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Original Article

Microbiology Section

Evaluation of Chromogenic Media in Detection of Vancomycin Resistant *Enterococci*

VIJAYA D.[1], VIJAYA S.[2], SANTHYA S.T.[3], YASHASWINI M.K.[4], MEGHA S.[5]

ABSTRACT

Introduction: Vancomycin resistant *Enterococci* have become important nosocomial pathogens. So it is necessary to monitor continuously such infections in the hospitals.

Materials and Methods: A total of 100 *Enterococci* isolated from 4489 various clinical samples were speciated and antibiogram was done according to standard laboratory methods. The efficacy of CHROMagar™ VRE (France) and Hicrome VRE (Himedia) in detecting VRE was evaluated using E- test (Himedia).

Results: Hicrome VRE and CHROMagar™ VRE showed sensitivity of 100% and specificity of 99% as compared to E-test.

Conclusion: In the present study VRE was not isolated. Prudent use of vancomycin and continuous surveillance for VRE will prevent the emergence of vancomycin resistant *Enterococci* in the locality in future. Identification of VRE by chromogenic media is rapid, easy to perform, cost effective compared to technically demanding, time consuming and costly conventional method.

Keywords: CHROMagar™, E-test, Hicrome VRE, Vancomycin resistant *Enterococci*

INTRODUCTION

Enterococci form the part of the normal flora in both the human and animal gastrointestinal tracts. These organisms have become notorious nosocomial pathogens, in spite of their limited virulence. This is related to their resistance to several antimicrobial agents and this resistance can be intrinsic (low level to penicillin, cephalosporins and aminoglycoside) as well as acquired resistance to glycopeptides with high level resistance to aminoglycoside. Vancomycin resistant *Enterococci* (VRE) was reported in 1988 by Uttley [1]. The first report of vancomycin resistant *Enterococci* (VRE) in India was done by Mathur in 1999, from New Delhi [2]. Later, various authors have reported prevalence of 1– 8.7% of VRE in India [3-5].

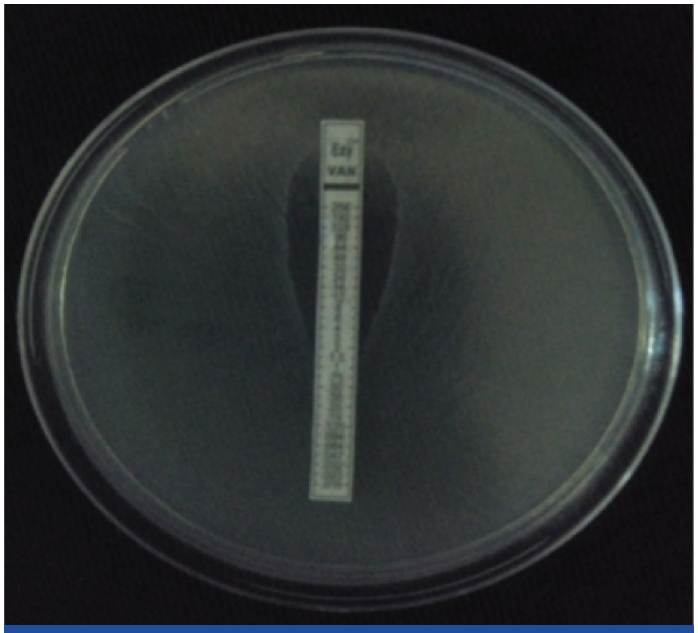
Many reports are available in the literature regarding the identification of vancomycin-resistant *Enterococci* (VRE) using conventional microbiological methods, which require time, resource and space. These standard methods are labour-intensive and require 48-72 h to give the result. Therefore, management of VRE infection relies on rapid and sensitive detection [6].

Chromogenic media are increasingly used as versatile tools in early differentiation and identification of VRE from clinical samples [7]. The present study was undertaken to evaluate the two different chromogenic media, CHROMagar™ VRE (France) and Hicrome VRE (Himedia, India) in detecting VRE in comparison with E- test (Himedia, India).

