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Genetic Determinants of Resistance to Fusidic Acid Among *Staphylococcus aureus* Isolates in Jordan

Rasha Aldasouqi [1], Luay F. Abu-Qatouseh [2], Eman F. Badran [3], Sameer A. Alhaj Mahmoud [4] and Rula Madhat Darwish [1,*]

[1] Department of Pharmaceutics and Pharmaceutical Technology, School of Pharmacy, The University of Jordan, Amman, Jordan
[2] Department of Pharmacology and Biomedical Sciences, Faculty of Pharmacy, University of Petra, Amman, Jordan
[3] Department of Paediatrics, Division Neonatology, School of Medicine, The University of Jordan, Amman, Jordan
[4] Department of Basic Medical Sciences, Faculty of Medicine, The Hashemite University, Zarka, Jordan

[*] *Corresponding author*: Department of Pharmaceutics and Pharmaceutical Technology, School of Pharmacy, The University of Jordan, Amman, Jordan. Email: rulamdarwish@yahoo.com

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Abstract

Background: Fusidic acid-resistant *Staphylococcus aureus* (FRSA) has been reported in many countries to have a remarkable difference in resistance determinants. Fusidic acid resistance is very important because it might lead to the failure of topical treatment, especially when it is used as empiric therapy. In addition, its resistance might be linked to other antibiotic resistances. The overall rate of fusidic acid resistance is still relatively low. However, there is an increase in the prevalence of clinical isolates of FRSA worldwide.

Objectives: We aimed to characterize FRSA isolated from Jordanian patients and evaluate the occurrence of the genetic resistance caused by *fusB* and *fusC*.

Methods: We conducted a prospective cross-sectional study to determine the prevalence and the resistance pattern of *S. aureus* to fusidic acid among Jordanian patients and healthy people. *Staphylococcus aureus* clinical isolates (n = 113) obtained from patients admitted to Prince Hamzah Hospital between February and July 2015 were compared with isolates (n = 288) obtained from healthy subjects. Conventional methods were used for the identification of *S. aureus* and further confirmations were done by the existence of the thermonuclease gene using polymerase chain reaction (PCR). Screenings of antibiotic resistance were performed using the disc diffusion method. The minimum inhibitory concentrations were calculated using the E-test. PCR was used to detect the presence of resistant genes.

Results: The FRSA frequency was significantly higher among clinical isolates (31.9%) than among isolates from healthy subjects (1%) and in methicillin-resistant *Staphylococcus aureus* (MRSA) (66.7%) than in methicillin-sensitive *Staphylococcus aureus* (MSSA) (33.3%). Of the FRSA isolates, 38.9% and 16.7% carried *fusB* and *fusC*, respectively, and they displayed low resistance compared to non-*fusB*, non-*fusC* FRSA isolates. The rate of FRSA was significantly ($P < 0.05$) higher among MRSA than among MSSA isolates (n = 24, 66.7% and n = 12, 33.3%, respectively). We found no association between fusidic acid determinants among MRSA and MSSA ($P > 0.05$).

Conclusions: A high occurrence of FRSA was detected in Jordanian clinical isolates of *S. aureus*, particularly among MRSA. Moreover, *fusB* was the predominant resistance determinant, with low-level resistance. Based on our findings, fusidic acid susceptibility testing is strongly recommended in medical laboratories. The restricted use of fusidic acid is advised.

Keywords: *Staphylococcus aureus*, Fusidic Acid, Drug Resistance, *fusB* and *fusC* Genes

1. Background

Staphylococcus aureus can be carried asymptomatically and it is one of the main causes of hospital and community-acquired infections (1). The extensive use of antibiotics for the treatment of *S. aureus* skin and soft tissue infections has caused selective pressure and eventually has given rise to multiple drug-resistant strains (2). Fusidic acid is an antibiotic often used in topical preparations for skin infections of *S. aureus*. It inhibits bacterial protein synthesis by binding to elongation factor G (EF-G), through which preventing its release from the ribosome and consequently, the elongation of nascent polypeptides (3). Fusidic acid-resistant *Staphylococcus aureus* (FRSA) has been reported in many countries to have a remarkable difference in resistance determinants. Fusidic acid resistance is very important because it might lead to the failure of topical treatment, especially when it is used as empiric therapy.

In addition, its resistance might be linked to other antibiotic resistances. The overall rate of fusidic acid resistance is still relatively low. However, there is an increase in the prevalence of clinical isolates of FRSA worldwide.

