RESISTANCE OF *STAPHYLOCOCCUS AUREUS* TO VANCOMYCIN IN ZARQA, JORDAN

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ABSTRACT

Background: Vancomycin has been widely used in the treatment of infections caused by Methicillin-Resistant *Staphylococcus Aureus* (MRSA). The emergence of Vancomycin-Intermediate and -Resistant *Staphylococcus Aureus* (VISA and VRSA, respectively) in various parts of the world has been of great concern in clinical settings.

Objective: This study was performed to evaluate the possible presence of VISA and VRSA in Zarqa, Jordan.

Setting: This study was done at Prince Hashem Hospital laboratory and clinical wards.

Patients and Methods: The Minimum Inhibitory Concentration (MIC) of vancomycin for 139 *Staphylococcus aureus* strains isolated between April 2002 and August 2004 was carried out according to the standards of the National Committee for Clinical Laboratory Standards (NCCLS) using the agar dilution method. Resistance of VRSA to vancomycin was checked by E-test. Disk diffusion method was also used to determine the susceptibility of strains to common antibiotics. Determination of oxacillin MIC was performed for VRSA with the agar dilution method according to the guidelines of NCCLS and the E-test.

Results: Using the disk diffusion test, most isolates (91.7%) were resistant to penicillin while the lowest resistance (10.9%) was to imipenem. Five of the 139 isolates had a vancomycin MI Cof e" 128 by agar dilution and E-test methods. All VRSA isolates were MRSA (MIC e" 256) and the majority were also highly resistant to other antibiotics tested.

Conclusion: This is the first report of isolation of VRSA in Zarqa, which calls for confirmation by reference laboratories and further epidemiological studies.

KEY WORDS: Staphylococcus aureus, MRSA, Vancomycin.

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INTRODUCTION

Staphylococcus aureus is a cause of hospital and community acquired infections.[1,2] In 1996, the first clinical isolate of *Staphylococcus aureus* with reduced susceptibility to vancomycin was reported from Japan.[3]

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Staphylococcus aureus continues to be a major cause of community-acquired and health-care related infections around the world.[4,5] The emergence of high levels of penicillin resistance followed by the development and spread of strains resistant to the semisynthetic penicillins (methicillin, oxacillin, and nafcillin), macrolides, tetracyclines, and aminoglycosides has made the therapy of staphylococcal disease a global challenge.[5,6]

In the 1980s, due to the widespread occurrence of MRSA, empiric therapy for staphylococcal infections (particularly nosocomial sepsis) was changed to vancomycin in many health-care institutions. Vancomycin use in many countries also increased during this period because of the growing numbers of infections with *Clostridium difficile* and coagulase-negative staphylococci in health-care

facilities.[7] Thus, the early 1990s saw a discernible increase in vancomycin use. As a consequence, selective pressure was established that eventually led to the emergence of strains of *Staphylococcus Aureus* and other species of staphylococci with decreased susceptibility to vancomycin, but in 1997 the first clinical isolate of *Staphylococcus aureus* with reduced susceptibility to vancomycin was reported from Japan.[3]

The vancomycin Minimum Inhibitory Concentration (MIC) result reported for this isolate was in the intermediate range (8 µg/mL) using interpretive criteria defined by the National Committee for Clinical Laboratory Standards (NCCLS).[8] This report was quickly followed by similar ones from other countries, including the United States,[9] Belgium,[10] Germany[11] and India.[12]

These strains were called Vancomycin-Intermediate *Staphylococcus Aureus* (VISA). The first clinical infection with Vancomycin-Resistant *Staphylococcus Aureus* (VRSA) (MIC e" 32µg/mL) was reported in July 2002 from Michigan[9] with a second case in Pennsylvania reported shortly thereafter.[13]

Though there have been only a few reports of VRSA, the high prevalence of MRSA and vancomycin use, both thought to be risk factors for VRSA, make the widespread dissemination of these organisms an alarmingly realistic possibility.[14]

Such resistance could result in serious clinical and public health consequences because, currently, few licensed alternatives to vancomycin are available to treat serious resistant *Staphylococcus aureus* infections.[15]

Furthermore, there is an equally alarming threat of the risk of transmission of these organisms between patients.[16] The emergence of VRSA underscores the need for programs to prevent the spread of antimicrobial-resistant microorganisms and to control the use of antimicrobial drugs in health-care settings.

