

Evaluation of different virulence factors and antibiogram of uropathogenic Escherichia coli (UPEC) isolated in a tertiary care centre

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ABSTRACT

Background: Escherichia coli (E.coli) is most common organism responsible for UTI because of its various virulence factors to break the inertia of mucosal barrier.

Objective: In this present study we aimed to access the different virulence factors and their correlation with multi-drug resistance.

Materials and Methods: This was a prospective study done at Burdwan Medical College and Hospital, West Bengal. A total 100 urine samples from clinically suspected UTI patients were processed by standard microbiological procedures. Fifty (50) E.coli were isolated on MacConkey agar showing significant bacteriuria from the 100 samples studied. The virulence factors studied were Haemolysis, Bio-film formation, Mannose sensitive/ resistant haemagglutination, serum resistance and antibiotic resistance pattern. For bio-film three methods – congoed agar, tube method and tissue culture plate method were compared. Haemolysis was observed on sandwich blood agar. Mannose resistance and sensitive haemagglutination and serum resistance were determined by standard methods. ESBL and MBL were detected by double disc diffusion method along with conventional antibiogram.

Result: Fifty E.coli were isolated from 100 urine sample in this study. Among these 30% showing haemolytic properties, 48% showing HA and 16% showing serum resistance. Three different methods of bio-film formation were evaluated. Antibiogram was plotted with MDR.

Conclusion: There is a need of periodic surveillance, antibiotic policy, careful use of empirical and target antibiotics, use of antibiotic coated catheter, standard guidelines on care of catheter. This will reduce incidence, chronicity and recurrence of Urinary Tract Infections.

Key words: Urinary Tract Infection, bio-film, virulence factors, drug resistance, antibiotic Policy

Introduction

Urinary tract infection (UTI) is microbial invasion and inflammation of the urinary tract. [1] It accounts for 25%- 40% of nosocomial infection. Escherichia coli (E.coli) is most common organism responsible for UTI because of its various virulence factors to break the inertia of mucosal barrier. [2] Certain strains of Escherichia coli are capable of hemolyzing red blood cells. This haemolytic property is associated with pathogenesis. The most important secreted virulence factor of

uropathogenic E. coli is a lipoprotein called α -haemolysin (HlyA), which is associated with upper UTIs such as pyelonephritis. [3] Biofilms are microbial communities of surface-attached cells embedded in a self produced extracellular polymeric matrix. [4] Biofilm consists Polysaccharide intracellular adhesion (PIA) coded by chromosomal ICA (intracellular adhesion) gene. [5] Biofilms have major role in antibiotic resistance. It includes trapping of antibiotics, diffusion barrier, impairment of drugs and biofilm can

facilitate plasmid exchange. ^[4] Bacteria inside biofilm can escape host immune system. ^[6] Type 1 and P fimbria are responsible for virulence. Fimbrial colonization factor antigens (CFAs) of E.coli have a major role in haemagglutination (HA) of human erythrocytes. Chromosome coded fimbria causing mannose sensitive haemagglutination and plasmid coded fimbria causing mannose resistant haemagglutination. ^[7] Serum resistance pattern in UPEC is an important virulence marker. Bacteria are killed by normal human serum through lytic activity of alternative complement system. A number of envelope components such as O side chain, lipopolysaccharides, acidic exopolysaccharides, K antigens and Outer Membrane Protein are able to protect the bacterial cell against complement. ^[8] In this present study we aimed to access the different virulence factors and their correlation with multi-drug resistance.

Materials and Methods

This was a prospective study done at Burdwan Medical College and Hospital, a tertiary care hospital of West Bengal. The study period was in the month of January, 2015. The study was approved by the Institutional Human Ethical Committee and informed written consent was taken from the patients before collection of samples. A total 100 urine samples from clinically suspected UTI patients were processed by standard microbiological procedures. It included both catheterised and non-catheterised patients. Samples were collected by standard method under complete aseptic conditions. Fifty(50) E.coli were isolated on MacConkey agar showing significant bacteriuria from the 100 samples studied. The virulence factors studied were Haemolysis, Bio-film formation, Mannose sensitive/ resistant haemagglutination, serum resistance and

