Re-evaluation of lunation of the activity of somantibacterial drugs against clinical isolates of Escherichia coli in al- najaf al- ashref governorate

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Abstract

Background: Escherichia coli is one of the most important bacterial pathogen that can cause several disease to human being . In our study we try to investigate the sensitivity resistance pattern of Escherichia coli against three antibiotics (Amikacin, Nalidixic acid and Cephalexin).

Methods: For this purpose we collected 51 clinical isolates of Escherichia coli from stool and urine of outpatient and inpatient patients from different wards of AL-SADER Teaching Hospital in AL-NAJAF AL-ASHRAf, IRAQ, and tested by culture and sensitivity test .

Results: The results appeared that Amikacin show the highest percentage of sensitivity (66.66 %) , while Cephalexin show the lowest percentage of sensitivity (6.34 %) .

Conclusions: we conclude that Amikacin is the most sensitive antibacterial drug for Escherichia Coli as compared with Nalidixic acid and Cephalexin, while Cephalexin is the most resistant antibacterial drug for Escherichia Coli as compared with Amikacin and Nalidixic acid.

key words: E. Coli isolates ,antibacterial activity.

Introduction

Antibiotics are frequently prescribed because of clinical suspension of infection while results of microbiological analysis are still pending .Empirical therapy may be influenced by microbiological culture results ,either by discontinuation of therapy in case of negative culture , or by broadening or narrowing the spectrum of antibiotics .[1]

The re-evaluation of folder antibiotics ,which are not widely used, can be conducted more rapidly as compared with development of totally new antimicrobial agent which takes several years.[2]

Escherichia coli (E .Coli) is one of the most common Gram- negative enteric bacteria that cause multiple diseases for the human being, like urinary tract infection and diarrheaial diseases that affect infants, children and young age group. During the last decade, it has become apparent that many enteric bacteria . In addition to their content of endotoxins ,it produce exotoxins of considerable medical importance .[3]

Some strains of E.Coli producer a heat –labile exotoxins that is under the genetic control of transmissible plasmid. The toxin is adsorbed to gangliosides at the brush border of the epithelial cells of the small intestine . There is stimulation of adenylate cyclase which in turn, result in great increase in the local concentration of cAMP . This mediator cause hyper secretion of water and chloride in to the gut lumen and inhibit re-absorption of sodium resulting in fluid loss . The gut lumen is distended with fluid and an explosive hyper-motility and diarrhea result that last only 1-3 days in most cases, E.Coli O157:H 7 considered as the most toxic strain of E.Coli for human beings .E.Coli especially O serotype 1,2,6,11,15and 75 is the commonest cause of urinary tract infection [3] Persons residing in endemic areas are likely to possess neutralizing antibodies and are less likely to develop diarrhea on exposure to toxin-producing E.Coli than are those without previous exposure to toxin.

Some strains of E. Coli produce a heat stable enter toxin, such e coli can cause mild diarrhea

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especially in children .Certain O serotype of E.Coli have been associated with outbreaks of diarrhea among neonates (e. g : O55,O111,O127) [4] The plasmid carrying gene of enter toxin production may also carry gene of synthesis of specific surface antigen (e.g. k 800) that are essential for attachment of E.Coli to intestinal epithelial cells.[5] This is important in the potential pathogenesis of such microorganisms. The production of enter toxin is unrelated to any specific serotype of E.Coli or to its ability to invade intestinal epithelial cells. But some serotype have been associated more frequently with enter toxin production than have others.[6]

Resistance to antibiotics is highly prevalent in bacterial isolates world wide particularly in developing countries [7] Routine monitoring of antibiotic resistance provides data for antibiotic therapy and resistance control[8] There is a considerable concern about the increasing levels of antibacterial resistance, result from a recent U.K survey show that for enter bacteria- the major urinary tract pathogen , whereas the development of totally new antimicrobial agent takes several years.The re-evaluation of older antibiotics which are not widely used ,can be conducted more rapidly[9]

