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Research Article

Prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) among *Staphylococcus aureus* collection at Sebha medical center

Khadija M. Ahmad[1,2,*], Almahdi A.M. Alamen[2], Fatima A. Atiya[3], Abdelkader A. Elzen[3]

[1*]*Department of Microbiology, Faculty of Medicine, Sebha University, 18758, Sebha, Libya.*
[2*]*Department of Microbiology, Sebha Medical center, Sebha, Libya.*
[3]*Department of Microbiology, Faculty of Science, Sebha University, P.O. Box 18758, Sebha-Libya.*

Abstract: The prevalence of multidrug-resistant *Staphylococcus aureus* has increased during the last few years in healthcare facilities, and methicillin-resistant *Staphylococcus* (MRSA) in particular has emerged as a serious nosocomial pathogen because it is difficult to destroy and treat. Therefore, this study was carried on to find out the frequency of MRSA among *S. aureus* isolates as well as to study their susceptibility profile. In this study, 43 strains of *S. aureus* were recovered from different departments at Sebha medical center and their antibiotic resistance profile was studied using Kirby Bauer disc diffusion method. Out of all 43 isolates, 16% were detected as MRSA using cefoxitin disk test. The strains that are resistant to erythromycin were further tested for inducible clindamycin resistance (ICR) using D-test. In this study, two strains showed ICR phenotype. While all isolates were 100% sensitive to vancomycin, the majority of isolates were resistant to ß-lactam group antibiotics. We observed that 14% of all isolates were resistant to ß-lactamase inhibitor. The response of *S. aureus* isolates to other antibiotics e.g. quinolone, aminoglycosides, tetracycline and macrolides was variable. In our study, it seemed to be vancomycin is the only antibiotic that still keeping its potency and it can be used for treatment of infections caused by multidrug-resistant MRSA.

Keywords: Opportunistic organism, *S. aureus*, MRSA, MDR, Clindamycin, D-test.

1. Introduction

Staphylococcus aureus is an opportunistic pathogen, which has become one of the most hospital-acquired pathogens [1,2]. *S. aureus* can be found as normal flora in healthy humans, but at the same time, it can be a leading cause of many diseases, including skin and tissue infection or in worse cases septicemia and infective endocarditis [3]. *S. aureus* in general and methicillin-resistant strains (MRSA) in particular are of clinical significance because they confer resistance to different groups of antibiotics that render the treatment more difficult. In fact, the MRSA is not only considered as nosocomial pathogen, but it has also been isolated from community settings [4]. MRSA first reported in 1961 shortly after the introduction of methicillin in the health facilitates [5] and thereafter became one of the most frequently isolated organisms worldwide [6,7].

S. aureus has ability to evolve its lifestyle and become a successful opportunistic pathogen through acquiring mobile genetic elements that code for virulence and antimicrobial resistance from other bacteria by horizontal gene transfer [8,9].

The resistance of *S. aureus* to methicillin and to all β-lactam antibiotics is mediated by *mecA* gene that codes for modified penicillin-binding protein (PBP2a), this gene is found on the staphylococcal cassette chromosome *mec* (SCC*mec*) [10,11,12,13].

The resistance of *S. aureus* and mainly MRSA against antimicrobial agents have recently become wider to involve quinolones, aminoglycosides, and macrolides [12,14,15,16]. The macrolides group (e.g. erythromycin) was an alternative drug for penicillin-resistant for a long time, but its usage has been limited during the last years because of the development of macrolides resistance [17]. Moreover, resistance to lincosamide (e.g. clindamycin), which is the drug of choice to treat skin and soft tissue infection caused by *S. aureus*, has also been detected [18,19].

Although glycopeptides, notable vancomycin was considered a cornerstone for treating the MRSA but resistance to this drug has unfortunately also developed

*Corresponding Author: Dr. Khadija Mohamed Ahmad 0000-0002-4230-3822.
E-mail: kady_sunrise@yahoo.com.
Phone No.: +218-918223767.

[20]. The presence of multiple resistant genes carried by MRSA strains considered one of the risk factors that participate in its spread.

