

Original article

Virulence factors and antimicrobial susceptibility among uropathogenic *Escherichia coli* – a study at a tertiary health care centre

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Abstract

Background and objectives: Large number of community-acquired and hospital acquired urinary tract infections are caused by *E. coli*. Uropathogenic *E. coli* carry chromosomally encoded virulence factors and show resistance to antimicrobial agents, which helps in their survival. The study depicts relation between presence of virulence factor and antimicrobial resistance.

Methods: *E. coli* isolates from urine of patients having symptoms suggestive of UTI were identified by standard microbiological methods. They were then subjected to tests for virulence factor detection i.e. hemolysin, haemagglutinin, serum resistance and gelatinase. The antimicrobial susceptibility testing was carried out using CLSI 2013.

Results: The number of *E. coli* showing presence of virulence factors were Haemagglutinin (28.5%), haemolysin (25.5%), gelatinase in (23%) and serum resistance (11%). Among community-acquired and hospital-acquired *E. coli* isolates, 16.05% and 73.02% were multi-drug resistant. Of the *E. coli* isolates, 61 (30.5%) were ESBL producers. 27.27% of serum resistant *E. coli* were multi-drug resistant followed by 23.53% of hemolysin producing *E. coli*.

Interpretation & Conclusion: Less number of *E. coli* isolates were found to be multi-drug resistant in presence of virulence factors. Maximum number (27.27%) of serum resistant *E. coli* were multi-drug resistant.

Keywords: *E. coli*, Hemolysin, Haemagglutinin, Serum resistance, Gelatinase, ESBL, Antimicrobial susceptibility, MDR

Introduction

Urinary tract infections (UTIs) are defined as diseases which are caused by the invasion of the genitourinary tract by microorganisms. *Escherichia coli* accounts for 70% to 90% of acute community-acquired uncomplicated infections in anatomically normal urinary tracts and is also responsible for 85% of asymptomatic bacteriuria (ABU) and for more

than 60% of recurrent cystitis.¹ It is now recognised that there are subsets of faecal *E. coli*, which can colonize periurethral area, enter urinary tract and cause symptomatic disease. These are currently defined as uropathogenic *E. coli* (UPEC).² Certain serotypes of *E. coli* consistently associated with uropathogenicity express chromosomally encoded virulence markers. The virulence factors include

different adhesins, hem-olysin production, serum resistance and siderophore production. These markers of UPE Care expressed with different frequencies in different disease states ranging from asymptomatic bacteriuria to chronic pyelonephritis.³ Also, the treatment of E. coli infections is increasingly becoming difficult because of the multidrug resistance exhibited by the organism. The present study was designed to determine the virulence factors in uropathogenic E. coli and their antibiotic susceptibility.

Materials and Methods

The study was carried out in Department of Microbiology, at a Tertiary Care Hospital from November 2012 to October 2014. Out of 300 urine specimen collected from clinically suspected cases of urinary tract infection presenting to various outpatient departments (community-acquired UTI) and from patients admitted to various wards in the hospital (hospital-acquired UTI), 200 E. coli from the symptomatic cases of urinary tract infection with significant bacteriuria were characterized. All the isolates were biochemically confirmed using standard methods.

Tests for Virulence factors :Test for detection of Haemolysin of E. coli was carried out according to Raksha et al^{2,4,5}The E. coli isolates were inoculated on 5% sheep blood agar and incubated overnight at 37°C. The indicator of hemolysin production is the ability of E. coli to produce Beta hemolysis on blood agar.

Test for detection of haemagglutininThe strains of E. coli were inoculated into 1% nutrient broth and incubated at 37°C for 48 hours for full fimbriation. The O group human red blood cells were washed thrice in normal saline and made up to a 3% suspension in fresh saline, used immediately or

within a week after storing at 3-5°C. The slide haemagglutination test⁶ was carried out on a multiple-concavity slide by adding one drop of the RBC suspension to a drop of the broth culture and rocking the slide to and fro at room temperature for 5 minutes. Presence of clumping was taken as positive for haemagglutination.

Test for detection of Serum resistance^{2,5}:Overnight culture of E. coli on blood agar plates were suspended in Hank's balanced salt solution. Equal volume of this bacterial suspension and serum (0.05 ML) were incubated at 37°C for 3 h. 10µl of this mixture was inoculated on blood agar plate and incubated at 37°C for 24 h and viable count was determined. The ability of bacterial isolate to survive to >1% of initial colony count suggests serum resistance.

