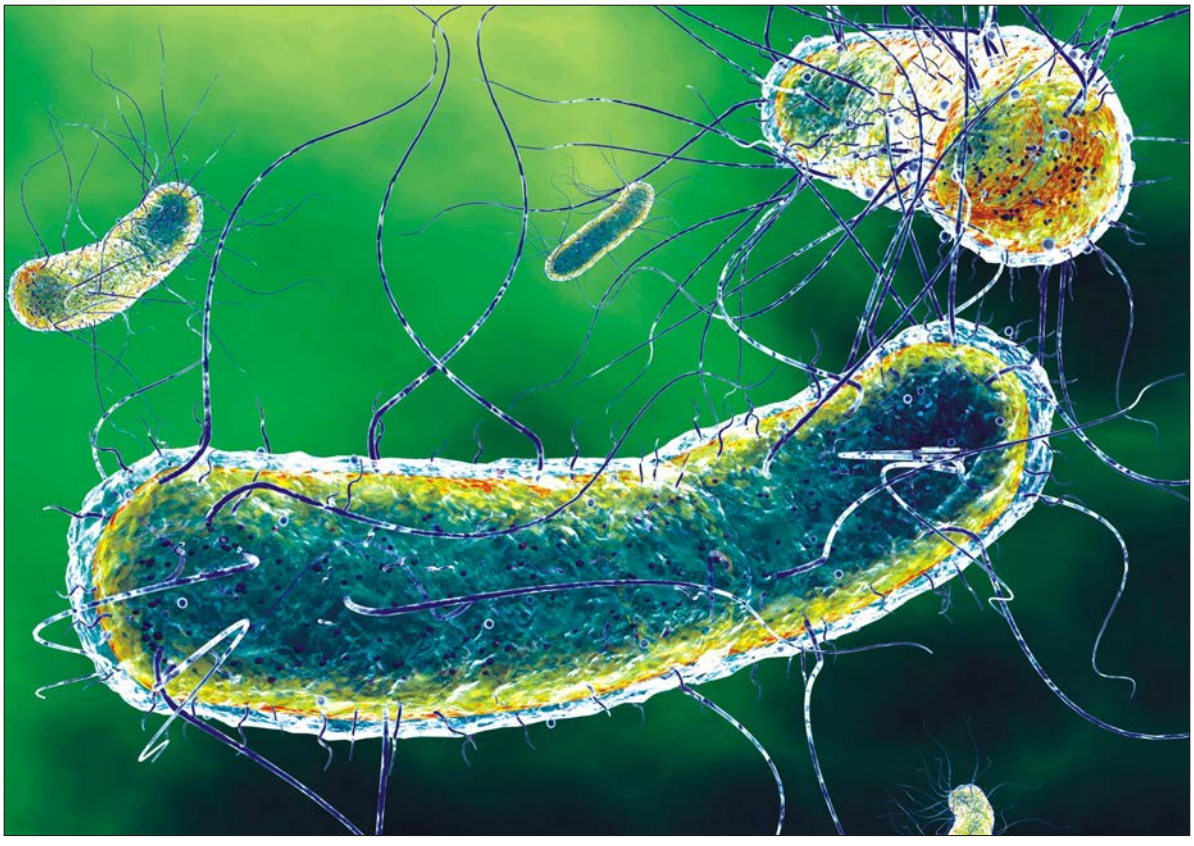

Current Research in Bacteriology

ISSN 1994-5426

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OPEN ACCESS Current Research in Bacteriology

ISSN 1994-5426
DOI: 10.3923/crb.2017.9.15

Research Article
Occurrence of Antibiotic Resistance and Virulent Factors in *Enterococcus faecalis* Isolated from Bush Meat Roasted and Sold along Road Sides in Ekiti State

