e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2022; 14(5); 578-585

Original Research Article

Comparative Evaluation of Vancomycin Disc Diffusion Method & Vancomycin Screen Agar with Vancomycin E-Test in the Detection of Clinically Significant MRCoNS.

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Received: 15-03-2022 / Revised: 23-04-2022 / Accepted: 25-05-2022
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Conflict of interest: Nil

Abstract

Background and Objectives: *Coagulase negative Staphylococci* (CoNS) are colonizers of the skin, anterior nares, and ear canals of human beings. They have long been considered as non-pathogenic but as a result of the combination of increased use of intravascular devices and increase in the number of hospitalized immunocompromised patients, they have become one of the important nosocomial pathogens. One of the characteristics of CoNS is their resistance to multiple antimicrobial agents. Some recent reports have pointed out high level of methicillin resistance and reduced susceptibility to vancomycin which is of utmost danger in this era of multidrug resistance.

Materials and Methods: A total of 100 Coagulase negative Staphylococci isolates from various clinical samples were collected. All clinical samples were processed as per the standard protocols and CoNS were identified and further characterized Susceptibility to antibiotics were tested by Kirby Bauer's disc diffusion method. Vancomycin screen agar was used for all MRCoNS isolates. MIC of vancomycin was performed by E-test.

Results: Of 100 isolates, 17 (17%) were MSCoNS and 83 (83%) were MRCoNS.91% isolates were susceptible to vancomycin disc method. 8 isolates grew on vancomycin screen agar and one isolate showed resistance to vancomycin using E-test. Whereas, one isolated showed intermediate susceptibility to vancomycin by E-test.

Conclusion: In our study, we have observed emergence of vancomycin resistant strain. Higher prevalence of MRCoNS and indiscriminate use of vancomycin are the risk factors and such strains can get disseminated in the hospital environments at an alarming rate. Hence as an alternative linezolid can be considered.

Keywords: MSCoNS, MRCoNS, E-Test, VSA

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Introduction

Coagulase negative Staphylococci (CoNS) are colonizers of the skin, anterior nares, and ear canals of human beings. They have long been considered as non-pathogenic but as a result of the combination of increased use of intravascular devices and increase in the number of hospitalized immunocompromised patients, they have become one of the important nosocomial pathogens . [1]

Even though CoNS is considered to be skin commensal, in recent years there has been a rise in CoNS associated infections in hospitals and community. CoNS infections which are hospital acquired are mostly being foreign body related infections, also designated as device associated health care-associated infections. The CoNS species commonly encountered in clinical specimens include Staphylococcus epidermidis, Staphylococc -us haemolyticus, Staphylococcus sapro-phyticus and Staphylococcus lugdunensis.

The exact mechanism of glycopeptide resistance among CoNS is still unclear. Heterogeneous susceptibility profiles, including reduced susceptibility for teicoplanin, may suggest some general predisposition to an intrinsic resistance to this antibiotic class. [2] Three phenomena leading to therapy failure after administration of glycopeptides have been discovered or postulated, mostly for S. aureus. (i) The first phenomenon is the development of so-called vancomycin-intermediate S. aureus (VISA) isolates and putative precursor subpopulations, termed heterogeneous VISA (hVISA) strains. [3] Since VISA isolates may also be resistant to teicoplanin, the term glycopeptide-intermediate S. aureus (GISA and hGISA, if heterogeneous) is also used. Their complex resistance mechanisms include cell wall alterations, resulting in reorganization and thickening, in addition to reduced autolytic activity. [4,5,6] Furthermore, hVISA and VISA may represent a bacterial evolutionary state favouring persistence in the environment of the host. [7] Also, cell wall thickening has been reported for glycopeptide-resistant CoNS (S. epidermidis and S. haemolyticus). [8,9] Some glycopeptide-resistant CoNS may possess an excess of glycopeptide-binding sites by virtue of the overproduction of cell wall peptidoglycan material. [9] Thus, one can consider that the basic mechanisms leading to a reduced susceptibility to glycopeptides may be similar in CoNS and S. aureus.

Although the exact mechanism of glycopeptide resistance in coagulase-negative staphylococci has not yet been elucidated, glycopeptide resistant strains of S. epidermidis and S. haemolyticus have been shown to differ considerably from glycopeptide-susceptible strains with respect to various parameters like cell wall composition and synthesis, binding to glycopeptides and even ultrastructural morphology. [10] Glycopeptide-resistant CoNS strains have been demonstrated to sequester glycopeptides like vancomycin and teicoplanin more efficiently than their glycopeptide sensitive counterparts at sites unassociated with the D-alanyl-D-alanine target. [11]

Some recent reports have pointed out high level of methicillin resistance, biofilm formation and reduced susceptibility to vancomycin & other glycopeptides which is of utmost danger in this era of multidrug resistance. This may explain the selection of multidrug-resistant isolates in hospital settings and the consequent failure of antimicrobial treatment.

