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Original Research Article

Virulence Factors Analysis and Antibiogram of Uropathogenic and Commensal *Escherichia Coli*: New Emerging Pattern of the Common Pathogen

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Abstract

Background: The most prevalent infectious disorders in children are urinary tract infections (UTIs), which include cystitis and pyelonephritis. 50% of nosocomial UTIs and up to 90% of community-acquired UTIs are caused by *Escherichia coli* (E. coli). Consequently, for both clinical and epidemiological consequences, it is crucial to identify E. coli strains and patterns of antibiotic resistance.

Methods: We examined 100 strains of *E. coli* that were found in stool samples from healthy children and urine samples from children under the age of 7 who had UTIs that were acquired in the community.

Results: We assessed the isolated *E. coli's* virulence factors (VFs) and drug sensitivity. The isolates' medication sensitivity levels for amikacin were 94%, nitrofurantoin was 90%, gentamicin was 66%, cefixime was 56%, nalidixic acid was 40%, and gentamicin was 28%. (cotrimoxazole). The prevalence of virulence factors varied between 2% for type 1 fimbriae and 18% for hemolysin and P fimbriae, according to laboratory investigations. Drug resistance was lowest for amikacin and highest for cotrimoxazole. Comparatively to non-pathogenic *E. coli*, uropathogenic *E. coli* were more likely to produce hemolysin and P-fimbriae.

Conclusion: Even though amikacin initially thought to be the preferred option for UTI in children, nitrofurantoin now seems to be practical and can be considered as the chosen choice for simple lower UTIs.

Keywords: *Escherichia coli;* Virulence factor; Drug resistance; Urinary Tract Infection.

Introduction

Many clinical symptoms caused by *Escherichia coli* include bacteremia, sepsis, meningitis, gastroenteritis, and urinary tract infections (UTIs). Moreover, it is the most prevalent facultative aerobic bacterium found in the gut flora of healthy people.[1,2].

Uropathogenic *E. coli* is a leading cause of the great majority of UTIs, and possesses a large variety of specialised virulence factors such as adhesins and toxins in addition to the typical ones [3].

Specific adhesin virulence factors in *E. coli* include Aggregative Adherence Fimbriae (AAF IIIII), Colonization Factor Antigens (CFA I-III), type 1 fimbriae, P-fimbriae, S-fimbriae, Bundleforming protein (Bfp), Intimin (non-fimbrial adhesion), and Dr-fimbriae [4]. The specific toxins present in *E. coli* include Heat Labile Toxins (LT I-II), Heat Stable Toxins (ST a-b), Shiga-like Toxins (Stx 1-2), Cytotoxins, Endotoxin (Lipopolysaccharide [LPS]), and Hemolysin. The iron-regulated gene, type 1 fimbriae, P and S fimbriae, Dr adhesins, afimbrial adhesins, and The most common adhesins found in uropathogenic *E. coli* are A homologue adhesins[5]. Type 1 fimbriae, which are generated by both pathogenic and commensal strains, are one of the most important VFs implicated in the development of a UTI, promoting extracellular binding to the host urothelium and invasion[6]. The majority of uropathogenic strains encode type 1 fimbriae for early urothelial attachment, which may also aid in microbial colonisation.

One-type fimbriae Inflammation may result from an *E. coli* infection via producing cytokines. Type 1-fimbriae are present in the majority of uropathogenic isolates [7]. Additionally, it was found that type I fimbriae helped the *E. coli* K-12 strain produce biofilms under static growth conditions[7]. P fimbriae is a key virulence factor associated with pyelonephritis caused by uropathogenic *E. coli.* In vivo adhesion to urothelial cells is thought to be mediated by P fimbriae, which also appear to cause an inflammatory reaction during renal colonisation[8]. P fimbriae may be involved in the recurrence or development of treatment resistance in UTI[9]. Several studies[10] explored the transition of *E. coli* from commensal to pathogen status. The ability of infections to diverge from commensals by gaining virulence-associated genes located on pathogenicity islands or plasmids is particularly intriguing.

The virulence factors in the genomes of pathogenic *E. coli* have been extensively studied, but less is known about the virulence factors in commensal species members. Understanding the virulence traits and antibiotic susceptibility of commensal *E. coli* isolates is necessary to select the appropriate empiric antibiotic therapy in secondary peritonitis brought on by intestinal perforation. To better understand the virulence factors in commensal isolates of *E. coli*, this study looked at the presence of two markers of virulence factors in *E. coli* isolated from healthy children's stools and compared the results with those obtained with *E. coli* isolated from children's urine during UTI. We also assessed the susceptibility of these isolates to various antibiotics in accordance with the presence of these virulence factors.

