Detection of Cefoxitin Resistant *Staphylococcus aureus* in Khartoum Hospitals, Sudan, 2011

***[1]Abbas, Israa, [2] Omer, Al Fadhil, [3]Ali, H.**

[1] Lecturer of Immunology and Medical Microbiology, Portsudan Alahlya College.
[2] MD, MRCP, FICTM, FIMBS, FRC Path, Am Bd, Professor in Medical Microbiology, Alneelain University
[3]Lecturer of Medical Microbiology, Alneelain University.
Correspondence to: ISRAA MOHAMMED ABBAS, MSc.
Lecturer of Immunology and Medical Microbiology, Portsudan Alahlya College
email: ISSO2411@GMAIL.COM Contact No: 00249916506712.

ABSTRACT

Objective: To detect resistance of *Staphylococcus aureus* isolated from clinical specimens against the antibiotic cefoxitin.

Materials and Methods: The study was carried out in Khartoum state, during the period of April-2011 to July-2011, 80 samples collected from clinical specimens, the bacteria was isolated on blood agar, Staphylococcus aureus was identified using the following: gram stain, catalase test, coagulase test, DNAse test, and tested for resistant to cefoxitin.

Result: About 65% of isolate were *Staphylococcus aureus* and 0% of which were resistant to cefoxitin.

Key Words: S.aureus, MRSA, Wound infection, Cefoxitin

{Citation: Abbas, Israa, Omer, Al Fadhil, Ali, H. Detection of cefoxitin resistant *Staphylococcus aureus* in Khartoum hospitals, Sudan, 2011. American Journal of Research Communication, 2015, 3(1): 73-83} www.usa-journals.com, ISSN: 2325-4076.

INTRODUCTION

Infection of methicillin resistant *Staphylococcus aureus* (MRSA) is an emerging world health problem. It can be develop in an open wound such as bed sore or when there is a

tube, such as urinary catheter that inters the body. Symptoms in serious cases include fever, severe headache and lethargy. MRSA can cause urinary tract infection, pneumonia, toxic shock syndrome and even death.

Staphylococcus aureus are gram positive cocci about 0.8-1 um in diameter, mainly joined in grape-like clusters, but some cocci are single and some in pairs, non motile and non capsulated, facultative anaerobe [1]. S.aureus ferment glucose, maltose, and usually mannitol producing acid and no gas. Catalase positive on media with 1% glucose, oxidase positive, nitrate reduced to nitrite, methyl red and Voges-Proskauer positive, indole negative. Most strains are liquefying Gelatin and coagulate serum[2].

It responsible for several of disease due to their powerful virulent factor, it cause food poisoning through the production of an enterotoxin, and pathogenicity is also associated with coagulase positivity, S. aureus can infect other tissues when barriers have been breached (e.g., skin or mucosal lining), this leads to Furuncles and Carbuncles (a collection of Furuncles). In infants, S. aureus infection can cause a severe disease [Staphylococcal Scalded Skin Syndrome (SSSS)] [3] .

The infections can be spread through contact with pus from an infected wound, skin-to-skin contact with an infected person by producing Hyaluronidase that destroys tissues, and contact with objects such as towels, sheets, clothing, or athletic equipment used by an infected person. Deeply penetrating S. aureus infections can be sever[3].

infection of a wound caused by physical injury of the skin as a result of penetrating trauma from plants, animals, guns, knives or other objects. Wounds break the continuity of the skin and allow organisms to gain access to tissues and cause infection.Wound infections are caused by the deposition and multiplication of microorganisms in the surgical site of a susceptible host, the most common causative organisms associated with wound infections include *Staphylococcus aureus* /MRSA , *Streptococcus pyogenes* , *enterococci* and *Pseudomonas aeruginosa.*[4]

Hospital strains of S. aureus are usually resistant to a variety of different antibiotics, a few strains are resistant to all clinically useful antibiotics except vancomycin and cefoxitin, and vancomycin/ cefoxitin -resistant strains are increasingly-reported in recent

year. Methicillin resistance is widespread and most methicillin-resistant strains are also multiply drug resistant (ex. vancomycin and cefoxitin) [5].

