Antimicrobial Susceptibility of *Escherichia Coli* isolated from patient with recurrent urinary tract infection in Kosti teaching Hospital -Kosti- Sudan

Shafie Abdulkadir Hassan[1, 2], Yousif Mousa Alobaid Ahmed[1, 3]

[1]Department of Medical Laboratory Sciences, Faculty of Medicine and Health Sciences, Jamhuriya University of Science and Technology, Mogadishu, Somalia
[2]Advance Medical Research, Jamhuriya Research Center, Jamhuriya University of Science and Technology, Mogadishu, Somalia
[3]Department of Microbiology, College of Medical Laboratory Sciences, University of El Imam El Mahdi, Kosti, Sudan
Correspondence: Shafie Abdulkadir Hassan, Department of medical laboratory sciences, Faculty of medicine and Health Sciences, Jamhuriya University of Science and Technology, Mogadishu, Somalia. Email: Shafie@just.edu.so, Tel: +252615206907

Abstract

Background: *Escherichia coli* are the greatest cause of primary urinary tract infections (UTI). Antimicrobial susceptibility testing provides information that allows physicians to select the most appropriate antimicrobial agents for treating a specific infection.

Objective: This study aimed to assess the current status of multidrug resistance among urinary *Escherichia coli* isolates in patients attending at Kosti teaching hospital.

Methodology and results: A total of 80 Preserved samples of *E. coli* were received which are collected from in and out-patients attending Kosti Teaching hospital in Sudan between September and January 2016. The samples were cultured on Cysteine lysine electrolytes deficiency (CLED) media and the bacterial isolates recovered were tested against Trimethoprim, Augmentin, Gentamycin, Tobramycin, Ceftazidime, Amikacin, Erythromycin, piperacillin, ciprofloxacin, Cefepime, Cefpodoxime. Using Kirby Bauer disc diffusion technique. (100%) and all *E. coli* isolates (100%) were found to be sensitive to Amikacin, Tobramycin and Gentamycin. In addition to 66.6% of isolates were susceptible to Ciprofloxacin. In addition, 50% of the isolates were sensitive to Piperacillin, Trimethoprim (33%), Augmentin, and Erythromycin (16%), however 16% showed the lowest percentage of susceptibility.

Conclusion and Recommendation

Considering the relatively high rates of UTI and drug resistance observed in this study, continued local, regional, and national surveillance is warranted. Antibiotics should only be issued when prescribed by physicians.

Keywords: *E. Coli*; antibiotic resistance. Urinary tract infection

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Introduction

In developing countries because of diarrhea 2.2 million people dies every year due to *E.coli* like coliform infection, every year 130–175 million patients suffer uncomplicated UTI worldwide and more than 80% them are due to *E. coli* (Malakar*,* 2014).

Escherichia coli have been documented as the most important pathogen associated with urinary tract infections in many countries (Samra *et al.,* 2001).

E.coli is common inhabitant of the human and animal gut but can also be found in water, soil and vegetation (Todar, 2014).

It is the leading pathogen causing urinary tract infection and is among the most common pathogens cause blood stream infections, wounds, otitis media and other complication in humans. *E.coli* is also the most common cause of food and water borne human diarrhea world-wide and in developing countries, causing many deaths in children under the age of five years (Todar, 2014).

E. coli can affect anybody who expose to its infections but some people easily get its infections depending upon some factors like age, weakened immune system, and eating habit. Generally children and older adults are more risky to get its infections, people having AIDS, cancer etc. are also easily able to get ill after ingestion of *E.coli* with food. Another important factor of its infection is type of food which includes undercooked hamburger, unpasteurized milk, apple juice or cider and soft cheeses made from raw milk (Malakar*,* 2014).

Clinical finding of *E.coli* are fever, water non bloody diarrhea sometimes with bloody diarrhea in condition with *EHEC* infection, abdominal cramping and symptoms of urinary tract infection (Levinson, 2014).

There were many studies performed by many investigators such as that by Kebira et al, 2009, who reported that the levels of resistance to antimicrobial agents among the 831 E.coli isolates analyzed are provided in Trimethoprim-sulfamethoxazole had the highest number of resistant isolates (64%) and Norfloxacin, Ciproxin®, Perfloxacin, Ceftriaxone shared the least number (5%). All *Escherichia coli* isolates (100%) were found to be susceptible to Ticarcillin, Peril/Tazo, Amikacin, Ofloxacin and Roceph. In addition, 96%of the isolates were susceptible to Ceftazidime/Fortum followed by 95% susceptible to Norfloxacin, Ciproxin and ceftriaxone. In addition, 94.6% of the isolates were sensitive to Perfloxacin, Cefuroxime (94%),Gentamycin (93%), Nalidixic acid (91%),and Cephalexin (87%).However, with Nitrofurantoin(77%) isolates were susceptible followed by Augmentin (75%); with Trimethoprim-Sulfamethoxazole, 36% showed the lowest percentage of susceptibility. Another study done by Sharma et al, NB. Hirulkar reported that cefotaxime was most sensitive antibiotic to *E coli,* more than 70% sensitivity towards piperacillin respectively.

E.coli are grown initially in blood agar plate and on differential media such as EMB agar or MacConkey agar which ferment lactose; feature help to distinguish *E.coli* from other lactose fermenting Gram negative rods, production of indole from tryptophan, dicarboxylic lysine use of acetate as it is only source of carbon and metal (Levinson, 2014).

Treatment of *E.coli* depends on site and on sensitivity pattern of the isolated organism. Diarrheal disease usually doesn't require treatment. However, the duration of the diarrhea can be shorted by antibiotics. Rehydration is essential (EL-mishad 2014).

