ORIGINAL ARTICLE

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

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

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Received on: 16. 02. 2022

Revised on: 01. 04. 2022

Accepted on: 05. 04. 2022

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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, *Staphyloccocus aureus*, *Staphyloccocus 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 (SCC*mec*) element, which is a combination of regulatory genes such as the *mec*A, C, I, and R gene complex, and the *ccr* (cassette chromosome recombinase) gene complex, encoding the recombinase gene (**Rahimi, 2016**). The *mec*A 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 f*usC* 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 Scc*mecA* 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 37^0C 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 *mecA* and *ermB* determinants were detected by PCR assays with primers and reaction conditions described previously **(Noha *et al.*, 2021).** To detect the presence of acquired fusidic acid resistance determinants (*fusB* and *fusC),* PCR assay reaction was performed. The DNAs were amplified for the *fusB gene* and the *fusC* gene using oligonucleotide primers BF (5'-ATTCAATCGGAAACCTATAATGA TA-3'), BR (5'-TTATATATTTCCGATTTGATGCAAG-3'), CF (50-TTAAAGAAAAAGATATTGATATCTCGG), and CR (50-TTTACAGAATCCTTTTACTTTATTTGG)

to generate amplicons of 292 and 332 bp from the *fus*B and *fusC* genes, correspondingly. The reaction PCR program conditions were denaturation step (94ºC for 3 min), followed by 35 cycles of (94ºC for 30 s), the annealing temperature of 57ºC for (30 s) for *fusC* and 60º C for (30s) for f*usB gene*, followed by 72ºC for (45 s) (**McLaws *et al.*, 2008; Xingmei *et al.*, 2017**).

3. Results:

3.1 Antimicrobial susceptibility testing

One hundred and one bacterial isolates were included in this study. Thirty-one were *S.aureus* and seventy were *S.epidermidis.* The most prevalent types of resistance were resistance to penicillin and fusidic acid. In this study, *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, *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 *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).

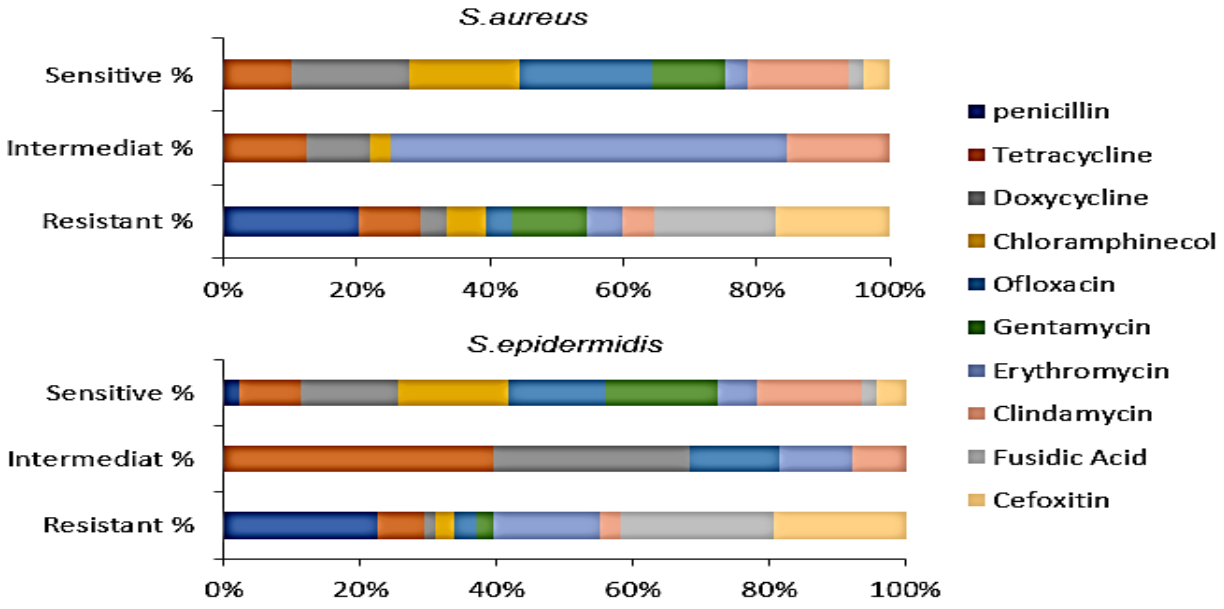

Figure 1: Antibiotic susceptibility pattern of *Staphylococcus aureus* and *Staphylococcus epidermidis* isolates recovered from acne vulgaris

