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Antibiotic Susceptibility and Resistance Patterns of *Staphylococcus aureus* Isolated from Pus Samples in Tobruk Medical Center at Tobruk, Libya

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ABSTRACT

Background: *Staphylococcus aureus* is a leading cause of purulent infections worldwide, with rising concerns about antibiotic resistance, particularly methicillin-resistant S. aureus (MRSA). This study aimed to determine the prevalence and antimicrobial susceptibility patterns of *S. aureus* isolated from pus samples at Tobruk Medical Center, Libya. **Methods:** A total of 127 pus samples were collected from patients with suspected *S. aureus* infections between April and December 2023. Specimens were cultured on Blood and Mannitol Salt agars. *S. aureus* isolates were identified using standard microbiological techniques (Gram stain, catalase, and coagulase tests). Antimicrobial susceptibility testing was performed against nine antibiotics (Levofloxacin, Clindamycin, Gentamicin, Doxycycline, Erythromycin, Penicillin, Ciprofloxacin, Cefoxitin, and Tetracycline) using the Kirby-Bauer disk diffusion method, following CLSI guidelines. **Results:** Out of 127 samples, 60 (47.2%) were culture-positive for *S. aureus.* The isolates exhibited high resistance rates to Penicillin (62.82%) and Erythromycin (58.97%). Cefoxitin disc testing identified a high prevalence of MRSA at 53.84%. In contrast, the highest susceptibility was observed to Gentamicin (57.69%), Ciprofloxacin (57.69%), and Doxycycline (51.28%). Levofloxacin also showed good activity with a susceptibility rate of 48.71%. **Conclusion:** This study reveals an alarmingly high prevalence of MRSA and multidrug-resistant *S. aureus* in pus isolates from our setting. Penicillin and Erythromycin are ineffective as empirical treatments. Gentamicin, Ciprofloxacin, and Doxycycline retained higher efficacy. These findings underscore the critical need for continuous antimicrobial resistance surveillance and stringent antibiotic stewardship policies to guide effective treatment and control the spread of resistant strains in Tobruk, Libya.

1. INTRODUCTION

Staphylococcus aureus is a leading human pathogen that causes an enormous burden of diseases ranging from superficial infections of the skin to deadly invasive diseases (Turner et al., 2019). Formation of abscesses with a pus collection that is an exudate composed largely of neutrophils with cellular debris but also with bacteria is one of the defining clinical presentations of infections with S. aureus.