Prevention strategies of this pathogen include; source protection, halogenous of water or boiling water for one minute is an important (Todar, 2014)

Method and Materials

This was a descriptive and cross sectional study isolated from patient with recurrent Urinary tract infection in Kosti teaching hospital.

Sample collection

Demographical data was collect from patients using a direct interviewing questionnaire covering information regarding name, age, gender and clinical symptoms.

Gram Stain and Microscopic Observation

Smears were prepared in clean sterile microscopic glass slide by spreading the loopful growth over a small area of the slide with a drop of water, and allowed to dry. The dry smears were fixed with the help of flame. Flooded the slide with crystal violet solution for one minute and then washed off with tape water. Secondly Iodine solution was added and then washed with tap water and drained. In the third step Slides were flooded with alcohol for 10 seconds and then washed with tap water. Drained the slide. Safranin was added in the fourth step for counterstain for 60 seconds. Drained the slide and after drying Microscopic observation was done under oil immersion where Gram Negative Bacilli were recovered.

Isolation and identification of *E. coli*

A loopful 0.01ml of urine sample was cultured on *Cystine Lactose Electrolyte-Deficiency* (*CLED)* agar and incubated for 24 h at 37˚C aerobically. Organisms were selected for inclusion in further study when they occurred as pure culture and in concentrations greater than 105 CFU/ml. Isolations and identifications were performed by use of biochemical tests using:

Indole Test

An inoculum from a pure culture was transferred aseptically to a sterile tube containing peptone broth and incubated at 37^0C for 24 hours. At the end of the incubation five drops of Kovac's reagents were added and presence of a red or red violet color in the surface alcohol layer of the broth indicated indole positive.

Kligler iron agar (KIA*)*

Using sterile straight wire an inoculum from pure culture was inoculated the test organism in slope medium. Presence of a yellow butt and slope indicated lactose fermentation.

Urease test

Using sterile straight wire an inoculum from pure culture was inoculated the test organism in urea medium and negative urease test registered.

Citrate utilization test

Using sterile straight wire an inoculum from pure culture was inoculated the test organism in Simmons's citrate medium (solid) , where the original green color of the medium turn to bright blue color (citrate positive)

After isolation of *E.coli,* the test organisms were sub cultured on peptone water containing 20% of glycerol and incubated aerobically at 37% overnight and preserved to the refrigerator.

Antimicrobial susceptibility testing

Kirby Bauer disc diffusion technique was used and 0.5 McFarland's 108/ml employed in inoculum suspensions preparation according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) (Murray *et al.,* 2003).

These discs include; Trimethoprim, Augmentin, Gentamycin, Tobramycin, Ceftazidime, Amikacin, Erythromycin, piperacillin, ciprofloxacin, Cefepime, Cefpodoxime, and were tested against the isolates.

The antibiotic sensitivity test was performed by disc diffusion technique using commercially available discs on Mueller Hinton agar plates.

Disc diffusion –modified Kirby- Bauer method

Three to colonies, of similar appearance of the organism to be tested were picked up by a sterile bacteriologic loop and emulsified in 1-2 ml of sterile saline or broth. The density of the bacterial suspension is read 620nm filter in calorimetry and dip a sterile swab in the suspension and the excess fluid were removed by pressing and rotating it against the side of the tube above the level of the suspension .Streak the swab evenly over the surface of the medium in three directions, rotating the plate to insure even distribution.

With the petri dish lid in place, allowed 3-5 minutes for the surface of the agar to dry and using sterile forceps, the appropriate antimicrobial discs were placed on the surface of the inoculated plate suitably spaced (25mm from disc to disc and 15mm from the rim). Plates are incubated at

37c for 18-24hr and then examined for the presence of zones of inhibition of bacterial growth around antibiotics discs using a ruler on the underside of the plate. According to the diameter of inhibition zone were determined if the organism is sensitive, moderately sensitive or resistant to the different antibiotics.

Results

For the Seven months of the study, a total of 80 Preserved samples of *E. coli* were received and sub cultured. *E. coli* was confirmed by growth characteristics in *CLED* media, Microscopic observation after gram stain and different biochemical tests.

Antibiotic susceptibility was done on *Muller Hinton agar media*, eleven numbers of antibiotics were used to check the results, and the antibiotics were. Trimethoprim, Augmentin, Gentamycin, Tobramycin, Ceftazidime, Amikacin, Erythromycin, piperacillin, ciprofloxacin, Cefepime, Cefpodoxime.

The levels of resistance to antimicrobial Agents among the 6 *E. coli* isolates analyzed as in (Table 3). Cefepime, Cefpodoxime, and Ceftazidime had the highest number of resistant isolates (100%) and all E. coli isolates (100%) were found to be sensitive to Amikacin, Tobramycin and Gentamycin. In addition to 66.6% of isolates were susceptible to Ciprofloxacin. In addition, 33.3% of the isolates were sensitive to Piperacillin and Trimethoprim. Augmentin and Erythromycin (16.6%), however 16.6% showed the lowest percentage of susceptibility.

Table (1) Respondents by Gender shows that the total of respondents was 6 persons, 2 of them was male that 33.3%, and 4 of them was female that 66.7%.

