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Prevalence of Methicillin-Resistant Staphylococcus Aureus among Staphylococcus Aureus Isolated at Tobruk Medical Center

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Abstract

The widespread emergence of methicillin-resistant *Staphylococcus aureus* (MRSA), as a common cause of infections is becoming a serious concern in global public health because it is difficult to destroy and treat. The objective of the present study was to find out the frequency of MRSA among *Staphylococcus aureus* isolates as well as to study their susceptibility profile to antibiotics. In this study, 249 strains of *Staphylococcus* were collected from microbiology department at Tobruk Medical Center from September 2023 to December 2024. A total of 200 (80.3%) were detected as methicillin-resistant Staphylococci isolates. Moreover, the results revealed that 112 (56.0%) of 200 were identified as MRS strains, while 88 (44.0%) were MRSA. Isolates were subjected to susceptibility testing using the disk diffusion method. The MRS and MRSA isolates showed high resistance to Oxacillin, Cefoxitin, and Erythromycin antibiotics, while showing high sensitivity to Gentamicin and moderate to high sensitivity to Ciprofloxacin, Clindamycin antibiotics. In Conclusion, the study highlights the widespread occurrence of MRSA and its multidrug resistance, prompting control measures and plans and ongoing Surveillance. Future studies must focus on a larger sample size and incorporate genomic analysis to better understand the colony relationships and resistance mechanisms of MRSA isolates.

Keywords: Patients, MRSA, MRS, prevalence

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انتشار المكورات العنقودية الذهبية المقاومة للميثيسيلين بين المكورات العنقودية الذهبية المعزولة في مركز طبرق الطبي

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الملخص

يُشكّل الانتشار الواسع لبكتيريا المكورات العنقودية الذهبية المقاومة للميثيسيلين، كسبب شائع للعدوى، مصدر قلق بالغ في مجال الصحة العامة العالمية نظرًا لصعوبة القضاء عليها وعلاجها. هدفت هذه الدراسة إلى تحديد مدى انتشار المكورات العنقودية والعنقودية الذهبية المقاومة للميثيسيلين بين عزلات هذه البكتيريا، بالإضافة إلى دراسة حساسيتهما للمضادات الحيوية. في هذه الدراسة، جُمعت 249 سلالة من المكورات العنقودية من قسم الأحياء الدقيقة في مركز طبرق الطبي، خلال الفترة من سبتمبر 2023 إلى ديسمبر 2024. تم الكشف عن 200 (80.3%) من هذه السلالات على أنها مكورات عنقودية مقاومة للميثيسيلين. علاوة على ذلك، كشفت النتائج عن أن 112 (56.0%) من أصل 200 من العزلات حددت على أنها عترات من العنقودية مقاومة للميثيسيلين (MRS)، بينما تم تحديد 88 (44.0%) منها على أنها عترات من العنقودية الذهبية مقاومة للميثيسيلين (MRSA). أُجريت اختبارات حساسية المضادات الحيوية على العزلات المقاومة للميثيسيلين باستخدام طريقة الانتشار القرصي. أظهرت عزلات المكورات العنقودية المقاومة للميثيسيلين (MRS) والمكورات العنقودية الذهبية المقاومة للميثيسيلين (MRSA) مقاومة للمضادات الحيوية أوكساسيلين، وسيفوكسيتين، وإريثروميسين، بينما أظهرت حساسية عالية للجنتاميسين، وحساسية متوسطة إلى عالية للسيبروفلوكساسين والكليندامايسين. في الختام، تُسلط الدراسة الضوء على انتشار المكورات العنقودية والعنقودية الذهبية المقاومة للميثيسيلين (MRSA) ومقاومتها للعديد من الأدوية، مما يدفع إلى وضع خطط للسيطرة عليها، وإجراء مراقبة مستمرة. يجب أن تركز الدراسات المستقبلية على حجم عينة أكبر، وأن تتضمن تحليل مادتها الوراثية لفهم أعمق لعلاقات المستعمرات وآليات مقاومة عزلات المكورات العنقودية الذهبية المقاومة للميثيسيلين.

