

## Antimicrobial susceptibility pattern for *Enterococcus* species colonizing the GIT of hospitalized and community patients

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### Abstract

**Objective:** To determine the species of *Enterococcus* prevalent in the gastrointestinal tract (GIT) of patients in hospitals and community and to determine the antimicrobial susceptibility pattern for the isolates.

**Materials and Methods:** *Enterococcus* species were isolated from stools and rectal swabs using selective media. Antimicrobial susceptibility pattern for 9 antibiotics were evaluated by Kirby-Bauer's disc diffusion method. Phenotypic characterization of vancomycin resistant enterococcus (VRE) isolate was done by determining the minimum inhibitory concentration (MIC) to vancomycin and teicoplanin by agar dilution method.

**Results:** Enterococcus species were isolated from gastrointestinal tracts among 74.4% of hospitalized patients and 90% of community patients. *E. faecium* was the frequent species isolated from both patients. Hospitalized patients had lesser number of other unusual *Enterococcus* species. Resistance to antibiotics was higher among isolates from hospitalized patients and 1.49% of hospitalized patients showed colonization with VanB phenotype VRE.

**Conclusion:** Gastrointestinal carriage of antibiotic resistant enterococci including VRE among hospitalized patients mandates the need for periodic surveillance among high risk patients in order to prevent infections and dissemination.

**Keywords:** Gastrointestinal carriage, *Enterococcus*, Antibiotic resistance, VRE, Hospitalized and community patients.

### Introduction

Enterococci species have gained significance as leading cause of hospital acquired bacteraemia, endocarditis and urinary tract infections. Of more than a fifty species in the genus, *E. faecalis* followed by *E. faecium* accounts for 80–90% and 10–15% of clinical infections respectively.<sup>1-3</sup> Infections by other unusual species including *E. casseliflavus*, *E. mundtii*, *E. gallinarum*, *E. durans*, *E. dispar* and *E. durans* are being reported from clinical specimens.<sup>4</sup> Enterococcal infections are a significant cause of concern because of their ability to resist wide range of antimicrobials by intrinsic and extrinsic resistance mechanisms.<sup>5</sup> Multiple drug resistant isolates contribute to increased length of hospitalisation, co-morbidities, increased mortality rates and inter and intra hospital spread.<sup>6</sup>

Enterococci are commensals of gastrointestinal tract and majority of the infections have found to be evolved from the patient's own flora. The selective pressure due to antibiotic administration perturbs the symbiotic relationship of GIT commensals resulting in proliferation of drug resistant isolates which then translocates to cause severe systemic infections.<sup>6,7</sup> Colonisation by vancomycin resistant enterococci and aminoglycoside resistant enterococci in the GIT are reported with the risk of developing blood stream infections and endocarditis. Hence, recognition of asymptomatic gastrointestinal reservoirs of antibiotic resistant *Enterococcus* isolates could be vital to prevent subsequent infections and dissemination leading to epidemics.<sup>1,8</sup>

Considering the dual role of enterococci as commensals and nosocomial pathogens, a study was aimed to investigate the presence of antibiotic resistance in intestinal colonizers among hospitalized patients and community patients. The findings of the study will help to generate epidemiological data in our geographical area, address the risk related to systemic infections besides insinuating the need for periodic surveillance to circumvent strain dissemination.

### Materials and Methods

The study was carried out in patients attending services of the Rajah Muthiah Medical College and Hospital, Annamalai University from June 2016 to December 2016. The study included faecal samples or rectal swabs obtained from 90 hospitalised patients and 60 community patients.

**Inclusion and Exclusion Criteria:** Hospitalized patients: Patients admitted to ICU, general medicine, paediatrics, OG and surgery wards with more than 5 days of hospitalisation were enrolled for the study. Community patients: Patients attending the OPD with or without diarrhoea, no history of prior hospitalisation for past 6 months and were not on antibiotic therapy for past 6 months were included in the study.

**Specimen Collection:** Samples were collected after obtaining informed consent from the patient or attendant. Stool specimens were collected in sterile, plastic wide mouth containers. The samples were transported to the Department of Microbiology, within 30 minutes and processed further. Rectal swabs were

obtained in sterile cotton swabs moistened with sterile saline. The swabs were transported in Cary-Blair transport media to laboratory within 30 minutes.