Gender	Frequency	Percent
Male	2	33.3
Female	4	66.7
Total	**6**	**100**

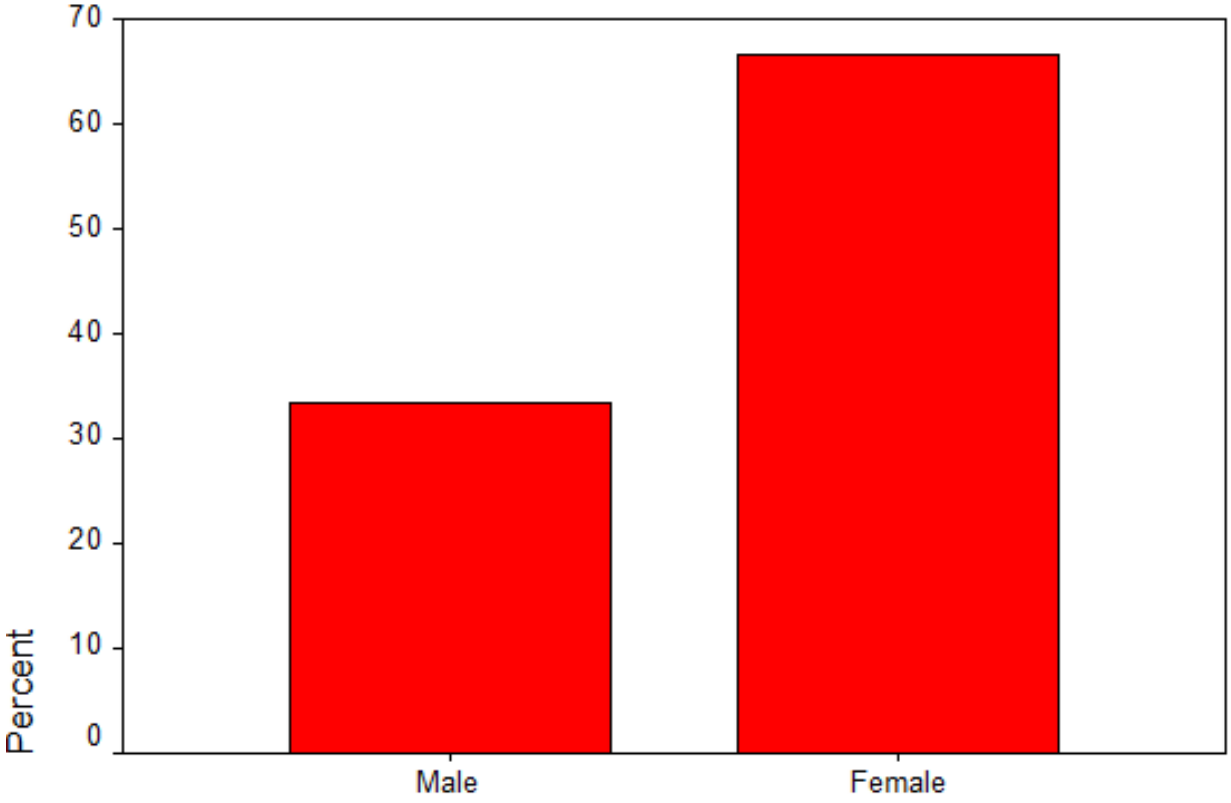

Figure (1) Respondents by Gender shows that the total of respondents was 6 persons, 2 of them was male that 33.3%, and 4 of them was female that 66.7%.

Table (2) shows Susceptibility of cefipime that the total of 6 Samples of E. Coli were resistance 100%

		Frequency	Percent
Valid	Resistant	6	100.0
	Sensitive	0	0
	Total	6	100.0

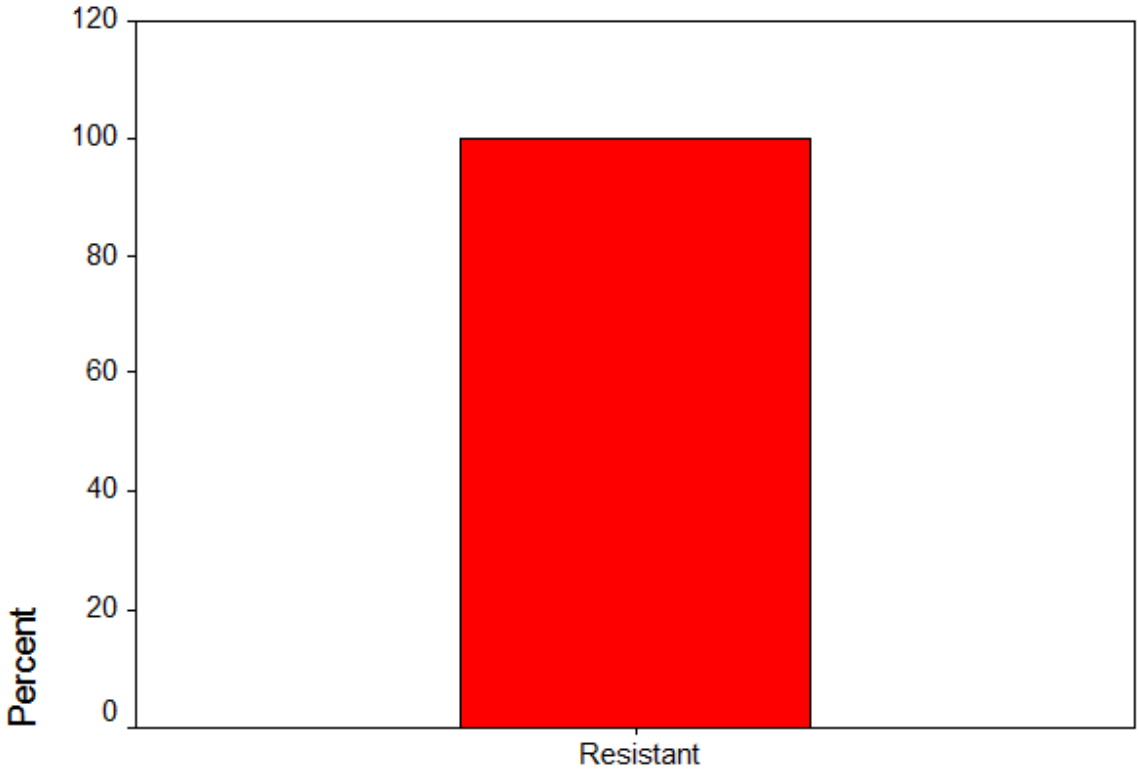

Figure (2) shows Susceptibility of cefipime that the total of 6 Samples of E. Coli were resistance 100%.

