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ORIGINAL ARTICLE

Evaluation of chromogenic VRE medium versus Conventional Vancomycin E test in Detection of Vancomycin Resistant Enterococci

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ABSTRACT

Background*: Enterococci have become resistant to a wide range of antibiotics which include glycopeptides like vancomycin. The rapid increase of vancomycin resistance enterococci (VRE) compromises physicians to treat infections caused by these strains as the therapeutic options for VRE infections are very limited.* ***Objectives****: To evaluate the efficacy of chromogenic VRE medium in detection and identification of vancomycin resistant enterococci.* ***Methodology****: the present study was conducted in Al Abbassia Fever Hospital, El Sayed Galal Hospital, Tanta University Hospital and Kafr El Sheikh General Hospital. Sixty enterococcal isolates were collected (thirty vancomycin susceptible, eight vancomycin intermediate resistant and twenty two vancomycin resistant isolates). Antibiotic susceptibility pattern for enterococcal isolates was done using disc diffusion method, chromogenic medium and MIC of vancomycin for vancomycin resistant and intermediate resistant isolates was determined by E test.* ***Results****: The most common isolated vancomycin resistant and intermediate resistant species was E. faecium (53.1%) followed by E. faecalis (40.6%). The highest rate of isolation of VRE was from urine (50.0%). VRE isolates were mostly recovered from ICUs (45.5), 41.7%, of the collected isolates were vancomycin resistant by disc diffusion method. Regarding E-test, out of fourty two vancomycin resistant and intermediate resistant by disc diffusion method 52.4% were resistant and 19% were intermediate resistant. HiChrome VRE had 100.0% sensitivity and 83.3% specificity in identifying vancomycin resistant enterococcal isolates.* ***Conclusion:*** *We can depend on chromogenic media in both detection and identification of vancomycin resistant enterococci.*

INTRODUCTION

Vancomycin-resistant enterococci (VRE) have emerged as important pathogens in many health care facilities. VRE may lead to extra intestinal infections, such as bacteremia and peritonitis[1]. Prevalence rates of VRE associated with serious clinical infections have increased worldwide over the past fifteen years [2]. An obstacle to control of the spread of VRE is the large, unrecognized cohort of patients with gastro intestinal VRE colonization[3]. Therefore, prompt accurate identification of patients with VRE is imperative. Thus to prevent further spread active control measures are increasingly being implemented in hospitals. A cornerstone of those control measures is the detection of non infected but gut-colonized patients that might serve as a source of the spread of VRE[4]. The most essential predisposing risk factor for infections brought about by VRE is the abuse or misuse of antibiotics. In patients colonized with low quantities of glycopeptide resistant Gram positive bacteria, antibiotic may permit overgrowth of these microbes with expanded danger of infection [5]. Detection of VRE colonization relied on culture techniques using selective/differential media [6]. Various chromogenic VRE agars appear promising for use in VRE stool screening [7]. Chromogenic VRE media can reduce turn around time to results through early visual colony identification of VRE direct from clinical samples [8]. Furthermore, molecular VRE screening methods decrease the time to identification but are costly. However, culture remains the screening method of choice for VRE stool screening [9]. Furthermore, chromogenic media are increasingly used as versatile tools in early differentiation and identification of VRE from clinical samples[8]

The aim of this study to evaluate the efficacy of chromogenic VRE medium in comparison to conventional vancomycin E test in detection and identification of vancomycin resistant enterococci.

METHODLOGY

The present study was conducted in Al Abassia Fever Hospital, El Sayed Galal Hospital, Tanta University Hospital and Kafr El Sheikh General Hospital during the period from February 2016 to

November 2016. Sixty isolates were collected from (38 males and 22 females) from different surgical and medical departments and ICUs. Their ages ranged from 15 years to 92 years. Twenty six isolates were obtained from urine samples while eighteen, eight, six, one and one isolates were obtained from wound, blood, endotracheal aspirate, ascitic fluid and cerebrospinal fluid respectively.

Confirmation of enterococcal isolates identification:

This was done by blackening around the colonies while culturing on bile esculin agar, Gram stained smear was done shows Gram positive cocci arranged singly, in pairs or short chains catalase negative.

