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Original Research Article

Enterococci: A journey of a successful pathogen

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Abstract

Introduction: A dynamic homeostasis is maintained between the host and native bacteria of the gastrointestinal tract in humans, but migration of bacteria from the gut to other organs can lead to disease or death. Enterococci, traditionally viewed as commensal bacteria are now acknowledged to be organisms capable of causing life-threatening infections in humans, especially in the nosocomial environment. The existence of Enterococci in such a dual role is facilitated by its intrinsic and acquired resistance to virtually all antibiotics currently in use.

Objective: The present pilot study was taken up to compare the multidrug resistance prevalence in commensal Enterococci and pathogenic Enterococci.

Material and methods: A total of 50 commensal Enterococci isolated from stool samples and 50 clinical samples yielding Enterococci were taken for the study. Antibiotic susceptibility testing was done using Kirby Bauer's disk diffusion method. Minimum inhibitory concentration of Vancomycin was tested by using E- strip.

Results: Among 50 commensal Enterococci, majority showed resistance to Ampicillin 50 (100%), Erythromycin 38 (76%), Clindamycin 30 (60%), higher level of resistance to high level Gentamycin 14 (28%), Linezolid 6 (12%), vancomycin 3 (6%). 23 (46%) isolates showed multi drug resistance (resistance to ≥ 3 categories of antibiotics). Among 50 clinical isolates, majority showed resistance to Ampicillin 50 (100%), Clindamycin 46 (92%), Tetracycline 46 (92%), Erythromycin 41 (82%), Linezolid resistance was seen in 8 (16%) and Vancomycin resistance in 5 (10%) clinical isolates. 48 (96%) showed multi drug resistance.

Conclusion: Boundary line between pathogenic and commensal Enterococci is blurred due to exchange of resistant traits. Regular screening of enterococcal isolates for resistance detection should

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be implemented. It is very important to consider infection control measures, screening of health care workers, surveillance cultures which can control spread of multidrug resistant Enterococci.

Key words

Antibiotic resistance, Commensal Enterococci, Multi drug resistance, Linezolid resistance, Vancomycin resistance.

Introduction

Until recently bowel commensals including Enterococci were perceived as bacteria with no harm. For long time, commensal Enterococci were frequently ignored when isolated in clinical laboratory. But recently Enterococci have attracted much attention due to its capability of causing variety of infections, especially in hospitalized patients and higher resistance to various antibiotics has led to understanding the importance of identification of Enterococcus [1]. Enterococci have evolved over the past century from being an intestinal commensal organism of little clinical significance to becoming the second most common nosocomial pathogen associated with significant morbidity and mortality [2].

Enterococci are frequently used as positive resistance indicator bacteria, because of their high prevalence in the feces of healthy population and their ability to harbor several resistance determinants [3]. Administration of antimicrobial agents affects both targeted pathogenic organisms as well as non-target commensals. Thus frequent antimicrobial use creates a pool of resistant commensal bacteria. This contributes to the general increase and dissemination of bacterial resistance worldwide and can be a source of resistance genes for pathogens [4].

Until recently commensal Enterococci represented an underappreciated means of assessing resistance [5]. But the ability of the organism to acquire newer traits makes it virulent to invade extra intestinal regions and cause infections [6]. With this background, the present pilot study was taken up to compare the multidrug resistance prevalence in commensal Enterococci and pathogenic Enterococci.

Material and methods

The present study was conducted in the Department of Microbiology, Mysore Medical College and Research Institute, Mysore. This study was cross sectional prospective study which included 50 isolates of Enterococci from stool samples of the patients who attended the outpatient departments in our hospital and other 50 isolates of Enterococci from various clinical samples such as burn wound swabs, ascitic fluids, surgical and non-surgical wounds, umbilical stumps, abdominal drain fluids, synovial fluids and Foley's catheters followed by subsequent urine specimens of the same patient, (except stool specimens) obtained in the Microbiology Department were processed for the isolation of Enterococci.

Enterococcal isolates were identified according to the standard protocols. Antibiogram was done using Kirby Bauer's disk diffusion test as per latest Clinical and Laboratory Standards Institute (CLSI) guidelines. The drug of different groups tested were Ampicillin (10 mcg), Amoxycalv (30 mcg), Erythromycin (15 mcg), Clindamycin (2 mcg), Ciprofloxacin (5 mcg), Tigecycline (15 mcg), high level Gentamycin (120 mcg), Cotrimaxozole (25 mcg), Tetracycline (30 mcg), Linezolid (15 mcg) and Vancomycin (30

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mcg). The isolates which showed resistance to three or more than three prototype of drugs were considered as multi drug resistant Enterococci [7]. The minimum inhibitory concentration levels of Vancomycin were determined by E strip as per latest CLSI [8].

