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Evaluation of the efficacy of antibiotics in the treatment of Staphylococcus aureus isolated from clinical cases in Al-Dawwaniyah hospitals

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***Abstract*---**Staphylococcus aureus, especially resistant to methicillin, is one of the Basic causes of hospital infection, its resistance to antibiotics has constituted a public health problem worldwide, due to the lack of studies related to the examination of sensitivity to antibiotics, especially in methicillin-resistant species in Iraq and in the city of Diwaniyah in particular. Therefore,The current study aimed to find out the prevalence rate antibiotic resistance in these bacteria isolated from different clinical sources in some hospitals in the governorate. During the research, 554 clinical samples (221 urine samples, 178 sputum samples, 100 burns samples and 55 injuries) for the period from the 11th of 2021 to the 3rd of 2022. Relying on the results of in vitro culture and conventional microscopic and biochemical assays, 100 isolates of Staphylococcus aureus were identified, using the cefoxetine disc spread method, as 80 isolates belong to MRSA. The results of the antibiotic susceptibility test were examined retrospectively. Using the method (Kirby Bauer) using 20 antibiotics used in the current study and it was found that (78%) Of these bacteria were multidrug resistant (MDR). And (22%) of these isolates are resistant to the widely used drug XDR, while the pattern of resistance to PDR was not identified in our study. The MAR value ranged from 0.05 to 0.75, which is an indication of excessive use of antibiotics in the place where the bacteria originated.

***Keywords*---**evaluation efficacy, antibiotics treatment, Staphylococcus aureus, clinical cases.

Introduction

Staphylococcus aureus, which belongs to the family Micrococcaceae, is a positive bacterium to Gram staining, positive for catalase, and positive for coagulease assay. Staphylococcus aureus. Common in nature, plants and humans.. These resistant isolates can become contagious in a variety of clinical situations ranging from superficial infections to severe life-threatening infections. Therefore, Staphylococcus aureus must be isolated frequently from both community-acquired and hospital-acquired infections.

Bacteria that are resistant to antibiotics methicillinare calledMRSA is a major cause of mortality and morbidity. MRSA can easily be transmitted from one infected person to another through the hands of employees and can lead to epidemics. Colonization MRSA is prevalent in hospitals. MRSA colonization rates range from 20% to 30%. Great efforts have been made towards the prevention of hospital-acquired MRSA infection, and many plans have been put in place to combat it until it remains widespread and widely in the community or health institutions. This study aims to know the percentage of antibiotic resistance in these bacteria and to determine the percentages of MRSA isolated from clinical samples in Al-Diwaniyah Governorate hospitals

Materials and Methods

During the period from the eleventh month of 2021 to the third month of 2022 retroactively. Culture samples were collected from patients from different locations, namely abscess, wounds, urine and sputum by routine methods. Clinical samples were cultured in blood agar medium and then incubated at 37°C for a period ranging from 18 to 24 hours. Isolates analyzed on blood agar at the end of this period were determined by morphology of the developing colony, Gram stain, catalase test, DNAase test and coagulation. Sensitivity assay for all isolates was performed using a spread-on-disc method by referring to CLSI standards. Statistically, an average test and comparison were performed to determine rates of resistance against methicillin and other antibiotics.

Results

During the current study, 554 different clinical samples were collected. 100 (18%) of these S. aureus isolates were obtained from burns, 55 (9%) from wounds, 178 (32%) from sputum, and 221 (39%) from urine. Table No. 1 shows the distribution of isolated isolates by isolation percentage and source

Table (1): distribution of bacterial isolates according to isolate sources

Sample type and source	total number of samples	NO.of samples container on the growth bacterial	NOr of samples devoid of the growth bacterial	number of isolates staphylococcus	number of isolates S. aureus

burns	100	44	56	28	11
wounds	55	44	11	18	11
sputum	178	112	66	66	24
urine infection	221	166	55	111	55
the total number	554	366	188	223	100
percent	100%	66%	34%	40%	18%

Bacterial sensitivity to antibiotics

100 isolates were tested Staphylococcus aureus is under study towards 20 antibiotics Using the disk spread method according to CLSI 2020 and CLSI 2016 and table (2) shows percentages of resistant, moderately resistant and sensitive bacterial isolates to antibiotics used.