Table (3) shows Susceptibility of Cefpodoxime that the total of 6 Samples of E. Coli were resistance 100%

		Frequency	Percent
Valid	Resistant	6	100.0
	Sensitive	0	0
	Total	6	100.0

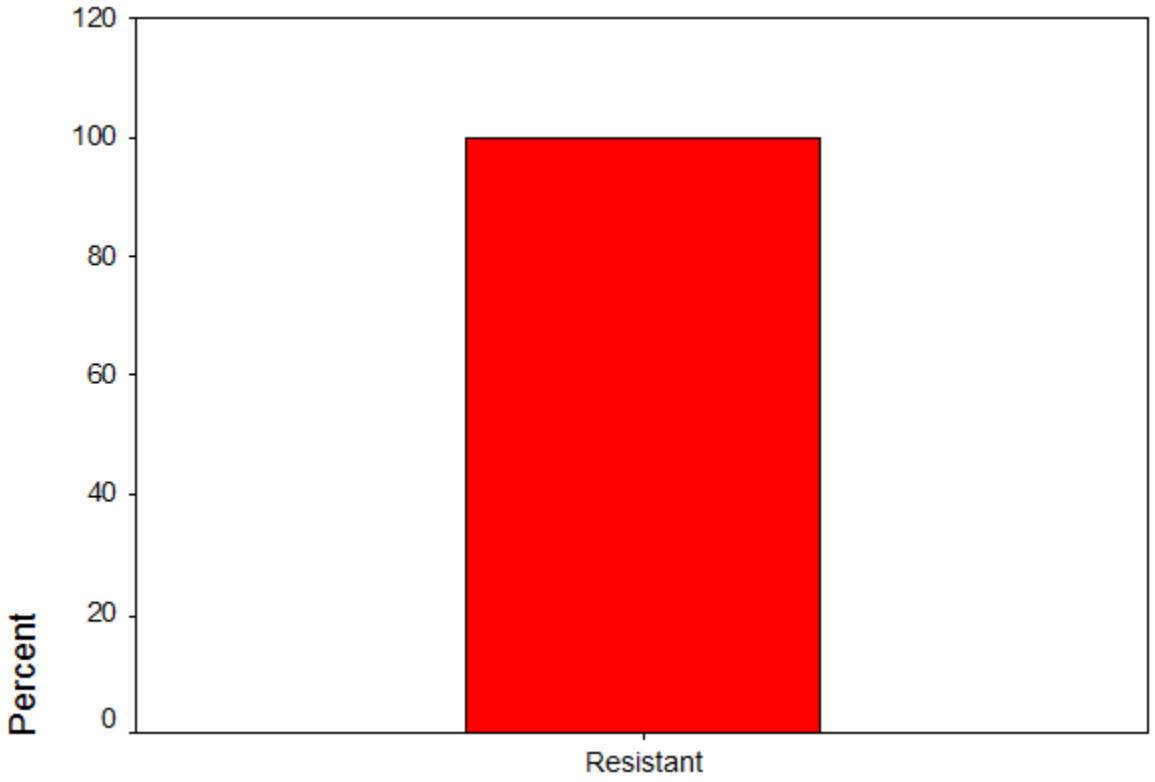

Figure (3) shows Susceptibility of Cefpodoxime that the total of 60 Samples of E. Coli were resistance 100%

Table (4) shows Susceptibility of Cefotaxime that the total of 6 Samples of E. Coli were resistance 100%