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Introduction

In recent years, the high rate of *staphylococcus aureus* infections has been considered a serious threat to patients. The first strain of methicillin-resistant *S. aureus* (MRSA) (MRS) was isolated in Europe in 1961. In later years, more resistant strains showed a wide pattern of resistance not only to ß-lactams but also to other antimicrobial groups such as aminoglycosides and macrolides [1]. MRSA was first recognized as being acquired from hospitalized patients, but the onset of MRSA infection outside the hospital setting, due to community acquired strains, has recently been described with increasing frequency [2]. Methicillin-resistant *staphylococcus aureus* includes any strain of *s. aureus* that has developed resistance to ß-lactam antibiotics. The organism acquires resistance via the incorporation of a mec A gene into its chromosome at a specific site, mec A encodes an alternative penicillin-binding protein that has low affinity towards semisynthetic penicillins, including methicillin, nafcillin and oxacillin agents [3]. Recently, WHO has enlisted MRSA, MRS as high-priority pathogens that immediately require a new class of antibiotics. It is a formidable pathogen capable of deploying a battery of virulence factors to inflict serious life-threatening disease in the healthcare setting and also in the community [4,5,6].

MRSA among healthcare and community settings and their antibiotic resistance patterns have extensively been studied in Libya [7,8,9,10,11,12,13,14,15, 16]. In Libya, little is known about the prevalence of MRSA, particularly in Tobruk. This study was therefore conducted to investigate the prevalence of MRSA among isolates collected from hospitalized patients and individuals attending the outpatient microbiology department between September 2023 and December 2024. The study also examined the antimicrobial susceptibility patterns of MRSA isolates.

MATERIALS AND METHODS

Study design and population

This study was done at the Microbiology Department at Tobruk Medical Center, and the samples were received from inpatients and outpatients. The bacteria were isolated from samples which had *Staphylococcus* in blood, urine, wound, pus aspirates, skin swabs, cerebrospinal fluid, and catheter tips during September 2023 to December 2024. A total of 249 samples were isolated and

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investigated as staphylococci, from which 200 samples were methicillin-resistant staphylococci.

Sample collection and Transport:

The samples were collected from the hospital wards and the outpatient clinic before starting antibiotics administration and using of sterile technique and choose of correct container with sufficient volume and labeling of samples and immediately were transported to the lab.

Isolation and Identification

The collected samples were cultured on blood agar to support *staphylococcus* growth, then were differentiated from streptococcus by catalase test after that coagulase test and Manitol salt agar was used to differentiate *staphylococcus aureus* from coagulase negative staphylococcus

Bacterial Antibiotic Susceptibility Testing

Antibiotic susceptibility test was done by disk diffusion technique according to standard operating procedure and CLSI guideline and the staphylococcus which resistance to cefoxitin disk detect as MRSA and MRS.

Statistical Analysis

The collected data were statistically analyzed using IBM SPSS Statistics software version 21. The contingency table for independence was run to verify the significant association between gender of patient and antibiotic Susceptibility pattern of s*taphylococcus aureus* between different antibiotics and susceptibility of *staphylococcus aureus.* The results were presented as frequency tabular manner.

Results

A total of 249 isolated staphylococcus strains were included in this study, of which 200 (80.3%) were identified as Methicillin resistant staphylococci. The results showed that 112 (56.0%) were identified as MRS strains, while 88 (44.0%) were MRSA. The distribution of bacterial types across different age group is presented in Table 1. Among MRS isolates, the highest frequency was observed in patients aged over 40 years (27.7%) followed by of those < 2 years old (22.3%) and 11-20 years old (20.5%). Similarly, the MRSA isolates were frequently observed in patients over 40 years (29.5%),

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followed by those aged 11-20 years (19.3%) and 21-30 years (17.0%).

Despite these variation, there was no statistically significant (**P>0.05**) association between **Staphylococcus strains** and age group.

Table 1. Distribution of MRS and MRSA strains in different age group

Staphylococcus strains		Age groups (years)						Total
		=<2	3-10	11-20	21-30	31-40	>40	
MAR	N	25	14	23	8	11	31	**112**
	%	22.3 %	12.5 %	20.5 %	7.1%	9.8 %	27.7 %	**100.0 %**
MARSA	N	9	13	17	15	8	26	**88**
	%	10.2 %	14.8 %	19.3 %	17.0 %	9.1 %	29.5 %	**100.0 %**
Total	N	34	27	40	23	19	57	**200**
	%	17.0 %	13.5 %	20.0 %	11.5 %	9.5 %	28.5 %	**100.0 %**
Significant level	X^2=8.76; P>0.05							

Table 2 shows the distribution of MRS and MRSA isolates according to gender. MRS isolates were slightly more common among females (51.8%) compared to males (48.2%). In contrast, MRSA isolates were predominantly found in males patients (61.4%), with a smaller proportion in females (38.6%). However, the association between **Staphylococcus strains** and gender was not statistically significant.