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Abstract

Background and Objectives: In Nigeria wild animals are hunted for meat mainly. Meanwhile, meat from wild animals are known to consist of pathogens apart from being contaminated by unhygienic environments (road sides) where they are mainly prepared and sold. The objective of this study was to investigate the occurrence of *Enterococcus faecalis* (*E. faecalis*) in samples of roasted bush meat prepared and sold along Ado-Ekiti-Ilesha road. **Materials and Methods:** A total of 182 roasted bush meat samples were collected in seven selected towns in Ekiti State, Nigeria between January and February, May and June, 2016 representing dry and rainy seasons, respectively. The samples were examined for the presence of enterococci within 2 h of collection. Standard methods were used to identify *Enterococcus faecalis,* determined its resistance to antibiotics and also determine the virulence factors in the sample. **Results:** A total of 91 (32.38%) out of 281 samples of roasted bush meat examined were contaminated with *E. faecalis.* The highest rate of contamination 61.22% was observed in samples collected from Igede-Ekiti while the least 6.70% was observed from samples collected in Ado-Ekiti. Rates of contamination among samples from other selected towns were, 40, 38.71 and 38.1% from Efon-Alaye-Ekiti, Iyin-Ekiti and Erio-Ekiti, respectively. Antibiotic susceptibility test results reveal that some of the isolates have acquired resistance to a number of antibiotics. High resistance rate was recorded against ampicillin 35.71%, followed by gentamicin 30.22%, ciprofloxacin 28.02% and ofloxacin 24.73%. The incidence of virulence factors was low in all the isolates with aggregation substance, haemolysin and gelatinase recording 7.69, 8.24 and 27.47%, respectively. **Conclusion:** The incidence of virulence factors in *E. faecalis* is an evidence of potential pathogenesis. The roasted bush meat screened from road sides in Ekiti State was contaminated with *E. faecalis.* There is need for strict monitoring and proper hygiene education for the food handlers in the study area.

Key words: Roasted bush meat, food hygiene, antibiotic resistance, *Enterococcus faecalis*, ampicillin

Received: September 28, 2016 Accepted: November 18, 2016 Published: December 15, 2016

Citation: Olawale Adetunji Kola, David Oluwole Moses, Onasanya Amos, Ajayi Ayodele Oluwaseun, Osuntoyinbo Richard Tope, Idris Olayinka Oluwatoyin and Oje Opeyemi James, 2017. Occurrence of antibiotic resistance and virulent factors in *Enterococcus faecalis* isolated from bush meat roasted and sold along road sides in Ekiti State. Curr. Res. Bacteriol., 10: 9-15.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Enterococci are contaminants of various foods, especially those of animal origin which could lead to food-borne diseases. Food borne diseases remain an important public health problem worldwide, one of the most significant food safety hazards is associated with foods from animals[1,2]. Meat is considered as the most important source of proteins consumed by humans. However, meat is the most perishable of all staple foods since it contains sufficient nutrient needed to support the growth of microorganisms[3]. It is a common practice in Ekiti State and many other parts of Nigeria to roast bush meats (meat of wild animals such as; antelope, grass cutter, deer and many others) and sell to motorists along highways. The hygiene practice in this business is usually poor due to the low level of hygiene education. To lower the incidence of food-borne diseases adequate interventions using the best available data on the distribution and reduction of risks is indispensable[4]. In that respect the understanding of the many risk factors between the point of production and the point of consumption and the ability to systematically target intervention efforts along this "farm-to-fork" continuum is necessary[4].

The possible sources of these bacteria are likely to come from the skin of the animal from which the meat was obtained. Other potential sources of microbial contaminations are the equipments used for each operation that is performed until the final product is eaten; the clothing and hands of personnel and the physical facilities are all implicated[5]. Retail cut could also result in greater microbial load because of the large amount of exposed surface area, more readily available water, nutrient and greater oxygen penetration available[6,7]. These conditions are favourable for microbial growth and proliferation which leads to spoilage and contamination of the meat[8,9]. Poor quality water is mostly used in preparation of bush-meats. For highly perishable food stuffs such as fresh bush meat, the threat of food poisoning is particularly high[10,11]. Poor sanitary conditions of catering establishments and presence of pathogenic organisms such as *Campylobacter, Salmonella*, *Staphylococcus aureus, Bacillus cereus, Escherichia coli*, *Enterococcus faecalis* have been reported to mainly cause food-borne infections[12].

