Prevalence of Scc-mecA, ermB, and fusidic acid-resistant genes in Staphylococcal species recovered from acne vulgaris in Egypt

Noha M. Hashem*, Alaa El-Din M.S. Hosnyb,c, Ali A. Abdelrahmand, Samira Zakeerd

1Pharmacist, Ministry of Health, Egypt, 2Department of Microbiology and Immunology, Faculty of Pharmacy, Cairo University, Egypt, 3Department of Microbiology and Immunology, Faculty of Pharmacy, MTI University, Egypt, 4Department of Microbiology and Immunology, Faculty of Pharmacy, Suez Canal University, Egypt

Received on: 16.02.2022
Revised on: 01.04.2022
Accepted on: 05.04.2022

*Correspondence Author:
Tel: +201003019064
E-mail address:
noha.mahmoud_22@yahoo.com

Abstract

A multidrug-resistant Staphylococcal species including Staphylococcus aureus and Staphylococcus epidermidis that colonizing patients with acne vulgaris were investigated for the presence of resistance determinants. Acne vulgaris patients are usually subjected to topical antibiotic treatment including erythromycin and fusidic acid as the first line of treatments, which have been associated with resistance development. In this study, we attempt to investigate the dissemination of resistance determinants exclusively related to fusidic acid antibiotics mainly the horizontally transmitted fusB and fusC genes among the SCCmecA harboring isolates using the polymerase chain reaction technique. Our results suggested amplified resistance to fusidic acid with a large abundance magnitude of fusC gene among methicillin-resistant Staphylococcus aureus isolates, while an increased abundance of fusB gene among Staphylococcus epidermidis isolates compared to other resistance determinants. Conclusion: patients with acne vulgaris who were subjected to a previous fusidic acid treatments should consider treatment with alternative antibiotics other than fusidic acid, to achieve maximum treatment benefits considering clindamycin and aminoglycoside.

Keywords: Acne vulgaris, Fusidic acid, Staphylococcus aureus, Staphylococcus epidermidis

1. Introduction:

Acne vulgaris is a common disorder involving the pilosebaceous unit. Roughly, 85% of young adults aged 12-25 have experienced acne vulgaris (Lynn et al., 2016). Acne frequently endures into adulthood, with 26% of women and 12% of men reporting acne in their 40s (Andrea and Zaenglein, 2018). The pathogenesis is multifactorial, including four key elements with interrelated mechanisms: hyperkeratinization of the follicular infundibulum, increased sebum production, inflammation, and microbial colonization. Patients with acne vulgaris are often colonized with Staphylococcus aureus (S.aureus), Cutibacterium acne, Staphylococcus epidermidis (S.epidermidis), and Malassezia furfur. These organisms are part of the skin microbiome which is thought to correlate with acne pathogenesis (Reham et al., 2016). Although S.aureus has been evidenced in many studies to possess high virulence, it is supposed that coagulase-negative staphylococci (CoNS) operate as a critical reservoir of antimicrobial resistance genes and resistance-associated mobile genetic
elements, that have the ability to mobile between staphylococcal species (Zhen et al., 2018). *Staphylococcus aureus* maintains the competence to subsist resistance to different classes of antimicrobial agents such as methicillin. In the Mediterranean area, methicillin-resistant *Staphylococcus aureus* (MRSA) were reported in Egypt, Jordan and Cyprus, where more than 50% recovered from blood culture were resistant to methicillin (Ahmed et al., 2019). Recently, it has been reported with an amplified magnitude in the community and so-called community-acquired MRSA (CA-MRSA) (Shymaa et al., 2010). CA-MRSA frequently carries genes for Panton-Valentine leucocidin (PVL), a harmful toxin that destroys bacterium-engulfing immune cells and also respiratory tissue, cytotoxins, and other pathogenicity factors (Shymaa and Alexander, 2017; Takano et al., 2008).

