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Original Article

Fusidic Acid Susceptibility in Staphylococci from Clinical Isolates at a Tertiary care Hospital in Lahore

Sheraz Hussain[1], Mariam Danish Iqbal[2], Tahir Naeem[3], Saadia Ijaz[4]

[1,2,4] Pathology Department. Shalamar Institute of Health Sciences, [3]Department of Pathology (Microbiology) University College of Medicine & Dentistry University of Lahore.

ABSTRACT

Background: Staphylococcus is a genus of Gram-positive bacteria in the family Staphylococcaceae. Bacteria in the genus staphylococcus are pathogens of men and other mammals. Under the microscope they appear in clusters (like a bunch of grapes). Phenotypically the clinical important species are classified into two groups on the basis of coagulase enzyme production (i.e. their ability to clot blood plasma). These gram positive cocci are responsible for severe morbidity and mortality as they cause community and hospital acquired infections.

Objective: To describe the susceptibility pattern among clinical isolates of Staphylococcus spp. using antimicrobial susceptibility test (disk diffusion method) to Fusidic acid.

Material & Methods: This was a cross-sectional study conducted at Shalamar Teaching Hospital. A total of 120 Staphylococci spp. were isolated from different clinical specimens collected over a 3 month period in the microbiology laboratory. Specimens were inoculated by standard techniques on to blood agar. Identification was done by colonial morphology, Gram staining and conventional biochemical testing. Antimicrobial susceptibility test was performed on these isolates. Fusidic acid resistance was measured by zone of inhibition around the antibiotic containing disk.

Result: Out of the 120 isolates, 38 were coagulase positive Staphylococci, 79% of which were sensitive to Fusidic acid and about 20 % resistant. Coagulase negative Staphylococci were 82 and out of these 45% were sensitive and 55% resistant to Fusidic acid.

Conclusion: There is an increasing resistance noticed against coagulase negative Staphylococci to Fusidic acid while resistance in coagulase positive Staphylococci is only 20% and comparable with previous studies.

Key Words: Staphylococcus, Fusidic Acid, Antimicrobial resistance, Coagulase negative staphylococcus, Staphylococcus aureus.

Introduction

There are two medically important genera of Gram-Positive Cocci. They include *Staphylococcus* and *Streptococcus*.[1] One of the earliest references to different species being named "*Micrococcus*" and, particularly, "*Staphylococcus*" in terms of pathogenicity was given in 1884 by Rosenbach, a German surgeon.[2]

Staphylococcal species are members of the family Staphylococcaceae. These gram-positive cocci occur in single forms, in pairs, short chains and in grape-like clusters. These catalase-positive cocci are differentiated on the basis coagulase reaction (their ability to clot blood plasma) that cause visible coagulum on slide & tube coagulase test.[3]

CORRESPONDENCE AUTHOR

Dr. Mariam Danish Iqbal

Pathology Department

Shalamar Institute of Health Sciences

Email: dr.mariam.danish@gmail.com

In medical microbiology the term coagulase positive staphylococci are synonymous with *Staphylococcus aureus*. *Staph aureus* colonizes mainly the nasal passages, but it may be found regularly in most other anatomical locations, including the skin, oral cavity, genitourinary tract and gastrointestinal tract.[4] *Staph aureus* and especially (MRSA) is an important cause of nosocomial and community-associated infections, and is notorious for health care associated infections. Coagulase-negative staphylococci (CoNS) are found as normal flora on the skin and mucous membrane of humans. It is commonly reported in immuno-compromised patients, and individuals with indwelling intravascular devices or implanted medical devices.[5]

Fusidic acid is an antimicrobial that is used as a topical agent for treating superficial skin infections caused by staphylococci, such as atopic dermatitis and impetigo.[6] This antimicrobial is used in systemic, skin, bone and soft tissue staphylococcal infections including coagulase negative staphylococci and strains that

shows resistant to penicillin & other antimicrobials. Fusidic acid was first isolated from a strain of Fusidium coccineum. It is steroid-like antibiotic belonging to the class of Fusidanes.[7] Fusidic acid inhibits bacterial protein synthesis by interfering with elongation factor G (EF-G) results in blockage of its release from ribosomes.[8] It has activity against a wide range of gram positive bacteria therefore has been used in the treatment of clinical conditions like bacterial conjunctivitis, Clostridium difficile colitis, cystic fibrosis surgical prophylaxis, ophthalmic and neurologic conditions[9], bone and joint infections.[10]

Fusidic acid resistant staphylococcus species has emerged in recent years due to its overuse and mutations in the organism.[8,9,10] So, determining the frequency of Fusidic acid resistance in patients will help the clinician to treat cases with a more appropriate drug. In this study we describe the susceptibility pattern among clinical isolates of Staphylococcus spp. using antimicrobial susceptibility test (disk diffusion method) to Fusidic acid at Shalamar teaching hospital.

