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Full Length Research Paper

A novel method "CHROMagar" for screening vancomycin-resistant enterococci (VRE) isolates

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Infection with vancomycin-resistant enterococci (VRE) is reported to be increasing and becoming a problem especially in health care systems with prolonged survival in the environment. In this study, we compared the performance characteristics of CHROMagar with conventional methods for detection of VRE in clinical urine specimens. A total of 7404 urine samples were entered in the study. All specimens were cultured by routine microbiological method and were simultaneously cultured in prepared CHROMagar plate for growth characteristics with positive and negative controls. Susceptibility tests were performed by disk diffusion method as recommended by Clinical Laboratory Standards Institute. We also used E-test minimum inhibitory concentration (MIC) for confirmation of VRE isolates. Total isolation rate was 22.19% of the tested specimens. *Enterococcus faecium* and *Enterococcus faecalis* were isolated in 10 (0.13%) and 72 (0.92%) cases, respectively while six were VRE belonging to admitted patients. Analyzed data of VRE cases revealed the results of all three applied methods. The antimicrobial susceptibility testing, E-test and CHROMagar were in agreement in all the six identified VRE cases. The obtained results in the present study indicate that CHROMagar method is easy to use, and is a cost- and time-effective procedure for the isolation of VRE especially in urine specimens.

Key words: Vancomycin-resistant enterococci (VRE), CHROMagar, admitted patients.

INTRODUCTION

Enterococci are normal flora of the intestinal tract in humans and animal. This organism may colonize in other anatomical sites of the body such as reparatory tract, vagina and biliary tract in healthy persons (Zhanel et al., 2003; Deshpande et al., 2007). *Enterococcus* species are Gram-positive and catalase -negative cocci. There are more than one dozen species of Enterococci; *Enterococcus faecalis* and *Enterococcus faecium* are the most important pathogens and account for approximately 85 to 90% and 5 to 10% of human infections respectively (Gin et al., 1996).*Enterococcus* species has been recognized as an important cause of hospital acquired infections in the mid 1970s. One reason for the rising pathohgenisity of the *Enterococci* may be due to the use of third generation cephalosporin, to which these organisms are intrinsically resistant (Swaminathan and Alangaden 2010). In 1988, the first vancomycin resistant enrococcus (VRE) was reported. VRE spread in many countries worldwide which caused significant nosocomial infections is a continuous problem especially in health care systems (Aznar et al., 2004). In a recent report of Centers for Disease Control (CDC), *Enterococcus* species was isolated from 12% nosocomial infection, and was the third most common pathogen isolated (NNIS, 2004; Furtado et al., 2006). Urinary tract infections (UTI) are the most common nosocomial infection caused by *Enterococci* in adults (Sonavan et al., 2008; Behroozi et al., 2010). The emergence VRE in Iran has revealed serious challenges for infection control committees in hospitals. Infections caused by VRE in Iran, like other countries, is associated with a high rate of mortality and morbidity. There are several reports regarding prevalence

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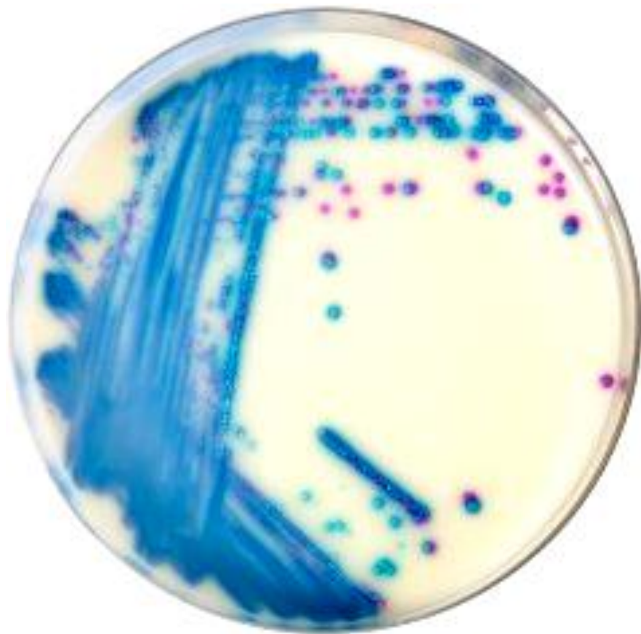