However, MRSA among healthcare and community settings and their antibiotic resistance patterns have extensively been studied in Libya [21,22,23,24,25,26,27,28,29,30,31], where they suggested that healthcare workers (HCW) could be a source of MRSA dissemination between the medical staff and directly to the patients. However, The HCWs may further spread this organism (MRSA) to their household members and thereby they increase frequency of community-acquired MRSA infection making the problem even worse [32]. It has also been found that the rate of MRSA has increased in Libyan hospitals during the last decades in patients with burn and infected surgical wounds [24,25]. Zorgani and his team have also isolated inducible clindamycin resistant Staphylococci from burn patients in Tripoli, Libya [33].

Despite all these studies that have been undertaken in Libya, yet very little is known about the prevalence of MRSA in south of Libya especially Sebha, and this project is considered as the first study carried in this area so far. Therefore, we in a present project focused on the prevalence of MRSA among isolates collected from hospitalized patients as well as people who attended outpatient department over a period of two years (January 2015-January 2017). The antimicrobial susceptibility pattern against different antibiotics was also studied in this project.

2. Material and Methods

2.1 Clinical Isolates

Between January 2015 to January 2017, 43 *S. aureus* isolates were collected from wound, pus, oropharyngeal, and screen swabs (nasal and neonate incubators). 29 strains of *S. aureus* were isolated from different hospitalized patients, while 9 isolates from patients attended outpatient department. The remaining was collected from neonatal incubators (2) and medical staff (3). After identification, all strains were given MA number and stored at -70^{0}C in our laboratory at Sebha medical center for further study. The details on each strain are available in Table 1. This study was done in Microbiology department, Sebha medical center. The clinical samples were grown on 5% sheep blood agar medium (Oxoid, England) and incubated overnight at 37^{0}C. *S. aureus* isolates were identified by (Gram stain, catalase test) and confirmed by cultured on Mannitol salt agar (Oxoid, England) and DNase plates (Oxoid, England).

2.2 Antimicrobial susceptibility testing

The confirmed *S. aureus* isolates were screened for their susceptibility to different antibiotics, according to CLSI [34] guidelines using Kirby Bauer disc diffusion method. A 0.5 McFarland standard suspension for each strain was prepared and used for all susceptibility tests. The bacterial suspension was performed on Mueller-Hinton agar (MHA) plate (Oxoid, England). The following antibiotics were used, Penicillin G (5µg), Ampicillin (10µg), Erythromycin (30mg), Vancomycin (30mg), Gentamicin (30µg), Ciprofloxacin (5µg), Ceftriaxone (30µg), Imipenem (10µg), Amoxicillin (25µg), Tetracycline (30µg), and Amoxicillin-clavulanic acid (30µg) (Bioanalyse®, Ankara, Turkey). The plates then were incubated overnight at 35^{0}C.

Table 1. Distribution of *S. aureus* isolates by departments used in this study.

Strain number	Department	Source
MA40	Neonate	Skin swab
MA52	Neonate	Rectal swab
MA58	Female surgical ward	Diabetic foot
MA69	Female surgical ward	Wound
MA70	Female surgical ward	Thigh abscess
MA81	Male surgical ward	Urinary catheter
MA101	Female surgical ward	Abscess
MA102	Female surgical ward	Wound
MA116	Male surgical ward	Leg abscess
MA142	Pediatric	Chest aspiration
MA151	Female surgical ward	Abscess
MA152	Male surgical ward	Wound
MA158	Male surgical ward	Diabetic foot
MA161	Male surgical ward	Abscess
MA162	Female surgical ward	Abscess
MA163	Female surgical ward	Abscess
MA164	Male surgical ward	Diabetic foot
MA172	Male surgical ward	Diabetic foot
MA173	Female surgical ward	Inguinal abscess
MA174	Female surgical ward	Breast abscess
MA180	Neonate	Incubator
MA181	Neonate	Incubator
MA183	Male surgical ward	Cellulitis
MA191	Neonate	Nasal swab
MA197	Female surgical ward	Axillary abscess
MA214	Ophthalmology	Swab
MA218	Neonate	Oropharyngeal swab
MA220	Neonate	Nasal swab
MA221	Neonate	Nasal swab
MA238	Neonate	Oropharyngeal swab
MA242	Male surgical ward	Wound
MA251	Male surgical ward	Postoperative wound
MA256	Male surgical ward	Abdominal abscess
MA258	Neonate	Oropharyngeal swab
MA263	Female surgical ward	Chest wall abscess
MA4	Outpatient department	Sputum
MA155	Outpatient department	Burn
MA160	Outpatient department	Abscess
MA171	Outpatient department	Abscess
MA85	Outpatient department	Genital abscess
MA98	Outpatient department	Nasal abscess
MA113	Outpatient department	Ear swab
MA153	Outpatient department	Abscess

