Vancomycin resistance among *Staphylococcus aureus* isolates in a rural setting, Egypt

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Abstract

Introduction **With the increased occurrence of methicillin resistant *S. aureus* (MRSA), the consumption of vancomycin, the drug of choice, has also increased. As a consequence, strains of *S. aureus* resistant to vancomycin have started to emerge. This study aimed to evaluate the level of vancomycin resistance among clinical and nasal *S. aureus* isolates in a rural town in Egypt.**

Methods **This cross-sectional study was held in the general hospital at the rural town of Kafr Eldawar in Egypt, during the period from January 2013 to January 2014. *S. aureus* isolates were collected from clinical samples and from nasal swabs.**

Results **Two hundred *S. aureus* isolates were collected, 80 (40%) from clinical samples and 120 (60%) from nasal carriage samples. Vancomycin resistant *S. aureus* (VRSA) was only detected in clinical samples, all collected from the outpatient clinic. Eleven VRSA isolates (13.8% of total *S. aureus* clinical isolates) and one strain of vancomycin-intermediate *S. aureus* (from nasal carriage) were detected. VRSA isolates were most resistant to ciprofloxacin (90.9%) and erythromycin (81.8%). Five isolates were resistant to all tested antibiotics: ciprofloxacin, clindamycin, erythromycin, linezolid, oxacillin, penicillin and trimethoprim-sulfamethoxazole. MRSA was found to constitute 43.8% of clinical *S. aureus* isolates. The MRSA colonization rate among community individuals was 43.6%, 42.9% among healthcare workers and 51.4% among patients.**

Conclusion **The prevalence of VRSA was high in clinical samples suggesting that there is a high level of VRSA strains in Egypt that goes undetected since most laboratories only use disk diffusion for detection of vancomycin resistance.**

Keywords **Antibiotic resistance, methicillin resistant *S. aureus*, vancomycin resistant *S. aureus***

Introduction

Vancomycin has been used as the drug of choice for methicillin resistant *S. aureus* (MRSA) infections. The rate of infections caused by MRSA has been steadily on the rise worldwide and as a result the consumption of vancomycin has also increased.[1] In 1997, the first case of vancomycin intermediate *S. aureus* (VISA) was reported in Japan.[2] From that time forward, more cases of VISA, and of vancomycin resistant *S. aureus* (VRSA) started to emerge throughout the world even in developed countries.[3]

While the morbidity rates of VRSA infections in the USA and Europe are still low, higher levels were identified in other regions in the world especially in developing countries. A dramatic increase in the percentage of VRSA was reported in India in 2011 compared to a previous study in 2006 (45% vs. 0.25%).[4] Like MRSA, isolated VISA and VRSA strains had been hospital-

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acquired, until cases of community-acquired VRSA were recorded.[5] In 2017 the World Health organization published a list of "antibiotic-resistant priority pathogens" that include VISA and VRSA.[6] The aim of this study was to determine the prevalence of *S. aureus* isolates resistant to vancomycin in clinical samples and nasal carriage.

Methods

This cross-sectional study was held in the general hospital at the rural town of Kafr El-Dawar in Egypt, during the period from January 2013 to January 2014. The study protocol was approved by the Ethics Committee at the High Institute of Public Health (HIPH) on 27 November 2012 (approval no. 32). An informed consent was obtained from all participants.

Collection and processing of samples

S. aureus was isolated from both clinical samples and nasal swabs. Clinical samples were collected from all inpatients admitted to all 3 surgical wards in the hospital; each ward contains 22 beds, two beds in each room. Clinical samples were also collected from outpatients visiting the surgical outpatient clinic. Any patient who suffered from an infection during the period of the study was included. Patients suffered from the following infections: abscesses, diabetic foot infections, postoperative wound infections, and skin infections. The sampling procedure was first described to each patient. The dressing was removed and the lesion was irrigated with sterile saline. Sterile cotton swabs, moistened with sterile saline, were used to collect samples. Sampling was done in a zigzag motion whilst rotating the swab between fingers, simultaneously.[7]