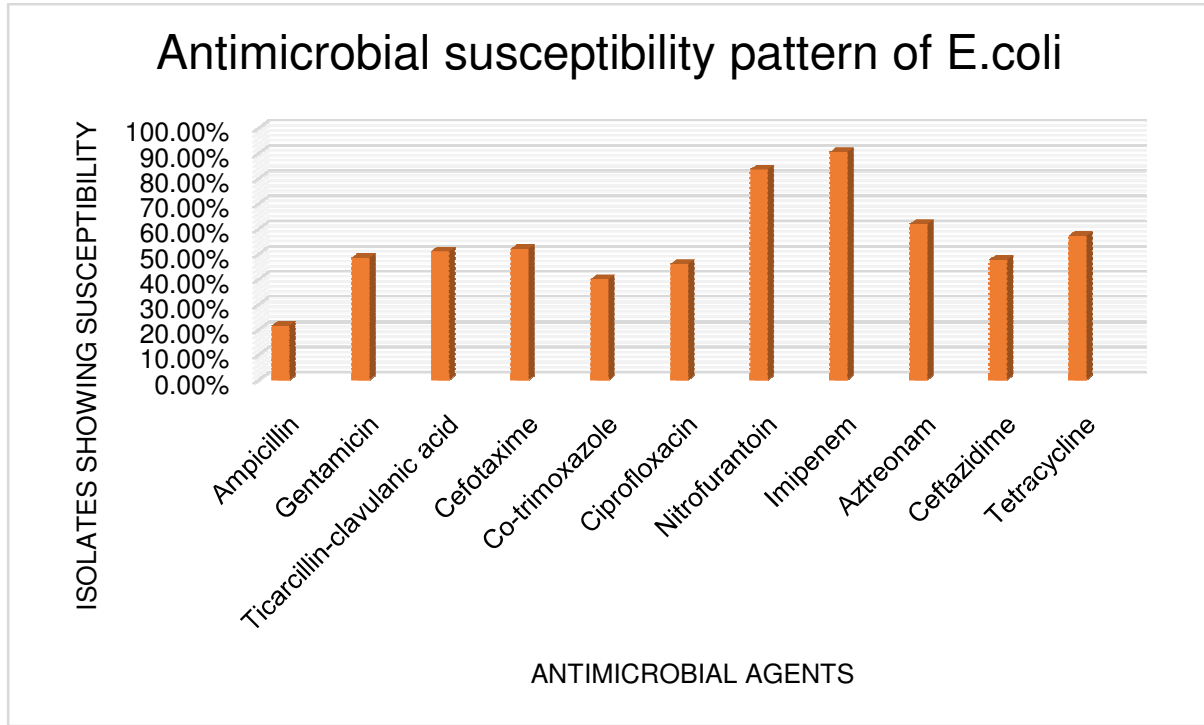
Gelatinase test⁷Gelatin agar plate was inoculated with test organism and incubated at 37°C for 24h. The plate was then flooded with mercuric chloride solution. The ability of bacterial isolate to produce opacity in the medium and the presence of zone of clearing around colony is indicative of gelatinase production.

Antimicrobial susceptibility test⁸ - Antimicrobial susceptibility testing was performed as per the CLSI guidelines (2013) by modified Kirby Bauer method.

