PREVALENCE OF ANTIBIOTIC RESISTANCE AMONG *ESCHERICHIA COLI* ISOLATED FROM DIFFERENT CLINICAL SAMPLES IN ERBIL CITY /IRAQ

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ABSTRACT

Escherichia coli. is an opportunistic nosocomial pathogen causing a variety of infections including urinary tract infections, gastrointestinal tract infections and vaginal tract infections, Antibiotic resistance in *Escherichia coli* is of particular concern because it is the most common Gram-negative pathogen in humans,the wild dissemination of antimicrobial resistance among bacterial populations is an increasing problem worldwide. Our study aimed to determine the antibiotic sensitivity pattern of *E. coli* isolated from different types. A total of 226 samples were collected from (urine, HVS and stool), collected from female 167 samples and from male 59 samples from privet Erbil hospital, Rabarin hospital and Rezgary hospital. *Escherichia coli.* isolated and identified by using microscopical, morphological, biochemical tests and Api and Vitek 2 compact system. Antibiotic susceptibility testing was performed by using Vitek 2 compact system according to the standard protocol aginst 13 antibiotecs which are (Gentamycin, Ciprofloxacin, Imipenem, Cefepime, Ampicillin/sulbactam, Ertapenem, Trimethoprim/sulfamethoxazole, Tobramycin, Ceftriaxone, Cefazolin, ceftazidime, Levofloxacin, Piperacillin/tazobactum). :117 total positive results of *Escherichia coli.* isolates isolated from 226 different clinical specimens (urine, HVS and stool). The highest percentage of *Escherichia coli.* isolated from female urine sample 3(53.84%), HVS 33(28.20%) and stool 3 (2.56%)while from male urine (15.38%) , when performing of antibiotic susceptibility the rate highest resistance were to ceftazidime 95 (81.1%) followed by Cefazolin 89 (76.0%), Ceftriaxone and Cefepime 87 (74.3%), Trimethoprim/sulfamethoxazole 77 (65.8%), Ampicillin/sulbactam 70(59.8%), respectively, In contract the highest effective antibiotic against *Escherichia coli* were Imipenem 113(96.5%),followed by Ertapenem 111(94.8%), Piperacillin/tazobactum 95(81.1%), Tobramycin 85 (72.6%), and Gentamycin 81 (69.2%) respectivly.

Keyword: E.coli,antibiotic resistance.

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INTRODUCTION

Few microorganisms are as versatile as *Escherichia coli*. An important member of the normal intestinal microflora of humans . But *E. coli* is more than just a laboratory workhorse or harmless intestinal inhabitant; it can also be a highly versatile, and frequently deadly, pathogen. Several different *E. coli* strains cause diverse intestinal and extraintestinal diseases by means of virulence factors that affect a wide range of cellular processes [1] . The urinary tract is one of the most frequent sites of bacterial infection in humans. Uropathogenic *Escherichia coli* (UPEC) strains are the leading cause of urinary tract infections (UTIs) and are responsible for greater than 80% of uncomplicated cases in adults. Infection of the urinary tract occurs in an ascending manner, with colonization of the bladder leading to possible kidney infection

