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# Prevalence of Methicillin-resistant Staphylococcus aureus in skin and soft tissue infections: single center study

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**Abstract**

**Introduction:** Skin and soft-tissue infections (SSTIs) are common diseases with a wide spectrum ranging from minor infections to life-threatening events.

**Aim of the study:** To analyze the characteristics of the SSTI lesions and detect the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and its antibiotic resistance among patients with SSTIs.

**Subjects and Methods:** This current descriptive study was conducted on 200 outpatients with SSTIs from the General Hospital in Fayoum, Egypt. Swabs were collected from the center of the infected soft tissue. Microbiological identification of S. aureus was done using routine microbiological methods, and primary identification of MRSA was performed by the cefoxitin disc diffusion method and culture on ORSAB (oxacillin resistance screening agar base) medium.

**Results:** The patients' ages ranged from 1 year to 78 years. Staphylococci were detected in 126 (62%) of the studied cases; 109 (54.5%) of them were Staphylococcus aureus, and MRSA was isolated from one-third of cases infected with S. aureus. The most common form of infection with MRSA was an abscess. Resistance rates to antibiotics were the highest with Amoxicillin-Clavulanic Acid (100%) and Cefepime (100%), while the lowest resistance rates were with Levofloxacin (2.4%), Clarithromycin (12.2%), Sulphamethoxazole/Trimethoprim (12.2%), Chloramphenicol (14.6%), and Linezolid (24.4%).

**Conclusion:** S. aureus was the most prevalent organism in SSTIs, with a high prevalence of MRSA. Antibiotic resistance was variable among MRSA isolates.

**Keywords:** ORSAB; SSTIs; Skin and soft tissue infection; MRSA.

## 1. Introduction

Skin and soft tissue infections (SSTIs) involve variable microbial infections of the skin, fascia, subcutaneous tissues, and muscles. They include a variable range of diseases, from minor ailments to necrotizing fasciitis and life-threatening events [1].

Non-life-threatening SSTIs should be treated in outpatient clinics, but life-threatening infections require sophisticated care. SSTIs have high rates of mortality and morbidity in hospitalized patients, and

physicians should properly manage SSTIs [2].

Family physicians have an important role in the early detection and proper antibiotic management of SSTIs. complicated and more serious infections may be excluded first, and any patient with signs of a systemic illness requires a full workup, including a complete blood count, C-reactive protein, and blood cultures [3].

Staphylococcus aureus is the second-commonest agent of healthcare-associated infections in the United States and the first pathogen to cause surgical site infections [4].

Methicillin-resistant Staphylococcus aureus (MRSA) is an opportunistic organism that causes infections ranging in severity from mild to serious life-threatening infections [5]. MRSA is also considered an important pathogen in both community- and hospital-acquired infections worldwide [6]. MRSA was reported for the first time in the United Kingdom in the 1960s [7]. Resistance to methicillin is due to an alteration in the penicillin-binding protein (PBP2a), which has a lower affinity for all penicillin's, carbapenems, and cephalosporins except cefazoline (the fifth generation) [8].

Over the last two decades, MRSA has become a major health problem. Although the prevalence of infection with MRSA in hospitalized patients recently declined, community-acquired MRSA increased and became a new challenge (9).

This study aimed to detect the prevalence of MRSA among patients with community-acquired SSTIs and its antibiotic resistance.

## 2. Subjects and methods

### *2.1.Subjects*

The current study was conducted on 200 outpatients from the General Hospital in Fayoum, Egypt. Included patients with soft tissue infection (e.g., cellulitis, skin abscess, infected surgical incision, infected traumatic wound, diabetic foot ulcer, decubitus ulcer, ischemic ulcer, infected bite) were of all age groups, both sexes. Patients with hospital-acquired infections or those who received antibiotics within the previous three days were excluded from the study.

### *2.2.Methodology*

#### *Sampling processing*

Specimens were collected from the center of the infected soft tissue using sterile syringes and sterile disposable cotton swabs (EIPICO Co., Egypt). The samples were transported within two hours to the microbiology lab of the medical microbiology and immunology department in the faculty of medicine at Fayoum University. Swabs were directly inoculated on Mannitol salt agar (MSA), MacConkey, and blood agar (Oxoid Ltd., Hampshire, UK) and incubated for 24 hours at 37°C.

Identification of staphylococci to genus level was done as described by Gang et al. (2000) Coagulase-positive staphylococci that yield yellow colonies on MSA were identified as *S. aureus* [10].

