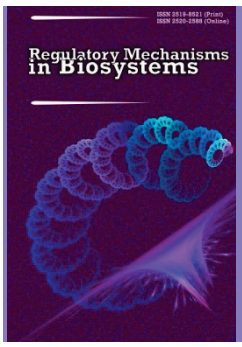

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Prevalence and molecular detection of methicillin-resistant *Staphylococcus aureus* isolates from infants and children with with folliculitis

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Infections of the skin and soft tissues due to MRSA are still relatively uncommon in babies. The ideal treatment for MRSA remains challenging. This study has been carried out to find out the antibiotics susceptibility pattern of MRSA bacteria causing community acquired folliculitis skin infections and to find the frequency of MRSA in an outpatient visiting hospital and private clinics in Mosul. *In vitro*, all *Staphylococcus aureus* isolates were investigated by antibiotic disk method and the gold standard of MRSA diagnostic testing, the molecular laboratory technique PCR to detect the *mecA* gene. Of 84 reported *S. aureus* isolates in the study period, 100% were MRSA and were *mecA* gene positive at 533bp. MRSA pathogenic strains were recorded as resistant to five antibiotics: pipracillin, ceftriaxone, cefixime, bacitracin, and cefotaxime (100%). However, erimethoprim/sulphamethoxazole, showed absolute sensitivity 84/84 (100%) for all *S. aureus* isolates. Also the prevalence of folliculitis among boys (75%) was higher than among girls. The antibiotic choice for *S. aureus* should be erimethoprim/ sulphamethoxazole for treating folliculitis.

Keywords: MRSA; folliculitis; bacterial skin infection; *mecA* gene; pipracillin; ceftriaxone; cefixime; bacitracin; cefotaxime; trimethoprim / sulphamethoxazole.

Introduction

Staphylococcus aureus is still common in the environment and in the natural flora of animals. It is a bacterium that is both pathogenic and commensal (Turner et al., 2019). It is consistently carried and is known to thrive as normal flora in the skin of roughly 20% of the worldwide population without causing any harm. Furthermore, 60% of people have it occasionally throughout their lives (Abazar et al., 2014; Nelson et al., 2015). If it has the chance to infiltrate tissue and the bloodstream, it is regarded as an opportunistic pathogen for both humans and animals (Bühlmann et al., 2008; Abazar et al., 2014). In general, it does not become contagious until it manages to penetrate the skin or mucous membrane through a puncture caused by a penetrating object (Japoni et al., 2004). Numerous illnesses have been linked to it, ranging from minor skin infections like boils, impetigo, folliculitis, cellulitis, scalded skin syndrome, furuncles, carbuncles, and abscesses to potentially fatal conditions like pneumonia, osteomyelitis, meningitis, endocarditis, and septicemia (Japoni et al., 2004). Around the world, the treatment of staphylococcal infections faces a threat from the emergence and spread of multidrug-resistant *S. aureus*. Research has shown that asymptomatic carrier children have been implicated in the spread of MRSA in the community (Hussain et al., 2001). To date, however, a thorough assessment of the precise prevalence of MRSA in Iraqi children has not been conducted. This study was conducted to evaluate the spread of MRSA in pediatric patients visiting the Al-Salam Hospital in Mosul. The antimicrobial susceptibility profile of a population of children was evaluated and the presence of MRSA was assessed. A comparison was made between the infections found in inpatients and outpatients. The correlation between the causative organism and various variables, including age, gender, and sample type, was evaluated. These play a significant role in infections caused by *S. aureus*. We think that by choosing the best therapeutic medication to treat *S. aureus*, this kind of research will help doctors provide first-line care.

The neonatal intensive care unit treats infants with serious diseases, who are very susceptible to infections or have surgical problems. Mainly they are admitted to the hospital quickly after birth, before the indigenous microbial flora has developed itself. As a result, those admitted to neonatal intensive care units are particularly vulnerable to hospital-associated germs such as methicillin-resistant *S. aureus* (MRSA). In the past few years, as strong infection control measures have been implemented in NICUs, the frequency of MRSA discovery has dropped, as has the rate of MRSA bacteremia infections.

Methicillin-susceptible *S. aureus* (MSSA) can be found in around 30% of healthy people, such as NICU healthcare professionals (Turner et al., 2019). As a result, MSSA continues to be a leading source of infections in infants' bloodstreams, skin, soft tissues, and surgical sites. Actually, MRSA bloodstream infections have decreased, while *S. aureus* bloodstream infections have not (Nelson et al., 2015). Bloodstream infection caused by diverse strains of *S. aureus* is one of the leading causes of reduced life expectancy in infants, a previous study reported no difference in complications or death between MRSA and MSSA infections in infants (Abazar et al., 2014). Thereby, it is critical to maintain control over both.

