

Characterization and Isolation of different strains (*E.coli*) from patients with Urinary Tract Infection

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ABSTRACT: This study discussed the isolation and identification of some antibiotic resistant *E.coli* isolates that causing urinary tract infection. Forty one bacterial isolates were resulted from 144 urine and fecal isolates that were randomly collected from 3 different hospitals in Al-Sharkia Governorate, Egypt.

These bacterial isolates were identified using conventional microbiological methods by biochemical characterization and. *E. coli* was isolated from each sample, and antimicrobial susceptibility testing of *E.coli* was done with fourteen antibiotics.

KEYWORDS: Escherichia Coli, Urinary Tract Infections, Antibiotic.

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I. INTRODUCTION

Escherichia coli is a Gram-negative, facultative anaerobic bacterium belonging to the Enterobacteriaceae family. The Gram-negative bacillus Escherichia coli (*E.coli*) dwells in the large intestine and is naturally removed in the feces. The urinary tract is the most prevalent site of *E.coli* infection, accounting for more than 90% of all uncomplicated urinary tract infections (UTIs). (Mahmoud and Saber 2022)

The prevalence of *E.coli* species isolated from hospitalized patients, factors associated with infection and antibiotics resistance patterns of the isolates were investigated.

Urinary Tract Infection (UTI) is one of the most common bacterial infectious diseases which causes considerable morbidity and costly health problems. *E.coli* " the most common pathogen causing UTI, is a highly heterogeneous group of extraintestinal pathogenic *E.coli* (ExPEC) which may carry a variety of virulence factors and belonging to different phylogenetic backgrounds.

II. Materials and Methods

2.1. Collection of Samples

A total of one hundred and forty four 144 different samples of urine and stool were randomly obtained from 3 different locations in Al-Sharkia government. The samples were collected and transported to the laboratory for microbiological analysis.

Table 1: Number of samples collected for the isolation of *E.coli* species

Hospital/Lab	Section	Sample type	No. of samples
The University Hospital	Urogenital care	14 Urine	14
	Intensive care unit	9 Urine	9
	Analysis lab	74 Urine 13 Stool	87
Al- Ahrar Hospital	Urogenital care	21 Urine 6 Stool	27
Sednawy Hospital	analysis lab	5 Urine 2 Stool	7
Total			144

2.2. Culture Conditions for Isolation of Bacteria

In case of urine samples; 1 ml from each sample was added into 9 ml of Buffered Peptone Water (BPW, HIMEDIA, M614-500G) and then incubated at 37°C overnight. A loopful from the pre-enriched broth was streaked onto MacConkey agar (MAC , HIMEDIA, M614-100G) plates and incubated at 37°C overnight then E.coli specific media Eosine Methylene Blue agar (EMB,HIMEDIA, M340-100G) and the plates were incubated at 37°C for 24 h.

In case of stool samples; 1gm from each sample was added into 5 ml of Buffered Peptone Water (BPW, HIMEDIA, M614-500G) and then incubated at 37°C overnight. Bacteria colonies observed after 24 hours were then sub-cultured into freshly prepared Nutrient agar in sterile petri dishes to obtain discrete colonies and were subsequently identified.

Presumptive colonies were then purified and identified using different biochemical tests.

2.3. Microscopic examinations

Microscopic examinations were carried out on the isolates for studying the shape, arrangement and reaction of the staining, which included Gram's stain, capsule stain, and the motility test of the isolated bacteria after subculture on the medium of the Nutrient broth at 37°C for 4 hours.

2.4. Biochemical tests

Biochemical tests were applied on bacterial isolates, which included gram staining, catalase, nitrate reduction, motility, urease, indole, methyl red, spore forming (Forbes, Sahm et al. 2007).

2.5. Gram's Reaction

On clean, grease-free slides, isolates were created as smears and heat-fixed. Following the addition of Gram's iodine for 60 seconds, two drops of Crystal violet were added. The slides were water cleaned, then decolorized with alcohol for 15 seconds before being water rinsed again. The decolorized slide was counter stained with Safranin for 60 seconds. It was rinsed off using water. The slides were air-dried and viewed under the microscope using oil immersion lens.

2.6. Analyze catalase:

A few drops of 3% H₂O₂ reagent were immediately applied to the bacterial growth on the agar plate culture. Catalase-producing organisms are discovered using this assay. By dissolving hydrogen peroxide into water and oxygen gas, this enzyme detoxifies it. The presence of oxygen gas bubbles obviously indicates the presence of catalase. (Lelliot and Stead 1987)

2.7. Nitrate reduction:

Durham's tubes were placed upside down, KNO₃ was dissolved in the broth, and they were sterilized for 20 minutes at 115°C. (Stanier, Palleroni et al. 1966)

2.8. Urease test:

Without being stabbed, the Christensen's medium's slope was heavily inoculated with the test bacterium. The contaminated tubes were kept in an incubator for 24 hours at 37 °C. Hydrolysis is the process by which the ammonia splits, turning the red color of urea from its purple pink hue. (Arora 2003)

2.9. Methyl red:

The bacterium was inoculated onto glucose phosphate peptone after a 24-hour incubation period at 37°C. Very little of the methyl red indicator was used. The hue yellow represented a successful test. (Koneman, Allen et al. 1997)

2.10. Gas Production:

The generation of carbon dioxide gas from glucose was crucial in identifying an isolate's homo-hetero fermentative nature. Inverted Durham tubes containing 10 ml of nutrient broth and pure culture isolates were incubated at 37° C for 48 hours.

