



Original Research Article

Characterization and antibiogram of Uropathogenic *Escherichia coli* from a tertiary care hospital in Eastern India

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ABSTRACT

Escherichia coli is one of the commonest uropathogens. It is frequently resistant to available antimicrobial agents. Aims and objectives: We aimed to study phenotypic features, virulence and antibiogram of urinary *E. coli* isolates recovered from urine samples of patients attending institute OPD and indoor, for 7 months. Characters like motility, colony characters, biochemicals were noted. Antibiogram was studied by Kirby Bauer disc diffusion method. Serum resistance assay was performed by first incubating bacterial suspension with normal saline and serum and subculturing a loopful of both on CLED, incubating and observing reduction in colony count. Hemolysis was checked by growing on blood agar. It was found that *E. coli* was the commonest agent causing urinary infection, constituting 29% of all uropathogenic bacteria followed in frequency by *Pseudomonas aeruginosa* and wild-type *Staphylococcus aureus*. Except one, all patients were adults. The mean age of patients affected by *E. coli* infection was 33.1 years. Infection was twice more common in females than males, and 21% of *E. coli* isolates were Multidrug Resistant. The isolates were mostly refractory to Ciprofloxacin and Cotrimoxazole, and least resistant to Piperacillin-Tazobactam and Nitrofurantoin. Ninety percent *E. coli* isolates were serum resistant and 10% were hemolytic. Conclusion: These observations are important with relevance to epidemiology, diagnosis and selection of empiric antibiotic therapy.

Keywords

Uropathogens,
Escherichia coli,
Antibiogram,
Serum resistance,
Hemolysis

Introduction

Escherichia coli is one of the commonest bacteria causing urinary tract infections (UTIs), especially in women[1]. Urinary tract infections have an enormous disease burden, causing about 150 million cases per annum worldwide[2].

These *E. coli* strains causing UTI are often multi drug resistant, i.e. refractory to 3 or more different classes of antibiotic agents[3]. Proper susceptibility data from a specified area is needed if empirical antibiotics are to be administered in this

group of patients. Identification is often challenging due to need of proper sample collection and transport, variation in phenotypic characteristics, like lack of motility, non-lactose fermenting variants and lack of microscopy and full battery of biochemical tests in peripheral laboratories[4]. Hence studies need to be done regarding atypical phenotype and antibiotic susceptibility pattern of these isolates. Serum bactericidal activity is protective against bacteremia and an indicator of alternate complement pathway activity and needs to be studied in a population[5].

The main aim of this study the prevalence of *E. coli* among uropathogenic bacterial isolates, their phenotypic features, serum resistance and hemolysis as markers of virulence and antibiotic resistance pattern.

And the objectives include studying the prevalence of *E. coli* among urinary isolates. To study the phenotypic attributes like serum resistance and hemolysis and biochemical tests of these *E. coli* isolates by simple, reproducible laboratory methods. Then to study the antibiogram by using Disc diffusion method.

Materials and Methods

This study was carried out in Department of Microbiology of the institute for 7 months, from February 2014 to September, 2014. Midstream, clean catch urine samples were collected in wide-mouthed, sterile, screw-capped universal plastic containers and received in the laboratory routinely from patients attending OPD (Out patient department) and also from indoor. The samples were inoculated on CLED (Cystine Lactose Electrolyte Deficient) agar plates with Bromothymol blue as indicator (HiMedia), with the help of a flame-

sterilised nichrome wire loop of 1 µl volume. All patients were native to Patna district or adjacent areas. Plates were

incubated aerobically at 37°C overnight.

After that, colonies, if present were counted manually. A colony count of >100 was taken to be significant, correlating with significant bacteriuria of >10⁵ CFU/ml. Gram stain was performed from the colonies. Subsequent processing was as follows: Catalase, motility and slide coagulase for Gram positive isolates and Oxidase, Hugh-Leifson's Oxidation-Fermentation (O/F) test, motility and standard biochemicals for Gram negative isolates, which were also observed for LF(Lactose fermenting) or NLF(Non-Lactose fermenting) colonies on CLED. Motility tests were performed by semisolid (0.5% agar) agar stab method. Biochemical tests for Gram negative isolates used were: Indole production in peptone water, Urease test on Christensen's Urea agar slant, Citrate utilisation on Simmon's citrate agar slant and acid/gas/H₂S production in TSI (Triple Sugar Iron)slant.

After identifying the pathogens, antibiotic susceptibility tests was done by Kirby Bauer Disc Diffusion method on Mueller-Hinton agar as per CLSI guidelines, using *Pseudomonas aeruginosa* ATCC 27853 as susceptible control strain[6]. The following antibiotic discs were used: Cotrimoxazole (25 µg), Amikacin (30 µg), Gentamicin (30 µg), Piperacillin-Tazobactam (100/10 µg), Nitrofurantoin (100 µg) and Levofloxacin (5 µg). The results and findings were noted. One hundred(100) consecutive urinary bacterial isolates were studied.

Serum resistance assay was done by incubating, 100 µl of bacterial suspension

of 0.5 Mac Farland standard with 300 µl of sterile normal saline (pH 7.2) and fresh, unheated human serum, respectively (2 sets

of tubes) at 37°C, for 90 minutes [7]. Then a

loopful from both sets were subcultured on

CLED, incubated overnight at 37°C and

colony counts of both were compared. A reduction in colony count after incubation in serum, to <1% was taken as serum sensitive or susceptible, and reduction by <90% as serum resistant.

Hemolysis was checked by subculturing the colonies on 5% sheep blood agar and incubating the plate aerobically and then observing for complete zone of clearing or hemolysis around colonies.