Table 2. Distribution of MRS and MRSA strains in different gender

Staphylococcus strains	Gender			Total
		Male	Female	
MAR	N	54	58	112
	%	48.2%	51.8%	100.0%
MARSA	N	54	34	88
	%	61.4%	38.6%	100.0%
Total	N	108	92	200
	%	54.0%	46.0%	100.0%
Significant level	X^2=3.43; P>0.05			

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A total of 173 isolates were tested for susceptibility to Oxacillin (OX), including 104 MRS and 69 MRSA strains (Table 2). Among MRS isolates, 66.3% (n=69) were resistant, and 33.7% (n=35) were sensitive. Similarly, 60.9% (n=42) of MRSA isolates were resistant, while 36.2% (n=25) were sensitive. Only 2 (1.2%) isolates showed intermediate susceptibility. Statistical analysis using the chi-square test showed no significant (P>0.05) difference in Oxacillin susceptibility **patterns** between the two groups of **Staphylococcus strains**.

Table 3: Oxacillin (OX) susceptibility patterns among MRS and MRSA strains

Staphylococcus strains		**Oxacillin**			Total
		Intermediate	Resistant	Sensitive	
MAR	N	0	69	35	104
	%	0.0%	66.3%	33.7%	100.0%
MARSA	N	2	42	25	69
	%	2.9%	60.9%	36.2%	100.0%
Total	N	2	111	60	173
	%	1.2%	64.2%	34.7%	100.0%
Significant level	X^2=3.29; P>0.05				

Concerning to Cefoxitin (FOX), Table 4 shows a total of 186 isolates were tested (104 MRS and 82 MRSA). Resistance rates were 63.5% in MRS (n=66) and 68.3% in MRSA (n=56). Sensitivity was observed in 35.6% (n=37) of MRS and 30.5% (n=25) of MRSA isolates, while intermediate responses were minimal (1 isolate in each group). Again, there was no statistically significant (P>0.05) difference in **susceptibility patterns** between the **Staphylococcus groups**.

Table 4. Cefoxitin (FOX) susceptibility patterns among MRS and MRSA strains

Staphylococcus strains		**Cefoxitin**			Total
		Intermediate	Resistant	Sensitive	
MAR	N	1	66	37	104
	%	1.0%	63.5%	35.6%	100.0%
MARSA	N	1	56	25	82
	%	1.2%	68.3%	30.5%	100.0%
Total	N	2	122	62	186
	%	1.1%	65.6%	33.3%	100.0%
Significant level	X^2=0.55; P>0.05				

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Regarding to Gentamicin (CN) antibiotic **susceptibility** test (Table 5). 129 isolates were tested (78 MRS and 51 MRSA). The results revealed that the sensitivity was highest for this antibiotic، with 83.3% (n=65) of MRS and 88.2% (n=45) of MRSA isolates. On the other hand; resistance rates were comparatively low 16.7% (n=13) in MRS and 9.8% (n=5) in MRSA. Only one isolate (from MRSA) exhibited intermediate susceptibility. Statistically insignificant (P>0.05) difference was observed in **susceptibility patterns** between the **Staphylococcus** groups.

Table 5. Gentamicin (CN) susceptibility patterns among MRS and MRSA strains

Staphylococcus strains		**Gentamicin**			Total
		Intermediate	Resistant	Sensitive	
MAR	N	0	13	65	78
	%	0.0%	16.7%	83.3%	100.0%
MARSA	N	1	5	45	51
	%	2.0%	9.8%	88.2%	100.0%
Total	N	1	18	110	129
	%	0.8%	14.0%	85.3%	100.0%
Significant level	X^2=2.66; P>0.05				

Table 6 shows Ciprofloxacin (CIPOR) susceptibility patterns among MRS and MRSA strains. Among the MRS isolates, 21.4% were resistant to ciprofloxacin, 8.7% showed intermediate susceptibility, and 69.9% were sensitive. Similarly, MRSA isolates exhibited 25.4% resistance, 8.5% intermediate susceptibility, and 66.2% sensitivity. No significant difference in Ciprofloxacin susceptibility between the two bacterium groups.

