



# **Detection and Antibiogram Profile of *Enterococcus* Species from Local Cheese Sample within Ikare and Akungba Akoko, Ondo State Nigeria**

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## **Author's contributions**

*The sole author designed, analyzed, interpreted and prepared the manuscript.*

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## **ABSTRACT**

*Enterococci* are part of the normal intestinal flora of humans and animals and are increasingly recognized as significant human pathogens and capable of causing major therapeutic challenges. The aim of this study was to isolate, identify and determine the antimicrobial susceptibility pattern of *Enterococcus* species in two local cheese sample collected from Akungba and Ikare. The isolation of *Enterococcus* was carried out using standard culture-based techniques. Antimicrobial susceptibility profile of the *Enterococcus* species was determined using disk diffusion method. A total number of 93 presumptive *Enterococcus* species were recovered from forty two different samples over a period of three months. The bacterial count observed on the Bile Aesculin agar ranged from  $1.5 \times 10^4$  cfu/ml to  $4.6 \times 10^4$  cfu/ml with Akungba sample having the highest bacterial load of  $4.6 \times 10^4$  cfu/ml. The Morphology and biochemical characteristics of suspected *Enterococcus* spp. isolated from the cheese sample revealed *Enterococci faecalis* as the isolated bacteria. The isolated *Enterococcus* species were tested against a panel of six antibiotics which include Penicillin G, Vancomycin, Tetracycline, Nitrofurantoin, Ciprofloxacin and Imipenem. It was observed that the isolates were susceptible to tetracycline, imipenem and ciprofloxacin while they are resistant to ciprofloxacin and vancomycin. The prevalence of *Enterococci* was confirmed with *Enterococcus faecium* and *Enterococcus faecalis* as the predominant species isolated in both cheese sample. The ability of *Enterococcus* species to survive a range of adverse environments allows multiple

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routes of cross-contamination of *Enterococci* in causing human disease, including those from food. Overall, greater understanding of the ability of *Enterococcus* species to survive stresses, of virulence traits and especially of increasing antibiotic resistance, is needed in order to fully appreciate the complexity of *Enterococcus* species in causing disease.

**Keywords:** *Enterococcus*; cheese; antibiotics resistance; environments; disease.

## 1. INTRODUCTION

*Enterococci* are Gram-positive bacteria ubiquitously found in the gastrointestinal tracts of mammals, as well as in soil and water [1]. Food, especially when eaten raw, is a possible intermediate vehicle for the transmission of multidrug and/or virulent *Enterococci* strains from animal origin that can persist in the human intestinal tract [2-4]. *Enterococci* are mainly opportunistic pathogen that are harmless in healthy individuals and mainly cause infection in patients who are in intensive care units, with underlying disease or who are immune-compromised [5]. *Enterococci* are ubiquitous bacteria which colonize different niches. The primary habitat is considered to be the gastrointestinal tract of animals and humans, thus via fecal contamination reach the raw milk and meat [6]. The wide distribution of the *Enterococci*, their resistance to high temperatures, tolerance to high salt concentration augmented their survival through the production process and implication that this group of organisms, may also be isolated from heat treated milk and dairy products, especially cheese made from raw milk [7]. *Enterococci*, present in raw milk, may develop during cheese making process and ripening period and may represent the predominant microflora found in cheese made from raw milk [8]. It is noteworthy that *Enterococcus* species are also predominant pathogens causing bovine mastitis, which affects udder health and milk quality [9,10]. Consequently, contamination of raw milk cheeses with animal, human or environmental *Enterococcal* strains during different stages of processing is likely to occur. Of note, different studies investigating the microbiological quality of raw milk cheeses confirmed the difficulties in obtaining safe raw milk cheeses for consumption in many countries all over the world [11] Within the genus *Enterococcus*, two species, *Enterococcus faecalis* and *Enterococcus faecium*, have emerged as opportunistic pathogens and are responsible for an increasing percentage of nosocomial infections, including bacteremia and intra-abdominal and urinary tract infections [12].

Recently, the evolution of hospital acquired multidrug-resistant *E. faecium* strains from *E. faecium* of animal origin has been elucidated [13] and indicates that *E. faecium* of animal origin can act as a donor of antibiotic resistance genes to other pathogenic *Enterococci*. The aim of this study was to carry out quality indices survey of *Enterococcus* spp. from the local cheese samples in Akungba Akoko, Ondo State, Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

Fresh and processed Wara and Nunu samples were collected from two different towns in Ondo State which includes; Akungba-Akoko and Ikare-Akoko.