antibiotic resistance pattern. Haemolysis was observed on sandwich blood agar. ^[3] Three methods of bio-film formation were compared. Congored agar (CRA), tube method (TM) and tissue culture plate (TCP) method. Optical density (OD) of biofilm was obtained by using micro ELISA autoreader. PIA reacts with congo red giving the black colour colony with dry crystalline consistency. In tube method comparison of adherence of dye was screened for differentiating bio-film producer and non producer. ^[9] Haemagglutination of human group O erythrocytes on VDRL slides were determined using 100 µl bacterial suspension, 100 µl erythrocytes and one drop PBS with and without 3% mannose. ^[10] Serum resistance was examined using pooled serum and Hank's balanced salt solution containing 0.1% gelatin. Samples (10 µl) were withdrawn after incubation for 60, 120 and 180 min at 37°C and were spread on blood agar plates. The plates were incubated for 18 hrs at 37°C and viable counts were determined. Susceptibility of bacteria and serum bactericidal activity was expressed as the percentage of bacteria surviving after 180 minutes in relation to the original count of bacteria determined at 0 minutes in the controls. ^[11] ATCC E.coli (25922) was used as control strain. Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method. ESBL and MBL were tested by double disc diffusion method. ^[12, 13]

Result

Fifty E.coli were isolated from 100 urine sample in this study. Thirty four patients were catheterised and 16 patients were non-catheterised. Female cases (38) were more in number than male patients (12). Fifteen (30%) isolates showed haemolytic properties and 8 (15%) showed serum resistance among the 50. [Table: 1, 4] Out

of 24 isolates tested strains positive for HA, 15 (31%) were MRHA positive and 9 (19%) were MSHA. [Table-3] Among the test for bio-film production, in CRA method 17 (34%) isolates show black colonies with a dry crystalline consistency. Results of TCP and TM method are enumerated in [Table: 2]

Final OD value of a tested strain was expressed as average OD value of the strain reduced by ODC value (OD= average OD of a strain-ODc). ODC means OD of negative control. Media without organism was used as negative control in TCP. Nitrofurantoin was the highest sensitive drug (94%). Among 50 isolates, 15 were ESBL producers and 8 were MBL producers. The study was statistically significant. P-value (<0.05) was determined by Epi info software system.

[Non bio-flim producer- OD ≤ ODC
 Weak bio-flim producer-ODC<OD≤2xODC
 Moderate bio-flim producer- 2X ODC<OD≤4 X ODC
 strong bio-flim producer-4XODC<OD] ^[14]

Table: 1 Haemolytic properties

Virulence Factor	Positive	Negative
Haemolysis	15(30%)	35(70%)

Table: 2 Serum resistance

Bio-film formation	TCP n (%)	TM n (%)
High	11(22)	10(20)
Moderate	20(40)	15(30)
Weak/none	19(38)	25(50)

Table: 3 Haemagglutination

Virulence Factor	Positive	Negative
Haemagglutination	24(48%)	26(52%)
MRHA	15(31.25%)	
MSHA	09(18.75%)	

Table: 4 Serum resistant

Virulence Factor	Positive	Negative
Serum resistant	08(16%)	42(84%)

Table: 5 Antibiotic sensitivity

	Nitrofurantoin	Amikacin	Levofloxacin	Cefotaxim	Ofloxacin	Co-trimoxazole	Colistin	Aztreonam	Meropenem	Cefepime-Tazobactam	Imipenem	Ceftazidime	Cefoxitin
E1	S	R	R	R	R	R	R	R	R	R	R	R	R
E2	S	S	S	R	S	R	R	R	R	R	R	R	R
E3	S	S	S	R	R	R	R	R	R	R	R	R	R
E4	S	S	S	R	S	S	R	R	S	S	R	R	S
E5	S	S	R	S	R	R	R	S	S	S	R	S	S
E6	S	S	S	R	S	R	R	S	S	R	R	S	R
E7	R	R	R	R	R	R	S	R	R	R	R	R	S
E8	S	S	R	R	R	R	S	R	S	R	R	R	R
E9	S	S	S	R	S	S	R	R	R	R	R	R	R