Hence, this study was undertaken to identify the clinical isolates of CoNS & analyse the virulence factors and to identify various drug resistance with special reference to vancomycin.

Materials and Methods

This study was conducted for a period of one year from March 2019 to March 2020 in the Department of Microbiology, Mysore

Medical College and Research Institute, Mysore. A total of 100 Coagulase negative Staphylococcus isolates from various samples were collected. Clinical data regarding age, sex, history of presenting illness, treatment history of antibiotics, duration of hospital stay and history of previous admissions was recorded.

Clinical samples encompassing blood, exudate, urine and body fluids specimens from the patients admitted or attending various outpatient departments.

Inclusion criteria includes clinically significant isolates of Coagulase negative Staphylococcus isolated from various clinical specimens. Exclusion criteria includes Staphylococcus aureus and other organisms were excluded.

Detection of Vancomycin susceptibility by Vancomycin screen agar

All isolates were screened for reduced susceptibility to vancomycin by growth on agar containing 6 µg/ ml of the antibiotic. [12] Isolates grown on this screen agar were further tested for MIC with E-test.

Detection of Vancomycin susceptibility by E-test

Principle: The gradient diffusion test uses a non-porous plastic strip that has been calibrated with minimum inhibitory concentration values that covers 15 two-fold dilutions, A pre-defined antibiotic gradient is immobilised on the surface of the strip that is opposite the MIC scale. When transferred to agar, antibiotic diffuses from the strip.

Procedure:

- With a nichrome wire 4-5 similar appearing colonies were emulsified in a test tube containing 4-5 ml of nutrient broth medium.
- The culture was incubated at 35^0C - 37^0C until it matched the turbidity of 0.5 MacFarland's standard.
- Mueller-Hinton agar plates were inoculated to obtain a uniform lawn of the test organism.
- The E-test was placed on the surface of the agar with an applicator, with the MIC scale facing up.
- Test was read after 18-24 hours of incubation at 37^0C.
- The MIC was recorded where a clearly defined zone of inhibition intersects the strip.

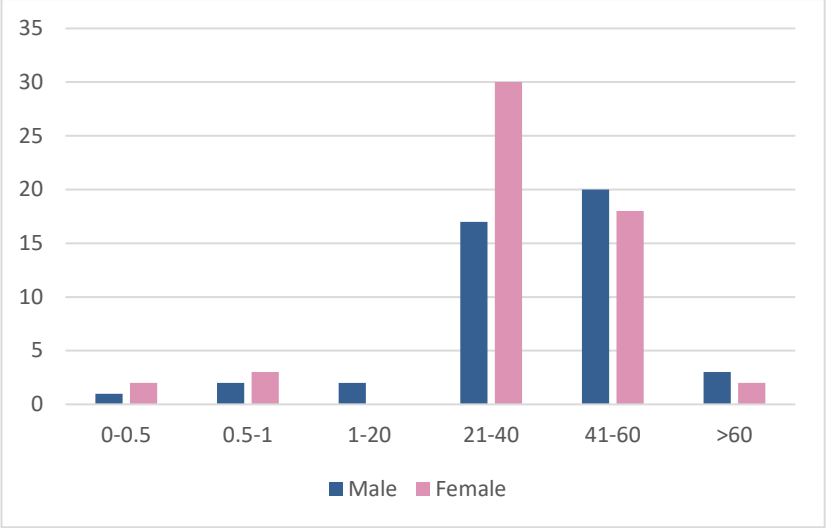
Interpretation:

The minimum inhibitory concentration (MIC) was checked by examining the plates for the lowest concentration of vancomycin that inhibited visible growth.

Vancomycin	Sensitive	Intermediate	Resistant
CoNS	≤4 µg/ml	8-16 µg/ml	≥32 µg/ml

Results

The study was conducted for a period of one year from March 2019 to March 2020 in the department of Microbiology, Mysore Medical College and Research Institute, Mysore. 100 Clinical isolates of CoNS, were isolated, studied for susceptibility to vancomycin among methicillin resistant CoNS by Kirby's disk diffusion test, vancomycin screen agar and MIC to vancomycin using E-strips. Antibiogram of other antibiotics were noted.



Graph 1: Graph showing age sex distribution

Out of 100 isolates, majority were isolated from subjects within age group of 21-40 years 47(47%), followed by 41-60 years 38(38%), >60 years 5(5%), 0.5-1 years 5(5%), 0-0.5 years 3(3%) and 1-20 years 2(2%).

