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Incidence of Multi-Drug Resistant *Escherichia Coli* Isolates from Blood and Urine in Kerbala, Iraq.

نسبة الاصابة لبكتريا الاشريشية القولونيةالمعزولة من الدم والادرار المقاومة لعدد من اصناف المضادات الحيوية في كربلاء. العراق

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Abstract

Escherichia coli is a common cause of urinary tract infection (UTI) and septicemia/ bactriemia. With the increase of antibiotic resistance among *E. coli* isolates, monitoring of the incidence of drug resistance became critical for appropriate empiric selection of antibiotic therapy. The aims of this study are to explore the drug resistance patterns among *E. coli* isolates from urine and blood in Kerbala, and to identify the antibiotics that might be considered appropriate for empirical treatment for UTI and Septicemia caused by *E. coli* in the study region. A total of 1637 culture results of urine (n=410) and blood (n=1227) were reviewed and analyzed for isolation and identification of bacteria , antimicrobial susceptibility testing, in addition, patients age and sex. Only single isolate per patients were considered in the study analyses. *E. coli* was isolated from 105 patients (62 from urine and 43 from blood samples). High resistance rates were documented to Ampicillin (95.7%), Cephalothin (94.0%), Amoxicillin-Clavulanic acid (89.09%), Cefixime (82.7%), Ceftriaxone (78.4%) and Cefotaxime (76.9%). In contrast, high degrees of sensitivity were detected to Imipenem (100%), Nitrofurantoin (90.2%), Chloramphenicol (81.8%) and Amikacin (76.0%). No significant differences were seen in susceptibility patterns among isolates from blood versus urine. Overall, 67 isolates (63.8%) were multi-drug resistant (MDR), that are resistant to more than 2 classes of antibiotics. Proportion of MDR isolates among urine samples was higher than among blood samples (69.4% versus 55.8%). In conclusion: *E. coli* displayed high resistance rates to several antibiotics. However, Imipenem, Nitrofurantoin, chloramphenicol and amikacin are appropriate for empirical treatment for UTI and Septicemia caused by *E. coli* inKerbala. Continuous surveillance of drug –resistance is highly recommended.

الخلاصة:

تعد بكتريا الاشريشية القولونية (*E coli*) سببا" شائعا" لالتهاب المسالك البولية (UTI) والانتانية الدموية / bactriemia. ونظرا" للزيادة الحاصلة في المقاومة للمضادات الحيوية بين عزلات هذه البكتريا، فقد أصبح من الضروري رصد حالات مقاومة العقاقير لاختيار العلاج الأنسب والمجرب من المضادات الحيوية. كانت أهداف هذه الدراسة تشمل الكشف عن أنماط المقاومة بين البكتريا (*E coli*) المعزولة من البول والدم في كربلاء، وكذلك التعرف على المضادات الحيوية التي قد تعتبر مناسبة في العلاج التجريبي لالتهاب المسالك البولية وتسمم الدم الناجم عن هذه البكتريا ضمن منطقة الدراسة . تم استعراض وتحليل ما مجموعه 1637 نتيجة من زرع البول (410) والدم (1227) لغرض عزل وتشخيص البكتيريا وكذلك اختبار الحساسية للمضادات الحيوية ، بالإضافة الاخذ بالنظر عمر وجنس المرضى. واظهرت النتائج بكتريا الاشريشية القولونية قد تم عزلها من 105 مريضا (62 من البول و 43 من عينات الدم). وقد تم توثيق معدلات مقاومة عالية للأمبيسلين (95.7٪)، سيفالوتين (94.0٪)، وحمض أموكسيسيلين-الكلافولانيك (89.09٪)، سيفيكسيم (82.7٪)، سيفترياكسون (78.4٪) وسيفوتاكسيم (76.9٪). في المقابل، تم الكشف عن درجة عالية من الحساسية للإميبينيم (100٪)، نتروفورانتوين (90.2٪)، كلورامفينيكول (81.8٪) وأميكاسين (76.0٪). كما لم تظهر النتائج وجود أي فروق ذات دلالة إحصائية في أنماط الحساسية بين العزلات من الدم مقابل البول. عموما، بينت النتائج ان 67 عزلة (63.8٪) كانت ذات مقاومة متعددة للأدوية (MDR)، التي هي مقاومة لأكثر من صنفين من المضادات الحيوية. وكانت نسبة MDR بين عزلات عينات البول أعلى من عينات الدم (69.4٪ مقابل 55.8٪). مما ذكر انفا نستنتج الاتي: هناك معدلات عالية للمقاومة للعديد من المضادات الحيوية. ومع ذلك فأن الإميبينيم، نتروفورانتوين، الكلورامفينيكول والأميكاسين ربما تعد مناسبة كعلاج تجريبي لالتهاب المسالك البولية وتسمم الدم الناجم عن الاشريشية القولونية في كربلاء.وبناءا على نتائج هذه الدراسة، ينصح بشدة المراقبة المستمرة للمقاومة للمضادات الحياتية.

