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Virulence characteristics and phylogenetic background of antibiotic-resistant *Enterococcus faecalis* from abattoir, poultry and clinical origin in Ado-Ekiti, Nigeria

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Enterococcus faecalis is a major cause of nosocomial infection in human and severe extra-intestinal infections in animals. _Enterococcus_ spp possesses the ability to acquire and spread genes linked with antimicrobial resistance which makes them an important nosocomial pathogen. This study aims to determine the distribution of antibiotic resistance and virulence genotype of _Enterococcus faecalis_ in animal, poultry and clinical sources. A total of 150 samples made up of poultry droppings, abattoir and clinical specimens were collected. Standard bacteriological methods were used in the isolation and characterisation of _E. faecalis._ Fifteen _E. faecalis_ isolates were examined for virulence determinant genes through quantitative Polymerase Chain Reaction analysis, while disc diffusion technique was used in determining the antibiotic-resistant pattern of the isolates. The highest prevalence of _Enterococcus faecalis_ was in poultry droppings (94.0%) and lowest in clinical samples (24.0%). Enterococcal surface protein _(Esp)_ was detected in 2/7, 0/5 and 0/3 of poultry, abattoir and clinical isolates, respectively. Antibiotic susceptibility pattern of the isolates indicated high resistant in poultry, abattoir and clinical samples as follows: Erythromycin (87.2%), (100.0%), (100.0%); Cloxacillin (72.3%), (90.5%), (91.7%); Cefuroxime (68.1%), (100.0%), (66.7%) and Augmentin (63.8%), (81.0%), (66.7%). High prevalence of antibiotic resistance and high virulence potential were observed among the _E. faecalis_ isolated. There is a need for constant epidemiological surveillance and strict enforcement of good hygiene practices in the study areas.

Key words: *Enterococcus faecalis,* antibiotic resistance, virulence, Nigeria.

INTRODUCTION

Enterococcus species have become one of the most common nosocomial pathogens, with patients having a high mortality rate of up to 61% (Shinde et al., 2012). They are of major importance in community-and hospital-

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acquired infections, and estimated to be responsible for approximately 12% of all nosocomial infections globally (Shinde et al., 2012). *Enterococcus faecalis* has been reported to be the most predominant enterococcus species, accounting for 80 to 90% of all clinical isolates (Shinde et al., 2012).

Enterococci occur in a remarkable array of environments, since they are able to grow and survive under harsh conditions. They can be recovered from water, soil, food, and a variety of animals, birds and insects (Murray, 2010). In humans, the major habitat of enterococci appears to be the gastrointestinal tract although isolated less frequently from other body sites. *Enterococci* are contaminants of various foods; especially those of animal origin and are found as members of the natural microbiota of a variety of fermented food products such as artisanal cheeses and fermented sausages. They reportedly play an important role in food processing (Giraffa and Skinner, 2010; Franz et al., 2011). The transmission of resistant enterococci or resistance genes takes place between humans and animals in a community (Klare et al., 2005). One possible explanation for animal to human transmission was the use of glycopeptide growth promoters like Avoparcin in feed animals (Klare et al., 2005).

E. faecalis is the most commonly isolated species from root canal samples with treatment failure, periapical lesions or chronic apical periodontitis (Sedgley et al., 2013). Its present potential threat to general health is not yet known because endodontic infecting microorganisms are localized in a restricted area within the root canal system and little is known on the extra radicular presence of *E. faecalis* (Sunde et al., 2012). It is able to colonize the oral cavity, particularly in patients with periodontitis or root canal infections associated with oral mucosal lesions and in immunocompromised patients (Pinheiro et al., 2006). It is frequently isolated from patients suffering from dental infections like periodontitis, gingivitis, teeth with failed endodontics and infected root canals.

E. faecalis has some virulence determinants such as gelatinase (*gel*A), aggregation substance (*asa*1), cytolysin *cyl*A, enterococcal surface protein (*esp*) and collagen-binding protein (*ace*) and could be the reason for its survival in a harsh environment of the root canal system. *E. faecalis* adheres to host cells, express proteins that allow it to compete with other bacterial cells, and alter host responses. It is able to suppress the action of lymphocytes, potentially contributing to endodontic failure and also able to share these virulence traits among species, thus, further contributing to its survival and ability to cause diseases (Jett et al., 2014).