Such pyogenic infections such as furuncles, carbuncles, and deep abscesses represent an important battleground between host defense strategies and bacterial virulence countermeasures (Powers & Wardenburg, 2023). Pus formation is a direct outcome of an unsuccessful resolution of the host's innate immune response. S. aureus establishes infection upon attachment to host tissues and extracellular matrix proteins via microbial surface components recognizing adhesive matrix molecules (Shukla et al., 2020). This invokes a vigorous inflammatory response and an overwhelming number of neutrophils to the infectious site. At the center of the organism's pyogenic prowess is its secreted toxin proteins, including the pore-forming α-toxin (Hla) and the bicomponent Panton-Valentine Leukocidin (PVL), which kill host cells, including neutrophils, releasing their cytoplasmic contents to add to pus formation (Spaan et al., 2023). In addition to that, S. aureus master's neutrophil subversion to facilitate its survival within these phagocytic cells culminating into their necrotic death with an overwhelming intensification of the inflammatory cascade and abscess growth (Dengler Haumeiter et al., 2024). One substantial contemporary challenge is a high prevalence of methicillin-resistant S. aureus (MRSA) lineages that are often implicated in recurrent and serious skin and soft tissue infections (Kourtis et al., 2019). An abscess's complex structure—consisting of a dead core with a peri-fibrin capsule surrounding it—naturally restricts antibiotics from penetrating, creating a sheltered niche that allows bacterial survival and contributes to treatment failure (Gonzalez et al., 2022). It is critical to break away from conventional antibiotics and to investigate new therapeutic interventions that directly abolish the virulence pathways leading to disease pathology. Staphylococcus aureus remains one of the most virulent human pathogens with a capability to induce a spectrum from benign skin and soft tissue infections (SSTIs) to lethal diseases such as bacteremia, endocarditis, and necrotizing pneumonia (Turner et al., 2019). It is an effective pathogen because it has a massive collection of virulence factors including adhesins, toxins, and immune-evasion molecules to facilitate colonization, invasion into tissues, and host injury. Features of S. aureus infection include abscess formation, which is a collection of pus comprising dead neutrophils, host cell debris, and bacterial cells. Pyogenic lesion development is a complex process initiated by bacterial adhesion to host tissues via microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) (Shukla et al., 2020). It causes an exuberant neutrophil-mediated inflammation. Of particular relevance is that S. aureus generates highly efficient cytolytic toxins such as alpha-toxin (Hla) and Panton-Valentine leukocidin (PVL) to kill neutrophils by lysing their membranes, thereby releasing their contents to contribute to the abscess's necrotic core (Spaan et al., 2023). Moreover, S. aureus's capability to survive within cells and later induce their necrotic death fuels the progression of the inflammatory cascade and growth of the abscess (Dengler Haumeiter et al., 2024). A structure comprising an abscess with a necrotic core with a fibrin capsule creates a physical barrier to antibiotic penetration as well as access to immune cells such that a sheltered niche is established to facilitate bacterial survival with such infections being notoriously recalcitrant to therapy (Gonzalez et al., 2022). This clinical challenge of S. aureus is also worsened by its exceptional ability to acquire resistance to antimicrobial compounds. Emergence and worldwide spread of methicillin-resistant S. aureus (MRSA), an organism that is resistant to all beta-lactam antibiotics, is a serious threat to public health. MRSA isolates have been related to hospitals or health-care facilities (HA-MRSA) but also gained a presence among community residents (CA-MRSA), presenting with recurrent serious skin infections (Kourtis et al., 2019). To counter such resistance, various antibiotics have to be introduced but rising resistance to such medications, such as macrolides, clindamycin, and even fluoroquinolones, is being increasingly documented across the globe with resultant treatment failures as well as excess costs associated with treating such infections. The aim of this study was to isolate and identify *Staphylococcus aureus* from pus samples obtained from patients at Tobruk Medical Center and to determine the antimicrobial susceptibility and resistance patterns of these isolates. This is followed by a statement of the purpose of the research issue or problem and/or set of questions you attempt to answer in your research**.**

2. METHOD

Study Population

This study involved 127 patients clinically suspected of *Staphylococcus aureus* infections who attended Tobruk Medical Centre. The cohort included 71 females and 56 males. Which showed 60 positive culture and 67 negative culture in samples collection. Patient ages ranged from neonates to adults older than 40 years.

Both pediatric patients under 20 years and adults above 40 were included to ensure a comprehensive demographic representation. All specimens obtained from various clinical specimens of patients between April 2023 to December 2023, were processed at the microbiology lab of the Tobruk Medical Center.

Specimen collection

The samples were collected using a sterile cotton swab, which was used for gentle swabbing of the inner surface of the infected area, Specimens were cultured on Blood agar and Mannitol salt agar plates and incubated at 37°C for 24hrs. Characteristic *S. aureus* colonies were identified by Gram stain, catalase, and coagulase testing according to standard bacteriological procedures.

Antimicrobial Susceptibility Testing

Susceptibility testing was performed briefly overnight cultures of isolates on Mueller Hinton agar incubated at 37 °C for 24 h were used for making bacterial suspensions in 0.85% NaCl and adjusted to McFarland 0.5 turbidity standards prepared suspensions were used for inoculation of the Mueller Hinton agar plate's surface, afterward the antibiotic discs were placed on the inoculated agar surface. After the incubation period of 24 h at 37°C, the inhibition zones around the discs were measured and interpretation of the inhibition zone values (S-sensitive, I-intermediate and R-resistant) was based on the standards guideline's instructions. Antibiotics discs used and their concentrations as the following Levofloxacin (30µg), Clindamycin (30µg), gentamycin (10µg), Doxycycline (30µg), erythromycin (5µg), penicillin (30µg), ciprofloxacin (5µg), cefoxitin (30µg) and **Tetracycline** (30µg).