Residents in and visitors to developing countries acquire antibiotic – resistance E.Coli as part of their normal flora [10] data show that the prevalence of resistance to most drug tested in E.Coli isolates is within the high range reported previously and has increased from 1986 to 1998 .The increase in prevalence of resistance antibacterial drugs were statistically significant .In most drugs tested ,the proportion of resistance isolates has increased rapidly ,so , the usefulness of antibacterial drug which is moderately effective in 1986 has been severely compromised .[10]

Amikacin is aminoglycoside antibacterial drug that act inside the cell by binding to the ribosome in such way that incorrect amino acid sequence are entered in to peptide chain. The abnormal protein which result are fatal to the microbe ,so it is a bactericidal drug .[11]

Cephalexin is one of cephalosporin's group of antibacterial drug that act by impair bacterial cell wall synthesis and hence are bactericidal drug , they exhibit time-dependent bacterial killing.[12]

Nalidixic acid is one of quinolones antibacterial drugs ,act principally by inhibiting bacterial (but not human) DNA gyrase ,so, preventing the super coiling of DNA , a process that is necessary for compacting chromosome s in to the bacterial cell, they are bactericidal and exhibit concentration-dependent bacterial killing.[13]

The present study was designed to investigate sensitivity and resistance pattern of Escherichia coli isolated from different patients against Amikacin, Nalidixic acid and Cephalexin.

Materials and methods

Escherichia coli isolates employed in this study was conducted at AL-SADER teaching hospital in AL-NAJAF AL-ASHREF, IRAQ during the period from 18-1-2006 to 18-4-2007. The sensitivity and resistance pattern of Escherichia coli isolated from the samples brought to hospital laboratory – Microbiology Unit , was determined against some of commonly used antibiotics using disc diffusion method at the hospital laboratory .

Samples comprised of stool and urine from outdoor patients and indoor patients from different wards of the hospital. A 153 samples were identified for sensitivity test against Amikacin , Nalidixic acid and Cephalexin types of antibiotics .

Methods

ISOLATION:
We select the patient from microbiological unit inside the hospital who referred for culture and sensitivity of their samples (stool and urine),then we cultured the samples on blood agar by taking loop full from 3 different places from the sample randomly to get high possibility rate of presence of bacteria and we fallow it for morphology and color of growth and we carry

biochemical test for the final diagnosis of the type of bacteria .We see that : they are red pinkish in color on maconky agar

Biochemical test :

Oxiadase test :

We immerse filter paper in detector oxiadase detector solution and then we added to it part of bacterial culture growth we want to examine it , by sterile stick . appearance of violet color within 10 – 60 seconds indicate that the test is positive. [14]

Catalase test:

We take part of bacterial growth in the culture media and put it on clean slide and added a drop of hydrogen peroxide 3% . release of pulps of oxygen gas indicate positive reaction. [8]

Urease test :

We lining the urea agar by slope line by bacteria we want to examine it " and incubate it for 24 hours by37^o c. The change in the color of agar from yellow to pink indicate the ability of the bacteria to produce urease enzyme. [9]

H2S production test :

We inoculate the clicker agar media by bacteria that we want to examine it by lining and incubate the culture by 37 c for 4 hours . Production of black precipitate in the bottom of the tube indicate production of H2s and the test is positive. [16]

Indol production test :

We inoculate the peptone agar media by bacteria we want to examine it and incubate it by 37 c for 48hours and then we added 0.5 ml from kovacs reagent which contain dimethyl amino benzaldehyde compound with good mixing , the appearance of red ring on the upper layer isoamyl alcohol indicate the reaction is positive. [14]

ANTIBIOTIC SENSITIVITY TEST:

We carry antibiotic sensitivity test for the clinical isolates from the samples of patient against number of antibiotic by using disc diffusion methods as described by Bauer et al (1966). [11]

Preparation of bacterial inoculums:

We prepare the bacterial inoculums for carrying antibiotic sensitivity test by taking loop full added and liner it on nutrient agar and incubated it on 37° c for 24 hour , and then we added to the bacterial culture 10 ml from nutrient broth and mixed the suspension for 30 seconds by using rotating mixture (Vortex) until we notice turbidity in bacterial suspension and we compare this turbidity with tubes containing the standard suspension McFarland which equal to 1.5 x 10 AS 8 cell ml as we added functional salt solution for the culture tubes of high turbidity until the turbidity become equal to the turbidity of McFarland tube.