Table (2): Percentages of susceptibility testing for 100 isolates

name of antibiotic	Resistant	Intermediate	Sensitive
Penicillin(P)	96%	0%	4%
Cefoxitin(FOX),	80%	0	20%
Moxifloxacin(MFX)	61%	5%	34%
Erythromycin(E),	59%	15%	26%
Azithromycin(azm)	57%	6%	37%
Levofloxacin(LEV)	42%	8%	50%
CLARITHROMYCIN(CL)	40%	0%	60%
Amikacin(AK)	35%	20%	45%
Clindamycin(CD),	35%	13	52%
Ofloxacin (OFX)	34%	7%	59%
Tetracycline(TE),	31%	0%	69%
Doxycycline(DOX)	22%	0%	78%

name of antibiotic	Resistant	Intermediate	Sensitive
Penicillin(P)	96%	0%	4%
Rifampin(RE)	19%	0%	81%
Norfloxacin(NOR)	15%	0%	85%
Trimethoprim(TMP)	15%	0%	85%
Gentamicin(GM)	13%	2%	85%
Trimethoprim-Sulfamethoxazole (SXT)	12%	0%	88%
Chloramphenicol(C)	5%	95%	95%

The results of the sensitivity test showed a clear variance in the response of the bacterial isolates under control The study of the antibiotics used, as the isolates showed the highest rates of resistance towards the antibiotic penicillin with a percentage of 96%, and this result was very close to what was found in a study (Akanbi et al. 2017) As bacteria were resistant to penicillin by 96,7 , the reason for this increased resistance may be due to its irregular and continuous use, which enhances the resistance of the stimulating bacteria, while resistance to the Chloramphenicol Where the results we obtained are the same as what was found in the study(Al-Zoubi et al. 2015) Which was 5% The results Show that the second highest percentage resistance after penicillin to the antagonist Cefoxitin,which is due to For cephalosporins,, the percentage of bacterial resistance to it was 80% which is lower than what was found in a study(Al-Hasnawy et al. 2019) in Babylon

As for macrolides antibiotics, the rate of resistance to erythromycin was 59% as it is very close to the study(Hashemzadeh et al. 2021) Where bacteria resisted this antibacterial by 58%, the reason for the high percentage of resistance as a result of its use These antibiotics are widely used to treat staphylococcal infection in Iraq, and this is an indication of resistance to modern macrolides such as Clarithromycin According to a study(Goyal et al. 2004), where the rate of resistance to the antibiotic Clarithromycin was 40%, and this result is consistent with what was reached(Zhou, Li, and Yan 2020).As for the antibiotic Azithromycin, where the results we obtained are 57%, the same as what was found in the study(Asbell, Sanfilippo, and Mah 2022) . As for the antibiotic Clindamycin, it recorded a resistance rate of 35%, which is higher than the percentage recorded in the study The same precedent(Assefa 2022) where the percentage was 19,8% .

As for the tetracyclines antibiotics represented by tetracycline and Doxycycline The isolates were resistant to tetracycline by 31% and to Doxycycline by 22% thus, The percentage of resistance to doxycycline was very close to what was

found by the researcher (Fri et al. 2020) (Gandhi et al. 2020), where the resistance rate was 30%, ,Staphylococcus aureus resists tetracyclines through two mechanisms: active influx by genes encoded by plasmid, and ribosomal protection by transposon or chromosomally encoded genes(Graber 2021). As for quinolone antibiotics and subsequent derivatives such as fluoroquinolones, the antibiotic ofloxacin recorded a resistance of 34%, and this is the same percentage that was obtained in a study(Adeoye-Isijola et al. 2020).

As for antibiotics by the two antibiotics, Ciprofloxacin and Norfloxacin, the isolates were resistant to them by 12% and 15%, respectively. while found by the researcher(Mohamed et al. 2020), where the resistance to the antibiotics ciprofloxacin and norfloxacin is 25% and 0%, respectively.As for the antibiotic Levofloxacin, the results we obtained are 42%, and this is close to the percentage obtained in the study(Preeja, Kumar, and Shetty 2021) where the antibiotic resistance rate is 41,2%. As for the resistance to the antibiotic moxifloxacin, where the results we obtained are 61%, and this is somewhat close to the percentage obtained in the study(Alseqely et al. 2021) where the rate of resistance to the antibiotic is 64%.