3. Ethic Approval

The Research Ethics committee (REC), University of Tobruk (UOT) reviewed discussed your application documents to conduct a study entitled:
I (Antibiotic Susceptibility and Resistance Patterns of Staphylococcus aureus Isolated from Pus Samples in Tobruk Medical Center at Tobruk)
In REC meeting held on 16/10/2025 we approved your request to conduct the study its present form (ethical approval Number NBC:009. H.25.31).
The REC expects to be informed about any changes in your study protocol during conduct of the study.
This letter constitutes ethical approval only and you should seek Consent from the Department separately.

4. RESULT

Out of the 127 samples processed, 60 were confirmed to be Staphylococcus aureus, yielding a culture positivity rate of 47.2%, as shown in Table 1. A total of 127 pus samples were collected from patients with suspected staphylococcal infections. The demographic distribution of the patients is summarized in Table 2. The cohort consisted of 71 (55.9%) females and 56 (44.1%) males. The majority of patients were under 20 years of age (38.73%), followed by those above 40 years (30.98%) and those aged 20-40 years (20.42%); this number is shown in Table 3.

All 60 S. aureus isolates were subjected to antimicrobial susceptibility testing against nine antibiotics. The susceptibility profile, including the frequency and percentage of susceptible (S), intermediate (I), and resistant (R) isolates, is detailed in Table 4. The highest resistance rates were observed against penicillin (62.82%) and erythromycin (58.97%). Testing with the cefoxitin disk, a surrogate marker for methicillin resistance, identified 53.84% (42/78) of the isolates as Methicillin-Resistant Staphylococcus aureus (MRSA). In contrast, the highest susceptibility was demonstrated to Gentamicin (57.69%) and Ciprofloxacin (57.69%), followed by Doxycycline (51.28%) and Levofloxacin (48.71%). A notable proportion of isolates showed intermediate susceptibility to ciprofloxacin (8.97%).

Table 1: Frequency Distribution of culture result of the sample.

Culture	Frequency	Percentage (%)
Positive	60	**47.2%** %
Negative	67	52.8%
Total	127	100%

Table 2: Frequency Distribution of the samples according to Gender.

Gender	Frequency	Percentage (%)
Females	71	**55.9** %
Males	56	**44.1**%
Total	127	100%

Table 3: Frequency Distribution of the samples according to Ages.

Age	Frequency	Percentage (%)
Less than 20	55	43.31%
20_40y	28	22.05%
Above 40y	44	34.65%
Total	127	100%

Table 4: shows effectiveness of antibiotics on *Staphylococcus aureus*.

	Frequency			Percentage (%)		
	S	R	I	S	R	I
FOX	31	42	2	39.74%	53.84%	2.56%
PG	1	49	0	1.28%	62.82%	0%
DO	40	14	0	51.28%	17.94%	0%
CN	45	27	1	57.69%	34.61%	1.28%
TE	39	26	1	50%	33.33%	1.28%
E	25	46	4	32.05%	58.97%	5.12%
DA	35	19	3	44.87%	24.35%	3.84%
CIP	45	21	7	57.69%	26.92%	8.97%
LEV	38	10	1	48.71%	12.82%	1.28%

5. DISCUSSION

The present study provides a critical snapshot of the antimicrobial resistance landscape of *Staphylococcus aureus* isolated from pus samples in Tobruk, Libya. The key findings reveal a high prevalence of *S. aureus* infection (47.2%) and an alarmingly high rate of methicillin resistance, posing significant challenges for clinical management.

The isolation percentage of S. aureus (47.2%) from pus specimens highlights its dominating position as a pyogenic pathogen within our community's healthcare. It is in agreement with reports from other areas finding S. aureus to be an established cause of skin and soft tissue infections (SSTIs) . Demographic results indicated that infections were highest among adults aged less than 20 years (38.73%), possibly because they have higher exposure rates, spend more time outdoors, or community-associated patterns of transmission occur more frequently among younger adults.

Most impactful finding of the present investigation is the high incidence of MRSA found in 53.84% of isolates. It is a very alarming figure which positions Tobruk among cities with some of the highest recorded MRSA percentages in the world. In comparison to Libya's previous work, our results are troubling. For example, a Tripoli investigation showed MRSA percentages of approximately 30% (Elahmer et al., 2017), whereas a Misurata investigation determined a percentage to be 41.6% (Zorgani et al., 2019). Our increased percentage might be a function of regional differences in antibiotic usage patterns, infection management strategies, or a possible emergence and establishment of a highly successful clone of MRSA present only throughout Eastern Libya.