Materials and Methods.

This study was a retrospective cross sectional in nature and was carried out in the Microbiology Laboratory, Pathology Department, Shalamar Teaching Hospital Lahore Pakistan. Duration of study was three months (October 2019 to December 2019).

All clinical specimens for culture such as wounds swabs, blood, pus, and urine with positive growth of Staphylococci were included in the study.

Samples were collected and inoculated according to the standard operating procedures of the Pathology Laboratory of Shalamar Teaching Hospital.[11] Identification of bacteria was done by morphology, gram staining, catalase test, Cefoxitin susceptibility disk test, Coagulase and DNase test were also performed.

Antibiotic sensitivity test was used to determine Fusidic acid resistance in *Staphylococcus species*. Disk diffusion testing and interpretation was done according to CLSI guidelines[12]. Approval was obtained from the Institutional Review Board of Shalamar Medical College/Shalamar Institute of Health Sciences Data was entered in computer software SPSS 20. The values of resistant strains of Staphylococci to Fusidic acid, and the sites they were collected from were calculated. These values were expressed as percentages.

Results

A total number of 120 samples were identified as Staphylococcal sp. from 1st October to 31st December in the Microbiology Department of Shalamar Institute of Health Sciences and Shalamar Teaching Hospital.

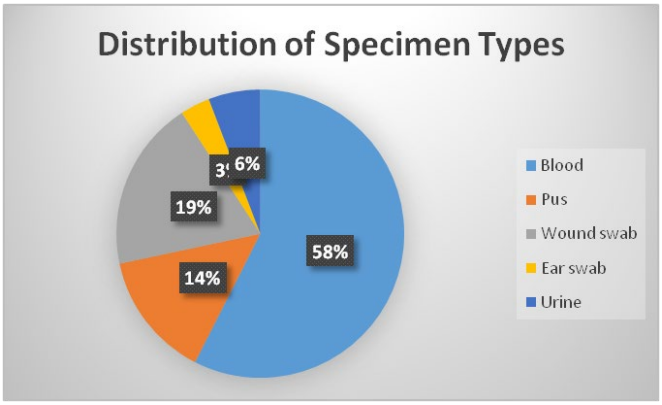

Figure 1. Distribution of specimen types

Table 1. Fusidic acid Susceptibility in different specimens

Specimen	Fusidic acid		Total
	Sensitive	Resistant	
Blood	31	38	69
Wound swab	16	7	23
Pus	13	4	17
Urine	4	3	7
Ear swab	3	1	4
Total	67	53	120

Table 2. Percentage of Fusidic acid Resistance and Sensitivity in Staphylococcus species

Coagulase positive Staphylococci

Fusidic acid	Frequency	Percent(%)
Sensitive	30	78.94
Resistant	08	21.05
Total	38	100.0

Coagulase negative Staphylococci

Fusidic acid	Frequency	Percent(%)
Sensitive	37	45.12
Resistant	45	54.87
Total	82	100.0

Discussion

Staphylococcus species, both coagulase negative and coagulase positive species are present ubiquitously in the environment and human skin. Both species can be found as normal human skin flora. As a part of the

skin flora the staphylococci are not inherently harmful to us. Staphylococci have become a threat to us now due to their development of antibiotic resistance. This pathogen can cause disease anywhere in the body, but mostly skin infections.[13]

Almost every person will have some type of Staphylococcal infections during his/her life time, ranging in severity from a mild food poisoning or minor skin infections to severe life threatening infections, such as toxic shock syndrome, and pneumonia.[14] Methicillin-resistant *S. aureus* (MRSA) is a highly infectious strain of the ordinary *S. aureus* bacteria that is resistant to many commonly used antibiotics. MRSA can cause widespread diseases which range from mild skin and soft tissue infection to more life threatening conditions such as sepsis. Its notable resistance towards many common drugs leads to a deadly outcome. MRSA were initially sensitive to Fusidic Acid, however recent literature[14, 15], as well as the current study indicate a rising trend towards resistance to this important antibiotic.