There are a number of fusidic acid resistance mechanisms among *S. aureus* and other staphylococci (4, 5). Numerous classes of *fus* genes have been recognized in fusidic acid-resistant *S. aureus*. The FusA class is linked to mutations in the chromosomal EF-G-encoding gene *fusA* that reduce the fusidic acid binding with EF-G ribosome complex (6). *FusA* mutations are mostly seen in the structural domain III of EF-G, and to a lower extent in domains I and V, and they are associated with high-level resistance (7). The *fusA*-small-colony variant (SCV) class is a subset of the FusA class in which mutations in *fusA* mostly appear in the structural domain V of EF-G, and some in domains I and III. The mutants of this class are the SCVs of *S. aureus* (8). They are characterized by slow growth and are implicated in chronic and relapsing infections (9).

Mutants of the FusE class carry mutations in *rplF*, which encodes ribosomal protein L6 that is situated at the interaction site with EF-G; these mutants also demonstrate the SCV phenotype and either hemin or menadione auxotrophy (10). In contrast, the FusB, FusC, and FusD classes harbor fusidic acid-resistance genes that yield proteins protecting EF-G from binding to fusidic acid and can be transmitted horizontally. In addition, they result in low-level resistance (11). The *fusB* gene is carried on different genetic elements (12); it can be present on the pUB101 plasmid, on a transposon-like element, or else in a pathogenicity island (4). The *fusC* and *fusD* genes are located on the chromosome in clinical isolates of different *Staphylococcus* species (13). The *fusC* gene has been associated with *S. aureus*, *S. intermedius*, and *S. epidermidis*, whereas *fusD* has been associated with *S. saprophyticus* and is the cause of intrinsic resistance of the bacteria to fusidic acid (4, 5).

2. Objectives

There is a lack of data concerning the prevalence of fusidic acid resistance amongst *S. aureus* isolates from Jordan. Therefore, the present study was performed to determine the rate and distribution of fusidic acid resistance, including *fusB* and *fusC* resistance genes, among clinical isolates from Jordan.

3. Methods

3.1. Collecting and Identifying Staphylococcus aureus Isolates

This prospective cross-sectional study was conducted using 113 clinical *S. aureus* isolates collected from adult Jordanian patients admitted to Prince Hamzah Hospital in Amman between February 1st, and July 30th, 2015. Of the 113 isolates, 63 (55.8%) were isolated from skin and soft tissues, 28 (24.8%) from respiratory secretions, including nasal swabs and sputum, 15 (13.3%) from blood, and seven (6.1%) from urine. Control nasal and skin sample specimens were obtained from 288 healthy adults from the community using sterile cotton swabs, and were placed in screw-capped tubes containing trypticase soy broth supplemented with 7% NaCl (14). Each isolate was cultured and identified presumptively as *S. aureus* by colony morphology, gram staining, and a set of biochemical tests, including catalase test, coagulase activity using rabbit plasma (Remel-Oxoid, Lenexa, UK), and mannitol fermentation. The isolates were further verified as being *S. aureus* by confirmation of the occurrence of the thermonuclease-encoding (*nuc*) gene by polymerase chain reaction (PCR) (15).

3.2. Testing for Antimicrobial Susceptibility

We detected the antimicrobial susceptibility to fusidic acid (FD/10 μg), penicillin G (10 unit), gentamicin (CN/10 μg), erythromycin (E/15 μg), teicoplanin (TEC/30 μg), cefoxitin (FOX/30 μg), vancomycin (VA/30 μg), clindamycin (DA/2 μg), and sulfamethoxazole-trimethoprim (SXT/25 μg) using the disc diffusion method in accordance with the guidelines of the Clinical and Laboratory Standards Institute (16). All antibiotics were obtained from Oxoid (Hampshire, UK). The methicillin-resistant *Staphylococcus aureus* (MRSA) phenotype was affirmed by the standard PCR for the *mecA* gene in the resistant isolates (15). The interpretive criteria for susceptibility and resistance to fusidic acid (10-μg disc) were the inhibition zones of $\geq$ 22 mm and $<$ 19 mm, respectively, according to Jones et al. (17). The minimum inhibitory concentration (MIC) of fusidic acid was determined using an E-test as the standard procedure in most clinical microbiology laboratories.

