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## Original article

# Study of vancomycin susceptibility pattern among *Staphylococcus aureus* isolated from superficial incisional surgical site infections

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## ABSTRACT

**Background:** *Staphylococcus aureus* (*S. aureus*), a well-known superbug, is the leading cause of surgical site infections (SSIs). High proportions of methicillin resistant *S. aureus* (MRSA) increased the use of vancomycin and this led to emergence of strains with reduced susceptibility to vancomycin. The present study aimed to study the pattern of vancomycin susceptibility among *S. aureus* isolates from cases of superficial incisional SSIs. **Methods:** Wound swabs, from 205 patients with superficial SSIs, were collected. *S. aureus* isolates were identified to the species level using conventional microbiological methods. Antimicrobial susceptibility testing was performed using modified Kirby-Bauer disc diffusion technique. *Staphylococcus aureus* susceptibility to vancomycin was assessed using E-test. **Results:** Out of 205 SSI cases, 73 (35.6%) were found to be caused by *S. aureus* representing the major causative organism followed by *Klebsiella* (23.9%), and *E. coli* (14.6%). Antimicrobial profile of *S. aureus* isolates revealed maximum sensitivity to tigecycline and linezolid. Methicillin resistant *S. aureus* (MRSA) represented 68.5% of isolates. E-test showed that all *S. aureus* isolates were sensitive to vancomycin. **Conclusions:** *Staphylococcus aureus* was the most common cause of SSIs and vancomycin still an effective treatment for MRSA. However, a higher degree of resistance to the commonly used antibiotics was observed, amplifying the need of strict adherence to rational antibiotic policy.

## Introduction

Surgical site infections (SSIs) are the most common type of healthcare-associated infections, accounting for 14-25% of the total healthcare-associated infections [1]. With a percentage of 24%, SSIs were reported as the most frequent nosocomial infection in Egypt followed by catheter associated urinary tract infections (20%) [2]. Surgical site infections are responsible for a prolonged hospital stay, long-term disability, and additional financial burden. They significantly reduce the potential

benefits of surgical interventions [3]. In Low- and middle-income countries, higher mortality rates are caused by SSI, as it is linked to 38% of deaths in patients with such infection [4].

Although there are global variations around the definition and classification of SSI, the Centers for Disease Control and Prevention (CDC) classified SSIs into superficial incisional SSI, deep incisional SSI, or organ/space SSI. Superficial incisional SSI is the most common type of SSIs,

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occurring within 30 days after the operation. It only involves skin or subcutaneous tissue of the incision and presented by pain, tenderness, warmth, erythema, swelling and/or pus formation [5].

Surgical site infections may be caused by endogenous and/or exogenous microorganisms that enter the surgical wound either primarily at the time of operation or secondarily after that [6]. Most SSIs are caused by endogenous microorganisms that appear on the patient's skin when the surgical incision is made [7].

Being one of the most common colonizing bacteria [8] and a highly virulent pathogen *Staphylococcus aureus* (*S. aureus*) represents the most common cause of SSIs [9]. In general, patients who are carriers of *S. aureus* have 2-9 times higher risk to develop SSI [10].

The prevalence of SSI caused by *S. aureus* has been increasing over the past few decades, predominantly due to continuous raising in the drug-resistant isolates. According to the CDC, the proportion of SSIs caused by *S. aureus* increased from 16.6% to 30.9% from 1992 to 2002 and the number of methicillin-resistant *S. aureus* (MRSA) isolates also elevated from 9.2%–49.3% [11]. High proportions of *S. aureus* isolates became resistant to all  $\beta$ -lactam antimicrobial agents excluding newer cephalosporin [12].

The current treatment options for MRSA infections include vancomycin, linezolid, trimethoprim-sulfamethoxazole and doxycycline, but vancomycin was considered the antibiotic of choice from the late 1980s [12]. Frequent use of vancomycin as the drug of choice for treatment of multidrug-resistant MRSA infections has led to the development of isolates with reduced susceptibility to vancomycin as vancomycin-resistant *Staphylococcus aureus* (VRSA), vancomycin-intermediate *S. aureus* (VISA) and heterogeneous VISA (hVISA) [13].

