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Comparison of Teicoplanin E-strip and Agar Dilution Methods against Clinical Isolates of Staphylococcus Species in a Rural Tertiary Care Centre

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A B S T R A C T

Staphylococcus are among the most frequently isolated organisms in clinical microbiology laboratories. Methicillin- resistance among *Staphylococcus* species has led to utilization of glycopeptides (Vancomycin and Teicoplanin) as the drug of choice for the treatment. Resistance to Teicoplanin is emerging among *Staphylococcus* species due to improper usage of this drug. Detection of Teicoplanin resistance is mainly done by disc diffusion method in the laboratories. Objectives are to detect and compare the resistant pattern of Teicoplanin by E-strip and agar dilution method of *Staphylococcus* species. Total of 100 samples of *Staphylococcus* species was included in the study. Teicoplanin resistant pattern were detected by E-strip method (Hi media) and by agar dilution method (Dilution range 0.5mg/ml to 128mg/ml) Results: Out of 100 *Staphylococcus* species, all the isolates were sensitive by E-strip method for Teicoplanin. By agar dilution method 95 were sensitive (≤8µg/ml), 2 were intermediate (≥16µg/ml) and 3 were resistant (≥32µg/ml) for Teicoplanin. Teicoplanin resistance detection by conventional disc diffusion method is difficult because of limited diffusion of its large molecule in agar (4). Results obtained in this study by comparing E-strip and Agar dilution is 95% concordant. Agar dilution method which is considered gold standard showed better performance compared to E-strip method, this could be due to different performance of commercially available gradient strips against staphylococci

Introduction

Staphylococcus are among the most frequently isolated organisms in clinical microbiology laboratories. They are known to cause minor skin and soft tissue infections to life threatening invasive infections involving different organ systems.(1) Treatment options mainly includes Penicillins, cephalosporins, Glycopeptides, etc. Methicillin- resistance among *Staphylococcus* species has led to utilization of glycopeptides (Vancomycin and Teicoplanin) as the drug of choice for the treatment in the recent days. Resistance to Teicoplanin is emerging among *Staphylococcus* species due to improper usage of this drug. Detection of Teicoplanin resistance is mainly done by disc diffusion method in the laboratories.(1) The objective of our study is to compare the E-strip method

and agar Microdilution for the detection of Teicoplanin resistance among Staphylococcal species.

Materials and Methods

Present study was conducted in the department of Microbiology, Adichunchangiri Institute of Medical Sciences. B.G.Nagara, Karnataka. A total of 100 Staphylococcal isolates obtained from various specimens were included in the study. Ethical committee clearance is obtained from institutional ethical committee.

Gram positive bacteria other than *Staphylococcus* species and Gram negative bacteria were excluded from the study. *Staphylococcus* species were isolated and identified from 100 clinical samples by conventional culture method in the laboratory. Antimicrobial susceptibility testing is done by Kirby-Beaur disk diffusion method. These isolates were further subjected for Teicoplanin disk diffusion E-strip and Agar dilution method.

E- Test[(2)]

Teicoplanin Ezy MIC™ Strip(0.016-256) obtained from Himedia were used for E-test. Staphylococcal pure cultures were used for preparing inoculum. 4-5 colonies were transferred to peptone water broth using an inoculation loop and incubated at 35-37˚C for 2-6 hours until light to moderate turbidity is obtained. Inoculum turbidity is compared with 0.5Mc Farland unit. These were used for lawn culture onto Muller-Hinton agar. Teicoplanin E-strips were then placed at the centre of the agar plates. Plates were then incubated at 35-37˚ C overnight. Then MIC are read the following day, where the Eclipse intersects the MIC scale on the Strip. *Staphylococcus aureus* ATCC 29213 was used as control.[(2)]

Interpretative criteria for Teicoplanin E-test [Himedia][(2)]

Sensitive	Intermediate	Resistance
≤8µg/ml	<=16 µg/ml	≥32 µg/ml

Agar dilution method[(3)]

Preparation of antibiotic stock solution.[(3)]

