

Original Article

Comparison of Disc Diffusion and E test methods for Antimicrobial Susceptibility Testing of Vancomycin in Coagulase Negative *Staphylococcus* Isolated from Blood Culture

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ABSTRACT

Background and Objective: Reliable methods of detection of antimicrobial resistance are of paramount importance in the treatment and management of infections caused by coagulase negative *staphylococci* (CoNS). The objective of the present study was to compare and evaluate the performance of disc diffusion and E test (Epsilon meter test) methods for antimicrobial testing of coagulase negative *staphylococci* in blood cultures.

Materials and Methods: A total of 77 isolates of coagulase negative *staphylococci* from blood cultures were included as test strains. Antimicrobial susceptibility testing was performed by disc diffusion method for vancomycin. Minimum inhibitory concentration (MIC) determination was performed by E test for the same antimicrobial agent. All tests were performed on Mueller Hinton media.

Results: Disc diffusion showed four resistant and 73 susceptible cases in examined strains with vancomycin. E test showed susceptibility of all strains to vancomycin. Decreased susceptibility to vancomycin were detected in 20 isolates in disc diffusion method but in 18 isolates by E test method

Conclusion: Disc diffusion test may be used as a preliminary screening method for susceptibility testing of coagulase negative *staphylococci*. E test is a simple, easy and reliable method for determination of resistance in coagulase negative *staphylococci*, however, cost and limited availability in Iran limits its use.

Key words: Coagulase negative *staphylococcus* (CoNS), Disc diffusion, E test

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Introduction

Infections caused by coagulase negative species of *staphylococci* usually occur in association with foreign bodies. *Staphylococcus epidermidis* is the species most frequently involved in such infections. *Staph. saprophyticus* is an important cause of bacteriuria, particularly in sexually active young females. *Staphylococcus hemolyticus*, although a relatively rare isolate in clinical specimens, can be resistant to vancomycin. The MIC obtained by E test has been shown to correlate well with those obtained by either agar or broth dilution for bacterial species. Disc diffusion is a simple method that provides a qualitative susceptibility result (1).

Two characteristics of this organism include production of a slim layer or biofilm that facilitates attachment to implanted medical devices and the ability to acquire resistance to most of the antimicrobial agents used in hospital environments (2). The number of infections caused by these pathogens has dramatically increased as management of patients in the neonatal intensive care units has become more dependent on invasive procedures and indwelling devices (3-5).

Treatment of CoNS infections is complicated by certain unique characteristics of these organisms. For this reason, vancomycin is usually the antibiotic of choice in the treatment of CoNS infections; however it has been reported that susceptibilities of clinically significant *staphylococci* to glycopeptide inhibiting antimicrobials such as vancomycin are decreasing (6-10). The mechanism for reduced susceptibility of *Staph. aureus* (11) and CoNS has not entirely been known (10;12).

Therefore, the goal of this study was to identify susceptibility of CoNS populating blood cultures of neonates, infants, and pediatric patients referred to Markaze Tebbi Koodakan (Children hospital affiliated with Tehran University of Medical Sciences) to vancomycin and to compare disc diffusion and E test methods in this regard.

Material and Methods

Test strains

Seventy seven isolates of coagulase negative *staphylococci* isolated from blood cultures of admitted patients in Markaze Tebbi Koodakan during 15 months from August 2006 to November 2007 were used in the study. The isolates were randomly

selected from a larger amount of isolates during this period. The strains were identified and confirmed by standard microbiological procedures.

Antimicrobial agents and media

Vancomycin discs (30 microgram) were obtained from Padtan Teb Co. (Iran) and E test strips were procured from Himedia Co. (India). Mueller-Hinton media was procured from Merk Co. (Germany).

Quality control strains

The control strains were obtained from Reference Laboratory. These included *Staph. aureus* ATCC 25923, 29213 for quality control of vancomycin antimicrobial discs and E test strips on Mueller-Hinton media respectively. For quality control of Mueller-Hinton media, *Enterococcus faecalis* ATCC 29212 and TMP-SMZ antimicrobial disc from Mast Co. (UK) were used. For quality control of TMP-SMZ antimicrobial discs, *E. coli* ATCC 25922 and also *Staph. aureus* ATCC 25923 were used. All of control test results were within standard range of NCCLS recommendation.

Susceptibility test methods

Disc diffusion

Well isolated colonies of coagulase negative *staphylococci* from 24 h blood agar plate were taken. A suspension of colonies with 0.9% normal saline was made, opacity adjusted to 0.5 McFarland and used for inoculation on Mueller-Hinton media as described previously. After the inoculated plates sufficiently dried, the discs were placed on the medium by sterile forceps, gently pressed and plates incubated at 37 °C for 24h. Mueller-Hinton media plates were 100 mm in diameter and contained 4 mm of media in depth. Each zone size was interpreted with reference to the NCCLS standards as susceptible (15 mm and above), or resistance (below 15 mm). We considered 15-16 mm as decreased susceptibility in this study.

