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Isolation and study of MRSA-resistant Staphylococcus aureus from clinical samples in Al-Diwaniyah Hospitals

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***Abstract*---**This study included the collection of 554 samples from different clinical sources and cases for patients of different ages, visit the different Diwaniyah hospitals for the period from November 2021 to March 2022. Staphylococcus aureus was investigated. The results showed the yield of 100 isolates of S aureus bacteria, which included 11 isolates from burns, 11 from wounds, 24 from sputum, and 55 from urine. MRSA was investigated using the method of spreading on the agar, and it was 80%, 50% urine, 25% sputum, 13% burns, and 11%wound. An antibiotic assay was done towards MRSA isolates, and the percentages were as follows: Penicillin 100%, Cefoxitin 100%, Moxifloxacin (MFX) 76.2%, Erythromycin 70%, azithromycin 63.7%, levofloxacin 51.5, CLARITHROMYCIN Amikacin 41, 38% 12, Clindamycin 287%, Ofloxacin 41.2%, Tetracycline 36.2%, Doxycycline 31.2%, Rifampin 23.7%, Norfloxacin 18.7%,Trimethoprim 18.7%, Gentamicin 13,3% Trimethoprim-Sulfamethoxazole 12 and Ciprofloxacin and 12% and Nitrofurantoin 10% and Chloramphenicol 6.1%, while the values of the MAR index ranged from 0.75 to 0.05

***Keywords*---**MRSA-resistant, Staphylococcus aureus, isolation.

Introduction

Staphylococci includes many types of pathogenic bacteria For both humans and animals, the aureus is. S is one of the most important types of bacteria that are pathogenic to humans. The most common at present (Shear 2012) which is responsible for a wide range of diseases is Methicillin-resistant Staphylococcus aureus.

MRSA (MRSA (aureus Staphylococcus) is a major health problem with a high and increasing risk all over the world because it causes many infections associated with hospitals and more in intensive care units and maternity wards, as well as in burn units (Hudson 2012). This is due to its possession of many factors of lethal virulence. Virulance factors that enable them to penetrate most of the body's natural barriers and the immune defence forces in the body and spread to the various tissues of the body easily. These factors include enzymes, toxins, surface proteins and components of the cell wall(Okba et al. 2022); thus, it is the largest Cause of hospital-acquired infection Nosocomial infection, which has become constantly increasing due to resistance of MRSA bacteria to most of the known classes of antibiotics (Yang et al. 2021). Infection with pathogens with multiple antibiotic resistance contributed to the higher mortality rate compared to sensitive strains, as it increased Those rates are in different countries The world and in large and varying proportions(Abadi et al. 2019) . MRSA is one of the most common bacteria in the world. opportunistic pathogens that have a great ability to cause They range from relatively simple skin infections to life-threatening systemic infections (infections due to conditions that are available when)(Bromley et al. 2021). Life - threatining systemic illness In view of the importance of MRSA bacteria in causing various infections in the body, and its increasing spread, the current study aimed to isolate MRSA bacteria. And studying its sensitivity to many different antibiotics

Materials and Methods

Study design and area

Al-Diwaniyah Governorate is located in southern Iraq. In this study, five hospitals, in different districts and districts, were included in the study. The study was conducted in the Public Health Laboratory, which serves as the national reference laboratory in Diwaniyah. Samples from inpatients with infected wounds, burns, urine and sputum (within 2 hours) were collected to the Microbiology Department of the Public Health Laboratory for treatment. Additional information was also collected on participants including age, gender, onset of lesion, previous antibiotic use, gender, and associated medical conditions. The study was conducted between the end of the year 2021 and the beginning of the year 2022.

Selection and exclusion criteria

The reviewers were examined by the attending physician for admission or expulsion from the study. The criteria for them to be included in the study included all inpatients suffering from burn infection, wound abscess, urine and sputum. The criteria included non-admission is to ensure that there was no informed consent.

Sample collection and processing

554 samples were collected from burns, surgical wounds, sputum and urine by sterile swabs. Sample collection sites were prepared using the Levine technique [23]. Double wound swabs were then taken from each location and purulent

discharge of the surgical wound, etc. were first taken in nutrient agar (NA) and blood agar for a whole day and then cultured in mannitol salt agar (MSA) (Oxoid Limited, UK) and incubated Aerobic for a full day for 24 hours in the incubator at 37 °C. Blood samples were inoculated with chocolate agar (California) (Oxoid Limited, UK) and then cultured in MSA.

After that, we microscopically investigated the bacterial colonies that showed typical characteristics of Staphylococcus aureus including golden yellow colonies on MSA for Gram staining, catalase test, DNase test and in the same conditions above. Mannitol-fermented bacteria, Gram-positive as they were shaped like grape-like clusters and exhibiting catalase positivity, were cultured in DNase agar (Oxoid Limited, UK) and incubated for 24 h at the 37 °C incubator. DNase agar plates were subsequently immersed in HCl (1N) (Oxoid Limited, UK). Isolates showing the ability to hydrolyze DNA were identified as S. aureus.