The coagulase negative staphylococci are opportunistic pathogens, they cause diseases in patients with implanted devices, such as joint prostheses, shunts, and with intravascular catheters especially in very young, old, and immunocompromised patients.[16] Due to the increase in hospital acquired infections due to CONS[16], it is important to have studies exploring the sensitivity patterns.[17] In the past CONS have been largely neglected because of the false notion that such bacteria cannot cause disease[18]. It is now known that CONS are severe pathogens and require increased infection prevention programs with hygiene discipline in hospitals.[18]

In the current study, 120 samples Staphylococcal sp were collected over a 3 month period.Number of coagulase positive staphylococci isolates resistant to Fusidic Acid was 21% and coagulase negative staphylococci isolates resistant to Fusidic Acid was 54 %. These results are comparable to a study conducted in a review article in 2021 by Hajikhani et al.[19] In a study conducted in Karachi in 2007, only 1% of caoagulase positive Staphylococci were found to be resistant to Fusdic Acid.[20]

A study published in 2013 in Pakistan found that 38% of their isolates were CONS.[21] Out of these about 46 % were from blood culture, about 37% from pus samples and wound swabs consisted of 17% of the samples. In their study Fusidic acid resistance among CoNs was (41.7 %).Present study shows that specimens isolated from blood culture were about the same as it was 58%, pus cultures were 14 %, wound swab were 19% of all cultures and the least amount of specimen which isolated staphylococci were urine with and ear swab with 3 %.

Another recent study from Karachi in 2019 performed antimicrobial resistance pattern for 13 different antibiotics including Fusidic acid, among clinical isolates of *Staph aureus* on various clinical samples[22]. Total 255 isolates were identified as Staphylococcus sp. Out of these two hundred fifty (250) were identified as *Staph aureus*. The Identified *Staph* aureus were also tested to differentiate between MSSA and MRSA. The study indicates a 59% resistance to Fusidic acid in Staph aureus, which is comparable with our study.

An article published in 2016 tested Fusidic acid sensitivity in isolates which were procured from an U.S. resistance surveillance program from 2008-2014. The isolates of Staphylococcus aureus were 99% sensitive and Coagulase negative Staphylococci were 90% sensitive.[23] Likewise an older study conducted in Malaysia in 2000, states that Fusidic Acid to be only 2.8% resistant. [24]

Conclusion

There is an increasing trend towards Fusidic Acid Resistance in Staphylococcal sp.(FRSS) These findings highlight the requirement for the implementation of (ongoing) surveillance and antibiotic stewardship measures to mitigate the emergence and spread of FRSS. Guidelines for infection control and education of clinicians on the proper prescribing of Fusidic Acid (FA) as well as the development of strategies for monitoring the effects of FA use should be instituted.

Competing interests

The authors of the study have no conflict of interest.

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Limitations of Study

1. Staphylococcal species were isolated only clinical specimen such as blood, urine wound swabs pus swabs ear swabs.
2. Due to the time constraints, total number of isolates is small. A larger study may be conducted to make more meaningful outcomes.

Molecular methods should be used whenever the facilities are available.

