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Isolation and Identification of Escherichia Coli in Uropathogenesis

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ABSTRACT

Introduction: Urinary Tract Infection (UTI) defines a condition in which the urinary tract is infected with a pathogen causing inflammation which is a common, distressing and occasionally life-threatening condition. UTI affects people of all ages and both genders. All patients with UTI are reported with asymptomatic bacteriuria. Females are more susceptible to UTIs compared to males. To ensure appropriate therapy, current knowledge of the organisms that cause UTIs and their antibiotic susceptibility. Methods: This study focused on the frequency of E.coli and their antibiotic susceptibility in different genders in the Derna District. Cultural and biochemical characterization of E.coli. Results: During this study period 200 samples were received, from these samples 180 bacterial isolates were obtained. Out of 180 isolates, 120 were identified as E.coli. Out of this predominant numbers were that of E.coli 120 (66.7%), followed by other bacterial isolated 60 (33.3%). this study shows that the highest E.coli isolated were from married females (93.3%) followed by males (6.7%). The isolated E.coli were sensitive to the various antibiotics. It shows 38.3% sensitivity towards Ciprofloxacin. Followed by levofloxacin and rapamycin which show 25.0% sensitivity, Ampicillin showed 11.7% the lowest sensitivity. Conclusion: Thus our study showed that there were statistically significant differences between the isolated E.coli and age, gender and marital status. We concluded that ciprofloxacin is a better antibiotic used for E. Coli.

1. INTRODUCTION

Urinary tract infections (UTIs) are the most prevalent illnesses seen in medical settings globally. Despite extensive attempts, around 150 million individuals worldwide continue to suffer from urinary tract infections, which cause major morbidity and expensive medical expenditures. Every year, the United States monitors more than 10 million office visits, 2 million department visits, and 100,000 hospitalisations for urinary tract infections (Draghijeva E, 1999; Leroy S, 2010).

UTIs induce a wide range of infections, including asymptomatic/symptomatic bacteriuria, as well as acute, chronic, and recurring infections (Prakash Kalra OM, 2009; Foxman B, 2000). This study represents a significant problem in the management of urinary tract infections (Foxman B, 2003). Children, women, the elderly, diabetics, and persons with uroliths and urinary catheters have a greater risk of infection (Cli Griebling TL, 2005). Urinary tract infections can be classed as complex or simple. Uncomplicated urinary tract infection (cystitis and pyelonephritis) occurs in healthy people who do not have a systemic urinary tract infection or kidney failure. Complex urinary tract infections are related with urinary tract blockages or defensive factors, including urine incontinence, urinary retention, immunosuppression, renal failure, pregnancy, and indwelling catheters or other drainage devices (Draghijeva E, 1999). Uropathogenic Escherichia coli (UPECs) are the primary cause of urinary tract infections, accounting for 75% of all cases (Draghijeva E, 1999; Foxman B, 2003). Because of its relevance, investigations have been undertaken on the UPEC virus (Griebling TL, 2005; Zhang L, 2003). As predicted, the UPEC virus cannot be described as a carrier of a specific gene, but rather as a combination of multiple genes depending on the places it colonises. Although UPECs and E. coli are related but not identical, UPECs are fatal, whereas the virus is not. Furthermore, identifying certain viruses, such as E. coli, is challenging due to the large number of identical species. coli bacteria in the urine samples of symptomatic individuals are adequate to diagnose UPEC (Johnson JR, 1989; Ovalle A, 2001). To colonise the urinary system, UPEC must first survive urine flow (difficult owing to adhesion and hardness), unstable pH levels, limited oxygen availability, and urea (Johnson JR, 1989; Wald E, 2004).Once in the urinary system, bacteria penetrate epithelial cells and multiply, creating intracellular bacteria (IBCs) (Schlager, 2004).

2. METHOD

This prospective cross-over study was performed in the Department of Microbiology lab, College of Medical Technology –Derna from Augustus 2022 to October 2022.

Sample collection

A total of 200 UTI (Urinary Tract Infection) cases were reviewed in this study from Augustus 2022 to October 2022. Midstream specimen of urine, preferably of the first morning void was collected from known UTI patients.