**Culture and Identification:** Rectal swabs and stool samples were inoculated on bile esculin agar and Pfizer selective enterococcus agar (Hi-Media Laboratories, India) and incubated at 37° C for overnight period. Colonies with dark brown halo and morphologically resembling enterococci were characterized by conventional test scheme including by Grams stain, catalase test, heat tolerance test, and arginine hydrolysis, ability to grow at 45° C and in the presence of 6.5% NaCl. Species identification of enterococci included test for pigment production, motility, efrotomycin (EFRO) test (Hi-Media Laboratories, India), acidification of MGP (1-)-methyl-alpha D-glucopyranoside) and sugar fermentation tests as recommended earlier.<sup>9,10</sup> Carbohydrate fermentation tests were done in Phenol red broth base medium containing 1% of carbohydrate-mannitol, sucrose, sorbitol, raffinose, pyruvate, arabinose, trehalose, lactose, xylose and ribose.

**Antimicrobial Susceptibility Testing:** The ability of antibiotics to inhibit the growth of gastrointestinal isolates of Enterococci was evaluated by Kirby-Bauer's disc diffusion method on Mueller Hinton agar using *E. faecalis* ATCC 29212 as quality control strain. The antibiotic discs included were penicillin [10 units], ampicillin [10 µg], gentamicin-high content [120 µg], streptomycin-high content [300 µg], erythromycin [15 µg], ciprofloxacin [5 µg], vancomycin [30 µg], teicoplanin [30 µg] and linezolid [30 µg]. The discs were purchased from Hi-Media Laboratories, India and stored at 4° C until use. The test was performed and the results were interpreted according to CLSI, 2012.<sup>11</sup>

**Phenotypic Characterization of Vancomycin Resistance:** Phenotypic characterization of vancomycin resistance was based on the minimum inhibitory concentration (MIC) of the isolates to vancomycin and teicoplanin antibiotics by agar dilution method. MIC was determined according to CLSI, 2012.<sup>11</sup> VanA phenotype was characterized by MIC value  $\geq 64$  µg/mL for vancomycin and  $\geq 16$  µg/mL for teicoplanin. Isolates with MIC value of 8 – 64 µg/mL for vancomycin,  $\leq 1$

µg/mL for teicoplanin were characterized as VanB phenotype.

### Statistical Analysis

Fisher exact test was applied to determine the significance of difference between the resistance levels using SPSS version 15.  $P \leq 0.01$  was considered as significant.

### Results

**Isolation Rate of Enterococci:** Gastrointestinal carriage of enterococci was screened among 150 hospitalized and community patients. One hundred and twenty one *Enterococcus* isolates were derived from stool and rectal swabs. *Enterococcus* was isolated from 67 of hospitalized patients and 54 of patients living in community.

**Distribution of *Enterococcus* Species among the Gastrointestinal Carriers:** *E. faecalis* and *E. faecium* were isolated from 41.8% and 59.6% of the hospitalized patients and 35.2% and 37% of the community derived patients respectively. Five species of unusual *Enterococcus* species were isolated from the study groups. The prevalence of *E. avium* and *E. raffinosus* were observed only among the patients from the community (Table 1), (Fig.1 – 4).

**Antimicrobial Resistance Pattern of Gastrointestinal Isolates:** Isolates from hospitalized patients demonstrated a higher percentage of resistance compared to the community patients (Fig 5). Resistance towards penicillin was found in 37% of isolates from both sources. Two vancomycin resistant enterococci (VRE) were isolated from hospitalized patients (Fig. 6).

**VRE Colonisation:** One VRE isolate was obtained from stool sample of a male, hospitalized patient aged 67, with history of diabetes, recent abdominal surgery, catheterization, intake of 3<sup>rd</sup> generation cephalosporin antibiotic and hospitalization for more than 10 days. The isolate was identified as *E. faecium* and was found resistant to penicillin, ampicillin, gentamicin, ciprofloxacin and linezolid. Phenotypic characterization of the VRE isolate revealed an MIC for vancomycin and teicoplanin as 64 µg/mL and 1 µg/mL respectively and hence belonged to VanB phenotype (Fig. 7, 8).

