Prevalence of Antibiotic Resistant Enterococci in Fast food Outlets in Osun State Nigeria.

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Abstract: Enterococci are opportunistic pathogens causing a variety of infections in man. Five hundred and twenty samples, from eleven fast food outlets comprising of food, table-top and palm of food handlers were examined for the presence of enterococci. Out of the 520 samples investigated, 211 (40.6%) were positive for enterococci. Among these samples, the highest contamination was recorded in the palm of the food handlers (51.7%), while the least was in the table-top (11.8%). Of all 211 strains of enterococci isolated, Enterococcus faecalis was the most predominant (55.0%), followed by E. faecium (30.8%) and E. gallinarum (14.2%). This study has revealed the prevalence of E. faecalis and other species of Enterococcus in the fast food outlets in Osun State, Nigeria. Although, the E. faecalis in this study showed acquired resistance traits to a number of antibiotics, they did not generally show resistance to the clinically important antibiotics, ampicillin or vancomycin, and a low incidence of resistance towards gentamycin and penicillin was observed. The presence of E. faecalis is an evidence of poor hygiene condition of some of the fast food outlets surveyed. Though, their identities were not revealed, the entries concerned were communicated on the need to comply with the rules of hygiene/good manufacturing practices. The results of this study reveal the need for inspection programme for catering premises for public health protection. [New York Science Journal. 2009; 2(7):70-75]. (ISSN: 1554-0200).

Keywords: Enterococci, Prevalence, Antibiotic resistance, Fast food outlets.

Introduction

Recent years have witnessed increased interest in enterococci not only because of their ability to cause serious infections such as endocarditis, bacteraemia, intra-abdominal and urinary tract infection (UTI), but also because of their increasing resistance to many antimicrobial agents (Moellering, 1992; Teixeira & Facklam, 2003). Enterococcus faecalis which is an indigenous flora of the intestinal tract, oral cavity and the genitourinary tract of humans and animals, are known to be relatively avirulent in healthy individuals, but have become important opportunistic pathogens, especially in hospitalized patients (Saxena et al., 2003). In Italian hospitals where active surveillance is operative, enterococci are the third most common cause of infections and cause mainly urinary tract infections (UTIs) and blood infections (Moro et al., 2001). Enterococci can readily be isolated from a range of food sources, including varieties of meat and dairy products. They are often a constituent of some mixed starter strains used commercially (Svec et al., 2001; Klein, 2003.).

Industrialization, mass fast-food production and human migration have disseminated and increased the incidence and severity of food-borne diseases world over (Klein, 2003). Eaton and Gasson (2001) in a study on the incidence of known virulence factors in medical, food and dairy starter Enterococcus strains, reveals that starter strains acquired additional virulence determinants from medical strains. Among the dozen of E. faecalis putative virulence factors reported, sets of known and potential virulence factors (e.g. aggregation substance, enterococcal surface protein [Esp], cytolysin toxin [Cyl], and gelatinase [GelE]) are widespread among various collections of isolates including food-associated isolates (Eaton and Gasson, 2001; Franz et al., 2001; Archimbaud et al., 2002; Creti et al., 2004). The findings that E. faecalis virulence genes are detected in food-associated isolates calls for safety assessment measures (Eaton and Gasson, 2001; Franz et al., 2001; Creti et al., 2004).

While many studies have assessed the diversity and antibiotic resistance (AR) of enterococci in food, the majority have focused on food before preparation and cooking (Franz et al., 2003; Giraffa 2002; Klein, 2003) during which many microorganisms and associated genes are likely destroyed. Only a few studies have evaluated enterococcal contamination in ready-to-eat foods (RTEFs), and these included cheese (Gelsomino et al., 2003; Giraffa, 2002), fanned sausages and produce (Franz et al., 2003; Johnston and Jaykus, 2004). However, RTEFs such as meals from fast-food restaurants that are very commonly consumed in developed countries, have not been assessed for the frequency and level of enterococcal contamination nor, as a source of a possible influx of AR and virulence genes to the resident microbial community in human digestive tract.

In this study the prevalence and diversity of enterococci in RTEFs (Fried-rice/Ioloif-rice, Chicken meat, Salad, Meatpie, Samosa); palm of food handlers and environment (table-top) from fast-food restaurants as well as enterococcal AR pattern were evaluated.

