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Research Article

Characterization of Food and Clinical Isolates of Enterococci

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Abstract

Members of genus *Enterococcus* are Gram-positive, catalase negative, facultatively anaerobic cocci, which can appear arranged in pairs or short chains. they can grow in a wide range of temperatures and in restrictive environment such as high salt content and low pH. The present research is to isolate, identify and to determine the *Enterococcus* species from food samples and clinical specimens. Secondly, this is to evaluate the comparative antibiotic susceptibility patterns and hemolytic activity between the food and clinical enterococcal species. In this study a total of 162 food samples were collected to screen for *Enterococcus* species. Of 162 samples, 55 yielded *Enterococcus* species. In clinical specimens, 14 isolates from urine samples were collected for comparative characterization between food and clinical.

Keywords: Enterococci; Microorganism; Nosocomial Infections

Introduction

Microbes – an Unseen Miracle in the world of living things that supports life process. Let there be no doubt about it – the microbes were here first, they cohabit the planet with us, and they will be here after we are gone. Microorganisms are ubiquitous. A vast and diverse microbial world occupies every nook and cranny of the globe, from the deepest depths of the ocean to the highest mountain peaks, living in the water we drink, soil and air that surround us, on and in the food that we eat, on and within our own bodies. In short, it inhabits every corner of the globe [1].

The most frequent human clinical infections that have been associated with enterococci include bacteremia, urinary tract infections, intra-abdominal and pelvic infections, burn wound, deep tissue infections, endocarditis and rarely been associated with meningitis and respiratory tract infections. Out of more than 29 species of genus Enterococcus, only two are suggested as responsible for these infections – Enterococcus faecalis and Enterococcus faecium.

Overuse and misuse of antimicrobials in food animals represent a public health risk as they contribute to the emergence of resistant forms of disease – causing bacteria. Such resistant bacteria can be transmitted from those food animals to humans, primarily via food. Like other Gram-positive bacteria, enterococci are 'intrinsically resistant' to a number of antibiotics like erythromycin, chloramphenicol, penicillin and vancomycin etc.

As a result, enterococci may have a 'dualistic effect'. On one hand they play a dominant role in various fermented products but on the other, some are considered as indicators of undesirable contamination or even as microorganisms carrying some pathogenic potential (Arizcun., *et al.* 1997).

The enterococcal levels in raw milk are variable. This variability is presumably influenced by factors such as region, climate, milk production levels, breed, and handling of the milk.

For a clinical perspective, enterococci have long been considered non pathogenic bacteria, until a multiple antibiotic-resistant strains were identified in the late 1970's. Since then and over the last three decades, enterococci are increasingly regarded as agents with potential pathogenicity in hospitalized patients ranking fourth (Day., *et al.* 2001) third (Ruoff., *et al.* 1999) or even second in the frequency of bacteria that can be isolated from patients in United States of America.

Materials and Methods

In this study, a total of 162 food samples from raw milk, packaged milk (processed and pasteurized) and milk products such as butter and cheese were collected aseptically from different vendors and shops and were transferred immediately to laboratory. Subsequently, all the food samples were subjected to standard microbiological processing so as to screen for the members of *Enterococcus* spp. The following methodologies were used to collect and

process the food samples and to isolate and identify *Enterococcus* species.

Collection and transportation of milk and milk products

Raw milk and packaged milk samples from vendors and shops (n=55), salted and pasteurized butter (n=50) and cheese (n=57) samples from five different retail shops were purchased and were transported to the laboratory. All the samples were stored at 4°C to retain originality of sampling conditions. Subsequently, each type of the food sample was microbiologically processed by carrying out serial dilution of the sample using recommended diluents and plating specific dilutions on appropriate media by spread plate method.

Collection and comparative analysis of clinical enterococci

A total of 14 enterococci isolated from urine samples were collected from the microbiology laboratory and were speciated, subjected to antibiotic susceptability testing and hemolytic activity determination. The antibiotics used to determine the susceptibility patterns of the clinical enterococci are given in Table-1. Subsequently, the antibiogram resistogram patterns of clinical enterococci were compared with the antibiotypes of food enterococci of this study. The findings were discussed and the detailed steps involved are described below:

- Microbiological Processing of Raw Milk, Packaged Milk, Butter and Cheese were processed by standard laboratory methods [2]
- The identification of enterococci from the non-enterococcal species, and subsequently its speciation was done using the standard test scheme (James Cappaccino and Natalie Sherman, 2004).