#### *Identification of MRSA*

Identification of MRSA was performed by using the disc diffusion method

with a cefoxitin (30 µg) disc by using the MSA plate, which was seeded with the tested organisms and incubated for 18 hours at 37°C. Oxacillin resistance was determined with the low-density inoculums (0.5 McFarland) at 37°C; all MRSA isolates showed cefoxitin inhibition zone diameters of less than 21 mm, and all MSSA isolates showed larger diameters> 22mm [11, 12].and/or oxacillin resistance screening agar base (ORSAB) medium (Oxoid Ltd., Hampshire, UK), yielded blue colonies on ORSAB medium [13].

***Antibiotic susceptibility test***

Antibiotic sensitivity tests were done for MRSA isolates by the Kurby-Bauer method as follows: Muller-Hinton plates (Oxoid Ltd, Hampshire, UK) were inoculated by swabbing of the tested organism after dilution with an equal amount of nutrient broth (Oxoid Ltd, Hampshire, UK) to obtain 0.5 McFarland onto the surface of agar plates. And antibiotic discs were applied. Then the plates were incubated for 24 hours at 37°C. The antibacterial activities of antibiotics were assessed by measuring the inhibition zones in mm [14].

The following antibiotic discs (Oxoid) were used: Cefoxitin (FOX) 30 µg, Erythromycin (E) 15 µg, Clindamycin (DA) 2 µg, Clarithromycin (CLR) 15 µg, Doxycycline (DO) 30 µg, Levofloxacin (LEV) 5 µg, Sulphamethoxazole/ Trimethoprim (SXT) 25 µg, Chloramphenicol (C) 30 µg, Linezolid (LNZ) 30µg, Meropenem (MEM) 10 µg, Vancomycin (VA) 30 µg, Amoxicillin/Clavulanic acid (AMC) 30 µg, Amikacin (AK) 30 µg, piperacillin/Tazobactam (TPZ) 110 µg, Cefepime (FEP) 30 µg [15, 16].

## 3. Results

The patient's ages ranged from 1 year to 78 years; 111 (55.5%) of them were males (**Table 1**). Regarding risk factor assessment, diabetes mellitus (DM) was present in about one-fourth of the patients (24.5%), while organ affection, smoking, and foreign bodies were less prevalent. Organ affection (impaired) and pregnancy were presented in 25/200 cases (12.5٪). Impaired cardiac functions were the most prevalent organ affection (8 cases, not shown in Figure 1). Fever was present in about half of the patients (48.0%); most of them were pediatricians (**Figure 1**).

**Table 1:** Demographic characters of the study population (N= 90).

| Variables | | Results |
|---|---|---|
| Age (years) | | 1-78 (25.3 ±19.9) |
| Gender | Male | 111 (55.5%) |
| | Female | 89 (44.5) |
| Residency | Urban | 100 (50%) |
| | Rural | 100 (50%) |

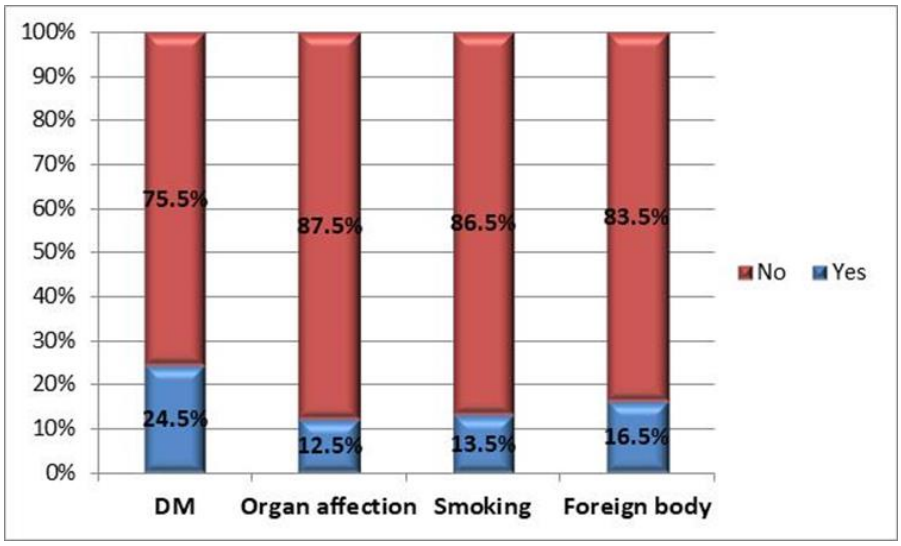


**Figure 1:** Assessment of Risk factors on the study participants.

About one-third of the patients had a hand infection, while the least affected site was a head infection. The majority of infections were in the form of abscesses in 139 cases (69.5%) (**Table 2**). *S. aureus* was 109 (54.5%) of the studied cases, MRSA were 41 (20.5%) of the studied cases, and 37.6% of S. aureus isolates. The most common form of infection with MRSA was an abscess (**Figure 2**).