MATERIALS AND METHODS

The present study was carried out in the Department of Microbiology, Adichunchanagiri Institute of Medical Sciences, BG Nagara from June 2013 to May 2014. The ethical committee of the institution granted approval for the study. Out of 4489 clinical samples screened, 100 were *Enterococci*. Isolates were identified and speciated. Further confirmation was done using Group D antisera (Histrep, Himedia India) and CHROMagar™ orientation agar (CHROMagar France).

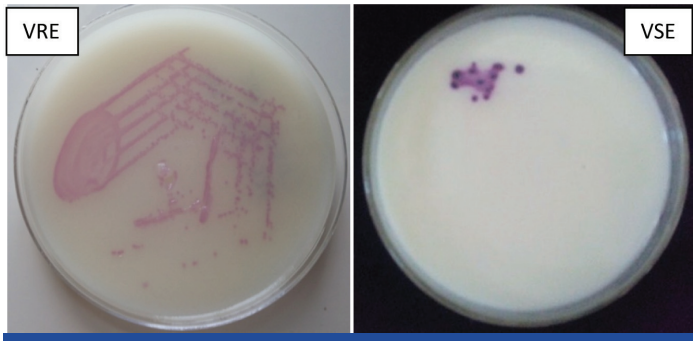
The minimum inhibitory concentration (MIC) for vancomycin is determined by E-test as shown in [Table/Fig-1] (0.016-256 µg/ml). The MIC ≥ 32µg/ml is considered as VRE based on the CLSI guidelines [8].



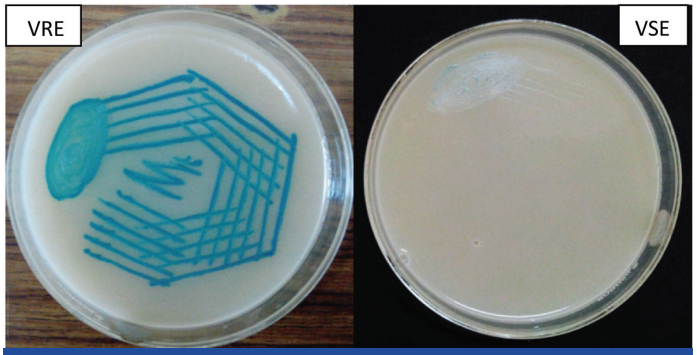
[Table/Fig-1]: Vancomycin E-test showing MIC of *Enterococci*

CHROMagar™VRE and Hicrome VRE were inoculated and incubated aerobically at 37°C. After 48 h of incubation, growth of *Enterococci* on these chromogenic media indicates VRE. On CHROMagar™VRE, used for the detection of Van A and Van B type transmissible resistance; vancomycin resistant *E.faecalis/E. faecium* produce pink to mauve coloured colonies, *E.gallinarum* and *E.casseliflavus* resistant to vancomycin produced blue coloured colonies and other *Enterococci* were inhibited [Table/Fig-2]. On Hicrome VRE agar, vancomycin resistant *E.faecalis* produced bluish green colonies and others were inhibited [Table/Fig-3].

E.faecalis ATCC 29212 and E.faecalis ATCC 51299 were used as susceptible and resistant control strains respectively. Identification of VRE by E- test was considered as reference method.



[Table/Fig-2]: CHROMagar™ showing VRE positive control and negative isolate



[Table/Fig-3]: Hicrome VRE showing VRE positive control and negative isolate.

Age Years	No of *Enteroocci*	Male	Female
0-20	10	7	3
21-40	44	15	29
41-60	29	12	17
≥ 61	17	9	8
Total	100	43	57

[Table/Fig-4]: Prevalence of *Enterococci* in relation to age and sex

Specimen	Total no	*E.faecalis*	*E.faecium*	*E.gallinarum*	*Enterococci* (%)
Urine	1682	31	28	4	63(3.75)
Exudates	716	15	7	0	22(3.07)
Sputum	434	3	2	1	6(1.39)
Blood	943	1	3	0	4(0.42)
Vaginal swab	570	2	1	0	3(1.05)
Body fluids	144	1	1	0	2(1.39)
Total	4489	53	42	5	100(2.2)

[Table/Fig-5]: *Enterococci species* isolated in relation to various clinical samples