As for the pathway folate antibiotics represented by Trimethoprim Sulfamethoxazole (SXT) and Trimethoprim (TMP) , the resistance to them was 12 % and 15%, respectively, Where the results we obtained are very similar to what was found in the study (Kwoji et al. 2017), where the resistance to the antibiotics (TMP) and (SXT) is 11%. The isolates showed the resistance to the antibiotic Nitrofurantoin is 9%, and this result is very close to the findings of the study(Chaudhary, Bisht, and Faujdar 2021), where the resistance rate was 10.5%. The decrease in bacterial resistance to nitrofurantoin is attributed to the multiple mechanisms of resistance development The limited use of this antibiotic to treat staphylococcal infection may limit the development of resistance to it.

As for the antibiotic Rifampin, the isolates showed a resistance rate of 19%, and thus it was higher than The percentage reached by researchers in the study (Al-Hasnawy et al. 2019) in Iraq, where it reached 9.5%, the aminoglycosides represented by the antibiotics Amikacin and Gentamicin, which have an effective effect against clinically important staphylococci through their association with the 30s ribosomal unit and their interference with protein synthesis, which leads to a fatal effect on bacteria(Khusro et al. 2021) the isolates showed the resistance to the antibiotic Gentamicin was 13%, and this result is very close to the findings of the study (Adzitey, Ekli, and Aduah 2020) where the resistance rate was 12,5%, While the isolates showed the percentage of resistance to the antibiotic Amikacin was 35%, and this result is consistent with of the study, (Ahmed et al. 2022)where the same resistance rate was,The widespread use from aminoglycoside therapy has led to the development of aminoglycoside-modifying enzymes AMEs, the more common mechanism of acquired resistance to these antibiotics in S. aureus, as well as other resistance mechanisms such as target modification, flux pumps, target mutants and mutants (Kong et al. 2020).

Here, it is necessary to explain the reasons for the difference in the proportions of antibiotic resistance in these bacteria in the studies. This is due to the excessive and indiscriminate use of drugs, as constant exposure to bacteria leads to

selection of strains that are resistant to these antibiotics as well as an increased chance of obtaining multi-resistant plasmids from other bacteria present in the gut through conjugation and transformation of different diagnostic methods and hospital policy in dealing with such cases. Furthermore, climatic and geographic health factors may also correlate with the relative variability of outcomes(Zainulabdeen and Dakl 2021) between different regions

Identification (MRSA)

MRSA isolates were phenotypically characterised by the modified Kirby-Bauer disc diffusion method according to the 2020 CLSI criteria using cefoxitin disc diffusion (FOXDD) at a concentration of (30µg)Which have an inhibition area of 21 mm or more for the cefoxitin tablet as MRSA strains, as the results showed that 80 isolates, or 80 % were MRSA, and only 20 isolates, or 20 % were (Methicillin-sensitive Staphylococcus aureus) MSSA as in the table (3),

Prevalence of Methicillin Resistant S. aureus

According to the results of (FOXDD) for the detection of mrsa, 80 isolates (80%) were classified as MRSA out of 100 isolates of S. aureus, and according to the data shown in Table (1), a discrepancy was found in the prevalence of MRSA strains among clinical samples, as it was found That 40% of these isolates were urine samples, 20% by sputum, and burns. by 11%, and between wounds by 9%. Recently, the spread of MRSA strains has increased and has become a great danger, especially in hospitals and health care centers, as many studies conducted in different regions in Iraq have reached rates close to what we have reached, where the proportions were as follows:

88% (Kadhim 2018)in Karbala, 91.6% in a study(Al-Hasnawy et al. 2019) in Babylon, 65.3% (Al-Mussawi et al. 2011) in Bsria . In addition, some studies used different diagnostic methods that affect the percentage of prevalence of these strainsMoreover, this discrepancy in the results obtained between the different governorates can be attributed to the different study designs. The results indicate that MRSA has become progressively dangerous because it has acquired a number of different genes that make it more resistant to antibiotics, and this reason has been confirmed by a study, and this reason was confirmed by(Machuca et al. 2022), in addition to the high ability of MRSA to multiply rapidly, which allows it to spread faster and develop more resistance to antibiotics(Smith and Andam 2021) (Kriegeskorte and Peters 2012) .

