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In Vitro Study of Fusidic Acid Susceptibility amongst Isolates from Various Swabs

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Abstract—***Fusidic acid is a common therapy for staphylococcal infections in Libya. The most common causes of superficial pyoderma are Staphylococcus aureus (77%) and Streptococcus pneumonia (2.2%), but reports have suggested high rates of resistance among clinical isolates. Susceptibility testing of S. aureus to fusidic acid is further complicated by the lack of consensus on mean inhibitory concentrations (MIC) and disk diffusion cutoffs to determine resistance. The purpose of this study was to determine the correlation between disk diffusion and Etest determined MIC susceptibility results in clinical isolates of S. aureus from Al-salem Medical laboratory. The swab infection rate was higher in males than in females. The most bacteria found were S. aureus, 57 isolates (77%) and Strep pneumoniae 9 isolates (12.2%). Almost isolated strains showed a high ratio of intermediate to fusidic acid. The susceptibility test for fusidic acid, found that 4% of Staph aureus isolates and Strep pneumonia isolates (1%) were susceptible to fusidic acid. The results of this study showed that there has been in vitro low susceptible of Gram positive cocci to fusidic acid. Therefore, fusidic acid is not recommended for use as Empirical treatment***

Keywords: ***impetigo, infections, dermatological infections, antibiotic resistance***

I. INTRODUCTION

The most common type of NIs are usually catheter associated (31%), 7 surgical site infections (SSIs) (17%), primary bloodstream infections (BSIs) (usually associated with the use of an intravascular device) (14%), and pneumonia (usually ventilator associated) (13%).7, 8 The story of fusidic acid can be likened to the proverbial saying “the stone the builders rejected has become the cornerstone.” Fusidic acid has been used in Europe as an antistaphylococcal agent since the 1960s. 4 With the increasing frequency of methicillin resistant Staphylococcus aureus (MRSA) worldwide, the need for more active anti-staphylococcal drugs is inevitable. Mode of action of fusidic acid by binds to bacterial ribosome, preventing polypeptide elongation and protein synthesis. 6 Fusidic acid, with favorable pharmacokinetics and pharmacodynamics, has the potential to fill this niche. It is available in intravenous, oral,and topical preparations and is widely distributed through the body, including areas such as bone, joint fluid, prostate, and abscesses, when given parenterally. 5

Based on its high efficacy against Staphy aureus,2 fusidic acid is often used as a topicaltreatment in skin and soft-tissue infections, and has been used widely against S. aureus over the last 20 years in the UK. Despite early reports of the rapid emergence of fusidic acid resistance in vitro, this has not been thought a major problem in clinical practice,3,4 although other recent reports suggest resistance may be escalating.2, 3Aim of this study was to examine the invitro activity of fusidic acid against bacterial isolates from various swabs

II. EXPERIMENTAL

A. Study area

The samples assembled from the wounds pus, burn; medical devices, tips and blood from male and female patients in the age group of 3 to 86 years with clinical characteristics suspected to be infected and are transmitted to the Microbiology Department of Al-Saleem laboratory, Benghazi, Libya between October 2021 and January 2022.

B. Sampling

All swabs specimens were collected aseptically and carried from patients by Stuart’s, Amies media. Collected blood samples were directly inoculated into brain

heart infusion (BHI) broth with the blood to broth ratio of 1:10 for up to 5 days and were immediately transported to the microbiology department. Age, sex, health status and use of

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medicines, infection type were recorded for all included patients.

C. cultures

Positive culture samples were directly inoculated onto blood agar and MacConkey agar, then incubated overnight at 37°C aerobically. Chocolate plates were incubated at 37 °C in microaerophilic conditions (containing 5% CO2). Moreover, 0.5 to 1 mL of the swab specimen was inoculated into a tube of nutrient broth. 12

D. Determination of bacterial isolates

Visual investigation of the bacterial colonies shows culture positivity. The supposed organism is then defined by Gram's stain and a series of biochemical tests, including an oxidase test, catalase test, Carbohydrate utilization test, Citrate utilization test, DNAase test, urease test, coagulase test, Analytical profile index (api) 20e test and Mannitol Salt Agar. 11

E. Direct Vitek 2 AST

The supposed strains' morphologically similar colonies were determined by biochemical assays utilising the Vitek system (bioMerieux, Hazelwood, MO). In brief, the cell density of the remaining bacterial suspension was modified to a density of 0.5 McFarland after dilution in 0.45% saline; 145µL of the bacterial suspension was drawn into 3 mL of 0.45% saline solution to further adjust the bacterial cell density. Vitek cards were inoculated with suspension vials and loaded into the Vitek automated reader-incubator. Vitek cards AST-GN13, AST-Gp67, and AST-Gp68 were utilized for gram negative bacteria, *staphylococci/enterococci/ streptococci* and *Strep pneumoniae*, respectively. 12