In the United States and many European countries, MSSA surveillance and decontamination have been shown to minimize MSSA infections in NICUs. Infectious diseases have a significant impact on neonatal survival due to inadequate reserve capacity. To this purpose, epidemiological research on MSSA in NICUs are critical. However, to our knowledge, the transfer of MSSA to infants born in aseptic settings has not been thoroughly investigated. Controlling *S. aureus* infections requires epidemiological data that includes MSSA transmission pathways in the NICU. As a result, this study conducted an epidemiological assessment of MSSA over a six-month period, including hospitalized neonates, parents, and healthcare workers who could be part of the transmission route (Bühlmann et al., 2008).

MRSA is a bacterium that causes infections in numerous hospitals. MRSA is a resistant bacterium that is extremely common in hospitals and

is a global causative agent of sickness and mortality, independent of the patient's age, climate, or geographic location (Japoni et al., 2004). Folliculitis and skin abscesses infection are pus-filled pockets in skin that are generated from bacterial infection, which are in some cases superficial or deep. Superficial folliculitis. In this condition the infection is restricted to the upper part of the pilosebaceous follicle. Most skin abscesses are produced by *S. aureus* and appear as pus-filled pockets on the skin surface. *Staphylococcus* strains resistant to previously effective antibiotics have become more widespread. This strain is known as MRSA (Cuny & Witte, 2005). Folliculitis is an inflammation of the hair follicles. It resembles a small red or white pimple near the base of the hair. There may be one or several infected follicles. Each infected follicle is itchy or slightly uncomfortable, but the individual does not feel sickness. *Staphylococcus aureus* obtains methicillin resistance by inserting the staphylococcal cassette chromosome (SCCmec), which contains the *mecA* gene, into the chromosome. This gene is responsible for coding an altered penicillin-binding protein, PBP-2a, that is not affected by current β-lactam antibiotics.

Our study aimed to find out the antibiotics susceptibility pattern of MRSA bacteria causing community acquired folliculitis skin infections and to find the frequency of MRSA in an outpatient visiting hospital and private clinics in Mosul and followed up with epidemiological molecular analysis of the collected isolates.

Materials and methods

Ethics approval. After each person agreed to participate in the study, the protocol was carried out in accordance with their consent and agreement.

Collection of samples and bacterial isolates. Over a period of 26 months, 84 swabs were obtained from babies and young children during routine screening in Al-Salam Hospital in Mosul city. These bacteria have been isolated from children suffering hair folliculitis with age 1 month – 7 years' old who attended the dermatology-clinic. A total of 84 patients with suspected folliculitis were included in the study: patients suffered from rash, itching, and pimples around hair follicles, burned skin and painful skin, and inflamed bumps.

Identification of methicillin-resistant S. aureus. All isolates underwent phenotypic characterisation through the application of standard bacteriological techniques, such as culturing on Mannitol salt agar (Merck Co., Germany) and a 24-hour incubation period at 37 °C. Subsequently, blood agar plates were used to plate all possible colonies of *S. aureus*. Gram stain morphology (displaying grape-like cluster purple cocci) and standard biochemical reactions, such as catalase, coagulase, and novobiocin sensitivity tests, were used to identify *S. aureus* grown colonies. The presence of β-heamolytic large white colonies suggested the presence of *S. aureus*, which was further identified based on this information (Abazar et al., 2014).

Separation and recognition. Within two hours of the collection, the specimens were processed in the microbiology lab (i.e., gram staining for microscopic inspection and bacterial culturing). A blood sample was taken right away, thoroughly mixed, and the brain heart infusion (BHI) bottle was screw-tightened after it was filled to a 1:10 v/v ratio. Following a 72-hour incubation period at 37 °C, the BHI bottles were sub-cultured on blood and MacConkey Agar plates. While cerebrospinal fluid (CSF) samples were streaked on MacConkey and chocolate agar, other samples, such as pus, urine, stool were also directly plated on blood and MacConkey agar. To isolate *S. aureus*, the growing colonies shown on blood and chocolate agar plates were further streaked on the enrichment medium, Mannitol Salt Agar (MSA). Whereas the MacConkey agar and MSA plates were incubated aerobically for 24 hours at 37 °C, the blood agar and chocolate agar plates were incubated anaerobically (5–10% CO_2). Gram staining was performed on selected colonies that had grown in MSA. For the purpose of *S. aureus* identification, only cocci bacteria were processed. Catalase and coagulase (slide and tube) tests were used with previously identified control strains to identify *S. aureus*.

