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 vancomcin, 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 eatries 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. 2010; 3(1):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, bacteremia, intra-abdominal and urinary tract infection (UTI), but also because of their increasing resistance to many antimicrobial agents (Moellering, 1992; Teixeira & Facklam, 2003).*Enterococcus* 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 a*l., 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 increase 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 a*l.,2003; Giraffa, 2002), famented 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/Jolof-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

before taken to the laboratory in an ice-packed container. The samples were processed for the isolation of *Enterococc*i within an hour after collection. They were plated on peizers *Enterococcus* selective agar which is a bile-esculin medium and incubated at 37^0C for 24 hours. Pure cultures of the isolates were kept on nutrient agar slants at 4^oC until used.

Identification and speciation

Identification and speciation of *Enterococcal* strains were done by using conventional physiological 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 *isolate*s 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 *Escherchia 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 states' 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.

Antibiotics	***E. faecalis*** (n = 116) **(%)**	***E. faecium*** (n = 65) **(%)**	***E. gallinarum*** (n = 30) **(%)**
Ampicillin	0	0	0
Tetracycline	47	12	3
Chloramphenicol	56	15	5
Penicillin	4	3	0
Ciprofloxacin	20	16	10
Erythromycin	35	40	15
Gentamycin	9	2	0
Vancomycin	0	0	0

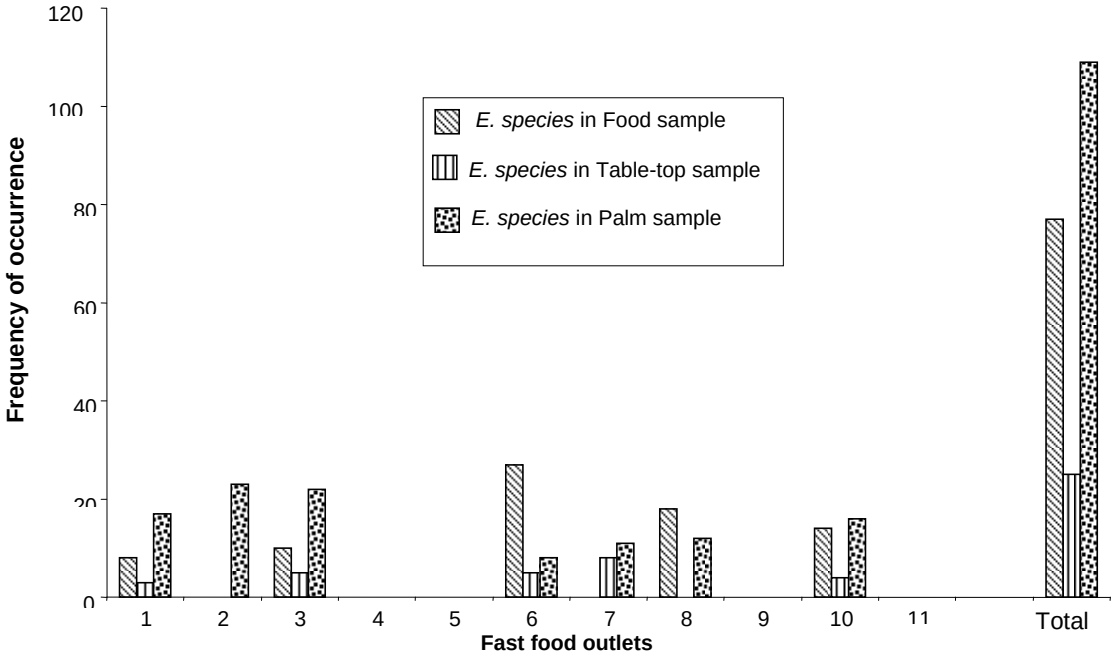

Figure.1: Distribution of *Enterococcus species* among various samples collected from fast food outlets.