Materials and Methods

This cross-sectional study was carried out in Meerat, Uttar Pradesh, at the Subharti Medical College, Department of Microbiology between July 2018 and December 2019. A total of 100 *E. coli* isolates were examined, 50 of which were uropathogenic *E. coli* that were isolated from young children with community-acquired UTIs and 50 of which were communal isolates taken from healthy children with no signs of UTI. Standard microbiological methods were used to identify the isolates [11,12].

Mannose resistant haemagglutination (MRHA) with human type A (3% v/v in PBS in the presence of 2.5% mannose) was explored as the indication of P fimbriae and mannose sensitive haemagglutination

(MSHA) as the indicator of type 1 fimbriae. *E. coli* was grown on MacConkey's agar plates and then inoculated into 5ml of phosphate-buffered saline to produce *E. coli* that is abundant in fimbriae. (PBS). The species was then incubated for 5 days at 37° C. in a pH 7.4 solution. Scraped off and subcultured on Casamino acids-yeast Extract Agar (CEA), the thin layer that had formed on the PBS surface was then incubated for 18 hours at 37°C. Five millilitres of group "A" positive human blood were obtained from the blood biobank, and the same amount of Alsever's solution was added. This procedure involved cleaning the blood sample with Alsever's solution three times before treating it with PBS and a 3% erythrocyte suspension. The CEA medium and *E. coli* colonies were mixed to produce a milky solution on a VDRL slide. Add a 3% suspension of erythrocytes in an equivalent volume and gently combine.

After being manually rotated for 3-5 minutes, the slide was examined for macroscopic haemagglutination within 10 minutes. On a slide, the colonies from CEA were emulsified in PBS, and MRHA was calculated by adding a drop of 2.5% mannose. This combination was then gently blended with an equivalent volume of 3% erythrocyte suspension. We rotated the slide for 3 to 5 minutes looking for HA.

Haemagglutination was classified as MRHA if it occurred to the same extent with and without D-mannose, and MSHA if it was inhibited in the presence of D-mannose[13].

After an overnight incubation on a 5% sheep blood agar plate, hemolysis activity was demonstrated by the presence of a zone of lysis around or beneath bacterial colonies[14].

Following the guidelines of the Clinical and Laboratory Standards Institute (CLSI), tests for susceptibility to amikacin (30 microgram), nitrofurantoin (300 microgram), gentamicin (10 microgram), cefixime (5 microgram), nalidixic acid (30 microgram), and cotrimoxazole (25 microgram) were performed using the disc diffusion method on Mueller Hinton agar.[15-17]

Two-tailed Fisher or Chi-square tests were used for statistical analysis with SPSS software (version 16). P value of < 0.05 was regarded to indicate statistical significance.

Results

In the uropathogenic group, b-hemolysis was identified in 9 isolates (18%) and in 41 (82%) of the isolates (Table1). Ten isolates out of the total number of specimens displayed γ-hemolysis, one of which belonged to the control group and the other nine to the uropathogenic group.

Table 1 : The Incidence of Hemolysis in *E. coli* Strains

Hemolysis type	Uropathogenic *E. coli* Number (%)	Enteric isolates *E. coli* Number (%)
γ-Hemolysis	41 (82)	49 (98)
β-Hemolysis	9 (18)	1 (2)
Total	50 (100)	50 (100)

Analyzed by Pearson Chi-Square ($X^2 = 7.111$, df = 1; P =0. 008)

As a result, isolates from the control group did not exhibit the same tendency to b-hemolysis as uropathogenic *E. coli*, which was likewise a statistically significant difference (P = 0.008). These specimens' hemolysis suggested the existence of type 1 or P fimbriae. Also examined as a virulence factor between two groups was agglutination (Table 2). Categories based on MRHA or

MSHA and agglutination negative isolates are shown in Table 2 data.

Agglutination positive isolates from the MRHA or MSHA column, including type 1 and P fimbriae, were pooled and compared to agglutination negative isolates using the Pearson Chi-Square test (χ^2 =1.19, df =1; *P* =0.275). As a result, there was no discernible difference in agglutination across the isolates in this study. Also, the total incidence of positive agglutination in the uropathogenic group was 10% (10 isolates), whereas MRHA made up 18% (9 isolates) and MSHA made up 2% (just one isolate). As a result, 2 percent of all the uropathogenic *E. coli* isolates examined had type 1 fimbriae, and 18 percent had P fimbriae. There were statistically significant differences between these virulence variables in the category of uropathogenic organisms (P = 0.002).

