Effect of Some Antibiotics on Uropathogenic *Escherichia coli* and Detection of Some Virulence Factors

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Abstract

In this study, three hundred urine samples were obtained from patients from both sexes suffering from urinary tract infections (UTIs), who have attending to Hilla Teaching Hospital, one hundred thirty four (134) male (44.6%) and one hundred sixty six (166) female (55.3%) with no age range. Out of the) 300)urine samples, only (47) (15.7%) isolates were found to be related to *Escherichia coli*. Hemolysin production by *E. coli* was studied and it was found that (19) (40.4%) out of (47) isolate of *E. coli* were able to produce hemolysin on blood agar as zone of hemolysis surrounding bacterial colonies, and other isolates of *E. coli* (28) (59.6%) have able to produce siderophore on M_9 media. Also, the antibiotic sensitivity test was studied on (47) isolates of *E. coli* and the results show that these isolates were resistance to Clindamycin (97.8%), (63.8%) for Cefotaxime and Cefaxime, Norfloxacine (59.5%), Naldixic acid (51.0%), Gentamycin (45.4%) and Amoxillin (44.6%, and all isolates were sensitive to Amikacin and Nitrofuranation (100%), (85%) for Chloramphenicol, (59.5%) for Streptomycin and Amoxillin (57.4%)

الخلاصة

تم في هذه الدراسه عزل ٤٧ عزله من بكتريا القولون من ٣٠٠ عينه من مرضى من كلا الجنسين يعانون من التهاب المجاري البوليه. وجد ان من ٤٧ عزله *E. coli* فقط (١٩) (٤٠,٤%) لها القابليه على انتاج الهيمولاسين على وسط اكار الدم اما باقي العزلات (٢٨) (٥٩,٦%) هي تمتلك نظام السايدروفورات.

ايضا درست فعاليه المضادات الحياتية على جميع عزلات *E. coli* فوجدت انها مقاومه للكلندماسين بنسبة (٩٧,٨%)، سيفاتاكزيم والسيفاكزيم بنسبه (٦٣,٨%)، نورفلوكساسين بنسبه (٥٩,٥%)، (٥١%) كانت مقاومه للنالدكسك اسد، جنتاماسين بنسبة (٤٥,٤%) والاموكسلين بنسبة (٤٤,٦%).

كما وجد ان جميع العزلات كانت حساسة للاميكاسين والنايتروفيورانيشن بنسبة (١٠٠%) ، (٨٥%) كلومفينيكول ، (٥٩,٥%) ستربتوماسين والاموكسلين كانت نسبة الحساسيه (٥٧,٤%).

Introduction

Escherichia coli is one of the most important enterobacteriaceae species; it is a gram negative rod, usually motile [1, 2].

E. coli is very important opportunistic pathogen associated with urinary tract infection [3].

Uropathogenic *E. coli* is bacterial infection of urinary tract in most cases; Uropathogenic *E. coli* (UPEC) cause 90% of UTIs in anatomically-normal unobstructed urinary tract. The bacteria colonize the feces or perianal region and ascend the urinary tract through the bladder with the aid of specific

adhesions. They are able to colonize the bladder [4].

E. coli produces much type of virulence factors such as production of hemolysin and siderophore [5].

Hemolysin is extracellular toxic proteins, which are produced by many gram-negative bacteria (e.g. *E. coli, Serratia spp., Proteus spp., Vibrio spp., Pasturella spp.* and *Pseudomonas aeruginosa*), and gram positive bacteria (e.g. *Streptococcus spp., Staphylococcus aureus, listeria spp., Bacillus cereus* and *Clostridium tetani*), all of which press a certain pathogenic potential [6].

UPEC strains usually produce siderophore that probably play an essential role in iron acquisition for bacteria during or after colonization [7].

Iron is essential for bacterial growth; the ability to acquire iron from the host is a prerequisite for establishment and maintenance of infections [8, 9].

Resistance to antibiotics is considered as virulence factor of pathogenic microorganisms to cause infection [10].

Multiple antibiotics resistance to useful class of antibiotics including the beta lactam, aminoglycoside and quinolones has generally emerged and this has been increasingly observed among a number of gram negative pathogen such as enterobacteriaceae bacteria [11].

