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RESEARCH ARTICLE

COMPARATIVE EVALUATION OF CHROMOGENIC AGAR WITH CONVENTIONAL E-TEST IN DETECTION OF VANCOMYCIN RESISTANT *ENTEROCOCCI*

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ABSTRACT

Background: The emergence of Vancomycin resistance *Enterococci* (VRE) is a cause of concern. They have become important nosocomial pathogen, which is very difficult to treat and control. So it is necessary to prevent their spread by early detection and monitor continuously such infection in hospital. **Objective**: The present study was undertaken to evaluate the two different methods - Epsilometer test (E- test) (Himedia, India) and HiCrome VRE Agar, Modified (Himedia M195, India) in detecting VRE. **Methods**: A total of 100 *Enterococci* isolated from various clinical specimens were speciated and antibiogram was done by using standard protocol. The efficacy of E- test (Himedia, India) and HiCrome VRE Agar, Modified (Himedia M195, India) in detecting VRE was evaluated .**Results**: In the present study, all the isolates were sensitive to Vancomycin. No VRE was isolated in my study. Hicrome VRE media and E-test showed sensitivity and specificity of 100%. **Conclusion:** In the present study VRE was not isolated. Identification of VRE by chromogenic media is rapid, easy to perform with reliable visual detection and cost effective compared to time consuming, technically demanding and expensive conventional method. Hence, HiCrome VRE Agar, Modified (Himedia M195, India) can be used as selective medium for isolation and differentiation of Vancomycin Resistant *Enterococcus faecalis* and *Enterococcus faecium* from clinical specimens.

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INTRODUCTION

Enterococci are one of the emerging nosocomial infections. They form the part of normal flora of intestinal tract of both human and animals (Winn, 2006). In 1988, Uttley *et al* were the first to report the isolation of vancomycin resistant *E.faecalis* and *E.faecium* in England. Shortly after the first isolates of vancomycin resistant *Enterococci* (VRE) were reported by investigators in the United Kingdom and France; similar strains were detected in hospitals located in the eastern half of the United States (Uttely, 1998). In 1990, Murray described the intrinsic and acquired resistance of *Enterococci* to antibiotics, methods of transfer of resistance and treatment of various diseases caused by *Enterococci.* He also noted strains exhibiting high level resistance to penicillin and ampicillin due to altered penicillin binding proteins strains producing β- lactamases (Murray, 1990). *Enterococci* exhibit both intrinsic and acquired resistance to aminoglycoside and cephalosporin.

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The emergence of vancomycin resistant *Enterococci* (VRE) in addition to the increasing incidence of high level aminoglycoside resistance (HLAR), presents a serious challenge for clinicians treating the patients with infections due to *Enterococci* (Winn, 2006; Murray, 1990; Sood, 2008). There are many studies available regarding the identification of Vancomycin resistant *Enterococci* (VRE) using conventional methods, which require trained workers, resources and time consuming to give the result. Hence, rapid and sensitive method is necessary for detection of VRE and its management (Tripathi, 2013). In recent days, Chromogenic media are increasingly used as tool in early identification of VRE from clinical specimens (Hajia, 2012). The present study was undertaken to evaluate the two different methods Epsilometer test (E-test) (Himedia, India) and HiCrome VRE Agar, Modified (Himedia M195, India) in detecting VRE.

MATERIALS AND METHODS

A total of 100 isolates from the clinical specimens like urine, pus, blood and body fluids were processed in the department of Microbiology, Adichunchanagiri Institute of Medical Sciences,

B.G.Nagara, for a period of one and half years. Ethical committee clearance was taken from the institution. The isolates were speciated and antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method. Confirmation of vancomycin susceptibility was done by the E-test and HiCrome VRE Agar, Modified (Himedia M195, India). The minimum inhibitory concentration (MIC) for vancomycin is determined by E-test. According to CLSI guidelines vancomycin MIC values were interpreted as follows:

- ≤ 4 µg/mL - sensitive
- 8-16 µg/mL - intermediate
- ≥32 µg/mL - resistant

Enterococcus with Vancomycin MIC of ≥32 µg/mL was taken as VRE (Clinical and Laboratory Standards Institute, 2013). HiCrome VRE Agar, Modified (Himedia M195, India) was inoculated directly by isolated colony prepared as a liquid suspension approximately equivalent to 0.5 Mc Farland turbidity and incubated aerobically at 37˚C.After 24 hours, media was observed for type of growth. *Enterococcus faecium* (VRE) ferments arabinose and cleaves the substrate thereby producing green colonies with yellow background. *Enterococcus faecalis* (VRE) does not ferment arabinose thereby producing blue colonies due to cleavage of chromogenic substrate. [Fig-2]. *E.faecalis ATCC 29212, E.faecalis (*VRE*) ATCC 51299 and E.faecium* (VRE) ATCC 700221 were used as control strains.

RESULTS

Out of 100 *Enterococcal* isolates, 20% of isolates showed an intermediate sensitivity to vancomycin by the Kirby Bauer disc diffusion method. These isolates were sensitive to vancomycin; with a MIC of less than 4µg/ml which was determined by E-test [Table1]. Vancomycin MIC range of *Enterococci* are shown in table 2. Table 3 shows the analysis of Chromogenic media; HiCrome VRE Agar, Modified (Himedia M195, India) with E-test.