and bacteremia., *Escherichia coli* being isolated far more frequently than any other organism. In about 10% of patients with UTI, two organisms may be present and both may contribute to the disease process. The presence of three or more different organisms in a urine culture is strong presumptive evidence of improper collection or handling of the urine specimen. However, multiple organisms are often seen in UTI in patients with indwelling bladder catheters [2] . UTI accounted for nearly 7 million office visits and 1 million emergency department visits, resulting in 100,000 hospitalizations. Nevertheless, it is difficult to accurately assess the incidence of UTIs, because they are not reportable diseases, This situation is further complicated by the fact that accurate diagnosis depends on both the presence of symptoms and a positive urine culture, although in most outpatient settings this diagnosis is made without the benefit of culture. Women are significantly more likely to experience UTI than men. Nearly 1 in 3 women will have had at least 1 episode of UTI requiring antimicrobial therapy by the age of 24 years. UTIs have become the most common hospital-acquired infection, accounting for as many as 35% of nosocomial infections, and they are the second most common cause of bacteremia in hospitalized patients. *Escherichia coli* are reported as one of the most common organisms found in the genital tract of non-pregnant (9–28%) and pregnant women (24–31%). Vaginal *E. coli* (VEC) strains are considered to be a reservoir for vaginal and/or endocervical colonization in pregnant women, and an important step in the development of urinary tract, intra-amniotic and puerperal infections through 'fecal-vaginal-urinary/neonatal' transmission [3,4]. *E. coli* is considered to be normally involved in these infections despite their polymicrobial susceptibility[5] When bacteria become resistant to an antibiotic, that medicine becomes less effective. Medical treatment of patients infected with these drug-resistant organisms can become more complicated, leading to longer hospital stays, increased health care costs and in extreme cases, untreatable infections. Without effective antimicrobials for care and prevention of infections, the success of treatments such as organ transplantation, cancer chemotherapy and major surgery would be compromised. Generally speaking, the growth of global trade and travel allows resistant microorganisms to spread rapidly to distant countries and continents through humans and food. [6]

Materials and Methods

Sample collection

A total of (226) samples were collected from three different sources (urine, stool, high vaginal swab). After collection all bacterial isolates were subjected to a series of confirming tests. Results showed that only (117) isolates were indicated as *Escherichia coli.* Clinical samples were collected from patients attending Rizgary hospital , Rabrin hospital &Privet Erbil Hospital in Erbil city during the period July 2016 to march 2017.

Midstream Clean Catch Specimen

This is the preferred type of specimen for culture and sensitivity testing because of the reduced incidence of cellular and microbial contamination. Patients are required to first cleanse the urethral area with a castile soap

towelette. The patient should then void the first portion of the urine stream into the toilet. These first steps significantly reduce the opportunities for contaminants to enter into the urine stream. The urine midstream is then collected into a clean container (any excess urine should be voided into the toilet). This method of collection can be conducted at any time of day or night. Since urine itself is a good culture medium, all specimens should be processed by the laboratory within 2 hours of collection, or be kept refriger-30 ated at 4°C until delivery to the laboratory and processed no longer than 18 hours after collection. Whenever possible, urine specimens for culture should be collected in the morning. It is advisable to ask the patient the night before to refrain from urinating until the specimen is to be collected [7]

Stool specimen collection

Feces passed directly into a clean, wide mouth container with a plastic spoon or stick and tight, leak-proof cover. Feces may also be collected from a sterile bedpan; however, the specimen is unsatisfactory if there is any contamination with urine or water. The specimen contains at least 5 g of feces. (In the case of liquid faeces, approximately 15ml collected). If the specimen is collected off site, it should be transferred immediately to a Cairy Blair vial. If stool is not readily obtainable, a rectal swab may be submitted. The swab is passed beyond the anal sphincter, carefully rotated and withdraw Stool specimens for culture transported immediately at room temperature to the laboratory. Stool samples examined and cultured as soon as possible after collection.Storage Refrigerated (2-8 °C) [8].

High Vaginal Swab Specimen collection

Cervical or high vaginal swabs are preferred to lower vaginal swabs. Specimens should be collected using sterile swabs and placed into Amies transport medium (+/- charcoal). Specimens should ideally be stored and transported in sealed plastic bags. Laboratory processing should occur as soon as possible after specimen collection. Specimens should be refrigerated if delays in processing over two hours are unavoidable [7]

Identification of Bacteria

Microscopic identification

The most basic technique used for classifying bacteria is based on the bacterium's shape and cell arrangement. The most ordinary shapes of bacteria include rod, cocci (round), and spiral forms. Cellular arrangements occur singularly, in series, and in groups.[8]*E.coli* is gram negative which mean red or pink colored and rod-shaped (bacilli) under microscope.