The range for Teicoplanin concentration for *Staphylococcus* species is 0.06-32mg/L. Diluent used is water and stored at 4˚C. Stock solution is prepared using formula

$$\frac{1000}{P} \times V \times C = W$$

Where P= potency given by the manufacturer(µg/mg)

V= Volume required (ml)
C=final concentration of solution(Multiples of 1000) (mg/L)
W= Weight of antibiotic(mg) to be dissolved in Volume V(mL)

A 50 ml of stock solution is prepared by dissolving 555.55mg of Teicoplanin antibiotic in 50 ml of diluent to obtain 10000mg/L of stock solution. This was filtered using antibiotic solution filters.

For further obtaining 1000 mg/L of antibiotic stock solution- 1ml of 10000mg/L solution is added to 9ml diluent

For further obtaining 100 mg/L of antibiotic stock solution - 100µl of 10000mg/L solution is added to 9.9ml diluent.

Preparation of antibiotic dilution range[(3)]

Dilution range required is 0.25 to 32 mg/L

Label 11 Universal containers as follows: 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25

From the 10000mg/L stock, dispense the following amounts with micropipette:

256µL into the container labeled 128
128µL into the container labeled 64
64 µL into container labeled 32
32 µL into the container labeled 16

From the 1000 mg/L stock, dispense the following amounts:
160µL into the container labeled 8
80µL into the container labeled 4
40µL into the container labeled 2

From the 100mg/L Stock, dispense the following amounts

200µL into the bottle labeled 1
100µL into container labeled 0.5
50µL into container labeled 0.25
No antibiotic is added to bottle labeled 0

Preparation of agar dilution plates[3]

20 ml of cooled molten nutrient agar(cooled at 50˚ C) is added to each container containing antibiotic solution. Mixed well and poured onto 90mm petri-dish. Allowed to set for 10 minutes and stored at 4-8˚C.

Preparation of inoculum[3]

Two to three colonies are taken and inoculated onto nutrient broth incubated at 35-37˚C 2-6Hrs, turbidity is adjusted to 0.5 McFarlands unit.

Inoculation[3]

Using a micropipette 1-2µl of bacterial suspension is inoculated onto the each media plate containing different antibiotic concentration. Inoculum is allowed to be absorbed into the agar before incubation. Inoculated plates are then incubated at 35-37˚C for 18-24hrs.

Reading and interpretation[3]

After incubation, ensure that all the organisms have grown on the antibiotic- free control plate.

The MIC is defined as the lowest concentration of antibiotic at which there is no visible growth of the organism. *Staphylococcus* ATCC Strain 25923 is used as control. MIC for control strain should be within plus or minus one or two fold dilution of the expected MIC.

Results and Discussion

A total of 100 Staphylococcal species were taken from various clinical samples. This includes *Staphylococcus aureus*, Coagulase negative *Staphylococcus* (CoNS), Methicillin resistant *Staphylococcus* (MRSA), Methicillin resistant Coagulase negative *Staphylococcus* (MRCoNS) (Table 1).

Among the 100 isolates, MRCoNS attributed to 45%, MRSA 21%, S.*aureus*18% and CoNS16%

All isolates were sensitive to Teicoplaninby E-strip method (Table 2 and Fig. 1).

By Agar dilution method, 95 samples were sensitive to Teicoplanin (≤8µg/mL), and 2 showed intermediate sensitivity pattern (≥16µg/mL) and 3 were resistant (≥32µg/mL) (Table 3 and Fig. 2).

