Re evaluation Of The Activity Of Some Antibacterial Drugs Against Clinical Isolates Of Staphylococcus Aureus In Al_Najaf Al_Ashref Governorate.

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الخلاصة :
خلفية البحث:المكورات العنقودية واحدة من اكثرانواع البكتريا المسببة للأمراض في الإنسان وطبيعة الإمراض الناتجة منها تمتاز بالمدى الواسع حيث تبدأ بالتهاب الجلد البسيط ويمكن أن تنتهي بتسمم الدم واحتمالية الموت .
هدف الدراسة: في هذه الدراسةا حاولنا اختبار مدى حساسية البكتريا العنقودية لأربعة أنواع من المضادات الحيوية وهي الجنتامايسين والكوترامیثوبریم والاموكسسلين والسيفالكسين ومن خلال العينات البالغ عددها 55 عينة من البول والفم والجروح والقيح من مرضى مستشفى الصدر التعليمي في مدينة النجف الاشرف – العراق في الفترة الممتدة من 9-1—2009 لغاية 8-3-2010 .
النتائج: أظهرت الدراسة أن الجنتامايسين كان أكثر أنواع المضادات الأربعة تحسسا من قبل البكتريا وبنسبة تحسس مقدارها 34.83% بينما أظهرت الدراسة أن والاموكسسلين اظهر أعلى نسبة من المقاومة(مقارنة بباقي الأنواع من البكتريا) ومقدارها 30%.
الاستنتاج: ان الجنتمايسين هو المضاد الحيوي الافضل والانسب لعلاج التهابات المكورات العنقودية في النجف الاشرف.
التوصيات:نوصي ان تقوم الدوائر المعنية باعادة تقييم دوري للمضادات الحيوية المستعملة بشكل دوري بالتعاون مع الباحثين في هذا المجال في كلية الطب.

Abstract :
Background: Staphylococcus aurous one of the most common pathogen that cause a wide range of infection started by simple skin infection and end by septicemias and high possibility of death .
The study aimed to: present study try to evaluate the sensitivity and resistance pattern of staphylococcus aureus against Gentamycin, Cotrimethoprim, Amoxicillin and Cefalexin .
Methods: A 55 isolates of staphylococcus aureus obtained from urine, pleural fluid, joint aspiration, ear, skin, and pus, of indoor and outdoor patient in AL-SADER teaching hospital, AL-NAJAF AL-ASHREF, IRAQ. From a period extended between 9-1-2009---- 8-3-2010.And each isolate was tested for these 4 antibacterial drug .
Results: and results showed that Gentamycin has the highest percentage of sensitivity by staphylococcus aureus (34.83%) while Amoxicillin showed the highest percentage of resistance by staphylococcus aureus (30%).
Conclusion: Gentamycin was the drug of choice in treatment of staphylococcus aureus infection in al-najaf al-ashref.
Recommendation: we recommend further evaluation about antibacterial sensitivity together with college of medicine.
Key words: staphylococcus aureus: antibacterial drugs

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INTRODUCTION:

Disease caused by *staphylococcus aureus* vary from localized inflammation and end by fatal generalized septicemia. For this reason it became very important for physicians to know the resistance and sensitivity pattern for the dangerous pathogens like *staphylococcus aureus* .

Staphylococcus aureus is one of the most versatile nasocomial and dangerous human pathogen since publication of its risk role in sepsis by ogston in 1880 and 1882 (1). In spite of the introduction of antimicrobial agents and improvements in the frequency and morbidity of staphylococcal disease in the twentieth century. staphylococci have persist as an important hospital and community pathogen. They are responsible for more than 80 percent of the suppurative diseases encountered in medical practice and are second to *E. Coli* as a cause of hospital acquired infections regard as a secondary pathogen after *E.coli*.(2)

The wide spread use of penicillin in 1950 s saw the spread of penicillin resistant staphylococcus aureus in hospitals . after this time methicillin and its derivatives became the drug of choice in treatment of infection caused by this organism. At the same time methicillin – resistant staphylococci were reported from USA and Italy and even before methicillin was widely used , a strain of S. aureus with natural resistance to this antibiotic was identified by jevans in1961. therefore methicillin-resistant .S. aureus emerged as major pathogen world wide.