Antibiotic susceptibility for enterococcal isolates by disc diffusion

Antimicrobial susceptibility testing was carried out according to modified Kirby-Bauer sensitivity testing technique [10]. Antibiotic discs used

Vancomycin (VA30 µg), Ampicillin (AMP10) , Chloramphenicol (C30), Ciprofloxacin (Cip5), Erythromycin (E15), Nitrofurantoin (F300), Penicillin G(P10), Rifampicin (RD5) , Levofloxacin(LEV5) and Norfloxacin (NOR10)

Determination of the minimum inhibitory concentration (MIC) for vancomycin by E-test:

The MIC ≥ 32µg/ml is considered as VRE based on the CLSI guidelines [11].

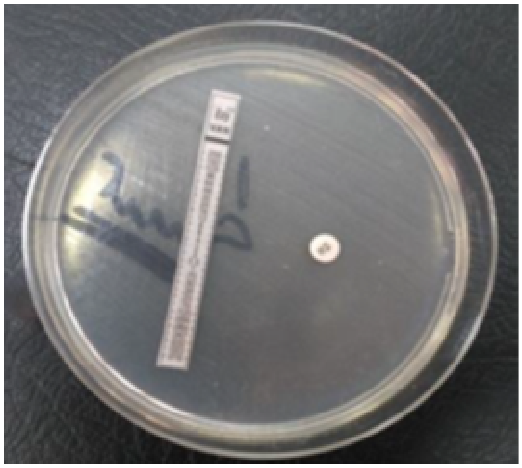

Fig. 1: Vancomycin resistant enterococcal isolate by E.test

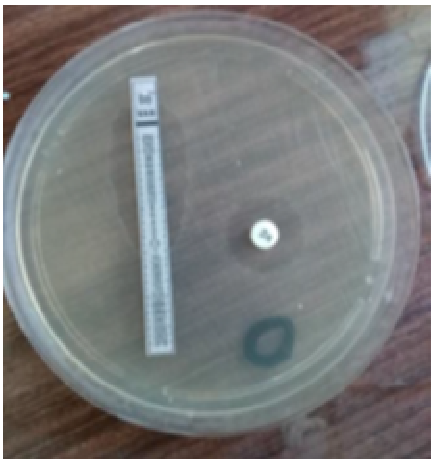

Fig. 2: Vancomycin sensitive enterococcal isolate by E.test.

The validity of the vancomycin E test strips together with vancomycin disks was first evaluated using ATCC 700221 and ATCC 29212 reference strains.

Culturing all collected isolates on HiChrome VRE agar modified plates

HiChrome VRE agar modified (patents pending) consists of a rich nutritive base including a variety of peptones. It also contains chromogenic mixture and a mixture of antibiotics including vancomycin (8 mg/l) which enable specific and selective growth of VRE and direct detection and the differentiation of *Enterococcus faecium (E. faecium)* and *Enterococcus faecalis (E. faecalis* through the characteristic color of colonies.

Enterococcus species possess the enzyme β-glucosidase which cleaves the chromogenic substrate in the medium to produce blue coloured colonies, *E. faecium* ferments arabinose and cleaves the substrate thereby producing green colonies with yellow background. *E.faecalis* does not ferment arabinose thereby producing blue colonies due to cleavage of chromogenic substrate.

Typical colonies of *E. faecium* and *E. faecalis* with acquired vancomycin resistance (VRE) were:

- A green colour with yellow background: *E. faecium* species
- A blue color: *E. faecalis* species

N.B: The reference control strains were the first isolates to be cultured on media to check its validity.

Fig. 3: Isolates of *Enterococcus faecium* (blue colonies)

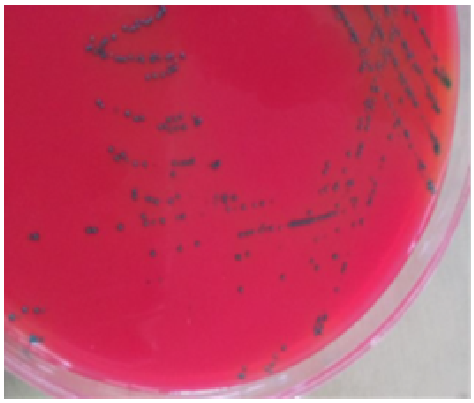

Fig. 4: *E.faecalis* on HiChrome VRE agar (Green colonies with yellowish background)

Statistical analysis of the data:

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0**.** Qualitative data were described using number and percent. Significance of the obtained results was judged at the 5% level.

