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Determination of Mininmum Inhibitary Concentrationof Ciprofloxcin Amoung β-Lactamaste Producing Isolates

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الخلاصة:

جمعت 200 عينة سريرية (الدم والقيح والبراز والجروح ومسحات المهبل) خلال الفترة من حزيران 2009 لغاية كانون الثاني2010، وشخصت 96 عزلة بكتيرية سالبة وموجبة لصبغة غرام بالأختبارات البكتريولوجية والبيوكيميائية.

كانت بكتريا *Escherichia coli* هي السائدة بنسبة (27.08%) تبعتها بكتريا *Salmonella typhi* بنسبة (12.5%) ثم بكتريا *Klebsiella pneumoniae* بنسبة (10.4%) وبكتريا *Proteus vulgaris* بنسبة (9.3%) وبكتريا *Staphylococcus aureus* بنسبة (8.3%).

أجري أختبار الحساسية ضد مضاد السايبروفلوكساسين بتركيز (5 µg) بطريقة الأنتشار بالأقراص وكان عدد العزلات المقاومة 22 عزلة بنسبة (23%) توزعت هذه العزلات فكان عدد بكتريا *E.coli* (10) عزلات وبكتريا *K.pneumoniae* (4) عزلات, تبعتها بكتريا *S.typhi* (3) عزلات، بينما كانت عدد عزلات*Shigella flexner* و*Enterobacter* المقاومة للسايبروفلوكساسين عزلتان فقط لكل منهما, وعزلة واحدة لبكتريا *S.aureus*. كانت العزلات المقاومة للسايبروفلوكساسين والمنتجة لأنزيم البيتالاكتاميز سبعة عزلات.

ان نتائج تحديد تركيز المثبط الأدنى للسايبروفلوكساسين للعزلات المنتجة لأنزيم البيتالاكتاميز *E.coli* 1 و *Enterobacter* 1 و *Enterobacter* 2 و *K.pneumoniae* و *S.flexneri* و *E.coli2* و *S.aureus* كانت (64, 32,32, 16 ,16,16, 8) مايكروغرام/ملليلترعلى التوالي.

Abstract:

Out of 200 clinical samples (blood ,pus,stool,urine,wound and vaginal swabs) were collected from Baghdad hospitals during June2009-January 2010, 96 Gram negative and positive bacterial isolates were identified by bacteriological and biochemical tests. *Escherichia coli* was the dominant bacteria (27.08%), followed by *Salmonella typhi* (12.5%), *Klebsiella pneumoniae* (10.4%), *Proteus vulgaris* (9.3%), and *Staphylococcus aureus* (8.3%).

Antimicrobial susceptibility test was done against ciprofloxacin disk (5µg) by disk diffusion method and the resistant isolates were 22 (23%);

distributed into *Escherichia coli* (10 isolates), *Klebsiella pneumoniae* (4 isolates), *Salmonella typhi* (3 isolates), *Enterobacter* and *Shigella flexnerii* (2 isolates) and *Staphylococcus aureus* one isolate. Seven ciprofloxacin resistant isolates were positive for β-lactamase production test (7.3%). Results of minimal inhibitory concentration of ciprofloxacin for positive β-lactamase producing isolates, *E. coli* 1, *Enterobacter* 1, *Enterobacter* 2, *K. pneumoniae, S. flexneri, E.coli* 2, and *S.aureus* were (64,32,32,16,16,16,8 µg/ml) respectly .
Keywords: Ciprofloxacin resistance bacteria, MIC, β-lactamase .

Introduction:

Antimicrobial resistance among enteric Gram negative bacteria is fast becoming a global public health concern with rapid increase in multidrug resistant organisms .Gram negative bacteria (GNB) are a common cause of urinary tract infections, neonatal sepsis and post surgical infections in hospitalized patients [1]. Resistance of Enterobacteriaceae to broad spectrum β-lactam antibiotics via ESBL production is an increasing problem worldwide [2].The fluoroquinolones have assumed an important role in the therapy of these infections, since they have a broad spectrum of activity, including Gram-positive and in particular Gram-negative bacteria[3,4]. Fluoroquinolones are potent antimicrobial agents used for the treatment of a wide variety of community- and nasocomial-infections. However, resistance to fluoroquinolones in Enterobacteriaceae is increasingly reported. Studies assessing the ability of fluoroquinolones to select for resistance have often used antimicrobial concentrations quite different from those actually acquired at the site of infection[5]. Fluoroquinolones are among the most commonly prescribed antimicrobials because of their broad-spectrum antimicrobial activity, and fluoroquinolone-resistant gram-negative pathogens have emerged worldwide. Quinolone resistance is traditionally mediated by the mutation of chromosomal genes encoding DNA gyrase and/or topoisomerase IV or by the mutation of genes regulating the expression of efflux pumps[6,7]

Gram-negative bacteria have adapted to broad-spectrum β-lactam antibiotics by modifying the substrate spectrum of common plasmid-mediated β-lactamases and by mobilizing resistance-promoting chromosomal β-lactamase genes into plasmids, allowing their spread to new hosts [8].