Several out-breaks were documented in many parts of the world caused by methicillin-resistant *S. aureus* , these epidemic strains was labeled as EMRSA (3) . Vancomycin has long been considered that antibiotic of last resort against serious and multi-drug resistant infection caused by Gram- positive bacteria , however ,Vancomycin resistance has emerged more recently in *staphylococcus aureus*. (4)

Hospital strains of S. aureus are usually resistant to a variety of different antibiotic . Few strains are resistant to all clinically useful antibiotic except Vancomycin , some worker have reported , however the presence of Vancomycin resistant strains.(5)

AIM OF THE STUDY:

The present study was designed to investigate sensitivity and resistance pattern of Gentamycin, Cotrimethoprim, Amoxicillin and Cefalexin against *staphylococcus aureu*s isolated from different patients .

MATERIALS AND METHEDS:

Staphylococcus aureus isolates employed in this study was conducted at AL-sader teaching hospital in AL-najaf AL-ashref, Iraq during the period from 9-1-2009 to 8-3-2010. The sensitivity and resistance pattern of *staphylococcus aureus* isolated from the samples brought to hospital laboratory – Microbiology Unit , was determined against some of commonly used antibiotics using disc diffusion method at the hospitals laboratory .

Samples comprised of urine, wound swab, ear swab, aural swab and pleural fluid from outdoor patients and indoor patients from different wards of the hospital. Out of 351 growth positive samples, 220 were *staphylococcus aureus* . These 220 *staphylococcus aureus* samples were identified for sensitivity test against Gentamycin, Co- Trimethoprim, Amoxicillin and Cefalexin types of antibiotics .

METHODS:

Preparation of culture agar :

1-Muller – Hinton agar:

Prepared as mentioned by (Oxoid) by dissolving 38 gm from the agar in one liter of distilled water , then boiling for one minute , and then sterilized by Autoclaving in 121 c for 15 minutes and then cooling to 50 C in water bath .

2-Blood agar:

Prepared by dissolving 40 gm from the agar in one liter distilled water and sterilized by autoclaving and then added 5% from blood to the agar after cooling the agar to 45 C.

3-Nutrient agar;

We dissolve 23 gm from the culture in one liter of distilled water and sterilized by autoclaving .

ANTIBIOTIC SENSITIVITY TEST:

the antibiotic sensitivity test for the clinical isolates was carried out for the samples of patient against number of antibiotic by using disc diffusion methods as described by Bauer et al (6):

Preparation of bacterial inoculums:

the bacterial inoculums were prepared for carrying antibiotic sensitivity test by taking loop full added of bacterial growth and liner it on nutrient agar and incubated it at 37 c for 24 hour . and then added to the bacterial culture 10 mill from nutrient broth and mixed the suspension for 30 seconds by using rotating mixture (Vortex) until notice the turbidity was noticed in bacterial suspension and we compare this turbidity with tubes containing the standard suspension McFarland which equal to 1.5 x $10^{n}8$ cell ml as 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 were 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 distance leaved in 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 the disc was fixed by forceps to ensure the fixing of the discs on the surface of the agar then plate incubated by inverse pattern in temperature of 37 c for 24 hours .

Isolation:

The samples were cultured on blood agar by taking a loop full from 3 different places of sample randomly to get high possibility rate

of presence of bacteria and fallowed it for morphology. and color of growth, whether they ferment lactose or not and carrying biochemical test for the final diagnosis of the type of bacteria .

they were yellow in color near gold color on blood agar with complete hemolytic .

Morphological criteria : they were spherical cells positive for gram stain arranged in grape- like arrangement and spherical in shape and gave negative oxiadase test and positive catalase test ,coagulase test and

growth on manitol salt agar and according to all these we diagnosed it as staphylococcus aureus bacteria .

RESULTS :

The result was recorded by measuring the diameter of zone of inhibition for each disc (which was the transparent zone, 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) (7) according to the listed tables as we classified the bacteria to :sensitive (S)
intermediate sensitivity (I)
resistant (R)
according to the zone of inhibition .

Table(1): Distribution Of Samples According To The Sensitivity

Antibiotic	sensitive	intermediate	resistant
Gentamycin	**15**	**13-14**	**12**
Co-Trimethoprim	**16**	**11-15**	**10**
Cephalexin	**18**	**15-17**	**14**
Amoxicillin	**29**	**21-28**	**20**

National Committee for Clinical Laboratory Standard (NCCLS) Samples:

Table (2):Distribution of the samples according to there types :

Type of sample	Wound swab	Ear swab	oral swab	Urine swab	Pleural fluid swab
Number of samples	**31**	**10**	**6**	**4**	**4**

Table (3):Distribution according to the total sex number of patient.