Table 1: Table showing MIC for vancomycin among MRCoNS

SL no.	Case	Vancomycin E- test zone (mm)
1	Infective endocarditis	48
2	Sepsis	12
3	SSI	1
4	SSI	1.5
5	Wound discharge	1
6	Sepsis	1.5
7	SSI	1
8	UTI	1

Table 2: Showing comparison of Vancomycin disc method, vancomycin screen agar and vancomycin E-test

SL no.	Clinical diagnosis	Vancomycin disc	Vancomycin screen agar	Vancomycin E-test
1	Infective endocarditis	R	GP	48
2	Sepsis	R	GP	12
3	SSI	R	GP	1
4	SSI	R	GP	1.5
5	Wound discharge	R	GP	1
6	Sepsis	R	GP	1.5
7	SSI	R	GP	1
8	UTI	R	GP	1

R- Resistant, GP- Growth present

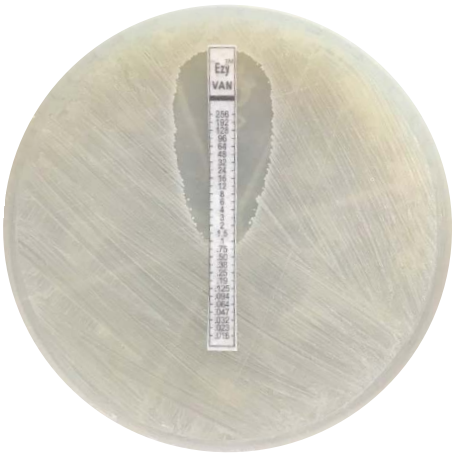


Figure 1: Picture showing MIC of 1.5 µg/ml for vancomycin by E-strip method

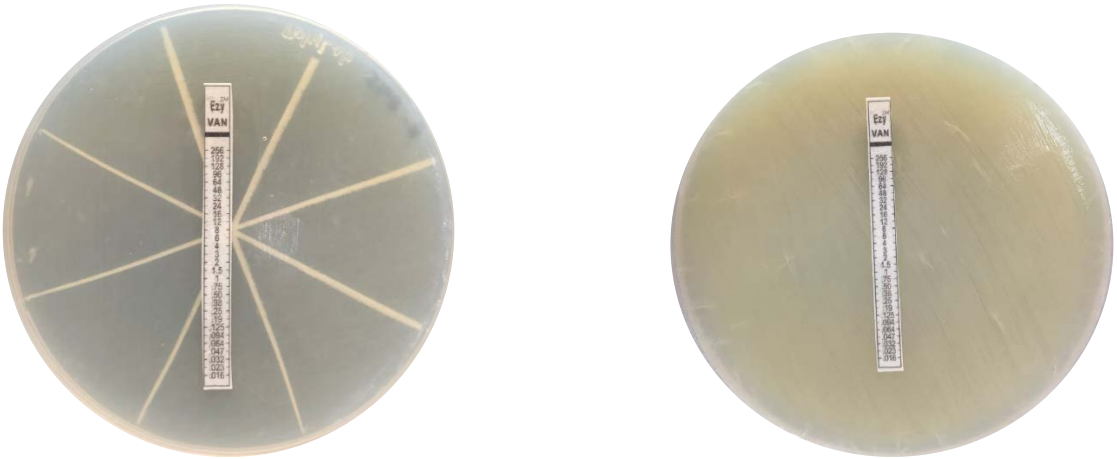


Figure 2&3: Picture showing MIC of 12 µg/ml 48 µg/ml for vancomycin by E-strip method

Discussion

CoNS are now the most common organism associated with various infections. Antimicrobial resistance acquisition in microorganisms has become a common microbiological research theme in the last few decades, particularly with Staphylococci. A significant evolution has been the acquisition of methicillin resistance in Coagulase negative staphylococci. Since CoNS and MRCoNS infections are increasing, emergence of vancomycin resistant strains of coagulase negative staphylococci, has caused concern that, nothing will be left in the antibiotic arsenal to treat patients infected by these strains. [12]

This study includes 100 clinical isolates of CoNS which were identified, speciated and were tested for methicillin resistance. This study has also compared susceptibility of MRCoNS to vancomycin using vancomycin disc method, vancomycin screen agar and E-test methods. 100 clinical isolates were speciated, antibiotic susceptibility pattern was detected, also all isolates were grown on vancomycin screen agar. For the isolates grown on vancomycin screen agar, susceptibility to vancomycin was confirmed by E-test.