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 *fusB* and *fusC* 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)

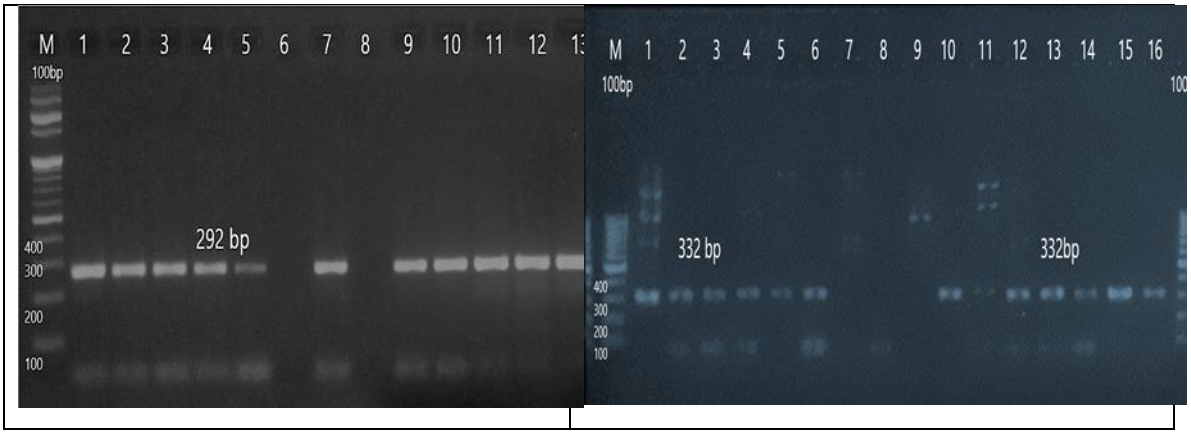

Figure 2: PCR gel electrophoresis shows amplicons of *fusB* gene products of *S. epidermidis* at 292bp and amplicons of *fusC* gene products of *S. aureus* at 332bp.

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

Resistant determinant genes	*S. aureus*		*S. epidermidis*	
	MRSA (n=26)*	***MSSA*** (n=5)*	***MRS*** (n=53)*	***MSS*** (n=17)*
mecA	**26**	**---**	**48**	**17**
ermB	**1**	**---**	**4**	**---**
fusC	**12**	**---**	**5**	**1**
fusB	**3**	**1**	**39**	**4**

*MRSA: Methicillin-resistant *S.aureus*, *MSSA: Methicillin-sensitive *S.aureus*, *MRS: Methicillin-resistant staphylococci, and *MSS: Methicillin-sensitive staphylococci.

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.

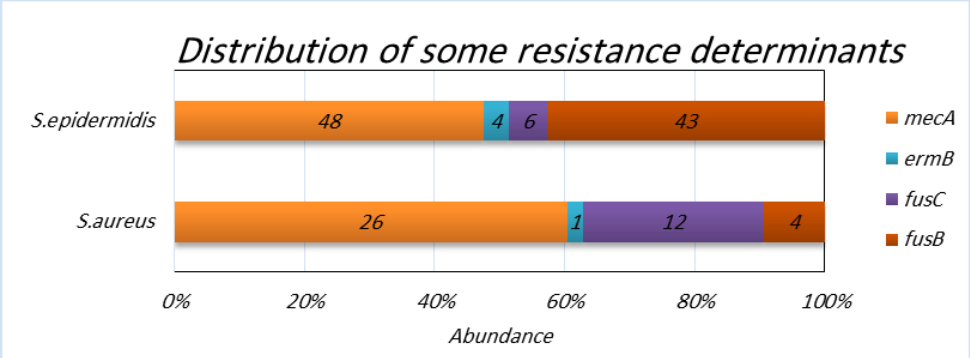

Figure 3: Distribution of selected resistant genes in clinical bacterial isolates from acne vulgaris

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.

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