العدد السادس والعشرين (26) –المجلد الثاني
تاريخ 30 – 9 – 2024
(ص 8 لـ ص 17)

مجلة جامعة الزيتونة الدولية للنشر العلمي
Zaytoonah University International
Journal for Scientific Publishing
Issue N: 26 – vol 2 / Issn:2958-8537

Isolation and identification of Methicillin Resistance Staphylococcus aureus from abscess, burns, and surgical wounds of the frequenter patients at Sebha Medical Center and AL-Gourda Health Center in Sebha, Libya

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Abstract:
Methicillin-resistant Staphylococcus aureus (MRSA) is one of the major causes of a variety of infections in hospitals and the community. Their spread poses a serious public health problem worldwide. Nevertheless, very little molecular typing data on MRSA strains is currently available in Libya. In this study, 236 specimens were sampled from abscesses, burns, and surgical wounds from patients in Sebha Medical Center and AL-Gourda Health Center between April and July 2024. From all of these specimens, Staphylococcus aureus was isolated from samples by culturing on nutrient agar, and mannitol salt agar and identified by morphological characteristics on selective media and biochemical characterization. Only 63 isolates (45.98 %) showed cultural and biochemical factors that indicate that they are S. aureus. The incidence rate of S. aureus among a male and female group of patients was 48 (76.19%) and 15 (23.81%) respectively. The prevalence of S. aureus among abscess, burns, and surgical wound specimen's patients were 23 (67.6%), 9 (26.5%), and 2 (5.6%) respectively. In addition, the susceptibility of these isolates to oxacillin and cefoxitin antibiotics was studied, and the results revealed that 34

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(53.96 %) of S. aureus isolates demonstrated high resistance to oxacillin and cefoxitin. The results reveal a possibility of a potential public health threat of MRSA.
Keywords: S. aureus; MRSA; coagulase; cefoxitin; oxacillin

Introduction:

Methicillin-resistant Staphylococcus aureus (MRSA) is one of the most successful modern pathogens. The same organism that lives as a commensal and is transmitted in both healthcare and community settings is also a leading cause of bacteremia, endocarditis, skin and soft tissue infections, bone and joint infections, and hospital-acquired infections. Staphylococcus aureus is one of the most common gram-positive pathogens that cause skin and soft tissue infections (SSTIs) [1,2]. It has been determined to be the most clinically significant species with widespread natural presence. Healthy people frequently carry it on their skin or in their noses as part of their normal body flora, which makes it simple to spread by air or fomites from patients or carriers [3,4]. It is also one of the infectious agents that cause the most morbidity and mortality on a global scale. This pathogen can cause many illnesses, from sepsis and pneumonia to moderately severe skin infections that can be fatal [2]. S. aureus produces a variety of virulence factors, such as the toxins hemolysins and leucocidins, immune-evading surface factors like protein A and capsule, and tissue-invading enzymes like hyaluronidase. Because successful lineages frequently differ from their ancestors at multiple loci, analyzing the success of strains from dominant clonal complexes can be difficult. [5]. Even though the majority of Staphylococcus aureus SSTIs are straightforward and can be treated with a brief course of sensitive antibiotics, some of these cases develop into invasive infections that can be fatal.[6]. Ceftaroline, ceftobiprole, dalbavancin, oritavancin, iclaprim, and delafloxacin are recent developments in new antimicrobials against MRSA and are all in various stages of clinical studies. [7-9]. Since it was first identified in a UK hospital in 1961, Methicillin-resistant Staphylococcus aureus (MRSA) has spread throughout the world [10,11] and poses a threat by causing serious infections in hospitals and the general public, which contribute to 60% more deaths than the non-resistant form of the infections [12]. Morbidity and mortality linked to MRSA are rising in both developing and developed countries. Compared to Methicillin-Sensitive Staphylococcus aureus (MSSA) infections, MRSA infections are linked to a nearly 50% higher risk of hospital deaths. [13]. Therefore, treating common staphylococcus infections becomes much more

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challenging if MRSA spreads throughout a community as a common form of Staphylococcus aureus [14]. The risk factors for acquiring MRSA are a weak immune system, diabetes,[15] intravenous drug use, use of antibiotics, hospital admissions, and nursing home patients.[16]. MRSA infection can be prevented by screening programs, handwashing, proper disposal of hospital gowns and wastes, judicious use of antibiotics, infective surfaces sanitizing [17]. and public health awareness programs [17]. In South Libya, limited information exists on the prevalence and drug susceptibility patterns of methicillin-resistant S. aureus isolated from clinical samples. Knowledge of local antimicrobial resistance patterns and MRSA in particular can be useful to physicians, clinical microbiologists, and public health officials within the country and across the region. main objective of this study was therefore to determine the prevalence of MRSA among patients attending the Sebha Medical Center and the Al-Qardah Health Center in Sebha, Libya.

