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DETECTION OF CREEPING MIC OF LINEZOLID AND VANCOMYCIN IN STAPHYLOCOCCI

Microbiology

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ABSTRACT

: In 1961, methicillin-resistant Staphylococcus aureus (MRSA) was first detected in United Kingdom, since then MRSA rapidly spread worldwide and drew much concern because it gives rise to serious problems in either hospital settings or community. Creeping minimum inhibitory concentration (MIC) of Vancomycin in MRSA isolates have raised doubts on the rationale of using it for treatment. Treatment failure is associated with an increase in the MIC as well as a decrease in the rate of bacterial killing. Linezolid is the only antibiotic with good activity against MRSA available as an oral formulation; making it desirable for outpatient treatment. It is safe and effective for use in children and newborns as well as adults.

KEYWORDS

MRSA, Vancomycin, Treatment failure, Linezolid, good activity.

INTRODUCTION:

Staphylococcus aureus and *coagulase negative Staphylococci* are one of the most common pathogens that lead to severe infections, including skin and soft tissue infection, pneumonia, bacteremia and endocarditis either in community settings or hospitals(1). In 1961,*methicillin-resistant Staphylococcus aureus* (MRSA) was first detected in United Kingdom, since then MRSA rapidly spread worldwide and drew much concern because it gives rise to serious problems in either hospital settings or community.(2). Creeping minimum inhibitory concentration (MIC) of Vancomycin in MRSA isolates have raised doubts on the rationale of using it for treatment. <1% of *S. aureus* and 2% of CONS are linezolid resistant. In 1997, the first strain of *S. aureus* with reduced susceptibility to Vancomycin and teicoplanin was reported from Japan. (9) Vancomycin is still used extensively for the treatment of oxacillin (methicillin)-resistant *Staphylococcus aureus* (MRSA) bacteremia, as well as other less serious MRSA infection. Linezolid is the first antibiotic of the oxazolidine class approved for clinical use for resistant S*taphylococcus aureus*. It is the only antimicrobial drug available which has proven high activity against multidrug resistant *Staphylococcus aureus* including the strain with reduced susceptibility to glycopeptides. Linezolid to be a better therapeutic option for infective endocarditis caused by multidrug resistant gram positive bacteria. Linezolid is used as an alternative to Vancomycin for MRSA meningitis and superior to Vancomycin as suggested by studies.

MATERIALS & METHODS:

All the *staphylococci* isolated from various clinical samples (pus,blood,urine,ET,Nasal swabs) at department of Microbiology, were included in the study from Jan-2016 to December-2017. The isolates were identified or confirmed by various standard biochemical tests and followed by Antibiotic susceptibility testing was done to detect the minimum inhibitory concentration of Linezolid and Vancomycin by Vitek 2 and E-test strips method.

- Vitek 2 automated culture ID and sensitivity system by using GPC catridges.
- By manual E-test done by using manufacturer protocol for two antibiotics (Linezolid and Vancomycin).

ANTIBIOTIC SUSCEPTIBILITY TESTING:

In this study the antibiogram of *Staphylococcus* is performed via Vitek 2 method for MIC determination. For the detection of most known intermediate and resistance of Vancomycin and Linezolid by E-strips is done.

BY VITEK 2 SYSTEM	E-TEST
Clindamycin	_
Erythromycin	_
Oxacillin	_
Tetracycline	_
Cotrimoxazole	_
Ciprofloxacin	_
Levofloxacin	_
Linezolid	Linezolid
Vancomycin	Vancomycin
Rifampicin	_
Teicoplanin	_
Gentamycin	_
Daptomycin	_
Tigecycline	_

DETECTION OF VANCOMYCIN & LINEZOLID INTERMEDIATE & RESISTANCE E-TEST:

The E-test method was performed according to the manufacturer's protocol. The 24 hour old culture plate with isolated beta hemolytic colony were picked up and processed for E-test method. Two Antibiotic E-stips were tested against the isolates. The antibacterial agents were Vancomycin (0.016-256 mcg/ml) and Linezolid (0.016-256mcg/ml). Muller Hinton Agar (MHA) plates were s-treaked evenly in three directions with a sterile cotton swab dipped into the standardized inoculums suspension. Plates were allowed to dry then antibacterial E-strips were placed onto the medium with a pair of forceps, the E-strips were aseptically removed from the packages and place on a dry clean surface of MHA plate to, and that the concentration maximum is nearest the rim of the plate. The whole length of the strip is to be in complete contact with the agar surface. The inhibition ellipse will form because the antibacterial agent will diffuse across the porous paper strip. If air pockets were seen underneath the strip, they were removed by pressing gently onto the strip (without moving the strip) with a pair of forceps, moving from the minimum concentration upwards. Small bubbles under the strip would not affect the results. Once applied, the strip was not to be moved because of the instantaneous release of drug into the agar.