2.3 Detection of MRSA by Cefoxitin disk

Resistance of *S. aureus* isolates to methicillin was determined by using a 30µg Cefoxitin disk. The plates were incubated at 35^{0}C for 18-24h. The results obtained from this experiment were interpreted according to

Clinical and Laboratory Standards Institute (CLSI) guidelines.

2.4 Inducible Clindamycin resistance screen test (D-shape test)

All *S. aureus* isolates that found to be resistant to erythromycin were further screened for inducible clindamycin resistance. Clindamycin (2µg) and erythromycin (15µg) disks (Bioanalyse®, Ankara, Turkey) were placed at a distance of 15mm (edge to edge) from each other. The plates were incubated at 35^0C for 18-24h. Appearance of D-shape zone in between the two disks and toward the clindamycin is considered positive for inducible clindamycin resistance.

3. Results and discussion

This study represents the prevalence of MRSA throughout different departments at Sebha Medical center, which is considered as the central hospital covering almost the majority of south Libya. We analyzed forty-three *S. aureus* strains collected from different departments at Sebha medical center, Libya. Further, 43 isolates were subdivided into two groups, 34 are hospital isolates (Inpatient (IP) and screen swabs) and 9 are from outpatient department (OP) (details in Table 1). All 43 isolates were subjected to antimicrobial susceptibility test. Initially, we could see that out of 43 strains, 84% and 88% were resistant to penicillin and ampicillin respectively (Fig. 1, Table 2, Fig. 2). This study showed that 14% of all isolates (IP & OP) are resistant to β-lactamase inhibitors (Augmentin). Among all isolates, 19% were resistant to ceftriaxone, 30% were resistant to tetracycline, 12% were resistant to gentamicin, 7% are resistant to ciprofloxacin, and 2% resistant to imipenem. Out of 43 isolates, seven isolates (16%) were confirmed MRSA positive using cefoxitin 30µg (Table 2). Four MRSA isolates were isolated from surgical departments and 2 from neonate while one strain was from outpatient department (OP-MRSA) (Table 3/Fig. 3). Among the whole collection, as noted from Table 3, the highest number of MRSA was from surgical departments (9%) followed by neonate (5%) whereas 2% was isolated from outpatients. Focusing on MRSA susceptibility pattern, we found all MRSA isolates resistant to ceftriaxone, 71% resistant to gentamicin, 43% resistant to ciprofloxacin, 43% resistant to erythromycin, and all of MRSA strains sensitive to tetracycline. However, all MRSA is sensitive to imipenem except MA238 (isolated from neonate), showed resistance phenotype. MA238 was only sensitive to tetracycline and vancomycin. Luckily, our study did not show any resistance to vancomycin and all 43 isolates (MRSA and MSSA) were 100% sensitive. The strains, that showed resistance to erythromycin (19%), were further studied for inducible Clindamycin test (D-shape test) (Table 4/Fig. 4A & B). Interpretation of the result was done according to Fiebelkorn [35], strains resistant to both erythromycin and clindamycin were considered to have constitutive clindamycin resistance (cMLSB). But when the strains showed flattening of the circular zone of inhibition toward clindamycin, it is considered inducible clindamycin resistance (iMLSB). The susceptible strains with circular zones around the clindamycin were considered to be clindamycin susceptible [35]. So, we observed two strains, MA162 (IP-MSSA) and MA4 (OP-MSSA), were iMLSB, where they exhibited resistance to erythromycin and sensitive to clindamycin with flattening or blunting of the inhibition zone toward clindamycin (D-shape positive) (Table 4/Fig. 4A). Only MA238 (IP-MRSA) was resistant to both erythromycin and clindamycin, which considered as cMLSB (Table 4/Fig. 4B) with no inhibition zone around them. Other erythromycin-resistant strains IP-MRSA (MA251, MA258) and IP-MSSA (MA81, MA180, MA181) were sensitive to clindamycin (Table 4) and according to Fiebelkorn, they considered to be MS.