The purpose of the present study was to determine the sensitivity of *Staphylococcus aureus* isolated from infected patients to common antibiotics and to evaluate the possible presence of VISA and VRSA in Zarqa.

PATIENTS AND METHODS

The study included 139 strains of *Staphylococcus aureus* isolated from clinical specimens obtained from 139 patients with infection at Prince Hashem Hospital, between April 2002 and August 2004.

Identification: *Staphylococcus aureus* was based upon colony morphology, positive gram stain, DNase, catalase and coagulase tests, and fermentation of mannitol. Antibiotic susceptibility tests were performed at the microbiology laboratory. Vancomycin was obtained from Sigma (USA, potency 1000 µg/mg) for the determination of MIC of 139 strains with the agar dilution method according to the procedure outlined by NCCLS.[8]

Vancomycin was incorporated into Mueller-Hinton agar in a Log 2 dilution series from 0.125 to 256 µg/mL. Inocula were prepared using direct colony suspension in 0.9% saline, to achieve a suspension equivalent to 0.5 McFarland standard, which results in 10^4 CFU per spot (5 to 8 mm in diameter) when 10 µL of each 1:100 diluted suspension was inoculated. Plates were incubated at 35°C for 24 hours. The MIC was defined as the lowest concentration of antibiotics to inhibit macroscopically visible colonies.

Determination of oxacillin MIC by agar dilution method was performed for vancomycin-resistant strains according to the same procedure using oxacillin obtained from Sigma (USA, potency 907 µg/mg).

Resistance to vancomycin and oxacillin was checked by E-test (AB biodisk, Sweden) according to the manufacturer's instructions.

Susceptibility: S. aureus to antimicrobial drugs was also determined by the disk diffusion method using the following disks for 120 strains: penicillin (10U), erythromycin (15 µg), oxacillin (1µg), tetracycline (30µg), gentamicin (10µg), cephalothin (30µg), amoxicillin-clavulanic acid (30µg), clindamycin (2µg) & imipenem (10µg).

Susceptibility of VRSA was tested using the aforementioned disks as well as vancomycin (30µg), trimethoprim-sulfamethoxazole (25µg), rifampin (5µg), cefazolin (30µg),

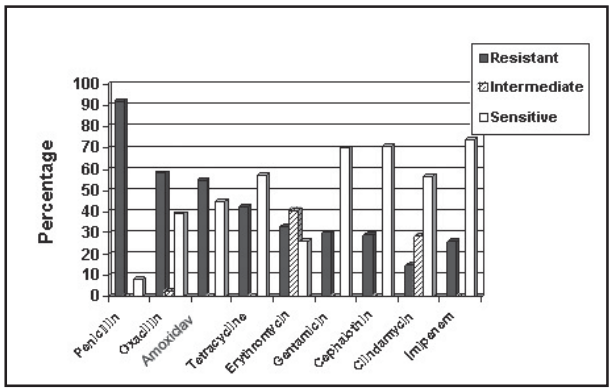

Figure-1: Disk diffusion test for 120 isolates of Staphylococcus aureus.

cephalexin (30µg), ciprofloxacin (5µg), and ceftriaxone (30µg) according to the procedure outlined by NCCLS.[17]

Susceptibility to amoxicillin and clindamycin was also determined by E-test (AB biodisk, Sweden) according to the manufacturer's instructions.

RESULTS

The results of the disk diffusion test using 9 antibiotics for 120 isolates of *Staphylococcus aureus* are shown in Figure-1.

Most isolates (91.7%) were resistant to penicillin while the lowest resistance was seen with clindamycin (15%). Minimum inhibitory concentration of vancomycin for 139 strains is shown in Table-I.