RESULTS:

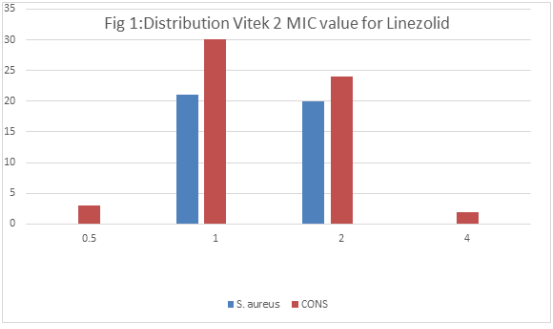
In the present study, a total of 100 staphylococcus isolates were collected. Out of 100 isolates, 53 isolates were isolated from pus samples, 32 isolates from Blood samples, 12 isolates from Urine samples and 2 isolates from ET samples and 1 isolate from nasal swab. Out of 100 samples 52(52%) were collected from male and 48(48%) were collected from female. The collected isolates were confirmed to be S. aureus and coagulase-negative staphylococcus by biochemical tests and were then subjected to antimicrobial susceptibility testing by Vitek 2. MIC to Vancomycin and linezolid was detected by E-strip method and vitek 2 system. Out of the 100 staphylococcal isolates 43 (43%) were S. aureus and 57 (57%) were CONS.

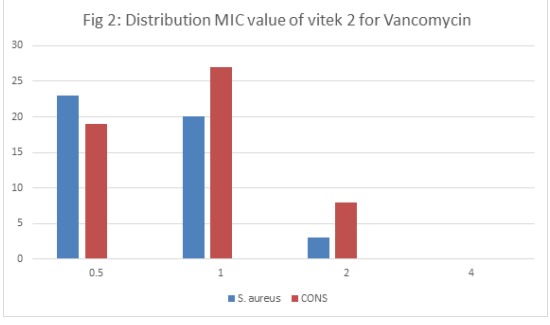
Antibiotic sensitivity carried out by Vitek 2 system showed the following sensitivity pattern.

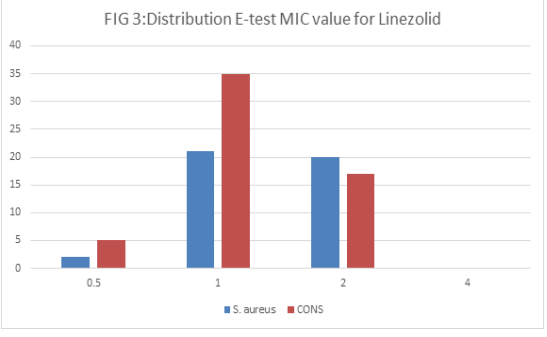
Table showing Antibiogram of Staphylococcal isolates:

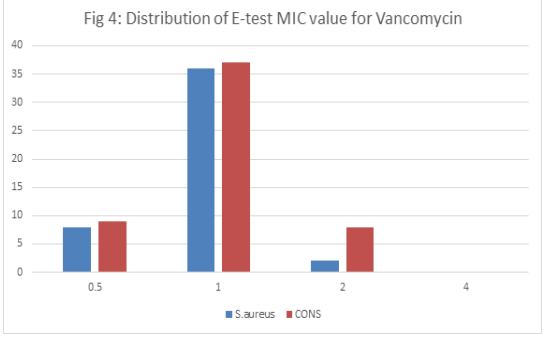
DRUGS	SENSITIVE	RESISTANCE	INTERMDIATE
Clindamycin	57 (57%)	35 (35%)	8 (8%)
Erythromycin	44 (44%)	50 (50%)	6 (6%)
Tetracycline	78 (78%)	17 (17%)	5 (5%)
Cotrimoxazole	71 (71%)	29 (29%)	-
Ciprofloxacin	35 (35%)	55 (55%)	10 (10%)
Levofloxacin	36 (36%)	19 (19%)	45 (45%)
Linezolid	100 (100%)	-	-
Teicoplanin	89 (89%)	4 (4%)	7 (7%)
Gentamycin	79 (79%)	15 (15%)	6 (6%)
Vancomycin	100 (100%)	-	-

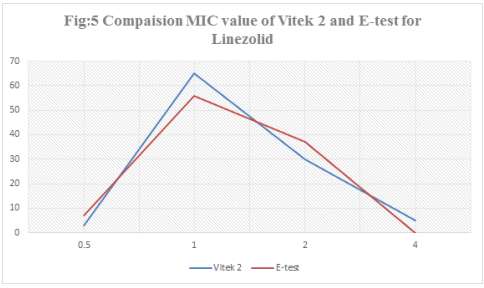
- In our study shows 100% of the isolates are sensitive to Linezolid, Vancomycin, Tigecycline, followed by 90% of the isolates are sensitive to Teicoplanin, 70% of the isolates are sensitive to Gentamycin, Tetracycline and Cotrimoxazole,50% of the isolates are sensitive to Clindamycin, below 50% of the isolates are sensitive to Ciprofloxacin, Levofloxacin and Erythromycin.