Enterococci are ubiquitous, occurring in traditional fermented food and dairy products, water surfaces, plants and animals[13-16]. The increased association of enterococci with human disease has raised concern about their use as probiotics[17,18]. *Enterococcus faecalis* and *E. faecium* are among the leading causes of nosocomial infections and may cause endocarditis, urinary tract infections and bacteraemia[12,19,20]. In the world, one of the most important food safety hazards is associated with undercooked meat and poultry[21]. Street vending of foods is a common characteristic of countries with high unemployment rates, low salaries and poor social security programme. Contaminated foods, from fresh red meat infected with microorganisms, can lead to consumer health problems. Ologhobo *et al.*[22] observed that microbial counts of chicken and beef suya (street sides roasted meat) were at levels that pose health problems to consumers. *Enterococcus faecalis* predominates among enterococci isolated from the environment and from human infections (more than 80%), while *E. faecium* is associated with the majority of the remaining infections[23].

The consumption of street vended food is frequently associated with diarrheal diseases which occur as a result of improper use of additives, the presence of pathogenic bacteria, environmental contaminants and disregard of good manufacturing and hygiene practices[24]. Sellers are often poorly educated, unlicensed, untrained in food hygiene and they work under crude unsanitary conditions with little or no knowledge of the causes of food-borne disease[25-29].

Antibiotic treatment eliminates vulnerable bacteria from the bacterial population, leaving resistant bacteria to grow and multiply. In *E. faecalis*, acquired elements, including antibiotic resistance genes, are estimated to represent over 25% of its genome[30-32]. Serious enterococcal infections are often difficult to treat since the organisms have a tremendous capacity to acquire resistance to penicillin, high concentration of aminoglycoside and vancomycin[9]. However, the aim of this study was to investigate the information about the prevalence of antibiotic resistant, virulence factor borne *E. faecalis* from food origin, especially ready-to-eat roasted bush meat is scanty particularly in the study area.

MATERIALS AND METHODS

Sampling: A total of 182 roasted bush meat samples were collected along road sides in 7 selected towns in Ekiti State, Nigeria. The samples were collected between January and February (dry season), May and June (rainy season) of 2016. The roasted bush meat samples were categorized into 3 [Fresh (0-6 h), semi-stale (a day) and stale bush meat (2 days and above)] and were aseptically collected. The samples were collected into sterile aluminum foil and transferred in an ice-packed container to the laboratory for analysis. Samples were kept frozen at -20°C whenever bacteriological analysis could not be performed within 24 h of collection[32,33]. The

samples were plated on Bile Aesculin Azide agar and incubated at 37°C for 24 h. Pure cultures of the isolates were kept on nutrient agar slants at 4°C until used. All chemical used are of analytical grades.

Identification and speciation: Bacterial colonies that produces black hallo on Bile aesculin azide agar (Oxoid) were suspected to be enterococci. The isolates were identified by colonial characteristics by Gram reactions, motility and oxidase tests. The ability of the isolates to utilize arabinose, inulin, lactose, mannitol, raffinose, sorbitol and sucrose were determined as described by Desai *et al.*[34] and Olawale *et al.*[35].

Antibiotics susceptibility test: Susceptibility of the recovered *E. faecalis* isolates to antibiotics was determined using disc diffusion method[36]. The isolates were tested against eight commercial antibiotic disks (Abtek Biologicals Limited) their concentrations in microgram in the discs were as follows: ampicillin (16), chloramphenicol (30), ciprofloxacin (5), gentamicin (10), linezolid (30), ofloxacin (8), tetracycline (30) and vancomycin (30).

Examination of virulence determinant factors: One hundred and eighty two isolates were examined for the expression of three putative virulence determinants factors, gelatinase, aggregation substance and haemolysin by phenotypic tests as follow.

Detection of gelatin hydrolysis: The method of Beceiro *et al.*[37] was used with slight modification to detect gelatinase production among the isolates. Briefly, nutrient agar supplemented with 0.4% by weight, of gelatin (BDH, Merck Chemicals Ltd., Nottingham, England, UK) with a final pH 7.2, was prepared and isolates were streaked on the plates and incubated for 48 h at 37°C. The plates were observed for growth and subsequently flooded with 10 mL of Frazier's solution (mercuric chloride, 15.0 g in 20 mL of 37% v/v hydrochloric acid, made up to 100 mL with distilled water). The plates which showed area of opaque layer with zone of clearance around the colonies were taken as positive for gelatin hydrolysis and an uninoculated plate was used as negative control.