Material and methods:

Collection of samples:

The study was conducted in the microbiology laboratory Department of Medical Laboratories at Wadi Al-Shati University, Libya, during the period of April 2024 to July 2024. A total of 236 specimens sampled from abscesses, burns, and surgical wound, were taken aseptically from the wound exudates after cleaning any remnants of ointments by using sterile cotton swabs. Each sample swab was properly labeled before transportation. Swabs were placed in a sterile plastic bag maintained in the cold chain (at 4oC) and immediately transferred to the Laboratory of the Department of Medical Laboratories at Wadi Al-Shati University, Libya

Samples processing:

Samples collected were directly inoculated on nutrient agar plates for purification of single colonies then streak pure colonies on Mannitol salt agar (MSA), a selective as well as differential media, and on blood agar for S. aureus. Plates were incubated aerobically at 37o C for 24 hours. This selective and differential media only allows Staphylococcus to grow and differentiates the growth of S. aureus from other Staphylococcus species attributable to a change in media color from pink to yellow because of Mannitol fermentation [18]. Mannitol fermentation is a specific activity of S. aureus and growth with no color change indicates any other Staphylococcus species while the growth of all other bacteria expected in the pus sample was repressed [19].

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Confirmation of colonies was done by Gram staining and biochemical profile (catalase, oxidase, and coagulase test).

Identification of MRSA:

All coagulase-positive S. aureus isolates were subjected to detect methicillin resistance by checking their susceptibility to oxacillin and cefoxitin. Identification of MRSA was done by Kirby Bauer disc diffusion method on Mueller-Hinton agar (MHA) plates. Suspensions of freshly revived bacterial culture were prepared in sterilized normal saline and turbidity was adjusted by comparing with 0.5 McFarland turbidity standards. A sterile swab was dipped in suspension and was swabbed thoroughly on Mueller-Hinton agar plates. Discs of 1µg oxacillin and 30µg cefoxitin were placed in equal distance and plates were incubated at 37oC for 24 hours. After incubation zones of inhibition were measured. The results were interpreted according to CLSI guidelines. Isolates showing no zone of inhibition or zone < 21mm for oxacillin and cefoxitin respectively were affirmed as MRSA [20]. Interpretive standards for antibiotics are shown in Table 1.

Table 1: Interpretive Standards for Oxacillin and Cefoxitin Antibiotics:

Antibiotic Disc Tested	Code	Disc quantity	Zone of inhibition (mm)		
			S	I	R
Oxacillin	OX	1µg	≥13	11-12	≤ 10
Cefoxitin	FOX	30µg	≥ 22	-	≤ 21

Note: S= susceptible I= Intermediate R= Resistant

Results:

Isolation and identification of S. aureus:

S. aureus was isolated from abscesses, burns, and surgical wounds of the frequenter's patients at Sebha Medical Center and AL-Gourda Health Center in Sebha, Libya, and characterized by biochemical tests. Out of 236 samples 137 isolates (58.05 %) of staphylococci and out of these isolates 63 (45.98 %) were S. aureus, which fermented mannitol salt agar, coagulase tested positive, catalase tested positive, oxidase test negative and had β hemolysis pattern on blood agar. Out of 63 S. aureus-positive samples, 48 (76.19%) and 15 (23.81%) were isolated from males and females, respectively. The ages of the study subjects ranged from 4 to 52 years, The Distribution of S. aureus in the toddler age group between (4 to 16 years) was the most 39 (61,9%) and it was the least in the oldest age group 24 (38.09%) for both genders. The rate of isolation for S. aureus

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was highest from abscess samples 36 (57.14%) followed by burns 21 (33.33%), and surgical wound 6 (9.52%) (Table 2).