Then the suspension was cultured by sterile cotton swab on the surface of Muller Hinton agar by homogenous way and buy average of 3 repetitions for each isolates and we leave the dishes for 5 minute to dry.

Application of discs :

The antibiotic discs was distributed on the surface of the culture media agar by using sterile forceps and by average of 5 discs for each dish and to prevent intervention between inhibition zones we leave distance not less than 24 millimeter between discs and another and distance not less than 10 millimeter between the border of the disc and the internal border of the Petri dish and we fix the disc by forceps to ensure the fixing of the discs on the surface of the agar then we incubate the dishes by inverse pattern in temperature of 37 c for 24 hours .

Reading of results :

The result readied by measuring the diameter of zone of inhibition for each disc (which is the transparent zone which is free from bacterial growth) and lie surrounding the antibiotic disc and included the diameter of the disc of antibiotic itself by millimeter by using graded transparent measure tool and the results was compared with the standard average for the antibiotic which mentioned in Quinn et al (1998) [18] according to the listed tables as we classified the bacteria to :

Sensitive (S)
Intermediate sensitivity (I)
Resistant (R)
according to the zone of inhibition .

Antibiotic	Sensitive	Intermediate	Resistant
Amikacin	17	15-16	14
Nalidixic acid	19	14-18	13
Cephalexin	18	15-17	14

National Committee for Clinical Laboratory Standard (NCCLS)

Morphological criteria : they are negative to gram stain and positive to indol test [14] and catalase test [14] .   negative oxiadase test and urease test [8, 9] positive production of H2s [16] and give green color in triple sugar iron test according to all these we diagnosed it as E.Coli bacteria .

Results

Samples:
Distribution of the samples according to there types :

Type of sample	stool	urine
Number of samples	20	31

Distribution of samples according to the total sex number of patient

male	19
female	32

Antibiotic discs

antibiotic	Concentration (microgram)	Source (company)
Amikacin	30	AL_RAZI center
Nalidixic acid	30	AL_RAZI center
Cephalexin	30	AL_RAZI center

Distribution of samples according to the sex of patient

Type of sample	Male	Female	Total
stool	12	8	20
urine	7	24	31
total	19	32	51

A 51 samples of E.Coli collected and 153 sensitivity test done153 , and the results was divided in to sensitive, intermediate, resistant .The results appear that total number of

sensitive test was 63 for all 3 antibiotics drugs, and the total resistant tests was 76 for all 3 antibiotics , while the rest (14) test show intermediate results.

Table 1: numbers of sensitive, intermediate and resistant test of Amikacin , Nalidixic acid and Cephalexin against E.Coli

.	Amikacin	Nalidixic acid	Cephalexin	Total
Sensitive	42= 66.6%	17=26.9%	4= 6.3%	63
Intermediate	7= 50%	2=14.2%	5=35.7%	14
Resistant	2=2.6%	32=42.1%	42=55.2%	76
Total	51	51	51	153

The results show that from a total of 51 test was done to AmikacinAgainst E.Coli ,it was appear that 42 (82.35%) show sensitive results, and 2 (3.92%) resistant,

while the rest 7(13..92%) was intermediate, as shown by table .(1),table . (2) below. And table .3.

Table (2) : The number and percentage of sensitivity of E. Coli to Amikacin , Nalidixic acid and Cephalexin .

Antibacterial drug	No. of sensitive test	percentage of sensitive test %
Amikacin	42	66.66%
Nalidixic acid	17	26.98 %
Cephalexin	4	6.34 %
	63	100 %

Table (3) : The number and percentage of the resistance of E.Coli to Amikacin, Nalidixic acid and Cephalexin

Antibacterial drug	No. of resistant test	percentage of resistant test %
Amikacin	2	2.63%
Nalidixic acid	32	42.16 %
Cephalexin	42	55.26 %
	76	100 %

From a total of 51 test done to Nalidixic acid against E. Coli ,it was appear that 17 (33. 33 %)show sensitive reaction and 32 (62.74 %) show resistance , while the rest 2 (3.29%) show intermediate results (table. 1 , 2 and 3)
From a total of 51 tests done for Cephalexin against E.Coli ,it was appear that 4 (7.84 %) show sensitive results and 42 (82.35 %) show resistance while the rest 5 (9.80 %) show intermediate sensitivity (tables 1, 2 and 3).