Detection of haemolysin production: Brain heart infusion agar (Oxoid) supplemented with 5% rabbit blood was used for the detection of haemolysin activity. Prepared plates were streaked with the isolates and incubated at 37°C for 24 h. After incubation clear zone around the colonies on the plate were recorded to produce beta-haemolysis[38].

Aggregation substance: Phenotypic expression of the *Asa1* gene was investigated using the method of Macovei and Zurek[39]. Each of *E. faecalis* strains was grown for 6 h at 37°C in Todd-Hewitt broth (Becton Dickinson, MA). The broth was then centrifuged at 6,000 rpm for 10 min and the pheromone-containing supernatant that induces pheromone-responsive plasmids was removed and autoclaved for 15 min. Tested isolates were grown in Todd-Hewitt broth (5 mL) for 6 h at 37°C. After incubation 1 mL of the supernatant from the isolates was added to each tube and incubated at 37°C overnight on a shaker at 150 rpm. Isolates that showed clumping were considered positive for aggregation substance expression. *Enterococcus faecalis* OGIRF served as positive control.

RESULTS AND DISCUSSION

A total of 91samples of 281 roasted bush meat examined were E. *faecalis* contaminated. The highest rate of contamination was recorded from Igede-Ekiti while the least was in samples from Ado-Ekiti. Rates of contamination among samples from other selected towns varies; 40, 38.71 and 38.1% from Efon-Alaye-Ekiti, Iyin-Ekiti and Erio-Ekiti, respectively (Table 1). The rate of *E. faecalis* contamination of bush meat (32.38% of 281) sold in the studied area was high. This could probably be due to the poor hygiene practices during bush meat processing. There was variation in contamination according to the freshness of the roasted bush meat samples. A total of 94.12% of stale samples were contaminated, followed by 55.60% of semi-stale samples while, 1.90% of the fresh samples were contaminated. It was observed that the longer the roasted meat exposed to the road sides environment the more contaminated it became. Similar reports of *E. faecalis* from animal meat had earlier been made[7,16,40]. Improper handling and poor hygiene could eventually affect the health of consumers[41-45]. Unhygienic nature of the production environment, bare hand touching of the meat by the sellers and some prospective buyers and poor storage conditions are likely responsible[22]. The traditional processing methods that are used in the preparation, inappropriate holding temperature and poor personal hygiene of food handlers are some of the main causes of contamination of ready- to-eat foods [46,47]. Also such foods are not effectively protected against flies and dust[48,49]. The results were in consonance with the report of Anihouvi *et al.*[50], who reported isolation of pathogenic bacteria from roasted meat sold on road sides Benin Republic. Macovei and Zurek[39], Eaton and Gasson[51] also reported incidence of *Enterococcus* in food.

Table 1: *Enterococcus faecalis* isolates from roasted bush meat sold along road side in Ekiti State

	Sources and number of samples							
Bush meat samples	Ado	Iyin	Igede	Aramoko	Erio	Itawure	Efon alaye	Total
Rain season samples	16	12	21	18	18	21	6	112
Dry season samples	29	19	28	20	24	30	19	169
Total samples examined	45	31	49	38	42	51	25	281
Number of contaminated samples	3	12	30	10	16	10	10	91
Rate of contamination (%)	6.70	38.71	61.22	26.32	38.10	19.61	40	32.38
Number of *E. faecalis* recovered	6	24	60	20	32	20	20	182