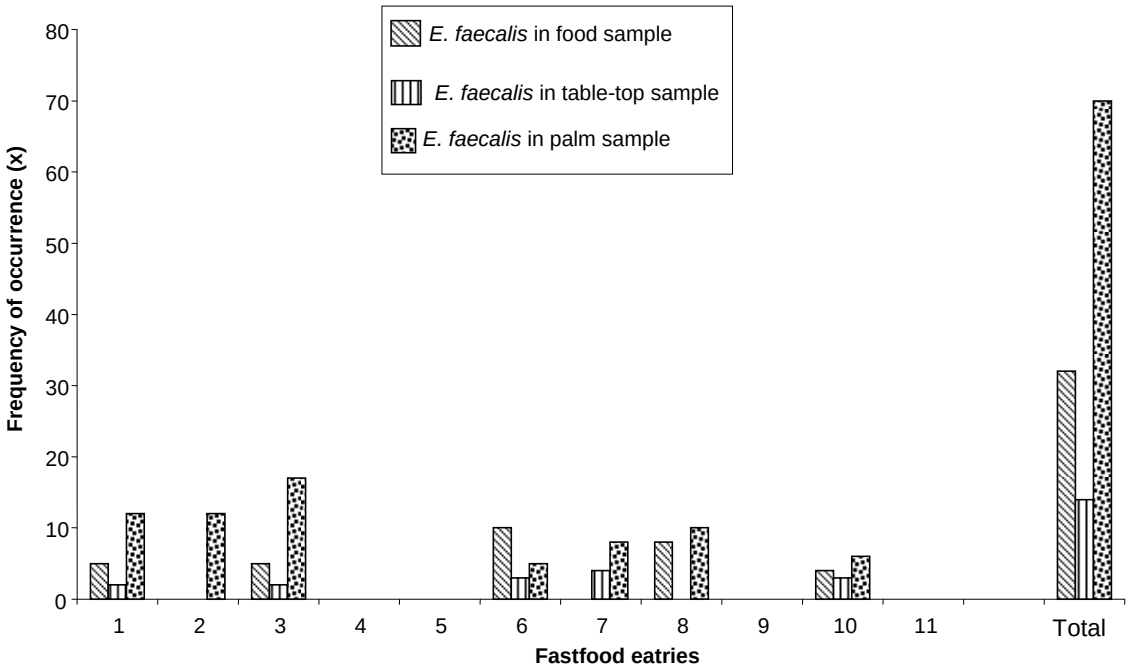

Figure.2:Ditribution of *E. faecalis* among the samples collected from fast food outlets