**Table 2:** Type and site of soft tissue infections (N=200).

| Site of soft tissue infections | | Frequency |
|---|---|---|
| Abdomen | | 12 (6%) |
| Anterior chest wall | | 2 (1%) |
| Arm | | 13 (6.5%) |
| Back of trunk | | 5 (2.5%) |
| Breast | | 5 (2.5%) |
| Face | | 16 (8%) |
| Foot | | 30 (15%) |
| Gluteal region | | 17 (8.5%) |
| Hand | | 62 (31%) |
| Head | | 10 (5%) |
| Hip | | 5 (5.6%) |
| Leg | | 90 (100%) |
| Neck | | 28 (31.1%) |
| Perianal | | 21 (23.3%) |
| Type | Abscess | 43 (47.8%) |
| | Diabetic foot | 42 (46.7) |
| | Infected wound | 5 (5.6%) |
| | Paronkia | 90 (100%) |
| | Surgical site infection | 28 (31.1%) |

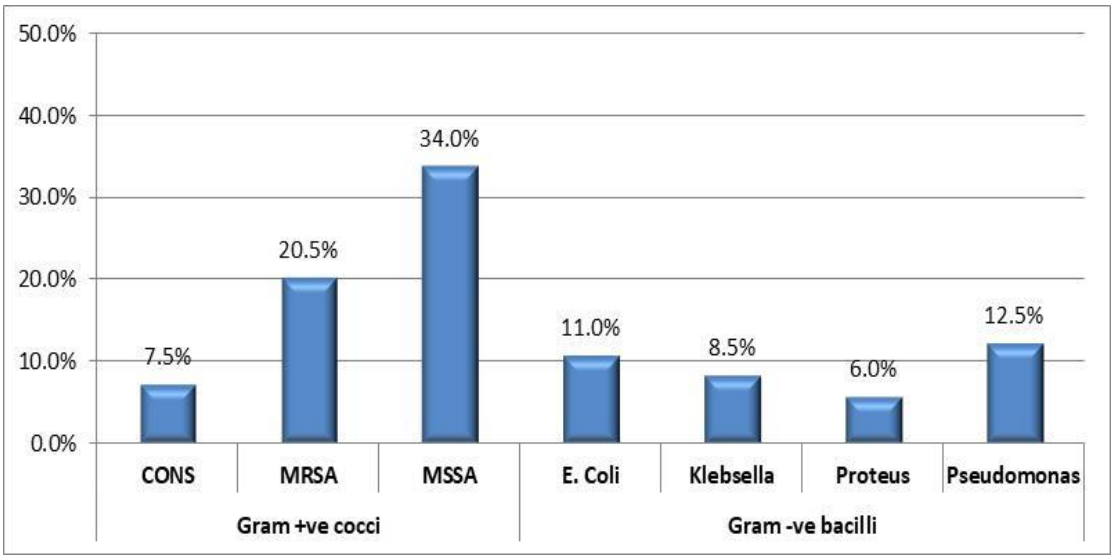


**Figure 2:** Microbial causes of soft tissue infections (N=200).

Among MRSA isolates, resistance rates were the highest with Cefoxitin (100%), Amoxicillin/Clavulanic Acid (100%), and Cefepime (100%), while the lowest resistance was with Levofloxacin (2.4%), Sulphamethoxazole/Trimethoprim (12.2%), Clarithromycin (12.2%), Chloramphenicol (14.6%), and Linezolid (24.4%) (**Table 3**).