Observations

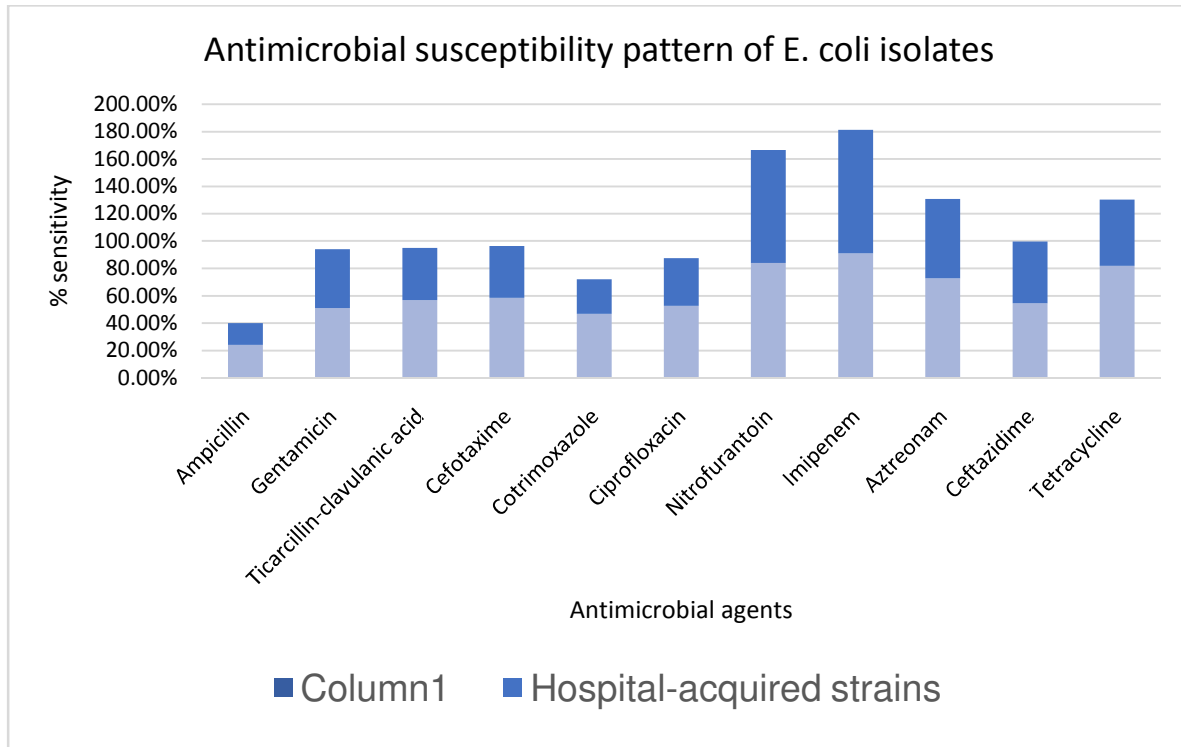
In a present study, E. coli was the commonest organism isolated (74.90%) from suspected cases of urinary tract infections. Of various virulence factors in E. coli, haemagglutinin was the most common identified (28.5%) followed by haemolysin in 25.5% and gelatinase in 23%. Presence of serum resistance was seen in 11% of E. coli. Multiple virulence factors (>4) were demonstrated in 18.5% of total E. coli.

Chart 1. Antimicrobial susceptibility pattern of E. Coli



E. coli (n=42) resistant to first line antibiotics were tested for sensitivity towards second line antibiotics.

Chart 2. Antimicrobial susceptibility pattern of E. coli among Hospital-acquired and Community-acquired group.



More than 80 % of both Community acquired (115/137) and hospital acquired (52/63) E. coli showed sensitivity to Nitrofurantoin. Community acquired E. coli showed similar sensitivity towards Cefotaxime, Ticarcillin-clavulanic acid, Ciprofloxacin and Gentamicin whereas more than 50% showed resistance towards Cotrimoxazole and Ampicillin. Among hospital acquired E. coli, more than 50% were resistant to Gentamicin, Cefotaxime and Ticarcillin-clavulanic acid, Ciprofloxacin, Cotrimoxazole and Ampicillin, in increasing order.

E. coli among both the groups, community-acquired and hospital-acquired, which were resistant to first line antimicrobial agents, showed 90% sensitivity to imipenem. 72.73% of community-acquired isolates were sensitive to aztreonam. This dropped to 58.06% among hospital-acquired isolates. Ceftazidime sensitivity was observed in 54.54% of community-acquired isolates and 45.16% of hospital-acquired isolates. Community-acquired E. coli showed sensitivity of more than 80% to tetracycline, whereas among hospital-acquired isolates, only 48.38% were sensitive.

Table 1. Distribution of E. coli showing Multi-drug resistance (resistance to 3 or more classes of drugs) and those not showing Multi-drug resistance among community-acquired and hospital acquired UTI groups

E. coli	E. coli showing Multi-drug resistance	E. coli not showing Multi-drug resistance
Community-acquired (n=137)	22 (16.05%)	115 (83.95%)
Hospital-Acquired (n=63)	46 (73.02%)	17 (26.98%)

Among community-acquired E. coli, only 16.05% showed resistant to 3 or more drugs among different class of antimicrobials, whereas 83.95% did not show multi-drug resistance.

Multi-drug resistance was observed in 73.02% hospital-acquired E. coli, 26.98% E. coli did not show multi-drug resistance.

Table 2. Out of total 200 E. coli tested by phenotypic confirmatory disc diffusion technique, 61 (30.5%) were found to be ESBL producers.