Identification of S. aureus by 16S rRNA gene PCR and sequence analysis. Bacterial isolates that were phenotypically suspected to belong to *S. aureus* were genotypically confirmed by amplifying there 16S rRNA via PCR and sending the PCR product for sequencing (Khaleel et al., 2023).

Antibiotic sensitivity test (AST). At first, all 48 isolates were tested for methicillin antibiotic resistance by disk diffusion method using Mueller Hinton agar (MHA). Resistance were determined by the absence of inhibition zone after 48 h of growth (Fig. 1). All MRSA resistant strains were tested for susceptibility to nitrofurantoin (F100), trimethoprim / sulphamethoxazol (SXT25), amikacin (AK10), chloramphenicol (C10), pipracillin (PRL100), ceftriaxone (CRO10), cefixime (CFM5), gentamycin (CN10), bacitacin (B10), trimetheprim (TMP10), and cefotaxime (CTX30) (Fig. 2) by disk diffusion testing on Mueller Hinton agar. After 48 hours of growth, the zone of inhibition was measured to assess susceptibility. The CLSI standards were followed in the interpretation of the data (CLSI, 2011. Performance standards for antimicrobial susceptibility testing; twenty-first informational supplement, M100-S21).

Detection of mecA gene by PCR technique for MRSA strains. Genomic DNA was extracted from staphylococci colonies that had been cultivated overnight on blood agar plates using a DNA extraction kit supplied from (Bioneer Co., Korea). The gradient thermal cycler (Eppendorf, Germany) was used to conduct the conventional PCR experiment. The 533 base pair (bp) fragment from mecA was amplified using the primers mecA-F: 5′-AAAATCGATGGTAAAGGTTGGC-3′ and mecA-R: 5′-AGTTCTGG AGTACCGGATTTGC-3′ (Bühlmann et al., 2008). 1 µL of genomic DNA (0.5 µg) was added to a final volume of 25 µL PCR mixture, which contained 0.7 µL of 10 µmol/L from each primer, 10 µL of sterile distilled water, and 12.5 µL of 2× Master Mix (Ampliqon, Denmark). The PCR thermal cycling protocol used for amplification was as follows: 94 °C for 3 min, followed by 33 cycles of 94 °C for 1 min, 53 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 6 min. The amplified PCR products were run on 2% agarose and stained with ethidium bromide (Abazar et al., 2014). According to the fact that MSSA strains lack the *mecA* gene; hence, the presence of this gene in any *S. aureus* isolate suggests the presence of MRSA (Hallin et al., 2003).

Results

This study focuses on babies and children aged from 1 month – 7 years (100%) who attended the private clinics in Mosul and had a positive superficial folliculitis. Eleven (13%) out of eighty four (100%) had a history of skin disease while the rest of them 73 (86.9%) showed negative history. The overall prevalence was high among boys (75%) and more common (Table 1).

The majority of MRSA pathogenic isolates from this study had an MRD resistance pattern to the antibiotics used. Resistance to five types of antibiotics was recorded to pipracillin, ceftriaxone, cefixime, bacitracin, and cefotaxime (100%). Nevertheless, different sensitive patterns were recorded for the rest of the antibiotics. Trimethoprim/sulphamethoxazole, however, was the only one that showed absolute effectiveness 84/84 (100%) fagainst all *S. aureus* isolates (Table 2). The other types of antibiotics showed different results, amikacin (95.2%), trimethoprim (90.4%), gentamycin (83.3%), and chloramphenicol (76.5%). Only nitrofurantoin showed low sensitivity reaction (16.5%).