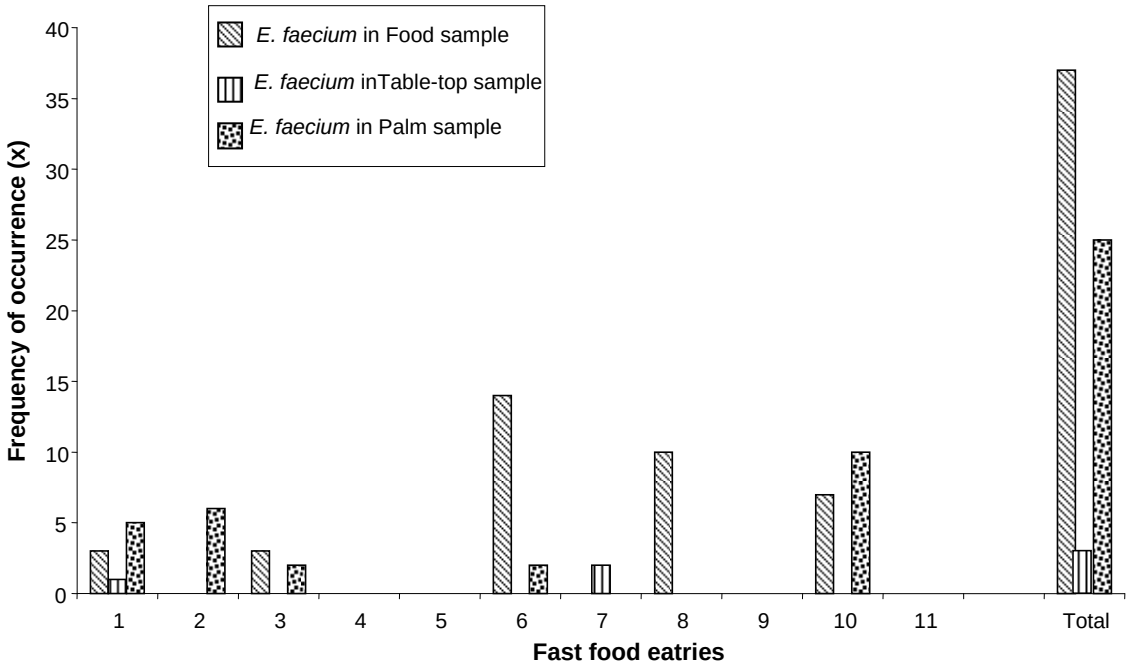

Figure.3: Distribution of *E.faecium* among various samples from fast food outlets

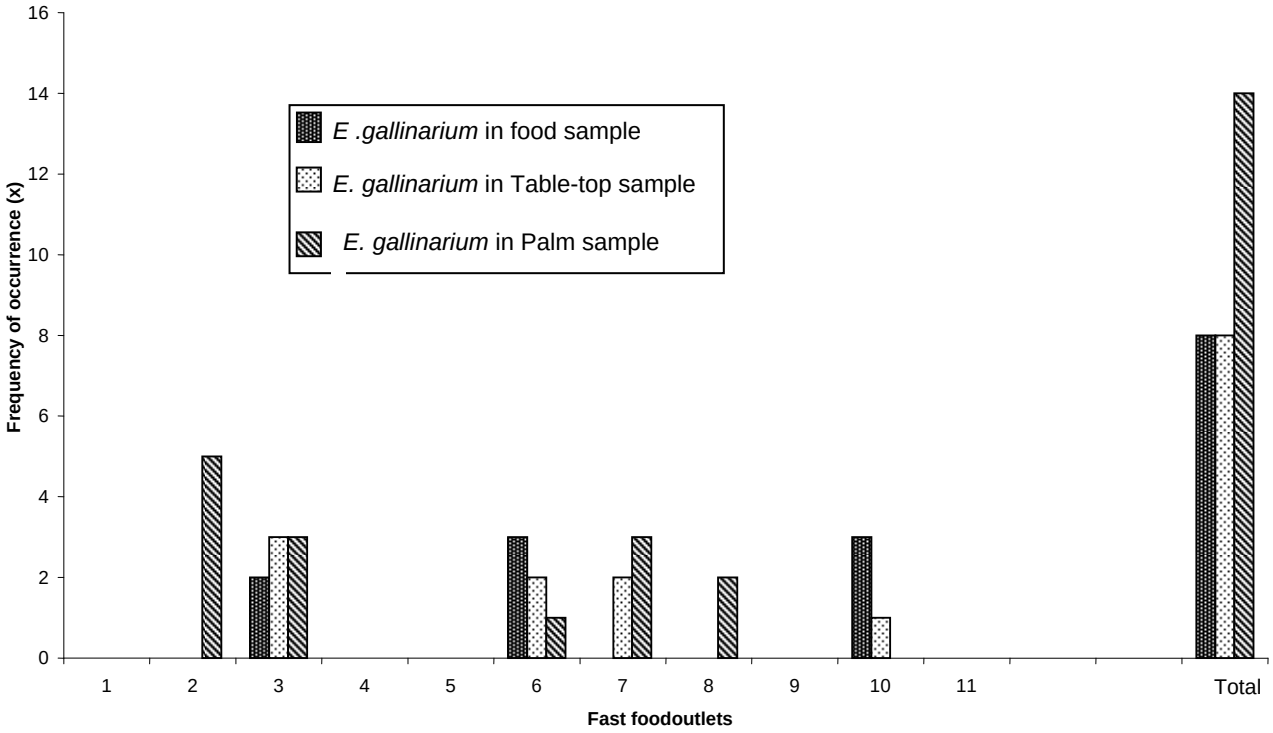

Figure 4: Distribution of*E.gallinarium* among samples from fast food outlets

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