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Detection of Cefoxitin-Resistant *Staphylococcus aureus* isolated from infected Sudanese Patients

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Abstract

Background: Resistant *Staphylococcus aureus* infections are worldwide, and are commonly difficult to treat. Its increasing frequency challenges clinicians and infection control teams throughout the world to seek better measures to combat this condition. Infection of cefoxitin-resistant *Staphylococcus aureus* can develop in wound bed sores or on urinary catheterization.

Objective: To detect cefoxitin-resistant *Staphylococcus aureus* isolated from infected Sudanese Patients.

Materials and methods: This study was carried out in Khartoum State (Sudan) during the period from April to July, 2011. 80 clinical specimens were collected; *Staphylococcus aureus* was cultured on blood agar and identified by the Gram stain, catalase test, coagulase test, and DNase test. The sensitivity of *Staphylococcus aureus* was tested against the antibiotic cefoxitin.

Result: About 65% of the clinical specimens investigated showed growth of *Staphylococcus aureus*. All strains tested were found sensitive to cefoxitin. Among diabetic patients, wound infections were more frequent (44.44%) in the age range 41-50 years; and among non-diabetic patients, wound infections were more frequent (50%) in the age range 41-60. Patients investigated were 55.6% males and 44.4% females among diabetic patients and were 75% males and 25% females in non-diabetic patients.

Conclusion: Since no cefoxitin-resistant *Staphylococcus aureus* was isolated from diabetic or non-diabetic infected patients, it is justifiable to use this antibiotic in treatment of infections caused by this organism.

Key words: Cefoxitin resistance, *Staphylococcus aureus*, Kirby-Bauer sensitivity testing.

Introduction

Staphylococcus aureus usually infects hospitalized patients who are old or very ill. Patients may be at more risk if they have had frequent or intensive use of antibiotics. Symptoms in serious cases include fever, severe headache and lethargy. *Staphylococcus aureus* can cause urinary tract

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infection, pneumonia, toxic shock syndrome and even death. Cefoxitin is a cephamycin antibiotic, often grouped with the second generation cephalosporins. The bactericidal action of cefoxitin results from inhibition of cell wall synthesis. It has *in vitro* activity against a wide range of Gram-positive and Gram-negative organisms. The methoxy group in the 7 α position provides cefoxitin with a high degree of stability in the presence of β -lactamases, both penicillanases and cephalosporinases, of Gram-negative bacteria. Cefoxitin has been shown to be active against many microorganisms, both *in vitro* and in clinical infections. It 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. The safety and effectiveness of cefoxitin in treating clinical infections have not been established in adequate and well-controlled clinical trials¹.

Most cases of nosocomial infections are acquired through exposure of hands of health care workers who are often transiently colonized with *Staphylococcus aureus* (*S. aureus*). Outbreaks may also result from exposure to a single long term carrier on environmental sources, but this mode of transmission is less common².

Investigations of *S. aureus* infections often require good isolation, identification, and susceptibility testing. In spite of the fact that resistant *Staphylococcus aureus* infections are quite prevalent in Sudan and very few data are available. This study was therefore performed to investigate the resistance of *Staphylococcus aureus* isolated from clinical specimens against the antibiotic cefoxitin. This is required to combat serious infections.

Materials and methods

This was a descriptive, cross-sectional, quantitative, case study aimed to investigate cefoxitin-resistant *Staphylococcus aureus* from clinical specimens. It was conducted in Khartoum Teaching Hospital and Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Al-Neelain University, Khartoum (Sudan). The population investigated was patients with wound infections. Other *Staphylococcus aureus* strains isolated from miscellaneous clinical specimens collected from Jabir Abu Al Ez Medical Center (Khartoum). All patients were informed about the aim of the study and a verbal consent was obtained. Permission to collect the specimens was taken from Director Khartoum Teaching Hospital, and approval to conduct the study was granted by Al-Neelain University, Khartoum (Sudan). Data were analyzed using SPSS program. Sample size was 50 wound swabs and 30 *Staphylococcus aureus* strains isolated from miscellaneous clinical specimens. Demographical and clinical data was collected using a structural questionnaire. Cotton- wool swabs were used to collect the specimens from patients by gently rolling the swab on the wound. Inoculation of samples was made under aseptic conditions near a Bunsen burner primarily on blood agar plates. The inoculated plates were incubated at 37°C for 24 hours. Growth showing characteristic golden yellow, white, hemolytic or non-hemolytic colonies was considered *Staphylococcus aureus*. Primary identification was performed by the typical *Staphylococcus aureus* colonial morphology (size, shape, color, and hemolysis) and the Gram positive reaction. Secondary identification was performed by catalase test, coagulase test, and mannitol salt agar test.