The occurrence of methicillin resistance is interrelated to the manifestation of the staphylococcal cassette chromosome mec (SCCmec) element, which is a combination of regulatory genes such as the mecA, C, I, and R gene complex, and the ccr (cassette chromosome recombinase) gene complex, encoding the recombinase gene (Rahimi, 2016). The mecA gene mainly encodes for a modified penicillin-binding protein (PBP-2A), with reduced affinity to B-lactam antibiotics which is responsible for this kind of antimicrobial resistance (Shymaa and Alexander, 2017). The infections caused by MRSA are challenging clinical treatment. MRSA is the most common nosocomial pathogen that causes infections, ranging from mild skin infections to severe and fatal necrotizing pneumonia (Fangyou et al., 2015). In many countries, topical antibiotics are used for treating superficial skin infections induced by staphylococci, such as atopic dermatitis and impetigo (McLaws et al., 2008). The most commonly used topical antimicrobial agents include macrolide, erythromycin, fusidic acid (FA), aminoglycoside, and mupirocin. Macrolides are widely involved in the treatment of skin and soft tissue infections. Resistance to macrolides progressively emerged laterally with their extensive use (Xingmei et al., 2017). The most prominent form of resistance to macrolides is ribosomal target modification induced by ermA, ermB, ermC, and erm33 genes (Coutinho et al., 2010). Fusidic acid is a steroid antibiotic employed predominantly as a topical agent for treating superficial skin infections caused by staphylococci. The main target of fusidic acid is the elongation factor G (EF-G), which is involved in protein synthesis. Fusidic acid stalls protein synthesis by inhibiting the turnover of elongation factor G (EF-G) from the ribosome, and resistance frequently develops through point mutation(s) in the chromosomal gene encoding EF-G (fusA), which typically confers high-level resistance. Additionally, the acquisition of factors that protect the translational machinery (encoded by plasmid-borne genes fusB or fusC) can prompt low-level resistance (Xingmei Liu, et al., 2017). Resistance to fusidic acid in *Staphylococcus aureus* and other staphylococci commonly arises either from mutations in fusA or following the horizontal acquisition of the fusB or fusC determinants (McLaws et al., 2008). Hereby this article is concerned with the prevalence of resistance specifically to fusidic acid, and also the occurrence of the SccmecA gene and ermB gene in clinical isolates of *S. aureus* and *S. epidermidis* harboring multidrug resistance from acne vulgaris patients in Egypt.

2. Methodology:

This study includes 101 acne vulgaris patients from the outpatient clinic of the Department of Dermatology at El Khanka Hospital. The study population is part of a larger cross-sectional (n=173), which has been described previously (Noha et al., 2021). Patients were included in the study during the period of March 2018 and December 2018. Information regarding medical treatment in the 3 months before sampling time was obtained from all patients.

2.1 Bacterial isolate collection

Bacterial swabs were taken from acne vulgaris pustules. *Staphylococcus* species were identified by plating samples on selective mannitol salt agar (MSA) (Himedia Laboratory M118-500G, Mumbai, India), and a single isolate was collected from each sample for further analysis. Isolates were identified as *S. aureus* and *S. epidermidis* by using Gram’s stain, catalase test, coagulase test, mannitol fermentation activity, hemolytic activity on sheep blood agar.

2.2 Antibiotic susceptibility testing

Disc diffusion method was performed according to the CLSI Guidelines 2019 (CLSI, 2019) on the following antibiotics: Tetracycline, Doxycycline, Erythromycin, Clindamycin, Penicillin, Gentamycin, Fusidic Acid, Ofloxacin, Cefoxitin, and Chloramphenicol. The inhibition zones were measured after 24hrs aerobic incubation at 37°C and the results were interpreted according to CLSI Guidelines 2019.

2.3 DNA extraction and detection of fusidic acid resistance determinants by PCR.
Bacterial DNA was purified with a Gene JET Genomic DNA Purification kit according to the manufacturer’s instructions. Dissemination of \textit{mecA} and \textit{ermB} determinants were detected by PCR assays with primers and reaction conditions described previously (Noha \textit{et al.}, 2021). To detect the presence of acquired fusidic acid resistance determinants (\textit{fusB} and \textit{fusC}), PCR assay reaction was performed. The DNAs were amplified for the \textit{fusB} gene and the \textit{fusC} gene using oligonucleotide primers BF (5’-ATTCAATCGGAAACCTATAATGATA-3’), BR (5’-TTATATATTCTTGATTTGATGCAAG-3’), CF (50-TTAAAGAAAAAGATATTGATATCTCGG), and CR (50-TTTACAGAATCCCTTTACTTTATTTTG).