**Table 3:** Antibiotic susceptibility among MRSA.

| Variables | Resistance | Intermediate | Sensitive |
|---|---|---|---|
| Cefoxitin (FOX) | 41 (100%) | 0 (0%) | 0 (0%) |
| Amoxicillin/Clavulanic acid (AMC) | 41 (100%) | 0 (0%) | 0 (0%) |
| Erythromycin (E) | 9 (22%) | 22 (53.7%) | 10 (24.4%) |
| Clindamycin (DA) | 10 (24.4%) | 19 (46.3%) | 12 (29.3%) |
| Clarithromycin (CLR) | 5 (12.2%) | 3 (7.3%) | 33 (80.5%) |
| Doxycycline (DO) | 5 (12.2%) | 9 (22%) | 27 (65.9%) |
| Levofloxacin (LEV) | 1 (2.4%) | 3 (7.3%) | 37 (90.2%) |
| Sulphamethoxazole/Trimethoprim (SXT) | 5 (12.2%) | 12 (29.3%) | 24 (58.5%) |
| Chloramphenicol (C) | 6 (14.6%) | 16 (39%) | 19 (46.3%) |
| Linezolid (LNZ) | 10 (24.4%) | 0 (0%) | 31 (75.6%) |
| Vancomycin (VA) | 27 (65.9%) | 0 (0%) | 14 (34.1%) |
| Meropenem (MEM) | 14 (34.1%) | 9 (22%) | 18 (43.9%) |
| Amikacin (AK) | 24 (58.5%) | 4 (9.8%) | 13 (31.7%) |
| piperacillin/Tazobactam (TPZ) | 37 (90.2%) | 0 (0%) | 4 (9.8%) |
| Cefepime (FEP) | 41 (100%) | 0 (0%) | 0 (0%) |

## 4. Discussion

SSTIs are a different spectrum of diseases that affect the skin or subcutaneous tissues and vary in localization and severity, mainly due to Gram-positive bacteria, especially S. aureus, with the emergence and diffusion of community-acquired MRSA [17]. Between 1999 and 2009, the percentage of SSTIs increased from 10% to 25% [18].

In the current study, *S. aureus* was detected in 109 (54.5%) of the studied cases, MRSA in 41 (20.5%) of the studied cases, and 37.6% of S. aureus isolates. In a study by Ray et al. (2013), SSTI episodes were identified. *S. aureus* was the most prevalent organism (about 80%), which was much higher than the current study, and 38% of S. aureus were MRSA, which was identical to the current study [18].

In SSTI episodes with a positive culture-confirmed organism, MRSA incidence increased from about 5% in 1998 to 10% in 2001 and 41% in 2005, then decreased to about 37% in 2009. and the most common form of infection by MRSA was abscesses [18]. In 2017, WHO published a vital global priority list to guide the discovery and production of new antibiotics. MRSA, which is a worrisome pathogen that causes complicated resistant infections, was number 5 on that priority list and was considered a public health priority in Europe [19].

In the current study, resistance rates were the highest with cefoxitin (100%), amoxicillin/clavulanic acid (100%), and cefepime (100%), while the lowest resistance was with levofloxacin (2.4%), sulphamethoxazole/trimethoprim (12.2%), clarithromycin (12.2%), chloramphenicol (14.6%), and linezolid (24.4%). This disagrees with the results of Zuma et al. (2017), who found a resistance rate of 78.7% for erythromycin, while the lowest was observed for chloramphenicol (19.7%) and linezolid (4.9%) [20]. The resistance rate was low for sulphamethoxazole/trimethoprim in the present study (12.2%); in a study by Zuma et al. (2017), the resistance rate was 11.5%.

### Conclusion

*S. aureus* was detected in more than half of the infections, and MRSA was detected in one-fifth of the cases. Diabetes was the most important risk factor for SSTIs with MRSA. Antibiotic resistance was variable among cases. Control of diabetes to decrease the risk of MRSA infection is essential. Antibiotic susceptibility tests should be done for all isolates.

**Ethical approval and consent to participate:** The study protocol was approved by the Research Ethics Committee, Faculty of Medicine at Fayoum University, Egypt, number 16 (Code: D68). Adequate precautions have been taken to safeguard data confidentiality during data collection, storage, analysis, and dispensation.

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**Conflicts of Interest:** All authors declare no conflict of interest.