Assay of hemolytic activity

The production of hemolysin was determined according to the method of Lanyi [3], by streaking out enterococcal isolates, grown on overnight at 37°C in brain heart infusion agar, on Blood agar plates supplemented with 5% of sheep blood. Plates were incubated at 37°C for 72 hours in aerobic condition, after which the plates were examined for hemolysis. The presence or absences of zones of cleaning around the colonies were interpreted as beta-hemolysis (positive) or gamma hemolysis (negative) activity, respectively. When observed, greenish zones around the colonies were interpreted as negative for the assessment of beta hemolytic activity (Tuisa., *et al.* 2003)

Antibiotic sensitivity test for the confirmed Enterococcal isolates

Antibiotic sensitivity test was performed for isolated Gram-positive *Enterococcus* species from food samples. A sterile cotton swab was dipped into each test enterococcal culture or cell suspension of the respective isolates whose turbidity was checked with 0.5 McFarland's standard and inoculated on the entire Muller Hinton agar surface of each plate first in a horizontal direction and then in a vertical direction to ensure the even distribution of organisms, using the swab. The agar surface allowed drying for 5 minutes. Picked up the appropriate antibiotic disc by the outer edge using a flamed, sterile forceps. The discs were pressed gently with the sterile forceps to ensure firm contact with the agar surface. Incubated all plates at 37^{0}C for 24-28 hours in an inverted position and the results were recorded (Jorgensen., *et al*. 2003)

Results

In this study, the members of *Enterococcus* species were screened from food samples specifically from milk and milk products. There are about 162 milk and milk products like butter and cheese were collected from various vendors and shops in Coimbatore. The enterococcal isolates of milk and milk products were compared with 14 enterococcal isolates of urine sample collected from the microbiology laboratory and the results were given in Figure 1 to 8.

The number of enterococci was found to be more (43.60%) in butter than other samples. A total of 55 *Enterococcus* species isolated from 162 milk and milk products (butter and cheese). An average of 21.80% of isolates from milk samples, 43.60% of isolates from butter samples and 34.50% of isolates from cheese samples [Figure 1]. The average distribution of *Enterococcus* species isolate from Milk, Butter and Cheese collected from various outlets, shops and vendors was shown in Figure 2 to 4.

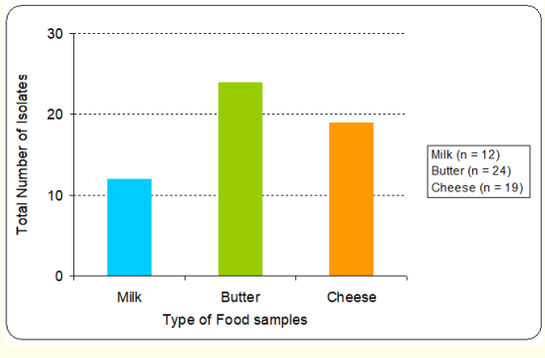

Figure 1: Distribution of *Enterococcus Species* in Different Food Samples.

Figure 2: Average Distribution of *Enterococcus Species* in Milk Samples.

Plate 1: Enumeration of *Enterococcus* Colonies on *M-Enterococcus* Agar.

Figure 3: Average Distribution of *Enterococcus Species* in Butter Samples.

Magnified form of *Enterococcus* colonies on *M-Enterococcus* Agar.

Figure 4: Average Distribution of *Enterococcus Species* in Cheese Samples.

Plate 2: *Enterococcal* growth morphology on methylene blue milk agar.

The enterococcal colonies on *M-Enterococcus* agar are shown in plate 1. The organism shows pin pointed colonies which turns brownish black on Kanamycin Esculin azide agar. Similarly, *Enterococcus* growth on *M-Enterococcus* agar, Methylene blue milk agar, Nutrient agar, Brain heart infusion agar and Ethyl Violet azide agar were shown in Plate 2,3,4 and 5.

The antimicrobial susceptibility testing of all food and clinical enterococcal isolates were found to have diverse antibiotypes [Figure 7 and Figure 8]. The Kirby-Bauer based antibiotic susceptibility patterns of food and clinical enterococci are shown in plate 6 and 7. All isolates of food and clinical specimen (100%) were

Plate 3: Enterococcal growth morphology on nutrient agar.

Plate 4: Enterococcal growth morphology on brain heart infusion agar.

Plate 5: Enterococcal growth morphology on ethyl violet azide agar.

Figure 5: Positive and negative biochemical characteristics of enterococcus species in food samples.

Figure 6: Positive and negative biochemical characteristics of enterococcus species in clinical samples.

identified to be susceptible to Chloramphenical. Altogether many different profiles were recorded on the basis of their antibiotic resistance.

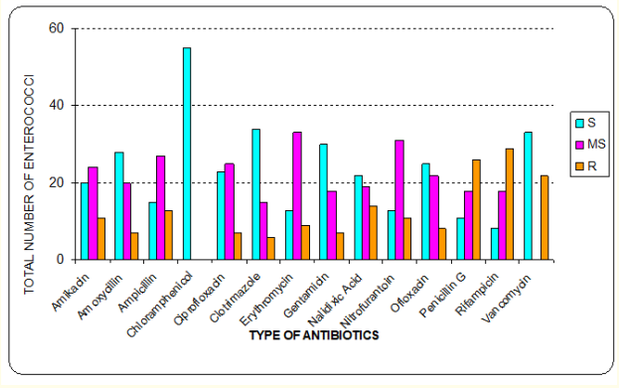

Figure 7: Antibiotypes of food enterorocci.

Figure 8: Antibiotypes of clinical enterorocci.

Plate 6: Antibiotypes of Food Enterococci based on Kirby-Bauer Method.