Results and Discussion

Out of a total of 700 urine samples received for culture in this period, only 200 yielded significant bacterial growth. *E. coli* was the most common urinary bacterial isolate among them , making up 50% of isolates (100 isolates out of 200).

Of all *E. coli* isolates, only 3 were from indoor patients, and 97 were OPD isolates. None of the patients were asymptomatic. *E. coli* was followed, in order of frequency by *Pseudomonas aeruginosa* (12%), *Staphylococcus aureus* wild type (9%), *Citrobacter koseri* (8%), *Enterococcus* spp. other than *E. casseliflavus* and *E. gallinarum* (6%), *Klebsiella pneumoniae* (4%), *Klebsiella oxytoca* (4%), Thymidine auxotrophic Small colony variants(SCV) of *Staphylococcus aureus* (4%),

Staphylococcus saprophyticus (2%) and *Moraxella catarrhalis* (1%).

Age predilection: Only 1 patient was minor (<18 years of age). All others having *E. coli* urinary infection were adults. The mean age of patients with *E. coli* urinary tract infection was 33.1 years.

Gender preponderance: *E. coli* bacteriuria was twice more common in females than males. In males, the mean age of affection was 43.3. years, while the same for females was 31. 1 years. Thus in females, younger age group showed more affection.

Phenotypic attributes: All *E. coli* isolates were motile except 1, oxidase negative, did not utilise Citrate and were Urease negative. Ninety five percent (95 out of 100 isolates) isolates were Lactose fermenting. One isolate (1%)fermented neither Lactose nor Sucrose. Four isolates (4%)were Non-lactose fermenter but sucrose fermenter. Four of the isolates (4%) had mucoid colony morphology. All others showed normal, smooth, low convex, lactose-fermenting colonies on CLED. Only five isolates 5(%) were Indole negative.

Antibiogram: It was found that the most effective antimicrobial agents for *E. coli* urinary infection were Piperacillin-Tazobactam and Nitrofurantoin (8% in vitro resistance to Piperacillin-Tazobactam and 3% resistance to Nitrofurantoin, respectively). The UPEC (Uropathogenic *E. coli*) Isolates were mostly resistant to Levofloxacin and Cotrimoxazole (47% and 62% resistance, respectively). The corresponding figure for Amikacin was 19%. Three isolates (3 %)of *E. coli* showed refractoriness to Nitrofurantoin. Multidrug resistance (in vitro resistance to 3 or more classes of antibiotics) was found in 21% isolates.

Serum resistance: By the serum bactericidal assay, it was seen that about 90% isolates (90 out of 100) of *E. coli* were serum resistant. These isolates thus showed capability of causing bacteremia.

Hemolysis assay: It was found that only 10 isolates (10 %) were haemolytic on blood agar. Thus the isolates were mostly non-hemolytic.

E. coli is the commonest uropathogenic bacterium as per most scientific reports, causing about 70-95% of all upper and lower UTIs[8]. UPEC (Uropathogenic *E. coli*) possesses many virulence factors like fimbrial adhesins, cytotoxins including hemolysins and iron uptake systems or siderophores[9]. Serum resistance is also a putative virulence factor of UPEC, by which the bacteria resist complement-mediated killing (alternate pathway) in the bloodstream. There is thus a strong positive correlation between serum resistance and the ability of the bacteria to invade bloodstream (bacteremia [10]. Serum bactericidal assay can be expressed as titres and correlates well with natural immunity[11]. Uropathogenic *E. coli* is frequently resistant to commonly used antibiotics like beta-lactams, fluoroquinolones and sulphonamides, and some studies quote this figure to be about 100% to Cotrimoxazole and amoxicillin[12]. In a study from South India, Ciprofloxacin resistance was observed in 73% of *E. coli* urinary isolates[13]. In that study, Meropenem, Amikacin and Nitrofurantoin were the most effective antibiotics against this pathogen[13].

Another report from North Israel mentions Cotrimoxazole resistance in vitro to be about 19% in their UPEC isolates[14]. Thus, in a setting where antibiotic therapy needs to be started empirically due to acute infection and immediate unavailability of susceptibility testing facility,

Fluoroquinolones or Cotrimoxazole are clearly not advisable. Cotrimoxazole empirical therapy is again not warranted if in any given area, in vitro resistance to this drug is found to be >10%[14]. Hemolysis, especially that caused by α -hemolysin is strongly proinflammatory, and is found in about 4% UPEC isolates as per other studies[15]. Thus in our study the isolates were not very virulent.

Our study highlights a few important points. Firstly, unless colony characters are observed minutely and full battery of biochemical tests put up and analysed properly, *E. coli* isolates can often be misidentified as *Klebsiella* spp. or *Citrobacter* spp. and others and such misidentification can frequently lead to improper antibiotic treatment. Secondly, in our study, we found that the most effective antibiotics in vitro against UPEC were Piperacillin-Tazobactam and Nitrofurantoin. Serum resistance was found to be quite high in our study, corroborating well with findings of other workers like Sharma *et al*, who found this figure to be about 88.6%, being the commonest observable virulence factor in uropathogenic *E. coli*⁽¹⁰⁾. Since serum resistant isolates mostly have the propensity to cause bacteremia following an episode of UTI, we propose that Piperacillin-Tazobactam can be used in those cases, at least in our area. Nitrofurantoin, being a luminal bactericidal agent and not absorbed systemically, can be reserved for serum-sensitive isolates, but will be ineffective if serum resistant isolates gain entry in blood from urinary tract. Thus serum bactericidal assay can act as the initial guide to choice of empirical antimicrobial therapy, at least in our area.

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