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The Use of E-Test Susceptibility Testing in Detection of Vancomycin-Resistant *Staphylococcus aureus*

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Abstract

Background: Vancomycin is considered the antibiotic of choice for the treatment of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA). Unfortunately, the incidence of vancomycin-resistant *Staphylococcus aureus* (VRSA) had increased in various parts of the world.

Objective: **To** use E-test susceptibility testing to detect vancomycin-resistant *Staphylococcus aureus*.

Materials and methods: A total of 50 *Staphylococcus aureus* (*S. aureus*) strains were isolated from clinical specimens. Minimum inhibitory concentration (MIC) of vancomycin susceptibility to *Staphylococcus aureus* isolates was determined by the agar diffusion and E-test susceptibility techniques.

Results: All methicillin-resistant *Staphylococcus aureus* (MRSA) isolates showed no inhibition zone to methicillin by the agar diffusion susceptibility technique. By the E-test technique, 21 strains (42%) of the MRSA investigated were found sensitive, 25 strains (50%) were intermediate sensitive, and only 4 strains (8%) were resistant to vancomycin.

Conclusion: The E-test is a standard method for detection of *Staphylococcus aureu*s strains resistant to vancomycin. Vancomycin susceptibility of *Staphylococcus aureu*s is decreasing and isolation of vancomycin-resistant *Staphylococcus aureus* had been reported.

Key words: Agar diffusion, E-test, Vancomycin, Methicillin, *Staphylococcus aureus*.

Introduction

Staphylococci are Gram-positive spherical cells, usually arranged in grape-like irregular clusters. They grow readily on many types of media and are active metabolically, fermenting carbohydrates and producing pigments that vary from white to deep yellow. Some are members of the normal flora of the skin and mucous membranes of humans; others cause suppuration, abscess formation, a variety of pyogenic infections, and even fatal septicemia. The pathogenic staphylococci often hemolyze blood, coagulate plasma, and produce a variety of extracellular enzymes and toxins. The most common type of food poisoning is caused by

a heat-stable staphylococcal enterotoxin. Staphylococci rapidly develop resistance to many antimicrobial agents and present difficult therapeutic problems [1].

The genus *Staphylococcus* has at least 35 species. The three main species of clinical importance are *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus*. *Staphylococcus aureus* is coagulase-positive, which differentiates it from the other species. *Staphylococcus aureus* is a major pathogen for humans. Almost every person will have some type of *Staphylococcus aureus* infection during a lifetime, ranging in severity from food poisoning or minor skin infections to severe life-threatening infections. The coagulase-negative staphylococci are normal human flora and sometimes cause infection, often associated with implanted appliances and devices, especially in very young, old, and immunocompromised patients. Approximately 75% of these infections caused by coagulase-negative staphylococci are due to *Staphylococcus epidermidis*; infections due to *Staphylococcus lugdunensis*, *Staphylococcus warneri*, *Staphylococcus hominis*, and other species are less common. *Staphylococcus saprophyticus* is a relatively common cause of urinary tract infections in young women. Other species are important in veterinary medicine [1].

Hospital unit's surfaces and environment may become contaminated by bacterial pathogens especially methicillin-resistant *Staphylococcus aureus* (MRSA). The organism is considered a major health problem in hospitals worldwide. The use of disinfectants is essential in infection control in hospitals and health care centers. Vancomycin can be used for treatment of MRSA, unfortunately some strains can mutate and resist vancomycin, therefore they become difficult to diagnose and treat [2].

The threat of vancomycin-resistant *Staphylococcus aureus* (VRSA) has been the topic of intensive research and discussion. Although VRSA remains extremely rare, there is a widespread concern that VRSA poses, by far, the greatest risk to patients, given the virulence of the organism [3].

The presence of *van A* gene in VRSA suggests that the resistance determinant was acquired from a vancomycin-resistant Enterococcus [4].

In fact, experimental transfer of the *van A* gene from enterococci to *Staphylococcus aureus* has been reported, and vancomycin can be used to treat MRSA infections in many cases[5].

Materials and methods

This is a laboratory-based, cross-sectional, descriptive, case study. It was carried out at Khartoum Teaching Hospital during the period from May to August, 2010. Inclusion criteria were clinical specimens of patients presenting with microbial infections. The data were analyzed using the Statistical Package of Social Science (SPSS) *version 10* program. Approval to conduct the study was given by Al Neelain University, Khartoum (Sudan). Permission to collect the specimens was granted by the Administration of Khartoum Teaching Hospital (Sudan). The sampling selected was a non-probability, convenience type. The sample size was 50 strains of *Staphylococcus aureus* isolated from clinical specimens collected from 50 infected patients. After complete purification, the susceptibility of all isolates was performed using the disc diffusion Kirby-Bauer technique. The turbidity of the bacterial suspension was compared to the Mc Farland turbidity standard. The isolates were streaked on Muller-Hinton agar. The antimicrobial discs used were methicillin (5 mcg) and vancomycin (30 mcg).