Urine culturing

Urine samples were cultured on blood agar and McConkey agar medium and incubated overnight at 37° C.Significant increase changed into evaluated as ≥ a hundred and five colony-forming devices CFU/mL of midstream urine.

Isolation (Scholes, 2000**)**

Urine samples were cultured on different media including Blood agar, and MacConkeys agar and incubated at 37°C for 24 hours. Thereafter bacteria were isolated and purified by streaking two times on the same media.

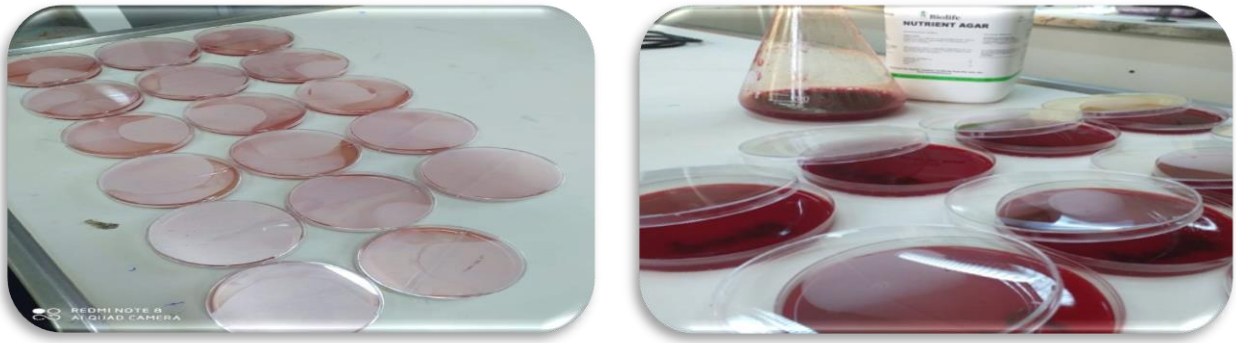

Figure 5: Media Preparation

Characterization and identification Morphological traits of colonies have been studied on MacConkey agar. Colour; length and fringe of colonies have been recorded after 24 hrs of incubation at 37°C.

Microscopic examination

A single colony of each isolate was fixed on a clean slide to study gram stain, under the light microscope.

Biochemical tests

The suspected isolates were subjected to TSI and the biochemical tests as mentioned by Macfadden (Lipsky BA, 1989), and as follows:

A- Catalase test

A single colony of each bacterial isolate was taken and smeared on a clean glass slide, then a drop of hydrogen peroxide (3 drops was flooded with 1.0 ml of 3% hydrogen peroxide. The presence of gaseous bubbles implies a fine result.

B- Oxidase test

This test was done using filter paper moistened with a few drops of a freshly prepared solution of tetramethyl- ρ-phenylenediamine-dihydrochloride. Aseptically, a clump of cells changed picked up from the slant boom with a sterile wood stick and smeared at the moistened paper. The development of a violet or pink colour inside 10 seconds suggests a fantastic result.

C- Indole test

The colony was inoculated into peptone water broth and incubated at 37°C for 24 hrs in a shaker incubator. After incubation, a few drops of Kovac's reagent have been added. The presence of a red-coloured ring shows an advantageous result.

D- Citrate utilization test (Simmon's Citrate slant)

A loopful of colonies became streaked onto a simmon citrate agar slant, after which incubated for twenty-four to forty-eight hrs at 37°C in the incubator. Change in medium shadeation to blue shadeation shows a high-quality result.

E-Methyl-red test

Colony was inoculated in MR-VP broth and incubated at 37°C for 24 hrs. After incubation, 3-four drops of methyl pink reagent have been added. Converting the media colour to purple is an effective result.

F-Vogas-Proskauer test

The colony was inoculated in MRVP broth and incubated at 37°C for 24 hrs. Then two drops of VP1 and four of VP2 were added. The appearance of red colour after 15 min indicates a positive result.

Antimicrobial sensitivity testing (Kirby-Bauer method)

The susceptibility of isolates to antibiotics was demonstrated by using nine specific antibiotics, including prescribed antibiotics that have been given by physicians. Isolates were placed on Mueller-Hinton agar by swabbing. After drying for approximately 5-10 min, Plates have been incubated for approximately 10-15 min at 37°C. Furthermore, involved antibiotic discs were adjusted on cultured plates with the usage of sterile forceps and incubated as inverted for twenty-four h at 37°C. After a single day of incubation, the diameter of every inhibition zone (together with the diameter of the disc) was measured and recorded in mm. The antibiotics used were (Ciprofloxacin, Levofloxacin, Rifamycin and Ampacllin).