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The antibiotic susceptibility testing was performed using Kirby-Bauer agar diffusion method. 0.5 % Mac Farland standard was used to match the inoculum turbidity. To obtain reproducible results, a standard number of bacteria (1.5×10^8 bacterial per ml) were used. It was prepared by direct touching of a colony with sterile loop and adjusted by using Mac Farland turbidity standard. 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 inoculum on all surface of the plate. Cefoxitin 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°C . After overnight incubation the diameter of each zone was measured and recorded in mm, using the ruler on the under surface of the plate. 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 should be taken as the area showing no obvious growth that was detected with unaided eye. The zone inhibition was interpreted according to the NCCS guide-lines as susceptible or resistant to cefoxitin³.

Results

Clinical specimens were collected from 80 patients. 50 of them were wound swabs and 30 were miscellaneous clinical specimens. Wound swabs patients were 34 (68%) diabetic patients and 16 (32%) non-diabetic patients. 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 patients was 5% (Table I).

Table (I) *Staphylococcus aureus* strains isolated

Specimen	Positive <i>S. aureus</i>	Total <i>S. aureus</i> isolated
Diabetic wound Swabs	18 (22.5%)	34 (42.5%)
Non-diabetic wound swabs	4 (5%)	16 (20%)
Other clinical specimens	30 (37.5%)	30 (37.5%)
Total	52 (65%)	80 (100%)

52 isolates of *Staphylococcus aureus* were tested for the sensitivity of ceftazidime. All isolates were found sensitive to the antibiotic ceftazidime, 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 among diabetic patients (Table II). Patients investigated were (55.6%) males and (44.4%) females among diabetic patients, and (75%) males and (25%) females among non diabetic patients.

Table II *Staphylococcus aureus* isolated according to age incidence

Age (years)	Diabetic patients	Non-diabetic patients
(31-40)	2 (11.1%)	0
(41-50)	8 (44.4%)	2 (50%)
(51-60)	4 (22.2%)	2 (50%)
(61-70)	4 (22.2%)	0

Discussion

18 (22.5%) *Staphylococcus aureus* strains were isolated from diabetic wound infected patients; and 4 (5%) from non-diabetic wound infected patients (Table I). This finding was similar to that reported by Weiglet who reported that 23% *Staphylococcus aureus* were isolated from diabetic infected wounds⁴.

This study showed that ceftazidime stands as a good antimicrobial for treatment of *Staphylococcus aureus* wound infections with a susceptibility of 100%. This finding agrees with Chin ying⁵ who reported a susceptibility of 100% and Peter Collignon⁶ who also reported a susceptibility of 100%.

However, this finding was higher than that reported by Astha Agarwal⁷ who reported a sensitivity of 94.44%; and it was also higher than Warren⁸ who reported a sensitivity of 95%.

Staphylococci are the main causative agents of nosocomial diseases. Over the last few years, the increase in the number of resistant staphylococci has become a major clinical problem. Accuracy and promptness in the detection of this resistance are of key importance in ensuring the correct antibiotic treatment in infected patients and control of resistant staphylococci in the hospital environment⁹.

From this study it may be recommended that isolation, identification, and antimicrobial susceptibility testing of *Staphylococcus aureus* should be performed routinely for patients with wound infections. Ceftazidime may be recommended as the antibiotic of choice for treatment of staphylococcal wound infections.

Conclusion: *Staphylococcus aureus* isolated was more frequent (42.5%) among diabetic patients aged 40 years and over. No ceftazidime resistant *Staphylococcus aureus* was isolated from diabetic

or non-diabetic wound infected patients. Cefoxitin is an excellent antibiotic for treatment of staphylococcal wound infections.

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