Gender	number	%
male	**36**	**65.4**
female	**19**	**34.6**

This table show distribution of male-female in all sample collected and their percentage**.**
Antibiotic discs:

Table (4): origin of antibiotic disc sensitivity tests

antibiotic	Concentration (microgram)	Source (company)
Gentamycin	**10**	**AL_RAZI center**
Co-trimethoprim	**25**	**AL_RAZI center**
Amoxicillin	**10**	**AL_RAZI center**
Cefalexin	**30**	**AL_RAZI center**

Table (5):Distribution of samples according to the gender of patient

Type of sample	Male	Female	Total
Wound swab	20	11	31
Ear swab	8	2	10
oral swab	5	1	6
Urine swab	1	3	4
Pleural swab	2	2	4
total	36	19	55

RESULTS:

A total of 220 sensitivity test was done to 55 isolates of *staphylococcus aureus* and the results was divided in to sensitive, intermediate, resistant The results appeared that number of total sensitive tests was 89 for all 4 antibiotics drugs, and the total resistant tests was 90 for all 4 antibiotics , while the rest (41) tests show intermediate results.

Table (6): resistance of some drugs against staphylococcus aureus..

	Genta.	Cotrimetho.	Amoxi.	Cefale.	Total
Sensitive	31	14	14	30	89
Intermediate	7	15	14	5	41
Resistant	17	26	27	20	90
	55	55	55	55	220

This table showed that from a total of 55 test was done to Gentamycin against staphylococcus aureus ,it was appeared that 31 (34.83%) showed sensitive results, and 17 (18.88%) resistant, while the rest 7 was intermediate, as shown by table no.(6). And table no. (7) below. and table no(.8).

Table (7) : sensitivity of Staphylococcus aureus to Gentamycin, Co-Trimethoprim, amoxicillin and Cefalexin .

Antibacterial drug	No. of sensitive test	Percentage of sensitive test (%)
Gentamycin	31	34.83 %
Co-Trimethoprim	14	15.73 %
Amoxicillin	14	15.73 %
Cefalexin	30	33.70 %
Total	89	100 %

This table show as the sensitivity of all group of antibiotics and the percentage of every one of theme.

Table:(8) :resistance of Staphylococcus aureus to Gentamycin, Co-Trimethoprim, Amoxicillin and Cefalexin .

Antibacterial drug	No .of resistant test	Percentage of resistant test %
Gentamycin	**17**	**18.88 %**
Co-Trimethoprim	**26**	**28.88 %**
Amoxicillin	**27**	**30.00 %**
Cefalexin	**20**	**22.22 %**
Total	**90**	**100%**

This table show as the resistance of all group of antibiotics and the percentage of every one of theme.

From a total of 55 test done to Cotrimethoprim against *Staphylococcus aureus* ,it was appear that 14 (15.73 %)show sensitive reaction and 26 (28.88 %) show resistance , while the rest (15) show intermediate results (table no:(6)and table no.(7)and table no:(8).

A 55 tests were carried out to amoxicillin against *staphylococcus aureus*, the result appeared that 14 (15.73 %) showed sensitive reaction , and 27 (30 %) show resistance , while the rest 14 show intermediate sensitivity as shown by table no. 6and table no. 7and table no.8.

We carried a 55 test to Cefalexin against *staphylococcus aureus* ,it was appeared that 30(33.70 %) showed sensitive reaction and 20(22.22 %) show resistance while the rest 5 show intermediate sensitivity as shown by table no . (6)and table no. (7)and table no.(8).

SENSITIVITY PATTERN OF staphylococcus aureus :

It was seen that Gentamycin has the highest percentage of sensitivity by staphylococcus aureus 34.83 % , while Cefalexin was The 2nd by 33.70 %, and both Co-Trimethoprim and Amoxicillin was the lowest by 15.73 %. (Figure no. 1).

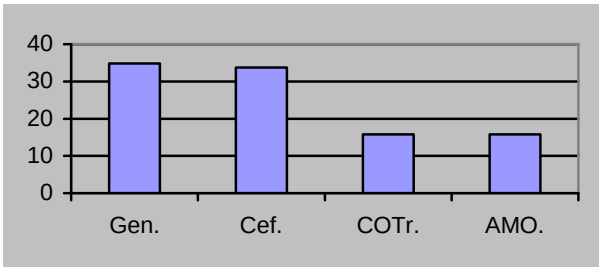

Figure no. (1): sensitivity pattern of staphylococcus aureus against Some antibiotics.

RESISTANCE PATTERN OF STAPHYLOCOCCUS AUREUS:

We see that Amoxicillin show the highest resistance percentage 30 % , and Cotrimethoprim was the 2nd by 28.88 % ,then Cefalexin by 22.22 % while Gentamycin was the lowest by 18.88 % (Figure no. 2)...

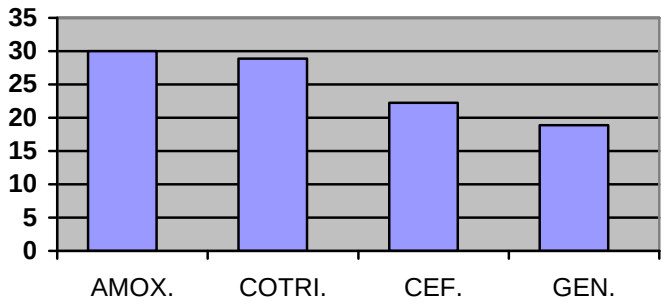

Figure no(.2): resistance pattern of *staphylococcus aureus* against Some antibiotics

Results distribution in both sensitive and resistant samples according to the sex of the patient as shown by table (9) and table number (10).

Table: (9): Distribution of sensitive tests according to type of antibiotic and sex of the patient

	Sex of patient					
	male		Female		Total	
	No.	%	No	%	No.	%
Gentamycin	20	64.52%	11	35.48%	31	1oo%
Co-trimethoprim	9	64.28%	5	35.72%	14	100%
Amoxicillin	10	71.43%	4	28.57%	14	100%
Cefalexin	18	60%	12	40%	30	1oo%
Total	57	64%	32	36%	89	100%

In this table we can recognize the number of sensitive test for every patient and their percentage from the total sensitive tests that will help as in known of their percentage in the total samples.

Table (10) :Distribution of resistant tests according to type of antibiotic and sex of the patient

	Sex of patient					
	male		. Female		Total	
	No.	%	No	%	No.	%
Gentamycin	8	47%	9	53%	17	1oo%
Co-trimethoprim	10	38.46%	16	61.54%	26	100%
Amoxicillin	13	48.14%	14	51.86%	27	100%
Cefalexin	14	79%	6	30%	20	1oo%
Total	45	50%	45	50 %	90	100%

In this table we can recognize the number of resistance. test for every patient and their percentage from the total sensitive tests that will help as in known of their percentage in the total samples

DISCUSSION:

Staphylococcus aureus was recognized as an important bacterial pathogen contributing towards hospital infection , globally , staphylococcus aureus causes localized infection spreading in to the blood stream (8). Despite the use of potent antibiotic , still high mortality exist in case of staphylococcus aureus infection. In the present study , the culture sensitivity pattern was assessed for staphylococcus aureus from ear, pleural fluid, aural, urine ,joint aspiration and pus and high resistance was recorded against Amoxicillin (30 %) fallowed by Cotrimethoprim by 28.88 % , Sddqui et al (9) reported a similar results while Kalssom et al (10) reported that resistance of staphylococcus aureus to Cotrimethoprim was more than (30%) . Our possible explanation for this high percentage of resistance to Cotrimethoprim was the excessive use of Cotrimethoprim without culture and sensitivity that may lead to increase in the resistance of staphylococcus .

Present study showed that *staphylococcus aureus* resist Cefalexin by 22.22 % , Mahmood (11) reported that 29% resistant pattern of isolates of staphylococcus aureus against Cefalexin .possible explanation for this relatively low results was due to uncommon use of Cefalexin as a drug of choice against *staphylococcus aureus* in our locality that lead to maintain activity (as compared with the others) of Cefalexin against staphylococcus aureus. In case of Gentamycin , present study showed that Gentamycin resistance pattern to *staphylococcus aureus* was 18.88 % while Namias (12)reported that Gentamycin resistance pattern against staphylococcus aureus was 30%,while Trejo(13) reported the percentage of Gentamycin resistance as 29.4%, and Sddqui report ed it by more than 20% , while Kalssom (10) reported it

by 22%. possible explanation to our low results(as compared with the others)percentage of resistance of Gentamycin to *staphylococcus aureus* was the small prescription and uncommon use of Gentamycin as first line antibiotic in treatment of staphylococcus aureus infection .Present study showed that Gentamycin was found to be the most effective against *staphylococcus aureus* (34.83%) from the 4 antibacterial drugs that included in our study, Kalssom (8) reported that sensitivity of Gentamycin was 41.99% .possible explanation for this relatively high percentage of sensitivity was uncommon prescription of Gentamycin for treatment of *staphylococcus aureus* in our locality ,in spite of the last years show increased in the use of Gentamycin for treatment of *staphylococcus aureus* infection.