SENSITIVITY PATTERN OF E. COLI
We see that Amikacin show the highest percentage of sensitivity by Escherichia Coli (66.66 %) , while Nalidixic Acid wasThe 2nd by (26. 98 %), and Cephalexin was the lowest by (6.34 %

RESISTANCE PATTERN OF E. COLI

We see that Cephalexin show the highest resistance percentage (55.26 %) , and Nalidixic acid was the 2nd by (42.16 %) , While Amikacin was the lowest by (2.63 %) .(Figure no. 2).

Discussion

E.Coli was recognized as an important bacterial pathogen contributing towards hospital infection specially diarrheaial diseases . It can cause infection of gastrointestinal tract and urinary tract infection which is one of the most common infectious disease in our locality specially in children and young age groups.

In the present study, the culture sensitivity pattern was assessed for E. Coli from stool and urine and high resistance was recorded with Cephalexin

(82.35 %) , fallowed by Nalidixic acid (62.74 %) , John et al [13]) reported that in Belgium in 2001 the resistance of Nalidixic acid by E.Coli was about 36 % while in Italy in 1998 reach about 38 %. Our possible explanation for the very high percentage of resistance to Nalidixic acid was :

1-excessive use of the antibiotics without culture and sensitivity.

2-Availability of these antimicrobial drugs for all people.

3-Easy to get antibacterial drugs without prescription paper.

Our study show that E.Coli resist Amikacin by 3.92 % which is very low percentage which can be explained by :

1- Unavailability of Amikacin in our locality .

2- Its injectable dosage form that lead to its limited distribution as compared with oral dosage form.

3-Non commonly used as compared with other types of antibiotics. specially for treatment of diarrheaial disease and urinary tract infections.

Our study show that Amikacin was found to be the most effective against E. Coli with sensitivity percentage of (82.35 %) such high sensitivity percentage should encourage our doctors in our locality (especially in Al-NAJAF AL-ASHREF) to use Amikacin as drug of choice or at least one of the first line drugs of treatment E.Coli .

Also our study reported that sensitivity of E.Coli to Nalidixic acid was

(33.33 %) , Iruka et al [14] reported that sensitivity to Nalidixic acid was (30.2 %) / this little increase in our percentage of sensitivity can be explained by uncommon use of Nalidixic acid as first line drugs for treatment of infection with E.Coli.

While about sensitivity of E.Coli to Cephalexin , our study show that it had a lowest percentage of sensitivity (7.84 %) , this very low percentage can be explained by ;

1-Cephalexin is an old antibiotic (1st generation Cephalosporin) that mean they used for long time for treatment of infection.

2-Its oral dosage form make him available for most of people very easy .

3-Excessive use of Cephalexin without culture and sensitivity .

Conclusion

1- Amikacin is the most sensitive antibacterial drug for E.Coli (82.35%) comparing to Cephalexin and Nalidixic acid.

2-Cephalexin is the most resistant antibacterial drug for E.Coli(82.35 %) Comparing to Amikacin and Nalidixic acid.

3-Physicians should take care of this side in antibacterial drug during the treatment of bacterial diseases.

References

1-B.Maraha ,et al :the impact of microbiological culture on antibiotic prescribing.clin. Microbiol. Infect. 6: 99- 102. 2000.

2-R.Skov et al : study of susceptibility of E.Coli. Journal of Antimicrobial Chemotherapy. 45: 920-921.2000.