Introduction

E. coli is the most common cause of Urinary Tract Infection (UTI) by gram-negative bacilli (1) and its most often isolated bacterial organism from blood cultures (2, 3, 4, 5). In many countries, *E. coli* displays high rates of resistance to recommended first- and second-line agents, such as penicillins, cephalosporins, sulfa drugs (1,3,6), and fluoroquinolones (7, 8) and is commonly associated with treatment failure (9, 10).

Due to constantly evolving antimicrobial resistant patterns, there is need for constant antimicrobial sensitivity surveillance (11). The World Health Organization (WHO) highlights the establishment of "effective, epidemiologically sound surveillance of antimicrobial resistance among common pathogens in the community, hospitals, and other health care facilities" as 1 of 2 fundamental public health priorities in efforts to confront antimicrobial drug–resistant organisms (11).

The emergence of multi-drug resistant *E. coli* makes the determination of antibiotic sensitivity patterns in periodic intervals is mandatory in each region for the clinicians to be aware of the emerging pathogens that pose a threat to the community, to provide safe and effective empirical therapies, develop rational prescribing practices and make policy decisions in a hospital and finally assess the effectiveness of all (12). Nowadays, bacterial drug resistance is an important problem and due to wide variations in bacterial drug resistance, results of studies and reports in one region or in one period of time are not necessarily true for other regions or periods of time. They are related with a series of social, environmental and technological changes (13, 14).

This study was conducted to explore the drug resistance patterns among *E. coli* isolates from urine and blood in Kerbala, and identify the antibiotics that might be considered appropriate for empirical treatment for UTI and Sepeticemia caused by *E.coli* in the study region.

Material and Method

During the period from January to September 2013, a total of 1637 of urine (n=410) and blood (n=1227) specimens were received and cultured at the microbiology laboratory of Al-Hussein Medical city hospital/ Karbala/ Iraq. Bacterial isolates were identified by standard microbiological techniques and diagnosis confirmed by use of API system.

The antibiotic susceptibility patterns of the organisms were performed by Kirby- Bauer's disk diffusion method (15)on Mueller Hinton agar plates and the results were interpreted according to guidelines of Clinical Laboratories Standards (CLSI) (16). The antibiotic discs that were used to identify the susceptibility pattern of the bacterial pathogens and their concentrations included: Ceftriaxone 30µg, Cefixime 5µg, Cefotaxime 30µg, Ceftazidime 30µg, Cephalothin 30µg, Chloramphenicol 30µg, Ciprofloxacin 5µg, Ampicillin 10µg, Amoxicillin-Clavulanic acid 20-10µg, Nitrofurantoin 300µg, Aztreonam 30µg, Imipenem 10µg, Amikacin 30µg, and Gentamycin 10µg.

All culture results and antibiotic susceptibility patterns were reviewed retrospectively and all repeated isolates were excluded from this study and only first visit isolate per patient was included in this study. In addition, demographic data such as sex, age and residency were collected and analyzed.

Data analysis

Descriptive analysis was performed using SPSS version 16.0 (SPSS Inc, Chicago, Illinois, USA). The frequency of susceptibility to each antibiotic was calculated for *E. coli* isolates. The chi-square test was used to compare different groups. *P value*less than or equal to 0.05 considered as statistical significant for all analyses. Frequency of multi drug resistant bacteria was found out as isolates resistant to more than two different classes of antibiotics were considered as multidrug resistant bacteria.

Results and Discussion

Out of 1637 cultured specimens, a total of 317 showed positive bacterial growth.Table 1 shows the detailed results of cultures and rates of *E. coli* culture positivity in the tested samples. *E. coli* was isolated from 105 samples (62 from urine and 43 from blood). Of the positive cases, the isolation rate of *E. coli* from urine was high (62 out of 121 positive cases, 51.2%), while the rate is lower from blood positive cases (43/196, 15.1%).

Table 1: Distribution of *E. coli* positive cultures among urine and blood samples during the study period.

Sample	Number of cultured samples	Number of positive cultures	Positive cultures for *E. coli*	*E. coli* isolation rates versus all tested samples	*E. coli* isolation rates versus all positive cases
Urine	410	121	62	62/410 (15.1%)	62/121 (51.2%)
Blood	1227	196	43	43/1227 (3.5%)	43/196 (15.1%)
Total	1637	317	105	105/1637 (6.4%)	105/317 (33.1%)

The detailed results of the susceptibility testing of the *E. coli* isolates to 14 antibiotics is displayed in Table 2. High resistance rates were documented to Ampicillin (95.7%), Cephalothin (94.0%), Amoxicillin-Clavulanic acid (89.09%), Cefixime (82.7%), Ceftriaxone (78.4%) and Cefotaxime (76.9%). In contrast, high degrees of sensitivity were detected to Imipenem (100%), Nitrofurantoin (90.2%), Chloramphenicol (81.8%) and Amikacin (76.0%). However, no significant differences were seen in susceptibility patterns among isolates from blood versus urine (data not shown).

Table 2: Antibiotic susceptibility patterns of *E coli* isolates.