The fluoroquinolones, ciprofloxacin and ofloxacin, have minimal gram-positive activity, but they are the most active against aerobic gram-negative bacilli. Limited microbial susceptibility and acquired resistance limit the usefulness of older agents in the treatment of staphylococcal ,streptococcal, and enterococcal infections[9]Beta-lactamase-producing bacteria (BLPB) can play an important role in polymicrobial infections. They can have a direct pathogenic impact in causing the infection as well as an indirect effect through their ability to produce the enzyme beta-lactamase. BLPB may not only survive penicillin

therapy but can also, as was demonstrated in *in vitro* and *in vivo* studies, protect other penicillin susceptible bacteria from penicillin by releasing the free enzyme into their environment. This phenomenon occurs in upper respiratory tract, skin, soft tissue, surgical and other infections [10] .

This study aims to determine the resistance against ciprofloxacin by different common pathogens and incidence of β-lactamase production.

Materials and Methods:

Sample collection and analysis:

The study was conducted on patients attending outpatient clinics at Baghdad hospitals, between June 2009-January 2010. 200 clinical samples (blood sample, pus, stool, urine, wound and vaginal swabs) were submitted to the clinical microbiology laboratory for culturing on blood agar and MacConkey, Salmonella-Shigella agar and mannitol salt agar plates from Oxoid. Only a single positive culture per patient was included in the analysis. The significant pathogens were identified by standard biochemical procedures by API system (Oxoid) [11].

Antimicrobial susceptibility testing:

Antimicrobial susceptibility testing for ciprofloxacin 5µg from (Oxoid) was performed using the disk diffusion method for all the isolates on Mueller-Hinton agar plates as described by [11,12]. Standard strains: *E.coli* ATCC (25922), *P.aeruginosa* ATCC (27853) and *S.aureus* ATCC (25923) were tested for antibiotic susceptibility.

Determination of Minimal Inhibitory Concentration:

The MICs were determined using the double dilution broth method as instructed by [11] .

Beta-lactamase production assay:

Production of beta-lactamase test was done for the isolates by using the rapid idometric method of WHO [13].

Results and Discussion:

Out of 200 clinical samples (blood sample,pus,stool,urine,wound and vaginal swabs) were collected,96 Gram negative and positive bacterial isolates were identified by bacteriological and biochemical tests as shown in (table-1).

Bacterial isolates	Number	%
Escherichia coli	26	27.08
Salmonella typhi	12	12.5
Klebsiella pneumoniae	10	10.4
Proteus vulgaris	9	9.37
Staphylococcus aureus	8	8.3
Pseudomonas aeruginosa	7	7.29
Enterobacter SPP.	5	5.2
Shigella flexnerii	4	4.1
Serretia marcescens	3	3.1
Acinetobacter baumannii	2	2.08
Pseudomonas fluorescens	2	2.08
Salmonella paratyphi A	2	2.08
Citrobacter SPP.	1	1.04
Enterococcus faecalis	1	1.04
Listeria SPP.	1	1.04
Pseudomonas putida	1	1.04
Shigella boydii	1	1.04
Streptococcus agalagte	1	1.04

Table-1: Numbers and percentage of bacterial Isolates *
Number of isolates= 96

E. coli was the dominant bacteria (27.8%), followed by *S. typhi* (12.5%), *K. pneumoniae* (10.4%), *P. vulgaris* (9.37%), and *S. aureus* (8.3%).
E.coli is worldwide the most frequent pathogen isolated from uncomplicated urinary tract infections (UTI) (70 - 95%) and, in bacteremia of nosocomial or community origin, it represents about the 15.5% and 42.1% of aetiologies, respectively [14].

Also *Klebsiella* spp.,especially *K.pneumoniae*, are involved in uncomplicated UTI for 5% and represent 4.1% of bacteremias,the mortality of nosocomial infections being more than twice that of community-acquired infection .

Enterobacter species are a common cause of several human diseases and are predominantly associated with nosocomial infections [15,16].