F. Antimicrobial susceptibility testing

Susceptibility of bacterial isolates to antibacterial drugs was carried out by the disk diffusion method to Analysis of the results was carried out following the recommendations of CLSI. 13 In brief, 4–6 morphologically similar bacteria colonies from clinical samples were collected with an inoculating loop and relocated into a tube including 5 mL of broth culture, gradually and carefully mixed until a relatively homogenous suspension was obtained, and incubated at 37°C. A sterile non - toxic dry cotton swab was utilized to streak a sample of the centralized inoculation on the Mueller Hinton agar plate. With the lid on, the inoculums were allowed to dry for 5–15 minutes.14

The antibiotic disks were distributed to the plates with sterile forceps at a distance of 15 mm from the edge and 24 mm apart from each other. After 24 hours of incubation at 37°C, the diameters of the zones of bacterial growth inhibition around the disks were measured to the nearest millimetre. The isolates were classified as sensitive (S), or resistant (R) utilising a standardized table. The inhibition zone of a ruler was measured in millimetres. 11

The susceptibility testing was carried out using Clinical and Laboratory Standards Institute recommendations. 11 MIC to fusidic acid were determined using Etests (BioM´erieux, AB Biodisk, Solna, Sweden) on Mueller-Hinton agar incubated for 24 h. Disk diffusion zone sizes were determined by direct colony suspension to 0.5 McFarland, the suspension inoculated to Mueller-Hinton plates with 10 µg fusidic acid disks (Biomerieux, AB Biodisk, Solna, Sweden), and the plate was read after incubation for 16–20 h at 35◦C. MRSA isolates were detected using either cefoxitin disk diffusion. A colony suspension equivalent to 0.5 McFarland was inoculated to Mueller-Hinton agar with a 30 µg cefoxitin disk (Oxoid, Basingstoke, UK) and interpreted after 16–20 h. MRSA was identified using a breakpoint of ≤21 mm zone size for cefoxitin disks. For the oxacillin Etests (AB Biodisk, Solna, Sweden), a 0.5 McFarland direct colony suspension was inoculated to Mueller-Hinton plates with 2.0% NaCl and interpreted after 24 hr incubation. An isolate with an MIC ≥ 4.0 µg/mL was considered oxacillin resistant. 11

G. Data Analysis

The data was analyzed by SPSS programs.

III. RESULTS AND DISCUSSION

A. Distribution table of different Wound pus and blood Infection by genders

The gender distributions of the patients with swab infection are reported in Table. 1 Concerning

gender, the swab infection rate was higher in males than in females.

TABLE. I. Distribution table of different swabs and blood Infections by genders**.**

Gender	Frequency	Percent
Female	26	35.1
Male	48	64.9
Total	74	100.0

B. Distribution of positive cases by age groups.

The study samples included in this study from <9 to 69 years old, majority of the subjects (21.6%) were in the 20-29 years old group.

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Strep pneumoniae	9	12.2
strep viridans	2	2.7
Total	74	100.0

TABLE II. Distribution of positive cases by age groups.

Age group	Frequency	Percent
0-9	5	6.8
10-19	8	10.8
20-29	16	21.6
30-39	14	18.9
40-49	9	12.2
50-59	10	13.5
60-69	9	12.2
70-79	2	2.7
80-89	1	1.4
Total	74	100.0

C. Distributions of bacterial infection in various swabs specimens Collected from Patients.

The most bacteria found were S. aureus, 57 isolates (77%) and Strep pneumoniae 9 isolates (12.2%). The World Health Organization (WHO), reported that in developing countries superficial pyoderma primarily caused by Staph aureus.

TABLE III. Distributions of bacterial infection in various swabs specimens Collected from Patient**s.**

Isolates	Frequency	Percent
MRSA	2	2.7
staph aureus	57	77.0
staph epidermidis	3	4.1
strep pyogens	1	1.4

D. Prevalence of different bacterial growth among female and male patients.

A total of 47 specimen culture results of patients with suspected infection during month's period were studied. The isolates and sex distribution of the patients are shown in Table 4. Bacteria isolation rate was in males 48 than males 26 had a positive bacterial culture result. *Staph aureus* was the most frequent isolate from male patients.

