SUMMARY

SUMMARY

Hospital bacterial infections (nosocomial infections) are important cause of morbidity and mortality in both developing and developed countries. Epidemiological typing of strains is important in an attempt to trace the source of contamination or to prevent patient to patient dissemination of strains. Epidemiological typing methods include biochemical typing (biotyping), serotyping, bacteriophage typing, antibiotic susceptibility typing (antibiogram), and bacteriocin typing.

Molecular typing techniques are widely used for studying the epidemiology of human, animal, and plant infections. These molecular typing techniques include DNA restriction endonuclease analysis, ribotyping, polymerase chain reaction (PCR), and pulsed field gel electrophoresis (PFGE).

Bacteriophage typing is laborious, of limited utility and available through only a few reference laboratories. Serotyping and bacteriocin typing can not discriminate between strains. Ribotyping is slow, more labor-intensive and involves working with probes. PFGE is a rather time-consuming.

In this study, we reported the restriction enyzme digestion (using *Hind III* enzyme) as a typing method and assess this method to detect genetic diversity in *Eschericia* coli (*E.coli*) (41 samples) in comparison with biochemical typing (biotyping) and antibiotic susceptibility typing (antibiogram).

Biochemical typing methods can be performed by different techniques; in the conventional techniques the enzymatic activity of bacteria can be detected by the changes in the pH using pH color indicator. The pH color indicator can not detect the pH changes accurately. In this study we used a novel technique based on the fluorescence of a pH sensitive fluorophore.

The antibiotic typing methods can be done by disc diffusion technique, the most common technique, and by dilution techniques (broth and agar dilution). The disc diffusion does not give the antibiotic concentration which inhibits the bacterial growth and the dilution techniques have time consuming technical steps.

In this study we used the E Test which is a novel method for measuring the minimum inhibitory concentration of antibiotics against bacteria. The E test comprise of thin impervious plastic carrier (5x50 mm) with a continuous exponential gradient of antibiotics immobilized on one side and a reading interpretive scale on the other. The antibiotic gradient can cover a broad concentration range corresponding to approximately 20 antibiotic dilutions.

Five different biochemical profiles were identified. These are biotype 1 (17%), biotype 2 (30%) which was the most common, biotype 3 (22%), biotype 4 (17%), and biotype 5 (14%). All of the bacterial samples enzymatically hydrolyzed the sugars Maltose, Arabinose, Trehalose, Fructose, Sorbitol, Mannitol, Glucose, and Lactose; while there was no enzymatic activity against Malonate, Urea, Inositol, Esculin, Tryptophan, Citrate, Arabitol, and Cellobiose.

Four different antibiotic susceptibility patterns were generated among the 41 bacterial samples of *E.coli* tested depending upon their susceptibilities to 7 different antibiotics. These antibiotics were Nitrofurantoin, Trimethoprim-Sulfamethoxazole, Norfloxacin, Ampicillin, Piperacillin, Cefotaxime, and Gentamicin.

The growth of the twenty bacterial samples present in pattern 1 was inhibited by Nitrofurantoin, Cefotaxime, Gentamicin, and Norfloxacin; and their growth was not inhibited by Ampicillin, Piperacillin, and Trimethoprim-Sulfamethoxazole.

Pattern 2 contained eight bacterial samples which their growth was inhibited by Nitrofurantoin, Cefotaxime, and Gentamicin; and their growth was not inhibited by Norfloxacin, Ampicillin, Piperacillin, and Trimethoprim-Sulfamethoxazole.

Also eight bacterial samples were present in pattern 3 in which their growth was inhibited by Norfloxacin, Cefotaxime, and Gentamicin.

The growth of the five bacterial samples present in pattern 4 was inhibited only by Cefotaxime and Gentamicin .

The antibiotic resistance patterns were pooled, and it was found that 31% of the bacterial samples their growth was not inhibited by Nitrofurantoin, 100 % not inhibited by Trimethoprim-Sulfamethoxazole, Ampicillin; and Piperacillin, 31% not inhibited by Norfloxacin, and 0% not inhibited by Cefotaxime and Gentamicin.

Seven DNA patterns were observed in restriction enzyme digestion. Seven bacterial samples were present in pattern 1 with two DNA fragments (bands) at 872 and 700 base pairs (bp). Pattern 2 included 7 bacterial samples with two bands at 1200 and 900 bp. Pattern 3 contained three bacterial samples with six bands at 1600, 1500, 1400, 1200, 600, and 400 bp. Pattern 4 included five bacterial samples with six bands at 1500,1400, 980, 860, 730, and 360 bp. Twelve bacterial samples were present in pattern 5 with six bands at 1300, 1100, 980, 800, 750, and 360 bp. Four bands, at 1200, 900, 800, and 360 bp were observed in pattern 6 which included four bacterial samples. Only three bacterial samples were included in pattern 7 with three bands at 1100, 1000, and 700 bp. There is a difference in intensity of bands within the same pattern or between the different patterns.

The use of restriction enzyme digestion was successful in detecting genetic differences in *E.coli* that could not be observed in biochemical and antibiotic susceptibility tests used to differentiate these strains.