Species	Ampicillin	Penicillin	Ciprofloxacin	Piperacillin	Gentamicin 120 µg	Vancomycin	Lenozolid	Teicoplanin
E.faecalis No.53	64.2	39.4	22.64	45.28	41.51	79.25	100	66.04
E.faecium No.42	52.4	23.8	23.8	40.48	47.62	11.43	100	59.52
E.gallinarum No.05	100	80	00	60	60	40	100	80
100	61	35	22	44	45	74	100	64

[Table/Fig-6]: Antibiotic susceptibility pattern of *Enterococci* by KBDDM (%)

RESULTS

Out of 4489 clinical samples studied, 100 (2.2%) *Enterococci* were isolated. [Table/Fig-4]: shows the prevalence of *Enterococci* in relation to age and sex. [Table/Fig-5]: shows the distribution of Enterococcus species among the various clinical samples. [Table/Fig-6]: shows the antibiotic susceptibility pattern of *Enterococci* by KBDDM (%). [Table/Fig-7]: shows the Vancomycin MIC range of *Enterococci*. [Table/Fig-8]: shows the analysis of Chromogenic media with E-test. [Table/Fig-9]: Shows study of VRE as reported by various workers.

MIC range µg/ml	*E.faecalis*	*E.faecium*	*E.gallinarum*	Total (%)
≤ 1	20	26	2	48
>1-2	27	13	3	43
>2-4	06	3	0	09
>4-256	0	0	0	0
Total	53	42	05	100

[Table/Fig-7]: Vancomycin MIC range of *Enterococci* isolates.

Test	Positive	True positives	False positives	False negatives	True negatives	Sensitivity %	Specificity%
HiChrome agar	1	0	1	0	99	100	99
CHRO Magar™	1	0	1	0	99	100	99

[Table/Fig-8]: Analysis of Chromogenic media with E-test

DISCUSSION

Enterococci have attracted much attention in the recent times due to their increased recognition as a cause of nosocomial "super-infection" in patients receiving antimicrobial agents. *Enterococci* have clearly emerged as a medically important organism in outbreaks of many nosocomial infections. An organism once considered a harmless commensal residing in the intestine has emerged as a multiple drug resistant, virulent pathogen accounting for more hospital borne infection [9].

Enterococci were isolated in 2.22% of the total specimen screened whereas Sreeja reported 0.23% [10]. In India, incidence of *Enterococcal* infection is not thoroughly identified. *E. faecalis* is the most prevalent species cultured from humans accounting for 80-90% of clinical isolates in other studies [11].

In the present study, maximum number of *Enterococci* were isolated from urine samples (3.75%) which is higher than Taneja (1.5%) [3] and Sreeja (1.58%) [10]. *Enterococci* were isolated from 3.07% of exudates and 0.42% of blood, whereas Sreeja has a higher rate of isolation from exudates (4.47%) and blood (1.1%) [10].

In the present study, *E.faecalis* were isolated more (53%), which is in comparison with other studies [3,6,11,12].

In the present study, *E. faecalis* and *E. faecium* showed almost similar sensitivity to various antibiotics by KBDDM. Resistance to various antibiotics among clinical strains of *Enterococci* species is a progressive and widely spreading problem. In the present study 55% of the isolate showed high level gentamycin resistance. Similar finding was found in Goshal, whereas Agarwal has reported 7.8% [12,13]. The higher rate of resistance in the present study is attributed to wider usage of broad spectrum antibiotics as this Hospital being a tertiary care Centre.

In the present study, 100% isolates were sensitive to linezolid is in comparison with the report of Gupta and Padmasini [14,15]. Linezolid was the first oxazolidinone to be available for clinical use in 2000. It has activity against both *E. faecium* and *E. faecalis*. Another advantage is that it can be administered both intravenously and orally [5]. The pattern of teicoplanin sensitivity in this study correlated with Gupta by disc diffusion method [14].

Vancomycin showed 26% of resistance by KBDDM as shown in the [Table/Fig-4]. By E-test, all *Enterococci* were sensitive to Vancomycin with MIC <4µg/ml. This proves the inaccuracy of KBDDM in detecting the susceptibility to vancomycin. Others have reported varying percentage of VRE in their studies which is shown in the [Table/Fig-9] [2,3,14-17].

