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Original Research Article

Utility of bile esculin azide agar for screening of stool samples for vancomycin resistant enterococci from patients with gut colonization

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ABSTRACT

Background: Due to increased prevalence of vancomycin resistant enterococci (VRE) in hospital settings as an important nosocomial pathogen, microbiology laboratories should be prepared with test protocol for prompt detection and reporting of these resistant organisms. This helps in appropriate treatment of patients without delay and implementation of infection control measures in order to prevent spread of such infections. With this background present study was conducted to demonstrate utility of bile esculin azide agar with vancomycin (BEAV) for screening of enterococci for vancomycin drug resistance.
Methods: Over a period of one year 200 stool samples were collected from hospitalized patients in a tertiary care hospital. Samples were inoculated on bile esculin azide agar with vancomycin (6ug/ml) to screen for vancomycin drug resistance in enterococci isolated from stool samples. Vancomycin drug resistance was confirmed by agar dilution method.
Results: Out of 200 stool samples collected from hospitalized patients, 13 (6.5%) samples showed growth on bile esculin azide agar with vancomycin (6 µg/ml). Of these 13 isolates, 12 (92.3%) isolates were confirmed as VRE by agar dilution method and demonstrated minimum inhibitory concentration (MIC) of ≥32 µg/ml and all 12 isolates were identified as *E. faecium*. One (7.7%) isolate grown on BEAV was identified as *E. gallinarum* and showed MIC value of 8 µg/ml.
Conclusions: Present study recommends use of bile esculin azide agar with vancomycin (6 µg/ml) as a screening medium for isolation of VRE from stool samples which usually carries mixed commensal flora of gastrointestinal tract.

Keywords: Agar dilution method, Bile esculin azide agar with vancomycin, VRE

INTRODUCTION

Enterococcus species is one of the important cause of nosocomial infections worldwide. There is increased concern regarding emergence of multidrug resistant enterococci especially about vancomycin resistance.[1] Vancomycin is one of the last choice of antibiotic for treatment of multi drug resistant enterococci.[2]

As per guideline of Hospital Infection Control Practices Advisory Committee (HICPAC), there is recommendation for setting up laboratory protocol for rapid detection and reporting of VRE in health care set up. It is also advised to perform routine surveillance cultures of stool samples of patients to identify the VRE colonized patients which act as the main reservoir of VRE in hospitals. Identifying such reservoir helps in implementation of hospital infection control measures to prevent spread of such resistant bugs.[2]

It is difficult to perform surveillance cultures of stool samples due to the fact that stool sample carries normal

commensal flora of gastrointestinal tract. It is cumbersome to isolate and identify VRE from such mixed culture.[3] There is need for a selective and highly sensitive screening medium for isolation of VRE from such samples. With this background present study evaluated bile esculin azide agar (BEA) with vancomycin (6 µg/ml) for screening of stool samples from patients with VRE colonization.

METHODS

A prospective cross sectional study was conducted over a period of 1 year from January 2013-December 2013 in a tertiary care hospital in B. J. Medical College, Pune Maharashtra. Institutional Ethical committee approval was obtained for the study (reference number-BMC/IEC/Pharmac/D0313005-05). Two hundred stool samples were collected from patients admitted in medical and surgical units not suffering from any infection and having underlying risk factors like malignancy, nutropenia, hepatorenal insufficiency, hypoalbuminemia, diabetes mellitus, prolonged hospital stay, use of immunosuppressants history of antibiotic exposure.

Preparation of medium

A dehydrated bile esculin azide (BEA) agar medium (Himedia Pvt. Ltd.) was used to prepare the media. 56.65 grams of medium for 1000 ml of distilled water was added and autoclaved at 121°C for 15 minutes for sterilization of media as per instructions of manufacturer.[3] Once BEA medium reached around 45°C, sterile solution of vancomycin (6 µg/ml) was added to the medium and plates were prepared.[4]

Stool sample processing

Total 200 stool samples were collected from hospitalized patients and they were directly inoculated on BEAV agar plates in laboratory. Plates were incubated at 37°C for 18-24 hours. After incubation, if any growth was observed along with blackening of the surrounding medium (Figure 1) then gram stain was prepared from it.

Figure 1: BEAV agar showing growth of *Enterococcus species* with blackening of surrounding medium.

If gram stain showed gram positive cocci (Figure 2) then, identification was done by observing colony morphology (Figure 3) and using conventional microbial tests like catalase test, motility, 6.5% NaCl tolerance, pyrrolidonyl arylamisase (PYR), mannitol fermentation, arabinose fermentation, arginine hydrolysis.[5]

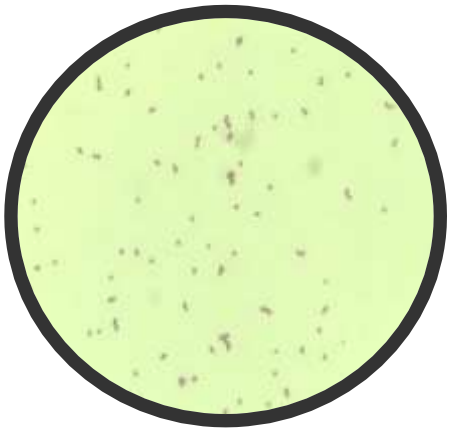

Figure 2: Gram stain of *Enterococcus species* showing oval to round gram positive cocci in pairs and short chains.