Nasal samples were collected from inpatients after 48 hours of admission, from healthcare workers in the hospital, and from community individuals visiting admitted inpatients. Nasal swabs were collected using a flexible sterile cotton swab, moistened with sterile saline. The swab was inserted 2 cm into each nostril and gently rotated for about 3 seconds.[8]

Upon arrival to the laboratory, clinical samples were cultured on blood and MacConkey agar plates while nasal swabs were streaked onto mannitol salt agar plates. All agar plates were aerobically incubated at 37°C for 24 hours. Mannitol salt agar plates were examined for any visible growth after 24 hours. If no growth occurred, they were reincubated for another 24 hours, with maximum incubation period of 72 hours.[9]

Identification and antimicrobial susceptibility

Isolated colonies were identified morphologically then examined microscopically by Gram staining. All isolates were subjected to full identification according to the standard microbiological methods.[9]

All isolates identified as *S. aureus* were tested for their antibiotic susceptibility using the disk diffusion method. Antibiotics tested were ciprofloxacin (CIP), clindamycin (DA), erythromycin (E), linezolid (LZD), oxacillin (OX), penicillin (PEN) and trimethoprim-sulfamethoxazole (SXT). For quality control, the susceptible control strain *S. aureus* ATCC 25923 was used.[10]

Vancomycin resistance was determined by MIC testing using the agar dilution method. Isolates that were vancomycin resistant by MIC test results were confirmed by E test.[11] For quality control, the control strains *S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used. Bacterial isolates were classified into VRSA, VISA, and VSSA according to the following MIC ranges VSSA ≤2 µg/mL, VISA 4-8 µg/mL and VRSA MIC ≥16 µg/mL.[10]

Types of samples and method of selection

All samples were collected consecutively from outpatients and inpatients of Kafr El-Dawar General Hospital until the required sample size was reached.

Statistical analysis

Data were analyzed using IBM SPSS 20, (IBM, Armonk, NY, USA). Descriptive statistics such as frequency, proportion, mean, standard deviation and median were used. The $p<0.05$

was taken as a cut-off for statistical significance and all tests were two-sided. The Chi-square test was used for testing the association of categorical data and in case of small cell frequency; the Monte-Carlo exact test was displayed. Comparison of resistance to different antibiotics was done using the Freidman test. Multiple comparison among antibiotics was conducted using Wilcoxon signed-rank tests with Bonferroni correction.

Results

During this study 240 clinical samples were collected. *S. aureus* was isolated from 80 samples (33.3%). Although we collected clinical samples from both inpatients and outpatients, *S. aureus* was detected only in outpatient samples (Table 1). Out of 960 nasal samples, 120 *S. aureus* isolates were retrieved and the total *S. aureus* nasal carriage rate was 12.5%. Nasal carriage samples included 55 isolates (45.8%) from community individuals, 37 (30.8%) from patients and 28 (23.3%) from healthcare workers (Table 2).

Table 1. *S. aureus* isolated from outpatients at Kafr El-Dawar General Hospital, 2013-2014

	Abscess No (%)	Diabetic foot infection No (%)	Postoperative wound infection No (%)	Skin infection No (%)	Total No (%)
S. aureus	42 (52.5)	16 (20.0)	5 (6.2)	17 (21.3)	80 (100)

Table 2. Distribution of isolation of *S. aureus* among nasal carriage groups, Kafr El-Dawar General Hospital, 2013-2014

Nasal carriage group	*S. aureus* nasal carriage				Total	
	Positive		Negative		No.	%
	No.	%	No.	%		
Community	55	12.4	387	87.6	442	46.0
Healthcare workers	28	15.6	152	84.4	180	18.8
Patients	37	10.9	301	89.1	338	35.2
Total	120	12.5	840	87.5	960	100.0

Two hundred *S. aureus* isolates were collected, 80 (40%) from clinical samples and 120 (60%) from nasal carriage. *S. aureus* isolates showed the highest resistance rate against penicillin (96%), while they were most sensitive to linezolid (95%), followed by clindamycin (94%), trimethoprim-sulfamethoxazole (89%), and erythromycin and ciprofloxacin (76% each). There was a statistically significant difference in the resistance to different antibiotics, Friedman $\chi^2_{(6)}$= 678.82, p<0.001 (Figure 1). There was a significant difference between penicillin and oxacillin on the one hand and all other antibiotics on the other hand (Wilcoxon signed-rank tests with Bonferroni correction, p<0.001).