The E-test (Liofilchem, Roseto Degli Abruzzi, Italy) consisting of a thin strip carrying a continuous gradient of fusidic acid was deposited on the surface of inoculated Mueller-Hinton agar plates in accordance with the instructions of the manufacturer; in this method, fusidic acid diffuses into the agar to generate its MIC value. This technique is less time-consuming, less expensive if limited drugs are tested, easy to execute, and suitable for testing of certain fastidious bacteria. Two *S. aureus* strains (ATCC 25923 and ATCC 43300) were used as control strains. Both control strains were obtained from Oxoid (Culti-Loops® Remel-Basingstoke-UK). Stock cultures of these strains were maintained at 4°C on slopes of nutrient agar. Cultures for experiments were prepared by transferring a sample from the

stock cultures into Mueller-Hinton broth (MHB) and incubating without agitation for 24 hours at 37°C. The cultures were diluted with fresh Mueller-Hinton broth to achieve optical densities corresponding to 2.0×10^6 colony forming units (CFU/mL). The fusidic acid MIC for *S. aureus* was grouped as recommended by the European Committee for Antimicrobial Susceptibility Testing (EUCAST)/British Society of Antimicrobial Chemotherapy (BSAC) criteria into susceptible where MIC ≤ 1 μg/mL and resistant where MIC >1 μg/mL (18).

3.3. Detecting of Fusidic Acid Resistance Determinants

All isolates that had fusidic acid MICs of > 1 mg/L were further verified for the occurrence of the *fusB* and *fusC* genes using the specific primers listed in Table 1 (6). The E.Z.N.A.® Bacterial DNA Kit (Omega Bio-Tek, USA) was used to isolate genomic DNA from an overnight culture according to the manufacturer's instructions. PCR amplification was performed according to Lannergard et al. (6).

Table 1. The Oligonucleotide Primers Used in the Study

Name	Sequence (5' - 3')	Product Size, bp
fusB		930
Forward	CGCCACTCAATGAGTGACGCT	
Reverse	CGGGAGGTGATGATGTTATGT	
fusC2		750
Forward	ATGAATAAAATAGAAGTGTATAAGTTTGTTAAA	
Reverse	CTATTTTATTTTAACAATAAATTCGTAAAGATT	

Thermocycling conditions were as follows: preliminary denaturation at 94°C for 5 minutes, and then 30 cycles of denaturation at 94°C for 30 seconds, followed by annealing at 55°C for 30 seconds, and final elongation at 72°C for 1 minutes. Electrophoresis on 1.5% agarose gels was done on the PCR products and then visualized under ultraviolet light after EtBr staining.

3.4. Data Analyzing

SPSS 22.0 (SPSS Inc., Chicago, USA) was used for statistical analysis. Contingency tables analysis (chi-square test) was used to assess variations in frequencies. The Fisher's exact test was applied with frequencies of less than five. Variations were considered significant at $P < 0.05$.

4. Results

4.1. Demographics

Demographic information of the sources of isolates is shown in Table 2. The healthy subjects were significantly younger than the patients.

Table 2. Demographic Information of the Sources of Isolates[a]

Characteristic	Patients (N = 113)	Healthy Adults (N = 288)
Sex		
Male	58 (51.3)	140 (49.7)
Female	55 (48.7)	148 (51.3)
Age, y		
< 25	19 (16.8)	288 (100)
26 - 50	47 (41.6)	0 (0)
≥ 51	43 (38.1)	0 (0)

[a]Values are expressed as No. (%).

Table 3. Distribution of Fusidic Acid-Resistant and Sensitive Isolates in Patients and Healthy Adults[a]

	Clinical Isolates (N = 113)	Healthy Control Isolates (N = 228)
FRSA	36 (31.9)	3 (1)
FSSA	77 (68.1)	285 (99)

Abbreviations: FRSA, fusidic acid-resistant *Staphylococcus aureus*; FSSA, fusidic acid-sensitive *S. aureus*.
[a]Values are expressed as No. (%).

4.2. Prevalence and Distribution of FRSA Isolates

Table 3 shows that the FRSA rate was significantly ($P < 0.05$) lower in the healthy adult control group (3/288, 1%) than in patients (36/113, 31.9%). The incidence of MRSA and methicillin-sensitive *Staphylococcus aureus* (MSSA) was detected in 53.1% (60/113) and 46.9% (53/113) of the patients, respectively.

The rate of FRSA was significantly ($P < 0.05$) higher among MRSA than among MSSA isolates (n = 24, 66.7% and n = 12, 33.3%, respectively) (Table 4). We found no association between fusidic acid determinants among MRSA and MSSA ($P > 0.05$).

Fusidic acid-resistant *S. aureus* isolates were significantly ($P < 0.05$) more abundant among clinical than among healthy control isolates (Table 3). While the distribution of FRSA clinical isolates was not statistically significant between sample types (Table 5). However, we found that all FRSA isolates from healthy individuals were from nose specimens.

4.3. Antibiotic Resistance Phenotypes of FRSA and FSSA

The percentage of FRSA isolates resistant to various other antimicrobial agents is shown in Table 6, which ranged from 0% for vancomycin to 100% for penicillin G. A high degree of resistance to erythromycin was observed, followed by clindamycin, gentamicin, and sulfamethoxazole-trimethoprim. The rate of resistance to teicoplanin was relatively low.