Materials and Methods

In this study, 300 urine samples were collected from patient from both sex suffering from urinary tract infection were admitted to Hilla Teaching Hospital.

Hemolysin production assay:

Hemolysin production was carried out by inoculating a blood agar medium with bacterial isolates at 37^{o}C for 24 hours. The appearance of a clear zone around the colonies referred to a complete hemolysis (ß- hemolysis). The appearance of greenish zone around the colonies referred to a partial hemolysis (α-hemolysis), whereas no change of zone referred to non-hemolysis (γ-hemolysis)[12].

Siderophore production assay:

M_9 media was prepared, and then supplemented with 2% agar. After sterilization in autoclave at 121^{o}C for 15 min. and cooling to 50^{o}C, 0.25% gm/l glucose (sterilize by filtration) and 200µm of dipyridyl were added to it, then organisms were inoculated into this media and incubated for 24 hr. at 37^{o}C. The results were seen if the growth of microorganism was present or not [13].

Antibiotic sensitivity assay by disk diffusion test:

It was performed by using a pure culture of previously identified bacterial isolate. The most effective antibiotic for each bacterial isolate was determined as recommended by [14].

1. The inoculums to be used in this test were prepared by adding 5 isolated colonies grown on blood agar plate to 5 ml of nutrient broth and incubated at 37^{o}C for 18 hours and compared with (0.5) McFarland standard tube.
2. A sterile swab was used to obtain an inoculums from the bacterial suspension, this inoculums was streaked on a Mueller-Hinton agar plate and left to dry.
3. The antibiotic discs were placed on the surface of the medium at evenly spaced intervals with flamed forceps or a disc applicator and incubated for 24 hours at 37^{o}C.
4. Inhibition zones were measured using a ruler and compared with the zones of inhibition determined by the National Committee for Clinical Laboratory Standards.

Table 1 Antibiotic disk

Antibiotic disk	*Symbol*	*Potency (mg)*	*Antibiotic disk*	*Symbol*	*Potency (mg)*
Amoxillin	AMC	30	Naldixic acid	NA	30
Cefotaxime	CTX	30	Gentamycin	CN	10
Cefaxime	CFM	5	Norfloxacine	NOR	10
Clindamycin	DA	2	Chloramphenicol	C	30
Streptomycin	S	10	Nitrofuranation	F	300
Amikacin	Ak	30	-	-	-

Results and Discussion

In this study, (300) urine samples were collected from patient of both sex that suffering from urinary tract infection were admitted to Hilla Teaching Hospital. These patients were distributed as one hundred thirty four (134) male (44.6%) and one hundred sixty six (166) female (55.3%) with no age range. These samples were subjected for culturing on selective media (EMB) to isolate *E. coli*.

From (300) urine samples, only 270 samples showed positive culture constituted (90%) whereas no growth was seen in the other (30) samples constituted (10%).

Among (270) isolates, only forty seven (47) (15.7%) isolate were found to be related to *E. coli* .These bacteria was isolated from (15) sample (32%) male and (32) sample (68%) female. The results were show in Table 2.

Table 2 bacterial *E. coli* isolate from patient suffering from UTIs

No. of urine sample	***Positive culture***	***M.O isolate***	***No. of Bacteria***	***Sex***
300 urine sample	270 (90%) isolate	*E. coli*	47 (15.7%)	Male 15 (32%) Female 32 (68%)

These results agreement with the results obtained by [15], show that a total of 300 urine samples with significant bacteriuria collect were analyses for present of enterobacteriaceae (187) urine samples comprising (68.9%) female and (31.9%) male included *E. coli* (15.5%), while the other are another microorganism of enterobacteriaceae.

Also reported that (220) urine samples in which *E. coli* was isolate (191) (87%) had significant bacteriuria, a total of (18) (9.4%) *E. coli* isolate were lactose fermentation colonies [16].

Who have pointed that *E. coli* is the determinant facultative bacterium in normal intestine flora[17]. It's however, responsible for majority of serious extra intestinal infections.

Urinary tract is among the most common site of bacterial infection both in commonly based and hospital patients [17].

Hemolysin and siderophore production:

Bacteria evolve a number of mechanisms for the question of iron from their environment, one of them is production of hemolysin which acts to release iron complexes to intracellular heme and hemoglobin. Iron can increase

disease risk by functioning as a readily a variable essential nutrient for invading microbial and neoplastic cells. To survive and replicate in hosts, microbial pathogens must acquire host iron [18].

Hemolysin production by *E. coli* was studied and it was found that (19) (40.4%) out of 47 isolate of *E. coli* were able to produce hemolysin on blood agar as zone of hemolysis surrounding bacterial colonies, and other isolates of *E. coli* (28) (59.6%) have able to produce siderophore on M_9 media. These results were showed in Table 3.

Table 3 show the hemolysin and siderophore production

No. of E. coli isolate	*Hemolysin production*	*Siderophore production*
47 (15.7%)	19 (40.4%)	28 (59.6%)

These results resemble with the results of [19]. How found that many strains of *E. coli* elaborate hemolysin responsible for zone of hemolysis surrounding bacterial colonies on blood agar. The present results suggest that hemolysin is produced early in the growth cycle, and production of hemolysin is mostly associated with pathogenic bacteria therefore considered important virulence factor [20].

The hemolysin is not essential during early infection but this factor is important at late stages of infection [21].

The hemolytic assay performed as an indicator of the pore forming ability of the toxins by measuring blood cells and lyses them by oligimerizing and forming pores [22].

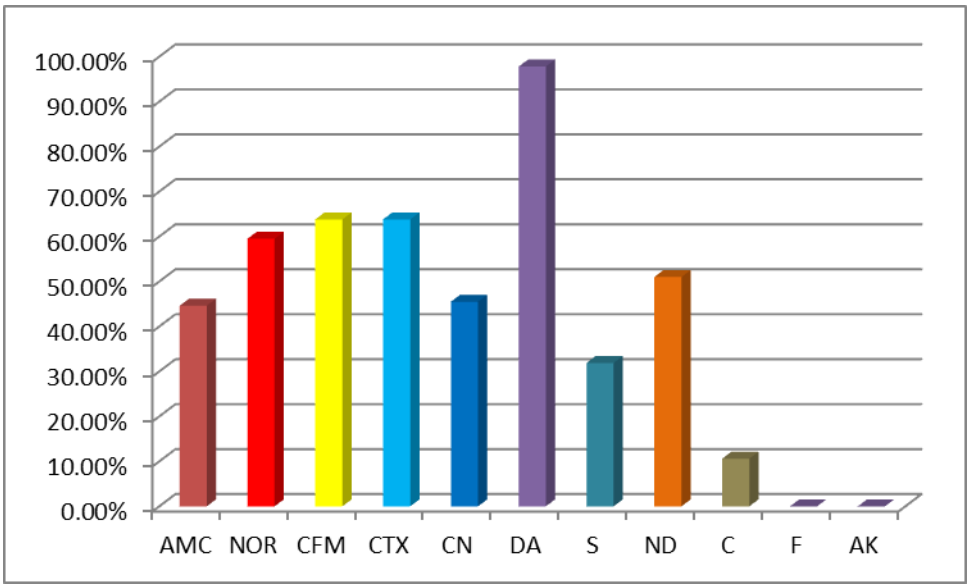
The siderophore production as virulence factor of *E. coli* isolate from patients suffering from urinary tract infections. This result suggests that the siderophore production positive strains can be considered as UPEC. Thus, although a great deal has been learned regarding *E. coli* virulence mechanism in UTI. Much remains to be learned and the practical application of our growing understanding of *E. coli* virulence factors to the prevention and treatment of UTI has to be continued [23].

In *E. coli*, the hydroxymate siderophore (aerobactin) is the most effective of the several iron chelation systems employed by the bacteria for iron acquisition. The siderophore (aerobactin) is commonly found in isolates from patients with UTI [24].

IreA and IroN are the recently identified siderophore receptors. Molecular epidemiologic evidence from several studies has demonstrated an increased prevalence of IroN among UTI isolates relative to fecal isolates. This evidence suggests that IroN functions as a siderophore receptor and is an urovirulence factor for UTI [24, 25].