Results

Total of 100 samples were processed. 50 isolates of Enterococci obtained from the stool samples were considered as commensal Enterococci. The other 50 Enterococci from the clinical samples were considered as pathogenic Enterococci. Ampicillin showed 50 (100%) resistance in both commensal and pathogen Enterococci. Majority of commensal Enterococci showed resistance to Erythromycin 38 (76%), Clindamycin 30 (60%), and Ciprofloxacin 22 (44%) as per **Graph - 1**. Commensal Enterococci showed higher level of resistance to high level Gentamycin 14 (28%), Linezolid 6 (12%), Vancomycin 3 (6%).

Among pathogen Enterococci, majority showed resistance to Clindamycin 46 (92%), Tetracycline 46 (92%), and Erythromycin 41 (82%) as per **Graph - 2.** High level Gentamycin showed resistance in 16 (32%), Linezolid in 8 (16%) and Vancomycin 5 (10%) of the isolates. Multi drug resistance in commensal Enterococci was as per **Table - 1**. Multi drug resistance in Pathogen Enterococci was as per **Table - 2**. Comparison of resistance pattern of various antibiotics in commensal and pathogenic Enterococci was as per **Graph - 3**.

Discussion

Antimicrobial agents are grossly misused in many developing countries including India leading to high selective pressure on microorganisms. Today, the emergence of bacterial strains which display resistance to a variety of drugs is a major cause of failure of treatment of infections worldwide and a serious concern to animal and public health.

In our study, commensal Enterococci have shown maximum resistance to Ampicillin 50 (100%), followed by Erythromycin 38 (76%), Clindamycin 30 (60%). Another study on commensal Enterococci has reported 100% resistance to Ampicillin, Erythromycin. Since Ampicillin is the drug of choice in the treatment of Enterococcal infections, the relatively high resistance of isolates in this study to Ampicillin is of great concern, especially in the case of endocarditis treatment [9]. Though we have not studied the risk factors, previous exposure to Ampicillin and urinary catheterization were found to be the major risk factors associated with the emergence of Ampicillin resistance Enterococci. Bladder catheterization has been shown to increase urinary Enterococcal colonization in patients with Ampicillin-resistant Enterococcal bacteremia and a gastrointestinal origin of urinary colonization has been indicated by plasmid analysis [10]. The use of antibiotics, whether for prophylaxis or chemotherapy, does not only affect the pathogenic bacteria but also the commensal bacteria. This maintains a pool of resistant bacteria with a pool of resistance genes in the population which further contributes to the general increase and dissemination of bacterial resistance and can be a source of resistance genes for pathogens [11, 12].

Bacteremia due to Vancomycin resistant Enterococci (VRE) is a significant complication in surgical patients and is associated with mortality rates ranging from 33% to 68% [13]. Although the prevalence of VRE infections in India is much lower than in the western world, it has been increasing in the past one decade. In our study of commensal Enterococci isolated from 50 fecal samples, 3 (6%) were found to be Vancomycin

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resistant. These finding mirrors, those of a study done by Vandana KE, where 3 (6.25%) were VRE [14]. 5 (10%) were found to Vancomycin resistant in pathogen Enterococci which is high compared to other reports from India. Glycopeptide resistance among our isolates is high, probably reflecting the increased use of Vancomycin in our hospital over the past few years. This fact highlights the importance of strict enforcement of antibiotic policies coupled with greater adherence to infection control measures to prevent emergence and spread of antibiotic resistant bacteria. Widespread use of vancomycin and extended-spectrum Cephalosporin in hospitals likely contributed to the emergence and dramatic increase of VRE over the past 20 years [15].

The prevalence of VRE has dramatically increased worldwide [16]. The National Nosocomial Infection Surveillance (NNIS) system in the USA has revealed a significant increase in the percentage of invasive nosocomial Enterococcus strains displaying high-level vancomycin resistance [17]. To a larger or lesser extent, non-microbiological factors such as antibiotic consumption (particular classes and in general); "colonization pressure", "understaffing", compliance with hand hygiene and other infection control measures also influence the development of VRE [18]. Nosocomial outbreaks of vancomycin-resistant Enterococci (VRE) are thought to occur when a patient already carrying VRE in his/her bowel sheds VRE, which are then transmitted by health care workers or via the environment to other patients. This model predicts that interventions based on screening and isolation of VRE-colonized patients, improved hand hygiene, and enhanced hospital cleaning will limit cross-transmission [19]. Millions of dollars are spent each year by health care systems trying to contain antibiotic-resistant bacteria and prevent cross-transmission [20]. The use of antibiotics as feed additives for growth enhancement in animals may be associated with the emergence of VRE. Enterococci can reach human consumers via food chain [21]. In countries using avoparcin, a glycopeptide antibiotic, as a growth promoter, Vancomycin-resistant Enterococci (VRE) are commonly found in the commensal flora of food animals, on meat from these animals and in the commensal flora of healthy humans despite very limited use of Vancomycin in hospitals [22].