		Frequency	Percent
Valid	Resistant	6	100.0
	Sensitive	0	0
	Total	6	100.0

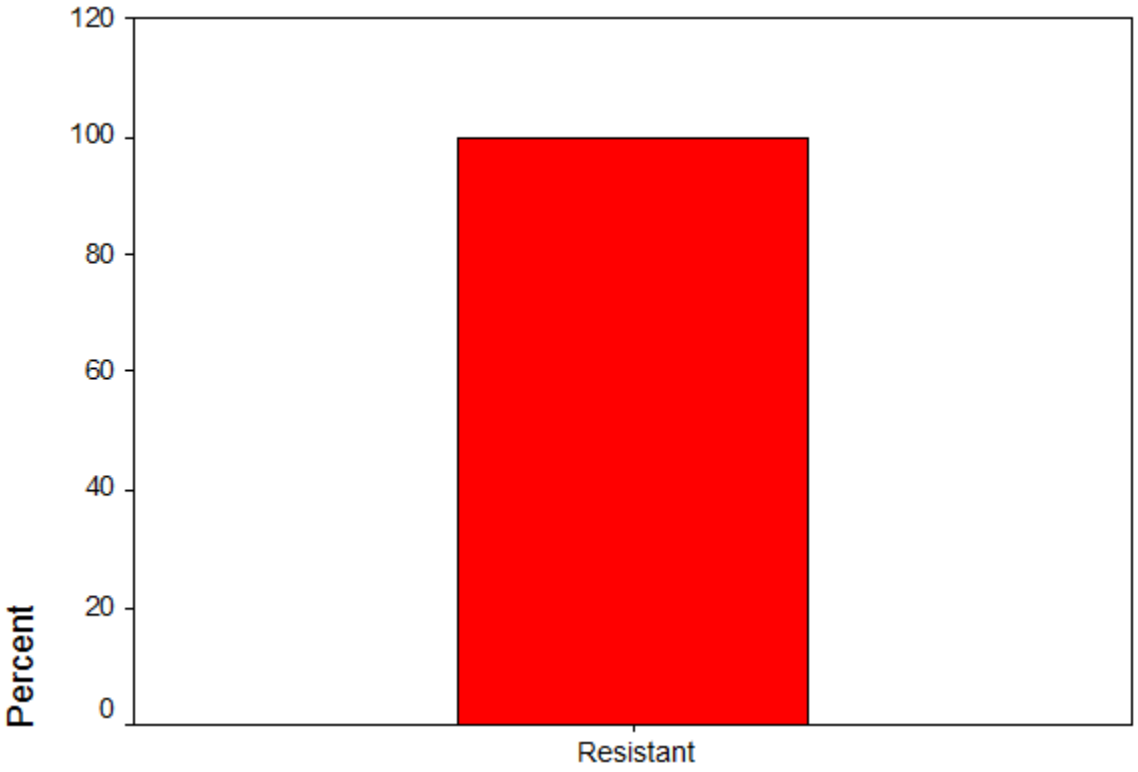

Figure (4) shows Susceptibility of Cefotaxime that the total of 6 Samples of E. Coli were resistance 100%

Table (5) shows Susceptibility of Trimethoprim that the total of 6 Samples of E. Coli 66.7 (4) were resistance and 33.3 (2) were sensitive.

		Frequency	Percent
Valid	Resistant	4	66.7
	Sensitive	2	33.3
	Total	6	100.0

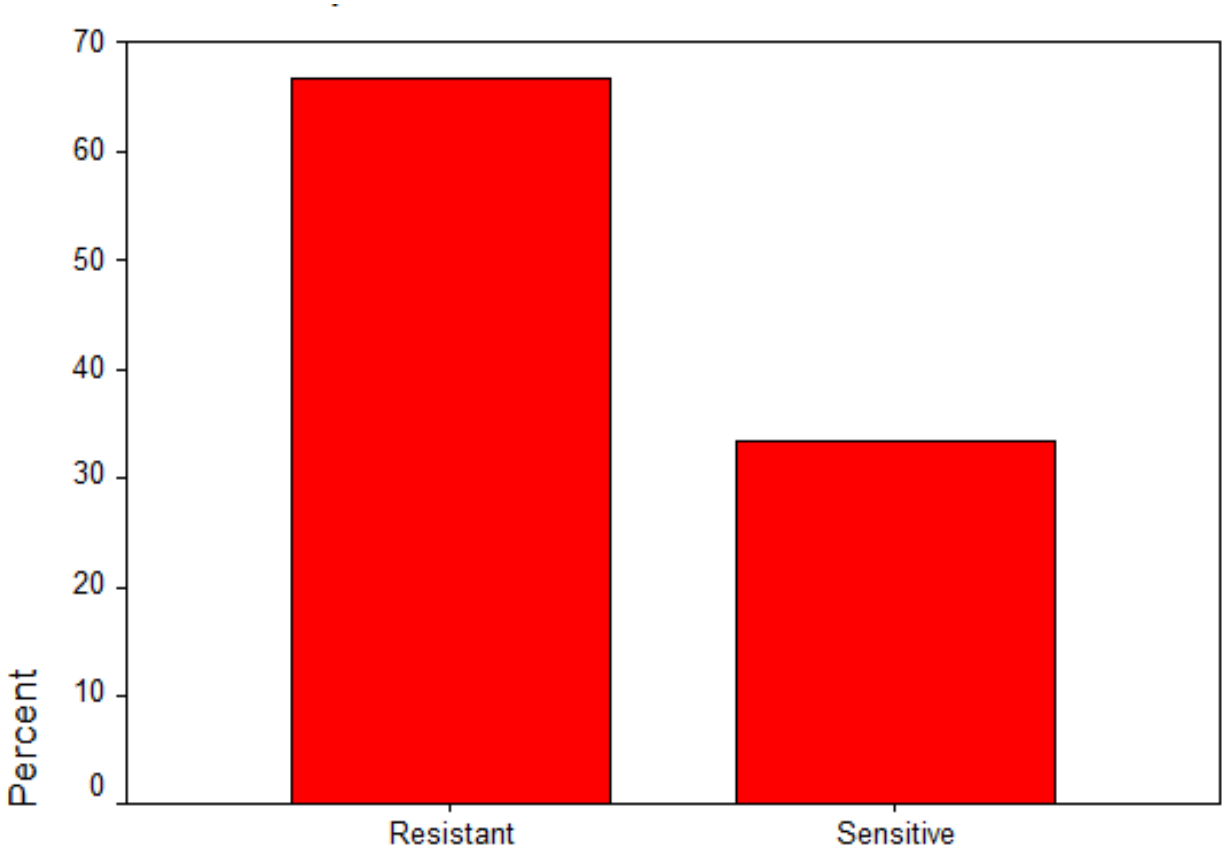

Figure (5) shows Susceptibility of Trimethoprim that the total of 6 Samples of E. Coli 66.7 (4) were resistance and 33.3 (2) were sensitive.

Table (6) shows Susceptibility of Amikacin that the total of 6 Samples of E. Coli 16.7 (1) were resistance and 83.3 (5) were sensitive.