2.11. Indole test:

The isolated bacteria were inoculated into peptone water and incubated at 37°C for 24 h. Then 5 drops of Kovac's reagent were dropping on the inner wall of the tubes. A deep red ring was positive reaction and negative one revealed yellow color at the alcoholic layer (Arora 2003).

III. RESULTS AND DISCUSSION

3.1. Prevalence of pathogenic bacteria

UTIs are caused by microbial invasion and subsequent multiplication in urinary tract. Eighty percent of the patients with UTI had bacterial etiology in this study. Although the infection rate was higher in female (87.5%) patients as compared to male (71.3%) (Sabir, Anjum et al. 2014)

The current study investigated the occurrence of E.coli in the examined urine and stool samples (n=144) collected from different hospitals at Sharkia Governorate.

Out of the examined samples, 41(28.5%) were suspected as E.coli spp. Biochemical tests and PCR examination confirmed that the obtained 20 isolates were identified as E.coli. The majority of the isolates 21.55% (n=31) were recovered from urine samples, while 6.95% (n=10) were identified in stool samples.

All the E. coli isolates were able to produce bright pink colonies on MacConkey agar, yellowish green colonies surrounded by an intense yellow green zone on BG agar and characteristic metallic sheen colonies on the EMB agar. Differences in colony morphology manifested by the isolates may be due to losing or acquiring some properties by the transfer of host or choice of host tissue as observed by (Dean 1990) and (Dubreuil, Fairbrother et al. 1991). In Gram's staining, the morphology of the isolated bacteria exhibited pink colored, small rod shaped, Gram negative bacilli and in the hanging drop technique all the isolates revealed motility as observed by several authors (Buxton and Fraser 1977). Reactions in TSI agar slant revealed yellow slant and butt with gas but no production of hydrogen sulphide gas was observed which supports the finding of (Buxton and Fraser 1977). In the present study almost all the isolates of E. coli fermented dextrose, maltose, lactose, sucrose and mannitol with the production of both acid and gas. (Ali, Rahman et al. 1998) also studied the biochemical characteristics of the different strains of E. coli isolated from different sources. They reported a little or no difference in these biochemical characters and stated that such similarity among the isolates might be due to presence of some common genetic materials. The results of Catalase, MR and indole test of the E. coli isolates were positive but V-P test was negative which are in agreement with the reports of (Buxton and Fraser 1977) and (Honda, Arita et al. 1982).

3.2. Phenotypic Characterization of isolated strain

Strains obtained from various sources displayed consistent phenotypic traits (Table 2). In contrast to Catalase, Nitrate Reduction, Indole and Methyl red give positive effect Urease and Gram stain give negative effect.