The used tests were

1 - Chi-square test

For categorical variables, to compare between different groups

2 - Fisher's Exact or Monte Carlo correction

Correction for chi-square when more than 20% of the cells have expected count.

RESULTS

The current study was conducted on 60 enterococcal isolates (22 vancomycin resistant isolates, 8 vancomycin intermediate resistant and 30 vancomycin susceptible isolates) plus 2 vancomycin control strains *VRE.faecium* and *VSE.faecalis* named (ATCC 700221 and ATCC 29212) respectively. Thirty seven stains were obtained from male patients and twenty three from female patients. Their ages ranged from 5 to 92 years. These isolates were first identified to genus level then the sensitivity of these isolates to different antibiotics were tested by disc diffusion method. The results obtained for vancomycin sensitivity by disc diffusion method were then checked by E.test. All isolates were cultured on HiChrome VRE agar modified to test its sensitivity in comparison to E. test in detection of VRE and detection of *VRE faecium*and *VRE faecalis.*

Table 1: Distribution of enterococcal isolates and VRE isolates in different hospital departments:

Department	***Enterococcal isolates (VSE, VIE) (n=38)***		***VRE isolates (n= 22)***		χ^2	***p***
	No.	%	No.	%		
ICU	20	52.6	10	45.5	0.311	0.856
Surgical wards	11	28.9	7	31.8		
Medical wards	7	18.4	5	22.7		

Table (1) shows that that high percentage of VRE (45.5%) were obtained from ICUs

Table 2: Distribution of enterococcal isolates and VRE isolates according to type of the specimen

Type of specimen	***Enterococcal Isolates (VSE,VIE) (n=38)***		***VRE isolates (n=22)***		χ^2	^{MC}p
	No.	**%**	**No.**	**%**		
Urine	15	39.5	11	50	2.026	0.944
Blood	6	15.8	2	9.1		
Wound	11	28.9	7	31.8		
Endotracheal aspirate	4	10.5	2	9.1		
Ascitic fluid	1	2.6	0	0.0		
CSF	1	2.6	0	0.0		

Table (2): shows that that high percentage of VRE (50%) were obtained from urine sample

Table (3): Antibiotic sensitivity of enterococcal (n=60) isolates to vancomycin by disc diffusion method according to CLSI guidelines

Vancomycin susceptibility	*Zone of inhibition (mm)*	*No. of isolates (n=60)*	*(%)*
Susceptible	≥ 17	18	**30%**
Intermediate	15 – 16	**17**	**28.3%**
Resistant	≤14	**25**	**41.7%**

Table (3): shows that twenty five isolate (41.7%) of the sixty enterococcal isolates were VRE by disc diffusion

Table 4: Determination of VancomycinMIC by E-test among VRE isolates detected by disc diffusion

Vancomycin susceptibility	*Vancomycin concentration (µg/ml)*	*No. of isolates (n=42)*	*(%)*
Susceptible	≤ 4	12	28.6**%**
Intermediate	8 – 16	8	19**%**
Resistant	≥ 32	22	52.4**%**

Table (4): shows that Twenty two isolates (52.4%) of the enterococcal isolates were VRE by E test.