Culture of Urine Samples

In order to obtain maximal yield, urine specimens where inoculated to several culture media after incubation overnight at 37°C. A positive urine culture is based on the growth of bacteria at a high number of colony forming units (CFUs).Urine culture results should be interpreted in conjunction with clinical symptoms of urinary tract infection (UTI). For clean-catch urine samples, a positive urine culture as indicated by the growth of bacteria greater than 100,000 CFUs/mL is suggestive of UTI liability of results is determined by the quality of the specimen and specimen collection, transport, and handling to the laboratory. The cultural characteristic of isolated bacterial colonies were identified. [9]

As following :

Vitek 2 compact system

The newly redesigned colorimetric Vitek 2 compact system, with updated advanced expert system (AES) (bioMerieux, Marcy l'Etoile, France) was evaluated for its accuracy and rapidity to identify clinical isolates and to detect several antimicrobial resistance[10] . Principles of the Vitek 2 is an automated microbiology system utilizing growth-based technology. This system accommodate the colorimetric reagent cards that are incubated and interpreted automatically. Overall, the Vitek 2 gave 95.8% of compatibility with the reference API strips (bioMerieux) in the identifications (ID)s of the Gram- positive cocci (GPC), Gram-negative rods (GNR), and yeasts. The accuracy was finally estimated to 98.3% through additional confirmatory tests. Also, > 90% of identifications of GPC and GNR were obtained within 7 hours of incubation. The most resistant isolates were identified within 12 hours of incubation. In conclusion, the new colorimetric Vitek 2 compact system with AES greatly improved is accuracy in species identification and detection of antimicrobial resistances, an it will be highly acceptable to clinical microbiology laboratory function[11].

Antimicrobial susceptibility test by Vitek 2 system

The system includes an AES that analyzes minimum inhibitory concentration (MIC) patterns and detects phenotypes for most organisms tested. This helps optimize laboratory efficiency for lean laboratory management. Rapid results allow clinicians to discontinue empiric therapy and prescribe targeted therapy, resulting in improved patient outcomes and enhanced antibiotic stewardship [12] . With its ability to provide accurate "fingerprint" recognition of bacterial resistance mechanisms and phenotypes, the AES is a critical component of Vitek 2 technology. The Vitek 2 card contains 64 microwells. Each well contains identification substrates or antimicrobial. Vitek 2 offers a comprehensive menu for the identification and antibiotic susceptibility testing of organisms. The Vitek 2 test card is sealed, which minimizes aerosols, spills, and personal contamination. Disposable waste is reduced by more than 80% over microtiter methods [13].

RESULTS

The incidence of *Escherichia coli* spp. in different clinical specimens

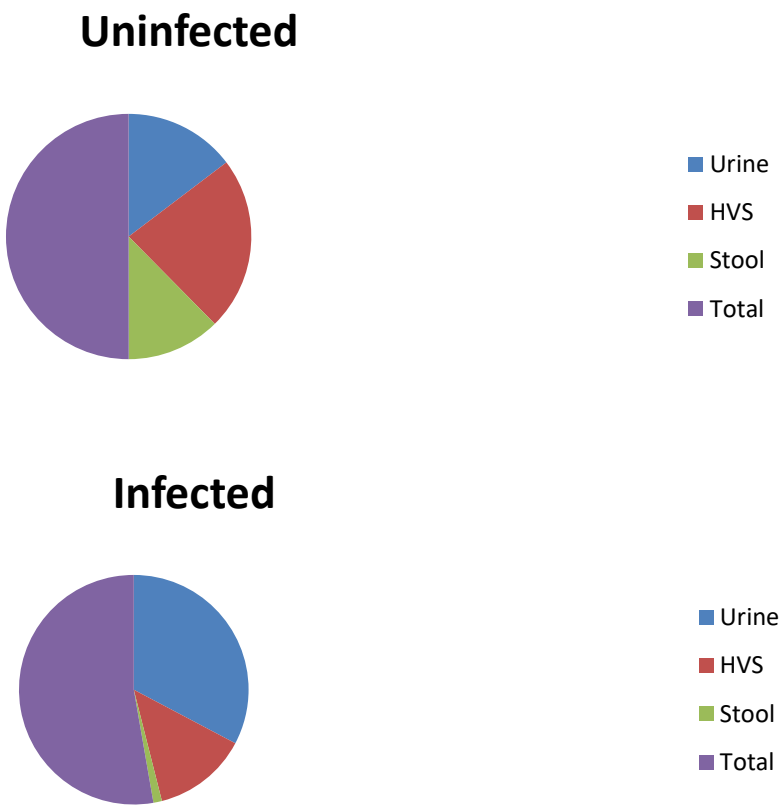
Out of 226 samples one hundred saventen *E.coli* isolates distribution according to their source of isolation as in table (1)and figure (1), our result showed that urine isolates are the most frequent encountered 81(35.84%), while for HVS 33(14.60%) and finally stool countered 3(1.32**%)**.