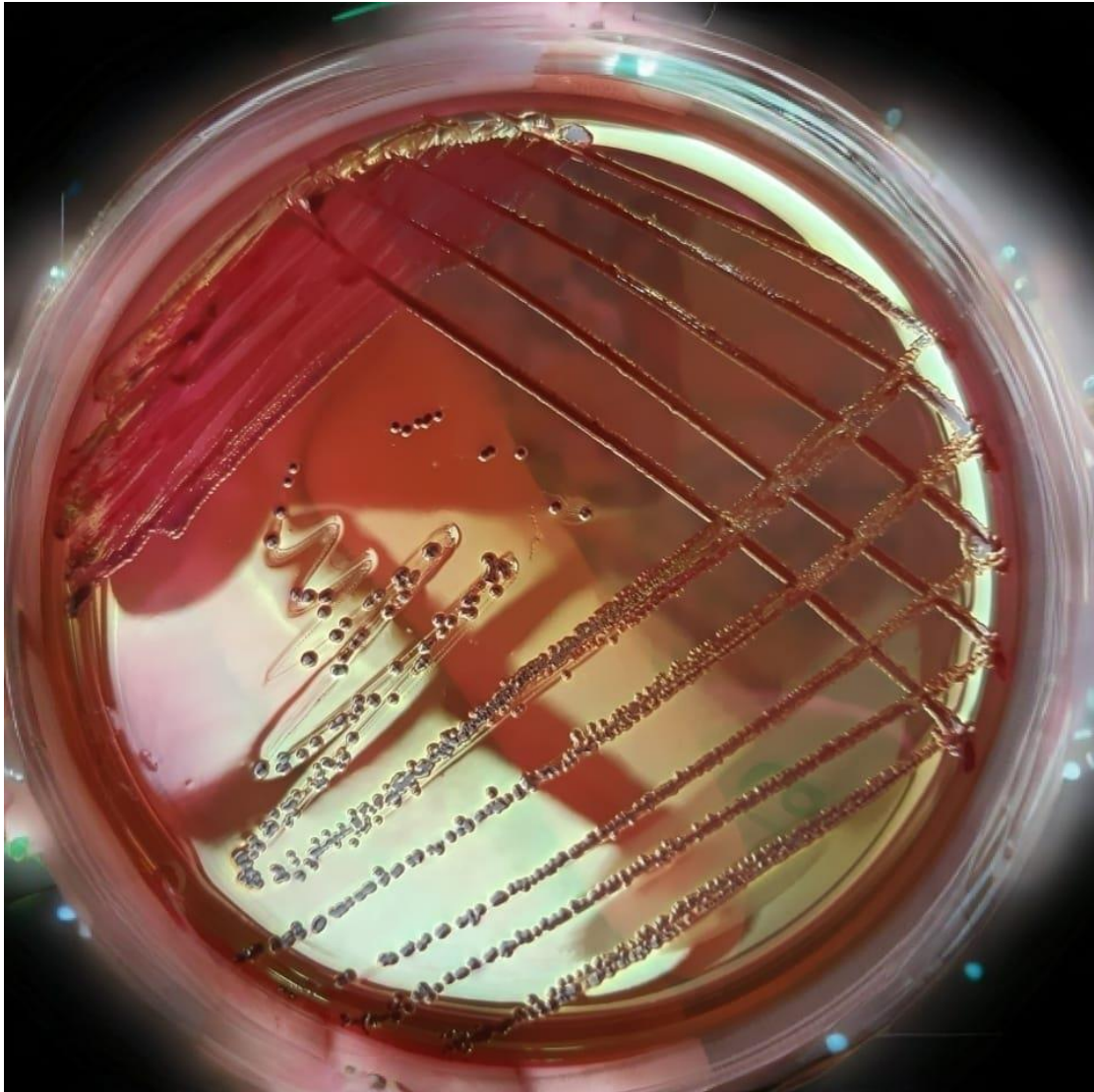

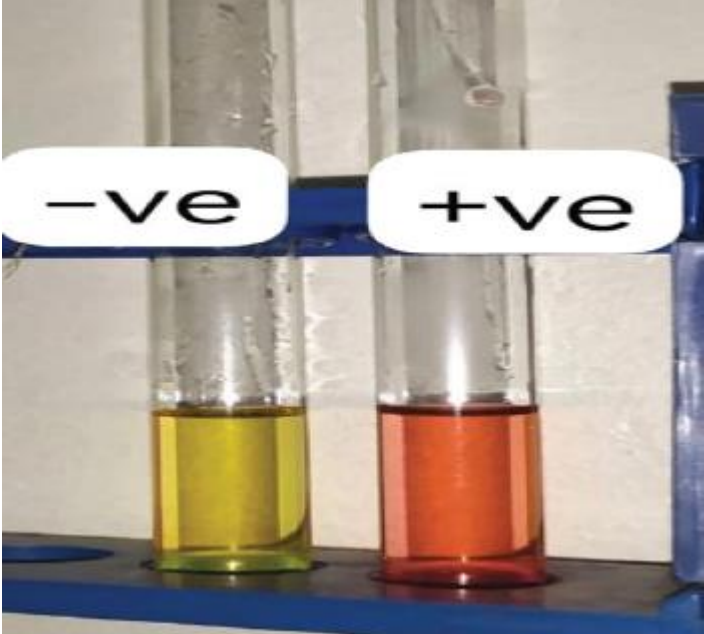



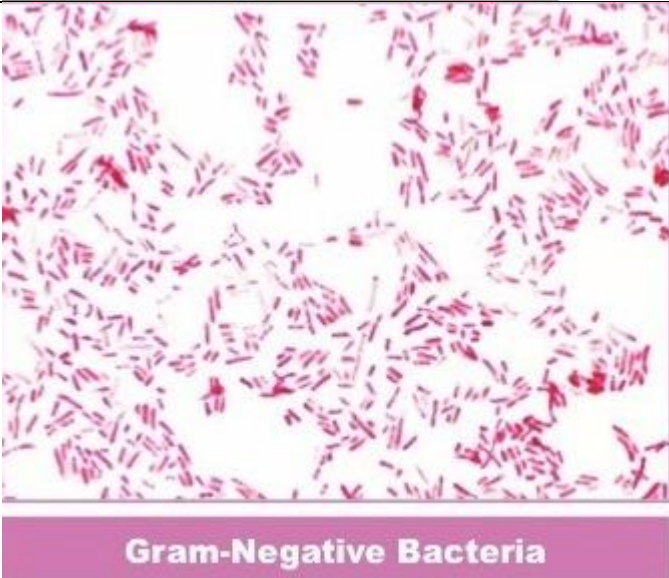


Figure 1: Bacterial cultivability after 24 h of incubation at 37 C0

Table 2: Growth and substrate utilization characteristics of isolated strain observed in this study.

Biochemical test	Result	Observation
Catalase	+ve	
Nitrate Reduction	+ve	

Urease	-ve	
Indole	+ve	

Methyl red	+ve	
Gram stain	-ve	

Conclusion:

From this results found the most prevalence bacteria found in urine and fecal samples is E.coli that were detected by biochemical and microscopic identification.

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