Table 4. Distribution of Fusidic Acid Resistance Determinants Amongst 36 MSSA and MRSA Clinical Isolates

	Total No. of Isolates (%)	No. of MRSA Isolates (%)	No. of MSSA Isolates (%)
fusB	14 (38.9)	9 (25)	5 (13.9)
fusC	6 (16.7)	5 (13.9)	1 (2.8)
Non-*fusB*, non-*fusC*	16 (44.4)	10 (27.8)	6 (16.7)
Total	36 (100)	24 (66.7)	12 (33.33)

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *S. aureus*.

Table 5. The Percentage of Fusidic Acid-Resistant *Staphylococcus aureus* Isolates in Four Sample Types[a]

Source of Isolates	FRSA Isolates
Skin and tissue	19 (16.8)
Respiratory secretions	12 (10.6)
Blood	3 (2.7)
Urine	2 (1.8)

Abbreviation: FRSA, fusidic acid-resistant *Staphylococcus aureus*.
[a]Values are expressed as No. (%).

The data in Table 6 show that the resistance of FSSA isolates to various other antimicrobial agents ranged from 0% for vancomycin to 100% for penicillin G. In general, lower degrees of resistance were noticed in FSSA isolates than in FRSA isolates.

4.4. Fusidic Acid Resistance

The results showed a significant ($P < 0.05$) relationship between fusidic acid resistance genes and resistance rate. Fusidic acid MICs ranged between 6 and $\geq$ 128 μg/mL. The majority of FRSA isolates (72.2%) displayed a low level of resistance (MIC $\leq$ 32 μg/mL), whereas 27.8% of isolates displayed an elevated level of resistance (MIC $\geq$ 128 μg/mL) (Table 7).

Amplification of the *fusB* and *fusC* genes with specific primers revealed that each resistant isolate carried a single resistance gene; 38.9% carried *fusB*, 16.7% carried *fusC*, and 44.4% of the isolates possessed neither *fusB* nor *fusC* (Table 4). Table 7 shows that most FRSA isolates harboring *fusB* and *fusC* (19/36 (52.7%) possessed a low degree of resistance (MIC $\leq$ 32 μg/mL). One isolate with a high level of resistance possessed the *fusC* gene. However, most isolates that did not have *fusB* or *fusC* showed a high degree of resistance (> 128 μg/mL). Interestingly, there was a statistically significant correlation between fusidic acid MICs and resistance genes ($P < 0.05$) (Table 7).

5. Discussion

A study in Jordan by Aqel et al. (15) revealed a high rate of infections with MRSA in both hospital and community-acquired diseases. In Jordan, fusidic acid is one of the conventional over-the-counter antibiotics increasingly used for the management of *S. aureus* skin and soft tissue infections. It is exclusively used topically either alone or in combination with anti-inflammatory agents, such as cortisone. The occurrence and mode of resistance of *S. aureus* clinical isolates to fusidic acid have not been studied in Jordan. We believe this is the first report on fusidic acid resistance gene determination among clinical *S. aureus* isolates from Jordan. Here, *S. aureus* phenotypic and genotypic characteristics were studied to explain the molecular mechanism underlying resistance.

The study revealed a significantly higher rate of fusidic acid resistance amongst clinical isolates than amongst isolates collected from healthy individuals, and it was comparably higher than those reported in other Arabic countries, such as Morocco (14%) (19). However, compared to European findings, the FRSA rate in Jordan is lower (31.9% vs. 64.9%), the occurrence of *fusB* is four times higher (38.9% vs. 10.1%), and the occurrence of *fusC* is similar (16.7% vs. 16.9%) (20). Interestingly, the FRSA rates were notably lower in the USA (0.3%), Canada, and Australia (7.0% for both countries), which can be explained by the fact that fusidic acid is not listed as a prescribed medication and is not yet authorized by the US Food and Drug Administration (21).