TABLE IV. Prevalence of different bacterial growth among female and male patients.

Isolates	gender		Total
	Female	Male	
MRSA	1	1	2
Strep pneumoniae	4	5	9
staph aureus	18	39	57
staph epidermidis	2	1	3
strep pyogens	1	0	1
strep viridans	0	2	2
Total	26	48	74

E. Distribution of clinical specimens.

The wound pus was the most samples from which bacteria were isolated, followed by ear swab, and least isolation was from Conjunctiva swab specimen.

TABLE V. Distribution of clinical specimens.

Sample	Frequency	Percent
Ear swab	6	8.1
Medical device tips swab	5	6.8
pus swab	59	79.7
Conjunctiva swab	4	5.4

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Sample	Frequency	Percent
Ear swab	6	8.1
Medical device tips swab	5	6.8
pus swab	59	79.7
Conjunctiva swab	4	5.4
Total	74	100.0

F. Susceptibility of Gram positive cocci to fusidic acid

Careful studies of MIC determination, and disk diffusion have demonstrated excellent correlation in measuring *S. aureus* resistance to fusidic acid, but these studies have led to slightly different interpretive criteria for classifying resistance. (15, 16)

In the present study, the antimicrobial resistance profile of isolates had been analyzed. Almost isolated strains showed a high ratio of intermediate to fusidic acid.

TABLE. VI Susceptibility of Gram positive cocci to fusidic acid

Susceptibility patterns	Frequency	Percent
I	45	60.8
R	25	33.8
S	4	5.4
Total	74	100.0

G. Antibiotic sensitivity, resistance and intermediate sensitivity of bacteria isolated to ceftriaxone

The drug resistance pattern differences among isolates grounded on colourful characteristics were estimated (Table 6). In view of that, there were no significant differences observed except for the instance types from which the strains were insulated. nearly the bacterial isolates were tested for vulnerability against ceftriaxone. Among the Gram-positive bacteria anatomized, the most resistant species to ceftriaxone were represented by *E. coli* and *S.aureus*. Utmost of *Escherichia coli* strains insulated from the whole instance were set up to be sensitive to the action of ceftriaxone in the present study. Else, other exploration finding reported that Escherichia coli displayed the loftiest resistance to ceftriaxone. (13, 14) *Staph aureus* strains were set up to be more susceptible than other bacteria strains to ceftriaxone which is inconsistent with former study in which utmost of the strains were resistant.(11) also, other exploration finding reported that Staph aureus displayed the loftiest sensitive to ceftriaxone. (12) still, it's in line with other studies conducted in different areas which reported the vulnerability of the strains towards the ceftriaxone.(15, 16)

H. Resistance pattern of isolated pathogens to evaluated antibiotics.

The susceptibility test for fusidic acid, found that 4% of *Staph aureus* isolates and *Strep pneumonia*e isolates (1%) were susceptible to fusidic acid. The results of this study indicate that Gram positive cocci are less sensitive to fusidic acid. The results of this study indicate that *S. aureus* is still intermediate sensitivity to fusidic acid and have somewhat good in vitro effectiveness. Low sensitive of *Staph aureus* isolate to fusidic acid in this study may be due to antibiotic usage. 9

TABLE. VII. Resistance pattern of isolated pathogens to fusidic acid antibiotic

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Isolates	FA			Total
	Intermediate	Resistant	Sensitive	
MRSA	1	1	0	2
Strep pneumoniae	5	3	1	9
staph aureus	33	21	3	57
staph epidermidis	3	0	0	3
strep pyogens	1	0	0	1
strep viridans	2	0	0	2
Total	45	25	4	74

I. Frequency of isolated pathogens from each nosocomial infection.

Staph aureus and Strep pneumoniae and were the most prevalent pathogens of wound pus contagion respectively. In the study of Rajabi et al., Acinetobacter spp and Klebsiella spp were the most common pathogens. (17)

TABLE IX. Frequency of isolated pathogens from each nosocomial infections.

Isolates	Ear swab	Medical device tips swab	Pus swab	Conjunctiva swab	Total

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MRSA	1	0	1	0	2
Strep pneumoniae	0	2	5	2	9
staph aureus	4	1	51	1	57
staph epidermidis	1	0	2	0	3
strep pyogens	0	0	0	1	1
strep viridans	0	2	0	0	2
Total	6	5	59	4	74

IV. CONCLUSION

The results of this study showed that there has been in vitro low susceptible of Gram positive cocci to fusidic acid. Therefore, fusidic acid is not recommended for use as Empirical treatment.

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