E test: The manufactures directions were followed while performing the test. Direct colony inoculations were performed on Mueller Hinton media same as disc diffusion method but E test strips were placed on media instead of antimicrobial disc. After incubation, a zone of inhibition was produced and the MIC was read directly from graduated E test strip at the lower limits of antimicrobial agent that inhibits growth of isolates of *staphylococci*. Readings were interpreted

as NCCLS recommendations (≤ 4 microgram was considered as susceptible). We considered 2-4 microgram as decreased susceptibility in this study.

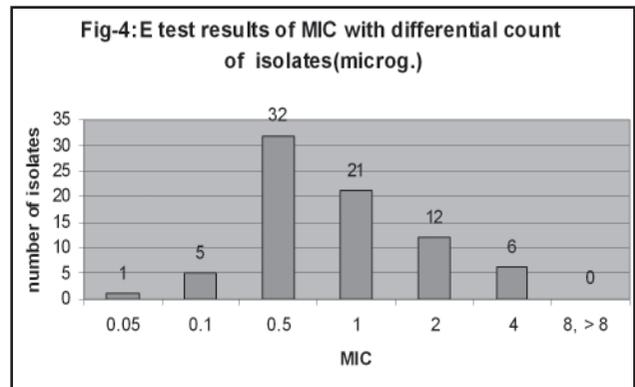
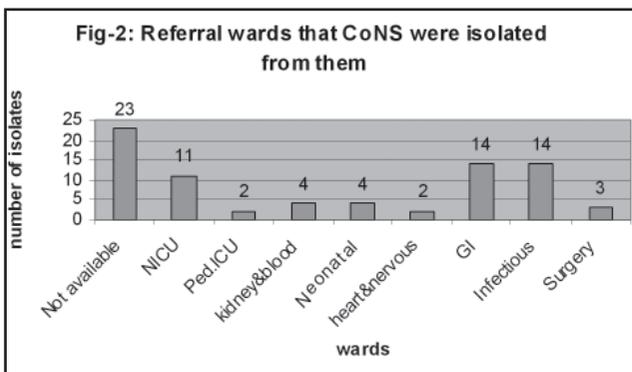
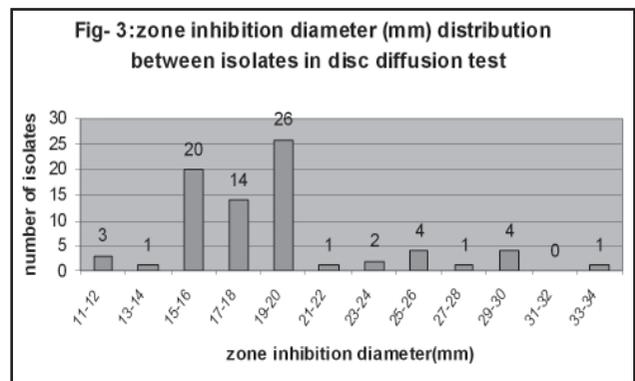
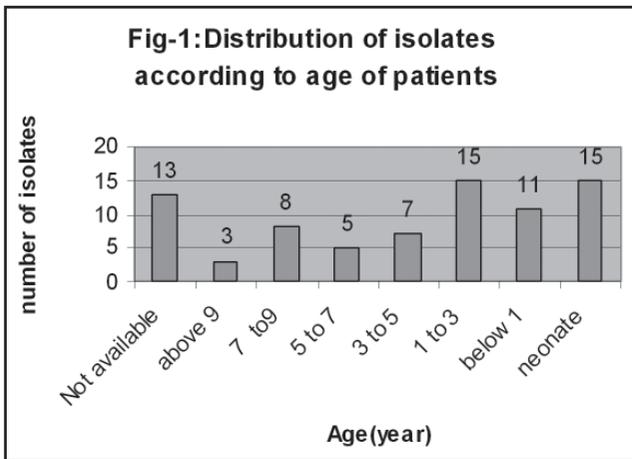
Results

A total of 77 isolates of CoNS were studied, sex distribution was as follows: 40 males, 21 females and 16 unknown. Age of patients is depicted in Figure 1. Isolates were mostly from infectious and gastrointestinal (GI) wards and also NICU (Neonatal Intensive Care Unit) and the least were from PICU (Pediatric Intensive Care Unit) and heart and nervous system wards (Figure. 2), and in 23 cases, the ward was unknown.

As shown in Figure 3, the least zone diameter in disc diffusion method was 11 mm and the mean zone diameter below 15 mm was 12.25 mm. The greatest zone diameter in disc diffusion was 33 mm and the mean diameter above 15 mm was 19.9 mm and 14 isolates have a zone inhibition of 15 mm.

As shown in Figure 4, the greatest MIC in E test results was 4 microgram in 6 isolates and 2 microgram in 12 isolates, the least MIC was 0.05 microgram in one isolate.