E10	S	S	R	R	R	R	R	R	R	R	R	R	R
E11	S	S	S	R	S	R	R	R	S	R	R	R	R
E12	S	S	S	S	S	R	R	R	S	R	S	S	S
E13	S	S	R	R	R	S	S	R	S	S	R	S	S
E14	S	S	S	R	S	R	R	R	R	R	R	R	R
E15	S	S	R	R	S	S	R	R	R	R	R	R	R
E16	S	S	R	S	R	S	R	R	R	R	R	S	R
E17	S	S	R	S	R	S	R	R	R	R	R	S	R
E18	S	S	R	S	R	S	R	R	S	R	R	S	S
E19	S	S	R	S	S	S	R	R	S	R	S	S	S
E20	S	S	R	S	R	S	R	R	S	R	S	S	S
E21	S	S	S	S	S	S	R	R	S	S	S	S	S
E22	S	S	R	S	S	S	R	R	R	S	R	S	S
E23	S	S	R	S	S	S	S	R	R	R	S	S	S
E24	S	R	R	S	R	S	S	R	R	R	R	S	R
E25	R	R	S	S	R	S	S	R	R	R	R	S	R
E26	R	R	S	S	R	S	S	R	R	R	R	S	R
E27	S	R	S	S	R	S	R	R	R	R	R	S	R
E28	S	R	S	S	R	S	R	R	R	S	R	S	R
E29	S	R	S	S	R	R	R	S	R	R	R	S	R
E30	S	R	R	S	R	R	R	S	R	S	R	S	R
E31	S	R	R	S	R	R	R	R	R	R	R	S	R
E32	S	R	R	S	R	R	R	R	S	S	R	S	R
E33	S	R	R	S	S	R	R	R	R	S	R	S	R
E34	S	R	R	S	S	R	R	R	R	R	S	S	R
E35	S	R	R	S	S	R	R	R	R	S	S	S	R
E36	S	R	R	R	S	R	R	R	R	S	R	S	R
E37	S	R	R	R	R	R	R	R	R	S	R	S	R
E38	S	R	R	R	S	R	R	R	R	S	R	S	R
E39	S	R	R	R	R	R	R	R	R	R	R	R	R
E40	S	R	R	R	S	R	R	R	R	R	R	R	R
E41	S	R	R	R	R	R	R	R	R	R	S	R	R
E42	S	R	R	R	R	R	R	R	R	R	R	R	R
E43	S	R	R	R	R	R	R	R	R	R	R	R	R
E44	S	R	R	R	R	R	R	R	R	R	S	R	S
E45	S	R	R	R	R	R	R	S	R	S	R	R	S
E46	S	R	R	R	R	R	R	R	R	S	S	R	R
E47	S	R	R	R	R	R	S	R	R	R	R	R	R
E48	S	R	R	R	R	R	R	R	R	S	R	R	R
E49	S	R	R	R	R	R	R	R	R	R	R	R	R
E50	S	R	R	R	R	R	R	R	S	R	R	R	R

Discussion

Urinary Tract Infections (UTIs) are one of the most common bacterial infections in humans. In this study 50 E.coli were

isolated showing significant bacteriuria. It fulfilled the Kass concept of significant bacteriuria including more than one pus cell or one organism per high power field

in uncentrifuged urine or more than 10 pus cells per high power field in centrifuged urine or Gram stain showing bacteria and semi quantitative culture showing more than 10^5 colonies per ml of urine. ^[15] In this study bio-film forming E.coli were more in number from catheterised patients. Bio-film also helps in the spread of antibiotic resistant traits in the nosocomial pathogens by increasing the mutation rates and by the exchange of the genes which are responsible for the antibiotic resistance. Thus ESBL and MBL producing organism are increasing. Bio-film is often responsible for chronic UTIs. ^[16] HlyA can induce the apoptosis of target host cells, including neutrophils, T lymphocytes, and renal cells, and promote the exfoliation of bladder epithelial cells. Approximately 50% of all cases of pyelonephritis, which leads to renal complications, are caused by HlyA. ^[17] Fimbria coded genes for HA produce persistence by supporting attachment on fibronectin of uroepithelial cells. ^[18] In our study 48% were HA, 16%serum resistant strain. Chronicity and recurrences depends on pathogenesis. More isolates were recovered from females as compared to males due to a short urethra and its proximity to the anal opening. It was seen that antibiogram varied in different virulent strain. It is helpful for developing guidelines for treatment. From the result of our study, nitrofurantoin (94%) appeared to be useful and could be considered as a choice for treating uncomplicated lower urinary tract infections. Aminoglycosides appeared to be best suited for complicated infections.

There is a need of periodic surveillance, antibiotic policy, careful use of empirical and target antibiotics, use of antibiotic coated catheter and standard guidelines on care of catheter. This will reduce incidence, chronicity and recurrence of Urinary Tract Infections.

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