The results for the molecular study confirmed *S. aureus* by sequencing gene with purity (98.1%) for all pathogenic isolates. All 84 isolates were confirmed as *S. aureus* strain N3 by 16S rRNA partial sequence. The nucleotide sequence of the 16S rRNA gene was homologous to the Gene Bank sequence (accession number: pp118306). MRAS results were confirmed by using PCR with detection of *mecA* gene with product size 533bp (Fig. 3) after antibiotic sensitivity disk method (Fig. 1). These antibacterial agents would not be reliable, as evidenced by the elevated MRSA isolation rate and resistance to ciprofloxacin, penicillin, ampicillin, erythromycin, cotrimoxazole, chloramphenicol, etc. It appears that vancomycin is the only antibiotic in the study that demonstrated 100% effectiveness against *S. aureus*. Vancomycin may therefore be the recommended medication for the treatment of MDR-MRSA infections. Nonetheless, regular testing and frequent monitoring of vancomycin sensitivity ought to be performed. Vancomycin's usefulness should be maintained by limiting its use. Only in situations with a definite need should it be administered. The screening test and MIC values are advised for early detection of impending resistance and tracking the response to therapy, even though all isolates were found to be vancomycin-sensitive.

The development of a clear antibiotic strategy and routine surveillance of nosocomial infections, which includes antiprogram monitoring for MRSA and MSSA, may be beneficial in lowering the incidence of MRSA infection. Thus, the study presents a way to support epidemiological research.

Discussion

Folliculitis is a common skin condition that develops when a hair follicle becomes inflamed. It appears as acne. The rash might itch or cause slight pain, and it manifests as little red or pus-filled pimples. Infant infection rates were quite low. Folliculitis is frequently seen on the thighs, arms, and buttocks. Redness on the skin might occasionally result from dark marks left behind after the rash has gone away. Although it is only found in hospitals and dermatology clinics, MRSA infection is known to be quite common among healthy children and people in the community (Grundmann et al., 2006).

Table 1
Selected socio-demographic characteristic of the study sample, Mosul City 2022

Demographic feature	No. (%)
Age	
1 month – 7 years	84 (100.0)
Nationality	
Arab	77 (91.6)
Kurd	7 (8.3)
History of skin diseases	
Yes	11 (13.0)
No	73 (86.9)
Gender	
Male	63 (75.0)
Female	21 (25.0)

Table 2
Antimicrobial sensitivity profiles of *S. aureus* MRSA isolates (n = 84)

Type of antibiotic	Resistance, n (%)	Intermediate, n (%)	Sensitive, n (%)
Nitrofurantoin (F 100)	40 (47.6)	30 (35.7)	14 (16.6)
Trimethoprim/sulphamethoxazole (SXT25)	0	0	84 (100)
Amikacin (AK 10)	0	4 (4.7)	80 (95.2)
Chloramphenicol (C 10)	5 (5.9)	13 (15.4)	66 (76.5)
Pipracillin (PRL100)	84 (100)	0	0
Ceftriaxone (CRO 10)	84 (100)	0	0
Cefixime (CFM 5)	84 (100)	0	0
Gentamycin (CN 10)	2 (2.3)	12 (14.2)	70 (83.3)
Bacitracin (B 10)	84 (100)	0	0
Trimethoprim (TMP 10)	0	8 (9.5)	76 (90.4)
Cefotaxime (CTX 30)	84 (100)	0	0

One of the most frequent causes of folliculitis is *S. aureus* bacterial infection of the hair follicles. On the skin, there are tiny pus-filled pimples that are red or white. In most cases, the damaged area heals (resolves) in a few days and is manageable at home. However, a dermatologist should be consulted for treatment of folliculitis in situations that are severe and persistent (Demos et al., 2013).

Pediatricians will likely suspect that an infection like folliculitis which is caused by MRSA should be treated by stronger or different antibiotics to treat the infection. The diagnosis of skin infection is made by the pattern of symptoms and physical examination findings. Trimethoprim / sulfamethoxazole (TMP-SMX or bactrim) were recommended as the best choice of drug for MRSA treatment infection (Hassoun et al., 2017). The complicated skin and soft tissue infection by MRSA in children was treated by newer antibiotics zyvox (linezolid), which are prescribed when other antibiotics such as bactrim or clindamycin aren't working (Li et al., 2017).