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) Qualitative records have been defined by the use of numbers and percentages. Kolmogorov-Smirnov checks have become used to affirm the normality of distribution Quantitative records were described using range (minimum and maximum), mean, famous deviation, and median. The importance of the received outcomes became judged on the 5% level.

3. ETHIC APPROVAL

For research to be considered ethical, it must receive approval from a scientific research ethics committee located in the same region where the research is being conducted.

4. RESULT

The management of UTI is very important because the prevalence of the pathogenesis and development of drug resistance caused by uropathogens are increasing in a higher magnitude. As per the reports documented by different countries, *E.coli* was found to be the most predominant uropathogenic isolated from patients with UTI and the development of multi-drug resistance among uropathogens that causes a complicated UTI. The urine specimens showed greater variation in color, odour, appearance and pH when they were subjected to physical examination. The urine specimens were subjected to different selective and differential media.

During this study period, 200 samples were received, and from these samples 180 bacterial isolates were obtained. Out of 180 isolates, 120 were identified as E.coli.
Out of this predominant numbers were that of E.coli 120 (66.7%), followed by other bacterial isolated 60 (33.3%). Fig(6)

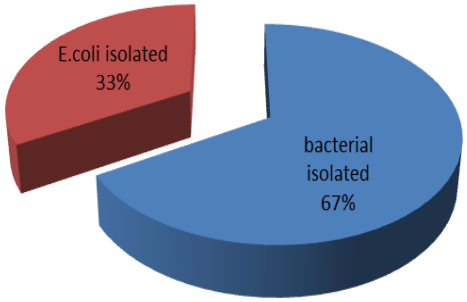

Figure (6): Percentage of E .coli from a total of 180 examined urine samples

The results of the distribution of the studied samples according to demographic data (n = 120) show that the highest E.coli isolated were from females (93.3%) followed by males (6.7%). Fig(7)

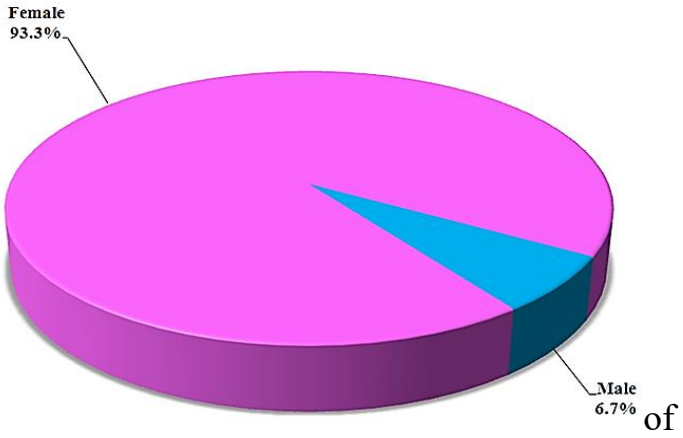

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Figure (7): Distribution of the studied samples according to gender

It is clear from table (1) that the patients in the age group ≤30 years showed the presence of the highest number of E .coli isolated 42.5%. Age groups 30-40 years and ≥40 years showed the lowest number of E.coli isolated were (30.8, 26.7%) respectively.

Table (1): Distribution of the studied samples according to age.

Age (years)	No.	%
<30	51	42.5
30 – 40	37	30.8
>40	32	26.7
Min. – Max.	18.0 – 60.0	
Mean ± SD.	33.25 ± 10.23	

It is evident from the figure (8) that, the highest percentage of E.coli isolated was revealed from married statute (63.3%).