Table 5: Detection of Vancomycin resistance among isolated enterococci by HiChrome VRE agar modified

HiChrome VRE Agar	*No. of isolates (n=60)*	*(%)*
Susceptible	28	46.7
Resistant	32	53.3

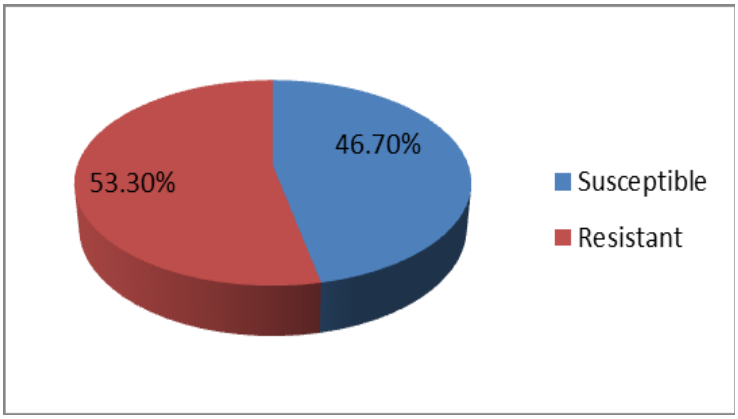

Fig. 5: Shows that out of 60 isolates 28 isolates (46.7%) were inhibited when cultured on HiChrome VRE agar modified while the rest 32 (53.3%) isolates show growth on it

Table 6: Sensitivity and specificity of HiChrome VRE agar and disk diffusion methods compared with E- test as a reference method

	E- test for vancomycin as a reference method				*Sensitivity*	*Specificity*	*PPV*	*NPV*	*Accuracy*
	Vancomycin susceptible (n=12)		**Vancomycin resistant (n=22)**						
	No.	**%**	**No.**	**%**					
HiChrome VRE Agar modified									
Vancomycin susceptible	10	83.3	0	0	100.0	83.3	91.7	100	94.1
Vancomycin resistant	2	16.7	22	100					
Disk for Vancomycin									
Vancomycin intermediate	8	66.7	3	13.6	86.4	66.7	82.6	72.7	79.4
Vancomycin resistant	4	33.3	19	86.4					

Table 6: shows that HiChrome VRE agar modified had 100% sensitivity, 83.3% specificity, 91.7% PPV, 100% NPV and 94.1% accuracy while disc diffusion had only 86.4% sensitivity and 66.7% specificity 82.6% PPV, 72.7% NPV and 79.4% accuracy in detection of vancomycin resistant enterococcal isolates.

DISCUSSION

Enterococci are members of the intestinal flora and are the most common aerobic Gram-positive cocci found in the large intestine of humans. They are the third most common cause of bacteraemia in the United States, as nosocomial pathogens. *E. faecalis* and *E. faecium* are the most common species encountered in the majority of human enterococcal infections, *E. faecium* is more likely to acquire resistance to glycopeptides, *E. faecalis* is linked more frequently to serious disease. [12] Colonized patients are a potential source of spread of organism to the hands of health care workers, the environment, and other patients. VRE colonized patients in hematology and oncology departments are at high risk of infection [13] .Regarding vancomycin resistant enterococci, the major problem is that the *vanA* and *vanB* vancomycin resistance determinants are transferable and can be transferred to other pathogens such as methicillin resistant *Staphylococcus aureus*, resulting in emergence of a highly dangerous pathogen difficult to treat with currently available antibiotics. Thus**,** reliable screening methods should be activated for early detection of VRE carriers and reduce the risk of VRE transmission in health-care settings[14]**.** Intensive care units are important source for the emergence and spread of antibiotic resistant pathogens because they house critically ill patients in confined environments where antibiotic use is extremely common. Furthermore, ICU patients and patients in oncology wards were found to be at increased risk of infection or colonization with VRE. This high presence of VRE in such wards could be explained by the intensive use of vancomycin[15]**.** As regards to our study results, we found that out of twenty two isolates ten were VRE isolates (45.5%), twenty out of thirty eight of enterococcal isolates other than VRE (52.6). were isolated from patients in the ICU department. That results clarified that VRE isolates are mostly isolated from ICUs and this agreed with a study that stated that the percentage of nosocomial infections caused by VRE increased from 0.3-7.9% which reflected an increase in the percentage of VRE infections in patients in ICU from 0.4-13.6% [16].