### 2.2 Sample Collection

Samples were obtained from Akungba and Ikare between July and September, 2018. Wara and Nunu samples were obtained using 40ml sterile screw capped plastic bottles and immediately placed on ice. Samples were thereafter conveyed to the laboratory for microbiological analysis within 2 h after collection.

### 2.3 Enumeration and Isolation of Bacterial Isolates

An aliquot of 0.1 µl of 10<sup>-4</sup> dilution of each sample was inoculated into the Nutrient agar (Oxoid, United Kingdom) and Bile Esculin Azide agar (Oxoid, United Kingdom) for heterotrophic and enterococci enumeration respectively. All Media were sterilized by autoclaving at 121°C for 15mins. After incubation, bacterial colonies were enumerated on the Nutrient agar and expressed as colony forming units per ml (cfu/ml). Black hallow colonies on Bile Esculin Azide agar were considered as presumptive enterococci colonies and were enumerated and expressed as cfu/ml. Thereafter, an average of 3 to 4 enterococci isolates was selected per positive plate and subcultured on fresh Bile Esculin Azide agar and incubated aerobically at 37 °C for 18-24 h.

Isolates were then purified on Nutrient agar and stored on agar slants at 4 °C until ready for further analysis.

### 3. RESULTS

The bacterial count observed on the Bile Aesculin agar ranged from  $1.5 \times 10^4$  cfu/ml to  $4.6 \times 10^4$  cfu/ml with Akungba sample having the highest bacterial load of  $4.6 \times 10^4$  cfu/ml while Akungba sample had the lowest microbial load with  $1.5 \times 10^4$  cfu/ml. The Morphology and biochemical characteristics of suspected *Enterococcus* spp. isolated from the cheese sample is shown in Table 1 which reveal *Enterococci faecalis* and *Enterococcus faecium* as the isolated bacteria. The biochemical tests

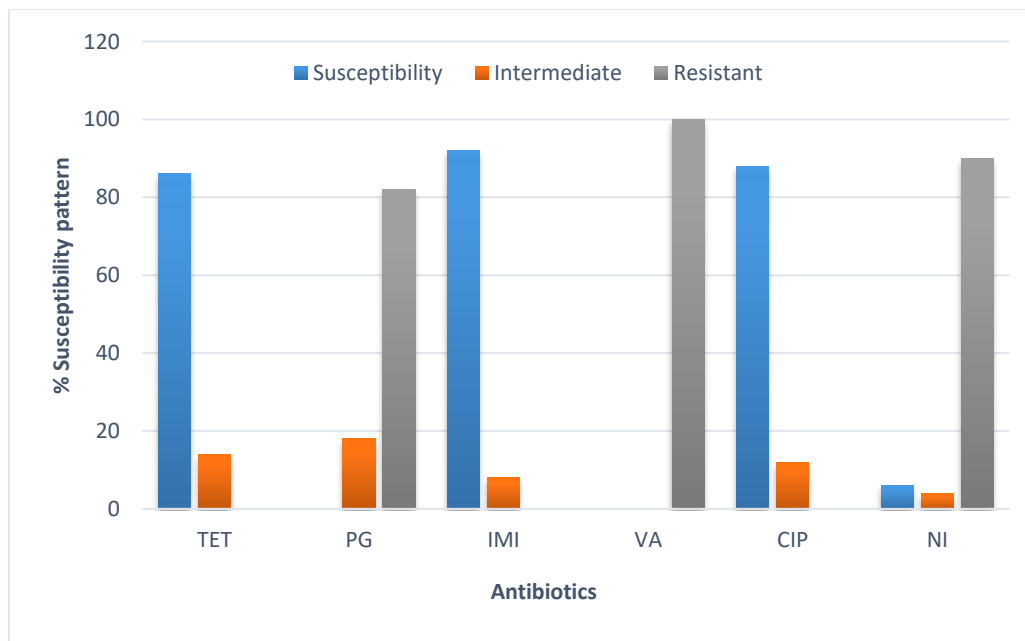
such as oxidase test and sugar tests such as lactose, glucose, maltose, mannitol, sucrose and fructose carried out and shown in the table also supported the identification of the presumptive isolates as listed in the table.