To generate amplicons of 292 and 332 bp from the \textit{fusB} and \textit{fusC} genes, correspondingly. The reaction PCR program conditions were denaturation step (94°C for 3 min), followed by 35 cycles of (94°C for 30 s), the annealing temperature of 57°C for (30 s) for \textit{fusC} and 60°C for (30s) for \textit{fusB} gene, followed by 72°C for (45 s) (McLaws \textit{et al.}, 2008; Xingmei \textit{et al.}, 2017).

3. Results:

3.1 Antimicrobial susceptibility testing

One hundred and one bacterial isolates were included in this study. Thirty-one were \textit{S.aureus} and seventy were \textit{S.epidermidis}. The most prevalent types of resistance were resistance to penicillin and fusidic acid. In this study, \textit{S.aureus} showed high resistance to fusidic acid with more than 90% resistance followed by cefoxitin resistance with 84%. Twenty-eight isolates were resistant to fusidic acid while only 3 isolates were susceptible. MRSA isolates were 26/28 of the FA resistant while only two isolates of FA resistant were MSSA. The resistance to the remaining antibiotics was as follows gentamicin, tetracycline, and erythromycin with 54.8%, 45.2%, and 25.8%, respectively. Meanwhile, \textit{S.epidermidis} showed increased resistance toward FA with 88.6% followed by cefoxitin and erythromycin with 75.7% and 61.4%, correspondingly. Sixty-two isolates of \textit{S.epidermidis} were resistant to FA while 8 isolates were susceptible. MRS isolates were 53/62 of the resistant FA, while 9/62 were MSS. Figure (1).

![Figure 1: Antibiotic susceptibility pattern of \textit{Staphylococcus aureus} and \textit{Staphylococcus epidermidis} isolates recovered from acne vulgaris](image-url)
3.2 Prevalence of antimicrobials resistance determinants by PCR

To determine the resistance genes in 31 S. aureus and 70 S. epidermidis PCR was conducted. We first detected mecA and ermB resistant determinants by PCR assay with primers and reaction conditions described previously (Noha et al., 2021). In brief, the mecA resistant determinant was dominant among all the isolates and recovered from all the MRSA isolates and was absent in the MSSA isolates. Meanwhile, it was recovered from 48/53 methicillin-resistant staphylococci while the remaining 5/5 failed to produce the band of 500 bp specific for the mecA gene.

While the ermB gene was only recovered from only 1 (3.2%) MRSA isolate which was multidrug-resistant and 4 isolates (5.7%) of S.epidermidis. In this study, the prevalence of FA resistance determinants fusC and fusB were determined in all FA resistant strains. The dissemination of the fusB gene was dominant in S.epidermidis isolates 39/53 of methicillin-resistant staphylococci (MRS) (73.5%) harbored the fusB gene. While in methicillin-sensitive staphylococci (MSS), the fusB gene was recovered from 4/9 MSS with (44.4%). However, the dissemination of FA resistance determinant fusC was infrequent, it was only recovered from 5/53 (9.4%) MRS, and only one isolate MSS showed the fusC gene. On contrary, the distribution of FA resistance determinant fusC was the most prevalent in S.aureus. It was recovered from 12/26 (46%) MRSA and was negative in MSSA however, the fusB gene was only detected in 3/26 (11.5%) MRSA and one MSSA isolate. Results of PCR amplicons of FA resistant determinants are shown in Figure (2). Gene distribution of bacterial isolates are shown in Table (1) and Figure (3).

Figure 2: PCR gel electrophoresis shows amplicons of fusB gene products of S. epidermidis at 292bp and amplicons of fusC gene products of S. aureus at 332bp.