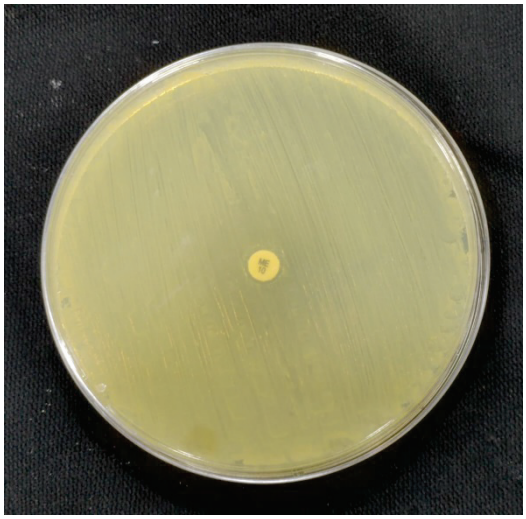

Fig. 1. MRSA culture grown on Mueller-Hinton agar media showing methicillin resistance against cefoxitin disc

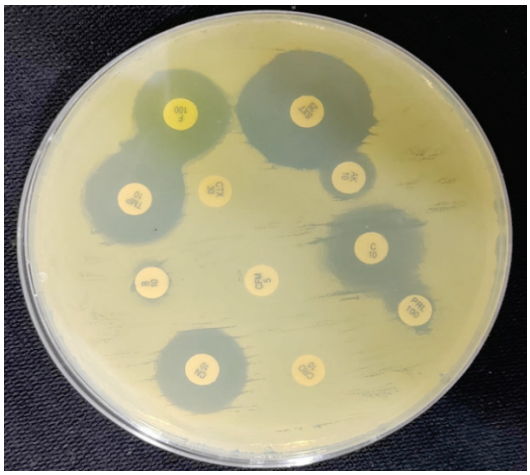

Fig. 2. Antibiotic susceptibility test: nitrofurantoin (F100), trimethoprim / sulphamethoxazole (SXT25), amikacin (AK10); chloramphenicol (C10), pipracillin (PRL100), ceftriaxone (CRO10), cefixime (CFM5); gentamycin (CN10), bacitracin (B10), trimethoprim (TMP10), cefotaxime (CTX30)

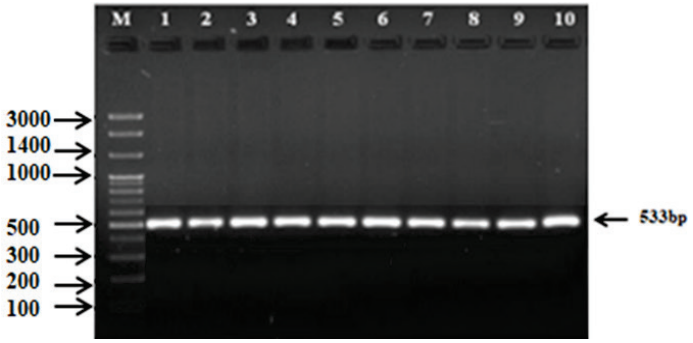

Fig. 3. PCR amplification of *mecA* gene in 10 selected isolates of *S. aureus*: lane 1–10: PCR product of *mecA* gene positive at (533bp); M: 3000 bp DNA size marker

The study on superficial folliculitis among pediatric patients, particularly focusing on MRSA infection, offers significant insights into prevalence, antibiotic resistance, and treatment implications. The findings shed light on several key aspects.

The study has several implications. First, the high prevalence of superficial folliculitis among 1 month to 7 years-old infants and children, especially boys, is identified. The distribution predisposes to the prevalence of the epidemiological meaning of such a condition and supports the necessity of understanding the pathology to make targeted interventions in healthcare provision for affected patients. Second, the association of superficial folliculitis with MRSA infection is noted. Considering the fact that MRSA is resistant to multiple antibiotics, obtaining the results indicates the necessity to choose alternative or simply stronger antibiotics than clindamycin. Third, the findings on antibiotic resistance help practitioners to make meaningful choices. Thus, although the sensitivity of trimethoprim/sulphamethoxazole reaches 100% of *S. aureus* isolates, other antibiotics also have good performance. Therefore, the consideration of local resistance when selecting treatment is relevant.

Furthermore, the study highlights the difficulty in addressing severe or persistent cases of folliculitis, especially when it is caused by MRSA. In some situations, medical practitioners might be forced to apply stronger or another type of antibiotics; therefore, there is the need for regular patient monitoring and suitable treatments. Nonetheless, there are potential limitations that should be acknowledged about the study, such as small sample size or biases, as well as future research areas. For example, a broader study may examine the entire range of factors that contribute to folliculitis and MRSA in children, and more importantly, investigate other treatment options that can adequately be used against another clinical issue.

In summary, the study provides valuable insights into the prevalence, antibiotic resistance patterns, and management considerations for MRSA-associated superficial folliculitis in pediatric patients. Understanding these aspects is crucial for guiding effective treatment strategies and mitigating the impact of antibiotic resistance on clinical outcomes.

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