The isolated *Enterococcus* spp were tested against a panel of six antibiotics and the results are tabulated based on the cattle farm in which the study was carried out. The antibiotics used were Penicillin G, Vancomycin, Tetracycline, Nitrofurantoin, Ciprofloxacin and Imipenem. It was observed that the isolates were susceptible to Tetracycline, Imipenem and Ciprofloxacin but they are resistant to Penicillin G, ciprofloxacin and vancomycin.

**Table 1. Morphology and biochemical characteristics of representative presumptive *Enterococcus* species recovered from cheese samples**

Isolates	Shape	Oxidase	Mannitol	Glucose	Sucrose	Fructose	Lactose	Probable identity
1	Cocci	-	Ag	Ag	Ag	Ag	Ag	<i>Enterococcus faecium</i>
2	Cocci	-	Ag	Ag	Ag	Ag	Ag	<i>Enterococcus faecium</i>
3	Cocci	-	Ag	Ag	Ag	Ag	Ag	<i>Enterococcus faecalis</i>
4	Cocci	-	Ag	Ag	Ag	Ag	Ag	<i>Enterococcus faecium</i>
5	Cocci	-	Ag	Ag	Ag	Ag	Ag	<i>Enterococcus faecalis</i>

Key:- = Negative; Ag = Acid gas positive



**Fig. 1. Antibiotic susceptibility profiles of presumptive *Enterococcus* species isolated from selected rivers**

Key: TET = Tetracycline; PG = Penicillin G; IMI = Imipenem; CIP = Ciprofloxacin; NI = Nitrofurantoin

#### 4. DISCUSSION

The genus *Enterococcus* is a diverse group of bacteria that have been reported in a variety of niches with gastrointestinal tract being their principal habitat [14]. The prevalence of *Enterococci* was confirmed with *Enterococcus faecium* and *Enterococcus faecalis* biochemically as the predominant species isolated in both cheese sample. The identification of *Enterococcus* species is vital because for many years *Enterococci* were believed to be harmless to humans and considered unimportant medically. Recently, *Enterococci* have become one of the most common nosocomial pathogens, giving a high mortality rate of up to 61 % [15]. The ability of *Enterococcus* species to survive a range of adverse environments allows multiple routes of cross-contamination of *Enterococci* in causing human disease, including those from food. The occurrence of resistant and virulent strains of *Enterococcus* species in farm animals could be detrimental to both the animals and the human population. The high counts of *Enterococcus* species obtained indicated that the cheese samples might have been contaminated by either the anthropogenic activities of the food handlers or the cattle is infected. This study provides information about the antibiotic resistance patterns of *Enterococci* isolated from cattle farms as well as their virulent potentials. Previous studies have shown that the occurrence of resistant strains of *Enterococcus* in cattle farms is due to the overuse of antibiotics in farm management [16]. The increasing concern about this organism is their occurrences and the spread antibiotic resistance genes (Lester *et al.*, 2011). The isolated strains of *Enterococci* were resistant to following antibiotics: penicillin G, vancomycin and nitrofurantoin. Bulajić and Mijačević [17] reported forty two strains of isolated *Enterococci*, which were subjected to antibiotic susceptibility testing. They reported that the isolated strains of *Enterococci* were resistant to the following antibiotics: penicillin, tetracycline, lincomycin, gentamycin, neomycin, erythromycin and chloramphenicol. In another study that was carried out in Thailand, a significant prevalence of MDR enterococci (10.3%) were Vancomycin resistant *Enterococcus* (VRE) isolates, in water samples including agricultural wells of food animal farms, canals and rivers [18]. Antibiotic resistance could be associated to the abuse of antibiotics due to over-the-counter medication and accessibility to patients devoid of doctor's prescription due to upsurge of pharmacies and patent medicine stores. In addition, consumption

of animal products treated with these antibiotics as growth promoters and prophylactic agents could have added to the resistance [19]. In order to evaluate the potential risk of food contaminated with resistant strains of *Enterococci* represent to human health, it is important to distinguish between intrinsic and acquired antibiotic resistance [20,21]. Further investigations are needed to address this question.

#### 5. CONCLUSION

Overall, greater understanding of the ability of *Enterococcus* species to survive stresses, of virulence traits and especially of increasing antibiotic resistance, is needed in order to fully appreciate the complexity of *Enterococcus* species in causing disease.

#### CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

#### ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

#### COMPETING INTERESTS

Author has declared that no competing interests exist.

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