After the bacterial isolates were subjected to antimicrobial susceptibility test against ciprofloxacin disk (5µg) by disk diffusion method the resistant isolates were 22 (23%); distributed into *Escherichia coli* (10 isolates), *K. pneumoniae* (4 isolates), *S. typhi* (3 isolates), *Enterobacter* and *S. flexnerii* (2 isolates) for each and *S. aureus* one isolate as shown in (table-2) .

Bacteria	No. of ciprofloxacin resistant isolates	%
E. coli	10	10.4
K. pneumoniae	4	4.16
S. typhi	3	3.12
Enterobacter	2	2.08
S. flexnerii	2	2.08
S. aureus	1	1.04

Table-2: Resistance ratio among bacterial isolates against ciprofloxacin.

Bacteria	MIC (µg/ml)
E. coli 1	64
Enterobacter 1	32
Enterobacter 2	32
K. pneumoniae	16
S. flexneri	16
E. coli 2	16
S. aureus	8

Table-3: Minimum inhibitory concentration of ciprofloxacin among β-lactamase positive isolates.

It was observed that (7.3%) of the tested isolates were beta- lactamase producers. This suggests possible abuse of these drugs, poor hospital attendance and the need for better enlightenment campaign against the use of drug without prescription [17] .In a recent study in China about 26.4% had a β-lactamase phenotype,the β-lactamase genes, which confer multi-drug resistance, are spreading among clinical bacterial isolates [18]. Increase in the beta-lactamase producing *S. aureus* and *E. coli* was above 80%.The increasing in prevalence of microorganism, particularly of beta-lactamase producing *E. coli* and *S. aureus* and ESLB is alarming situation [19]. Most of the studies refer the resistance to β-lactam antibiotics to the production of beta-lactamase by plasmids in *E.coli* and *Klebsiella* [20].

Results of minimal inhibitory concentration of ciprofloxacin for positive β-lactamase producing isolates, *E. coli* 1, *Enterobacter* 1, *Enterobacter* 2, *K.pneumoniae, S. flexneri, E. coli* 2, and *S.aureus* were (8,32,32,16,16,8 µg/ml) respectly as in table-3.

The quinolone resistance mechanism described most frequently, both in *Shigella* spp. and in other microorganisms, involves the presence of mutations in the quinolone target site. As described previously for Enterobacteriaceae antibiotic consumption trends in a particular region may influence the resistance

profiles of local bacteria. Quinolones have been used as the drugs of choice for treating shigellosis in some areas. The widespread use of these agents might account for the high percentage of quinolone-resistant isolates recovered from the feces of travelers to India [21].

There are quite a few reports of strains of *Shigella* spp. harbouring different types of Extended Spectrum β-lactamases (ESBLs) in developed and developing countries in Asia,the World Health Organization (WHO) currently recommends fluoroquinolones as the therapy of shigellosis.

The emergence of ciprofloxacin resistance in *Shigella* has shifted the attention to cephalosporins [22]. The close relationship between ESBL production and ciprofloxacin resistance is particularly worrisome because the first reported instance of plasmid-mediated ciprofloxacin resistance has been in an isolate of *K. pneumoniae* also possessing an ESBL, suggesting that patient-to-patient transmission of such strains occurred [23]. Clinics and Hospitals in Benin City, Nigeria, were screened for quinolones resistance gene and the results revealed that (23.9%) of isolates were resistance to quinolones with a MIC ≥4μg/ml for ciprofloxacin [24]. There was a significant increase in the resistance of the ESBL-producing isolates to ciprofloxacin compared to those we reported previously (from 55% to 72%) for *E.coli* and *K.pneumoniae*. The elevated MIC of ciprofloxacin in *E. coli* ≥ 32mg/ml and *K. pneumoniae* ≥64mg/ml indicates that in these species resistance to ciprofloxacin is strongly associated with ESBL production [25]. A recent study in Oman showed that ciprofloxacin is 27.02%, 21.95%, 16.66%, 72.22% and 44.44% resistant to *E.coli*, *S.aureus*, *S. typhi*, *K. pneumoniae* and *P. aeruginosa* respectively [26] ,but in the current study the results of ciprofloxacin resistance showed that *E.coli* resistance was (10.4%), *K.pneumoniae* (4.16%),*Salmonella* (3%) and *S.aureus* (1%). These differences may be referred to samples, kinds of hospitals and locations. *In-vitro* disk diffusion method was used to evaluate the growth of inhibition of pathogens, since Bauer-Kirby disk diffusion technique is a simple, reliable, and reproducible way to assess the antimicrobial susceptibilities. [27].

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