		Frequency	Percent
Valid	Resistant	1	16.7
	Sensitive	5	83.3
	Total	6	100.0

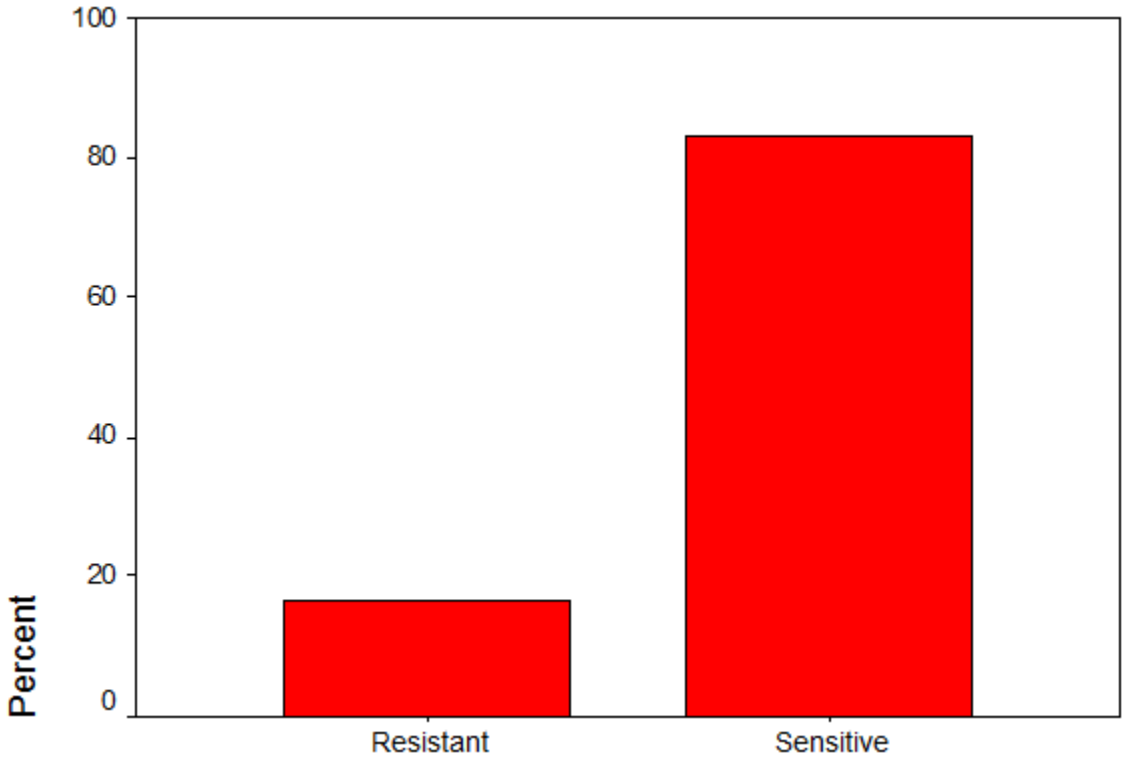

Figure (6) shows Susceptibility of Amikacin that the total of 6 Samples of E. Coli 16.7 (1) were resistance and 83.3 (5) were sensitive.

Table (7) shows Susceptibility of Piperacillin that the total of 6 Samples of E. Coli 83.3 (5) were resistance and 16.7 (1) were sensitive.

		Frequency	Percent
Valid	Resistant	5	83.3
	Sensitive	1	16.7
	Total	6	100.0

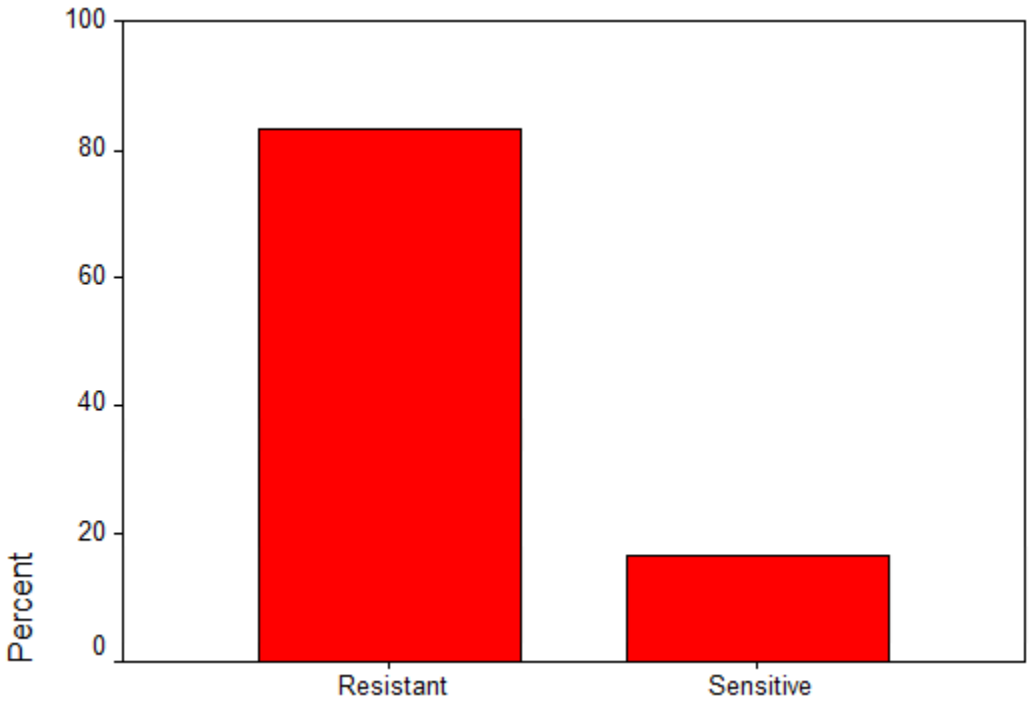

Figure (7) shows Susceptibility of Piperacillin that the total of 6 Samples of E. Coli 83.3 (5) were 0resistance and 16.7 (1) were sensitive.

