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Determination of virulence factors of *Escherichia coli* isolated from urinary tract infection patients

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Abstract

Urinary Tract infections are one of the most common bacterial infections in human. On the present study urine sample of suspected UTI patients were included. Total 23 samples were found to be positive for bacterial infection in which predominant species was *Escherichia coli* 13 (56.52%) and 10 (43.48%) were found to be other bacterial species i.e. *Proteus spp.* 4 (17.3%), *Citrobacter spp.* 3 (13%), *Klebsiella spp.* 1 (4.3%), *Enterobacter spp.* 1 (4.3%), and *Staphylococcus aureus* 1 (4.3%). The isolates identified as *Escherichia coli* were screened for virulence factors namely cell surface hydrophobicity, haemolysin and gelatinase production by recommended methods. Among 13 *E. coli* 8 (61.54%) were showing cell surface hydrophobicity, 10 (76.92%) were haemolysin positive and nil result for gelatinase production was observed. The study reveals hydrophobicity and α - haemolysin production in *E.coli* strains which are important virulence factors in the pathogenesis.

Keywords: *Escherichia coli*, Urinary Tract infection, Hydrophobicity, Virulence factor, Haemolysis.

1. Introduction

Urinary tract infection is one of the most frequent causes of illness in humans and common both in the community and hospitalized patients. Most of the UTIs are caused by few genera of bacteria of which *Escherichia coli* is the predominant bacterial agent. *Klebsiella pneumonia* and other enteric gram negative rods are the most common bacterial agents of UTI [14]. UTI affects patients in all age groups and both sexes [6]. *Escherichia coli* have been documented as the most important pathogen associated with urinary tract infections in many countries [12].

Escherichia organisms are gram-negative bacilli that exist singly or in pairs. *Escherichia coli* are facultative anaerobic with a type of metabolism that is both fermentative and respiratory. Optimal growth of *Escherichia coli* occurs at 37 °C but some laboratory strains can multiply at temperatures of up to 49 °C. They are either non motile or motile by peritrichous flagella. *Escherichia coli* are a major facultative inhabitant of the large intestine.

The ability of *Escherichia coli* to cause urinary tract infections depends largely on several virulence factors, which help in the survival of *Escherichia coli* under adverse conditions present in those sites. The virulence of individual strains in a given infection is determined by the presence and actual expression of the virulence genes present in them, and also by the environmental conditions in the host [9].

The treatment of *Escherichia coli* infections is increasingly becoming difficult because of the multidrug resistance exhibited by the organism [13]. *Escherichia coli* have ability to produce ESBLs in large quantity. These enzymes are plasmid borne and confer multiple drug resistance, making urinary tract infection difficult to treat [3].

The knowledge of drug resistance pattern in a geographical area and the formulation of an appropriate hospital antibiotic policy will go a long way in the control of these infections. Therefore, it is necessary to know the antibiotic susceptibility pattern of pathogenic *Escherichia coli* to select the correct antibiotics for proper treatment of infections caused by it [13].

2. Material and Methods**2.1 Collection and transport of sample**

Urine sample was collected from the patients suspected for urinary tract infection. Midstream urine was collected aseptically in a sterilized container. It was necessary to process the urine in the laboratory within one hour of collection.

2.2 Isolation

The initial isolation of pathogenic strains from different urine samples was done on MacConkey's Agar. Then it was incubated at 37 °C for 48 hrs [13].

2.3 Identification of isolates

The isolates were identified on the basis of characters as given in Bergey's Manual of Systematic Bacteriology [7].

2.4 Detection of virulence factor

Virulence factors of *Escherichia coli* were determined by:-

a. Salt aggregation test

In this test one loop full of bacterial suspension made in phosphate buffer (pH 6.8) was taken and mixed with equal volume of ammonium sulphate solution of different molarity, i.e., from 0.3125 M to 5.0 M on a glass slide and observed for 1 min. while rotating. The highest dilution of ammonium sulphate solution giving visible clumping of bacteria was scored as the salt aggregation test value. Strains showing aggregation in 0.002 M phosphate buffer alone (pH 6.8) was considered auto aggregative. *Escherichia coli* strains that were SAT value ≤ 1.25 M were considered hydrophobic [11].

b. Haemolysin production

Plate haemolysis test was done for the detection of α-haemolysin produced by *Escherichia coli*. The bacterial culture was inoculated on to blood agar and incubated overnight at 37 °C. Haemolysin production was determined by the presence of the clear zone of complete lysis of erythrocytes around the colony [13].

c. Gelatinase test

In gelatinase production test was used Gelatine agar. The plates were inoculated with test organism and incubate at 37 °C for 24 hrs. Then the plate was flooded with mercuric chloride solution. Development of opacity in the medium and zone of clearing around colonies was considered positive for gelatinase [4].

2.5 Statistical analysis

The data recorded during the course of investigation was statistically analysed by using Z-test and chi square (χ^2) test then conclusion was drawn [1].

3. Results and Discussion

3.1 Isolation of Bacteria from urine samples

In the present study total 50 patients were studied for the presence of bacteria in their urine samples. Out of the 50 urine samples, 23 (46%) showed positive results for bacterial infection and rest of the samples i.e. 27 (54%) showed no more infections but in few cases they showed non bacterial infection either fungal or yeast [16]. Further among 23 positive patients, 7 (30.43%) bacteria were isolated from male patients whereas 16 (69.57%) were obtained from female patients. Similar findings were reported by Bhowmick and Rashid (2004) [2], Jha and Bapat (2005) [8] and Kebira *et al.* (2009) [10] where they showed 30%, 48.8%, and 24% incidence respectively. Whereas Bhowmick and Rashid (2004) [2] and Kebira *et al.*, (2009) [10] were reported that females presented the highest prevalence of the cases compared to male.