Table (3): Distribution of MRSA isolates by source of isolate

sample source isolates	Number of MSSA	The number of MRSA
urine	15	40
sputum	4	20

wounds	1	9
burns	0	11
Total	20	80

When examining the sensitivity to antibiotics, MRSA strains in particular showed higher resistance than MSSA strains to all the used antibiotics. The highest resistance to cefoxitin was shown by 100% and the least resistance to chloramphenicol was 16%, while MSSA strains showed the highest resistance to the antibiotic. Penicillin with a percentage of 16% and less resistance to 8 antibiotics, namely Cefoxitin, Doxycycline, Norfloxacin, Trimethoprim, Trimethoprim-Sulfamethoxazole, Ciprofloxacin and Chloramphenicol, at a rate of 0% as in the table below

Table (4) comparing the rates of resistance shown by MRSA and MSSA isolates

name of antibiotic	Resistant MRSA(%) N=80	Resistant MSSA(%) N=20	The total number of resistant isolates
Penicillin(P)	80(100)	16(80)	96%
Cefoxitin(FOX)	80(100)	0(0)	80%
Moxifloxacin(MFX) \	61(76,2)	0(0)	61%
Erythromycin(E),	56(70)	3(15)	59%
azithromycin(azm)	51(63,7)	6(30)	57%
levofloxacin(LEV)	41(51,2)	1(5)	42%
CLARITHROMYCIN	33(41,2)	7(35)	40%
Amikacin(AK)	31(38,7)	4(20)	35%
Clindamycin(CD),	23(28,7)	12(60)	35%
Ofloxacin (OFX)	33(41,2)	1(5)	34%
Tetracycline(TE),	29(36,2)	2(10)	31%
Doxycycline(DOX)	25(31,2)	0(0)	22%
Rifampin(RE)	19(23,7)	0(0)	19%
Norfloxacin(NOR)	15(18,7)	0(0)	15%
Trimethoprim(TMP)	15(18,7)	0(0)	15%

name of antibiotic	Resistant MRSA(%) N=80	Resistant MSSA(%) N=20	The total number of resistant isolates
Gentamicin(GM)	11(13,3)	2(10)	13%
Trimethoprim-Sulfamethoxazole (SXT)	12(15)	0(0)	12%
Ciprofloxacin(CIP)	12(15)	0(0)	12%
Nitrofurantoin(F),	8(10)	1(5)	9%
Chloramphenicol(C)	5(6,1)	0(0)	5 %

Criteria for Defining MDR, XDR and PDR in S. aureus

According to the results of sensitivity testing for 20 antibiotics covering 10 classes of antimicrobials which include aminoglycosides, ansamycin, fluoroquinolone, folate pathway inhibitors, tetracyclines, phenicol, nitrofuranins, macrolides, lincosamides and penicillinase sensitivity, the isolates were classified as MDR/XDR/PDR. Multidrug resistance (MDR) is defined as an acquired deficiency of at least 1 factor in 3 or more classes of antimicrobials. XDR is defined as the absence of sensitivity to at least one agent in all but two or less classes of antimicrobials. Pandrug resistance (PDR) was defined as the lack of sensitivity to all agents in all classes of antimicrobials. As stated in(Eatemadi et al. 2021) . the results showed that (n = 22 / 100, 22%) were XDR and (n = 39 / 100, 78%) was MDR. No PDR is observed in our study, and this same percentage was obtained in a similar study in Iran(Moosavian, Baratian Dehkordi, and Hashemzadeh 2020) The MAR index of an isolate is defined as the number of antibiotics the bacteria were resistant to divided by the number of antibiotics the isolate was exposed to (a MAR index greater than 0.2 indicates that bacteria originating from an environment in which they often used antibiotics(Titilawo et al. 2015). Values ranged fromThe MAR index in this study ranged from 0,75 to 0,05 for 80 MRSA isolates While the MAR index values ranged from 0,40 to 0,05 for 8 isolates of MSSA as shown in table (5)

TABLE (5) MAR index values for MSSA and MSRA isolates

No. of strain MRSA	No. of strain MSSA	MAR index value	No.of resist antibiotic
2	0	0,75	15
2	0	0,70	14
1	0	0,65	13
4	0	0,60	12

3	0	0,55	11
6	0	0,50	10
12	1	0,40	8
9	2	0,35	7
8	2	0,30	6
17	0	0,25	5
11	3	0,20	4
4	4	0,10	2
1	3	0,05	1

These results indicate that there is a wide use of antibiotics and a high selective pressure in Society The MAR index values obtained in this study indicate that a high percentage of Bacterial isolates were exposed to many antibiotics, MAR values were high in this study.Makes it necessary to conduct a sensitivity test to antibiotics before using them, as it will limit the spread of Resistant isolates in hospitals as well as in the community In addition, this procedure helps in Practical use of antibiotics

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