Materials and Methods

A total of 520 samples comprising; food, table-top and palm were collected from eleven fast food outlets in major towns and cities of Osun State. Samples were collected twice every week, for a period of three Months. Food samples were collected into sterile specimen bottles with tight screw caps while, both table-top and palm samples were collected with separate sterile swab stick. All samples were immediately labeled

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Identification and speciation

Identification and speciation of Enterococcal strains were done by using conventional biochemical tests devised by Facklam and Collins (1989) and other biochemical tests as described by Desai et al., (2001).

Antibiotics Susceptibility Test

Susceptibility of the Enterococci isolates to antibiotics was determined using disc diffusion method (NCCLS, 2000). The antibiotic multidisc containing Ampicillin (16μg), Penicillin (8μg/ml), Tetracycline (30μg/ml), Chloramphenicol (30μg/ml), Ciprofloxacin (5μg/ml), Erythromycin (8μg/ml), Gentamycin (10μg/ml) and Vancomycin (30μg/ml) (Abtek Biological Lts, U.K.) were used.

Results and Discussion

Two hundred and eleven strains of Enterococci were isolated from a total of 520 various samples from fast food outlets. The prevalence rate was 40.6%. The most frequent sources of Enterococci were found to be palms of the food handlers (51.7%), followed by food items (36.5%), while the least was table-top (11.8%) (Fig.1). Among 211 isolates of Enterococci, only 116 (55.0%) were E. faecalis, 65 (30.8%) were E. faecium, while the rest 30(14.2%) were E. gallinarum. The distribution of isolates among the various samples examined is presented in fig. 2 - 4 respectfully. This result follows the distribution pattern of enterococcal isolates from food/fast-food restaurants; with E. faecalis predominates in the studies of Macovei and Zurek, (2007). Antibiotic susceptibility test results reveal that most of the isolates have acquired resistance to a number of antibiotics. High resistance rate was recorded against chloramphenicol, followed by tetracycline, erythromycin and ciprofloxacin. This confirms the findings that AR E. faecalis strains are detected in food-associated isolates (Eaton and Gasson, 2001; Franz et al., 2001; Creti et al., 2004) Low incidence of resistance was recorded for the isolates to gentamycin and penicillin; while total susceptibility of the isolates was observed for ampicillin and vancomycin.

Many strains of Enterococci can act as opportunistic pathogens causing variety of infection leading to disease of economic and public health importance (Murray, 1990). The implication of the results obtained in this study has shown that E. faecalis and other Enterococcus species are common contaminants in Nigerian fast food outlets. These findings suggest that some of these canteens are of poor hygiene quality. This is similar to the works of Costa-Cruz et al., (1995), who reported isolation of E. faecalis in food canteens in Brazil; and Macovei and Zurek, (2007). Eaton and Gasson, (2001) also, reported incidence of Enterococci in food. Agboola, (2007) had previously reported the isolation of E. faecalis in clinical specimens in Nigeria. However, much has not been documented on incidence of E. faecalis in food or canteens in Nigeria. The contamination must be attributed to the poor hygiene practices of the food handlers which include non-disinfection of their hands before and after food processes, upon returning from toilet, lack of disinfection of table-tops, before and after daily use by the customers. This postulation is supported by the previous report on the isolation of Salmonella species and Escherichia coli from hands of food vendors and food canteens in Nigeria (Famurewa and Moro, 1989; and Famurewa et al., 2003).

The results of antibiotic resistance test on Enterococci strains isolated from palm of food handlers and other samples further, suggested that human diseases associated with Enterococci infection (such as gastrointestinal disorder) may be a common problem in Nigeria but, not reported or misdiagnosed all this while. Therefore, in the interest of public health safety, the fast food eateries operator must comply with rules of hygiene at all time. The regulatory agencies of foods, like national agency for food and drug administration control agency (NAFDAC) and state’s ministry of health should be aware of E. faecalis in our fast food outlets, for the purpose of re-enforcement of conformation with microbiological standard in canteens, because of their capacity to spread diseases easily within a large population.

Table 1: Resistance of E. faecalis, E. faecium and E. gallinarum strains isolated from fast food outlets samples to selected antibiotics.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>E. faecalis (n = 116) (%)</th>
<th>E. faecium (n = 65) (%)</th>
<th>E. gallinarum (n = 30) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>47</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>56</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Penicillin</td>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>20</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>35</td>
<td>40</td>
<td>15</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>9</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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Figure 1: Distribution of *Enterococcus species* among various samples collected from fast food outlets.

Figure 2: Distribution of *E. faecalis* among the samples collected from fast food outlets.
Figure 3: Distribution of *E. faecium* among various samples from fast food outlets

Figure 4: Distribution of *E. gallinarium* among samples from fast food outlets
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