## References


1. Stevens DL, Bisno AL, Chambers HF, Dellinger EP, Goldstein EJ, Gorbach SL, Hirschmann JV, Kaplan SL, Montoya JG, Wade JC; Infectious Diseases Society of America. Practice guidelines for the diagnosis and management of skin and soft tissue infections: 2014 update by the Infectious Diseases Society of America. Clin Infect Dis. 2014;59(2):e10-e52. doi: 10.1093/cid/ciu444.
2. Stephens JM, Gao X, Patel DA, Verheggen BG, Shelbaya A, Haider S. Economic burden of inpatient and outpatient antibiotic treatment for methicillin-resistant *Staphylococcus aureus* complicated skin and soft-tissue infections: a comparison of linezolid, vancomycin, and daptomycin. Clinicoecon Outcomes Res. 2013;5:447-457. doi: 10.2147/CEOR.S46991.
3. Martin P, Nunan R. Cellular and molecular mechanisms of repair in acute and chronic wound healing. Br J Dermatol. 2015;173(2):370-378. doi: 10.1111/bjd.13954.
4. Weiner LM, Webb AK, Limbago B, Dudeck MA, Patel J, Kallen AJ, Edwards JR, Sievert DM. Antimicrobial-Resistant Pathogens Associated With Healthcare-Associated Infections: Summary of Data Reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011-2014. Infect Control Hosp Epidemiol. 2016;37(11):1288-1301. doi: 10.1017/ice.2016.174.
5. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015;28(3):603-661. doi: 10.1128/CMR.00134-14.
6. Serray B, Oufrid S, Hannaoui I, Bourjilate F, Soraa N, Mliji M, Sobh M, Hammoumi A, Timinouni M, El Azhari M. Genes encoding adhesion factors and biofilm formation in methicillin-resistant *Staphylococcus aureus* in Morocco. J Infect Dev Ctries. 2016;10(8):863-869. doi: 10.3855/jidc.8361.
7. Jevons MP. "Celbenin" - resistant Staphylococci. Br Med J. 1961;1(5219):124–145.
8. CDC. Centers for Disease Control and Prevention. Laboratory Testing for MRSA. (2017).
9. Sader HS, Mendes RE, Jones RN, Flamm RK. Antimicrobial susceptibility patterns of community- and hospital-acquired methicillin-resistant *Staphylococcus aureus* from United States Hospitals: results from the AWARE Ceftaroline Surveillance Program (2012-2014). Diagn Microbiol Infect Dis. 2016;86(1):76-79. doi: 10.1016/j.diagmicrobio.2016.06.017.
10. Gang RK, Sanyal SC, Bang RL, Mokaddas E, Lari AR. Staphylococcal septicaemia in burns. Burns. 2000;26(4):359-366. doi: 10.1016/s0305-4179(99)00170-9.
11. Felten A, Grandry B, Lagrange PH, Casin I. Evaluation of three techniques for detection of low-level methicillin-resistant *Staphylococcus aureus* (MRSA): a disk diffusion method with cefoxitin and moxalactam, the Vitek 2 system, and the MRSA-screen latex agglutination test. J Clin Microbiol. 2002;40(8):2766-2771. doi: 10.1128/JCM.40.8.2766-2771.2002.
12. Clinical and laboratory standards institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement.

CLSI document M. wayne, PA. 2019; 22: 99- 122.
13. Ben Nsira S, Dupuis M, Leclercq R. Evaluation of MRSA Select, a new chromogenic medium for the detection of nasal carriage of methicillin-resistant *Staphylococcus aureus.* Int J Antimicrob Agents. 2006;27(6):561-564. doi: 10.1016/j.ijantimicag.2006.03.011.
14. Patton T, Barrett J, Brennan J, Moran N. Use of a spectrophotometric bioassay for determination of microbial sensitivity to manuka honey. J Microbiol Methods. 2006;64(1):84-95. doi: 10.1016/j.mimet.2005.04.007.
15. Clinical and laboratory standards institute (CLSI). Performance standards for antimicrobial susceptibility testing: 22 informational supplemented. CLSI document M. wayne, PA. 2012; 22: 100-22.
16. Clinical and laboratory standards institute (CLSI). Performance standards for antimicrobial susceptibility testing: 22 informational supplemented. CLSI document M. wayne, PA. 2015; 22: 100-22.
17. Principi N, Argentiero A, Neglia C, Gramegna A, Esposito S. New Antibiotics for the Treatment of Acute Bacterial Skin and Soft Tissue Infections in Pediatrics. Pharmaceuticals (Basel). 2020;13(11):333. doi: 10.3390/ph13110333.
18. Ray GT, Suaya JA, Baxter R. Microbiology of skin and soft tissue infections in the age of community-acquired methicillin-resistant Staphylococcus aureus. Diagn Microbiol Infect Dis. 2013;76(1):24-30. doi: 10.1016/j.diagmicrobio.2013.02.020.
19. Silva V, Almeida F, Carvalho JA, Castro AP, Ferreira E, Manageiro V, Tejedor-Junco MT, Caniça M, Igrejas G, Poeta P. Emergence of community-acquired methicillin-resistant Staphylococcus aureus EMRSA-15 clone as the predominant cause of diabetic foot ulcer infections in Portugal. Eur J Clin Microbiol Infect Dis. 2020;39(1):179-186. doi: 10.1007/s10096-019-03709-6.
20. Zuma AVP, Lima DF, Assef APDC, Marques EA, Leão RS. Molecular characterization of methicillin-resistant *Staphylococcus aureus* isolated from blood in Rio de Janeiro displaying susceptibility profiles to non-β-lactam antibiotics. Braz J Microbiol. 2017;48(2):237-241. doi: 10.1016/j.bjm.2016.09.016.