Enterococci having vancomycin resistance are being reported from different parts of the world with increasing frequency, although the epidemiology of these microorganisms varies widely in different geographical areas. During 1989-1997, the National Nosocomial Infections Surveillance System reported that the percentage of (VRE) in nosocomial infections increased from 0.4% to 23.2% among patients in intensive care units and from 0.3% to 15.4% among patients in noncritical care units. Since 1997, rates of vancomycin-resistant enterococci have continued to increase in both clinical settings [17]. Enterococci have become the second or third leading cause of nosocomial urinary tract infections (UTIs), wound infection and bacteremia in the United States. Enterococci is responsible for 16% of nosocomial UTIs and up to 10% of all UTIs what makes UTIs the most common infections encountered by enterococci [18]. In our study the highest rate of VRE isolated were from urine representing 50% of the isolates followed by wound representing 31.8%. Similar study concluded that the urinary tract was the most common site of entercoccal infection (53.7%), which often occurred after instrumentation of the patient's urinary tract followed by wound infection [19].The major reason for the survival of enterococcus in hospital environment is their intrinsic resistance to several commonly used antibiotics and perhaps more important their ability to acquire resistance to all currently available antibiotics, either by mutation or through the transfer of plasmids and transposons[20]**.** Some enterococci are intrinsically resistant to β-lactam antibiotics and many aminoglycosides while some of them acquired multidrug resistance to tetracycline, erythromycin, chloramphenicol and fluoroquinolones. [20]

In our study out of the 60 enterococcal isolates collected in our study 83.3% were resistant to penicillin G , 91.7% were resistant to ampicillin , 91.7% were resistant to erythromycin and 31.7 % were resistant to chloramphenicol. Similar results were obtained by a study that showed that enterococcal resistance to ampicillin was 100%. [21].

Our study showed that twenty five out sixty (41.7%) isolates were vancomycin resistant, seventeen out of sixty (28.3%) were vancomycin intermediate and eighteen (30%) were vancomycin susceptible. Regarding E-test, twenty two out of fourty two (52.4%) isolates were susceptible to vancomycin, eight out fourty isolates (19%) were intermediate and twelve out of fourty two isolates (28.6%) were resistant to vancomycin. When comparing different methods used for detection of vancomycin resistance, disk diffusion method demonstrated (86.4%) sensitivity and (66.7%) specificity when compared with E-test. Similar results were obtained by a study that showed (77.3%) sensitivity and (65%) specificity for disk diffusion in detection of resistance to vancomycin. So they concluded that the disk diffusion method is neither reliable nor satisfactory [22]. Therefore, a routine MIC monitoring of important antibiotics like vancomycin has to be done, before reporting it as resistant or intermediately sensitive [23]. For proper control of infections encountered by VRE strains, the rapid and

accurate detection of VRE colonization is essential. Conventional culture methods that depend on colony morphology, biochemical characteristics and antibiotic susceptibility testing may consume up to 5 days at the expense of patient management. Several chromogenic media have been developed recently to face this obstacle [12]. In our study, we tested the efficacy of chromogenic medium HimediaVRE in isolation and identification of vancomycin resistant enterococci. In the current study, out of 60 isolates 28 isolates were inhibited when cultured on HiChrome VRE agar modified while the rest 32 (53.3%) isolates show growth on it. Hi ChromeVRE agar (HIMEDIA-India) demonstrated (100%) sensitivity and (83.3%) specificity in detection of VRE. Our results agree with a study that found that the sensitivity of chromogenic media in detection of VRE was (96.9%) and specificity was (99.4%) [24]. Similar to another study that evaluated two types of chromogenic VRE media named (CHROMagarTM VRE and Hicrome VRE Performance) in detecting VRE in comparison with E-test and stated that those media have sensitivity of 100% and specificity of 99% [25].

CONCLUSION

The obtained results in this study indicated that HiChrome VRE agar method is an easy to use, cost-and time-effective procedure for the isolation of VRE. Furthermore, Chromogenic media may be a promising rapid method in detection of vancomycin resistant enterococci together with identification of isolated bacteria accurately to species level.

The increase in the rate of prevalence of the Enterococcus species and the emergence of vancomycin resistant enterococci, emphasize the importance of the implementation of appropriate infection control measures to limit the nosocomial spread of these enterococcal species in any nosocomial setting.

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