Table 6. Ciprofloxacin (CIPOR) susceptibility patterns among MRS and MRSA strains

Staphylococcus strains		Ciprofloxacin			Total
		Intermediate	Resistant	Sensitive	
MAR	N	9	22	72	103
	%	8.7%	21.4%	69.9%	100.0%
MARSA	N	6	18	47	71
	%	8.5%	25.4%	66.2%	100.0%
Total	N	15	40	119	174
	%	8.6%	23.0%	68.4%	100.0%
Significant level	X^2=0.38; P>0.05				

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Resistance to clindamycin was observed in Table 7. The results revealed that 45.4% of MRS isolates and 39.7% of MRSA isolates were resistant، while sensitive isolates of MRS and MRSA were 52.6% and 60.3%, respectively in. Only 2.1% of MRS isolates showed intermediate susceptibility. No significant difference between MRS and MRSA strains in their **Clindamycin susceptibility patterns**.

Table 7. Clindamycin (DA) susceptibility patterns among MRS and MRSA strains

Staphylococcus strains		Clindamycin			Total
		Intermediate	Resistant	Sensitive	
MAR	N	2	44	51	97
	%	2.1%	45.4%	52.6%	100.0%
MARSA	N	0	25	38	63
	%	0.0%	39.7%	60.3%	100.0%
Total	N	2	69	89	160
	%	1.3%	43.1%	55.6%	100.0%
Significant level	X^2=2.00; P>0.05				

A marked difference was observed in Erythromycin susceptibility between MRS and MRSA isolates (Table 8). Resistance to Erythromycin was significantly (P<0.001) higher among MRS isolates (85.2%) compared to MRSA isolates (51.2%). Intermediate susceptibility was seen to be 1.9% among MRS and 12.2% of MRSA isolates, while sensitivity was in only 13.0% of MRS compared to 36.6% of MRSA isolates. This difference of Erythromycin susceptibility between MRS and MRSA isolates was statistically significant (P<0.001).

Table 8. Erythromycin (E) susceptibility patterns among MRS and MRSA strains

Staphylococcus strains		Erythromycin			Total
		Intermediate	Resistant	Sensitive	
MAR	N	2	92	14	108
	%	1.9%	85.2%	13.0%	100.0%
MARSA	N	10	42	30	82
	%	12.2%	51.2%	36.6%	100.0%
Total	N	12	134	44	190
	%	6.3%	70.5%	23.2%	100.0%
Significant level	X^2=26.75; P<0.001				

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Discussion

This study inspected the prevalence and characteristics of methicillin-resistant*Staphylococcus aureus (*MRSA) isolates that collected from Tobruk Medical Center. A total of 200 samples were isolated from patient age ranged from less than 2 years to more than 40 years [17]. Also the results showed that 112 of 200 (56.0%) were identified as MRS strains, while 88 (44.0%) were identified as MRSA. The results revealed that the high percentage of prevalence of MRS ((26) and MRSA, (29.5%) were observed in patient with age more than 40 years. Regarding to gender; the highest percentage of MRSA was observed in male patients (54, 61.4%), While the highest percentage MRS was observed among female patients (58, 51.8%) [18]. The Suggests that MRSA continues to be a major public health concern, particularly in hospitals [19].

Antibiotic exposure testing showed that MRSA isolates exhibited high resistance to Oxacillin, Cefoxitin, and Erythromycin, while remaining sensitive to Ciprofloxacin, **Clindamycin** and Gentamicin. These findings align with previous studies [20], emphasizing the need for continuous assessing of antimicrobial resistance patterns [21].

Interestingly, Oxacillin and Cefoxitin observed the lowest sensitivity to MRS and MRSA as it illustrates in table (3); this could be attributed to low resistance in Staphylococcus may result from genetic loss or down regulation of resistance genes [22], or due to development shifts in response to antibiotic overuse that favor less-resistant but move fit stains [23]. The discovery of the mecA gene in MRSA isolates verifies the molecular basis of methicillin resistance [24]. Our findings emphasize the importance of implementing strict infection control measures and antibiotic management programs, especially in health care settings. Further move, regular testing and molecular observation of MRSA strains are crucial for early detection and containment of outbreaks. Although these insights, our study had some limitation including [eg, small sample size, limited geographic area (Tobruk), lack of whole-genome Sequencing I, which may affect the applicability of results [25]. Future Studies must focus on a larger sample size and incorporate genomic analysis to greater understand the Clonal relationships and resistance mechanisms of MRSA isolates.

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Conclusion

In Conclusion, the study highlights the widespread occurrence of MRSA and its multidrug resistance, prompting control stages and plans and ongoing Surveillance.

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