The diameter of the inhibition zone was measured in mm and compared with the Clinical Laboratory Standards Table to determine the organism's susceptibility. The E-test susceptibility was performed as follows:

a) A lawn of *Staphylococcus aureus* was inoculated onto the surface of Muller-Hinton agar plate and vancomycin E-test strip (100 mcg) was laid on top.
b) After 24 hours of incubation, the drug diffuses out into the agar, producing an exponential drug gradient.
c) An elliptical zone of inhibition was produced and the point at which the ellipse meets the strip gave the reading for vancomycin minimum inhibitory concentration (Fig. 1).

Interpretation: Sensitive (≤ 2 mg/l). Intermediate (> 2 – 16 mg/l). Resistant (> 16 mg/l)

Fig. 1 Vancomycin E-test susceptibility

Results

50 isolates of *Staphylococcus aureus* were tested for the sensitivity of methicillin and vancomycin using the agar diffusion Kirby-Bauer and E-test techniques. All methicillin-resistant *Staphylococcus aureus* strains investigated, were found resistant to methicillin (100%) by the agar diffusion method, and they were found sensitive (21/42%) to vancomycin by the E-test method. Also E-test method detected a susceptibility rate (25/50%) for vancomycin-intermediate *Staphylococcus aureus* and a susceptibility rate (4/8%) for vancomycin-resistant *Staphylococcus aureus* (Table 1).

Table (1): Susceptibility of methicillin-resistant *Staphylococcus aureus* as detected by the agar diffusion and E-test techniques

Susceptibility	Agar diffusion method		E-Test method
	Vancomycin	Methicillin	Vancomycin
Number sensitive	28 (56%)	0 (0%)	21(42%)
Number intermediate	0 (0%)	0 (0%)	25 (50%)
Number resistant	22 (44%)	50(100%)	4 (8%)
Total	50(100%)	50(100%)	50(100%)

Discussion

Infections caused by methicillin-resistant *Staphylococcus aureus* had been associated with high morbidity and mortality rates. Vancomycin was the main antimicrobial agent available to treat serious infections associated with MRSA. Unfortunately vancomycin susceptibility of *Staphylococcus aureu*s deceased and isolation of vancomycin-intermediate and resistant *Staphylococcus aureus* were recently reported in many countries[6].

In 2003, Saderi and his colleagues reported that five out of 139 strains of *Staphylococcus aureus* isolated in Tehran were vancomycin-resistant *Staphylococcus aureus* (VRSA) with an MIC ≥ 128 mg/l[7].

Also Emaneini and his co-workers studied the MIC of 164 *Staphylococcus aureus* strains against vancomycin in a teaching hospital in Tehran. They found that 97.5% of the isolates were sensitive (MIC ≤ 2 mg/l); and only one strain was resistant (MIC ≤ 256 mg/l). They also claimed that isolation of VRSA in Tehran calls for the implementation of a regional and nationwide surveillance system to monitor presence of these strains in other regions in Iran[8].

In this study, using the E-test technique, 21 strains (42%) of the MRSA investigated were found sensitive, and only 4 strains (8%) were resistant to vancomycin (Table 1).

There was increasing evidence that strains with a vancomycin MIC of 4mg/l behave similarly in clinical setting to VRSA strains and clinical failure may generally result if treatment with vancomycin is continued[9].

Stein (2007*)* in Washington reported three isolates of *Staphylococcus aureus* as intermediate sensitive to vancomycin (VISA)[10]. In this study 25 isolates of *Staphylococcus aureus* strains were found vancomycin intermediate sensitive (Table 1).

VISA and VRSA isolates are not usually detected by the agar disc diffusion method or by the automated methods. E-test is the standard method for detection of VISA and VRSA[11]. Hence the use of E-test in the present study conforms to the international standards.

In 2006, the American Center for Disease Control and Prevention (CDC) recommended the use of the MIC method plus the vancomycin screen agar technique for detection of VISA and VRSA[12]. Because of that two different methods had been performed in this study to identify vancomycin resistance in *Staphylococcus aureus*.

From this study, it may be recommended that physicians should include vancomycin susceptibility tests in their strategies for managing patients with *Staphylococcus aureus* infections. Also health authorities should encourage an active infection control policy to

prevent the spread of *Staphylococcus aureus* vancomycin-resistance in healthcare facilities. Conclusion: As compared with the agar diffusion technique, the E-test was the acceptable and standard method for detection of *Staphylococcus aureu*s strains resistant to vancomycin. Vancomycin susceptibility of *Staphylococcus aureu*s was decreasing and isolation of vancomycin-intermediate and resistant *Staphylococcus aureus* had been reported in many countries.

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