Table 1: Distribution of selected resistant gene in clinical bacterial isolates from acne vulgaris

<table>
<thead>
<tr>
<th>Resistant determinant</th>
<th>S. aureus</th>
<th>S. epidermidis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MRS* (n=26)</td>
<td>MSSA* (n=5)</td>
</tr>
<tr>
<td>mecA</td>
<td>26</td>
<td>---</td>
</tr>
<tr>
<td>ermB</td>
<td>1</td>
<td>---</td>
</tr>
<tr>
<td>fusC</td>
<td>12</td>
<td>---</td>
</tr>
<tr>
<td>fusB</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

4. Discussion:

In our previous study (Noha et al., 2021), we reported amplified dissemination of the mecA gene among CA-MRSA and MRS, these isolates were multidrug-resistant. However, the mecA gene was absent in MSSA isolates and they were susceptible to a variety of antibiotics. This phenomenon can be correlated to the absence of SCCmec elements, that contain additional drug resistance genes carried on integrated plasmids and transposons for kanamycin, tobramycin, bleomycin, tetracycline, as well as transposon Tn554 (carrying ermA gene) responsible for inducible macrolide, lincosamide, and streptogramin (MLS) resistance (Saber et al., 2017). The high resistance to fusidic acid in this study indicated that fusidic acid may not be appropriate to treat S. aureus and S.epidermidis infections induced by resistant strains causing acne. Meanwhile, low resistance to clindamycin and macrolides indicated that these two antibiotics remained effective for treatment of acne caused by staphylococci. The latest data suggest a global spread of fusC resistance determinant among MRSA isolates with an association with the epidemic ST239 clone in Taiwan (Lin et al., 2014), besides the emergence of different MRSA harboring the fusC gene in New Zealand following the amplified application of fusidic acid since 2000 (Williamson et al., 2014). Earlier data from the UK have shown that fusidic acid resistance in MRSA bacteremia isolates increased from 1.8% to5.5% (Ellington et al., 2015). In the U.S. and European collections, the fusC gene was more prevalent than the fusB gene in S. aureus strains (Castanheira, Mendes et al., 2010). Another study in Denmark showed a high resistance prevalence of the fusC gene with (65%) S. aureus from atopic dermatitis patients as these patients are frequently treated with topical fusidic acid. These statements are in agreement with our finding in which the most prominent horizontally transferred FA resistant determinant was the fusC gene among S.aureus with (46%) isolated from acne vulgaris. Other causes of resistance may be due to the fusA point mutations and horizontally transferable genes including the fusB, and the fusD genes. In this study, the fusB gene was infrequent in S.aureus that is also comes in agreement with the previously mentioned studies (Castanheira, Mendes et al., 2010; Williamson et al., 2014; Ellington et al., 2015). The high resistance prevalence to FA in S. epidermidis has been subjected to strong fusidic acid selection pressure, which is probably a result of topical application of the drug to the skin. In a previous study, the fusB gene was detected in 18 of the 23 S.epidermidis resistant isolates, and two further isolates were found to carry the fusC gene (McLaws et al., 2008). In another study, The total resistance rate of FA in CoNS from skin flora was 37.3%, which was lower than those recovered from hospitalized patients in Taiwan (48.9%) but is still higher than CoNS recovered from hospitals in the United States (7.2%), Canada (20.0%), Australia (10.8%) or some European countries (Greece, Israel, Italy, Poland, Spain and Turkey, 12.5% to 32.0%) (Castanheira, Mendes et al., 2010; Castanheira, Bell et al., 2010). In this study, the fusB gene among MRS was dominant with (73.5%) compared to the fusC gene that was only found in 5 MRS isolates with (9.4%). As with S.aureus additional sources of resistance may be due to the fusA point mutations and by the horizontally transferable genes including the fusC, and the fusD genes.

![Figure 3: Distribution of selected resistant genes in clinical bacterial isolates from acne vulgaris](image)
5. Conclusion:

Fusidic acid resistance has been disseminated among superficial *Staphylococcus* species that have been treated with topical application of fusidic acid. Resistance to this antibiotic in *S. epidermidis* is commonly the result of carriage of the *fusB* determinant, while resistance in *S.aureus* is frequently related to the occurrence of *fusC* gene determinant among other resistant determinants.

6. References:


