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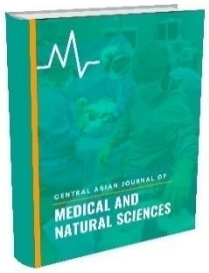

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ISOLATION AND IDENTIFICATION OF *ESCHERICHIA COLI* FROM CHILDREN WITH URINARY TRACT INFECTION IN KIRKUK CITY

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Abstract: Introduction: Urinary tract infections (UTIs) are among the most common infections worldwide. Uropathogenic Escherichia coli (UPECs) are the main causative agent of UTIs. UPECs initially colonize the human host adhering to the bladder epithelium. Adhesion is followed by the bacterial invasion of urothelial epithelial cells where they can replicate to form compact aggregates of intracellular bacteria with biofilm-like properties.

Aim: Isolation and identification of Escherichia coli from urinary tract infection of children and antibiotic susceptibility test by disc diffusion method for Escherichia coli.

Materials & methods: 120 samples will collect from urine of urinary tract infection. These samples will identify on different culture media such as MacConkey agar their biochemical tests will examine. After identification, E. coli isolates will keep in LB broth supplemented with 20% glycerol at -20°C for further use.

Results: A total of 120 samples were taken from patients, with an average of 40(33.3%) samples for males and 80 (66.7%) for females. The results showed that 37(30.8%) of the samples showed positive E. coli growth. About antibiotic susceptibility test E. coli showed total resistance (100%) to Amoxicillin/clavulanic acid and Ampicillin. 50% resistance to Nalidixic. Otherwise, E. coli showed 40%, 20%, 60%, 20% and 50% resistance to Imipenem, Amikacin, Gentamicin, Azithromycin and Cefepime respectively.

Conclusions: The current study showed that Escherichia coli are one of the causative agents of urinary tract infection.

Key words: UTIs, E. coli, antibiotic susceptibility, Azithromycin, Amikacin.

Introduction

Bacteria commonly cause urinary tract infections when the bacteria enter the urinary tract [1]. In most cases, the host defense system removes the bacteria before they can cause any symptoms [2]. However, due to some underlying conditions, the body is not able to fight off the bacteria and an infection occurs. Urethritis occurs when the urethra is inflamed, if the bladder is inflamed, cystitis occurs and if the kidney tissue is inflamed, pyelonephritis occurs [3]. Urinary tract infections can be grouped as either uncomplicated (acute) or complicated. Uncomplicated UTIs will occur in people who are healthy with no anatomical or nervous urinary tract anomalies. Male urinary tract infections apart from infants and elderly infections are not considered normal and require urological investigations [2]. Men experience UTI infrequently due to the anatomical distance between the anus and urethral meatus and also because of a longer urethra as compared to women. A drier perimeatal environment in men and prostatic secretions also aid in some antibacterial activity [4]. Frequent agents identified to cause UTI include; Enterobactericeae coli, *Klebsiella Pneumoniae*, *Proteus Mirabilis*. *Enterococcus Faecalis* and *Staphylococcus Saprophyticus* [5]. The enteric bacterium E. coli is documented as the reason for more than 80% of uncomplicated community-acquired UTI (UPEC) [6]. These bacteria normally inhabit the lower intestines as normal flora but when they enter the urinary tract, they form the basis for a UTI [7-8]. Other pathogens commonly associated include; *Staphylococcus saprophyticus*, *Klebsiella* species, *Proteus mirabilis*, and *Enterococcus faecalis*. Complicated UTIs caused by bacteria such as Neisseria gonorrhea and Chlamydia trachomatis are associated with compromise in the urinary tract or host defense including; urinary blockade, urinary retention caused by nervous disorders, low immunity, kidney failure, kidney transplantation, and [5]. E. coli species have many virulence factors that allows the bacteria to cause infection and disease, the most important of these diseases being urinary tract infections (UTIs). The reason why some strains of the bacteria have the ability to overcome the immune system and cause disease is their encoding genes that are found on specific locations known as pathogenicity islands (PAIs) [9].