The global prevalence of VRSA, VISA and hVISA isolates is 1.5%, 1.7%, and 4.6%, respectively. In Egypt the overall prevalence of VRSA and VISA was estimated to be 5.5% and 0.5% respectively [13, 14]. Little data are available about the current epidemiology of VRSA. Hence, the objective of the study was to study the pattern of vancomycin susceptibility among *S. aureus* isolates from cases of superficial incisional SSIs.

## Materials

The study protocol had been revised and approved by the Research Ethics Committee of the Faculty of Medicine, Ain-Shams University from the ethical point of view, according to guidelines of the International Council on Harmonization and the Islamic Organization of Medical Sciences, the United States Office for Human Research Protections and the United States Codes of Federal Regulations.

## Study population

This study was conducted in the Department of Microbiology at Central labs of Military Forces, Egypt from July 2019 to December 2020. The study population included 205 patients with surgical site infections at different departments of Kobry El-kobba military hospitals. These patients were selected according to the definition of the **Centers for Disease Control and Prevention** [15]. An informed consent was obtained from all patients before inclusion in the study.

## Inclusion criteria

A patient who has at least one of these criteria was considered a case of SSI: A superficial surgical wound with purulent discharge, organism isolated from aseptically obtained culture of wound exudates, or at least one of the signs or symptoms of infection like pain or tenderness, localized swelling, redness, or heat.

## Exclusion criteria

Wounds that characterized by cellulitis only without any discharge, a stitch abscess alone, or a localized stab or pin site infection were excluded.

## Specimen collection

Two wound swabs were collected from each patient using Levine method with aseptic precautions according to **Swanson et al.** [16]. Each swab was then placed into a sterile transport medium (**APTACA Spa, Italy**), labeled with patient name and date, and then sent to the lab for processing within 4 hours at room temperature according to **Robinson et al.** [17].

## Methods

### Sample processing

Direct Gram-stained film, culture, and identification of isolates were performed according to **Public Health England** [18].

**Identification of *S. aureus* isolates** to the species level was done according to **Becker et al.** [19]. Colonies of *S. aureus* were identified by Gram

staining, catalase and coagulase E-tests, and subculture on mannitol salt agar.

#### Antimicrobial susceptibility testing

All *S. aureus* isolates were subjected to antibiotic susceptibility testing on Muller-Hinton agar plates according to the Kirby-Bauer disk diffusion susceptibility test protocol [20]. The antimicrobial containing discs, supplied by Oxoid, UK, included amikacin (AK 30µg), ceftiofur (FOX 30µg), oxacillin (OX 1µg), tetracycline (TE 30µg), erythromycin (E 15µg), gentamycin (CN 10µg), penicillin (P 10µg), ciprofloxacin (CIP 5µg), clindamycin (CD 2µg), chloramphenicol (C 30µg), Trimethoprim-sulfamethoxazole (TS 25µg), nitrofurantoin (NI 300µg), linezolid (LZD 30), and tigecycline (TEC 15). Methicillin resistance isolates (MRSA) were detected using ceftiofur (30 µg) and oxacillin (1µg) discs. Interpretation of the results was done according to the **Clinical and Laboratory Standards Institute (CLSI)** [21].

#### Vancomycin susceptibility testing

Epsilometer Test (E-test) was performed to determine the Minimal Inhibitory Concentration (MIC) for detecting vancomycin resistance. E-test was done and interpreted according to **CLSI** [21], using vancomycin E-Test Strips with a range of 0.016-256 µg/mL (**biofilchem®, Italy**). Interpretation of the E-test results was done according to the **CLSI** [21].

#### Statistical analysis

Complete data were entered to a PC using Statistical package for Social Science (SPSS 25). Descriptive statistics were used to present the antimicrobial susceptibility patterns. Frequencies and percentages were used to summarize descriptive statistics.

## Results

Of 205 patients with SSI enrolled in this study, the majority 170 (82.9%) were males, with a male to female ratio of 4.8:1. The ages of participants ranged from 10 to 76 years with a mean age of 38.6 years. 49.7% of study participants were in the age group of 17-39 years and only 17.6% of the patients were above 60 years. 32 (15.6%) of cases were from cases of external fracture fixation. While colectomies and other orthopedic surgeries represent 11.2% and 9.8% of cases respectively (**Table 1**).

