



Original Research Article

Isolation and Identification of *Escherichia Coli* (*E. coli*) From Children Suspecting Urinary Tract Infection (UTI)

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ABSTRACT

Urinary Tract infection is the second most common bacterial infection in children. Classic symptoms and signs were poor feeding irritability or fever combined with emesis and apparent abdominal pain. The most frequent etiology continues to be *Escherichia coli* (*E. coli*). The present study was undertaken to isolate the *E. coli* from children suspecting urinary tract infection of different age groups and sex. Identification and confirmation of these bacteria was done based on microscopic, cultural and biochemical characteristics and we also studied their antimicrobial susceptibility patterns. The study was conducted at department of Medical Laboratory Technology, Doon (PG) paramedical college, HNB Garhwal University, Dehradun, India between October and November 2011. The study encompassed of 83 subjects. The children, aged 11-15 were selected for study in absence of clinical sign and symptoms of UTI. Urines samples were collected and examined and organisms were identified by conventional methods and antimicrobial testing was done according to Kirby Bauer's method on all isolates. Specific morphological characteristic of *E. coli* strains were gram negative and rod shaped. *E. coli* strains showed pink colored colonies on MacConkey's agar medium while white creamy color colonies on the nutrient agar medium. Specific biochemical characteristic of strains were oxidase negative, H₂S positive, nitrate-reduction positive, phenylalanine-deaminase negative, malonate negative, β-galactosidase positive, Methyl-Red positive and Voges-Proskauer negative. Thirty seven strains showed more than 10⁵cfu/ml urine sample on MacConkey agar medium and EMB agar medium. *E. coli* strains showed specific morphological, cultural and biochemical characteristic of *E. coli*. The increase in incidence of bacteriuria in female subjects was observed in all three age groups (60.00%), (66.66%), (62.50%) as compared with male subjects were (40.00%), (33.33%), (37.50%) respectively. All *E. coli* strains were sensitive to Norfloxacin, Roxithromycin, Chloramphenicol, Amikacin, Gentamicin, Azithromycin, Ciprofloxacin but the strains were resistant against Tetracycline (TE/30μg), Erythromycin (E/15μg).

Key words: *Escherichia coli*, Urinary Tract Infection, Bacteriuria, Bacterial strain

INTRODUCTION

Urinary Tract Infections (UTIs) are the second most common bacterial infection in children with greatest level of morbidity after those of the respiratory tract. [1]

Asymptomatic bacterium, the presence of significant number of bacteria in the urine of asymptomatic patient, has been the subject of several long term studies in the school aged children. Many children with

asymptomatic bacterium will have intermittent episodes of symptomatic bacterium.^[2] UTI is a very common disorder among all age groups and found both in men and women. By the age of seven 8.4% of girls and 1.7% of boys will have suffered at least on episode.^[3] Classic symptoms and signs are present in older children, but these features are often absent in infants, toddlers, and pre-schoolers. In infants, nonspecific symptoms of poor feeding, irritability, or fever may be combined with emesis or apparent abdominal pain. Fever is often the only sign of UTI by physical examination in young infants.^[4,5] The most frequent etiology continues to be *Escherichia coli* and approximately 85% of urethrocystitis is caused by *E. coli*.^[6] *Proteus mirabilis* and *Klebsiella pneumoniae* are less frequent offenders. Less commonly, enterococci including *Gardnerella vaginalis* and *Ureaplasma urealyticum* are known agents in UTIs. Gram-positive organisms are even less common in which *Group B streptococcus*, *Staphylococcus saprophyticus* and *Staphylococcus haemolyticus* are probable organisms.^[7] Main aim of present study is isolation and characterization of pathogen from urine of children. Screening and identification of bacterium was undertaken with the belief that early detection of infection and identification of structural abnormalities coupled with appropriate management might lead to prevention of pyelonephritis, renal damage and other complications.

MATERIALS AND METHODS

The study population included native residents of Dehradun Valley and surrounding areas and those who have migrated from other parts of Uttaranchal, India. A total of 83 individuals participated in the present study conducted between October and November 2011. The clinical examination consisted of a personal

interview. Recruitment of participants for the investigation was performed by principal investigators. The research is carried out accommodating all principles of Helsinki declaration, verbal and written consent was taken to individual subject during sample collection. Those children, aged 11-15 were selected while those who were suffering from UTI were excluded for the study. The mid-stream urine samples collected from all the children were transported to the laboratory within half an hour to one hour. The specimens were examined microscopically for the presence of pus cells, RBC and casts. 10ml of urine was transferred to sterilize centrifuge tube and centrifuged at 2000 rpm for 10 min to get bacterial pallet. After centrifugation, loopful of inoculums was taken and streaked on the sterilized MacConkey agar medium. The plates were incubated at 37°C for 24-48hrs. Then the plates were examined after overnight incubation to quantify the organisms present. The colony count was evaluated and organisms were identified by conventional methods and antimicrobial testing was done according to Kirby Bauer's method on all isolates. Following antibiotics were used: Norfloxacin (10µg), Roxithromycin (15µg), Chloramphenicol (30µg), Amikacin (30µg), Gentamicin (10µg), Tetracycline (30µg), Azithromycin (15µg), Ciprofloxacin (5 µg), Erythromycin (15 µg). For the identification and confirmation of isolates microscopic and biochemical test were done.