Four resistant isolates by disc diffusion method show susceptibility by E test method. The isolates with decreased susceptibility in E test results (MIC= 2-4 microgram) were mostly from NICU, GI, and infectious wards. In our study from 77 isolates of CoNS, four isolates have zone diameter within resistance zone in disc diffusion test (below 15 mm) but three of them have a MIC of 4 microgram in E test results and one of them has a MIC of 2 microgram. Thus, all of them showed susceptibility by E test method. As shown in Figure 3, most isolates have a zone inhibition of 19-20 mm in disc diffusion tests but there was also 20 isolates with decreased susceptibility to vancomycin with a zone diameter of 15-16 mm. As shown in figure 4, most isolates had a MIC of 0.5 microgram in E test results but 18 isolates had a MIC of 2-4 microgram that showed decreased susceptibility. E test strip is shown in figure 5. Disc diffusion in this study had a specificity of 94.8% as compared to E test method for the diagnosis of susceptibility to vancomycin with a confidence interval of 89.7-99.8%.



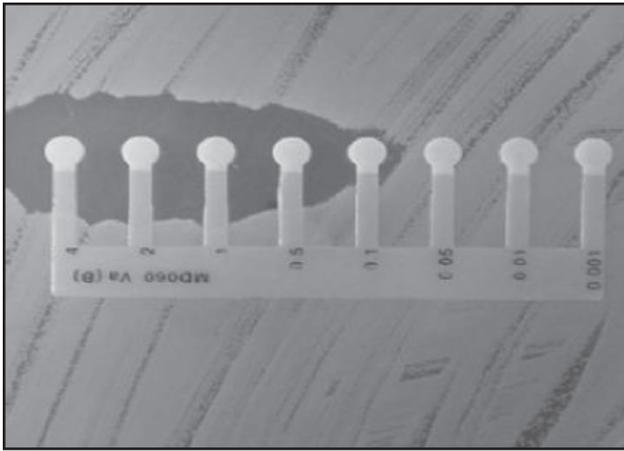


Figure 5. E-test strip on Mueller Hinton Media after bacterial inoculation

Discussion

Coagulase negative *staphylococci* (CoNS) can cause significant clinical diseases, generally following a breakdown of normal host defenses (including the presence of a catheter or prosthesis), or less commonly, in individuals with deficient defenses ((3,5,13-15). CoNS are the leading cause of bacteremia in the NICU settings, where immature infants rely on invasive devices for their care. Venous catheters have been implicated in more than one-half of the cases of CoNS bacteremia in NICUs (16-19). Multidrug resistance is a common characteristic of the CoNS, especially among those recovered during hospitalization. Despite the widespread use of vancomycin since its release in 1956, virtually all CoNS have remained susceptible to the concentrations of vancomycin expected to be achieved in serum and although resistance was first reported in clinical isolates of *S. epidermidis* (20) and *Staph. haemolyticus* ((14,21) in the 1980s, this phenomenon has not become widespread to date. However, recently we and others have noted increasing vancomycin MICs for clinically important staphylococcal species, both *Staph. aureus* and CoNS (6-10). Kimberly et al have shown that colonization with CoNS and decreased vancomycin susceptibility was significantly associated with vancomycin exposure, especially of long duration (above 10 days). In that study the vancomycin MICs at which 50% and 90% of isolates were inhibited for the total population of CoNS were 1.0 and 2.0 microgram/ml respectively and none of the predominant species were exquisitely susceptible to vancomycin (MICs below

0.25 microgram/ml) (22). E-test can conveniently identify the heterogeneously vancomycin resistant *Staphylococci* similar to the agar dilution method and is superior to the paper disks (23). The mechanism of reduced vancomycin susceptibility in CoNS is unclear but may be related to the selection of resistant subpopulations under pressure of antimicrobial exposure (21,24-26).

In our study in comparison with the study of Kimberly et al (22), we have decreased CoNS susceptibility to vancomycin in 20 (26%) and 18 (23%) isolates in disc diffusion and E test methods respectively, but Kimberly et al showed a total of 3.9% of CoNS isolates with decreased vancomycin susceptibility (MIC>2 microgram/ml), thus it seems that susceptibility is lower in our study. This may be due to increased use of vancomycin in recent years. Thus, further studies are suggested for evaluation of susceptibility of this organism to vancomycin in future to follow up their antimicrobial susceptibilities and the reason of their new behaviors. In our study, there are more CoNS isolated from GI, neonatal and infectious wards that implicate more instrumental intervention in these wards.

Conclusion

Our results showed that disc diffusion has a specificity of 94.8% as compared to E test method in diagnosis of susceptibility to vancomycin with a confidence interval of 89.7- 99.8%, so disc diffusion is a reliable method for determining antimicrobial susceptibility, however E test is a more precise method in determining susceptibility of antimicrobial tests, especially when disc diffusion shows decreased susceptibility or resistance to vancomycin in coagulase negative *staphylococci*.

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