Among 205 patients, *S. aureus* was isolated from 73 (35.6%) of surgical wound sites becoming the most commonly isolated organism followed by *Klebsiella* 49 (23.9%) isolate. Other predominant organisms which were isolated were *Escherichia coli* 30 (14.6%) and *Pseudomonas aeruginosa* 25 (12.2%). Methicillin resistance was detected in 50/73 (68.5%) of *S. aureus* isolates (**Table 2**).

The antimicrobial profile of the *S. aureus* isolates is shown in **table (3)**. Antimicrobial susceptibility testing of *S. aureus* isolates revealed that all were resistant to penicillin (100%). A high degree of resistance to ciprofloxacin (64.4%), erythromycin (63%), and clindamycin (43.8%) was detected. The overall resistance among MRSA strains was higher than that among MSSA strains. All *S. aureus* isolates were susceptible to tigecycline and linezolid (**Table 3**).

The MIC of vancomycin for all isolates was tested by E- test strip (**Figure 1**). **Table 4** summarizes the results of E-test, the MIC ranged from 0.38 to 1 µg/ml for all 73 *S. aureus* isolates which were susceptible to vancomycin including those three isolates that showed resistance using the disc diffusion method.

**Table 1.** Demographic data of the studied patients.

Demographic data	Patients	Number (%) Total = 205
Age (years)	10-16	20 (9.7%)
	17-39	102 (49.7%)
	40-59	47 (22.9%)
	≥60	36 (17.6%)
Gender	Male	170 (82.9%)
	Female	35 (17.1%)
Surgical procedure	External fracture fixation	32 (15.6%)
	Colectomy	23 (11.2%)
	Orthopedic surgery with prosthesis	20 (9.8%)
	Other procedures	130 (63.4%)

**Table 2.** Characterization of bacterial isolates obtained from patients with surgical site infections.

Organism	Number of isolates (%)
<i>Staphylococcus aureus</i>	73 (35.6%)
MRSA	50 (68.5%)
MSSA	23 (31.5%)
<i>Klebsiella species</i>	49 (23.9%)
<i>Escherichia coli</i>	30 (14.6%)
<i>Pseudomonas aeruginosa</i>	25 (12.2%)
<i>Acinetobacter species</i>	9 (4.4%)
<i>Coagulase negative staphylococci</i>	8 (3.9%)
<i>Proteus species</i>	8(3.9%)
<i>Enterococci</i>	3 (1.5%)
<b>Total</b>	<b>205 (100%)</b>

**Table 3.** Antimicrobial susceptibility pattern of *Staphylococcus aureus* isolates: (%) of resistant isolates by disc diffusion method.

Antimicrobial	<i>S. aureus</i> (n=73)	MSSA(n=23)	MRSA(n=50)
Amikacin	13.69%	8.7%	16%
Tetracycline	23.3%	8.7%	30%
Ciprofloxacin	64.4%	39.1%	76%
Erythromycin	63%	47.8%	70%
Clindamycin	43.8%	30.4%	50%
Gentamycin	35.61%	8.7%	48%
Trimethoprim/ sulfamethoxazole	19.2%	8.7%	24%
Chloramphenicol	11%	8.7%	12%
Nitrofurantoin	6.8%	4.3%	8%
Penicillin	100%	100%	100%
Cefoxitin	68.5%	0%	100%
Oxacillin	68.5%	0%	100%
Tigecycline	0%	0%	0%
Linezolid	0%	0%	0%