Figure 1. VRE colony in CHROMagar plate. VRE is revealed in pink color and the rest in blue color.

of VRE in Iran (Saifi et al., 2010; Fatholazadeh et al., 2006). However, isolation and rapid detection of VRE in clinical specimens is time consuming in microbiology laboratories. Introducing rapid methods such as using chromogenic media helps for rapid isolation and identification of VRE from clinical specimens such as urine (Peltroche-Llacsahuanga et al., 2009; Kallstrom et al., 2010).

Chromogenic media are increasingly being used as versatile tools in early differentiation and identification of bacterial isolates from clinical specimens such as urine. The aim of this study was to compare the performance characteristics of CHROMagar with conventional methods for detection of VRE in clinical urine specimens.

MATERIALS AND METHODS

Specimens

This retrospective study was carried out in summer 2010 at Milad Hospital. A total of 7404 urine samples consecutively collected by midstream method and/or catheter catch urine samples obtained from patients attending the various hospital wards, intensive care units (ICUs) and from other high-risk groups, such as oncology, transplant, and HIV patients, were used in the study, as well as collected samples from out-patients.

Culture

All specimens were cultured on blood and MacConkey agar. These media were prepared in the laboratory using commercially available dehydrated media and identified by recommended deferential tests (Merk, Co. Germany), and prepared according to the manufacturer's recommendations, then dispensed into plate and checked for sterility and performance. They were stored at 2 to 8°C till use.

All urine samples were simultaneously cultured on prepared CHROMagar plate for growth characteristics (CHROMagar Co, France) (Figure 1). Positive controls, VRE ATCC 700221 (pink to mauve) and two negative controls [*Escherichia coli* (ATCC 25922 as a no growth control] and *Enterococcus gallinarum* (ATCC 49573 as a blue colony) were used on each day of testing. The plates were incubated as recommended by the manufacturer. The cultures with no growth within 48 h or colonies with blue color had no further workup.

Antibiotic susceptibility testing

All isolates were identified to species level by routine bacteriological methods. Susceptibility testing was performed by disk diffusion method as recommended by Clinical Laboratory Standards Institute (CLSI). For the quality control of susceptibility tests, *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC29213, *E. faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC27853 strains were used. All urine isolates were tested for resistance to ampicillin, penicillin, tetracycline, norfloxacine, nitrofurantoin, and vancomycin (CLSI, 2006).

RESULTS AND DISCUSSION

A total of 7404 patients' specimens were received from both out-patients and admitted patients during the study period. Total isolation rate was 22.19%. *E. faecium* and *E. faecalis* were isolated in 10 (0.13%) and 72 (0.92%) cases, respectively from various urine specimens. Other isolated bacteria were *E. coli, Klebsiella pneumoniae, Klebsiella ozaenae, Proteus mirabilis, Klebsiella oxytoca, Enterobacter aerogenes, Enterobacter cloacae, Citrobacter koseri, Citrobacter freundii, Acinetobacter lwofii, A. bumani*, *P. aeroginusa, S. aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus,* Strep group B, non *E. faecium* and *E. faecalis* species. Candida species was causative agents in 6.41% of UTI cases (Table 1).

Six VRE out of 82 isolates were identified among the isolated *E. faecium* and *E. faecalis.* Therefore, the rate of VRE was 7.31% among these isolates belonging to admitted patients in our study. All the six VRE were identified by the antimicrobial susceptibility testing, E-test and CHROMagar, and had the same susceptibility pattern. The appearance of colony morphology and its color are presented in Figure 1.