Cefoxitin is a cephamycin antibiotic developed by Merck & Co., Inc., often grouped with the second−generation cephalosporins. It is also known as mefoxin. The bactericidal action of Cefoxitin results from inhibition of cell wall synthesis. Cefoxitin has in vitro activity against a wide range of gram-positive and gram-negative organisms. The methoxy group in the 7α position provides Cefoxitin with a high degree of stability in the presence of beta-lactmases, both penicillinases and cephalosporinases, of gram-negative bacteria[6].

Cefoxitin exhibits in vitro minimum inhibitory concentrations (MIC's) of 8 mcg/mL or less for aerobic microorganisms and 16 mcg/mL or less for anaerobic microorganisms against most (≥ 90%) strains however, the safety and effectiveness of Cefoxitin in treating clinical infections due to these microorganisms have not been established in adequate and well-controlled clinical trials[6].

In 2005, in USA, Warren JA and his colleagues studied confirmation of oxacillin-resistant *Staphylococcus aureus* isolates using Cefoxitin disk diffusion, One hundred Vitek-2 identified oxacillin resistant S. aureus (ORSA) isolates were tested by oxacillin screen-plate (OXA-plate) and cefoxitin disk (FOX-disk) for confirmation of oxacillin resistance. Ninety-five of 100 confirmed oxacillin resistant by both, 3 of 5 were oxacillin susceptible by both and mecA negative, and 2 to 5 were oxacillin susceptible by OXA-plate, resistant by FOX-disk and mecA positive. The FOX-disk out-performed the OXA-plate for ORSA confirmation and can be performed using standard laboratory techniques[7].

Despite the importance of cefoxitin in treatment of *Staphylococcus aureus* infection very little researches were carried in Sudan.

The goal of this study is to isolate *Staphylococcus aureus* from clinical specimens and to determine the sensitivity against cefoxitin.

MATERIALS &METHOD

This study was descriptive cross sectional study. Conducted in patient with wound infection attending Khartoum hospitals for treatment. A total of 50 patients with

wound infection and 30 isolate from other clinical specimens were included in this study. Data was collected by direct interviewing questionnaire.

Ethical clearance

Approval had been taken from faculty of medical laboratory science (Al Neelain university), and ministry of health ethical board.

1. Vocal consent had been taken from all patients.

2. The participant informed into their simple language about the infection, aim of the research and benefits of the study.

Procedure

Sample Collection

Cotton- wool swabs were used to collect the specimens from patients, by gently rolling the swab in the wound.

Inoculation

Under aseptic conditions near a Bunsen burner the samples were inoculated primarily on blood agar.

Incubation

The inoculated plates were incubated at 37° C for 24 hours.

Isolation

Growth showing characteristic golden yellow, white, hemolytic or non hemolytic colonies was considered *Staphylococcus aureus* . Plates were stored in refrigerator for further investigation.

Identificationof S.aureus

1-Colonial morphology: The size, shape, color, and hemolysis of colonies were reported.

2-Gram stain: Gram positive reaction of S.aureus was noted.

3-Catalase: Using an applicator stick a portion from center of a well isolated colony was transferred to the surface of a glass slide. 2 drops of 3% hydrogen peroxide were added. Positive control (S.aureus) and negative control (Streptococcus) were included in this test.

4-Coagulase test: A drop of physiological saline was placed on the center of a glass slide and a loop-full of the organism was gently emulsified in the normal saline. A drop of human plasma was added, the slide was tilted back and forth, and observed for 10 seconds for formation of granular precipitate of white clumps.

5-Mannitol salt agar: The organism was sub-cultured on mannitol salt agar, incubated at 37° C for 24 hours, and observed for mannitol fermentation (a yellow color develops on fermentation).

Antibiotic sensitivity test

Antibiotics susceptibility test was done for isolates of S. aureus, using Kirby-Bauer agar diffusion method. Antibiotic tested was cefoxitin[8], procedure done as follow:

1-Mac Farland standard

The turbidity standard 0.5 of 1% w/v barium chloride solution was added to 99.5 ml 1% sulphuric acid solution and mixed to get 0.5 % McFarland standard. A small volume of the turbid solution was transferred to a screw-cap bottle and stored in darkness at room temperature [8].