Inspite of introducing Teicoplanin for more than two decades, correct dosage of Teicoplanin remains controversial, as does the need for routine monitoring of serum levels[4]It is important to use a susceptibility test

method that can reliably detect resistance and predict patient outcome. Unfortunately, detection of Teicoplanin resistance by conventional disc diffusion methods is difficult because of limited diffusion of its large molecule in agar[5], moreover resistance demonstrated by one method is often not confirmed by another.[6]

In our study, the occurrence of Teicoplanin resistance(≥32µg/ml) considering agar dilution method as gold standard is 3%(3/100 isolates), Which is in concordance with Ana paula et al 8.5%(9/106)[7] and Wong et al [8] showed similar rates(7.4%)

Incidence of Teicoplanin resistance is more with MRCoNS, which is accounted for 4.44% (2/45) and MRSA 4.76% (1/21), which is similar to the study of Ana paula et al and Wong *et al.,* where the maximum isolates which showed Teicoplanin resistance are CONS. This result can be worrisome because CoNS are the most frequent Gram-Positive agents isolated from nosocomial bacteremias[9] andare also prevalent as endogenous microbiota in humans.[10] These facts in association with antibiotic pressure, especially by use of vancomycin owing to the high prevalence of oxacillin resistance among these organisms, probably would be contributing to the establishment and maintenance of the glycopeptides resistance in hospital. [7]

Table.1 Organisms isolated from different clinical specimens

Organisms	Number of organisms isolated
MRCoNS	45
MRSA	21
S.*aureus*	18
CoNS	16

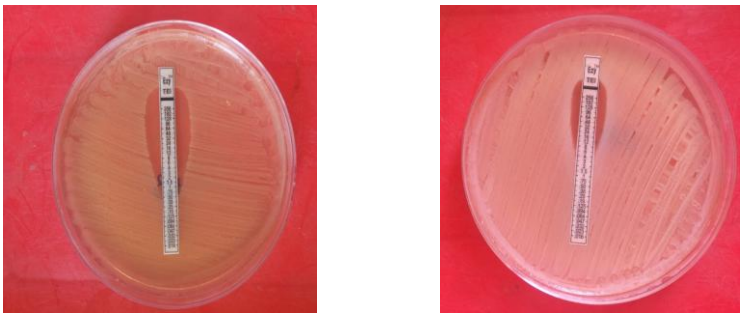
Table.2 Sensitive and resistant pattern among different species of *Staphylococcus* tested by E-Strip method

Organisms	Sensitive	Resistant
MRCoNS	45	0
MRSA	21	0
S. *aureus*	18	0
CoNS	16	0

Table.3 Sensitive and resistant pattern among different species of *Staphylococcus* tested by agar dilution method

Organisms	≤8µg/ml (S)	≥16µg/mL (I)	≥32µg/ml (R)
MRCoNS	42	1	2
MRSA	20	0	1
S.*aureus*	18	0	0
CoNS	15	1	0
Total	**95**	**2**	**3**

Fig.1 E-strip method showing sensitivity pattern a) MIC -0.5 and b) MIC-4



(a) (b)

Fig.2 Agar dilution Method. Dilution range from 0.5µg/ml(to the right) to 128µg/ml (left first image)

In our study, standard E- test method failed to detect low-level glycopeptide resistance.[11] E-strip method showed 95% concordance with agar dilution for Teicoplanin sensitivity. This is in concordance with ELOISA *et al*., study which showed 88% categorical agreement with M.I.C.E

In conclusion, our study showed E-strip picking less teicoplanin resistance strain compared to agar dilution method which is considered as gold standard for *Staphylococcus* species, which is similar to Eloisa et al study. Thus we must be aware of different performance of commercially available gradient strips against *Staphylococcus* species.[3]

In the present study Hi media E -Strips results were interpreted based on manufacturers and CLSI guidelines.[2] Though it is less cumbersome procedure compared to agar dilution method, it failed to detect low level Teicoplanin resistance, as different commercially available gradient strip shows different performance for *Staphylococcus* species.

Disc diffusion test cannot be used for determining Teicoplanin resistance as it is showed in the study of Charlesworth et al [4] that only 20-29% of the teicoplanin diffuses in to agar after 6 hrs of incubation compared to 72-74% of vancomycin. [5] As large molecules, these agents diffuse slowly. The E-test in contrast, only requires limited antibiotic diffusion into the agar to produce a stable and continuous antibiotic gradient

beneath the strip and appears more reliable, especially with high inoculum test. However standard E-test method (0.5 McFarland standard inoculum) can fail to detect low level Teicoplanin resistance.[12]

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