Table (8) shows Susceptibility of Gentamycin that the total of 6 Samples (100) were sensitive

		Frequency	Percent
Valid	Sensitive	6	100.0
	Resistant	0	0
	Total	6	100.0

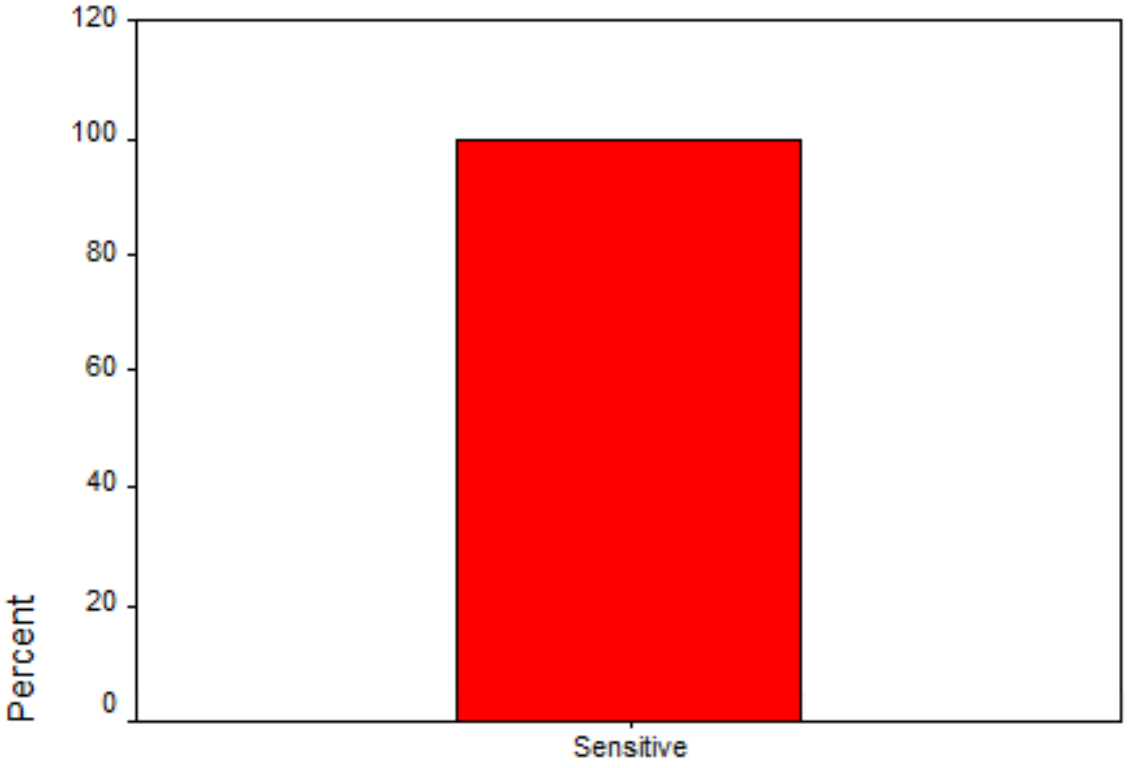

Figure (8) shows Susceptibility of Gentamycin that the total of 6 Samples (100) were sensitive.

Table (9) shows Susceptibility of Ciprofloxacin that the total of 6 Samples of E. Coli 83.3 (5) were resistance and 16.7 (1) were sensitive.

		Frequency	Percent
Valid	Resistant	2	33.3
	Sensitive	4	66.7
	Total	6	100.0

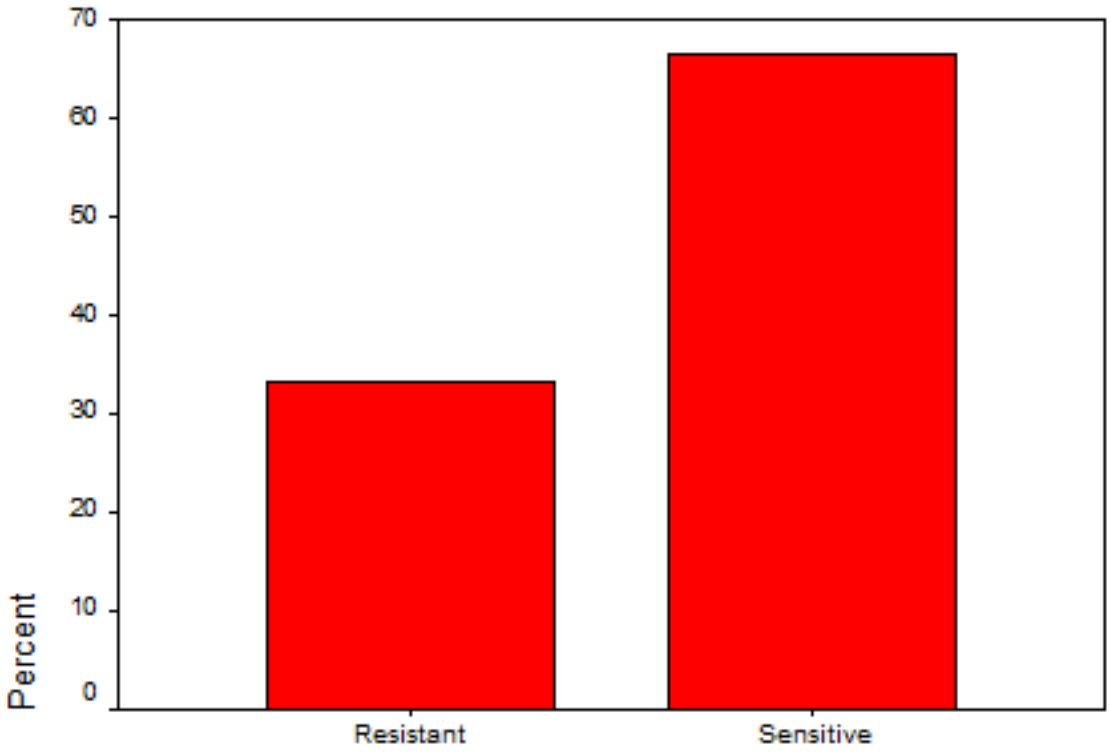

Figure (9) shows Susceptibility of Ciprofloxacin that the total of 6 Samples of E. Coli 33.3 (2) were resistance and 66.7 (4) were sensitive.