RESULTS

Fig. 1 shows the distribution of children among the various age groups and sex distribution among each age group. The age distribution was done in three groups; infants (ages 4 weeks-1 years), preschooler (ages 1-5 years) and school-aged child (ages 6-11 years). Eighty three samples were analyzed. Thirty seven (44.57%) strains

showed more than 10^5 cfu/ml urine sample on MacConkey agar medium and EMB agar medium. Selection of strain was based on gram staining, growth on selective medium and biochemical tests. Among 37 bacteriuria subjects, 13 (35.15%) were male and 24 (64.86%) were female. The difference in

incidence of bacteriuria was noticed in male and female. The increase in incidence of bacteriuria in female subjects was observed in all three age groups (60.00%), (66.66%), (62.50%) as compared with male subjects were (40.00%), (33.33%), (37.50%) respectively.

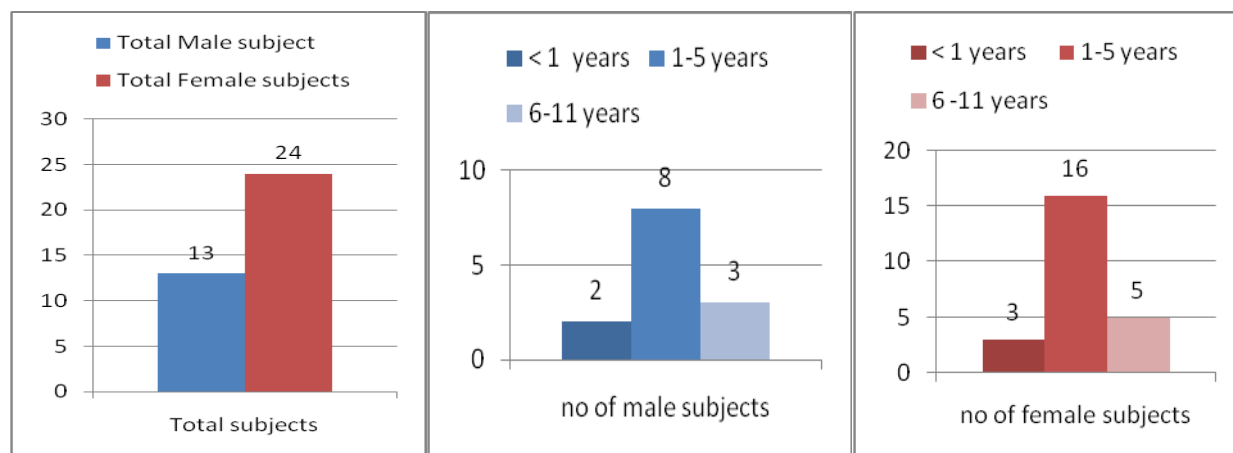


Figure 1: Sex, age of UTI positive children

Table 1 shows morphological and biochemical characteristics of *E. coli*. Specific morphological characteristic of *E. coli* strains were gram negative and rod shaped. *E. coli* strains showed pink colored colonies on MacConkey's agar medium, white creamy color colonies on the nutrient agar medium, slightly yellowish green color with metallic sheen colonies on the endo agar medium, metallic sheen around dark center colonies on the Eosine methylene blue medium while red rose color colonies on deoxycholate lactose agar medium. Specific biochemical characteristic of strains were oxidase negative, Indole positive, methyl red positive and voges-proskauer negative, catalase positive, H_2S positive, nitrate-reduction positive, β -galactosidase positive, phenylalanine deaminase negative and malonate negative. No *E. coli* strains were able to solubilize

gelatin and all the *E. coli* isolates were unable to utilize citrate. All the strains were produced gas and acid in sucrose, glucose and lactose broths. All the stains produce acid in Mannitol broth. No *E. coli* strains hydrolyzed Urea. All *E. coli* strains were produced acid in litmus milk and did not utilize starch. All the strains failed to grow in KCN. All the *E. coli* strains showed β -galactosidase positive.

Fig. 2 reveals the antibiotic sensitivity pattern of isolated *coli* strains. All the *E. coli* strains were sensitive to Norfloxacin (NF/10 μ g), Roxithromycin (AT/15 μ g), Chloramphenicol (CH/30 μ g), Amikacin (AK/30 μ g), Gentamicin (GM/10 μ g), Azithromycin (AZK/15 μ g), Ciprofloxacin (CP/5 μ g) but the strains were resistant against Tetracycline (TE/30 μ g), Erythromycin (E/15 μ g).