Table 2: Demographic characteristics

Parameters	Inference
Total samples, n	236
S. aureus, n (%)	63 (45.98 %)
Age (years)	4–16 years: 39 (61.9%)
	over 16 years: 24 (38.09%)
Gender, n (%)	Male: 48 (76.19%)
	Female: 15 (23.81%)
types of clinical specimens n (%)	Abscess samples: 36 (57.14%)
	Burns samples: 21 (33.33%)
	Surgical wound samples: 6 (9.52%)

Prevalence of MRSA among S. aureus:

MRSA were detected by their sensitivity to oxacillin and cefoxitin as performed according to the CLSI guidelines. It was observed that out of 137 isolates, 63 (45.98 %) were coagulase-positive *S. aureus* isolates, 34 (53.96 %) isolates were MRSA and the remaining 29 (46.03%) isolates were MSSA. Prevalence of MRSA was recorded. 76.19%, and 23.81% in Sebha Medical Center and AL-Gourda Health Center respectively. Among MRSA the highest number was observed in abscess specimens 23 (67.6%) followed by burns specimens 9 (26.5%) and the least was surgical wound specimens 2 (5.6%) as depicted in Table 3. The predominance in abscess and burns specimens could be due to exposure of the wound to microorganism in the environment and S. *aureus* present on the skin as commensal makes the wound more prone to infection [21].

Table 3: Methicillin-resistant S. aureus association with different types of clinical specimens

Specimen type	MRSA N (%)
Abscess	23 (67.6%)
Burns	9 (26.5%)
Surgical wound	2 (5.6%)

Discussion:

The prevalence of S. aureus and methicillin-resistant S. aureus (MRSA) is now widespread in the majority of nations. diseases and outbreaks caused by S. aureus and

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MRSA have been on the rise in many nations, while the prevalence of these diseases varies geographically and amongst institutions within a particular area [22, 23]. This study used a variety of sample types to examine the prevalence of S. aureus and MRSA among patients at Sebha Medical Center and AL-Gourda Health Centre in Libya. All S. aureus-contaminated samples have the potential to serve as MRSA reservoirs and disseminate the disease across the neighborhood. As a result, there is a chance that the MRSA infection that has been acquired in the community will spread swiftly [24]. However, an increase in the prevalence of MRSA was observed to correlate with the increased age of patients [25]. However, the difference in the prevalence of MRSA between the groups examined in this study was noticeable. where the prevalence rates of S. aureus in the age categories evaluated in this study varied, as highlighted by the finding that the rate in the age group (4–15 years) was higher than the rate in the age group (above 16 years), We also observed differences in prevalence rates between S. aureus males and females, 76.19% and 23.81%, respectively. In the present investigation, MRSA was found in 53.96% of the S. aureus isolates examined. Our findings are higher than previous reports in Benghazi. Najat Bouzid et al. [26] They reported the spread of MRSA in hospitals. They observed a median of 31% for MRSA within isolates of S. aureus MRSA was detected in clinical samples examined from different patients in the present investigation, The observed high prevalence of MRSA in our study may be due to the high rate of certain antibiotics use either due to availability or cost-effectiveness issues.; however, the highest prevalence rate of MRSA was found among S. aureus from patients with burns (68.4%) and surgical wound infections (54%). Zorgani et al. [27] reported MRSA in 54.2% (65/120) of S. aureus isolated from burn patients in the Burn and Plastic Surgery Center in Tripoli, Libya. Others have reported similar findings [28,29]. The variation in prevalence might be due to variation in the study subjects, study conducted time, and the method employed for the detection of S. aureus. Given the fact that staphylococci can be transmitted from one person to another by direct or indirect contact, the rising prevalence of MRSA is becoming a grave issue in the current state of medical care

Conclusion:

In conclusion, the problem of antibiotic resistance is severe in Libya and appears to be on the rise. Where he is MRSA was frequently detected in Libyan hospitals. Accordingly, a major component of future policies for preventing and controlling

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antimicrobial resistance in Libya should be educating healthcare workers, pharmacists, students, and the general public and encouraging them to understand that antibiotic resistance is a serious health problem. Many international agencies (particularly the WHO), scientific societies, and other institutions provide excellent and accurate educational resources on the subject for free. Such agencies can be consulted, and their educational resources should be used as guidelines. In addition, local scientific and cultural societies, sports clubs, mosques, schools, universities, welfare and correctional centers, and the media should be involved in such programs. The crisis of antimicrobial resistance in Libya has reached a stage that requires the Official agencies to join forces in addressing this issue.

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