2-Inocula preparation and application

To obtain reproducible results, a standard number of bacteria ($1.5x10^8$ bacterial per ml) was used. It was prepared by direct touching of a colony with sterile loop and the growth was adjusted by using Mac Farland turbidity standard [8].

Within 15 min after preparing the inocula, a sterile swab was tipped into the inoculum, excess inoculum was removed by pressing and rotating the swab firmly against the side of the tube above the level of liquid. The swab was streaked over the surface of the

Muller Hinton agar plate three times, the plate was rotated through an angle of 60°each time to ensure good distribution of inoculums on all surface of the plate [8].

3-Discs application

The antimicrobial discs were placed on the inoculated plates using a sterile forceps. Each disc was pressed gently down to ensure even contact with medium, and plates were placed inverted in an incubator at $37^{O}C$ [8].

4-Reading and interpretation

After overnight incubation, the diameter of each zone was measured and recorded in mm, using the ruler on the under surface of the plate. Zone of inhibition was uniformly circular and had a confluent lawn of growth. The diameters of zone were recorded to the nearest millimeter. The end point of inoculation was generally judged by the naked eye at the edge where growth starts. The zone margin is the area showing no obvious growth that was detected with unaided eye. The result of the zone inhibition was interpreted according to the critical diameters given in the most recent NCCS, documents, showing the test organism as either susceptible or resistant to the antibiotic that have been tested[8].

Statistical analysis

The results were analyzed using SPSS program.

RESULTS

Clinical specimens were collected from 80 patients. 50 of these specimens were wound swabs collected from patients attending Khartoum Teaching Hospital. The remaining 30 were miscellaneous clinical specimens collected from Jabir Abu Al Ez Medical Center. Wound swabs patients were 34 (68%)diabetic patients and 16 (32%) were non-diabetic, (Table I).

Out of the 80 specimens investigated, *Staphylococcus aureus* was isolated and fully identified in 52 (65%) specimens, and 28 (35%) samples showed no growth of

Staphylococcus aureus. The frequency rate of *Staphylococcus aureus* among diabetic wound swab patients was 22.5 % and that of Non-diabetic wound swabs patient s was 5%, (Table II).

52 isolates of *Staphylococcus aureus* were tested for the sensitivity of cefoxitin using Kirby-Bauer agar diffusion technique. All isolates were found sensitive to the antibiotic cefoxitin, and none of them had a phenotypic resistance to this antibiotic.

Wound infections were more frequent (44.44%) in the age range (41-50) years in diabetic patients and (50%) in the age range (41-50 and 51-60), (table III).

Patients investigated were (55.55%%) males and (44.44%%) were females in diabetic pateint and (75%) male and (25%) female in non diabetic patient, (table IV).

Table I : Specimens investigated

Specimen	Diabetic patients		Non-diabetic patients		Total	
	No.	%	No.	%	No.	%
Wound swabs	34	42.5 %	16	20 %	50	62.5%
Mis. Clinical specimens	0	0 %	30	37.5%	30	37.5 %
Total	34	42.5 %	46	57.5 %	80	100%

Table II: *Staphylococcus aureus* isolated

Specimen	Positive *S. aureus*		Negative *S. aureus*		Total	
	No.	%	No.	%	No.	%
Diabetic wound Swabs	18	22.5 %	16	20 %	34	42.5%
Non-diabetic wound swabs	4	%	12	15%	16	20%
Clinical specimen	30	37.5 %	0	0	30	37.5%
Total	52	65.0 %	28	35 %	80	100%

Table III distribution of S.aureus wound infection in diabetic and non diabetic patient among age

Age	Diabetic patients		Non diabetic patients	
	NO	%	NO	%
(31-40)	2	11.11%	_	_
(41-50)	8	44.44%	2	50%
(51-60)	4	22.22%	2	50%
(61-70)	4	22.22%	_	_

Table IV distribution of S.aureus wound infection in diabetic and non diabetic among gender

Gender	Diabetic patients		Non diabetic patients	
	NO	%	NO	%
Male	10	55.55%	3	75%
Female	8	44.44%	1	25%

DISCUSSION

22.5% *Staphylococcus aureus* was isolated from diabetic wound infected patients and 5% from non diabetic wound infected patients (Table II).This finding was similar to that reported by Weiglet (2005)who reported that 23% *Staphylococcus aureus* were isolated from diabetic infected wounds[9] .