The morbidity and mortality of UTIs the uropathogenic *E. coli* (UPEC) are gathering more attention. UPEC have long been recognized as distinct clones of *E. coli*, which exhibit specific characteristics such as virulence associated traits, distinctive O antigens, genotypes and multidrug resistance. A total of (226) samples were collected from three different sources (urine, stool, Human vaginal swab). After collection all bacterial isolates were subjected to a series of confirming tests such as microscopic and macroscopic tests (Gram stain, biochemical test and vitek 2 system).

Table (1) The incidence of *Escherichia coli* spp. in different clinical specimens:

PATIENTS	No. and % of *E.coli* isolates			
	URINE	HVS	STOOL	TOTAL
	Number. and percentage			
INFECTED	81	33	3	117
	35.84%	14.60%	1.32%	51.76%
UNINFECTED	32	50	27	109
	14.15%	22.12%	11.94%	48.23%
TOTAL	113	83	30	226
	50%	36.72%	13.27%	100%

Figure (1): The incidence of *Escherichia coli* spp. in different clinical specimens.



Results showed that only (117) isolates were indicated as *Escherichia coli*. Total of 117(51.76%) *Escherichia coli* positive out of 226 from different clinical samples , urine sample appeared to be the most dominant specimen than other specimens the total of urine in our study 81(35,84%) is lower than those reported by Paul *et al*., [14] in Kerala who were found *E. coli* causing urinary tract infection was (60.64%). Our study is higher than Hammoudi [15] in Baghdad who were found (22.2%) *E. coli* causing urinary tract infection. The isolated percentage of high vaginal swab 33 (14.60%) in our study is higher than Khamees[16] in USA Who were collected 310 HVS and founded that 39 (13.8%) of *E.coli* isolated in female vagina, our results is lower than those reported by Ahmad and Ali[17] in Erbil city in 2014 who were found42 (57.5%)*E. coli* causing vaginaitis. The high rate of Urinary tract infection on our results are due to environments, various rate of diabetes, kidney stones, use of indwelling catheter for long duration, and heavy use of antibiotics which can destroy the natural normal flora of the urinary tract.

Distribution of *Escherichia coli* in relation with gender in different clinical specimens

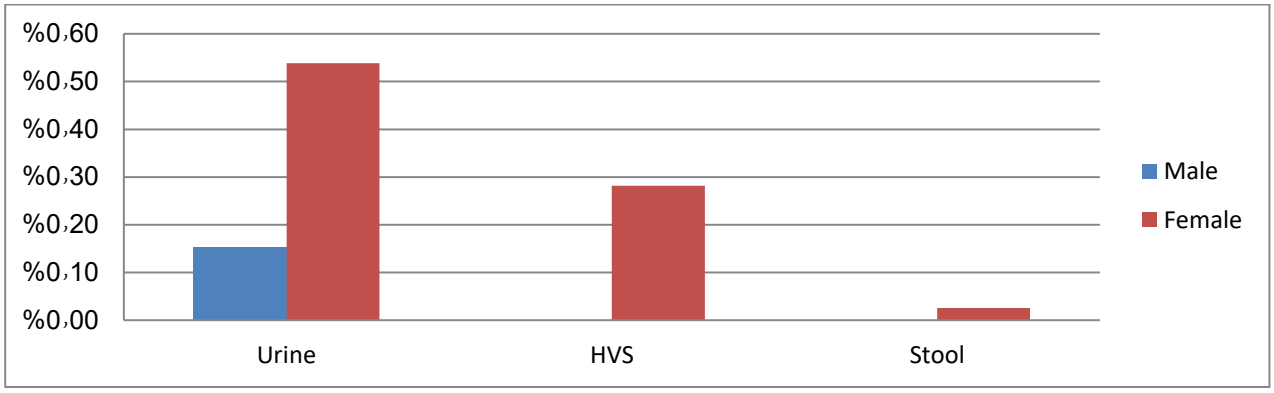
After the interpretation of the data we found that the prevalence of *E.coli* was analysed according to person's gender in table(2) and figure(2) among the 117 positive growth 18 (15.38%) were from males and 99 (84.61%) from females. In the present study the higher rate of *E.coli* was found in females compared to males on the other hand the highest rate of *E.coli* causing UTI was found in female urine 63(63.63%) compared to 18 (15.38%)in male urine, while we don't isolated any bacteria from male stool.

After the interpretation of the data we found that the prevalence of *E.coli* was analysed among the 117 (51.76%) positive growth among 81(69.23%) positive growth of *E.coli* isolated from urine and 18 (15.38%) were from male' s urine and 63 (53.84%) from female's urine. In the present study the higher rate of *E.coli* was found in females compared to males. Our results higher than that recorded by Ali *et al*., [18] in Erbil city who found that 26 (6.52%) of *E.coli* isolates are males. Also our study is lower than study done by Aftab *et l* [19] in US who found that (71.3%) of *E.coli* isolates are males. Our results of stool sample wear in females(3.03%) and males(0%),. Also our study is lower than study done by Shakya *et al* [20] in India who found that (42%) from females and (58%) from males fecal isolates.

Table (2) Distribution of *Escherichia coli* in relation with gender in different clinical specimens:

Patients	**No and % of *E.coli* isolates**			**Total**
	Urine	**HVS**	**Stool**	
	Number and %			
Male	**18**	**0**	**0**	**18**
	15.38%	**0**%	**0**%	**15.38**%
Female	**63**	**33**	**3**	**99**
	53.84%	28.20%	2.56%	**84.61**%
Total	**81**	**33**	**3**	**117**
	69.23%	**28.20**%	**2.56**%	**100**%

Figure (2): Distribution of *Escherichia coli* in relation with gender in different clinical specimens



The higher incidence of urinary tract infections in females is due to unique anatomical features of the female genitourinary tract, which include a shorter urethra and the more proximal location of the urethral meat us to the anus makes it easy for bacteria to ascend in the urinary tract.

The number and percentage of antibiotic resistance among *E.coli*

The antibiotics resistance patterns of one hundred seventeen *E.coli* isolates were screened for their resistance to thirteen widely used antibiotics. The antibiotic susceptibility patterns are shown in table (3). The most sensitive and the most resistents patterns of *E. coli* isolates. Most of isolates 81.1%

resistant to ceftazidime and only 76.0% were resistant to Cefazolin, 74.3% were resistant to Ceftriaxone.

Table (3) The number and percentage of antibiotic resistance among *E.coli*

Antibiotic	No .Resistance	% Resistance	No. Sensitive	% Sensitive
AMPA/S	70	59.8%	47	40.1%
TZP	22	18.8%	95	81.1%
CAZ	95	81.1%	22	18.8%
CFZ	89	76.0%	28	23.9%
CRO	87	74.3%	30	25.6%
CEP	87	74.3%	30	25.6%
ETP	6	5.1%	111	94.8%
IPM	4	3.4%	113	96.5%
GM	36	30.7%	81	69.2%
TOB	32	27.3%	85	72.6%
CIP	42	35.8%	75	64.1%
LVF	45	38.4%	72	61.5%
SXT	77	65.8%	40	34.1%

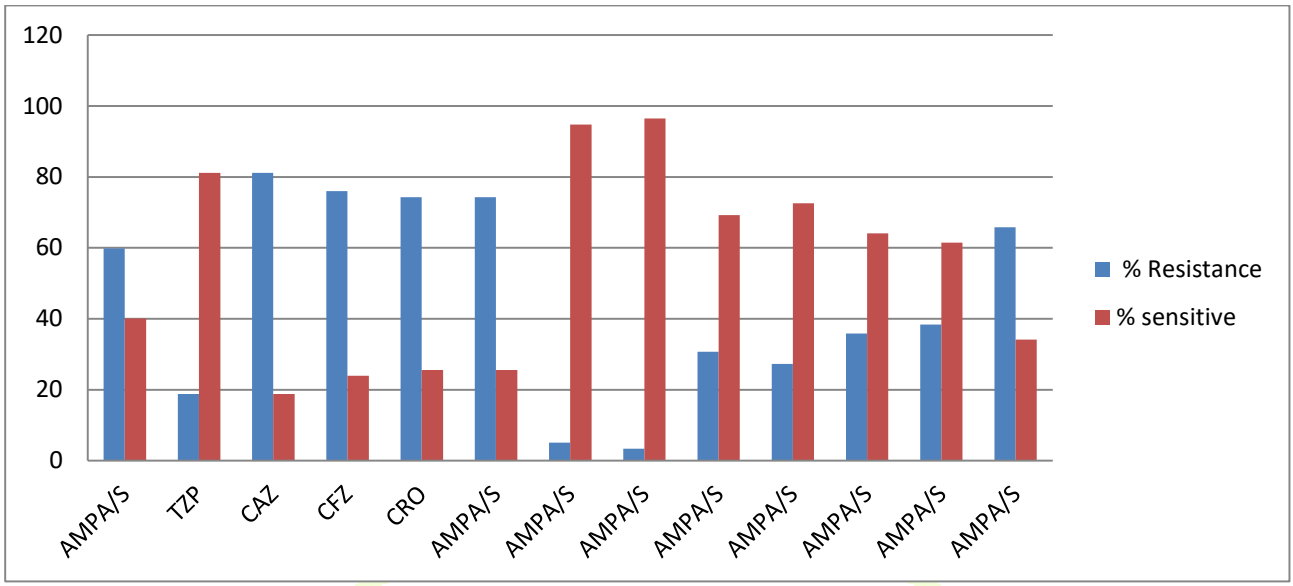


Figure (3): The number and percentage of antibiotic resistance among *E.coli*

96.5% of *E.coli* were sensitive to Imipenem followed by 94.8% were sensitive to Ertapenem and 81.1% of the strains were sensitive to Tazobactam, Our results higher than results were recorded by Otiliaet [21] pure results higher in Romania who found that 50% were sensitive to Imipenem. And lower than the results were recorded by Ezzaddin and Alkhateeb [22] in Erbil city they found that 95.2% sensitive to Imipenem. The second most frequent sensitive observed in this work was to Ertapenem, our results lower than results obtained by Regina *et al*., [23] in Brazil who reported that 100% of *E.coli* isolates were sensitive to Ertapenem. And also results obtained by [24] in Mosul City who reported that 96.8% of *E.coli* isolates were sensitive to Tazobactam. On the other hand, most of the strains 81.1% were resistant to Ceftazolin and only 76% were resistant to Ceftazidime, 74.3% of the strains were resistant to Ceftriaxone. Our results disagree with [23] in Brazil who founded that 8.1% resistant to Cefazolin and higher than results obtained by Al- Shabaki [24] who reported that 59.1% with *E.coli* isolates were resistant to Cefazolin in Mosul city and agree with the result recorded by [22] who found 91.7% resistant to Ceftazidime in Erbil city and disagree with result recorded by Shakya *et al*.,[25] is 14% in Ujjain city India. The percentage of antibiotic resistant in *E.coli* to Ceftriaxone is 74.3%, disagree with the results also were recorded by Shakya *et al*., [25] 13% in Ujjain city India. The high rate of resistance to Ceftazolin, Cetazidime, and Ceftriaxone may reflect the fact that these are the most commonly prescribed antibiotics in hospital and also the most easily available in the community without prescription, and so subject to abuse and misuse of antibiotic. This high resistant may be also due to the