Results of antimicrobial susceptibility testing for the six VRE isolates are shown in Table 2. All isolates were resistant to penicillin and tetracycline, while they were susceptible to nitrofurantoin.

Total rate of identified *E. faecium* and *E. faecalis* was 1.05% of all received specimens and 4.99% in isolated organisms. All isolated VRE by routine method were directly cultured in CHROMagar media causing more rapid results.

Enterococci, especially *E. faecium*, are known as

Table 1. List of isolated organisms.

Isolated organism	Number
E. coli	752
K. pneumoniae	87
Proteus mirabilis	7
K. oxytoca	11
K. ozaenae	7
E. aerogenes	12
E. cloacae	7
C. koseri	3
C. freundii	3
A. bumani	7
A. lwofii	3
P. aeroginusa	57
S. aureus	125
S. epidermidis	6
S. saprophyticus	11
Strep group B	92
Non *E. faecium* and *E. faecalis* species	22
Candida species	431
Total	1643

Table 2. Antimicrobial susceptibility results of VRE isolates.

Isolates	Am	RA	FM	P	V	TE	NOR
E. faecium No.1	R	R	S	R	R	R	R
E. faecium No.2	R	R	S	R	R	R	R
E. faecalis No.1	S	S	S	R	R	R	R
E. faecalis No.2	S	S	S	R	R	R	R
E. faecalis No.3	S	S	S	R	R	R	R
E. faecalis No.4	S	S	S	R	R	R	R

Am, ampicillin; P, penicillin; TE, tetracycline; NOR, norfloxacine; FM, nitrofurantoin; V, vancomycin; S, sensitive; R, resistant.

nosocomial pathogens, and are resistant to many antibiotics. Therefore, current treatment of choice is vancomycin, although its resistant rate is increasing to vancomycin. However, this finding was reported in a previous study (Rahbar et al., 2007). These organisms have been isolated from nearly all health care facilities, including monitoring devices, public toilet seats, and so many other medical equipments (Bonten et al., 1996, 2001). On the other hand, susceptible hosts are at high risk for VRE colonization, including those who are severely ill and those receiving multiple and prolonged courses of antimicrobial agents (Bonten et al., 1998).

Current method relies on the isolation of the organisms and provides pure culture at the first step, then identifies its resistance to the vancomycin in the second day. Therefore, it cannot be performed directly on the clinical specimens. Routine susceptibility test based on CLSI method was unreliable for detection of vancomycin resistance upon primary isolation, especially in low-level inducible resistance (Pendle et al., 2008). However, basic method for detecting VRE is the incorporation of esculin into the vancomycin containing base medium, which provides a presumptive identification at the genus level because all *Enterococci* hydrolyze esculin.

PCR is also applied to identify vancomycin resistant gene with high sensitivity and specificity (d`Azevedo et al., 2009; Seo et al., 2011). This method cannot detect VRE at species level and can be performed only in those laboratories that have the necessary facilities to run the test (d`Azevedo et al., 2009). Otherwise, using molecular methods does not have any cost benefit especially in developing countries.

Another advantage of the CHROMagar is in admitted patients where the routine screening and identification methods were used. At present, Centers for Disease Control (CDC) recommends the screening of stool or

rectal swab in health care setting in order to identify VRE because it is recommended to separate these VRE positive patients from other patients to reduced contamination rate and transfer of the organisms. Therefore, applying a rapid screening method can be very helpful in this regards (HICPAC, 1995).

It is probable that the use of rapid screening method based on chromogenic media can identify VRE within 24 h and provide reliable results for clinicians for the urine specimens; previous studies were mostly based on stool specimens.

Conclusion

The obtained results in this study indicate that CHROMagar method is an easy to use, cost-and time-effective procedure for the isolation of VRE especially in urine specimens.

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