References

1. MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); [updated 2020 Jun 24]. Staph infections in the hospital; [updated 2021 August 24; reviewed 2021 Aug 25; cited 2022 Jul 29]; [about 5 p.]. Availablefrom:https://medlineplus.gov/ency/patientinstructions/000449.htm
2. Becker K, Heilmann C, Peters G. Coagulase-negative staphylococci. Clin. Microbiol. Rev. 2014; 27(4):870-926.
3. Udo E, AlFouzan W, Boloki H, Musaileem WA, Verghese T. Investigation of fusidic acid resistance determinants in methicillin-resistant Staphylococcus aureus obtained in Kuwait hospitals. J Infect Public Health. 2020;13(2):324
4. Sakr A, Brégeon F, Mège J-L, Rolain J-M, Blin O. Staphylococcus aureus Nasal Colonization: An Update on Mechanisms, Epidemiology, Risk Factors, and Subsequent Infections. Front Microbiol. 2018; 9:2419-2420. https://doi.org/10.3389/fmicb.2018.02419
5. Grace J-UA, Obaro SK, Olayinka BO, Onaolapo JA, Hassan-Hanga F, Munir H, et al. Staphylococcus aureus Bacteraemic Infection in Blood Culture of Children Attending Different Hospitals in Northern Nigeria. Poster presented at : 11th World Congress of the World Society for Pediatric Infectious Diseases; Nov 2019; Manilla, Phillipines. doi :10.13140/RG.2.2.15117.08166
6. Curbete MM, Salgado HR. A critical review of the properties of fusidic acid and analytical methods for its determination. Crit Rev Anal Chem. 2016; 46(4):352–60.
7. Curbete MM, Salgado HRN. A critical review of the properties of fusidic acid and analytical methods for its determination. Critical reviews in analytical chemistry. 2016; 46(4):352-60.
8. Michalik, M., Samet, A., Podbielska-Kubera, A. et al. Coagulase-negative staphylococci (CoNS) as a significant etiological factor of laryngological infections: a review. Ann Clin Microbiol Antimicrob 19, 26 (2020). https://doi.org/10.1186/s12941-020-00367
9. Yu F, Liu Y, Lu C, Lv J, Qi X, Ding Y, et al. Dissemination of fusidic acid resistance among Staphylococcus aureus clinical isolates. BMC Microbiol. 2015; 15:210.
10. Long, BH. (2008). Chapter 2. Fusidic acid in skin and soft-tissue infections. Acta Dermato-venereologica - Acta Dermato-Venereol. 88. 14-20. Doi: 10.2340/00015555-0387
11. Baird D, Staphylococcus : cluster forming gram positive cocci. In : Mackie & McCartney Practical Medical Microbiology. 14th Ed. Ed by Collee JG, Fraser AG, Marmion BP, Simmons A, pp245-262, Churchill Livingstone Elsevier, Kundli, INDIA. 2015.
12. European comittie on antimicrobial sensitivity testing (EUCAST). Clinical breakpoints – breakpoints and guidance. 2022. Cited on 15th November 2021. Available fromURL https://www.eucast.org/clinical_breakpoints/
13. Dadashi M, Hajikhani B, Darban-Sarokhalil D, van Belkum A, Goudarzi M. Mupirocin resistance in Staphylococcus aureus: a systematic review and meta-analysis. J Glob Antimicrob Resist. 2020; 20:238–47.
14. Grace J, Olayinka BO, Onaolapo JA, Hassan-Hanga F, Munir H, Fey PD, et al. Methicillin resistance and biofilm formation of Staphylococcus epidermidis in blood culture isolates from children under five: A multicenter study in Nigeria. Clin Microbiol.Rev. 2018; 7(6). Doi : 10.4172/2327-5073.1000321.
15. Akinkunmi EO, Adesunkanmi A-R, Lamikanra A. Pattern of pathogens from surgical wound infections in a Nigerian hospital and their antimicrobial susceptibility profiles. Afr. Health Sci. 2014; 14(4):802-9.
16. Zafar A, Ejaz H, Ahmed JM, Javed A. Frequency and Antimicrobial Susceptibility of Staphylococcus Aureus isolated from pus Samples of Paediatric patients. Pak J Med Health Sci 2012. 2012; 6(1):32-5.
17. Jonathan Asante, Daniel G. Amoako, Akebe L.K. Abia, Anou M. Somboro, Usha Govinden, Linda A. Bester, and Sabiha Y. Essack.Microbial Drug Resistance.Aug 2020.951-970.http://doi.org/10.1089/mdr.2019.0381
18. Michels R, Last K, Becker SL, Papan C. Update on Coagulase-Negative Staphylococci—What the Clinician Should Know. Microorganisms. 2021; 9(4):830. https://doi.org/10.3390/microorganisms9040830
19. Hajikhani, B., Goudarzi, M., Kakavandi, S. et al. The global prevalence of fusidic acid resistance in clinical isolates of Staphylococcus aureus: a systematic review and meta-analysis. Antimicrob Resist Infect Control 10, 75 (2021). https://doi.org/10.1186/s13756-021-00943
20. Perwaiz S, Barakzi Q, Farooqi BJ, Khursheed N, Sabir N. Antimicrobial susceptibility pattern of clinical isolates of methicillin resistant Staphylococcus aureus. JPMA J Pak Med Assoc. 2007;57(1):2
21. Ehsan MM , Memon Z , Ismail MO, Fatima G. Identification and antibiotic susceptibility pattern of coagulase-negative staphylococci in various clinical specimens. Pak J Med Sci. 2013; 29(6):1420-1424. doi:10.12669/pjms.296.4064
22. Hanif E, Hassan SA. Evaluation of antibiotic resistance pattern in clinical isolates of Staphylococcus aureus. Pak J Pharm Sci. 2019 Jul; 32(4(Supplementary)):1749-1753. PMID: 31680068.
23. Farrell DJ; Mendes RE; Castanheira M; Jones RN. Activity of Fusidic Acid Tested against Staphylococci Isolated from Patients in U.S. Medical Centers in 2014. 2016. Antimicrob. Agents Chemother. 60 (6); 3827-3831. doi:10.1128/AAC.00238-16
24. Rohani MY, Raudzah A, Lau MG, Zaidatul AAR, Salbiah MN, Keah KC, et al. Susceptibility pattern of Staphylococcus aureus isolated in Malaysian hospitals. Int J Antimicrob Agents. 2000;13(3):209–13

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CONTRIBUTION OF AUTHORS	
Author	**Contribution**
Sheraz Hussain	A,B,C
Mariam Danish Iqbal	A,C
Tahir Naeem	A,C
Saadia Ijaz	C

KEY FOR CONTRIBUTION OF AUTHORS:

A. Conception/Study/Designing/Planning
B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion