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Microbiology

DETECTION OF VIRULENCE FACTORS OF UROPATHOGENIC ESCHERICHIA COLI FROM DIFFERENT PATIENT GROUPS IN A TERTIARY CARE HOSPITAL.

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ABSTRACT

Background : Urinary tract infection is one of the most important cause of morbidity and mortality and most commonly caused by bacterial infections. Escherichia coli is the most frequent urinary pathogen. Number of virulence factors have a role in the virulence of Uropathogenic E.coli that are absent in non pathogenic E.coli.

Materials and Methods: 300 E.coli strains from symptomatic UTI patients of different groups were screened for virulence factors like haemolysin, Mannose Resistant (MRHA) and Mannose sensitive haemagglutination (MSHA) by recommended methods. Antimicrobial susceptibility pattern were also recorded.

Results: Hemolysin production (35.8%) and MRHA (29.9%) indicating the presence of P fimbriae were more in E.Coli isolates from Nephrology patients. 3rd Generation Cephalosporins showed a increased resistance rates.

Conclusion: Early Detection of these virulence markers is reasonably easy which will prevent complications like persistent and recurrent infection. Antimicrobial susceptibility pattern guide the clinician to provide appropriate antimicrobial therapy.

KEYWORDS : Uropathogenic Escherichia coli, Mannose Resistant Haemagglutination, Urinary tract infection.

Introduction:

Urinary tract infections are the most common entities encountered in medical practice and it affects all age groups from new born to old age. It is responsible for many complications such as premature babies, hypertension and renal failure[1]. In India, UTI accounts for 9.3 million doctor visits and nosocomial UTI accounts for more then 1 million cases[5]. More than 90% of UTI are caused by Escherichia coli[2] and 10 to 20% by coagulase negative staphylococcus and 5% or less caused by Klebsiella, Proteus mirabilis, Enterococcus and Pseudomonas aeruginosa[5]. In rare cases Candida albicans can cause UTI.

UPEC strains cause 75-90%[4] of community acquired and about 50% of Nosocomial UTI[3]. Bacterial pathogenicity plays a major role in host pathogen interaction that lead to UTI. Virulence markers of Uropathogenic E.Coli includes flagella, aerobactin, hemolysin, adhesins (P fimbriar& type 1 fimbriae) K Capsule, cytotoxic necrotizing factor, cell surface hydrophobicity, siderophores and resistence to serum killing[6]. These virulence factors favor the development of cystitis, urethritis, phelonephritis, bacteremia and septic shock

Among the virulence factors of UPEC, production of fimbriae, (Type 1 finbriae and Pap Fibriae) &hemolysin are more important. Attachement by fimbriae and subsequent secretion of hemolysin causes desteuction of urinary tract cells[7].

Hence,the present study is to demonstrate the presence of virulence factors like type P fimbriae, type 1 fimbriae and hemolysin in symptomatic UTI patients of Nephrology, Urology, medicine and paediatric departments along with antimicrobial susceptibility pattern.

Materials and Methods

This study was conducted in Coimbatore medical college hospital, Coimbatore for a period of 1 year from February 2014 to January 2015. A total of 300 Escherichia coli isolates in symptomatic cases of UTI (both outpatients & inpatients) from various department like Nephrology, Urology, Medicine and Paediatric patients were studied for detection of virulence factors of UPEC in Microbiology Diagnostic laboratory after obtaining approval from ethical committee. Sample collection and processing done as per standard protocols. Escherichia coli were isolated and identified as described by Bailey & Scott with modifications.

Detection of virulence factors:

Fimbriae (pili) are thin, are hair- like surface adhesive organelles made of protein sub units. In 1960s, JP Deguid described different types of fimbriae

Type 1 Fimbriae:

Single most commonly expressed virulence factor by more than 80% of all UPEC. Type 1 pili possess adhesins whose ability to mediate haemagglutination is blocked in the presence of D- Mannose (MSHA)

Type P Fimbriae:

Second most common virulence factor of UPE C also named as PAP – Pyelonephritis – associated pili. It is derived from the ability of p fimbriae to bind specifically to the p blood group antigen which contains a D- galactose – D galactose residue[8]. Type P Pili possess adhesins which agglutinate erythrocytes in the presence of D – Mannose. (MRHA)

Haemaaalutination:[12]

The haemagglutination was detected by clumping of erythrocytes by fimbriae of bacteria in presence of D-mannose. The method followed was according to Siegfred et al. Bacteria grown on TSI agar medium were sub cultured onto Mac- Conkey's plates and incubated at 37°C overnight. E.coli grown on MA plates were inoculated into 5 mL of phosphate buffered saline pH 7.4 (PBS) and incubated for 5 days at 37°C to get fimbriae enriched E.coli. The pellicle formed on the surface was noted and sub cultured onto colonization Factor Antigen (CFA) agar and incubated overnight at 37°C. Five milliliter of group A positive venous blood was collected using disposable syringe from a voluntary donor and added to an equal amount of Alsever's solution. This was washed three times and 3% erythrocyte suspension was made with PBS. Controls used were ATCCE.coli 25922.The procedure of Siegfred et al was modified and carried out on VDRL slides instead of microtitre plates.

Standard uropathogenicE.coli:

E.coliATCC 25922 was used as controls for detection of virulence markers.