spontaneous and there are no control on take the drugs, and about 50% of it given to outpatients without physicians prescription are from outside of hospital, as well as the occurrence of any infection in the organ of patient, they taking antibiotics without culturing and determination of antibiotic susceptibility for its side effect, the emergence of different types of antibiotic.The bacterial isolates revealed remarkable variation in their resistance to antibiotics used, but in general most isolates of *E.coli* were multidrug resistance to more than four antibiotics, E13, E50, E65 were resistance to all antibiotics as Shawn in table 4.

In present study, the majority of strains (16) isolates of *E. coli* shows resistant to 5 antibiotics, and only (15) strains of *E. coli* were resistant to 6 antibiotics, (14) strains of *E. coli* were resistant to 10 antibiotics out of 117(51,76%) strains of *E.coli*. Our result agree with Polse [26] in Zakho city from Iraq who founded that (32) of *E. coli* strains were resistant to 5 antibiotics, and only (30) of *E. coli* strains were resistant to 6 antibiotics, (28) of *E. coli* strains were resistant to 10 antibiotics out of 468 (50.0%).

On the other hand, (16) of *E. coli* strains were sensitive to 8 antibiotics, and only (15) of *E. coli* strains were sensitive to 7 antibiotics, (14) of *E. coli* strains were sensitive to 3 antibiotics out of 117 (51,76%) strains of *E.coli*. Our result disagree with Kibret and Abera , [27] in Romania who found that (159) of *E. coli* strains were sensitive to 8 antibiotics, and only (156) of *E. coli* strains were sensitive to 7 antibiotics , (154) of *E. coli* strains were sensitive to 3 antibiotics out of 160(80.5%). Although the emergence of antimicrobial resistance is invariably associated with antimicrobial use, the multiple mechanisms of resistance, the frequency of gene exchange in the natural environment, and the nonspecific nature of many resistance mechanisms make developing resistance-specific strategies to reduce individual resistance phenotype complicated and fraught with potential deleterious unintended consequences. Efforts to reduce overall antimicrobial exposure, for example, through organized efforts to identify appropriate minimal lengths of therapy, hold greater promise for reducing resistance. When a patient does not benefit from antimicrobial therapy chosen on the basis of clinical presentation, additional investigations are needed to determine the etiologic agent or exclude noninfectious diagnoses. To optimize an accurate microbiological diagnosis, clinicians should ensure that diagnostic specimens are properly obtained and promptly submitted to the microbiology laboratory, preferably before the institution of antimicrobial therapy Miyagi *et al.*,[28] .

Although the emergence of antimicrobial resistance is invariably associated with antimicrobial use, the multiple mechanisms of resistance, the frequency of gene exchange in the natural environment, and the nonspecific nature of many resistance mechanisms make developing resistance-specific strategies to reduce individual resistance phenotype complicated and fraught with potential deleterious unintended consequences. Efforts to reduce overall antimicrobial exposure, for example, through organized efforts to identify appropriate

minimal lengths of therapy, hold greater promise for reducing resistance.