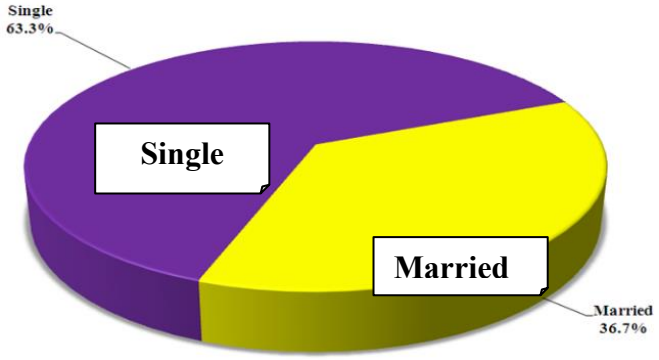

Figure (8): Distribution of the studied samples according to marital status

The plates were observed for zone formation around the discs and the diameter of the growth inhibition zone was measured and recorded in mm.
The isolated E.coli were sensitive to the various antibiotics. It is shown from table (2) 38.3% sensitivity towards Ciprofloxacin. Followed by levoflaxocin and rifamycin which show 25.0% sensitivity, Ampicillin showed 11.7% the lowest sensitivity.

Table (2): Distribution of the studied samples according to sensitivity of antibiotics.

Type of antibiotics	No.	%
Ciprofloxacin	46	38.3
Levofloxacin	30	25.0
Rifamycin	30	25.0
Ampicillin	14	11.7

5. DISCUSSION

Urinary tract infections (UTIs) are extreme infections worldwide (Moreno E, 2008). Most of the research nation that there may be a resistance of gram-poor especially members of Enterobacteriaceae to antibiotics in their different kinds, especially β-lactams antibiotics (Moreno E, 2008).
During this study period, 200 samples were received, and from these samples, 180 bacterial isolates were obtained. Out of 180 isolates, 120 were identified as E.coli. Out of this predominant numbers were that of E.coli 120 (66.7%), followed by other bacterial isolated 60 (33.3%). This result is consistent with most of the previous studies (22, 23). The percentage of E.coli conforms to what (Pitout JD, 2005) had come up. The reason behind the spreading of E.coli is that it has many pathogenicity factors like flagella that help it to move and adhesion (P fimbriae, haemolysin and K capsule), in addition to its outer membrane (Svanborg C, 2005).
The results of the distribution of the studied samples according to demographic data (n = 120) show that the highest E.coli isolated were from females (93.3%) followed by males (6.7%). Overall, UTI is more prevalent among females than males, attributable to the proximity of the urogenital tract to the anus in females, the duration of the male urethra, and the antibacterial hobby of prostatic fluid in men (Lipsky BA, 1989), Gupta K, 2005). Functional, hormonal, and anatomical adjustments that arise in the course of being pregnant predispose pregnant ladies to UTI (Ovalle A, 2001). The patients in the age group ≤30 years showed the presence of the highest number of E .coli isolated was 42.5%. Age groups 30-40 years and ≥40 years showed the lowest number of E.coli isolated (30.8, 26.7%) respectively. the relationship between sexual activity and UTI established in younger women. (Johnson JR, 1991).

Also in this study, the highest percentage of E.coli isolated was revealed from a single statute (63.3%). During intercourse, bacteria gain access to the UT by colonizing and ascending. (Mobley HL, 1994))

The results of the susceptibility test showed found the most effective antibiotic in inhibiting bacterial growth was Ciprofloxacin (38.3%). These consequences have critical medical implications. Thus, these antibiotics do best in the case of Urinary tract infections. Ampicillin showed 11.7% the lowest sensitivity.

The reasons for the enumerated resistance spread of antibiotics in the study may be traced to the Random and illogical use of those antibiotics with the aid of the transient and everlasting sufferers of the hospital (Kuehn MJ, 1992, Lane MC, 2007). The energetic manner to save you the enumerated resistance of the drug with the aid of using pathogens is the logical use of antibiotics in addition to being restricted to the specialized physician orders which supply the best benefit of antibiotics, in addition to the financial expenditures (Johnson JR, 1998).

6. CONCLUSION

Among the Gram-negative bacteria, UPEC is the pathogen most frequently associated with UTIs. E.coli, which colonizes the urinary tract, may ascend towards the bladder to cause cystitis. Left untreated, microorganisms ascend the ureters to the kidney. Thus our study showed that there were statistically significant differences between the isolated E.coli and age, gender and marital status. We concluded that ciprofloxacin is a better antibiotic used for E. coli.

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