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Isolation of Uropathogenic *Escherichia Coli* and Study of Its Virulence Traits

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Abstract: Uropathogenic E. coli (UPEC) is responsible for approximately 90% of urinary tract infections [1]. Abuse and improper prescribing policy of antibiotics causes remarkable increase of antibiotic resistance pattern among the E. coli isolates from UTI[2]. These types of resistance are associated with genetic mutation and intra or inter species transfer of resistance gene through plasmid[3]. Therefore regional studies on pattern of antibiotic sensitivity are very much necessary to overcome this problem. Considering the majority of UTI cases caused by E. coli and increasing use of antibiotics followed by growing resistance in bacteria and emerging MDR strains, the present study was conducted to identify the UPEC and also investigate the drug resistance pattern of those E. coli strains collected from clinical sample.

Keywords: Uropathogenic E. coli, Virulence traits, Phenotypic assays

Introduction

Urinary tract infections (UTIs) are a major public health concern in developing countries. Most UTIs are caused by *Escherichia coli*, accounting for up to 90% of community-acquired UTIs (CAUTI). The origin of these strains is frequently the patient's own intestinal flora. A subset of fecal *E. coli* having the virulence factors which enable them to colonise periurethral area, enter urinary tract, and cause symptomatic disease are defined as uropathogenic *E. coli* (UPEC). Virulence factors of UPEC include the ability to adhere to uroepithelial cells and certain specific serotypes O and K antigens are resistance to phagocytosis and bactericidal action of normal serum. Other factors known to contribute to the virulence are the production of α hemolysins, colicins, aerobactin, cytotoxic necrotizing factor, and cell surface hydrophobicity[4]. Incidence of UTI was more common in females than in males. Piatti *et al.*,[5] also reported a higher prevalence of UTI in female (77%). The reasons for the high prevalence of the UTIs in females can be due to the anatomical structure of the urogenital tract having short urethra, presence of normal flora in vagina,menstrual cycle and pregnancy. This work was conducted to study the incidence of *E.coli* in local cases of urinary infections and characterize these isolates with reference to drug resistance, and virulence factors.

Materials and Methods

Urine samples were collected from 50 patients suffering from urinary tract infection who were admitted to hospitals in and around Coimbatore. Samples were transported in sterile screw capped containers under ice cold conditions. *E. coli* strains were isolated from the samples following standard procedures. The samples were inoculated in Mac Conkey agar and lactose fermenting colonies were selected. These colonies were sub cultured and stored in nutrient agar slants for further confirmation. Using standard biochemical tests *E.coli* cultures were identified[6].

Antibiotic susceptibility testing

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Then antibiotic susceptibility test was done on Mueller Hinton agar by Kirby Bauer method[7]. The following antibiotic discs were used. Amikacin (30 µg), Nitrofurantoin (300 µg), Ciprofloxacin (1 mg), Norfloxacin (10 µg), Rifamipicin (5 µg), Gentamycin (30µg),Steptomycin (10µg),Kanamycin (15µg). Antibiotic discs were obtained commercially from Himedia. The diameter of zone of bacterial growth inhibition surrounding the disc (including the disc) was measured and compared with the standard for each drug.

Phenotypic assays to determine virulence factors

Hemolysin production.

The detection of hemolysin was performed by analysing the hemolytic zone observed after overnight growth at 37°C on sheep blood (5%) agar[8].

Mannose Resistant Fimbrial Haemagglutination

Different concentrations of ammonium sulphate (0.02-4.0M) were prepared in 0.02M sodium – phosphate buffer (pH 6.0) containing methylene blue. Equal volumes of bacterial suspension (5×10^9 cells/ml) and solution of ammonium sulphate at different concentrations were mixed with tooth picks on hydrophobic paper[9]. The concentration of ammonium sulphate at which bacterial aggregation occurred was scored immediately after mixing and confirmed next day by examination of the dried-up mixtures.

Cell Surface Hydrophobicity

All the *E.coli* isolates were tested for their hydrophobic property by using different molar concentrations of ammonium sulphate in VDRL tile; 40 µl of bacterial suspension in PBS was added in each of the wells containing 1 M, 1.4 M and 2 M ammonium sulphate. Clumps were seen by naked eyes. Strains were considered hydrophobic, if they aggregated in the PBS concentration of ≤1.4 M[10].

Serum Bactericidal Assay

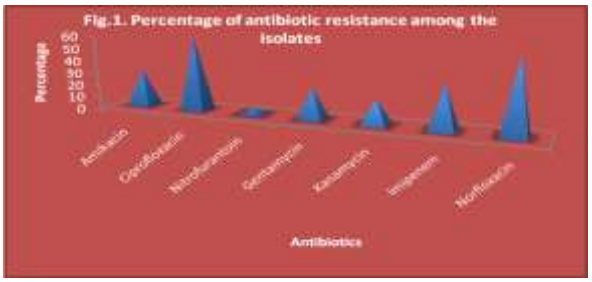
This assay was done according to Siegfried *et al.*[10]. Overnight cultures of *E. coli*, grown at 37°C on Muller Hinton Agar (MHA), were harvested and the cells were suspended in Phosphate buffered saline (PBS). Test tubes were used for incubation of bacterial suspensions (0.05 ml) with serum (0.05 ml). Control tubes contained 0.05 ml of PBS instead of serum. The tubes were mixed by shaking for a minute and incubated at 37°C in an incubator. Samples (10 µl) were taken at 0 minute and after incubation for180 min at 37°C and spread on MHA. The plates were further incubated for 18 h at 37°C. Susceptibility of bacteria to serum bactericidal activity expressed as the percentage of bacteria surviving after 180 min.