The emergence of Linezolid resistant Enterococci is a dangerous fact [23]. In our study, Linezolid resistance was 6 (12%) and 8 (16%) in commensal and pathogen Enterococci respectively. Though Linezolid has been used in clinical practice for a relatively short period of time, there are already several reports of Linezolid-resistant Enterococci, which is a matter of concern. As Linezolid is the final resort, sometimes even for VRE it is recommended to do a susceptibility testing of clinically significant gram-positive pathogens before starting Linezolid therapy so as to shorten the course of Linezolid treatment [24].

In our study, the multi drug resistance in commensal and pathogen Enterococci is 46% and 96% respectively. The emergence of multi-drug resistant Enterococci has lead to a scenario which is almost as bad as the preantibiotic era since many of these multi-drug resistant (MDR) strains have developed resistance to practically all available antibiotics [25].

Community awareness of the issues involved in antibiotic therapy is poor and this is compounded by over the counter drug availability and self medication. Various contributing factors other than these included like the combination of poverty and ignorance making the ground perfect for the development of resistance. Patient's pressure, aggressive marketing by pharmaceutical companies, lack of

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uniformity among the physicians to follow antibiotic policy, causing the recurrence of the disease also have added to the practice [26]. Effective control of multiple-drug resistant Enterococci will require better understanding of the interaction between Enterococci, the hospital environment, and humans, prudent antibiotic use and better contact isolation in hospitals and other patient care environments [27].

Antimicrobial resistance is a natural biological phenomenon that often results from antibiotic pressure in humans, animals and the widespread use of disinfectants in farm and household chores. When it gets amplified many times, it results in serious public health concerns and long term shifts in resistance levels [22]. In essence, the situation is alarming. This emerging threat has to be tackled at the initial phase itself, which could be done through active surveillance of antimicrobial resistance in the community. Education of the professionals and public, accessibility of microbial investigation and its results to the practitioners to rationalize the choice of antimicrobial therapy is also required to combat this problem.

Last but not the least, co-ordination of the surveillance of the antibiotic resistance in human and animal health sectors along with regulating the antibiotic use, restriction of antibiotic as growth promoters in animals should be equally considered. Multi-drug resistant Enterococci have become a serious threat to public health. Enterococci with raised MIC to penicillin and high level resistance to amino glycosides are being reported. Although the incidence of vancomycin resistance was low, its presence is a cause of concern. It is important to maintain regular surveillance of antibiotic susceptibilities so that changes in their pattern can be detected early.

Conclusion

Boundary line between pathogenic and commensal Enterococci is blurred due to exchange of resistant traits. In our study multidrug resistant traits are seen in both commensal and pathogenic Enterococci with various percentages. So it becomes necessary to Screen-Isolate-Destroy Enterococci at various levels. At a minimum, a successful program for control of multidrug resistant Enterococci requires effective surveillance to identify colonized and infected patients. Equally important is renewed vigor in the search for additional drugs, accompanied by the evolution of new therapeutic paradigms less vulnerable to the cycle of drug introduction and drug resistance.

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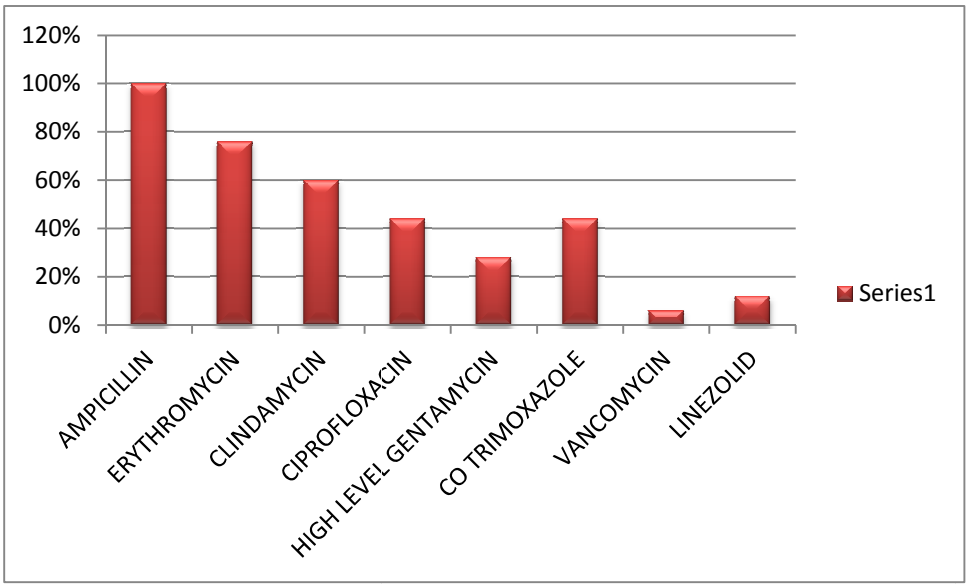
Graph - 1: Drug resistance in commensal Enterococci.



