





Methicillin and Inducible Clindamycin-Resistant *Staphylococcus aureus* Isolated from Postoperative Wound Samples

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Abstract

The present study aimed to investigate the incidence of Methicillin-Resistant *Staphylococcus aureus* strains (MRSA) and inducible clindamycin resistant *S. aureus* (ICRSA) among postoperative wound infected patients. A total of 94 *S. aureus* strains were isolated by conventional laboratory methods from 135 swab samples collected from post-operative wound infected patients in Khartoum State hospitals. The isolated strains were screened for MRSA by using cefoxitin disc. ICRSA strains was detected by D-test and their susceptibility to antimicrobial agents was done by modified Kirby-Bauer's disc diffusion method. Ninety-four *S. aureus* isolates were screened for MRSA strains, we found 42 (45%) of isolates were MRSA and 52 (55%) of strains were methicillin-sensitive *S. aureus* (MSSA) phenotype. The incidence of ICRSA, constitutive clindamycin resistant (CCRSA) and erythromycin resistant (ERSA) strains among *S. aureus* isolates were 15.9% (15/94), 9% (8/94) and 2.12% (2/94), respectively. ICRSA resistant strains were slightly more frequent among MRSA, when compared with MSSA strains (16.67% (7/42) vs. 15.38% (8/52)). In addition, 33% of ICRSA strains were found resistant to both co-trimoxazole and gentamicin, while, 23% of strains were resistant to vancomycin. This study concluded that MRSA strains was nearly accounted a half of clinical isolates, which need more attention by improving hospitals environment health quality. ICRSA isolates were detected within both MRSA and MSSA strains and the D test must be implemented as routine susceptibility test to avoided clindamycin treatment failure.

Keywords: *Staphylococcus aureus*, wound, MRSA, inducible clindamycin, D-test.

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INTRODUCTION

Staphylococcus aureus (*S. aureus*), a leading cause of infection in hospitals and within communities worldwide¹. It is one of top most common bacterial causing wound infections² and other serious skin and soft tissue infections³. *S. aureus* is unusual pathogen that can persist in hostile environmental conditions as well as in present of antimicrobial drugs⁴.

Methicillin-resistant *S. aureus* (MRSA) was emerged 1960⁵ and still causing of significant morbidity and mortality globally⁶. It was reported that MRSA is responsible more than 50 of *S. aureus* associated health-care-acquired infections, including postoperative wound infection⁷. MRSA are resistance to multiple drugs and only susceptible to glycopeptide antibiotics such as vancomycin⁸, nevertheless, vancomycin-resistant *S. aureus* (VRSA) was emerged in 2002 leading to limited treatment options⁹.

Clindamycin, considered as a good alternative antibiotic for treatment of MRSA infection, but inducible clindamycin (ICRSA) and clindamycin resistant strains have been reported¹⁰. Thus, the present study aimed to determine the incidence of MRSA and clindamycin-resistant *S. aureus* in patients with post-operative wound infection.

MATERIALS AND METHODS

This cross sectional study was carried out in the microbiology laboratory at faculty of Medical Laboratory Sciences (FMLS), Omdurman Islamic University during the period from October 2017 to May 2018. A total of 135 postoperative wound swabs were collected from different hospitals in Khartoum State, including Soba University Hospital, Al-Ribat University, Fedail Hospital and Yastabshiroon Hospital.

The samples were processed by using conventional laboratory methods and 94 strains of *S. aureus* were isolated. The susceptibility of *S. aureus* isolates to antimicrobial agents including cotrimoxazole (1.25/23.75µg), vancomycin (30µg), gentamycin (10µg), erythromycin (15µg), Clindamycin (2µg) and ceftiofur (30µg) (Bio-analyte, Turkey) was screened by modified Kirby-Bauer's disc diffusion method¹¹. Briefly, bacterial colonies were harvested and suspended

in sterile normal saline to density equilibrium to 0.5 McFarland turbidity standard. The bacterial suspension was inoculated over entire surface of Muller Hinton agar plate (Hi-Media-India) by using sterile cotton swab. Next, antimicrobials discs were replaced in the inoculated plates and the diameter of inhibition zone was measured and interpreted after incubation at 35°C for 18–24 hours, according to the manufacturer instructions. MRSA strains were identified by their resistance to ceftiofur and the isolates yielded inhibition zone ≥ 20 mm were considered as MRSA¹².

ICRSA strains were detected by D test as method previously described¹³. Briefly, the D-test was done by placing the erythromycin (15µg) disc in distance approximately 15–20 mm from clindamycin (2µg) disc on Mueller-Hinton agar plate inoculated with *S. aureus* isolated strains. Next, the plates were incubated for overnight at 35 °C. After that the plates were examined for the flattened zone of inhibition around clindamycin disc, which is look like D-letter shape that indicating a positive ICRSA (Fig. 1). The isolates were resistance to both clindamycin and erythromycin considered as CCRSA, whereas, isolates were only resistance to erythromycin (ERSA) described as moderate sensitive.

Standard *S. aureus* ATCC 25923 strains was used to determined quality of antimicrobial discs and laboratory procedures. Obtained data was analyzed by Statistical Package of Social Sciences Standard Program (SPSS) version 21. 0.