Materials & Methods

Specimen Collection

120 samples will collect from urine of urinary tract infection. These samples will identify on different culture media such as MacConkey agar and Cetrimide agar, and their biochemical tests will examine. After identification, *Escherichia coli* isolates will keep in LB broth supplemented with 20% glycerol at -20°C for further use.

Bacterial Identification

Bacteria were diagnosed based on the following aspects:

Morphological diagnosis and media characteristics

Colonies of *Escherichia coli* were diagnosed based on the culturing characteristics of the colonies growing on MacConkey agar and Blood agar. The developing colonies were isolated and purified by taking a single colony and replanting it by streaking method on MacConkey agar medium again to ensure the purity of the isolated colonies.

Microscopic Examination

Bacterial colonies were diagnosed based on the morphological characteristics of the germ cells under the microscope through the nature of their interaction with the gram stain, which shows the type of interaction and the shape and arrangement of the germ cell.

Cultural characteristics:

With the use of a 10X magnification, the morphological traits of isolated colonies were extensively examined.

Biochemical reaction and motility test:

1. Catalase production test
2. Oxidase test
3. Indole production test
4. Methyl red test
5. Voges-proskauer test
6. Citrate utilization test
7. Urease (Christensen's) production test

Antibiotic susceptibility test by disc diffusion method (Kirby Bauer test)

The NCCLS consensus effort consistently updates the performance of this procedure, making it one of the best standardized exams.

Results

Samples distribution

The 120 samples used in this study obtain from urine samples. According to the findings, optimal cultured media like blood agar, mannitol agar, and MacConkey agar produced positive results for bacterial growth in 37(69.2%) of the total samples, as shown in table (1).

Table (1): Distributed of study samples accordin to growth

Gender Results	Male (with average age: 20-45 years)		Female (with average age: 16-41 years)		Total
	Number	%	Number	%	
Negative growth -ve	28	18.1	68	81.9	83(69.2%)
Positive growth +ve	12	32.4	25	67.6	37(30.8%)
Total	40	33.3%	80	66.7%	120 (100%)

Identification

On blood agar, mannitol agar, chocolate agar, and MacConkey agar, the morphology, diameter, and shapes of bacterial isolates were determined. Additionally, the outcomes of the biochemical identification were confirmed by microscopic and biochemical examinations, which included the particular tests for each type. The outcomes of Vitek-2 and the biochemical tests were exact duplicates. The findings revealed two types of isolation: 9(19.3%) of mixed bacteria and 46(80.7%) of single bacteria.

Table (2): bacterial isolates groups in the current study

Microbial isolates	No.	%
Single bacterial isolates	46	80.7
Mixed bacterial isolates	9	19.3
Total	57	100

Identification of Gram-negative bacteria

Escherichia coli

When grown on the EMB media, *E. coli* appeared to have a green metallic sheen and formed small, dry pink colonies (fig. 2). When tested using the IMVC method, the results were negative for the Fox Perseure test, negative for the citrate test, and positive for indole and red methyl germ, which is a bacterium that fermented sugars and produced the acid and gas, but they were negative for the urease test. In order to ascertain the presence of *E. coli*, the presumed colony on McConkey agar was successively subcultured onto Eosin Methylene Blue (EMB) agar. Presumably chosen *E. coli* were the greenish-black colonies with a metallic sheen on EMB agar.

Table (3): biochemical tests of gram negative isolated

Biochemical tests	Catalase	Kliguler test	Motility	H_2S	Simmons citrate	V-P	M-R	Indol	EMB	Oxidase
E. coli	-	-	-	-	-	-	+	+	+	-

Figure (1): E. coli colonies on MacConky agar

Antibiotic Susceptibility Test

E. coli showed total resistance (100%) to Amoxicillin/clavulanic acid and Ampicillin. 50% resistance to Nalidixic. Otherwise, *E. coli* showed 40%, 20%, 60%, 20% and 50% resistance to Imipenem, Amikacin, Gentamicin, Azithromycin and Cefepime respectively as shown in table (4).