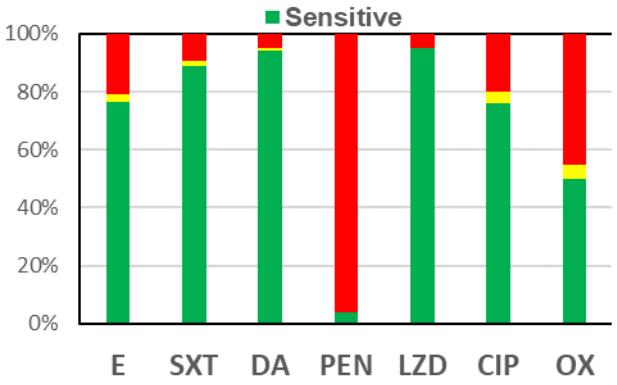


Figure 1. Antibiotic resistance among 200 *S. aureus* isolates

CIP – ciprofloxacin; **DA** – clindamycin; **E** – erythromycin; **LZD** – linezolid; **OX** – oxacillin; **PEN** – penicillin; **SXT** – trimethoprim-sulfamethoxazole.

The overall methicillin resistance was 45% (90 strains). The resistance to methicillin among clinical samples and nasal samples was comparable (43.8% and 45.8%, respectively); Pearson Chi-square=0.63 (p=0.730), i.e., not significant. The percentage among nasal isolates was slightly higher among patients (51.4%) than in the community (43.6%) and in healthcare workers (42.9%); Pearson Chi-square=0.76 (p=0.944), i.e., not significant.

VRSA was only detected in clinical samples. Eleven VRSA isolates were detected, constituting 13.8% of the total *S. aureus* clinical isolates. Two of the 11 isolates had an MIC of ≥16 µg/mL, 6 had an MIC of ≥32 µg/mL and 3 showed a high MIC of ≥64 µg/mL. However,

one strain of VISA was detected among the strains collected from nasal carriage from a healthcare worker (0.5%) – Figure 2. VRSA isolates were most resistant to ciprofloxacin (90.9%) followed by erythromycin (81.8%), sulfamethoxazole-trimethoprim, clindamycin and linezolid (63.6% each). Five isolates were resistant to all tested antibiotics.

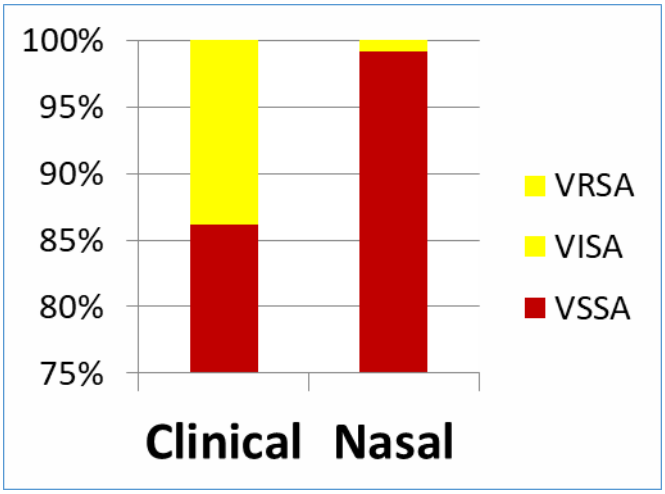


Figure 2. Susceptibility of 200 *S. aureus* isolates to vancomycin according to the type of sample

VISA – vancomycin intermediate *S. aureus*; **VRSA** – vancomycin resistant *S. aureus*; **VSSA** – vancomycin sensitive *S. aureus*.