Type of Antibiotic	Susceptibility pattern			Total number of tested isolates
	Resistant N (%)	Sensitive N (%)	Intermediate N (%)	
Ceftriaxone	51 (78.4%)	14 (21.5%)	0 (0%)	65
Chloramphenicol	10 (18.1%)	45 (81.8%)	0 (0%)	55
Gentamycin	27 (30.3%)	50 (56.1%)	12 (13.4%)	89
Cefixime	77 (82.7%)	16 (17.2%)	0 (0%)	93
Ciprofloxacin	20 (25.9%)	54(70.12%)	3 (3.8%)	77
Cefotaxime	50 (76.9%)	14 (21.5%)	1 (1.5%)	65
Ampicillin	88 (95.65%)	3 (3.26%)	1 (1.08%)	92
Ceftazidime	36 (57.14%)	24 (38.09)	3 (4.76%)	63
Amoxillin-Clavulanic acid	49 (89.09%)	6 (10.90%)	0 (0%)	55
Nitrofurantoin	5 (9.80%)	46(90.19%)	0 (0%)	51
Imipenem	0 (0%)	30 (100%)	0 (0%)	30
Amikacin	6 (24.0%)	19 (76%)	0 (0%)	25
Cephalothin	47 (94.0%)	3 (6.0%)	0 (0%)	50
Aztreonam	19 (55.88%)	10(29.41%)	5 (14.70%)	34

Antimicrobial resistance in *E. coli* has been reported worldwide and increasing rates of resistance among *E. coli* is a growing concern in both developed and developing countries (17, 18) . A rise in bacterial resistance to antibiotics might be due to the fact that most of the patients are given antibiotics before bacteriological investigation (19). In addition,self medication is very common as the medicines are available at the counter, subsequently, this may complicates

treatment of infections.Results of susceptibility profiles of *E. coli*varied in various populations and environments (20).

In the current study, taken as a whole, *E. coli* isolates showed high resistance rates to antibiotics. The results is in conformity with other studies (21, 22). More than 95% and 94% of *E. coli* isolates were resistant to Ampicillin and Cephalothin, respectively. The high rate of resistance toAmpicillinwas also reported in several other places of the world (21, 23, 24).High resistance rates was also seen to Amoxicillin-Clavulanic acid, Cefixime, Ceftriaxone, Cefotaxime, Ceftazidime (89.09%, 82.7%, 78.4%, 76.9%, 57.14%, respectively). Inversely, very high sensitive rates was seen toImipenem (100%), Nitrofurantoin (90.19%), Chloramphenicol (81.8%), and Amikacin (76%), showing a good opportunity for the use of these drugs for blood stream and urinary tract infections. These findings are in agreement with other studies (22, 25, 26).

Isolates resistant to more than two different classes of antibiotics were considered as MDR.As could be seen in table 3, 67 isolates (63.8%) were multi-drug resistant (MDR)(24 isolates isolated from blood culture and 43 isolates isolated from urine culture). Accordingly, the proportion of MDR isolates among urine samples was higher than among blood samples (69.4% versus 55.8%), however, this difference was statistically insignificant.

Table 3: Distribution of Multi-drug resistant (MDR) *E.coli* isolates among urine and blood samples.

		Drug resistance		Total
		MDR (%)	Non-MDR (%)	
Sample	Blood	24 (55.8)	19 (44.2%)	43
	Urine	43 (69.4%)	19 (30.6%)	62
Total		67	38	105
Chi-square (*p-value)*		0.21		

Table 4,shows classification of the *E. coli* isolates according to the number of antibiotics classes resistance. Only 3 isolates (2.9%) were sensitive to all antibiotic tested. Majority of isolates were resistant to 3 or more antibiotics (MDR) and as follows; 27 isolates were resistant to 3 classes, 27 isolates were resistant to 4 classes of antibiotics, 10 isolates were resistant to 5 classes of antibiotics, 3 isolates were resistant to six classes of antibiotics.Similarly, a high rates ofMDRamong *E. coli* isolates werealso recorded by other studies elsewhere (27, 28).

Table 4: Classification of the *E coli*isolates according to their number of resistance to antibiotic classes.

Number of Phenotypic Drug Resistance			
		No. of isolates	Percent
Non MDR	Sensitive to all tested classes	3	2.9
	One ClassResistance	11	10.5
	Two classes Resistance	24	22.9
MDR	Three ClassesResistance	27	25.7
	Four classesResistance	27	25.7
	Five Classes Resistance	10	9.5
	Six Classes Resistance	3	2.9
Total		105	100.0

In conclusions, the results of this study indicate high resistance rate of *E coli* isolates for several antibiotics like Ampicillin, Cephalothin, Amoxicillin- Clavulanic acid, Cefixime, Ceftriaxone, Cefotaxime, Ceftazidime. On the other hand Imipenem, Nitrofurantoin, Chloramphenicol, and Amikacinwere found to be the most effective antibiotics against *E. coli* isolates and, thus, might be considered appropriate for empirical treatment of *E. coli* in the study area. Periodic monitoring of antibiotic sensitivity is imperative to detect any changing patterns. The multiple resistance of isolates to some antibiotic classes are of great public health concern and calls for caution in the indiscriminate use of antibiotics on humans.The result indicate the presence of high resistant rates for different classes of antibiotics.

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