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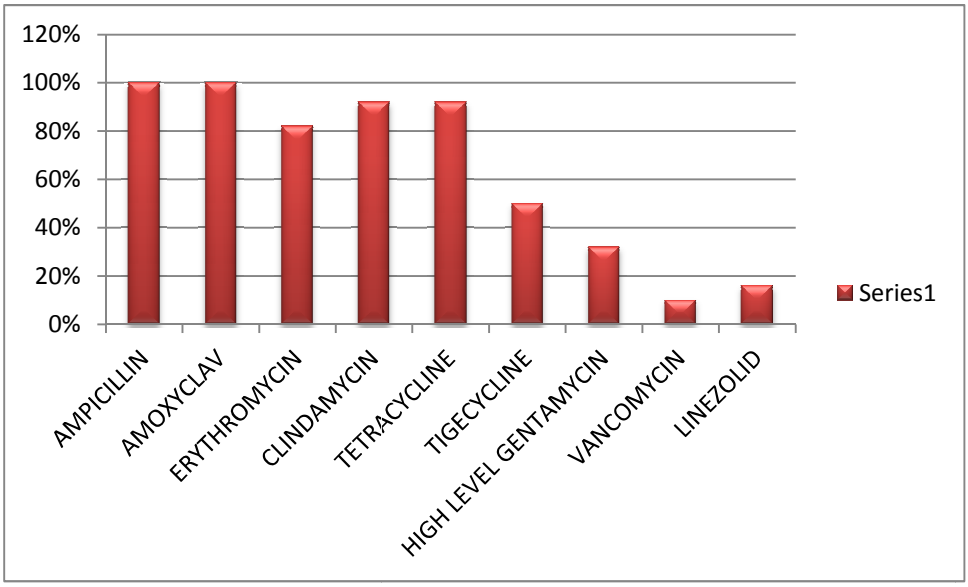
Graph - 2: Drug resistance in pathogen Enterococci.



Graph - 3: Comparison of resistance pattern of various antibiotics in commensal and pathogenic Enterococci.

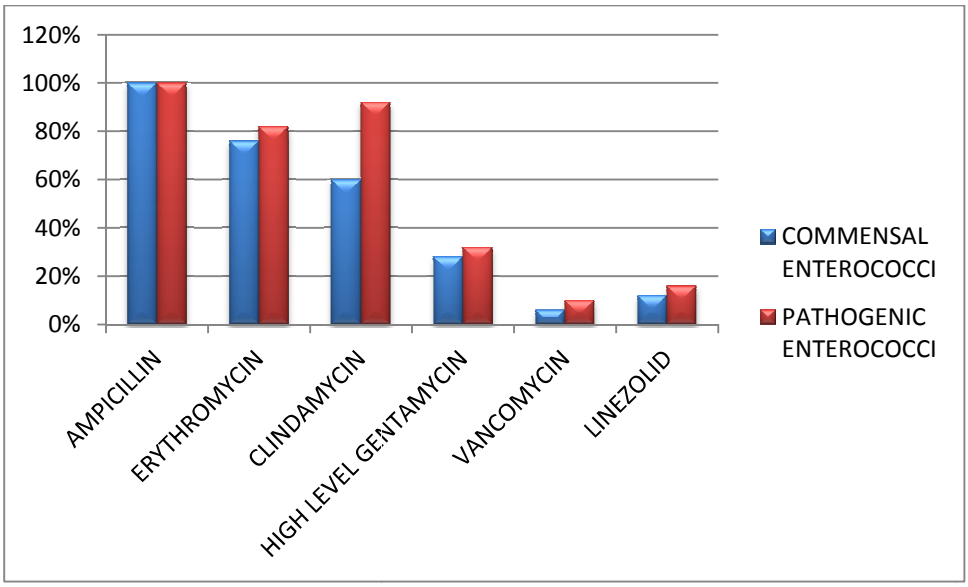


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Table – 1: Multi drug resistance in commensal Enterococci.

Number of isolates	Multi drug resistance
23 (46%)	Resistance to ≥ 3 categories of antibiotics
8(16%)	Resistance to ≥ 4 categories of antibiotics

Table - 2: Multi drug resistance in pathogen Enterococci.

Number of isolates	Multi drug resistance
48(96%)	Resistance to ≥ 3 categories
38 (76%)	Resistance to ≥ 4 categories
24 (48%)	Resistance to ≥ 5 categories

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