Prevalence of Vancomycin Resistant *Enterococci* (VRE) in Catheter Associated Urinary Tract Infections(CAUTI) with special reference to biofilm formation

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Abstract

Introduction: *Enterococci* have been recognized as an important cause of hospital-acquired infections. They are currently the second most common organisms recovered from nosocomial urinary tract infection. Emergence of Vancomycin resistance has been a threat in the hospital settings. Biofilm formation is also an important virulence factor contributing to drug resistance.

Aim: To know the prevalence of VRE in device infections CAUTI and role of biofilm formation in the associations of CAUTI.

Materials and Methods: Total 100 Catheter-Associated Urinary Tract Infection (CAUTI) cases were included. Controls comprised of 50 cases of UTI but non-catheterized. Bacteriological identification and susceptilibity testing were done as per standard protocol.

Results: Overall culture positivity in CAUTI was 32%. *Enterococci* (40.62%) were the predominant pathogens followed by *Esch.coli* (37.50%). VRE prevalence in current study was 18.75%. Strong biofilms were mainly produced by resistant isolates(30.76%). Biofilm formation by VRE was statistically significant (p value= 0.01124). VRE infection was also more common in CAUTI (p value= 0.000019).

Conclusion: VRE detection and its control entail an aggressive approach which involves strict adherence to standard infection control practices by the hospital personnel. Role of the Microbiology Laboratory plays important role in the Detection, Reporting and Control of VRE. Collaboration between the laboratory and the infection-control program will definitely play an important role in this.

Keywords: *Enterococci,* VRE, CAUTI, Biofilm.

Introduction

Enterococci have been identified as an important cause of various nosocomial infections. They are able to colonize the host tissues, resist the immune system and produce a wide range of infections. These pathogenic effects are mediated by various *Enterococcal* virulence factors.

The most frequent infection caused by *Enterococci* is urinary tract infection.[1] In addition, UTI is also reported as a frequent focus of infection for *Enterococcal* bacteraemia.[2,3] Persons with predisposing factors like who have been instrumented, or are on broad spectrum antibiotics for some infection, have structural abnormalities of urinary tract and/or have recurrent UTIs, the rate of urinary infection rises. Catheter-associated urinary tract infection (CAUTI) is one of the most common healthcare-associated infection.[4] Though *Escherichia coli* has been reported as the leading cause of CAUTI in various studies, *Enterococci* is also gaining importance in the causation of CAUTI.[5,6] The *Enterococcus* spp., especially *E.faecalis* and *E.faecium*, account for 15% to 30% of CAUTI. The worldwide rise of *Enterococcal* infections, since the late 1980s, is of particular concern due to the emergence of Vancomycin Resistance.[7]

According to CDC's National Nosocomial Infections Surveillance System (NNISS) survey conducted in 1993, nosocomial VRE infections increased from 0.3% to 7.9%.[8] Since then there has been remarkable increase in prevalence of VRE globally.[9] Multiple factors predispose a person to infection with VRE such as underlying co morbid conditions and previous VRE colonization or infection.[10-12]

The emergence of VRE has been attributed to the imprudent use of cephalosporins, vancomycin, noncompliance with infection control measures and use of invasive devices like catheters. [12] While the catheter in situ, colonization plays a major role in developing catheter-associated urinary tract infection. *Enterococci* are capable of producing biofilms, which consist of a population of cells attached irreversibly on various biotic and abiotic surfaces, encased in a hydrated matrix of exopolymeric substances. Biofilm plays a significant role in colonization and provides an opportunity for the bacteria to develop drug resistance. *Enterococc*i take advantage of this property to establish persistent infections in the urinary tract.[13,14] High antimicrobial concentrations are required to inactivate organisms growing in a biofilm, as antibiotic resistance can increase 1,000 fold.[15]

Considering all above factors, the current study was conducted to assess the prevalence of Vancomycin Resistant *Enterococci* (VRE) in urine samples of patients clinically suspected to have CAUTI and to find the association of biofilm formation with the spread of resistance, so that necessary preventive actions can be taken to curtail the major outbreaks in hospital settings.

Materials and Methods

This was a prospective laboratory-based study conducted to find out the prevalence of VRE in cases of catheter-associated urinary tract infections (CAUTI) and evaluation of its association with biofilm formation. Institutional Ethics Committee (IEC) clearance was obtained before beginning the study.

The study was conducted in the Department of Microbiology at a tertiary care hospital and included samples from patients admitted to the ICU and wards of various specialties in the hospital. Total of 100 urine samples were collected from patients catheterized and suspected of having infection. Urine samples were collected after appropriate informed consent. Controls comprised of 50 mid-stream urine samples from cases of clinically suspected UTI but not catheterized.