Figure 3: Blood agar showing colony morphology of *Enterococcus species.*

Vancomycin drug resistance confirmation

Agar dilution method was used for confirmation of vancomycin resistance in enterococci isolated on BEAV agar.[6,7] MIC values for vancomycin were noted and interpreted as per clinical laboratory standard institute (CLSI) guidelines. Vancomycin MIC value of ≥32 µg/ml was considered resistant, 8-16 µg/ml as intermediate resistant and ≤4ug/ml was considered sensitive. ATCC *E. fecalis* 29212 was used as a reference strain in the study (Figure 4).[6]

Microsoft excel was used for statistical analysis of the study results.

Figure 4: Agar dilution method for VRE.

RESULTS

Out of total 200 stool samples screened, 13 (6.5%) samples showed growth along with blackening of the medium on BEAV agar. By species level identification 12 (92.3%) out of 13 isolates were identified as *E. faecium* and one (7.7%) isolate was identified as *E. gallinarum* (Figure 5).

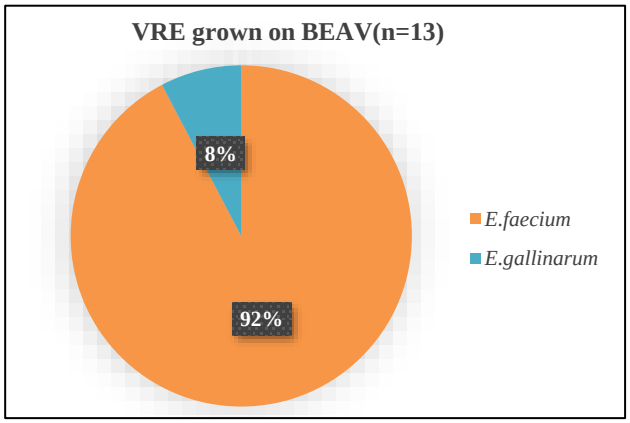

Figure 5: showing species distribution of enterococci isolated on BEAV.

Table 1: MIC distribution of VRE isolates.

VRE strains (n=13)	BEAV MIC values (µg/ml)	Agar dilution method MIC values (µg/ml)
***E. faecium* (12; 92.3%)**	≥6	64-256
***E. gallinarum* (1; 7.7%)**	≥6	8

Agar dilution method confirmed vancomycin drug resistance in all 12 isolates of *E. faecium*. Single isolate of *E. gallinarum* demonstrated intermediate drug resistance to vancomycin (Table 1). Age of the patients with VRE colonization ranged from 41-60 years out of which males were predominant.

DISCUSSION

Bile esculin hydrolysis is one of the important identification test for *Enterococcus species.* In bile esculin azide agar, black colour produced is due to esculin hydrolysis which releases esculetin that combines with iron in the medium to form a black coloured phenolic iron complex.[8]

Presence of azide in the medium inhibits growth of gram negative bacteria which are predominant bacterial flora of gut. This is the major advantage of using this medium. Other gram positive cocci like *Staphylococcus species* and diptheroides can grow on this medium but they can be easily distinguished by colony morphology, catalase test and Gram stain.[4] One more medium recommended for isolation of VRE is campylobacter blood agar with vancomycin.[9] The main drawback of this medium is that it supports growth of gram negative bacilli because of which it is difficult to isolate enterococci. VRE cannot be distinguished on this medium due to lack of characteristic colony morphology of enterococci.

E. fecalis and *E. faecium* are the most common *Enterococcus species* isolated from clinical samples.[10] In present study high level vancomycin resistance was correctly detected by BEAV and agar dilution method in 12 enterococci from stool sample. All the isolates of VRE with high level vancomycin resistance were *E. faecium*. *E. gallinarum* has intrinsic low level (8-16 µg/ml) vancomycin drug resistance which is chromosomally mediated and is non-transferrable. Therefore, it does not have implications on hospital infection control measures.[11] This low-level resistance was also picked up by BEAV in present study in single isolate of *E. gallinarum*, making this medium highly sensitive for screening vancomycin resistance.

BEAV acts as a selective and differential medium for isolation of VRE from stool samples which are usually contaminated with normal commensal flora of gut which helps in rapid and accurate identification of VRE in laboratories.[12] This significantly reduces time for reporting and ultimately treatment given to the patient. Infection control measures can also be implemented promptly to avoid spread of infection in hospital set up.

Limitations of the study were results of BEAV could not be compared with any other screening agar for VRE. Findings were confirmed by MIC method.

CONCLUSION

The findings in the present study recommend BEAV agar as a sensitive screening medium for isolation of VRE from specimens which are usually contaminated with commensal bacterial flora like stool samples. Rapid and accurate test protocol should be in place in microbiology laboratories where there is isolation of even a single case

VRE in hospital setting which has long term implications on infection control measures for nosocomial infections.

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Ethical approval: The study was approved by the Institutional Ethics Committee (reference number-BMC/IEC/Pharmac/D0313005-05)

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