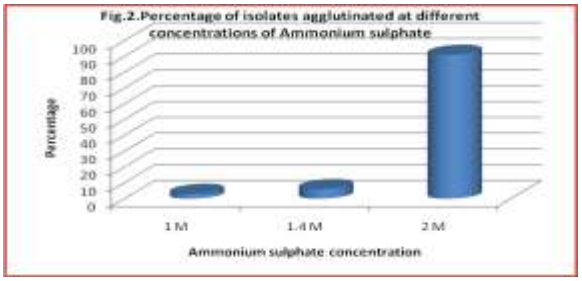
Serogrouping

Typing of somatic antigens was performed at the National Salmonella and *Escherichia* Centre, Central Research Institute, Kasauli, India, using antisera against O antigens - O1 to O173.

Results and Discussion

Fifty urine samples were collected from UTI patients. 30 numbers of *E.coli* isolates were isolated and identified. The uropathogenic strains showed highest resistance rates to Genamycin and Ciprofloxacin (fig 1). Multiple drug resistance was observed. Our studies was similar to the previous studies done by Maheswari *et al.*,[4] and Naveen and Mathai[11].





Hemolysin Production

Among the 30 isolates 6 were α hemolytic, 15 were β hemolytic and 9 were Y hemolytic.

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Hemolysin production is associated with human pathogenic strains of *E. coli*, especially those causing more clinically severe forms of UTI[12]. It is toxic to a range of host cells in ways that probably contribute to inflammation, tissue injury and impaired host defenses[13]. In the present study, 50 % *E. coli* isolates produced hemolysin. In other studies conducted by Raksha *et al.*[14], Siegfried *et al.*[15], Hughes *et al.*, [16], Shruthi *et al.*,[17], hemolysin production was detected in 41.36% and 59.6%, 59.7% and 41.9% isolates respectively.

Mannose Ressistant Fimbrial Haemaggultination

The strains were considered mannose resistant when agglutination occurred at a concentration above 2.0 M ammonium sulphate. Amongst urinary *E.coli* strains (30), MRHA adhesins were found in (16) strains. Mannose resistant haemagglutination (MRHA) is used to indicate P fimbriae. In this study, majority of the strains were able to agglutinate. This shows the presence of P fimbriae that contributes for virulence. This concept has been supported in many researches, e.g., Seigfried *et al.*,[15] , Vagarali *et al.*,[18] , Raksha *et al.*, [14] , Kauser *et al.*,[19] have reported the incidence of MRHA *E. coli* isolates as 23%, 25%, 30.9%, 30% respectively. In the present study also the rate of MRHA positive *E. coli* isolates was higher.

Cell Surface Hydrophobicity

The high hydrophobicity of the bacterial cell surface promotes the adherence of the bacteria to various surfaces like the mucosal epithelial cells. In the present study, 90% of the isolates were hydrophobic (fig.2). A study by Sharma *et al.*, 2007 demonstrated that out of 152 *E. coli* isolates, 36 (23.7%) isolates were hydrophobic, 132 (86.8%) were serum resistant and only 4 were positive for protease. Raksha *et al.* [14], demonstrated that among 220 urinary isolates, 91 (41.36%) were hemolytic, 68 (30.9%) showed MRHA, 58 (26.36%) were cell surface hydrophobicity positive and 72 (32.72%) were serum resistant.

Serum Bactericidal Assay

Among the UPEC isolates 63% showed resistance to the bactericidal action of the serum. During zero minute of incubation, there was 100% growth both in control (other *E.coli* strain from the lab) and the test strains. After three hours (180 minutes) the growth rate dropped to 30 % in the test strains and the test strain did not grow. Growth was noticed among the 63% isolates in UPEC. In other studies, Kauser *et al.*, [19] and Sharma *et al.*, [20] have demonstrated the serum resistance in 49.5% and 86.8% of the urinary *E. coli* isolates. Hughes *et al.*, [16] stated that the increased degree of serum resistance is associated with increased virulence of the organisms.

Serogrouping

Serogroups like O11, O33, O1, O6, O18, O15 and O75 are the predominant serogroups identified. Previous studies also show the association of these strains with uropathogenecity.

Virulence factors of *E. coli* affect the pattern of drug resistance in these isolates. Therefore, the knowledge of virulence factors of *E. coli* and their antibiotic sensitivity pattern will help in better understanding of organism and in treatment of UTI.

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