Globally, our MRSA prevalence is much higher than 1.8% found in Norway (NORM/NORM-VET 2023) or ~25% average among several Western European nations (ECDC, 2023), emphasizing an egregious gap in AMR burden. It is more comparable to levels found in some other Middle Eastern and North African (MENA) nations, including Egypt (50-60%) (Abdullahi et al., 2022) and Saudi Arabia (40-50%) (Al-Hamad et al., 2021), indicating common regional issues such as indiscriminate antibiotic usage and cross-transmission.

This high prevalence of MRSA precludes therapeutic choices from the beginning because it grants resistance to all beta-lactam antibiotics. It was also supported by extreme Penicillin resistance (62.82%), making it effectively useless for empirical therapy. It is a near-universal resistance phenomenon worldwide for S. aureus because of almost ubiquitous penicillase presence. Likewise, high Erythromycin resistance (58.97%) is consistent with patterns of macrolide resistance amongst S. aureus isolates globally as well as throughout the MENA region.

Alternatively, isolates showed promising susceptibility to several principal antibiotics. Gentamicin (57.69% susceptible) and Ciprofloxacin (57.69% susceptible) were most efficient agents in vitro. Such high efficiency of gentamicin is corroborated by data of a survey carried out in Zawia, Libya (Ghenghesh et al., 2016), where also high susceptibility to aminoglycosides existed. But it contradicts information from some countries where aminoglycoside resistance among MRSA is increasing. Our susceptibility to Ciprofloxacin (57.69%) is significantly higher than commonly cited levels of resistance for MRSA globally. There have also been various fluoroquinolone-resistant international clones of MRSA. Our finding would then either mean that fluoroquinolones were less vigorously utilized about our environment or local clones have yet to acquire these resistances. But an intermediate level for CIP (8.97%) is a caution that resistance is potentially rising.

Doxycycline also showed good activity (51.28% susceptible). It is a useful finding because tetracyclines such as doxycycline are routinely prescribed for ambulatory treatment for SSTI. Such a percentage is acceptable for a few North African nations but lower than high degrees of susceptibility (>80%) that were commonly observed in nations located in Northern Europe.

High resistance rates found here could be explained by various reasons common to Libya and such countries as uncontrolled over-the-counter antibiotic sales, self-medications, and partial treatment courses. Even the surrounding of an abscess itself described in introduction creates a haven for bacterial survival with associated additional difficulties for treatment and possible enrichment with resistant clones (Gonzalez et al., 2022).

Empiric antibiotic therapy for bacterial infections is a widespread phenomenon throughout Libya and beyond. Unfortunately, with hardly any recent local data available regarding antibiotic resistance patterns, such practices will be inefficient and again select for resistant organisms. Little data exist regarding S. aureus susceptibility patterns, particularly from purulent infections from Tobruk City, Eastern Libya.

Our current work thus attempts to isolate and characterize S. aureus isolates from pus specimens among participants to Tobruk Medical Center and establish their antimicrobial susceptibility patterns. Results will provide definite up-to-date local data regarding MRSA prevalence and trends of resistance to form a nucleus to clinicians to allow optimum selection of empirical therapy, to hospitals to adopt effective infection control methodology, and to policymakers to establish effective antibiotic stewardship programs to stem antimicrobial resistance tide.

6. CONCLUSION

this study demonstrates a high prevalence of *S. aureus* and a disturbingly high rate of MRSA in pus isolates from patients at Tobruk Medical Center. The findings paint a picture of significant multidrug resistance, with Penicillin and Erythromycin being largely ineffective. The results provide actionable data for clinicians in Tobruk and Eastern Libya. Gentamicin, Ciprofloxacin, and Doxycycline appear to be the most suitable choices for empirical antibiotic therapy of suspected *S. aureus* SSTIs in this region. However, the high MRSA burden strongly advocates for a policy shift towards routine culture and susceptibility testing to guide definitive therapy. Ultimately, these findings sound an alarm for the urgent implementation of robust antibiotic stewardship programs and continuous surveillance systems to monitor the evolution of AMR. Public health initiatives to educate both clinicians and the public on the rational use of antibiotics are paramount to curbing the spread of these resistant and dangerous pathogens.

Conflict of interest:

None declared

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