In catheterized patients, after cleaning with an alcohol pad clamp placed on the catheter, then with all aseptic precautions urine was aspirated with syringe and needle directly from the part of the tubing proximal to the clamp and collected in a sterile wide mouthed container. The samples were immediately transported to the Microbiology laboratory after filling the lab requisition form.[16]

For the collection of a urine specimen from controls, clean catch mid-stream specimens were collected. Patients had been given clear instructions regarding collection of sterile specimen.[16] After collection of samples, semi-quantitative culture on Blood agar and MacConkey agar were carried out. The *Enterococcal* isolates were confirmed by Gram's staining, catalase production, growth in nutrient broth containing 6.5% NaCl, aesculin hydrolysis in presence of 40% bile salts.[16] Vancomycin resistance in the isolated *Enterococci* was determined by Kirby-Bauer disc diffusion method on Mueller-Hinton agar by using 30µg vancomycin disc and results were interpreted as per CLSI guidelines.[17]

Biofilm production among a population of Vancomycin Resistant *Enterococci* (VRE) and Vancomycin Susceptible (VSE) *Enterococci* was investigated using simple tube method described by Christensen et al.[18] A loopful of the resistant *Enterococcal* isolate was inoculated in 10 ml of trypticase soy broth with 1% glucose in test tubes and then incubated at 37^0C for 24hours. After incubation, growth medium from the tubes was discarded. Each tube was washed with phosphate buffer saline (pH 7.3) to eliminate the unbound bacteria. Tubes were then stained with crystal violet (0.1%). Excess stain was removed and tubes were washed using deionized water. Tubes were air dried in an inverted position and observed for biofilm formation. Biofilm formation was considered positive when a visible film lined the wall and bottom of the tube. Ring formation at the liquid interface was not indicative of biofilm formation. Tubes were examined and the amount of biofilm formation was scored as 1-weak/none, 2-moderate or 3-strong/high.[18]

Observations and Results

In the current study, a total of 100 clinically suspected Catheter-Associated Urinary Tract Infection (CAUTI) cases were included. Simultaneously, to know the association of VRE with catheterization, 50 controls were included who were clinically suspected to have urinary tract infection but were non-catheterised.

All the observations and results were analysed further using statistical software. Chi-square and P-value were calculated by using InSilico software to evaluate the statistical significance of association of catheterization with VRE prevalence. All observations were summarised in different tables and graphs accordingly, which are elaborated in this section further.

Table 1 shows culture positive CAUTI cases and their bacteriological profile. Overall culture positivity was 32% for catheter-associated UTI. *Enterococcus* (40.62%) was the predominant organism isolated, *Escherichia coli* (37.50%) as the second most common organism, followed by *Staphylococcus aureus* (9.38%).

Total 6 isolates of *Enerococci* were vancomycin resistant, therefore overall VRE prevalence among culture positive cases was 18.75%. (Table 2) VRE prevalence was found to be more in males (30.67%) as compared to females (15.38%). Amongst these, the maximum number of VRE cases were found to be in the age group above 45 years (23.07%). Table 3, summarizes biofilm formation as seen by test tube method. Total 4 (30.67%) VRE cases were strong biofilm formers whereas none of the vancomycin sensitive isolates demonstrated strong biofilms.

Table 1: Micro-organisms isolated from culture positive cases

Micro-organisms	Number of isolation(%) (n=32)
Enterococci	13 (40.62%)
Escherichia coli	12 (37.50%)
Staphylococcus aureus	3 (9.38%)
Coagulase negative staphylococci	1 (3.13%)
Candida spp.	1 (3.13%)
Pseudomonas aeruginosa	1 (3.13%)
Klebsiella pneumoniae	1 (3.13%)

Chart 1: Micro-organisms isolated from culture positive cases

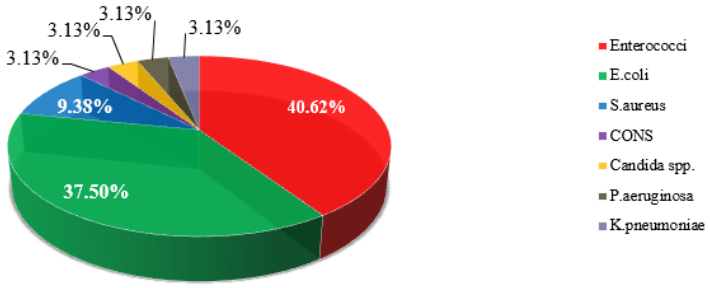

Table 2: No. of VRE isolates

Enterococcal resistance among the Culture positive cases	No. of Isolates (n=32)
VRE(Vancomycin Resistant Enterococi)	6(18.75%)
VSE(Vancomycin Susceptible Enterococci)	7(21.85%)