This study showed that cefoxitin stands as a good antimicrobial for treatment of *Staphylococcus aureus* wound infections with a susceptibility of 100 % this finding agree with Chin Ying (2007)[10] who reported 100% and Peter Collignon (2005) who also reported 100%[11].

This finding is higher than that reported by Astha Agarwal (2008)[12] who reported 94.44% were sensitive, Warren JA (2005) who reported 95%were sensitive[13].

CONCLUSIONS

As our survey showed, we could conclude that cefoxitin stands as a good antimicrobial for treatment of *Staphylococcus aureus* wound infections with a susceptibility of 100 %.

RECOMMENDATIONS

we recommend to avoid clinical complications from MRSA infections, and this is done by consider MRSA as a potential pathogen in patients with suspected S. aureus infections in the community setting, isolation and identification of bacteria from infected patient should be done and followed by antimicrobial susceptibility testing, and prevent spreading S. aureus or MRSA skin infections by practice good hygiene.

ACKNOWLEDGEMENTS

By the grace of Almighty Allah and his help I completed this study, all praise to Him. My gratitude goes to Prof. Al Fadhil Al Obeid Omer ,my supervisor who guides me to complete this work . All appreciation to the staff of Medical Microbiology Department (Al NeelainUniversity) who handed me a lot of favors specially Dr: Waleed Eldaif and Us: Khalid Inan. Finally special thanks to patients who were so cooperative and hospitable despite their pain.

References

1-McCartney and Mackie.In practical medical microbiology,14th edition.Churchill Livingstone, New York, 1990.

2-Omer E. Review of medical microbiology. First edition.1990.

3-World HealthOrganization. Pathogenicity of Staphylococcus aureus. 6th edition. Geneva, 1997.

4-Peacoc, S., de Silva, I., and Lowy, F. Wound infection causes and trends.*J Med* 2001; 344:605-610.

5-World HealthOrganization. antimicrobial susceptibility of Staphylococcus aureus. 6th edition. Geneva, 1997.

6-Boyle-Vavra, S., and R. and Daum, S.Cefoxitin role in microbiology.*Lab. Investig*2007; 87:3-9.

7-Brown, D. F. J. Detection of methicillin/oxacillin resistance in staphylococci. *Journal of Antimicrobial Chemotherapy* 2001; 48: 65–70.

8-Cheesbrough, M.Medical laboratory manuals for tropical countries. 2nd edition. Cambridge university press, England, 1989.

9-Weigelt, J., Itani, K., Stevens, D., Lau, W., Dryden, M., and Knirsch, C., Linezolid versus vancomycin in treatment of complicated skin and soft tissue infections. *Antimicrob Agents Chemother*. 2005;49:2260-6.

10-Sakoulas, G., Gold, H. S., Venkataraman, L. et al. Methicillin-resistant Staphylococcus aureus: comparison of susceptibility testing methods and analysis of mecA positive susceptible strains. *Journal* of *Clinical Microbiology* 2001 ;39:46–51.

11-Boutiba-Ben Boubaker, I., Ben Abbes, R., Ben Abdallah, H., Mamlouk, K., Mahjoubi, F., Kammoun, A., Hammami, A. & Ben, R. S. Evaluation of a cefoxitin disk diffusion test for the routine detection of methicillin-resistant Staphylococcus aureus. *Clinical Microbiology Infection* 2004;10: 762–765.

12-Chambers, H. F. Methicillin resistance in staphylococci:
molecular and biochemical basis and clinical implications. *Clinical Microbiology Reviews*1997 ;10: 781–91.

13-Brown, D. F. J. Detection of methicillin/oxacillin
resistance in staphylococci. *Journal of Antimicrobial Chemotherapy* 2001; 48: 65–70.