A Comparative Study of Different Antibiotics Against Escherchia Coli Isolated From Urinary Tract Infection By Minimal Inhibitory Concentration Test

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Abstract

Background: Urinary tract infection (UTI) is one of the most common bacterial infections around the world. UTI is usually caused by *Escherichia coli* (*E. coli*), a type of bacteria commonly found in the gastrointestinal tract (GI).

The aim of this study: To detected the Minimal Inhibitory Concentration (MIC) of Augmentin, Ciprofloxacin, Doxycycline, Levofloxacin and Amikacin against uropathogenic *Escherichia coli* (UPEC) by broth microdilution method.

Method: thirty – one urine sample from confirmed UPEC were collected from patient in Al-Hussein Teaching Hospital and Al-kafeel Super-Speciality Hospital. Cases with mean age 24.38 ± 15.80 (range from 1 to 65 years) during the period from June 2019 to September 2019 in Karbala city. The gender is 16 female (51.6%) and 15 male (49.4%). To determine the (MIC) of Augmentin, Ciprofloxacin, Doxycycline, Levofloxacin and Amikacin against (UPEC) by broth microdilution method. The method depended on Clinical and Laboratory Standards Institute (CLSI2015) (M07-A10).

Result: The results showed that these samples had high resistance to Augmentin (Amoxicillin - Clavulanic Acid) (100%) while the resistance was less to Ciprofloxacin (80.6%) and low resistance to Doxycycline (0%), Levofloxacine (3.2%), and Amikacin (3.2%). The results also showed the highest sensitivity to Amikacin 4 mg / l, by (58.1%).

Conclusion: UPEC more susceptible to amikacin and doxycycline (inhibitor of protein synthesis).UPEC high resistance to augmentin ® amoxicillin - clavulanic acid (inhibitor of bacterial cell wall synthesis) while the resistance was less to ciprofloxacin and levofloxacin (inhibitor of bacterial nucleic acid synthesis).

Keyword: Urinary tract infection, Uropathogenic *Escherichia coli*, Minimal Inhibitory Concentration.

دراسة مقارنة من المضادات حيوية المختلفة ضد الإشريكية القولونية المعزولة من خمج المسالك البولية عن طريق اختبار تركيز المثبط الأدنى. م.م حسين عبد علي مجد صادق، مرتضى ماجد ياسين، زيد احمد محمود، باقر عبد الامير عبد الصاحب

الخلاصة

الخلفية: خمج المسالك البولية (UTIs) هي واحدة من أكثر أنواع العدوى البكتيرية شيوعًا حول العالم. يحدث اصابات المسالك البولية عادة بسبب الإشريكية القولونية (E. coli) ، وهي نوع من البكتيريا توجد عادة في الجهاز الهضمي.

الهدف من هذه الدراسة: تحديد تركيز المثبط الأدنى (MIC) للمضادات الحياتية لأوجمنتين وسيبر وفلوكساسين ودوكسيسايكلين وليفوفلوكساسين وأميكاسين ضد الإشريشيا القولونية المسببة للأمراض البولية (UPEC) بطريقة التخفيف الدقيق للمرق.

الطريقة: تم جمع 31 عينة مؤكدة من UPEC من المرضى في مستشفى الحسين التعليمي ومستشفى الكفيل التخصصي . الحالات بمتوسط أعمار 24.38 ± 15.80 (تتراوح من 1 إلى 65 سنة) خلال الفترة من حزيران 2019 إلى أيلول 2019 في مدينة كربلاء. كان الجنس 16 أنثى (51.6٪) و 15 ذكر (49.4٪). اعتمدت الطريقة على معهد المعايير السريرية والمختبرية (M07-A1) (CLSI201).

النتيجة: أظهرت النتائج أن هذه العينات لديها مقاومة عالية لأوجمنتين (أموكسيسيلين - حمض كلافولانيك) (100٪) بينما كانت المقاومة أقل للسيبر وفلوكساسين (80.6٪) ومقاومة منخفضة للدوكسيسيكلين (0٪) وليفوفلوكساسين (3.2٪). وأميكاسين (3.2٪). كما أظهرت النتائج أعلى حساسية لأميكاسين 4 ملجم / لتر بنسبة (58.1٪). الخلاصة: الاشريشيا القولونية المسببة لخمج المسالك البوليه أكثر حساسية للأميكاسين والدوكسيسيكلين (مثبط تخليق

البروتين). و تبدي مقاومة عالية للأوجمينتين ® أموكسيسيلين - حمض الكلافولانيك (مثبط تخليق جدار الخلية البكتيرية) وأقل مقاومة للسيبروفلوكساسين والليفوفلوكساسين (مثبط تخليق الحمض النووي البكتيري). **الكلمات المفتاحيه**: اصابات المسالك البولية، الاشريكية القولونية الممرضة للجهاز البولي، التركيز المثبط الأدني

Introduction

Urinary tract infections (UTIs), which are mainly caused by uropathogenic Escherichia coli (UPEC), are one of the most common bacterial infections.[1]. In urinary tract infection, *Escherichia coli* remains the predominant uropathogen (80%) isolated in acute community-acquired uncomplicated infections, followed by *Staphylococcus saprophyticus* (10% to 15%). *Klebsiella*, *Enterobacter*, enterococci and *Proteus* species, rarely cause uncomplicated pyelonephritis and cystitis [2] This infection affects all ages and both sexes[3]. These infections are much more common in girls and women than in boys and men [4]..Immunodeficiency and urogenital tract anatomical abnormalities have been considered the essential risk factors for increased UTI [5].

Escherichia coli is a very diverse genus of bacteria that both humans and many other animal species naturally find in the intestinal tract. [6]. *E. coli* is a Gram-negative, rod-shaped oxidase-negative, bacterium from the family *Enterobacteriaceae*. It is able to grow both condition aerobically and anaerobically, preferably at 37°C, and can either be motile or nonmotile , with peritrichous flagella[7]. to break the inertia of the mucosal barrier the UPEC expresses a many of virulence factors [8]. virulence factors of UPEC strains have an increase of both structural in cell wall (as fimbriae, pili, , flagella) and can be secreted (endotoxins and exotoxin, iron-acquisition systems) that enhance to their ability to cause infection [9].

Depending on the function that is inhibited by the agents, the mechanism activity of antimicrobial agents may be categorized, typically involving the inhibition of ribosome role, cell wall synthesis, nucleic acid synthesis, cell membrane function and foliate metabolism[10].

Antimicrobial susceptibility testing is a daily task in clinical microbiology laboratories worldwide[11]. Dilution methods are used to detect the minimum inhibitory concentrations (MICs) of antimicrobial agents and the reference methods for

antimicrobial susceptibility testing are MICs. Microorganisms are evaluated in the dilution process for their ability to make noticeable growth in broth microtitration plate wells (broth microdilution) including serial antimicrobial agent dilutions. [12].to increase the throughput of broth macrodilutioncan used Microdilution attempts by conducting one test using 12 different antibiotic agents in a 96-well plate[13]. However, because less quantities of the tested intermediates, reagents and agents are used, the microdilution method is more economical and less laborious than the total dilution method. Thus, miniaturization has made the fine dilution method more practical and popular in testing conventional antimicrobial agents[14]

The relative rate of Escherichia coli multidrug resistant has risen over the past few years. The data on the resistance radiation trend of the bacterial strains in a geographical region will help guide the proper use of antibiotic drugs. The expression of an effective hospital antibiotic strategy in the management of these infections would also go a long way.[15].

Method

Study Design

This was a laboratory-based cross-sectional study on confirmed uropathogenic *Escherichia coli* isolated from urine sample during period June 2019 to September 2019 in Karbala city. This study is restricted by working at laboratory and there is not close contact with patients. Ethic's approval was obtained from College of Pharmacy 1829 in 17 July 2019. All wasted materials and equipment's were collected, packaged then sterilized by autoclaving. The study was accomplished from **June 2019 to March 2020** at Medical Research Laboratory College of Medicine, University of Kerbala. All cases diagnosed as uropathogenic *E. coli* by specialist microbiologist and their data were collected from Al-Hussein Teaching Hospital and Al-kafeel Super-Specialty Hospital.

Clinical Sample

Thirty-one (31) samples which store from June 2019 to September 2019 were included. The clinical data were obtained from microbiological report which contained: all age group at diagnosis which ranged from 1year to 65 years old, gender (male 15 and female 16).

Preparation of inoculum by broth culture method

Four well-isolated colonies of the similar morphological type from a media plate culture were selected for each sample. From top of each colony of *E. Coli* was contacted with a loop and the growth was carry into a tube containing 5 mL of broth. The broth was incubated at $35-37^{\circ}$ C in the incubator until the growth reaches a turbidity equal to or greater than that of a 0.5 McFarland standard (usually 2–6 hours). The culture was adjusted with sterile broth to give a turbidity equivalent to the McFarland 0.5 standard. This results in a suspension containing approximately 1×10^{8} CFU/mL for *E. coli*. In order to give a final organism density of $5 * 10^{5}$ cfu / mL, the inoculum prepared above was diluted in broth. 0.1 mL of suspension of the organism was transferred to a tube containing 9.9 mL of broth, giving an inoculum density of $1 * 10^{6}$ cfu / mL, which results in a final inoculum of $5 * 10^{5}$ cfu / mL / mL when mixed with an equivalent amount of antimicrobial solution in tubes or wells."

Five antibiotics amikacin,, ciprofloxacin, doxycycline, levofloxacin and augmentin (amoxicillin & clavulanic acid) which were used in this study . Antimicrobial agent's stock solutions were prepared at concentrations of at 1280 μ g/mL. (38.4 mg of each antimicrobial were dissolved in 30 ml of appropriate solvent). Stock solutions were stored at -20° C in the freezer refrigerator Antimicrobial agents were dissolved according to its water solubility, directions provided by [16] The method involves preparing two-fold dilutions of the antimicrobial agent (e.g. 64, 32, 16, 8, 4, 2,1,0.5,0.25and 0.125 μ g/mL) in a liquid growth medium dispensed in tubes containing a minimum smaller volumes using 96-well microtitration plate (microdilution)). The broth microdilution method to determine MIC for the *E. coli* isolates was carried out in accordance with guidelines [17].

Statistical analysis

Data were analyzed with International Business Machines Corporation (IBM) (Armonk, New York, United States) Statistical Package for the Social Science (SPSS) Software Version 22 for Windows (GraphPad Software, San Diego, California, USA

Result

Thirty – one urine (31) samples of confirmed UPEC infections were collected in this work. The cases with mean age 24.38 \pm 15.80 (range from 1 to 65 years) figure1. Sixteen females (51.6%) and fifteen males (49.4%) figure1 and 2. The current study examine the antimicrobial effect of five antibiotics which be a member of five different classes of antibiotics with different conduct of action. The different antibiotics be seen MIC values ranging between 64µg/ml and 0.125 µg/ml The MIC value is described as the lowest antibiotic concentration that inhibits a microorganism's visible growth after overnight incubation figure 2 and 3. The results showed that these isolates were found to be resistant to augmentine 100%, ciprofloxacine 80.6% Also the results showed the UPEC sensitive 58.1% for amikacin at 4 µg/mL, 64% for levofloxacin at 8 µg/mL, and doxycycline 54.8% at 16µg/mL





Figure 1: Distribution of age range in this study





Figure3: The visible growth of UPEC after co-incubated with Augmentin after incubated at 35-37°C in ambient air for 24 hours without agitation.



Figure 4: The growth of UPEC after co-incubated with Amikacin at 35-37°C in ambient air for 24 hours without agitation.



Figure 3: The MIC $\,$ of 31 sample of UPEC after tested with five antibiotics at 9 different conc. (ranged from 0.125 μ g /mL to 64 μ g /mL).

Discussion

In microbiology laboratory, an important work of the clinical is the performance of antimicrobial susceptibility testing of considerable bacterial isolates. The purpose of testing is to check prospective drug resistance in common pathogens and to ensure susceptibility to drugs of choice for specific infections[14].In 21st century the public health problems is the Antimicrobial resistance (AMR) has shown that the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi no longer susceptible to the regular medicines used to treat them [18]

In this study ,The Augmentin effect on UPEC (β -lactam/ β -lactamase) inhibitor combinations were in the same line with the previous Spanish study[19]. The present results were much higher than that reported by Sudan study which revealed the UPEC isolates displayed resistance to amoxicillin-clavulanic acid (50.4%) at $30\mu g/ml[20]$.the result close to Pakistan study which record 71% (105 out of 148) resist to amoxicillin-clavulanic acid[21]. Augmentin is indeed a drug comprising amoxicillin (antibiotic) and clavulanic acid a non-antibiotic compound. Clavulanic acid is able to inhibit beta-lactamase enzyme thereby extend the antibacterial action of the amoxicillin component of augmentin equable amongst producing bacteria the penicillinase.[22].The resist of UPEC may be the environment-borne *E.coli* species may capture antibiotic resistance genes from other environmental species before infection.

In this study the resistance to Ciprofloxacin was (80.65%). These results were close to Pakistan study which showed that (62%) of UPEC isolates were resistant to ciprofloxacin (45 out of 72) [23]. this result was approximately the same result of Norway study demonstrated that resistance was (76%) of UPEC(80 out of 105) [24]. An Sudan study revealed that 58.4%(125 out of 214) of UPEC isolates were resistant to ciprofloxacin which is less than present study [20]. Ciprofloxacin, the derivatives from fluoroquinolone show best action against Enterobacteriaceae and *Ps. aeruginosa*, and their spectrum also includes staphylococci but not streptococci. organisms resistant to other antibiotics ,Ciprofloxacin may be used in such treatment ; it can also be used in combination with a b-lactam or aminoglycoside antibiotic[25].

In this study the resistance to levofloxacin was (3.23%), which was close to Japanese study which recorded 16% (49 out of 312) [26]. this result is far with Korean study which recorded34.97 /% (178 out of 509) [27] and Nepal study which recorded 32.93% (717 out of 2173)[28]. To treat respiratory and urinary tract infections the fluoroquinolones (a subset of the quinolones) are a group of broad spectra, bactericidal antibiotics with a similar mode of action by blocking DNA gyrase or topoisomerase-IV which are the most important enzymes for replicatation and transcription of bacterial DNA synthesis [29]. This property may be the reason for the low level of UPEC resistance to this antibiotic.

In this study all UPEC were sensitive to Doxycycline (0.0% resistance), this result was in the same line with result of South Ethiopia which record resistance to Doxycycline (0.0%). The result in this study is Far with Pakistani study which recorded resistance to Doxycycline 66.6% (214 out of 321) and Equatorial Guinea 78.4% (67 out of 86). As a bacteriostatic agent, doxycycline has been used as a broad-spectrum that inhibits bacterial protein synthesis by targeting the 30S ribosomal subunit of both gram negative bacteria and gram-positive [30]

The UPEC more sensitive to antibiotics Amikacin 58.1% (18 out of 31) at $4\mu g/mL$ and 19.4% (6 out of 31) at $8\mu g/mL$ this result which compared with Pakistani study which recorded 94% (71 out of 74) [23] Other studies in Equatorial Guinea close with this study result which recorded 95.1% (68 out of 72) [31] other Turkish sudy revealed that 100% (53 cases) of UPEC isolates were susceptible to Amikacin [32] and South Ethiopia100% (44 cases) [33]. Amikacin enhances resistance to some, but not all, types of aminoglycoside modifying enzymes, as it has fewer sites of modification [25].

Conclusion:

UPEC more susceptible to amikacin and doxycycline (inhibitor of protein synthesis).UPEC high resistance to augmentin ® amoxicillin - clavulanic acid (inhibitor of bacterial cell wall synthesis) while the resistance was less to ciprofloxacin and levofloxacin (inhibitor of bacterial nucleic acid synthesis).

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