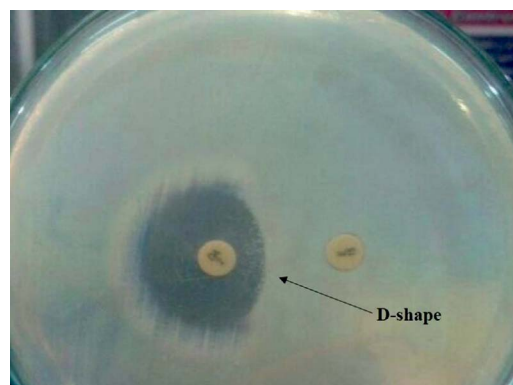


Fig. 1. Double disc test. The flattening zone of inhibition in the form of D-shape around clindamycin disc proximal to erythromycin disc, indicating for inducible clindamycin resistance.

RESULTS

During study period 94 *S. aureus* strains were isolated from non-duplicate 135 postoperative wound swab samples. These isolates were screened for drug-resistant phenotypes, namely, methicillin resistant and inducible clindamycin resistant *S. aureus* and their resistance patterns to different types of antimicrobial agents.

Ninety-four of *S. aureus* isolates were screened for methicillin-resistant phenotype by using ceftiofur, we found that 45% (42) isolates were methicillin resistant strains (MRSA), while 55% (52) were methicillin-susceptible strains (MSSA) (Table 1).

Table 1. Frequency of methicillin-resistant *S. aureus* (MRSA) isolates (n = 94)

Phenotype	Frequency	Percentage
MRSA	42	45%
MSSA	52	55%

MSSA; Methicillin susceptible *S. aureus*

The frequency of ICRSA, CCRSA and ERSA strains among *S. aureus* isolates were 15.9% (15/94), 9% (8/94) and 2.12% (2/94), respectively, (Table 2). ICRSA were slightly more frequent among MRSA when compared MSSA strains (16.67% (7/42) vs. 15.38% (8/52) (Table 2). Fifteen ICRSA

Table 2. MLSB resistance phenotypes among *S. aureus* isolates

Strains	ERSA	ICRSA	CCRSA
<i>S. aureus</i> (n = 94)	2.12%	15.9%	9%
MRSA (n = 42)	0%	16.67%	5%
MSSA (n = 52)	3.8%	15.38%	11.5%

ERSA: only resistance to resistance to erythromycin; ICRSA: Inducible clindamycin resistant *S. aureus*; CCRSA: constitutive clindamycin resistant *S. aureus*.

Table 3. Antimicrobial susceptibility profiling of inducible MLSB resistant *S. aureus* strains (n = 15)

Antibiotic	Susceptible	Resistant
Vancomycin	73% (11/15)	27% (4/15)
Gentamicin	67% (10/15)	33% (5/15)
Co-trimoxazole	67% (10/15)	33% (5/15)

strains were tested for susceptibility to 3 different types of antimicrobial drugs, they displayed that 33% of inducible clindamycin strains were resistant to both co-trimoxazole and gentamicin, whereas, 23% of strains were resistant to vancomycin (Table 3).

DISCUSSION

Postoperative wound infection is most common type of hospital acquired infections¹⁴ which need more attention particularly with increasing the incidence of multi-drugs bacteria. *S. aureus* is most predominate etiology of postoperative wound infection¹⁵. MRSA is most common *S. aureus* strains that causing greater than a half of *S. aureus* associated healthcare-acquired infection⁷. In our study 45% (42/94) of *S. aureus* were resistant to methicillin. This prevalence rate was higher than previous reported in India (34%)¹⁶, Nepal (25.1%)¹⁷, Brazil (32%)¹⁸ and in Egypt (15.8%)¹⁹. However, it was lower than documented in Taiwan (57.7%)²⁰ and in Eritrea (59%)²¹.

Clindamycin was found to be effective for treatment of invasive MRSA and MSSA infections²². Nonetheless, emergency of *in vitro*-false clindamycin susceptible *S. aureus* strains among erythromycin resistant strains may resulting in treatment failure²³. This study showed that the incidence of ICRSA, CCRSA strains was 15.9% (15/94) and 9% (8/94), respectively. In addition, ICRSA isolates were relatively more frequent among MRSA 16.7% (7/42), when compared MSSA 15.38% (8/52). Our findings were in concordance with the study reported by Deotata *et al.* noted that 14.5% of isolates were ICRSA and 3.6% were CCRSA²⁴. They also found that ICRSA strains were more frequent among MRSA than MSSA strains²⁴. Prabhu *et al.* documented that ICRSA strains was low in prevalent compared with CCRSA strains (10% and 18%, respectively). Nevertheless, ICRSA was found to be highly frequent among MRSA than MSSA²⁵.

The present study demonstrate that ICRSA isolates exhibited resistance to multiple antimicrobial agents as 33% of isolates were resistance to both co-trimoxazole and gentamicin. Previous report showed that 42.86% of ICRSA strains were resistant to co-trimoxazole and 21.43% were resistant to gentamicin²⁶. Gupta *et*

al. found 44.44% of ICRSA strains were resistant to co-trimoxazole, while 27.77% were resistant to gentamicin²⁷. Interestingly, several previous studies documented that all isolated ICRSA strains were sensitive to vancomycin^{26,27}. However, our study revealed that 27% of isolated strains were resistance to vancomycin. This variation in antimicrobial susceptibility of isolated strains might be due to antimicrobial agents treatment policies used in each country²⁸.

In summary the present study revealed that MRSA strains was account nearly a half of clinical of isolates, which need more attention by implementing and continuous improving hospitals infection control protocol. In addition, we found that the inducible clindamycin resistance strains were frequent within both MRSA and MSSA strains, therefore, the D test must be implemented as routine susceptibility test to prevent clindamycin treatment failure.

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CONFLICT OF INTEREST

The authors declares that there is no conflict of interest.

AUTHOR'S CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study does not contain any studies performed within human participants or animals.

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