Antimicrobial sensitivity test

A standard method for Kirby Power disc diffusion was performed using Muller-Hinton Agar (MHA) plate technology (Oxoid, Basingstoke, Hampshire, England) according to CLSI guidelines A bacterial suspension equivalent to 0.5 McFarland turbidity standard was prepared for inoculation. Standard antimicrobial tablets representing multiple drug classes mentioned above were used. The plates were incubated at 37 °C for a full day 24 h in Muller-Hinton agar (MHA) with 2% NaCl. The diameter of the inhibition zone was then measured by the digital ruler for each antimicrobial and interpreted as resistive (R), medium (I), and sensitive (S) by doing a comparison with mecA negative (S. aureus ATCC 29213) and mecA positive (ATCC). 33591)

Ethical Consent

Ethical approval was obtained by the ethical committee of the Iraqi Ministry of Health (MoH) and Al-Qadisiyah College of Science. The purpose of the study was explained to each of the volunteers, and written consent was obtained from each person later. We kept the results confidential throughout the study period.

Result

Isolate rate of Staphylococcus aureus

In this study, 554 of the above-mentioned clinical sources were collected, it was found that 366 (66%) showed bacterial growth and 188 (34%) did not show growth. Additionally, 100 (18%) samples were also positive for S. aureus. All discharge samples showed, . It should be noted here that the cure rate of S. aureus bacteria from all four types of samples (wounds, burns, urine and sputum) was significantly associated with a value of 0.005 Table1.

Table (1) the number and percentage of positive and negative samples taken from different clinical cases

Culture results	number of samples	percentage
Positive cases	366	66%
negative cases	188	34%
Total	554	100%

Spread of MRSA

The prevalence of MRSA was obtained to be 80 (80%). The recurrence rate of MRSA in male patients was 55% versus 45% in female patients. in addition to , . Moreover, the frequency of MRSA in patients with burns, wounds, urine and sputum was 40, 20, 11 and 9 percent Table2.

Table (2): Distribution of MRSA isolates by source of isolate

sample source isolates	Number of MSSA	The number of MRSA	Value
urine	15	40(40%)	0,05
sputum	4	20(20%)	0,05
wounds	1	9(9%)	0,05
burns	0	11(11%)	0,05
Total	20	80(80%)	0,05

Table No. 3 shows the sex ratio

GENEDR	MSSA	MRSA
Male	12(60%)	44(55%)
Female	8(40%)	34(45%)

Organism resistance file

Antimicrobial susceptibility test results indicated 13 (15.9%) Penicillin 100%, Cefoxitin 100%, Moxifloxacin (MFX) 76.2%, Erythromycin 70%, azithromycin 63.7%, levofloxacin 51.5, CLARITHROMYCIN Amikacin 41, 38% 12, Clindamycin 287% 41.2%, Tetracycline 36.2%, Doxycycline 31.2%, Rifampin 23.7%,

Norfloxacin 18.7%, Trimethoprim 18.7%, Gentamicin 133% Trimethoprim-Sulfamethoxazole 12 and Ciprofloxacin and 12% and Nitrofurantoin 10% and Chloramphenicol 6.1%, respectively, as in Table No. (4).

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%
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 %

Multidrug resistance

Among the isolates of Staphylococcus aureus bacteria, where there is the highest resistance obtained from 2 isolates that resisted 15 antibiotics, while it was less resistant than one isolate where it resisted only an antibiotic as in Table No. (Table 7). We can describe the isolates as MDR/XDR/PDR. Multidrug resistance (MDR) is described as resistance to a single antibiotic in 3 or more classes of antimicrobials. XDR is defined as the absence of sensitivity to at least one antibiotic in all but two or fewer classes of antimicrobials used. Pandrug resistance (PDR) was defined as hyposensitivity to all antibiotics in all classes of antimicrobials. As mentioned in (Eatemadi et al. 2021). The results in this study showed that (n = 22/100, 22%) was XDR and (n = 39/100, 78%) was MDR. And no PDR was observed in our current study, and the value of MAR, which is described as the process of dividing resistant antibiotics, divided by the number of total antibiotics, is often less or equal to one, and its value ranged in our study between 0, 75 and 0.05 as table (5).