**Table 1: Distribution of *Enterococcus* species among the gastrointestinal carriers:**

Enterococcus species	Hospitalized patients (n=67)		Community patients (n=54)		Total (n= 121)	
	No. of isolates	Percentage	No. of isolates	Percentage	No. of isolates	Percentage
<i>E. faecium</i>	31	59.6	20	37	51	42.1
<i>E. faecalis</i>	28	41.8	19	35.2	47	38.8
<i>E. gallinarum</i>	2	3	7	13	9	7.4
<i>E. casseliflavus</i>	4	6	2	3.7	6	5
<i>E. raffinosus</i>	0	0	2	3.7	2	1.7
<i>E. avium</i>	0	0	2	3.7	2	1.7
<i>E. hirae</i>	2	2.9	2	3.7	4	3.3

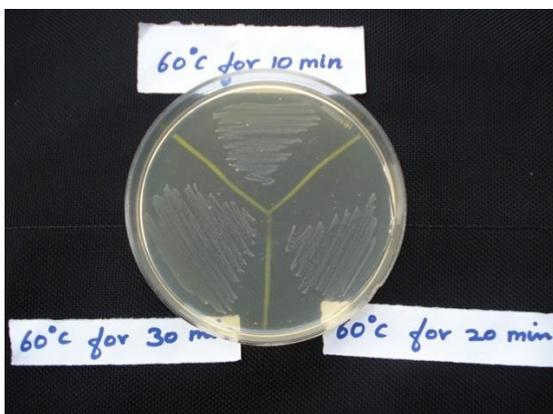


Fig. 1: Heat tolerance test for *Enterococcus* identification



Fig. 2: Tests for *Enterococcus* identification  
Glucose fermentation, Bile esculin +, Growth at 45 °C and in the presence of 6.5% NaCl

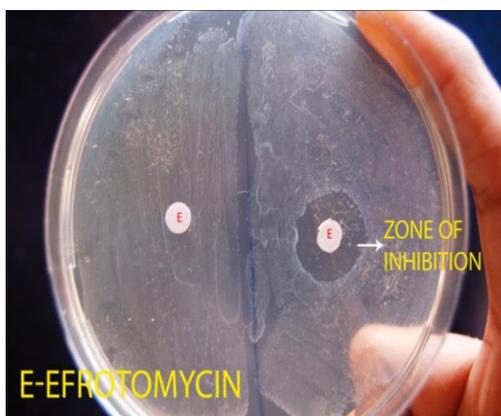


Fig. 3: Efro test



Fig. 4: Carbohydrate fermentation tests

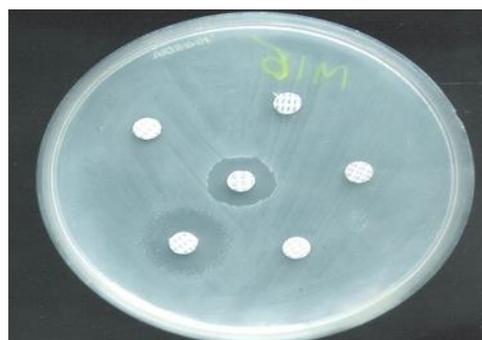


Fig. 5: Disc diffusion test  
Presence and absence of zone of inhibition around the antibiotic discs

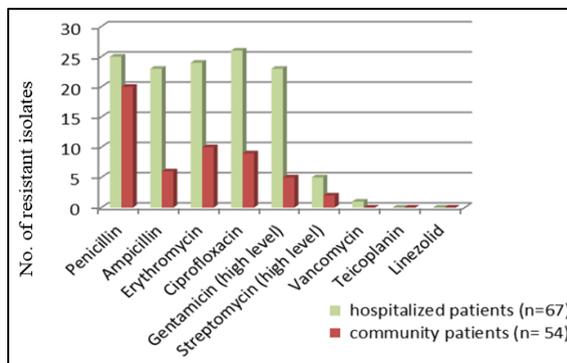


Fig. 6: Antimicrobial resistance pattern of gastrointestinal isolates

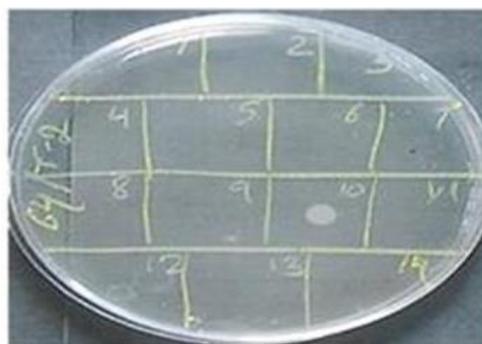
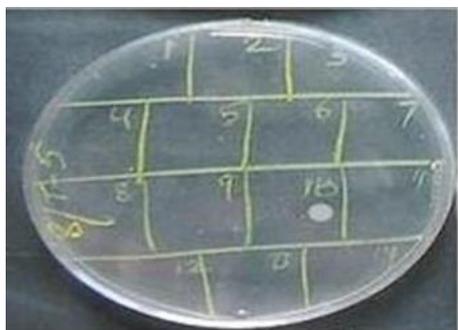