This corroborates that excessive antibiotic use exerts a selective pressure and increases the rate of resistance, and thus might affect the treatment regimen and narrow the choice of antibiotics effective against *S. aureus*. The resistance rate of FRSA isolates to antibiotics tested was overwhelmingly higher than that corresponding to the FSSA isolate. However both groups showed high susceptibility to vancomycin and teicoplanin. The retained susceptibility to vancomycin and teicoplanin correlates with the restricted use of the drug and indicates its usefulness. It is also worth mentioning that the percentage of FRSA isolates was significantly different among various sample types. A possible explanation is that skin commensal staphylococci

Table 6. Comparison of the Resistance Profile Between FRSA and FSSA Isolates

	Antimicrobial Agent							
	VA	TEC	SXT	CN	DA	E	FOX	P
Resistance of FRSA isolates, %	0	9.10	24.2	45.50	54.50	66.70	72.7	100
Resistance of FSSA isolates, %	0	2.70	13.3	14.20	32.70	43.4	53.10	100

Abbreviations: CN, gentamicin; DA, clindamycin; E, erythromycin; FOX, cefoxitin; FRSA, fusidic acid-resistant *Staphylococcus aureus*; FSSA, fusidic acid-sensitive *S. aureus*; P, penicillin G; TEC, teicoplanin; VA, vancomycin; SXT, sulfamethoxazole-trimethoprim.

Table 7. Distribution of FRSA Isolates According to Fusidic Acid Resistance Determinants and MIC[a]

Resistance Determinant	Total Isolates	No. of Isolates		P Value
		Low-Level Resistance, MIC $\leq$ 32	High-Level Resistance, MIC $\geq$ 128	
fusB	14 (38.9)	14	0	0.0078
fusC	6 (16.7)	5	1	
Non-*fusB*, non-*fusC*	16 (44.4)	7	9	
Total	36 (100)	26 (72.2)	10 (27.8)	

Abbreviation: MIC, minimum inhibitory concentration.
[a]Values are expressed as No. or No. (%).

may be a major source for fusidic acid resistance genes as suggested by Wei-Chun Hung et al. (22). A successful clone of FRSA circulating in the hospital could account for clonal expansion, thus spreading the resistance.

The study showed a high prevalence of *fusB* and *fusC* genes among isolates, with *fusB* being more prevalent than *fusC*. Previously, *fusB* was the predominant element causing fusidic acid resistance among 73.2% and 90% of *S. aureus* isolates in China and the Netherlands, respectively (23, 24). In contrast, Elazhari et al. reported that *FusC* was the most known fusidic acid resistance element among *S. aureus* from Casablanca (25). In Australia, New Zealand, the USA, and some European countries, *fusC* was the most common fusidic acid resistance gene (20, 21). In Canada, *fusB* and *fusC* occur at the same rates amongst *S. aureus* isolates (20, 21). Studies in Taiwan revealed that 84% of fusidic acid-resistant MRSA isolates had *fusA* mutations (20, 21, 26).

We found a significant association between the genetic determinants and the level of fusidic acid resistance. All *fusB* and *fusC* carrying isolates had low levels of resistance. The majority of the isolates that lacked *fusB* and *fusC* genes presented high fusidic acid resistance. This is in accordance with the results of Chen et al. (26) showing that generally isolates with *fusA* mutations were highly resistant to fusidic acid (MIC $\geq$ 128 μg/mL), whereas isolates with other determinants (*fusB* or *fusC*) had low-level resistance (MIC $\leq$ 32 μg/mL) (26). The present study had several limitations; it was a single-hospital study and a limited number of samples were collected over a period of only six months. To reflect the trend in infections caused by FRSA strains in the region, we need a multicenter study involving all types of healthcare setups for a longer period. In addition, we did not evaluate the presence of potentially new mutations in *fusB*, *fusC*, and *fusA*. Finally, there were no data on antibiotic use history of the patients. Thus, further investigation is warranted.

5.1. Conclusions

In conclusion, the rate of fusidic acid resistance is high amongst clinical isolates of *S. aureus*, particularly among MRSA isolates in Jordan. FRSA isolates in Jordan presented unique epidemiological characteristics, with a high incidence of *fusB*-carrying isolates. Furthermore, the majority of the isolates with acquired resistance genes had a low level of resistance to fusidic acid. Based on the findings of this study, further investigations and comparative studies should be performed in various patient groups and clinical conditions. Research to examine the presence of potentially new and novel mutations in the *fusA* gene is recommended. Antimicrobial susceptibility testing for fusidic acid is strongly recommended in medical laboratories. The restricted use of fusidic acid is advised.

Footnotes

Authors' Contribution: Rula Madhat Darwish and Luay F. Abu-Qatouseh developed the original idea and the protocol, abstracted, analyzed, and interpreted the data and prepared the manuscript. Rasha Aldasouqi prepared the samples and performed the experiments and together with Eman F. Badran and Sameer A. Alhaj Mahmoud abstracted the data and prepared the manuscript.

Conflict of Interests: It is not declared by the authors.

Ethical Approval: Ethical approval for the study was obtained from the Institutional Committee for the study of scientific research at the Prince Hamzah Hospital, Amman, Jordan.

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