Table No. 6 shows the numbers of resistant antigens for each isolate

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

name of antibiotic	Resistant MRSA(%) N=80	The total number of resistant isolates
Trimethoprim(TMP)	15(18,7)	15%
Gentamicin(GM)	11(13,3)	13%
Trimethoprim-Sulfamethoxazole (SXT)	12(15)	12%
Ciprofloxacin(CIP)	12(15)	12%
Nitrofurantoin(F),	8(10)	9%
Chloramphenicol(C)	5(6,1)	5 %

TABLE (6) MAR index values for MSSA and MSRA isolates

No. of strain MRSA	MAR index value	No.of resist antibiotic
2	0,75	15
2	0,70	14
1	0,65	13
4	0,60	12
3	0,55	11
6	0,50	10
12	0,40	8
9	0,35	7
8	0,30	6
17	0,25	5
11	0,20	4
4	0,10	2
1	0,05	1

Discussion

Gentamicin 13,3%(M and Ali 2018; Wichelhaus et al. 2002) and Nitrofurantoin 10%(Babakir-Mina et al. 2013) and Chloramphenicol 6.1%(Ahmed et al. 2012; Blumberg et al. 1991; Fayyaz et al. 2013), A test was conducted in order to know the antibiotic resistance of MRSA isolates, and the percentages were as follows: Penicillin is 100%, which is the same results obtained in the study(Abdolmaleki et al. 2019) and differed with the results of (Dehkordi et al. 2017), which was 90% , cefoxitin 100%, which is the same as the results of the researcher (R and Vysakh 2013), as for Moxifloxacin 76.2%, and regarding erythromycin 70%, the results were very close to (Rahi et al. 2020) where it was 71%, and for azithromycin 63.7% the results are somewhat close Of the results of (Hoşbul et al. 2013), which recorded a resistance of 62%, and it shows us that levofloxacin 51.5% is the same as the results of (Antonov et al. 2015) who got 51%, and clarithromycin 41% and amikacin 38% are the same results as(Tiwari et al. 2009), and Clindamycin 28.7% was different from(Siberry et al. 2003) the score of 44%, ofloxacin 41.2% was different from the score of 40%(Hamdad et al. 2006), tetracycline 36.2% close to the score of 36%(Sun et al. 2015), doxycycline 31.2%(Sun et al. 2015; Simor et al. 2007), rifampin 23.7% same as results(Wichelhaus et al. 2002)which were 23%, norfloxacin 18.7% that differ from (M and Ali 2018) he record 22%,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 , 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%.Gentamicin was 13.3%, which contrasts with a study that recorded 67%(Miró et al. 2009) And as for the percentage, it was recorded for the antibiotic 10% Nitrofurantoin, which is the same in a study(Miró et al. 2009; Arora et al. 2010) As for Chloramphenicol, it scored 6.1%, which is close to a result with the researcher (Udo et al. 2006)who scored 4.1%.

Restriction

Although this research is in the process of presenting data from an environment where information about antibiotic resistance is very limited, we must acknowledge some difficulties or limitations. Data generalizability may be compromised by sampling biases - the true case burden for these hospitals remains unknown. Also, the data should not be generalized to the entire Iraqi state. Moreover, the distinction between HA and MRSA and between CA and MRSA is not very clear. Therefore, the actual source of infection is not completely certain. In addition, we did not collect data on the combination of atlamour such as, but not limited to, length of stay and hospital stay, area of patient residence, history of antimicrobial use of the examined patient, and contact with livestock, among other things. The associated PCR examination was also not performed. Despite the limitations described, the goal here remains that these studies provide important vital information about MRSA burden and associated AMR patterns. Therefore, the data we collect have an important role to play on the quality of patient care (high frequency of MRSA-positive bacteria here is a sign of

poor patient care), the choice of experimental antibiotics, and the need for ongoing studies of antimicrobial resistance (Rinartha et al., 2018; Suryasa et al., 2021).

Conclusion

Antibiotic resistance is an intractable and growing public health problem in Iraq and most countries, and it has been suggested to conduct a study of local studies. However, the epidemiology of antibiotic resistance in bacteria in the region is poorly understood. This same situation is true of MRSA. In this study, we provided data on recurrence in MRSA in Al-Dawwaniyah hospitals. In contrast to a previous study, the current study found very alarming levels where MRSA isolates (80%) are highly resistant to other antibiotics (penicillin in particular). This indicates a significant increase after 2020. The increase observed here highlights the need for a comprehensive system in operation to monitor and contain drug resistance. The tracking system should be able to collect data on emerging antimicrobial resistance trends, report infections from various health care sectors in the governorate (acute, long-term, ambulatory) and veterinary care across Iraq, and identify patients at risk for these bacterial infections. Lethality, among other things. This information can be used to design further studies for infection control and optimal use of antimicrobial agents in Iraq. We understand the difficulty of implementing these suggestions but only in the long-term. For now, an initial reassessment of current infection control practices and implementation of more effective practices (that is, screening for MRSA carriers, isolating or pooling individual patients, health care workers in colonies, and ongoing environmental decontamination, among others) should suffice. other). Priority should also be given to investing in laboratory infrastructure and allied personnel in order to limit the spread of this germ.

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