Discussion

In the present study, even though we collected clinical samples from inpatients and outpatients, *S. aureus* was only detected in outpatients' samples which means they were all community-acquired isolates. *S. aureus* isolates were most susceptible to linezolid and clindamycin. Higher levels of resistance were reported in India, where MRSA was found to constitute 45% of *S. aureus* isolates. Similar prevalence rates of MRSA were reported in Egypt among skin and soft tissue infections, where 47.4% of the isolated *S. aureus* strains were reported as MRSA.[4,12] Unfortunately, until the end of the study, there was no working microbiological laboratory in the hospital, so antibiotics were prescribed without performing a susceptibility test and were given empirically. This empiric use of antibiotics may not only lead to sub-optimal and ineffective treatment, but it may also select for antibiotic-resistant bacteria.[13]

In the present study, 51.4% of patients and 42.9% of healthcare workers were colonized with MRSA. A similar MRSA colonization rate was reported by Moniri et al. (52.6%).[14] The problem with this high carriage rate among both groups is the high risk of the development of subsequent MRSA infections either through transmission from healthcare workers to patients or through auto-infection.[15] The prevalence of MRSA in clinical and nasal carriage samples was comparable, probably because the studied infections were mostly from the outpatient clinic (i.e., mostly community-acquired MRSA). However, oxacillin disks were used in this study instead of cefoxitin so this can be considered as a limitation in the study.

Detection of vancomycin resistance is essential not only for an optimal therapy, but also for infection control measures and epidemiological purposes.[1] As recommended by the Clinical & Laboratory Standards Institute (CLSI), the agar dilution method was used for MIC determination for the detection of VISA and VRSA strains in this study. Most microbiology laboratories in Egypt depend only on the disk diffusion method to determine *S. aureus* susceptibility to vancomycin, which does not give reliable results. It can leave many VISA/VRSA isolates undetected and they will give inhibition zones with sizes similar to those of the vancomycin-susceptible ones.[16] In the present study, MIC determination detected 5.5% of the *S. aureus* isolates as VRSA and 0.5% of them as VISA. The prevalence of VRSA among clinical cases was 13.8% while none of the nasal carriers had VRSA. A lower prevalence rate was reported in other developing countries but they used the disk diffusion method, which means that the actual prevalence of VRSA might be different.[17,18] Gohniem et al. reported an even higher resistance rate (20.68% VISA and 20.68% VRSA) indicating that resistance rates are on the rise.[19] On the other contrary, Amr et al. reported a lower incidence (8.8%) of VRSA among clinical isolates. This difference is probably due to different geographical locations of both studies in Egypt.[20]

Differences in the antibiotic resistance patterns vary widely between different geographic regions. Many factors contribute to those patterns including local infection control programs implemented, antibiotic prescribing policies and epidemiology of the studied strains themselves.[1] In developing countries the problem is more challenging due to frequent dispensing of antibiotics at drug stores without prescription.[21] Also, use of antibiotics without performing antibiotic susceptibility testing and the thus inappropriate prescribing of antibiotics that was observed in this study. Other factors include the uncontrolled use in agriculture and livestock.[22]

Fortunately, none of the patients or community individuals were colonized with VISA or VRSA. Only one healthcare worker was colonized with VISA (0.5%). A comparable finding was reported by Ploy et al. in France, where 3.4% of healthcare workers were VISA nasal carriers.[23]

Available data regarding the antibiotic susceptibility pattern of VRSA is sparse. All the detected VRSA and VISA strains were also methicillin-resistant.[24] An alarming observation was that five out of the eleven VRSA isolates in the present study showed resistance to all the tested antibiotics, including linezolid. Higher linezolid resistance (40%) in VRSA strains was also reported in Egypt.[20] Also, in another study linezolid was less effective against VRSA than other antibiotics.[25] Sanchez Garcia et al. reported that linezolid resistance probably develops on exposure.[26]

Conclusion

The prevalence of VRSA was high in clinical samples (13.8%) collected from community-acquired infections suggesting that there is a high level of VRSA strains in Egypt that goes undetected since most labs only use disk diffusion for the detection of vancomycin resistance. Infection control measures and antimicrobial stewardship efforts should focus both on community and hospital resistant strains.

Authors' contributions statement: NE was responsible for collection of data, samples and the laboratory work included in the study; she was also involved in literature search and writing of the article. MA was responsible for the analysis and interpretation of the results; AEKA was responsible for the conception and design of the research plan; she was also responsible for writing and revising the article. All authors read and approved the final version of the manuscript.

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