Table 2: Incidence of Mannose Resistant and Mannose Sensitive Haemagglutination in *E. coli* Strains

Hemolysis Type	Mannose Resistant Haemagglutination Number (%)	Mannose Sensitive Haemagglutination Number (%)	Without Agglutination Number (%)
Uropathogenic *E. coli*	9 (18)	1 (2)	40 (80)
Enteric isolates *E. coli*	4 (8)	2 (4)	44 (88)
Total	13 (13)	3 (3)	88 (88)

2nd and 3rd columns (type 1 and P) have been combined and analyzed by Pearson Chi-Square (X^2=1.19, df=1; P=0.275)

4 percent of the isolates were MRHA and 2 percent were MSHA, while 12 percent of the control group displayed positive agglutination. (Type 1 fimbriae and P fimbriae). There were no statistically significant changes between these virulence factors in the control group (P = 0.294). In our investigation, P fimbriae was the most frequent virulence factor, and it was more frequent in the uropathogenic group than in the control group.

Antibiotic susceptibility testing was done in accordance with the distribution of virulence factors in commensal and uropathogenic *E. coli* isolates. Another virulence trait present in these isolates was hemolysis. Our findings demonstrated a correlation between increased uroepithelial attachment ability and resistance to the bacterial death process in *E. coli*, which produces hemolysis. Antibiotic susceptibility patterns were examined in isolates with positive (referred to as b hemolysis) and negative (referred to as γ hemolysis) hemolysis.

The non-susceptible isolates were created by combining the resistant and intermediate isolates. Due to their frequent prescription in our area, nitrofurantoin, amikacin, cotrimoxazol, nalidixic acid, cefixime, and gentamicin discs were used for assessing antibiotic susceptibility. Table 3 provides a summary of the pattern of susceptibility to these drugs. Drug sensitivity was reported to be 100% (amikacin), 90% (nitrofurantoin), 70% (gentamicin), 60% (cefixime), 80% (nalidixic acid), and 40% for isolates with hemolysis (cotrimoxazole). Except for nalidixic acid, which revealed a statistically significant difference between the two groups, no changes between hemolysis positive and hemolysis negative isolates were found, as shown in Table 3. (P value 0.014). Among isolates that tested positive for b-hemolysis, amikacin and cotrimoxazol showed the lowest and greatest levels of resistance, respectively. We also looked at each isolate of *E. coli's* antibiotic susceptibility based on whether or not it agglutinated.

The susceptibility patterns for the isolates with positive agglutination were 93% (amikacin), 87% (nitrofurantoin), 68% (gentamicin), 75% (cefixime), 18% (nalidixic acid), and 25%. (cotrimoxazole). Among the isolates that tested positive for agglutination, amikacin and nalidixic acid showed the lowest and greatest rates of resistance, respectively.

Table 3: Isolates of Hemolysis Uropathogenic and Enteric *E. coli* Strains and Their Susceptibility to Common Antimicrobial Agents

Agent	γ -Hemolysis			β-Hemolysis			P value
	Resistant	Intermediate	Sensitive	Resistant	Intermediate	Sensitive	
Nitrofurantoin	4 (4.4)	6 (6.7)	80 (88.9)	0 (0)	1 (10)	9 (90)	p>0.99
Amikacin	1 (1.1)	2 (2.2)	87 (96.7)	0 (0)	0 (0)	10 (100)	p>0.99
Cotrimoxazole	61 (67.8)	3 (3.3)	26 (28.9)	6 (60)	0 (0)	4 (40)	p>0.482
Nalidixic acid	49 (54.4)	8 (8.9)	33 (36.7)	2 (20)	0 (0)	8 (80)	P=0.014
Cefixime	32 (35.6)	4 (4.4)	54 (60)	4 (40)	0 (0)	6 (60)	p>0.99
Gentamicin	9 (10)	8 (8.9)	73 (81.1)	1 (10)	2 (20)	7 (70)	P=0.414

Resistance and Intermediates have been combined and the Fisher Exact test has been used

Except for nalidixic acid (P=0.048), there was no noticeable difference between the tested antibacterial medications when the resistant and intermediate isolates from each group (with positive and negative agglutination) were combined and evaluated as non-susceptible isolates. (Table 4).