Distribution of MSCoNS and MRCoNS among isolates

In the present study of 100 clinical isolates of CoNS, majority of the isolates were MRCoNS 83 (83%) and 17 (17%) were MSCoNS. A study by Koksal F et.al. has reported isolation rates of MRCoNS as 67.5% and MSCoNS as 32.5% from

various clinical specimen. [13] Another study by Sabal ME *et.al.* reported 62.5% MRCoNS which correlates with the present study. [14] The high rate of MRCoNS could be because of frequent contact with healthcare facility or due to autoinoculation or due to recurrent colonisation from family reservoir.

Vancomycin screen agar

In our study among 83 MRCoNS, 8 isolates grew on vancomycin screen agar. A study by Pinheiro L et al. showed that 51 (47.7%) isolates were able to grow on agar plates containing 4 μg mL-1 of the antibiotic, whereas three (2.8%) isolates were able to grow on agar containing 6 μg mL-1.15 In Another study by Ma XX et.al. to screen for potentially vancomycin-resistant strains among the 17 teicoplanin-non-susceptible CoNS strains, they carried out a one-step resistance selection experiment using vancomycin-containing agar. And found that all CoNS strains could grow on selection agar with 4 μg/ ml. [15]

MIC of Vancomycin

In the present study MIC of vancomycin was tested using E-test methods for those isolates grown on vancomycin screen agar. MIC of 8 isolates were checked by vancomycin E-test. Four isolates had MIC of 1μg/ml, two isolates had MIC of 1.5 μg/ml, one isolate had MIC of 12 μg/ml and one isolate had MIC of 48 μg/ml.

Under the guidelines of the National Committee for Clinical Laboratory Standards, CoNS for which the vancomycin MIC is 4.0 μg/ml are reported as susceptible. [16,17] CoNS with reduced susceptibilities to vancomycin, resistance appears to develop in a stepwise fashion and is related to the duration of vancomycin administration. [17 18] Given the high prevalence of multidrug-resistant CoNS and the emergence of reduced vancomycin susceptibilities among other gram-positive organisms, the detection of vancomycin hetero-resistance among CoNS may be of clinical relevance.

A study by Maria CGDL *et.al*. had Forty-two isolates with vancomycin MIC 2 μg/mL (35 isolates at 2 μg/mL, 5 isolates at 3 μg/mL, and 2 isolates at 4 μg/mL), which correlates with our study which had 8 isolates with MIC ≥ 2 μg/ml. [19] The emergence of decreased vancomycin susceptibility among staphylococci has led to evaluations of susceptibility tests performed by clinical laboratories to avoid imprecise vancomycin MIC determinations. Although BMD is the reference method for MIC determination, the E-test is an attractive option, as it is easy to perform. [20]

As per the reports available from the world 3-11 per cent CoNS isolates have decreased susceptibility to vancomycin. In our study, 2% CoNS isolates were observed with decreased susceptibility which is higher as compared to other studies.

In our study among all 8 isolates grown on vancomycin screen agar, 6 of them showed sensitive range in E-test and one isolate showed intermediate range. A study by Mashaly GE-S *et.al.* showed all 9 isolates susceptible to vancomycin, but few isolates showed intermediate range. [21] The notable exception is the study by Froggatt et al., in which 42% of Staphylococcus haemolyticus isolates were intermediately resistant (MIC 6.25 μg/ml) to vancomycin.

Because of the small number of cases, determining risk factors for the development of reduced vancomycin susceptibility among coagulase-negative staphylococci is difficult. Thus far, all resistant strains have been recovered from patients in acute care hospitals. There was no common underlying illness in all the cases, although two of the five patients were on peritoneal dialysis. Exposure to a glycopeptide antibiotic would certainly appear to play an important role, as four of the five reported patients received at least 30 days of vancomycin before a less susceptible isolate was recovered.

Comparison of disc diffusion test, vancomycin screen agar and E-test for vancomycin susceptibility

In the present study we found a significant P value of < 0.005 in the detection of vancomycin susceptibility by disc diffusion test, vancomycin screen agar and MIC by E strip method. Nine isolates showed resistance with vancomycin disc method out of which only 8 grew on vancomycin screen agar. And by E-test method, five isolates had MIC of 1µg/ml, two isolates had MIC of 1.5 µg/ml, one isolate had MIC of 12 µg/ml and one isolate had MIC of 48 µg/ml.

Conclusion

In our study, we calculated the sensitivity of both vancomycin disc method and vancomycin screen agar to be 100%. Whereas, specificity of vancomycin disc method was 41.17% and that of vancomycin screen agar was 50%. As vancomycin screen agar has more specificity than vancomycin disc method, vancomycin screen agar can be used as a screening method for vancomycin resistance which can be confirmed with E-test. In our study, we have observed emergence of vancomycin resistant strain. Higher prevalence of MRCoNS and indiscriminate use of vancomycin are the risk factors and such strains can get disseminated in the hospital environments at an alarming rate. Hence as an alternative linezolid can be considered.

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