Fig. 7: Phenotypic characterization by agar dilution for vancomycin



**Fig. 8: Phenotypic characterization by agar dilution for teicoplanin**

## Discussion

The gastrointestinal tract exhibiting resistant enterococci acts as reservoir of infections.<sup>3</sup> In our study enterococci were isolated from 74.4% of hospitalized patients and 90% of community patients. Previous investigations of stool samples from hospitalized patients showed the prevalence of enterococci around 71.2% - 77%.<sup>12,13</sup> Among community patients the GIT colonization of enterococci ranged from 85.5% - 100%.<sup>14,15</sup> Similar observations in our study supports the fact that usage of broad spectrum antimicrobials in the hospital may have altered the GIT flora leading to their lower isolation rates.<sup>16</sup>

*E. faecium* was the frequently isolated species from both hospitalized and community patients however, its isolation percentage was higher among hospitalised patients (59.6%) compared to community patients (38.9%). Reports on isolation of *E. faecium* ranging from 60.5%-71% among hospitalized patients and 29%-39.6% among community patients by other workers is in accordance with our study.<sup>13,17</sup> It could be comprehended that the intrinsic nature of drug resistance associated with *E. faecium* compared to other species could have offered a selective advantage to emerge in higher numbers.<sup>1</sup> The unusual *Enterococcus* species frequently isolated from stool samples are *E. avium*, *E. raffinosus*, *E. durans*, *E. mundtii* and *E. casseliflavus*.<sup>12,13,17</sup> In our study three and five species of unusual enterococci were obtained from hospitalized and community patients respectively. The isolation of lesser number of unusual *Enterococcus* species among hospitalized patients is a clear indication of the role of antibiotics against other *Enterococcus* species which are well known for their heightened susceptibility to antibiotics.<sup>1</sup>

Resistance rate towards penicillin antibiotic was found to be same (37%) among isolates from both sources. Similar resistance rates for penicillin among isolates from both sources could be attributed to the frequent use of penicillin in the community for the empirical treatment of infectious diseases. Enterococci possess an intrinsic resistance (chromosomally coded) to betalactam antibiotics by producing penicillin-

binding protein 5 (PBP5) which has low affinity to the drug and continues peptidoglycan synthesis.<sup>3</sup>

The resistance percentage to ampicillin, erythromycin, ciprofloxacin and gentamicin antibiotics were higher among the isolates from hospitalized patients and this increased levels was found statistically significant ( $P \leq 0.01$ ) compared to community derived isolates. Resistance to high level gentamicin is by production of aminoglycoside modifying enzymes whose action eliminates the possibility of synergistic treatment of enterococcal infections with cell wall inhibiting agent-ampicillin or vancomycin.<sup>18</sup> Considering the characteristic nature of enterococci to survive in harsh environment outside the GIT and its possibility to be transmitted via health care workers, the GIT colonization by drug resistant *Enterococcus* among hospitalized patients raises serious concern in our setting.<sup>19</sup>

VRE colonization was observed in 1.49% of hospitalized patients in our study. Investigations on VRE colonization from Belgium, UK, Netherland and Finland have reported a prevalence of 2-3.5% among hospitalized patients.<sup>21-22</sup> The risk factors for VRE colonization as described by Safdar et al., 2002 including long term hospitalization, surgery, catheterization, exposure to 3<sup>rd</sup> generation cephalosporin correlates well with the history of VRE colonized patient in our study.<sup>23</sup> This signals the need for surveillance among high risk groups to implement infection containment measures.

## Conclusion

Gastrointestinal carriage of antibiotic resistant *Enterococcus* species was marked among the hospitalized patients compared to community counterparts. This proves the role of selective pressure of antibiotics in altering the GIT flora which may precipitate serious clinical infections. Occurrence of VRE isolate and existence of increased number of isolates expressing resistance to antibiotics used for synergistic treatment mandates measures for surveillance at least among high risk groups. The study coerces the need for stringent infection control procedures in order to prevent infection and dissemination of resistant isolates colonizing intestinal tract of hospitalized patients.

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