DISCUSSION

Enterococci are commensals of the gastrointestinal tract of human beings. Over the past two decades they have become important nosocomial pathogens probably due to inherent resistance to antibiotics (cephalosporins), ability to adhere to

Table 1. Vancomycin susceptibility testing

Sensitivity pattern	Kirby- Bauer disc diffusion method (%)	E-test (%)
Sensitive	80	100
Intermediate Sensitive	20	0
Resistant	0	0

Table 2. Vancomycin MIC ranges of *Enterococcal* isolates by E-test

MIC range µg/mL	*E.feacalis*	*E.faecium*	Total (%)
≤4	74	26	100
8-16	0	0	0
≥32	0	0	0
Total	74	26	100

Table 3. Analysis of Chromogenic media with E-test

Test	positive	True positive	False positive	False negative	True negative	Sensitivity (%)	Specificity (%)
E test	0	0	0	0	100	100	100
Hicrome VRE agar, Modified	0	0	0	0	100	100	100

Figure 1. Vancomycin E-test showing MIC of *Enterococci*

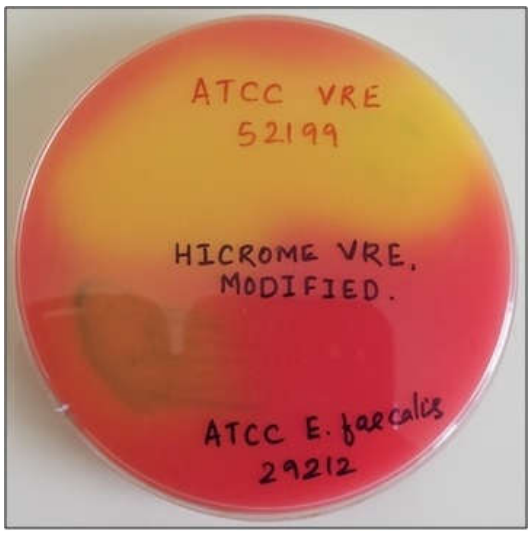

Figure 2. HiCrome VRE agar, Modified Himedia M195

indwelling medical devices, and ability to survive adverse environmental conditions (Qamer, 2003). The emergence of Vancomycin resistance *Enterococci* (VRE) is a cause of concern, since they are very difficult to treat and control (Desai, 2001). In the present study, 20% of *Enterococcal* isolates showed intermediate resistance by Kirby Bauer disc diffusion method as shown in the Table 1. By E-test, all *Enterococcal* isolates were sensitive to Vancomycin with MIC less than 4µg/ml. This shows the inaccuracy of Kirby Bauer disc diffusion method in detecting the susceptibility to vancomycin. In this study, all the isolates were sensitive to Vancomycin, where as Mathur P *et al* (2003) reported 1% of VRE, Ranghdale VA *et al* (2008) reported 11.2 % of VRE and Parameswarappa J *et al* (2013) reported 36% of VRE (Mathur, 2003; Ranghdale, 2008 and Parameswarappa, 2013). No VRE was isolated in my study, due to less sample size. In the present study, HiCrome VRE Agar, Modified (Himedia M195, India) showed sensitivity of 100% and specificity of 100%, similar to Hajia *et al* (2012) who reported 100% sensitivity and specificity, Jenkins *et al* (2011) showed sensitivity and specificity of 98% and 95% respectively using a different Chromogenic media (Hajia, 2012 and Jenkins, 2011). Conventional E-test depends on time consuming method, which involves detection of organism in pathological specimens by inoculating to one or more culture media followed by identification of its resistance to the vancomycin on 3rd or 4th day by using Muller - Hinton agar. Hence, rapid, sensitive, accurate and cost efficient methods for detection of VRE are required. Chromogenic substrates have proved to be a powerful tool in the identification of microorganisms due to the detection of specific enzymes produced by the target microorganism. These enzymes cleave the chromogenic substrate that points up the microorganism by color differentiation of the grown bacterial colonies. Chromogenic media incorporating Chromogenic enzyme substrates and antimicrobial agents have become available for detection of VRE. Chromogenic media (HiCrome VRE Agar, Modified Himedia M195) is available as dehydrated media or in ready-to-use formats, which can be used for routine screening and identification of VRE in hospitalized patients, thereby routine supervision will prevent the spread of VRE among patients (Hajia, 2012). Further it helps in its speciation and can be used directly for clinical specimens whereas E-test cannot be performed for the same, as it requires time lag methods. Thus, chromogenic media is simple, rapid, easy to perform with reliable visual detection and cost effective compared to time consuming, increased work load and technically demanding conventional method.

Conclusion

Enterococci are the important nosocomial pathogens, with the emergence of VRE it has become one of the major threat in healthcare setting. The early detection of VRE using proper methods will help in the effective therapy and preventing its emergence and spread. Chromogenic media has higher accuracy in the detection of VRE and can be integrated in the routine screening. The present study suggests that HiCrome VRE Agar, Modified (Himedia M195) offers an excellent and time saving method for the reliable detection of VRE and its speciation as opposed to conventional E-test.

Conflict of Interest

There is no conflict of interest.

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