	Enterococci studied	VRE %	*E.faecalis* %	*E.faecium* %	Others %
Vijaya D	100	0	0	0	0
Padmashini [15]	43	4.6%	72.8%	16.3%	6.9%
Vidyalakshmi [16]	600	4	0	100	0
Baragundi Mahesh [11]	120	7.5	22.2	44.44	33.33
Gupta [17]	100	2	50	50	0
Neelam Taneja [3]	144	5.55	12.5	62.5	25
Purva Mathur [2]	444	1.12	100	0	0

[Table/Fig-9]: Study of VRE as reported by various workers

Risk factor for VRE is from exposure to VRE positive patients and lengthy hospital stay. Organ transplantation and hemodialysis patients form the high risk groups, mostly by stool of patient contaminating the environment. Outbreak of VRE can occur from fabric sheets and transferred by staffs' hands. Vancomycin resistant *Enterococci* have been shown to be capable of surviving on dry surfaces in the hospital for upto four months [18].

In the present study, CHROMagar™ VRE and Hicrome VRE showed sensitivity of 100% and specificity of 99%, whereas, Llacsahuanga reported sensitivity of 98.2% and specificity of 96.5% and Hajia reported 100% sensitivity and specificity for CHROMagar™VRE correlating with the present study [7,19]. Jenkins showed sensitivity and specificity of 98% and 95% respectively using a different Chromogenic media [20].

Conventional E-test relies on isolation of the organisms as a first step, then identification of its resistance to the vancomycin on 3rd or 4th day. Therefore, rapid, sensitive and inexpensive methods for detection of VRE are needed. Chromogenic media incorporating Chromogenic enzyme substrates and antimicrobial agents have become available for detection of VRE. E-test cannot be performed directly on clinical specimens, whereas Chromogenic media can be used. Another advantage of CHROMagar, is it can be used for routine screening and identification of VRE in hospitalized patients, thereby routine surveillance will prevent the spread of VRE among patients [7].

Advantage of chromogenic media is that it is rapid, simple, easy to perform, cost effective compared to time consuming, laborious and technically demanding conventional method.

CONCLUSION

Enterococcus infection should be of concern for health care institution. The early detection of VRE will help in the effective therapy and infection control measures, to prevent the spread of VRE. Chromogenic media has higher sensitivity and specificity in the detection of VRE and can be incorporated in the routine screening. The present study indicates that Chrom agar (Hicrome & CHROMagar™VRE) in detection of VRE is simple, rapid, easy to perform and cost-effective compared to conventional E- test.