Method of detection of ESBL	E. coli isolates n=200 (%)
Phenotypic confirmatory disk diffusion method	61 (30.5%)

Table 3. Comparison of Antibiotic susceptibility of ESBL producers and non-producers to various non-beta-lactam antibiotics among E. coli isolates

Antimicrobial agents	ESBL producer (n=61)		ESBL non-producer (n=139)	
	Susceptible		Susceptible	
	Number	Percentage	Number	Percentage
Nitrofurantoin (NIT)	29	47.54	138	99.28
Gentamicin (G)	20	32.78	77	55.39
Cotrimoxazole (CO)	18	29.50	62	44.60
Ciprofloxacin (CI)	22	36.06	77	55.39

Table 4. Association of virulence factors and MDR (resistant to 3 or more classes of drugs) E. coli isolates

Virulence Factors	MDR positive E. coli isolates (%)	MDR negative E. coli isolates (%)
Haemolysin (n=51)	12 (23.53%)	39 (76.47%)
Haemagglutinin (n=57)	9 (15.78%)	48 (84.22%)
Serum resistance (n=22)	6 (27.27%)	16 (72.73%)
elatinase (n=46)	7 (15.22%)	39 (84.78%)

Among haemolysin producing E. coli, 23.53% (12/51) were found to be multi-drug resistant. Six out of 22 (27.27%) of serum resistant E. coli were multi-drug resistant. This figure dropped to 15.78% where 9 out of 57 of hamagglutinin positive E. coli and 15.22% where 7 out of 46 gelatinase producing E. coli were multi-drug resistant.

Discussion

E. coli strains isolated from urinary tract infections were investigated for possession of virulence factors and antimicrobial susceptibility. Virulence factors enable E. coli to colonise selectively the mucosal uroepithelium, proceeding from lower urinary tract to involving renal cavities. The capacity of E.coli to produce virulence factors contribute to its pathogenicity. These virulence factors enable some members of the normal flora to elicit an infection by overcoming the host defence mechanisms.⁹

Colonization of urinary tract with hemolytic strains of E. coli is more likely to develop into severe form of infection. Hemolysis, though not essential for establishment of acute pyelonephritis, may contribute to tissue injury, survival in renal parenchyma and entry into blood stream.¹⁰ In present study, 25.5% of E.coli strains produced haemolysin, of these, 23.53% were MDR. Haemolysin production as a virulence factor by urinary isolates of E.coli has been shown by previous workers.^{2,10} E. coli strains causing urinary tract infections were observed to agglutinate human erythrocytes despite the presence of mannose and this was mediated mainly by fimbriae.² Fimbriae mediate

the ability of E. coli to adhere to the uroepithelium, thereby resisting elimination by the flow of urine.¹⁰ In our study, 28.5% of E.coli strains showed presence of haemagglutinating fimbria. Serum resistance is the property by which the bacteria resist killing by normal human serum due to the lytic action of complement system. In the present study, 23% of UPEC isolates produced gelatinase and 15.22% of them were found to be associated with MDR. Antimicrobial susceptibility pattern was studied for isolates of E. coli. Resistance was observed towards Ampicillin, Cotrimoxazole, Ciprofloxacin, Gentamicin, Cefotaxime, and Ticarcillin-clavulanic acid, in that order. Resistance to common antibiotics has also been reported by other workers.^{11,12,13} Maximum number of isolates were resistant to Ampicillin (79.5%) and sensitive to Imipenem (9.5%) and Nitrofurantoin (16.5%). These results are consistent with the previous studies on drug resistance in E. coli.^{11,14} Hospital-acquired E. coli strains showed more resistance towards antimicrobial agents than community-acquired strains. The findings were consistent with other workers.¹⁵ Also, 73.02% of hospital-acquired E. coli isolates showed multi-drug resistance as opposed to 16.05% of community-acquired isolates. Increasing rate of ESBL production as observed in our study may be due to the selective pressure imposed by extensive use of antimicrobials. The indiscriminate use of cephalosporins is responsible for the high rate of selection of ESBL

producing microorganisms. These results are consistent with the other studies on ESBL detection.^{16,17}

Several bacteria, including *Escherichia coli*, construct a multiple-antibiotic-resistance efflux pump that provides the bacterium with resistance to multiple types of antibiotics.¹⁸ *E. coli* (23.53%) were found to be multi-drug resistant. Serum resistance and hemolysin production were two important

virulence factors associated with resistance to antimicrobial agents. Although virulence factors and antimicrobial resistance may confer increased fitness for extraintestinal infections in humans, they may do so via mutually exclusive pathways and distinct populations.¹⁹ However, we observed that in the presence of virulence factors, presence of drug resistance is less.

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