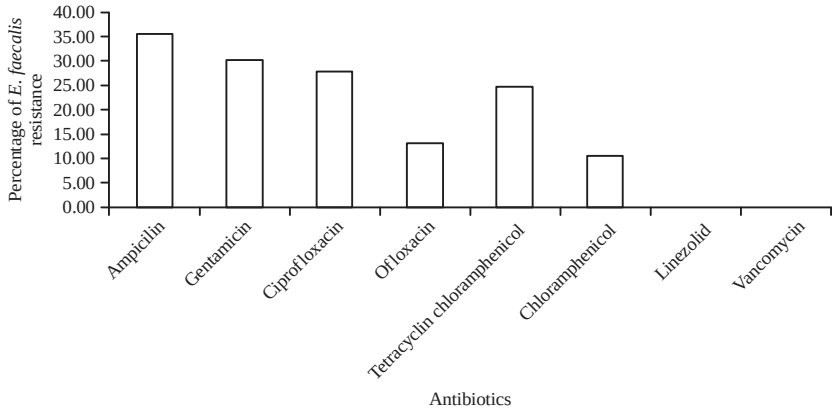

Fig. 1: Resistance pattern of *Enterococcus faecalis* isolates to antibiotics

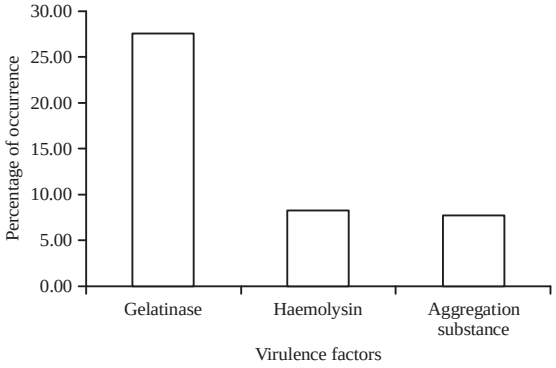

Fig. 2: Frequency of occurrence of virulence factors in *E. faecalis* from roasted bush meat

Antibiotic susceptibility test results (Fig. 1), revealed that some of the isolates have acquired resistance to a number of antibiotics. High resistance rate was recorded against ampicillin 35.71%, followed by gentamicin 30.22%, ciprofloxacin 28.02% and ofloxacin 24.73% while, total susceptibility of the isolates was observed for linezolid and vancomycin.

High resistance rate recorded against some antibiotics confirmed the fact that the isolates had prior exposure to antibiotics[17,51-53]. The antimicrobial resistance of *E. faecalis* should not only be of treatment concern but also virulence of the organism should be considered according to Damborg *et al.*[33] and Jorgensen *et al.*[40]. Many strains of enterococci can act as opportunistic pathogens causing variety of infections leading to disease of economic and public health importance[54].

Phenotypic expression of putative virulence factors was low in all the isolates. Aggregation substance 7.69%, haemolysin 8.24% and gelatinase 27.47% were detected though at different rates (Fig. 2). The incidence of gelatinase production among food *E. faecalis* strains in this study was much lower than the findings for clinical strains[53,55]. This value was also lower than the 56% incidence among nine *E. faecalis* strains recovered from food by Eaton and Gasson[51].

Meanwhile the incidence of virulence gene in *E. faecalis* strains in this study is an evidence of potential pathogenesis[7,53]. Previous studies have shown that phenotypes such as haemolysin and aggregation substance, which are encoded by *E. faecalis* pheromone-responsive plasmids, are related to pathogenicity and enhance the virulence of enterococci in animal models[37,45,56]. The implication of the results obtained in this study has shown that *E. faecalis* and other enteric bacteria are common contaminants in the roasted bush meat produced and sold along road sides in Ekiti state. The results of antibiotic resistance and virulence factors tests on *E. faecalis* strains

isolated from the roasted bush meat further could contribute to enterococcal infections in human[9,18]. Therefore, in the interest of public health safety, the roasted bush meat handlers must comply with rules of hygiene at all time and better still the meat should be further processed before consumption.

CONCLUSION

This study reveals high level of potentially virulent and antibiotic-resistant *E. faecalis* contamination in roasted bush meat produced and sold along road sides in Ekiti state. Hence, the product could not be considered as safe. Poor hygiene and bad food manufacturing practices may have been factors responsible for the contamination. Consequently, there is a need to educate roasted bush meat processors/sellers on good hygiene and manufacturing practices.

SIGNIFICANCE STATEMENTS

This study discovered that most roasted (ready-to-eat) bush meat sold in the study locations were contaminated with *E. faecalis*. The strains of the isolates were antibiotic resistant and possess virulence factors. This study will help the consumers of such product to know the danger it poses and also that it may serve as a vehicle or reservoir of infectious diseases.

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