**Table 4.** Vancomycin MIC of *S. aureus* isolates by E-test.

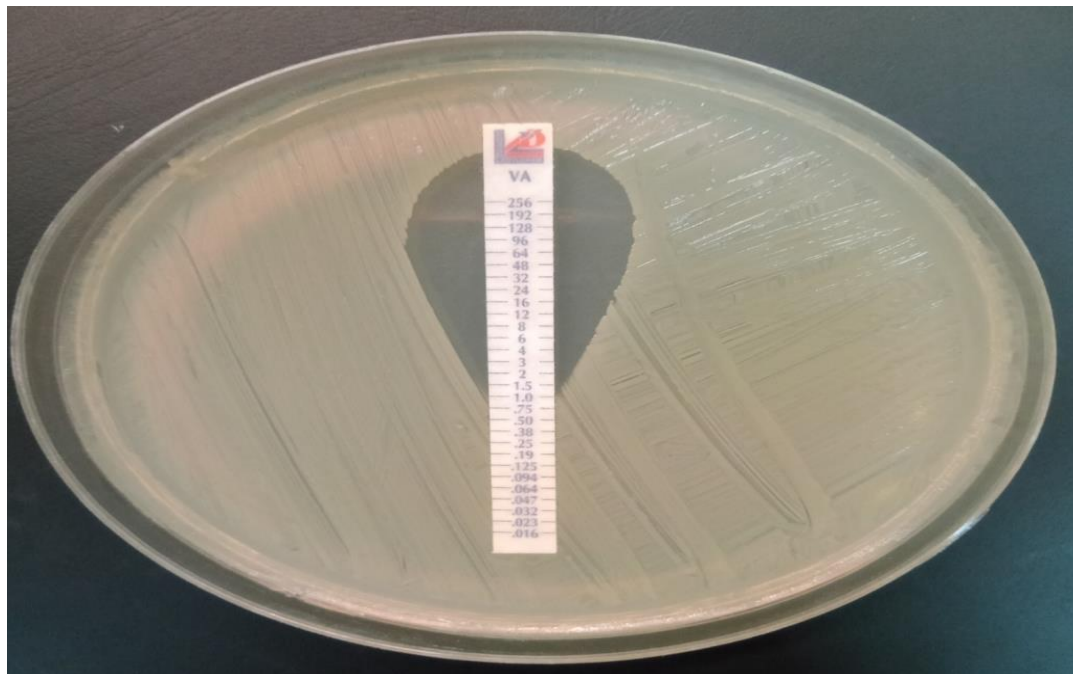
MIC ( $\mu\text{g/ml}$ )	MSSA (n=23)	MRSA (n=50)
$\leq 0.25$	–	–
<b>0.38</b>	1(4.3%)	4(8%)
<b>0.5</b>	16(69.6%)	12(24%)
<b>0.75</b>	4(17.4%)	6(12%)
<b>1</b>	2(8.7%)	27(54%)
<b>1.5</b>	–	–
<b>2</b>	–	–
<b>4</b>	–	–
$\geq 8$	–	–
MIC50*	0.5	1
MIC90*	0.75	1
S%	100	100

\*MIC50: the minimum concentration at which 50% of the isolates were inhibited.

\*MIC90: the minimum concentration at which 90% of the isolates were inhibited.

MRSA, methicillin resistant *S aureus*.

MSSA, methicillin sensitive *S aureus*.

**Figure 1.** Testing of vancomycin susceptibility by E-test (MIC=1 $\mu\text{g/ml}$ ).

### Discussion

Although SSI is one of the preventable causes of nosocomial infections, it is an important cause of health care associated infections (HAIs) worldwide [10]. Despite the advances in surgical techniques and a better understanding of the

pathogenesis of wound infection, management of SSIs remain a significant concern for surgeons and physicians in a healthcare facility [22].

Surgical site infections have serious implications for the surgeon, patient, and institution

increasing the treatment period, causing an economic burden, and doubling the risk of patient mortality [3]. *Staphylococcus aureus* which constitutes the most common cause of SSI is characterized by a rapidly spreading resistance to the available array of antimicrobials and this potentiates the existing problem [23].

In the present study, 17.6% (36) of studied patients aged more than 60 years which was in agreement with **Misha et al.** [24] who reported that 20.8% of the studied patients were above 60 years. Most of other studies reported SSI more in older patients. **Negi et al.** [25] reported that 51.8% of their patients aged >50 years. Despite advancing age is an important risk factor for SSI development, the higher number of young patients in this study can be explained by the high rate of flow of this age group in military hospitals. The predominance of male patients was seen in this study with male: female ratio of 4.8:1 and this finding was in tandem with the previous studies by **Mukagendaneza et al.** [26] and **Juyal et al.** [10] who reported male: female ratio of 3.8:1 and 3.9:1 respectively.

