing challenges to its treatment.
- The spread of resistance in Enterobacteriaceae is complicating treatments of serious infections.
- ESBLs are a group of enzymes produced by bacteria that causes multi drug resistance to β-lactam antibiotics in gram-negative bacteria.
- ESBLs are often located on plasmids that are transferable and between bacterial species.
- β-lactamases is proven to be one of the leading cause of resistance to β-lactam antibiotics among gram-negative bacteria.
- Most ESBL-producing bacteria can be divided into three groups: TEM, SHV and CTX-M types.
- Increase in the prevalence of SHV and CTX-M type of ESBLs imposes burden on the clinical use of third generation cephalosporins, penicillin, monobactams for the treatment of infections.

MATERIALS AND METHODS

- Patients’ urine samples were cultured on Cysteine Lactose Electrolyte Deficient (CLED) agar & MacConkey agar and incubated for 24 – 48 hrs.
- All the bacterial isolates were identified based on their cultural characteristics, lactose and various biochemical utilization tests.
- API 20E Test strips were used for the biochemical utilization tests.
- Antibiotic sensitivity test – were used to detect their antibiotic sensitivity pattern with the list of antibiotics (Tab.1)
- Combination disc diffusion test – the antibiotic discs Cefotaxime, Cefotaxime + Clavulanic acid, Cefnazidime and Cefazidime + Clavulanic acid were used to detect and confirm the ESBL strains of the bacterial isolates.
- The ESBL strains were subcultured and used for plasmid DNA extraction by acid-alkaline lysis method.
- The extracted plasmid DNA was quantified by Nano drop method and used for performing PCR by using two gene primers such as bla<sub>SHV</sub> and bla<sub>CTX-M</sub> (Tab.2).
- PCR amplification - The PCR master mix have given in the table-3 and the amplification program was set up on Veriti 96 well Thermal Cycler (Applied Bio-systems) (Fig.1).
- The program of gene amplification is given in the table & figure.
- The amplified DNA fragments were separated and identified in the 2.3% agarose gel electrophoresis by using UV transilluminator Gel Doc XR.

RESULTS

- In this study, 237 urine samples were reported for microbiological examination.
- 65 ESBL producing bacteria and 172 β-lactam sensitive bacteria were isolated from that urine samples.
- The Figure 2 & 3 shows the double disc diffusion test and combination disc test which are used to confirm the ESBL producing bacteria.
- Based on the gram staining reaction, lactose utilization, cultural characteristics and various biochemical utilization, all the 65 ESBL producing isolates were identified as Escherichia coli, Klebsiella pneumoniae and Citrobacter species (Fig.4 & 5), and their antibiotic sensitivity have given in the table.4.
- The E. coli and K. pneumonia are the predominant isolates in the study sample.

Detection of bla<sub>SHV</sub> & bla<sub>CTX-M</sub> genes

- The extracted plasmid DNA concentration was maintained ideally around 50 ng/μl with 260/280 as 1.6 - 2.0.
- Out of that 65 samples, 50 plasmid DNA were selected and were used to detect the presence of the genes bla<sub>SHV</sub> and bla<sub>CTX-M</sub>.
- The targeted amplicons of 1018 bp bla<sub>SHV</sub> and 544 bp bla<sub>CTX-M</sub> were observed (Fig.6).
- The bla<sub>CTX-M</sub> gene was identified in 14 DNA samples and the bla<sub>SHV</sub> gene was identified in 02 DNA samples.
- In 08 DNA samples both the genes were identified (Tab.5).
- Neither of these two genes were present in the remaining 26 amplified DNA samples.

DISCUSSION

- In the present study, E. coli is the most frequent ESBL producing bacteria followed by Klebsiella pneumoniae and Citrobacter species.
- From a total of 237 urinary isolates, 65 were ESBL producers; E. coli being 81.5%, Klebsiella pneumonia 15.4% and Citrobacter species 3.1% (Fig.7).
- 48% of the DNA samples contain the bla<sub>CTX-M</sub> and bla<sub>SHV</sub> genes either alone or both and 52% of the DNA samples neither carry bla<sub>SHV</sub> nor bla<sub>CTX-M</sub> genes, but they are ESBL positive.
- The individual prevalence of the bla<sub>CTX-M</sub> and bla<sub>SHV</sub> gene in a DNA sample is 58.3% and 8.3% respectively and the combined prevalence of the both genes in an individual DNA sample is 33.3%.

CONCLUSION

- This current study highlights the prevalence of ESBL producing E. coli, K. pneumoniae and Citrobacter species, which causes urinary tract infections in patients attending Thumbay hospitals, U.A.E.
- The gene bla<sub>CTX-M</sub> gene is predominantly detected (58%) in the E. coli and Klebsiella pneumoniae.
- Further studies are required to find the prevalence rate of other gene variants of ESBLs such as OXA 48, TEM and NDM 1, etc. in the Thumbay hospitals, U.A.E.

REFERENCES


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