Antibiotic sensitivity test:

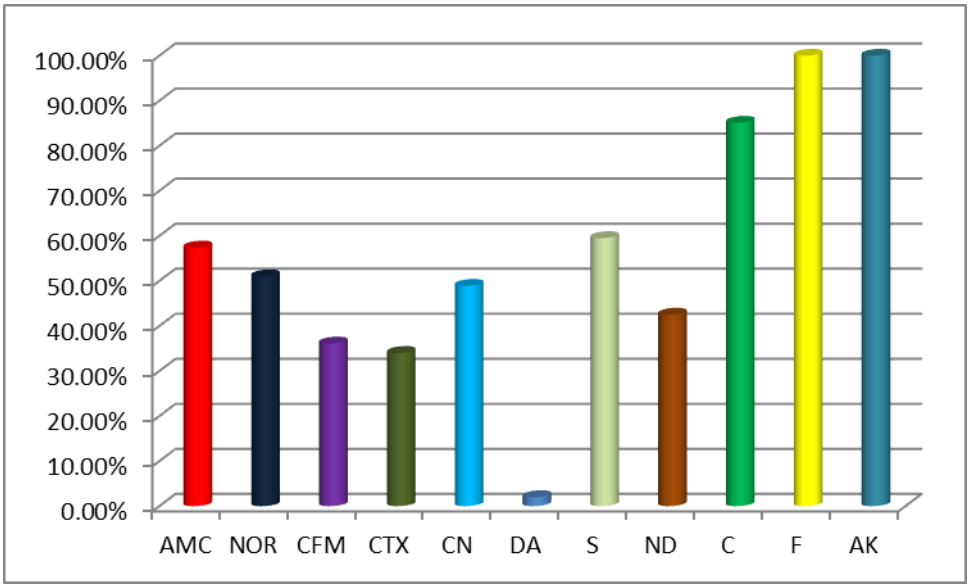
The antibiotic sensitivity test was studied on 47 isolates of *E. coli* and the results show that these isolates were resistance to Clindamycin (97.8%), (63.8%) for Cefotaxime and Cefaxime, Norfloxacine (59.5%) Naldixic acid (51.0%), Gentamycin (45.4%) and Amoxillin (44.6%) as show in Figure (3-1).



CTX= Cefotaxime, AK= Amikacin, CN= Gentamycin, S= Streptomycin, AMC= Amoxillin,CFM=Cefaxime, DA= Clindamycin, NA= Naldixic acid, NOR= Norfloxacine, C= Chloramphenicol, F= Nitrofuranation.

Figure 1 show antibiotic resistance for *E. coli* isolates

And some isolates were sensitive to Amikacin and Nitrofuranation (100%), (85%) for Chloramphenicol, (59.5%) for Streptomycin and Amoxillin (57.4%) as show in Figure 2.



CTX= Cefotaxime, AK= Amikacin, CN= Gentamycin, S= Streptomycin, AMC= Amoxillin, CFM=Cefaxime, DA= Clindamycin, NA= Naldixic acid, NOR= Norfloxacine, C= Chloramphenicol, F= Nitrofuranation.

Figure 2 show antibiotic sensitive for *E. coli* isolate

The results were resembled to study of [26] who found that the bacterial *E. coli* isolate from patients suffering from UTIs are resistance to Naldixic acid 88%.

The Norfloxacine intermediate resistance and the most effective antibiotics against *E. coli* were found to by Streptomycin [27].

But the results in this study not resemble to study by [28] who found that from 102 isolate of *E. coli* 60-79% were resistance to chloramphenicol.

Amikacin in this result is identical with those obtained [9]. However some reports have mentioned that treatment failure with Amikacin has been attributed to long term treatment [29].

Clindamycin can direct inhibit ribosomal activity and thus would be expected to inhibit synthesis of bacterial protein toxin [30].

Two studies were conducted on the effect of quinolones antibiotics on *E. coli*; these studies suggest that Naldixic acid cause loss of Pathogenicity Island and investigated the capacity of quinolones to induce partial loss of Pathogenicity Island in Uropathogenic *E. coli* [31].

The wide spread availability of antibiotics UTI remain the most bacterial infection in human. Antibiotics resistance may develop in uropathogen due to frequent misuse of antibiotics [28].

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