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REFERENCES

[1] Uttley AHC, Collins CH, Naidoo J, George RC. Vancomycin resistant *Enterococci*. *Lancet.* 1988;1:57-58.
[2] Mathur P, Kapil A, Chandra R, Sharma P, Das B. Antimicrobial resistance in *Enterococcus faecalis* at a tertiary care centre of Northern India. *Indian J Med Res.* 2003;118:25-28.
[3] Taneja N, Rani P, Emmanuel R, Sharma M. Significance of vancomycin resistant *Enterococci* from urinary specimens at a tertiary care center in Northern India. *Indian J Med Res.* 2004;119:72-74.
[4] Kaur N, Chaudhary U, Aggarwal R, Bala K. Emergence of VRE and their antimicrobial sensitivity pattern in a tertiary care teaching hospital. *J Med Biol Sci.* 2009;8:26-32.
[5] Praharaj IS, Sujath, Subhash CP. Phenotypic and genotypic characterization of vancomycin resistant *Enterococcus* isolates from clinical specimens. *Indian J Med Res.* 2013;138:549-56.
[6] Tripathi A, Shukla SK, Singh A, Prasad KN. A new approach of real time polymerase chain reaction in the detection of vancomycin resistant *Enterococci* in detection of vancomycin resistant *Enterococci* and its comparison with other methods. *Indian J Med Microbiol.* 2013;31(1):47-52.
[7] Hajia M, Rahbar M, Zadeh MM. A novel method "CHROMagar" for screening vancomycin – resistant *Enterococci* (VRE) isolates. *African J Biotech.* 2012;11(41):9865-68.
[8] Clinical and Laboratory Standards Institute. Performance standards for Antimicrobial susceptibility supplement testing. *Twenty third informational supplement.* 2013;M100:33:1.
[9] Giridhara PM, Ravikumar KL, Umapathy BL. Review of virulence factors of Enterococcus: An emerging nosocomial pathogen. *Indian J Med Microbiol.* 2009;27(4):301-05.
[10] Sreeja S, Babu PRS, Prathab AG. The Prevalence and the characterization of the *Enterococcus* species from various clinical samples in a tertiary care hospital. *J Clin Diag Res.* 2012;6(9):1486-88.
[11] Nelson RRS, McGregor KF, Brown AR, Amyes GB, Young HK. Isolation and characterization of glycopeptides resistant enterocci from hospitalized patients over a 30-month period. *J Clin Microbiol.* 2000;38:2112-16.
[12] Ghoshal U, Garg A, Tiwari DP, Ayyagari A. Emerging vancomycin resistance in *Enterococci* in India. *Indian J Pathol Microbiol.* 2006;49(4):620-22.
[13] Agarwal VA, Jain YI, Pathak AA. Concomittant high level resistance to penicillin and aminoglycosides in *Enterococci* at Nagpur, Central India. *Indian J Med Microbiol.* 1999;17:85-87.
[14] Gupta V, Singla N. Speciation and antimicrobial susceptibility pattern of *Enterococci* from a tertiary health care center of North India. *J Clin Diag Res.* 2007;1(5):385-89.
[15] Padmasini E, Padmaraj R, Ramesh RR. Detection of vancomycin resistant *Enterococci* with vanA genotype in clinical isolates from a tertiary care center. *Indian J Med Microbiol.* 2014;32(1):89-101.
[16] Mahesh BC, Sonth SB, Solabannavar SS, Chidanand SP, Yemul V. Species prevalence and antimicrobial resistance pattern of *Enterococcal* isolates in a tertiary health care center. *J Clin Diag Res.* 2010;(4):3405-09.
[17] Vidyalakshmi PR, Gopalakrishnan R, Ramasubramanian V, Ghafur KA, Nambi PS, Thirunarayana MA. Clinical, epidemiological and microbiological profile of patients with vancomycin-resistant *Enterococci* from a Tertiary Care Hospital. *J Global Infect Dis.* 2012;4(2):137-38.
[18] Ramadhan AA, Hegedus E. Survivability of vancomycin resistant *Enterococci* and fitness cost of vancomycin resistance acquisition. *J Clin Pathol.* 2005;58:744-46.
[19] Llacsahuanga HP, Top J, Heynemann JW, Lutticken R, Haase G. Comparison of two Chromogenic media for selective isolation of Vancomycin Resistant *Enterococci* form stool specimens. *J Clin Microbiol.* 2009;47(12):4113-16.
[20] Jenkins SG, Raskoshina L, Schuetz AN. Comparison of performance of the novel Chromogenic spectra VRE agar to that of Bile esculin azide agar and Campylobacter agars for the detection of Vancomycin-Resistant *Enterococci* in fecal samples. *J Clin Microbiol.* 2011;49(11):3947-49.

PARTICULARS OF CONTRIBUTORS:
1. Professor and Head of Depratment, Department of Microbiology, AIMS, B.G.Nagar, Karnataka, India.
2. Assistant Professor, Department of Microbiology, AIMS, B.G.Nagar, Karnataka, India.
3. Post Graduate, Department of Microbiology, AIMS, B.G.Nagar, Karnataka, India.
4. Post Graduate, Department of Microbiology, AIMS, B.G.Nagar, Karnataka, India.
5. Post Graduate, Department of Microbiology, AIMS, B.G.Nagar, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Vijaya D,
Professor & Head of Department, Department of Microbiology, AIMS, B.G. Nagara, Karnataka-571448, India.
Phone: +91-94820 09120, E-mail: vijayadanand @ rediffmail.com

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