No. of isolates	No. of resistant	% of resistant	No. of sensitive	% of sensitive
E1	10	76.92%	3	23.07%
E2	2	15.38%	11	84.61%
E3	4	30.76%	9	69.23%
E4	7	53.84%	6	46.15%
E5	10	76.92%	3	23.07%
E6	4	30.76%	9	69.23%
E7	1	7.69%	12	92.30%
E8	1	7.69%	12	92.30%
E9	8	61.53%	5	38.46%
E10	2	15.38%	11	84.61%
E11	0	0%	13	100%
E12	10	76.92%	3	23.07%
E13	4	30.76%	9	69.23%
E14	5	38.46%	8	61.53%
E15	1	7.69%	12	92.30%
E16	6	46.15%	7	53.84%
E17	1	7.69%	12	92.30%
E18	0	0%	13	100%
E19	9	69.23%	4	30.76%
E20	5	38.46%	8	61.53%
E21	7	53.84%	6	46.15%
E22	9	69.23%	4	30.76%
E23	9	69.23%	4	30.76%
E24	0	0%	13	100%
E25	4	30.76%	9	69.23%
E26	9	69.23%	4	30.76%

E27	5	38.46%	8	61.53%
E28	5	38.46%	8	61.53%
E29	9	69.23%	4	30.76%
E30	9	69.23%	4	30.76%
E31	9	69.23%	4	30.76%
E32	4	30.76%	9	69.23%
E33	10	92.30%	3	23.07%
E34	4	30.76%	9	69.23%
E35	6	46.15%	7	53.84%
E36	1	7.69%	12	92.30%
E37	6	46.15%	7	53.84%
E38	6	46.15%	7	53.84%
E39	5	38.46%	8	61.53%
E40	6	46.15%	7	53.84%
E41	4	30.76%	9	69.23%
E42	10	76.92%	3	23.07%
E43	4	30.76%	9	69.23%
E44	7	53.84%	6	46.15%
E45	5	38.46%	8	61.53%
E46	6	46.15%	7	53.84%
E47	6	46.15%	7	53.84%
E48	3	23.07%	10	76.92%
E49	5	38.46%	8	61.53%
E50	13	100%	0	0%
E51	1	7.69%	12	92.30%
E52	10	76.92%	3	23.07%
E53	4	30.76%	9	69.23%

E54	6	46.15%	7	53.84%
E55	4	30.76%	9	69.23%
E56	0	0%	13	100%
E57	11	84.61%	2	15.38%
E58	6	46.15%	7	53.84%
E59	5	38.46%	8	61.53%
E60	7	53.84%	6	46.15%
E61	2	15.38%	11	84.61%
E62	10	76.92%	3	23.07%
E63	1	7.69%	12	92.30%
E64	5	38.46%	8	61.53%
E65	13	100%	0	0%
E66	6	46.15%	7	53.84%
E67	5	38.46%	8	61.53%
E68	5	38.46%	8	61.53%
E69	5	38.46%	8	61.35%
E70	5	38.46%	8	61.35%
E71	5	38.46%	8	61.35%
E72	2	15.38%	10	76.92%
E73	7	53.84%	5	38.46%
E74	5	38.46%	8	61.53%
E75	6	46.15%	7	53.84%
E76	0	0%	13	100%
E77	10	76.92%	3	23.07%
E78	0	0%	13	100%

E79	8	61.35%	5	38.46%

When a patient does not benefit from antimicrobial therapy chosen on the basis of clinical presentation, additional investigations are needed to determine the etiologic agent or exclude noninfectious diagnoses. To optimize an accurate microbiological diagnosis, clinicians should ensure that diagnostic specimens are properly obtained and promptly submitted to the microbiology laboratory, preferably before the institution of antimicrobial therapy And most of E. coli isolates were similar to the finding showed that most of *E. coli* isolate were resistance to more than 4 antibiotics Kibret and Abera, [27] .

CONCLUSIONS

There is a need to emphasize the rational use of antimicrobials and strictly adhere to the concept of "reserve drugs" to minimize the misuse of available antimicrobials in medicine. In addition, regular antimicrobial susceptibility surveillance is essential.

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