Haemolysin :

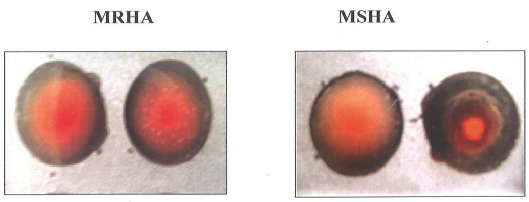
The cytolytic protein toxin secreted by most haemolytic E.coli isolates is known as alpha haemolysin. Alpha Haemolysin was detected by determining a zone of lysis around each colony on 5% sheep blood agar plates after overnight incubation.

Antibiogram:
Done by Kirby-Bauer method of agar disc diffusion method as per CLSI standards

RESULTS:
300 Escherichia coli isolates obtained from both out patients and hospitalized patients belonging to the departments of Urology, Nephrology, Medicine and Paediatrics. The positive cultures were obtained from 60.8% (n= 182) female and 39.2% (n= 48) male patients within the age group of 2 - 70 years. Both sexes were equally affected below 10 and above 50 years of age (45 to 55 %) . Females were more commonly affected during the reproductive age group (70%). The females predominate with female to male mean ratio of 1.5:1)

After confirming E.coli by cultural methods and biochemical reactions, the phenotypic characteristics like MRHA of 3% human erythrocytesin the presence of mannose indicating type P fimbriae, MSHA of 3% human erythrocytes indicating type 1 fimbriae and haemolysin production of 300 E.coli isolates were studied. The E.Coli isolates were more from Paediatrics and Medicine departments than from Urology (20%) and Nephrology(18%).Significantly more isolates from (29.9%) Nephrology followed by (19.2%) isolates from Paediatric patients exhibited MRHA of 3% human erythrocytes indicating the presence of P fimbriae. However, MSHA of 3% human erythrocytes indicating type 1 fimbriae was present equally in all the four groups of patients. Hemolysin production was also significantly higher in (35.8%) Nephrology patients when compared to others. All the above 3 virulence factors were more in isolates of E.coli from (88.1%) Nephrology patients.

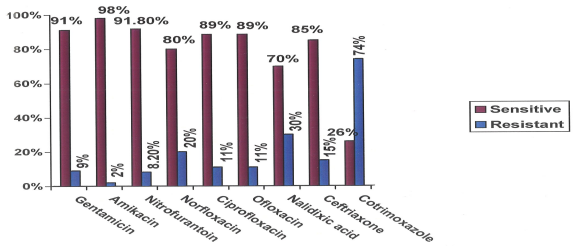
HAEMAGGLUTINATION TEST:



VIRULENCE FACTORS IN VARIOUS DEPARTMENTS

Virulence marker	Urology No: %	Nephrology No: %	Paediatrics No: %	Medicine No: %
MRHA	9 (12.2)	20 (29.9)	20 (19.2)	16 (12.8)
MSHA	21 (28.4)	15 (22.4)	23 (22.1)	18 (14.4)
Hly	12 (16.2)	24 (35.8)	29 (27.9)	26 (20.8)
Total	42 (56.8)	59 (88.1)	72 (69.2)	60 (48)

ANTI MICROBIAL SUSCEPTIBILITY PATTERN



Antibiogram: In this study, Sensitivity of 3rd generation Cephalosporins (85%), quinolones (89%), Nalidixic acid (70%) and Cotrimoxazole (26%) showed increased resistant rates.

DISCUSSION
UTI is a result of interaction between an uropathogen and the host. Bacterial infections of the urinary tract are the commonest cause of both community acquired and nosocomial infections. Worldwide studies have revealed a preponderance of E coli in urinary isolates i.e., 65.3% in Japan , 69% in Italy , 74% in Sweden , 75% in England and up to 90% in USA and as high as 94% in Israel.

Virulence Factors: In the present study, E.coli causing UTI in Nephrology patients had expressed more number of virulence markers i.e 88.1% in total. Out of this, the virulence markers of E.coli like p fimbriae (29.9%) detected by MRHA of 3% human erythrocytes and hemolysin (35.8%) were more in Nephrology patients. This could be due to the ability of P fimbriated E.coli to bind to the digalactoside expressed on renal tubular epithelium which results in upper UTI. Hemolysin contributes to tissue injury and favours the survival of E.coli in the renal parenchyma. The type! fimbriae detected by MSHA of 3% human erythrocytes was present equally in the isolates of all four groups of patients as both type 1 and type P fimbriae help in adherence of E.coli to uroepithelial cells in the lower urinary tract.

Rebecca Naveen et al [13] had reported hemolysin production (40.7%) and MRHA (42.4%) whereas Raksha et al [11] reported 41.36% haemolytic and 30.9% showed MRHA. A study by Johnson et al [8] showed MRHA in 58% of urinary isolates .

Antibiotic Sensitivity Pattern : In the present study, 3rd Genertion Caphalosporins like Cephotaxime and Ceftriaxone showed that 84.6% of E.Coli isolates were susceptible. the highest sensitivity rate of 99.8% was reported by Dr.Sanaali et al, 97% by Maria et al [15] others had reported 91% by Hooton et al [16], 89.8% by Rebecca et al [13], 82% by Acharya et al[1].

CONCLUSION:
Thus it is concluded that, the methods of detection of these virulence markers is reasonably easy and screening them in microbiological laboratory is a worthwhile, since, there is a high prevalence of antimicrobial resistance among uropathogens, thereby it helps in early detection and appropriate antimicrobial therapy of urinary tract infection which will reduce the morbidity.

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