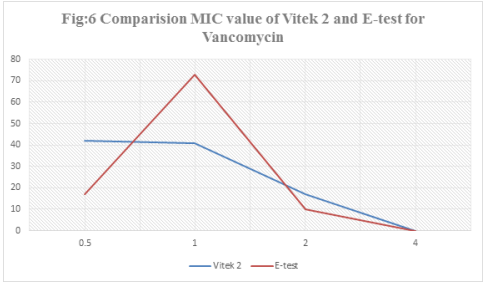
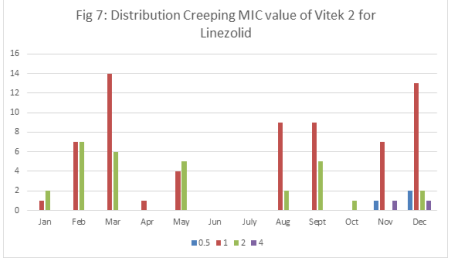


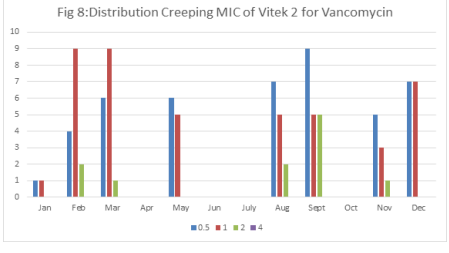
Table 1: Comparison of MIC value of Vitek 2 for Linezolid

MIC value of Vitek 2 for Lz	2016 MIC value		2017 MIC value		Variation
0.5	1	(S)	3	(S)	2%
1	59	(S)	65	(S)	6%
2	35	(S)	30	(S)	5%
4	5	(S)	2	(S)	3%
Total	100		100		16%

Table 2: Comparison of MIC value of Vitek 2 for Vancomycin

MIC value of Vitek 2 for Va	2016 MIC value		2017 MIC value		Variation
0.5	42	(S)	45	(S)	3%
1	41	(S)	44	(S)	3%
2	17	(S)	11	(S)	6%
4	00		00		0%
Total	100		100		12%





DISCUSSION:

In the present study, an attempt was made to detect in-vitro activity of Linezolid and Vancomycin on Clinical isolates of *Staphylococcus aureus* and *CONS* at JSS Hospital, Mysore. Our study shows that out of 100 strains, all 100 (100%) were sensitive by E-test and Vitek 2 for detection of Minimum Inhibitory Concentration of Linezolid and Vancomycin. The present study compares the efficacy of two different test methods; i.e. E-test method and Vitek 2, Linezolid and Vancomycin sensitivity by detection of Minimum Inhibitory

concentration by E-test method. In our study 100 isolates were collected, of *Staphylococcus aureus* and *CONS* were collected from Pus, Blood, Urine, ET and NS samples from which sensitivity to linezolid and Vancomycin was detected by E- test method and in Vitek 2.The testing of resistance and intermediate to Linezolid and Vancomycin against *S. aureus* and *CONS* is important due to the challenge of emerging resistance as the strains resistant to Linezolid and Vancomycin are difficult to detect accurately with many of the recent susceptibility-testing methods.

In our study none of the isolates showed resistance or intermediate to Linezolid and Vancomycin by E-test method. In our study MIC for Linezolid by Biomurieux Vitek -2 compact was found to be in the range of 0.5-4µg/ml and all the isolates were found to be sensitive to Linezolid and Vancomycin. In our study MIC for Linezolid and Vancomycin by E-test method was found to be in the range of 0.5 µg/ml–2 µg/ml. In the present study susceptibility to Linezolid and Vancomycin by E-test method and Vitek 2 is in complete correlation (100%). The results of our study at JSS Hospital Mysuru, Linezolid and Vancomycin were found to be very active against the S. aureus, CONS and VISA strains and appear to be a potentially useful drug for infections caused by Staphylococcus aureus (23). Therefore, it could be a suitable therapeutic option for the treatment of highly resistant nosocomial infections but it should not be used empirically without proper laboratory evaluation. For future, we recommend that to deal with the ever-increasing antimicrobial resistance, it is necessary to monitor resistance patterns carefully and continuously.

Conclusion

Antimicrobial resistance is now acknowledged as a serious public health issue not only in hospital settings but also in the community .The prevalence of *S. aureus and CONS* isolates that are resistant to antimicrobial agents is increasing globally. Thus, the aim of this study was to analyze the pattern of resistance and intermediate to the antimicrobial drugs and more significantly for Linezolid and Vancomycin in *S. aureus* and *CONS,* which may be likely to proclude antibiotic treatment.

The need of MIC determination for susceptibility pattern testing of *S. aureus* and *CONS* is a better method because as it was found that the isolates which were sensitive for E-test even had the resistant MIC for Linezolid and Vancomycin respectively. We conclude that the surveillance to *S. aureus* and *CONS* susceptibility pattern is crucial for monitoring the development of antimicrobial resistance. When we try to look at creeping MIC for Linezolid and Vancomycin there was mild increasing in MIC in few strains but overall the creeping MIC was not found to be statistically significant.

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