Moreover, when low doses of antibiotics are used against bacteria , they inhibit the growth of susceptible bacteria , leaving the smaller number of already resistant bacteria to thrive and grow. These bacteria spread their resistant strain to other previously non resistance cells then eventually affecting other cells (11).

The study documented the importance of staphylococcus aureus as important Gram-positive pathogen , and increasing resistance in commonly used antibiotics . although the high cost and inappropriate use of antibiotics have been documented and the long course s of prophylactic antibiotics may lead to increase resistance to antimicrobials, increase incidence of drug reaction and increased money cost.

CONCLUSION:

Gentamycin was the most sensitive antibacterial drug by staphylococcus aureus.

Amoxicillin was the most resistant antibacterial drug by staphylococcus aureus in our study.

There is no difference in total number of resistant patient between male and female .

The male show higher number of sensitive test as compared with female .

RECOMMONDATIONS:

Physicians should take care of this side in antibacterial drugs during treatment of bacterial diseases.

REFERENCES:

1- Lowy, F. D. " Staphylococcus aureus infection" new eng. J . Med ., 339,520-532.

2- "Resistance pattern of clinical isolates of staphylococcus aureus against five groups of antibiotic " Kalssom farzana et al . journal of research (science) zakariya university , Pakistan . vole .17 no. 1 January 2006 . pp19-26.

3- Kellkar, R. (2002)"Methicillin resistant Staphylococcus aureus an expensive battle with the most versatile human pathogen. Bhj: journal /1997/3901-jan.

4- Boneca , I.G., Chiosis,G.(2003) " Vancomycin resistance : occurrence mechanisms and strategies to combat it " Expert Open there targets ,7 , 311-328.

5- Shakibaie , M.R, Mansouri , S. and Hakak , S. (2003) " Plasmid pattern of antibiotic resistance in beta- lactamase producing staphylococcus aureus isolated from hospital in Karman, Iran." AIM: 9922.

6- Bauer ,A,W; Kirby W.M.M, Sherries. J. C. and Truck M,(1966) : Antibiotic Susceptibility testing by standardized Single disc Method AM. J. Clin.Pathol..945: 493-496 .

7- Quinn. P. j. carter M .E. Markey, B, and Carter G.R.(1998) Clinical Biology C. V, Mosley Company U.S.A.

8-Espersen, F.“ Identifying the patient risk for staphylococcus aureus blood stream infection’s. Chemotherapy , 7,11-17.

9- Sddqui , F.M. , Bint-e- Masood , Noor-us- saba , samad , A. , Qayyum , M. and Qazilbash, A.A.(2002)” Antibiogram sensitivity pattern of Methicillin –resistant staphylococcus aureus isolation from pus samples” Pakistan Journal of biological science , 5, 491-493.

10- Kalssom Farzana, and Abdul hameed . “ Resistance pattern of clinical isolates of staphylococcus aureus against five groups of antibiotics” Journal of Research, Multan , Pakistan. Vol. 17,No. 1, January 2006.pp 19-26.

11- Mahmood, A. ,Rafique, S., Qayyum, M., Qazilbash, A.A.(2001) “Prevalence of nasocomial and community – based Methicillin – resistant staphylococcus aureus (MRSA)” Pak. J. Med. Res., 40, 86-89.

12- Namias,N.,Harvill,.S., Ball, S., McKinney, M.G., Salomone, J.P., Sleeman,D. and Civetta, J.M. “ Empiric therapy of sepsis in the surgical intensive care unit with broad - spectrum antibiotic for 72 hours does not lead to the emergence of resistant bacteria” Journal of trauma injury infection and critical care, 45,887-891.

13-Carig, W.A. “Pharmacokinetic/ Pharmacodynamic parameters: rationale for antibacterial dosing of mice and men”. Clin.Infect.Dis.26,1-12.