3.2 Incidence of *Escherichia coli* in urine sample

Among the 50 urine samples 23 (46%) bacteria were isolated. On the cultural, morphological and biochemical analysis it was found that out of these 23 isolates 13 (56.52%) were confirmed as *Escherichia coli* and 10 (43.48%) were found to be other bacterial species i.e. *Proteus spp.* 4 (17.3%), *Citrobacter spp.* 3 (13%), *Klebsiella spp.* 1 (4.3%), *Enterobacter spp.* 1 (4.3%), and *Staphylococcus aureus* 1 (4.3%). On statistically analysis it was found that there was non significant difference in male and female urine samples between incidence of *Escherichia coli* and other organisms. There was highest prevalence of *Escherichia coli* in urinary tract infection in comparison to other bacterial species. The presence of other bacterial infections rather than *Escherichia coli* had been reported by Vasquez and Hand (2004) [15], Jha and Bapat (2005) [8], Ehinmidu (2003) [5], and Bhowmick and Rashid (2004) [2], that *Escherichia coli* is a species which is majorly involved in urinary tract infection.

Table 1: Incidence of *Escherichia coli* in urine sample

Total no. of patients	Total no. of isolates	Organisms					
		<i>Escherichia coli</i>	Other suspected bacteria				
			<i>Proteus spp.</i>	<i>Citrobacter spp.</i>	<i>Klebsiella spp.</i>	<i>Enterobacter spp.</i>	<i>S. aureus</i>
50	23	13 56.52%	4 17.3%	3 13%	1 4.3%	1 4.3%	1 4.3%

3.3 Occurrence of *Escherichia coli* with respect to their age and sex

Maximum incidence of *Escherichia coli* 4 (30.76%) was observed in the age group of 21-30 years. In this age group females were found to be more infected by *E. coli* (3/4 i.e. 75%) whereas male showed only 25% incidence i.e. 1/4. In the age group of 11-20, occurrence of *Escherichia coli* was observed to be 2 (15.38%) in which 1 (50%) in male and 1 (50%) in female. In between the age group of 21-30 yrs there

was high prevalence of the patients mainly the females, which is exactly comparable with the findings of Bhowmick and Rashid (2004) [2], and Jha and Bapat (2005) [8]. In our study higher rate of UTI in case of <10 yrs ages and >60 yrs ages was observed. UTI in >60 yrs is very usual but UTI in <10 yrs ages can be explained as having structural abnormalities, obstruction of the urinary tract that placed them at higher risk of UTI.

Table 2: Occurrence of *E. coli* in patients with respect to their age and sex

Age group	No. of patients +ve for <i>E.coli</i>	Sex	
		Male	Female
10-20	2	1 (50%)	1 (50%)
21-30	4	1 (25%)	3 (75%)
31-40	3	1 (33.33%)	2 (66.67%)
41-50	1	0 (0%)	1 (100%)
51-60	2	1 (50%)	1 (50%)
61-70	1	0 (0%)	1 (100%)
Total	13	4 (30.77%)	9 (69.23%)

3.4 Detection of Virulence factor

The obtained isolates were screened for the presence of different virulence factor such as salt aggregation test, haemolysin production and gelatinase production. The obtained results are as follows:

a. Salt aggregation test

In salt aggregation test 8 (61.54%) *Escherichia coli* were found to be hydrophobic, among which 2 (25%) were isolated from

male and 6 (75%) were from female samples. In those 8 *Escherichia coli* only 2 (15.38%) were showed auto aggregative characters. In those 2 *Escherichia coli* 1 (50%) isolated in male urine sample and 1 (50%) in female. On statistical analysis it was found that there was non significant difference between male and female. Similar findings reported by Wojnicz (2007) [16], Raksha *et al.* (2003) [11] and Sharma *et al.* (2007) [13].

Table 3: Incidence of hydrophobicity of *E.coli* in different patients

Total no. of <i>E.coli</i>	Total isolates +ve for salt aggregation test	SAT +ve isolates		Total isolates -ve for salt aggregation test	SAT -ve isolates	
		Male	Female		Male	Female
13	8 61.54%	2 25%	6 75%	5 38.46%	2 40%	3 60%

b. Haemolysin production

In haemolysin production test out of 13 *E. coli* isolates 10 (76.92%) showed positive results for α -Haemolysis and 3 (23.08%) *E. coli* gave negative results. Out of 10 *E. coli* isolates, 3 (30%) were isolated from male patients and 7(70%)

were from female patients. Other *E. coli* did not produce haemolysin. On statistical analysis it was found that there was non significant difference between male and female. This result is exactly comparable with the findings of Wojnicz (2007) [16] *i.e.* 77%.

Table 4: Incidence of Haemolytic *E. coli* in different patients

Total no. of <i>E.coli</i>	Total isolates +ve for α -Haemolysin production	Haemolysin production +ve isolates		Total isolates -ve for Haemolysin production	Haemolysin production -ve isolates	
		Male	Female		Male	Female
13	10 76.92%	3 30%	7 70%	3 23.08%	1 33.33%	2 66.67%

c. Gelatinase test

In gelatinase production test among the 13 *E. coli*, none of the isolates showed positive results. Sharma *et al.* (2007) [13] also used gelatinase test to detect virulence factor of *E. coli* and in their study they found that 6.9% *E. coli* gave positive result for gelatinase test.

3.5 Conclusion

Therefore on the basis of above mentioned facts it can be finally concluded that *E. coli* is one of the important cause of UTI in the patients. The presence of different virulence factors indicates an urgent need for proper detection and diagnosis of the causal organisms along with their virulence factors; hence a

detailed study on this aspect is required to solve this problem.

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