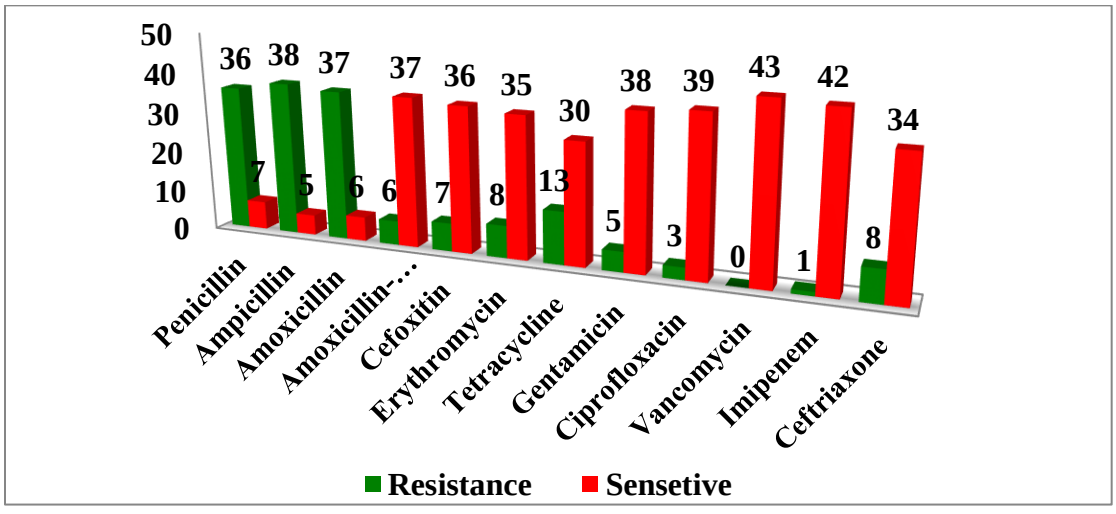

Fig. 1. Antibiotic Resistance profile of *S. aureus* isolates to different commonly used antibiotics.

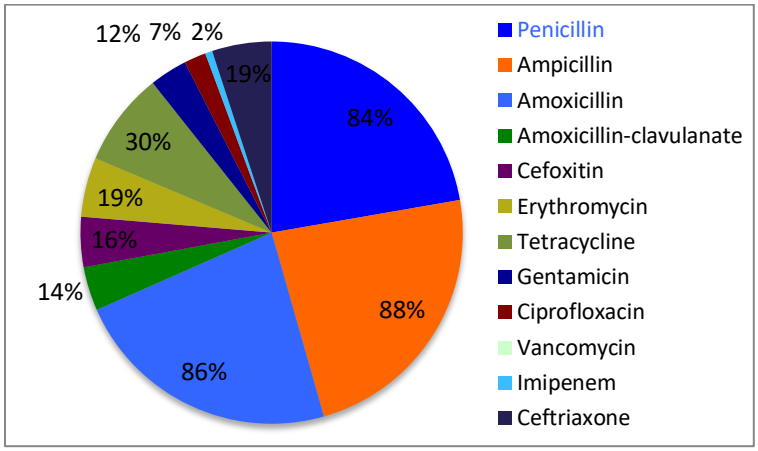

Fig. 2. Antibiotic resistance rate of *S. aureus* isolates.

Table 2. Susceptibility pattern of all 43 *S. aureus isolates* to different groups of antibiotics.

Antibiotics	Sensitive	Intermediate	Resistance	Resistance rate
Penicillin	7		36	84%
Ampicillin	5		38	88%
Amoxicillin	6		37	86%
Amoxicillin-clavulanate	37		6	14%
Cefoxitin	36		7	16%
Erythromycin	35		8	19%
Tetracycline	30		13	30%
Gentamicin	38		5	12%
Ciprofloxacin	39	1	3	7%
Vancomycin	43		0	0%
Imipenem	42		1	2%
Ceftriaxone	34	1	8	19%

Table 3. Distribution of MRSA according to hospital departments.