3- Ernest Jawetz, Joseph L. Mel nick , Edward A. Adel berg " Review of medical microbiology. p.230. 1980

4- Calva JJ, Sifuentes – O sorino J . " Antibacterial resistance in fecal flora : longitudinal community based surveillance of children from urban Mexico ." antimicrobial agents chemotherapy .; 40: 1699-1702. 1996

5- Lamikanra A, Okeke IN . A study of the effect of the urban and rural divided on the incidence of antibiotic resistance in Escherichia coli . Biomed.; 55; 91-7. 1997

6- Hoge CW et al. " Trends in antibiotic resistance among diarrhea pathogens isolated in Thailand over 15 years." Clin. Infect. Dis.; 26: 341-5. 1998

7- Hart CA, Kariuki S. " Antimicrobial resistance in developing countries " Med J.; 317; 647-50. 1998

8- O'Brien .T. " The global epidemic nature of antimicrobial resistance and need to monitor and manage locally ." Clin. Infect. Dis.; 24; supply. 1 ; s 2-8. 1997

9- L B jerager, et al. " In vitro study of the susceptibility of Escherichia coli to mecillinam" Journal of Antimicrobial chemotherapy . 45, 920- 921. 2000.

10-Susan . T. et al :antibiotic resistance in E.Coli.   Emergining   Infectious Disease.Vol.6,No.4.Jul-Aug.2000.

11-P.N.Bennett, M. J. Brown: Clinical Pharmacology: page : 223-225.2003

12- P.N. Bennett, M.J .Brown: Clinical Pharmacology: page : 221.2003.

13- P.N .Bennett, M.J. Brown: Clinical Pharmacology: page : 232-233.2003.

14-Baron .E.L. and Fine gold S.M. Bailey and Scotts : Diagnostic Microbiology .8Th ed. C. V. Mosby comp. U.S.A.1990.

15- Cruickshank R. et al: Medical Microbiology Vol .II :The practice of medical microbiology 12th ed. London .Churchill Livingstone . 1975

16- Lennette . E. et al :American society for microbiology 4th ed. Washington .D.C. 1985.

17- Bauere A.W. Kirby w. m. Sherries .C. and truck .M: antibiotic susceptibility testing by standarazied single disc method Am. J. Clin. Pathol. :945:493-496. 1966.

18- Quinn D .J. Carter M.E. Markey B. and Carter G.R .:Clinical Biology C.V. Mosby company U.S.A. 1998.

19- John M. et al: Integrating Escherichia coli antimicrobial susceptibility data from multiple surveillance programs . Margining infectious disease Vol 11.nmumber 6. June. 2005.

20- Iruka N. et al: Antibiotic resistance in Escherichia coli from Nigerian students 1986-1998 . Emergencies infectious disease . Vol. 6, NO.4. 2000.

الخلاصة

الاشريشيا القولونية واحدة من أهم أنواع البكتريا التي تسبب الأمـراض للإنسـان خصوصـا أمـراض الإسـهال والتـهابـات الأمعـاء والمجـاري البوليـة و لغـرض دراسـة مـدى حساسـيتها ومقاومتهـا للمضـادات الحيويـة مثـل الاميكاسـين والناليدكسـيك أسيد وكذلك السيفالكسين قمنا بأجراء الدراسة الحالية على ٥١ عينة من عزلات البكتريا من عينـات البول والغـائط لمجموعـة من المرضى الراقدين في مستشفى الصدر التعليمي في محافظة النجـف الاشـرف أو المـراجعين للعيـادة الاستشــارية لــنفس المستشفى وقمنا بـإجراء اختبـار الحساسـية لكـل عزلـة من هذه العـزلات البكتيريـة تجـاه هـذه الأنـواع الثلاثـة مـن المضـادات الحيوية ، وقد أظهرت الدراسـة إن الاميكاسين هو أكثر أنواع

المضادات الحيوية تحسسا من قبل البكتريا المعنية مقارنة بباقي الأنواع المشمولة بالدراسة الحاليـة وبنسبة مئويـة مقدارها (٦٦.٦٦ %) بينما كان السيفالكسين اقل الأنواع تحسسا من قبل البكتريا بنسبة قدرها(٦.٣٤ %) مقارنـة ببـاقي الأنواع الثلاثة من المضادات الحيوية المشمولة بالدراسة.

وخرجنا من هذه الدراسة باستنتاج أن الاميكاسين هو المضاد الحيوي الأكثر تحسسا من قبل الاشريشيا القولونية إذا ما قورن بالناليدكسيك أسيد أو السيفالكسين بينما السيفالكسين هو المضاد الحيوي الأكثر مقاومة من قبل الاشريشيا القولونية إذا ما قورن بالاميكاسين أو الناليدكسيك أسيد.