Table 3: Biofilm formation assessment

Sensitive/ Resistant	Grading for biofilm formation			Total	Chi-square value	P value
	0/1(none/mild)	2(moderate)	3(strong)			
VRE	2(15.38%)	0	4(30.67%)	6	8.9762	0.01124
VSE	2(15.38%)	5(38.46%)	0	7		
Total	4(30.67%)	5(38.46%)	4(30.67%)	13		

Table 4: Distribution of isolates among the cases and controls

	Cases (CAUTI)	Controls	Chi-square value	P value
VRE	6(18.75%)	0	24.5162	0.000019
VSE	7(21.88%)	2(6.45%)		
Other	19	29		
No growth	68	19		
Total	100	50		

Discussion

In India, infection due to VRE has been on the rise in recent years. Most of the cases are hospital associated, urinary tract infections being one of them and maximum are catheterized. In India, there are very few studies carried out to know the prevalence of VRE in different hospital settings. In the view of the emerging VRE infections and scarce data in medical literature, the present study was conducted to evaluate the prevalence of VRE in CAUTIs.

In the current study, culture positivity rate was 32% with *Enterococci* being the most common pathogen. Isolation rate of *Enterococci* (40.62%) was much higher as compared to Nandini et al,[6] Yonitet al,[19] Vyawahare et al[20] and Karina et al[21] all of which have isolation rates around 3-7% in CAUTIs. Isolation rate was slightly higher as compared to the study by Desai et al.[22] Increased hospitalization rates along with an rise in catheterization, may be a reason for increased prevalence of CAUTI in hospitalized patients.

Table 1 summarizes the detailed bacteriological spectrum of CAUTI in this study. In this study, *Enterococcus spp.* (40.62%) was the commonest organism isolated from CAUTI followed by *Esch.coli* (37.50%). In various studies, *Esch.coli* has been found to be the most common organism associated with CAUTI.[6,20,21] Though initially, *Enterococcus spp.* was not a predominant pathogen in CAUTI and other nosocomial infections, recently it has emerged as a predominant pathogen in different nosocomial infections. It was the second most common organism after *Esch.coli*, isolated from culture in CAUTI in different hospital settings.[23,24]

Overall VRE prevalence among the culture positive cases in the current study is 18.75% (Table 2). There are a handful studies carried out in India to know the exclusive prevalence of VRE in CAUTI.[25] Wavare et al[26] documents the dramatic increase in vancomycin resistance among *Enterococci*. As most of the urinary tract infections are catheter-associated, this highlights

the need for further studies to estimate the prevalence of VRE exclusively associated with CAUTI in India.

Advancing age is one of the predisposing host factors for the development of CAUTI. Maximum VRE cases were isolated from the age group 46-70 years (15.38%), followed by ≥70 year's (7.69%). It suggests that age group may play a role in increased prevalence of VRE in CAUTI.

The significance of biofilm formation in the causation of CAUTI observation summarized in the table 3. Although both VRE and VSE are biofilm producers, it was found that strong biofilms were predominantly formed by resistant isolates(30.76%).This difference in biofilm formation by VRE and VSE was statistically significant by Fischer's exact test (p value= 0.01124). This can be a reason for increased isolation of VRE from catheterized patients or patients with any other invasive device. Biofilm formation plays a role in colonization as well as adaptation to hospital environment. Also, the presence of biofilm may be the reason for higher antibiotic resistance and its nosocomial spread. Similar findings were observed by Oli et al,[27] Biswas et al[28] which reports statistical significance of biofilm formation by VRE. This may put forward a genetic linkage between the biofilm and VRE genes. There are few studies which are in discordance with our findings. Banerjee et al[29] did not found any correlation between biofilm formation and antimicrobial resistance, especially Vancomycin resistance in *Enterococci*. Lack of larger study, might be responsible for lower biofilm formation by VRE in above studies.

To find out association of invasive device manipulations such as urinary catheterization in the spread of VRE, controls were also analyzed along with the cases of CAUTI(Table 4). Only 2(6.45%) of the 50 controls were isolated *Enterococci* and which were vancomycin sensitive. On statistical analysis we came to conclusion that VRE prevalence is more common in CAUTI which is statistically extremely significant (p value= 0.000019). We did not came across much studies in India which are carried out to know the prevalence of VRE solely in CAUTI, though there were few isolated studies in the world such as Tedja et al[24].

Conclusion

We conclude that VRE were major contributors among *Enterococcal* infections especially among CAUTI. Biofilm formation act as a major contributing factor in establishment of *Enterococcal* infection.

Taking these aspects into consideration, it is important to implement infection control measures, effective use of microbiological investigations, rational use of antimicrobial combinations and education of hospital staff. Clinicians should also take into consideration the changing trend in etiology of CAUTI.

This study signals the emergence of VRE and highlights the importance of screening for vancomycin resistance. Analysis also highlights development of vancomycin resistance is credited to their ability to produce biofilms.

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