Table (10) shows Susceptibility of Tobramycin that the total of 6 Samples of E. Coli (100%) were sensitive.

		Frequency	Percent
Valid	Sensitive	6	100.0
	Resistant	0	0
	Total	6	0

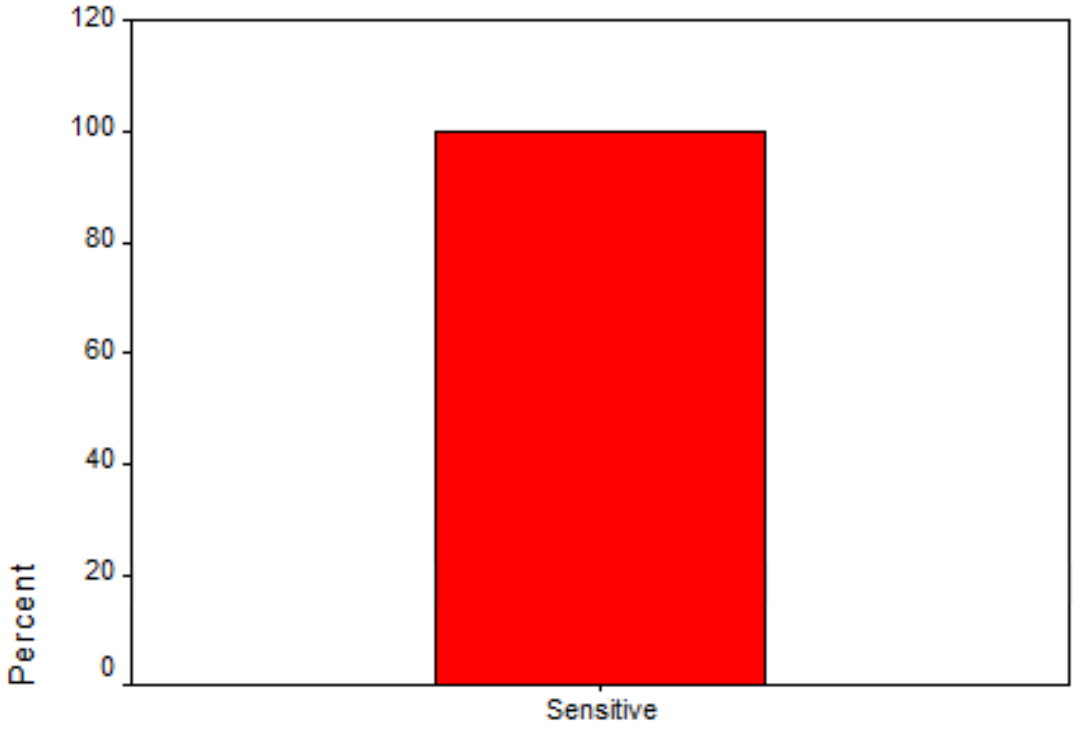

Figure (10) shows Susceptibility of Tobramycin that the total of 6 Samples of E. Coli (100%) were sensitive.

Discussion

Antimicrobial resistance in E Coli has increased worldwide and its susceptibility patterns show substantial geographic variation as well as difference in population and environment. The isolate number of *E coli* in the present study is 6 Samples and it was commonly isolated from the preserved *E. coli* samples (80).

In this study the overall resistance of *E.coli* to antimicrobials was high.

The study done by S. Sharma et al showed that cefotaxime was most sensitive antibiotic to *E coli* but our study showed that cefotaxime is one of the most resistant antimicrobials of *E coli*, others are Cefepime and Cefpodoxime.

The drug resistant pattern based geographical area may varies due to different factors like over dose of specified drug, environmental factors etc. The study of S. Sharma was done at Mangalore-India where as our study done at Kosti White Nile state- Sudan.

Study done by Kibera et al in Kenya showed that Amikacin, Gentamycin and Tobramycin was most sensitive antibiotics in *E coli* isolated from urine samples (96%), This study was agreement to our study which are 100% sensitive to gentamycin and Tobramycin and 83.3% sensitive to amikacin. Another study of NB Hirulkar from Indore found more than 70% sensitivity towards piperacillin, in our study it is 33.3% sensitive.

Study done in Slovenia showed high level of resistant in E.coli 80% to erythromycin also our study showed 84% resistant erythromycin among E coli isolates.

Conclusion

The resistance displayed by the bacteria, E coli are indicated indiscriminate use of these antibiotics, it may due to patients self-treating behavior from pharmacy without any knowledge of dose sensitive resistant knowledge which warrants the initiation of steps to prevent public health hazard. Time to time the sensitive resistant nature of bacteria may change due to over use of drugs for which time to time study is necessary.

Recommendations

The conclusion of our study result from that *E.coli* is resistant most of tested antibiotics so we recommended the hospitals

- To do AST before prescribing antibiotics
- To train the staff for culture and sensitivity testing.
- To establish Antimicrobial stewardship in hospitals.

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