MRSA/ MSSA/ Department		Number of MRSA/MSSA	Rate (%)
MRSA	Surgical wards (MA69, MA263, MA172, MA251)	4/43	9%
	Neonate (MA238, MA258)	2/43	5%
	Outpatient (MA171)	1/43	2%
MSSA (All departments)		36/43	84%

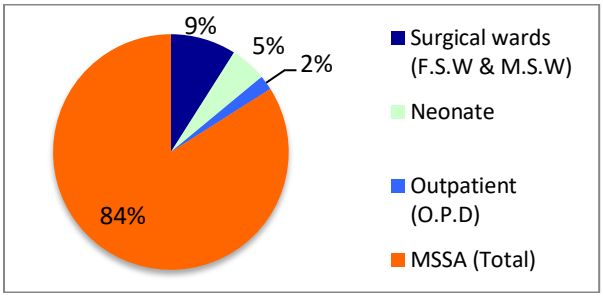

Fig. 3. Frequency of MRSA strains by departments.

The current study was conducted at Sebha medical center, which located at south of Libya and represents the biggest hospital in this area. The healthcare workers with improper hand hygiene are reported as a source of transmission of hospital-acquired pathogens among hospitalized patients. In addition, migration has also been reported as one of the risk factors of multidrug-resistant organism transmission. Heudorf and his group in 2016 [36] have found that 9.8% of the refugees in Germany were colonized with MRSA. Further, Ravensbergen has reported a similar result in 2017 [37], where he found that 10% of Asylum seekers in the Netherlands were MRSA positive compared to general patient population rate, which was 1.3%.

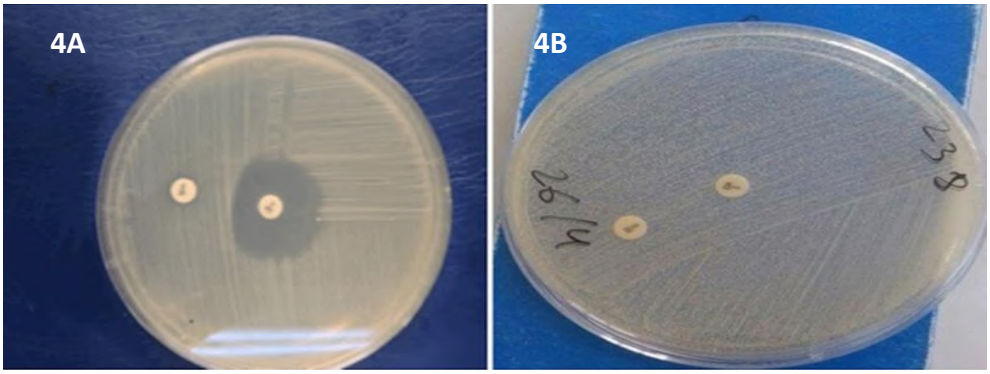

Fig. 4A. Inducible macrolide-lincosamide-streptogramin B (D-test positive). B: constitutive macrolide-lincosamide-streptogramin B resistance.

In Libya, especially after revolution 2011, overflow of immigrants importing multidrug-resistant organisms, war-injured patients with lack of health services, suboptimal infection control and improper antibiotic prescription all might have contributed to prevalence of MRSA and other multidrug-resistant pathogens. After the revolution of February 2011, 51 Libyan injured soldiers were transferred to major incident hospitals in Utrecht, Netherland. A 10% was detected as MRSA among all 51 injured people, and MDR was found in 59% [38].

Based on studies conducted in Libya, the number of MRSA has increased during the last years, particularly in surgical ward and burn patients [24,21]. Furthermore, Zorgani, 2009 [21] has reported that 18% of the *S. aureus* isolates collected from healthcare workers at six different hospitals were MRSA. Our data showed the number of MRSA isolated from hospitalized patients is higher than the isolates from outpatient, 18% and 11% respectively. With respect to the sample size, this result is similar to the one found by Wareg and his team in 2014 [27], when around 511 strains of *S. aureus* were collected between October 2009 and November 2010. Interestingly, similar to results obtained by Buzaid, 2011 [24], we found that the majority of MRSAs are from surgical department.