It has been regularly noted that *S. aureus* continues to be the single most common bacterial species in the primary etiology of SSIs with prevalence rates ranging from 4.6% to 54.4% [10]. In the present study, *S. aureus* comprised of 35.6% of SSIs, a finding which was in tandem with the previous studies by **Krishna et al.** [27], **Bhattacharya et al.** [28], **Juyal et al.** [10], and **Tefera et al.** [12] who reported *S. aureus* percentages of 31.3%, 31.3%, 45.3%, and 29.3% respectively.

The predominance of *S. aureus* in SSIs seen in this study and many other studies is most likely associated with endogenous source as the organism is a member of the skin and nasal flora colonizing between 30% and 50% of the world population [8]. Other possible sources include contamination from environment, surgical tools, or from hands of healthcare workers [9].

Surgical site infections caused by *S. aureus* are mainly due to its predominant role in HAI and emergence of MRSA strains which constituted 25% of *S. aureus* strains [29]. In this study methicillin resistance was detected in 68.5% of *S. aureus* isolates. This finding was in tandem with the studies conducted by **Bhalla et al.** [30], **Ahmed et al.** [31], and **Tefera et al.** [12]. In which 78%, 66.7%, and 45.1% of the *S. aureus* isolates respectively were MRSA. In contrast, lower incidences of 9.6%,

15.7%, and 25.45% of MRSA have been reported by **Naik et al.** [32], **Negi et al.** [25], and **Bhattacharya et al.** [28] respectively.

The variation in incidence of MRSA among different studies may be due to difference in the location and time period of the study, the types of studied patients, and the infection control polices in different medical settings in addition to the compliance of the health care workers to these polices. Referral hospitals have higher incidences as most of their patients were previously admitted in other facilities and received previous antimicrobial therapy, which increases the risk of acquiring resistant strains.

In the present study, most *S. aureus* isolates showed resistance to most tested antimicrobials, with 100% resistance to penicillin, (64.4%) to Ciprofloxacin, (63%) to Erythromycin, and (43.8%) to Clindamycin. These findings agree with those reported by **Bhalla et al.** [30] and **Ahmed et al.** [31] who reported 97% and 100% resistance to penicillin, 50% and 66.7% resistance to Ciprofloxacin, and 62% and 66.7% resistance to Erythromycin respectively. In this study, the percentages of drug resistant strains were higher in MRSA than MSSA isolated strains. These findings were explained by the antibiotic misuse and the inappropriate surgical antimicrobial prophylaxis which reaches between 30 to 90% of prophylaxis therapies [10].

All the *S. aureus* isolates, in the present study, were sensitive to Tigecycline and Linezolid. This was in agreement with **Juyal et al.** [10] and **Bhalla et al.** [30] who reported 100% sensitivity for both Tigecycline and Linezolid. The results of antimicrobial susceptibility testing can be of relevant clinical use in the development of antibiotic policy guidelines for hospitals.

Regarding vancomycin MIC results in this study, all *S. aureus* isolates were sensitive with MIC ranging between 0.38 and 1  $\mu$ g/ml.

Similar results of 100% vancomycin sensitivity were reported in many previous studies, **Negi et al.** [25], **Juyal et al.** [10], **Bhalla et al.** [30], and **Ahmed et al.** [31].

In contrast, **Tefera et al.** [12] reported a percentage of VRSA of 14.1%. Also, 11 VRSA isolates (13.8%) were detected in clinical samples from rural Egypt by **ElSayed et al.** [14]. This variation of results among different studies can be attributed to the geographical distribution, different

study populations and time periods. These findings necessitate an active surveillance program in Egypt.

### Conclusion

*Staphylococcus aureus* is the most common pathogen for SSI (35.6%). The proportion of MRSA was very high in the present study (68.5%) and no VRSA strains were detected. Resistance to the more commonly used antibiotics increased.

Therefore, prompt prevention and control measures must be ensured for MRSA high risk population. Strict adherence to a firm antibiotic policy based on local data obtained by surveillance studies is needed. A national level multi-central study involving large numbers of samples and different geographic parts of country is recommended to provide more significant results and the actual situation of *S. aureus* susceptibility to vancomycin, in Egypt.

### Disclosure of potential conflicts of interest

The authors report no conflicts of interest. All authors of this study have participated in the article preparation. All authors have approved the final article.

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