Table (4): Antibiotic susceptibility test of *E. Coli*

Antibiotics	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	%
AMP (10mg)	R	R	R	R	R	R	R	R	R	R	100
AMC (10/20mg)	R	R	R	R	R	R	R	R	R	R	100
CN (10mg)	R	R	R	S	R	R	S	S	R	S	60
CPM (30mg)	R	R	R	R	S	S	S	R	R	S	50
IMI (10mg)	S	R	S	S	R	R	R	S	S	S	40
AK (30mg)	S	S	R	S	S	S	S	S	S	R	20
AZM (15mg)	S	S	S	R	S	S	R	S	S	S	20
NA (30mg)	S	R	R	S	R	S	R	S	S	R	50
TMP (5mg)	S	R	S	S	S	S	R	R	S	S	30
CIP (5mg)	R	R	R	S	S	S	S	S	S	R	40

R=Resistant, S=Sensitive, AMP= Ampicillin, AMC= (Amoxicillin and Clavulanic acid (Augmentin)), CPM=Cefepime, IMI=Imipenem, CN=Gentamicin, AK=Amikacin, AZM=Azithromycin, NA=Nalidixic acid, TMP= Trimethoprim.

Discussion

Samples distribution

The 120 samples used in this study obtain from urine samples. According to the findings, optimal cultured media like blood agar, mannitol agar, and MacConkey agar produced positive results for bacterial growth in 37(69.2%) of the total samples. *S. aureus* and *E. coli*, two of the most prevalent bacteria that were isolated from semen, were the main organisms with the most detrimental effect on sperm quality [10-11], and *S. epidermidis* may be crucial in sperm impairment caused by infertility [12]. Therefore, before using an assisted reproductive technique, those pathogenic bacteria in the semen should be eliminated using antibiotics or other antimicrobial agents. By colonizing and contaminating the male urogenital tract, bacterial invasion of the male reproductive system harms spermatozoa and contributes to a decrease in sperm quality. This is the most divisive issue [13]. By causing motile sperm agglutination, restricting acrosome reactions, and changing cell shape, microorganisms can directly impair male reproductive function in males, or they can do so indirectly by producing reactive oxygen species in the inflammatory response to infection [14-15]. Our findings were similar with the study implemented by Isaiah et al. [16] found that bacteriospermia was connected with sperm quality, particularly sperm count. Also, gram positive bacteria have a poor influence on urine. The results of this study correspond with those of Al-Saadi and Abd [17], who found a decrease in the number of sperm with normal morphology in the Staphylococcus infected group. One the other hand, study done by Merino, et al., [18] record no decrease in number of normal sperm of infected group. This variation could be related to the diverse bacterial species identified, which have varied effects on sperm morphology.

Antibiotic Susceptibility Test

Penicillins are bactericidal substances that prevent the synthesis of bacterial cell walls. The isolated *E. coli* in this study had a 100 percent penicillin and ampicillin resistance rate. Other studies [19] also reported similar findings, particularly for the -lactamase producers. The prolonged use of penicillins may be to blame for the low susceptibility to these drugs. Additionally, earlier studies found that ampicillin has no longer any effect on urinary tract pathogens [20]. *E. coli* infections are typically treated with cephalosporins, particularly second and third generations. Only 16.2% of the participants in this study showed sensitivity to ceftriaxone, which was less than the 28.1%, 50%, and 24% reported in other studies [19, 21-22]. Additionally, resistance to cefotaxime and cephalexin was 94.1% in non-B-lactamase producers and 100% in those who were, which is consistent with the findings of another study by Jirachai, et al, [23]. The fact that cephalosporins are readily available from pharmacies in our area without a prescription and are relatively affordable antibiotics may help to explain the high resistance to these drugs. Additionally, these medications are occasionally administered insufficiently to treat various infections, which may cause a high level of resistance to develop. When it comes to aminoglycosides, gram-negative bacilli infections are typically treated with them. According to other researchers' findings [24-26], amikacin actually demonstrated a high sensitivity percentage (80%), whereas other studies [22, 27], revealed a lower sensitivity, which may be related to the widespread use of aminoglycosides. Additionally, the sensitivity to gentamicin was 61.7%, which is comparable to what other studies have reported [24, 28].

Conclusions

The results of the current study showed Escherichia coli are the main causative agent of urinary tract infections. Escherichia coli had a total resistance (100%) to Ampicillin, while Amikacin and Cefotaxime were the most effective antibiotics in the current study against bacteria.

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