Table 1: Biochemical characterization of different isolated E. coli strains

Tests/Characteristics	<i>E. coli</i>																		
	HE 1	HE 2	HE 3	HE 4	HE 5	HE 6	HE 7	HE 8	HE 9	HE 10	HE 11	HE 12	HE 13	HE 14	HE 15	HE 16	HE 17	HE 18	
Gram staining	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Morphology	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MacConkey Agar	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endo Agar	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EMB Agar	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Deoxycholate lactose Agar	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nutrient Agar Medium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase Activity	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Catalase Activity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Indole test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Methyl Red Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VogesProskauer test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gelatinase Activity	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Citrate Utilization	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid and Gas Production in Sucrose Glucose, and Lactose broths	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid production in mannitol broth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urea Hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H ₂ Sproduction in TSI	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitrate Reduction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PhenylalanineDeaminase Production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lysine Decarboxylase activity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malonate Utilization test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid reaction in Litmus milk	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Starch hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth in KCN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
β- galactosidase test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tests/Characteristics	<i>E. coli</i>																		
	HE 19	HE 20	HE 21	HE 22	HE 23	HE 25	HE 26	HE 27	HE 28	HE 29	HE 30	HE 31	HE 32	HE 33	HE 34	HE 35	HE 36	HE 37	
Gram staining	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Morphology	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MacConkey Agar	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endo Agar	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EMB Agar	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Deoxycholate lactose Agar	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nutrient Agar Medium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase Activity	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Catalase Activity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Indole test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Methyl Red Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VogesProskauer test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gelatinase Activity	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Citrate Utilization	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid and Gas Production in Sucrose Glucose, and Lactose broths	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid production in mannitol broth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urea Hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H ₂ Sproduction in TSI	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitrate Reduction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PhenylalanineDeaminase Production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lysine Decarboxylase activity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malonate Utilization test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid reaction in Litmus milk	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Starch hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth in KCN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
β- galactosidase test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

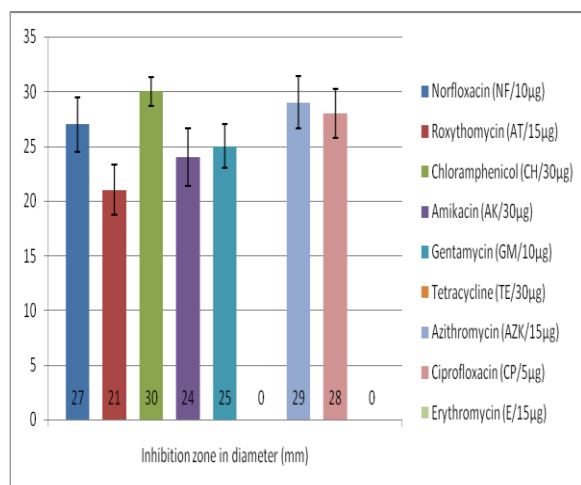


Figure 2: Intrinsic antibiotic sensitivity on the *E. coli*

DISCUSSION

Our study isolated *E. coli* strain and measured more than 10^5 colony forming units/mL in urine sample that indicated children were UTI positive. Similar finding was concluded in the study done in 2010 by Moyo et al., He mentioned the significant bacteriuria ($> 10^5$ CFU/ ml) was found in 42/200 (21%) specimens and out of 42 isolates, the most commonly isolated bacteria were *E. coli*. [8] All isolated *E. coli* strains were gram negative rod under microscope. On the MacConkey's agar medium, all strains ferment lactose. On the Eosine methylene blue medium, all strains produce metallic sheen around dark center colonies. On the Endo agar all strains were produce slightly yellowish green color with metallic sheen colonies. Red rose color colonies were developed in the Deoxycholate lactose agar. On the nutrient agar medium white creamy color colonies were appeared. All Biochemical test confirmed that the isolated strains were *E. coli*. Similar observation has been reported in Bergey's manual of determinative Bacteriology. [9] Our resulted showed that isolated strains were *E. coli* and children age group 1 to 5 year and girls are more affected by the disease similar to those found by Williams et al., (2006) reported that by seven years of age, 8 percent of girls and 2

percent of boys will have at least one episode [10] and Naseri and Alamdaran in 2007, also by reported similar findings. [11] Previously, in one study of 7-year-old school entrants, 7.8% of the girls and 1.6% of the boys had symptomatic UTI verified by urine culture (Hansson and Jodal, 2004). [12] Again, Abdulhadi et al (2008) reported UTI is common in pediatric practice and important cause of morbidity and mortality in children. [13]

CONCLUSION

Thirty seven *E. coli* strains showed more than 10^5 cfu/ml urine. All strains showed specific morphological, cultural and biochemical characteristic of *E. coli*. Evidence of bacteriuria was higher amongst female children's of different ages compared to the counterpart male children. It indicates the increased susceptibility of female to Urinary tract infection.

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