NCCLS guidelines define staphylococci for which the MIC of vancomycin is d" 4 µg/mL to be susceptible, while isolates for which the MIC is 8 to 16 µg/mL are intermediate and those for which the MIC is e" 32 µg/mL are resistant.[8] Of the 139 *Staphylococcus Aureus* isolates, 134 (96.4%) were considered susceptible and 5 (3.6%) resistant to vancomycin. These 5 strains had also vancomycin MIC e" 256 µg/mL by E-test; therefore they were considered VRSA strains. All isolated VRSA were resistant to oxacillin in the disk diffusion test, E-test and agar dilution method.

These strains were also resistant to 16 antibiotics used in the disk diffusion method, while intermediately resistance to ciprofloxacin and ceftriaxone in two strains and susceptibility to ciprofloxacin in two other strains were observed.

DISCUSSION

Since first being reported in 1997, the threat of vancomycin resistance in *S. aureus* has been the topic of intensive research and discussion. Although vancomycin resistance in S. *aureus* remains extremely rare, there is widespread concern that vancomycin-resistant *S. aureus* poses, by far, the greatest risk to patients, given the virulence of the organism.[16]

We wanted to assess and compare it with the first report of heterogeneous resistance to vancomycin in Thailand and an early warning for the possible emergence of vancomycin resistance in *S. aureus* in Southeast Asia.[18]

The presence of van A genes in VRSA suggests that the resistance determinate was acquired from a vancomycin-resistant *Enterococcus*.[13] In fact, experimental transfer of the van A genes from enterococci to *S. aureus* has been shown previously.[19]

The Center for Disease Control and Prevention (CDC) recommends contact precautions when caring for patients with VRSA; therefore, clinical microbiology laboratories must ensure that they are using susceptibility testing methods that will detect these organisms and that they are saving potential resistant strains for confirmatory testing. In addition, more systematic surveillance for VRSA will enhance the ability of the public health system to rapidly address this resistant pathogen. Using proper infection-control practices and good antimicrobial agent

Table-I: MICs of vancomycin for 139 isolates of Staphylococcus aureus.

MIC (µg/mL)	*No. of strains*	*Percentage*
0.125	1	0.72
0.25	4	2.88
0.5	43	30.94
1	77	55.39
2	9	6.47
4 – 64	0	0
128	1	0.72
256	4	2.88
Total	*139*	*100*

management will help limit the emergence and spread of antibiotic-resistant microorganisms, including VRSA.[13]

The present study describes the first clinical isolates of VRSA in Zarqa Jordan. These 5 isolates were all resistant *in vitro* to several antimicrobial agents, including penicillin, erythromycin, oxacillin, tetracycline, gentamicin, cephalothin, amoxicillin-clavulanic acid, clindamycin, imipenem, trimetho prim-sulfamethoxazole, rifampin, cefazolin, and trimethoprime. Resistance of VRSA to many antimicrobial agents has been reported by other studies, including two VRSA isolates from the United States.[13,20]

However these reports have shown that VRSA has remained susceptible to trimethoprim-sulfamethoxazole, rifampin, and tetracycline, whereas our five VRSA were resistant to these antibiotics. The same result was reported in a recent study in which all the isolates of VRSA (n = 6) were resistant to penicillin, trimethoprime, tetracycline, gentamicin, erythromycin, and ciprofloxacin but resistance to rifampicin and clindamycin was seen in 83.3% and to trimetho prim-sulfamethoxazole in 66.6%.[21]

A study by Denis et al showed that the proportion of hetero-VISA strains was 0.1% of *S. aureus* and 0.4% of MRSA strains, whereas the proportion of VISA strains was 0.1% of *S. aureus* and 0.3% of MRSA strains.[10]

However, in the present study, two strains were intermediately resistant to ciprofloxacin and ceftriaxone and two other strains were also susceptible to ciprofloxacin. Linezolid and quinupristin/dalfopristin were recently approved by FDA and are antimicrobials with activity against glycopeptide-resistant Gram positive microorganisms such as VRSA.[13,15]

CONCLUSION

We described the first clinical isolates of VRSA in Zarqa that call for further epidemiological studies to define whether VRSA is endemic in the community and, on a larger scale for the implementation of a regional and nationwide surveillance system to monitor antimicrobial resistance trends in Jordan. However, reference laboratory confirmation of isolated VRSA is recommended.

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