In this study, we could see that a few strains were sensitive to β-lactam group and this is because they do not produce β-lactamase, while 37 out of 43 were sensitive to β-lactamase inhibitors (amoxicillin/clavulanate). The resistance to β-lactamase inhibitors (14%) in this study was mainly by MRSA. This finding, which is not surprising, has been reported many years ago by Brumfitt [39] and has been confirmed by other studies [40]. Our study revealed that majority of MRSA was resistant to β-lactams (penicillin, ampicillin, and ceftriaxone), β-lactamase inhibitor (amoxicillin/clavulanate), aminoglycoside and quinolones (ciprofloxacin). This observation is in agreement with the same finding, reported by other researchers [41,42,43,44,24].

On the other hand, some published studies reported that 0% resistance of MRSA to ciprofloxacin and 5% to gentamicin [27], but ours showed 43% of MRSA were resistant to ciprofloxacin and 71% to gentamicin. Furthermore, we noticed that this resistance to ciprofloxacin and gentamicin is only exhibited by MRSA but not MSSA strains. For this reason, quinolones, which have previously been used for MRSA treatment [45,46], are not recommended anymore and this finding is supported by other studies [47].

Luckily, between all MRSA and MSSA isolates enrolled in this study, only a single strain was resistant to imipenem, MA238. Such a very low resistance to imipenem suggesting it can still be used for treatment of MRSA.

For many years, vancomycin was considered as the golden antimicrobial agent against multidrug-resistant MRSA, but regrettably, it has also developed resistance. In contrast, to study undertaken in the same area in 2015, Sebha, where they found that 90.5% of *S. aureus* were resistant to vancomycin [48], all our strains showed the opposite and were 100% sensitive to vancomycin. This observation is consistent with other studies [43]. Contrarily, some studies had reported resistance of MRSA to vancomycin [49,50,51].

However, the emergence of β-lactams resistant *S. aureus* strains in the last few years have led to introducing other antibiotics to eradicate *S. aureus* infections, for instance, macrolide, lincosamide and streptogramin B (MLSB) [35]. Nevertheless, resistance to macrolides may also be acquired through either active efflux encoded by *msrA* or modification of enzymes encoded by *ermA* or *ermC* genes (macrolide, lincosamide and streptogramin B resistance (MLSB)) [52]. In this study, out of 43 isolates, 8 (19%) were resistant to erythromycin. Among 8 erythromycin-resistant strains, 2 (5%) detected positive for inducible clindamycin resistance (ICR) and gave D shape. The false clindamycin susceptibility result may mislead the clinicians to use this antibiotic in the treatment of Staphylococcus infections. This misinterpretation can happen if the isolates were not tested for ICR. Therefore, to avoid the failure of clindamycin therapy, the microbiologist should routinely perform this simple test.

In relation to MRSA and MSSA, our study did not detect inducible clindamycin resistance among MRSA rather they were predominant in methicillin-susceptible *S. aureus* isolates, while the constitutive phenotype was observed in MRSA only. This finding is in line with other studies where they also reported higher rate of ICR in MSSA compared to MRSA [2]. In general and regardless MRSA or MSSA, Our data showed the rate inducible clindamycin resistance is higher than constitutive clindamycin resistance and a similar observation has also been reported by Ajantha [53]. Conversely, Fiebelkorn [35] reported higher number of Constitutive resistance compared to inducible resistance and Nikam has also reported a similar result [54].

Thus, according to these results with prevalence of MDR, we have only a few options for treatment of MRSA detected in this study. The reason behind this fast spread of multidrug-resistant organisms perhaps is due to self-medication and improper use of commonly prescribed antibiotics. The worldwide prevalence of multidrug resistance among MRSA strains and other hospital-acquired pathogens has become of critical concern and consider as a major public health problem.

Our study is not surveillance, but rather it highlights the main problem in our hospital and this progressing nosocomial infection problem will increase the morbidity if not the mortality rate.

4. Conclusion

Apparently, *S. aureus* has a remarkable ability to acquire multiple antibiotic resistance, and a new implementation of effective management against multidrug-resistant organisms must urgently be proposed. Further, Hospital infection control and prevention with the proper education to minimize the spread of MRSA should be taken into consideration. Therefore, Healthcare workers and patients before admission must routinely be screened for MRSA and other nosocomial pathogens.

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