Table 4: Evidence of Positive or Negative Agglutination in Uropathogenic and Enteric *E. coli* Strains and Susceptibility to Common Antimicrobial Agents

Agent	Agglutination Positive			Agglutination Negative			P value
	Resistant	Intermediate	Sensitive	Resistant	Intermediate	Sensitive	
Nitrofurantoin	0 (0)	2 (12.5)	14 (87.5)	4 (4.7)	5 (6)	75 (89.3)	p>0.99
Amikacin	1 (6.2)	0 (0)	15 (93.8)	0 (0)	2 (2.4)	82 (97.6)	P=0.411
Cotrimoxazol	12 (75)	0 (0)	4 (25)	55 (65.5)	3 (3.5)	26 (31)	P=0.711
Nalidixic acid	12 (75)	1 (6.3)	3 (18.8)	39 (46.4)	7 (8.4)	38 (45.2)	P=0.048
Cefixime	4 (25)	0 (0)	12 (75)	32 (38.1)	4 (4.8)	48 (57.1)	P=0.181
Gentamicin	2 (12.5)	3 (18.7)	11 (68.8)	8 (9.5)	7 (8.4)	69 (82.1)	P=0.303

Resistance and Intermediates have been combined and the Fisher Exact test has been used

Our findings indicated that among isolates with positive hemolysis and agglutination, nitrofurantoin was the most sensitive agent, followed by amikacin.

Discussion

UTIs are among the most common infections in neonates and young children. An earlier analysis by Kausar *et al*. carried out in Iran between 2007 and 2009 discovered high rates of tetracycline, ampicillin, and nalidixic acid resistance among the isolates. They came to the conclusion that *E. coli* is the most common type of bacterial infection that causes urinary tract infections and that it has a high rate of resistance to many commonly prescribed antimicrobial medications. They came to the conclusion that understanding antibiotic sensitivity can assist consultant doctors in determining the appropriate course of treatment for patients with UTIs and also minimise the issues related to serious UTI in young children[18].

Another essential characteristic of uropathogenic *E. coli* is its capacity to agglutinate human red blood cells in the presence of mannose. In the current study, we investigated how the virulence characteristics of various *E. coli* species relate to antibiotic resistance. Virulence factors were discovered in 12% of the commensal isolates in the current investigation, which was fewer than the 20% observed for uropathogenic isolates. Pfimbriae and fimbriae type 1 were distributed differently in commensal and uropathogenic *E. coli* isolates, with a significantly higher prevalence in the latter. Additionally, Gulsun and colleagues discovered that pathogenic *E. coli* caused MRHA more frequently than nonpathogenic *E. coli* in patients with recurrent UTI. They contend that the coexistence of MRHA and MSHA promotes the virulence of *E. coli*[19]. The second study by Kausar *et al*. (2009) found that when pathogenic *E. coli* was isolated from symptomatic UTI patients and compared with enteric isolates from individuals who looked to be healthy, 21%, 30%, and 36%, respectively, presented with hemolysin, MRHA, and MSHA. Hemolysin, MRHA, and MSHA, on the other hand, were all found in 16%, 4%, and 8%, respectively, of the isolates from the controls group. Between the two groups, there was no appreciable difference in hemolysin production. They observed a considerable difference between the UTI and control groups with MRHA. They discovered that 80% of pathogenic *E. coli* species have one or more virulence factors [18].

The distribution of virulence factors among pathogenic *E. coli* was studied by Jalali *et al*.[20]. According to his research, 27% of the 173 *E. coli* isolates from UTI patients had fimbriae type 1, 23% had P fimbriae, and 23% had both. In addition, 20% of people are thought to have MSHA and MRHA. According to our research, hemolysis was stronger in uropathogenic *E. coli* than in the control group, and uropathogenic strains were also more likely to have hemolysin. Conversely, commensal isolates tended to γ hemolyze more frequently. However, this distinction lacked statistical significance. Our observations also emphasise the significance of hemolysin as a virulence factor in uropathogenic *E. coli*, as other researchers have previously observed.[21–22].

Hemolysis, a symptom that is substantially more common in uropathogenic isolates, was present in 100% of the isolates under examination. Only 96.7% of commensal *E. coli* isolates with this virulence factor were amikacin-sensitive, though. Amikacin sensitivity was 93.8% for isolates that had a positive agglutination response and 97.6% for isolates that had a negative agglutination response.

Conclusion

In conclusion, this study findings showed that *E. coli* hemolysin and P fimbriae, both of which are common among uropathogenic *E. coli,* are important for the bacteria's ability to stick to uroepithelial cells. Despite the fact that amikacin appears to be the favoured treatment for UTI in children, this study's findings suggest that nitrofurantoin may be preferable for milder UTIs with no problems.

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