Plate 7: Antibiotypes of Clinical Enterococci based on Kirby-Bauer Method.

From this study, by using the basic test we identified E*nterococcus faecalis, Enterococcus faecium* and *Enterococcus durans* from the food (milk and milk products) and clinical isolates.

Discussion

In this study, we observed the prevalence of *Enterococcus faecalis, Enterococcus faecium* and *Enterococcus durans* from milk and milk products. Among the isolates, *Enterococcus faecalis* was the most predominant species [4]. The dominant species in the cheese and the milk was *Enterococcus casseliflavus* (Gelsomino, 2001), although *Enterococcus faecalis* and *Enterococcus faecium* have been reported to be the most common species of *Enterococcus i*n cheese [5,6].

Beta hemolysis on blood agar was exhibited exclusively by strains of *Enterococcus faecalis*. This property of *Enterococcus* faecalis has been mentioned in several reports and *Enterococcus faecalis* strains show frequent loss of hemolytic activity depending upon the source of blood used in blood agar medium (Priya., *et al*. 2005). The isolates of milk, butter and cheese were compared with clinical isolates for various biochemical and sensitivity tests and found significantly higher prevalence of beta-hemolysis in clinical isolates and supported by the results of previous studies [7]. Analysis of beta-hemolytic behaviour among enterococcal isolates showed a higher prevalence in *Enterococcus faecalis* and *Enterococcus faecium*, a result that correlates well with the leading role of these species as causes of enterococcal infections (Jett., *et al*. 1994, Johnson., *et al*. 1998, Mundy., *et al*. 2000) [8-17].

In both food and clinical isolates single, double and multiple antibiotic patterns were observed and antibiotic resistance strains are much higher in clinical samples compared to that of food. Further, the antibiotic susceptibility of food enterococci isolates against different antibiotics was found to be to unique from that of clinical *Enterococcus* species. The findings of this study have been compared with the reports made by many authors. Lopes., *et al*. (1999) have reported that a number of 605 enterococcal isolates screened from milk and cheese samples collected from four different Portuguese registered designation origin (RDO) areas. Further a total number of 187 cheese enterococcal isolates was identified and reported through a survey carried out by Huys., *et al*. 2004.

The susceptibility to Gentamicin (10 mcg/disc) was observed in our study is 54% which is similar to the observation of around 42% of 90 isolates of *Enterococcus* species reported to be susceptible to Gentamicin having a concentration of 10 mcg per disc by Lopes., *et al*. (2003).

The clinical isolates of Enteroroccus species of about 40% were positive for vancomycin resistant, the same findings was reported by Kathryn L. Ruoff., *et al*. 1990, Karine Gambarotto., *et al*. 2000, Shabnam Qamer., *et al*. 2003.

Among the food samples, Enterococci were found to be higher in butter compared to that of other samples. Raw milk and milk products contain more enterorocci that processed and packaged milk and milk products. Beta hemolytic strains were found to be higher in clinical isolates compared to that of food isolates. In both food and clinical isolates, single, double and multiple antibiotic pattens were observed and antibiotic resistant strains are much high in clinical isolates compared to that of food isolates. The hemolytic potential and antibiotic resistance of *Enterococcus* species may be due to change in food, chemical, half life of drugs and genomic adaptability.

The observation of this study shows that it needs further molecular techniques to identify the different type of *Enterococcus* species observed both in food and clinical samples for its genetic variations related to its presence, resistance and susceptibility to various environments. This may help to identify the nosocomial pathogenic effect of the *Enterococcus* species.

Summary and Conclusion

In this study a total of 162 food samples (milk and milk products) were collected from Coimbatore to screen for *Enterococcus* species. Of 162 samples, 55 yielded *Enterococcus* species. As much

as 12 of 55 *Enterococcus* species were isolated from milk and 24 of Enterococcus species from butter and 19 of *Enterococcus* species were isolated from cheese samples. *Enterococcus* species were isolated commonly from unpreserved raw milk, home made butters and cheese samples. About 14 clinical isolates from urine samples were collected from Microlab, R.S.Puram, Coimbatore for comparative characterization between food and clinical isolates

In the present study, to evaluate the pathogenic potential of the isolate using hemolytic assay in both food and clinical isolates of enterococci were assessed. Hemolytic behavior among clinical enterococcal isolates is higher when compared with food samples like Milk, Butter and Cheese.

The results show that enterococci were found to be higher in butter compared to that of other food samples. Similarly enterococci were more in raw milk and milk products than processed and packaged milk and milk products. Single, double and multiple antiobiotic pattens were observed in both food and clinical isolates. In clinical isolates the antibiotic resistant strains were much higher compared to that of food isolates.

The antibiotic resistance and hemolytic potential of *Enterococcus* species may be due to change in food, chemical, half life of the drugs and genomic adaptability. The recent attention focused on the *Enterococcus* is likely to continue in the foreseeable future. This study reveals that the genetic analysis is essential to identify possible epidemiological linkage among antibiotic resistant enterococci isolated from food and clinical infections.

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