A contributing factor to the pathogenesis of *E. faecalis* is its evolving resistance to antibiotics. For instance, resistance to vancomycin is now widespread and common among members of the genus, which leaves few options for disease management (Rehaiem et al., 2014). There is a dramatic increase in antibiotic resistance of *Enterococcus* species worldwide highlighting the need for a greater understanding of this genus, including its ecology, epidemiology, and virulence (Ndubuisi et al., 2017).

However, little information is available on the virulence potentials of enterococci from different sources. There is a need to examine the occurrence of putative virulence factors by phenotypic and genotypic approaches in *E. faecalis* strains isolated from different sources such as abattoir, poultry and clinical origin in Ado-Ekiti, Ekiti State, Nigeria. More investigation on the potential virulence determinants and susceptibility patterns of different antibiotics would be useful in understanding their roles in disease causation. This study seeks to determine the frequency and distribution of antibiotic resistance and virulence genotypes of *Enterococcus faecalis* from poultry, animal and clinical sources.

MATERIALS AND METHODS

Collection of samples

One hundred and fifty samples were collected from three different sources, between August and November 2017. The sample collected was made up of poultry droppings (50), Abattoir (50), and Clinical specimens (50). Clinical specimens from wound infection, pus exudate, sputum and throat swab were collected from the Medical Bacteriology and Parasitology laboratory Department, Ekiti State University Teaching Hospital, Ado-Ekiti. Animal samples were collected from selected farms within Ado-Ekiti metropolis, including Afe Babalola University Farm, Ado-Ekiti, Nigeria and Elongated farms Ltd., Ado-Ekiti. Samples were collected in line with the procedure described by Diego et al. (2016).

Isolation and identification of *E. faecalis*

Microscopy and culture

Sample swabs were inoculated directly onto sterile plates of Bile esculin agar (Titan Biotech Ltd, Bhiwadi-301019, and Rajasthan, India). Swabs samples were processed within 6 h of collection, inoculated onto plates and incubated aerobically at 37°C for 24 h. The plates were examined for colonies with a characteristic dark reddish colour which is the presumptive feature for the identification of *Enterococcus* spp. on Bile esculin agar (Ekuma et al., 2016).

Discrete colonies of the isolates were picked from plates, subcultured onto MacConkey agar and incubated overnight at 37°C after which discrete colonies were picked and stored on nutrient agar slant at 4°C as stock. The isolates were identified using standard biotyping methods (Barrow et al., 2004; Fawole and Oso, 2001; Olutiola et al., 1991). Microbial surface colony count was carried out on the primary plate to determine the microbial load of each sample. Identification of *E. faecalis* was done using Gram stain and biochemical tests such as Catalase test, Motility test, and Esculin hydrolysis and heat resistance.

Antibiotics susceptibility testing

Susceptibility testing was carried out on Muller Hinton agar using disc diffusion method according to Clinical and Laboratory Standard Institute (CLSI, 2016; Yilema et al., 2017). The following antibiotics

disks (Abtek Biologicals, and Oxoid Ltd) with their concentration (in µg) which were previously identified from previous studies were used: Ceftazidine (30 µg), Cefuroxime (30 µg), Gentamycin (10 µg), Ceftriaxone (30 µg), Erythromycin (5 µg), Cloxacillin (5 µg), Ofloxacin (5 µg) and Augmentin (30 µg). A sterile wire loop was used: 3-5 well-isolated colonies were picked and mixed in nutrient broth. The prepared turbidity was matched with a turbidity standard (0.5 McFarland) to have an equal suspension.

A sterile swab was used to inoculate the suspension by streaking on the prepared and dried Muller Hinton agar plate evenly. Afterward, it was allowed to stay for 3-5 min. Sterile forceps were used to place the antimicrobial discs on the inoculated plates. Within 30 min after applying the disc, the plate was incubated at 35°C for 16-18 h. By using meter rule on the underside of the plate, diameter of each zone of inhibition was measured in millimetre (mm). Zone diameter for ATCC 25922, a recommended reference strain for antibiotic susceptibility testing was compared with National Committee for Clinical Laboratory Standards (NCCLS) published limits; interpretative charts were used to interpret the zone sizes of inhibition (Adesida et al., 2017). Results were recorded as susceptible or resistant based on the zones sizes of each of antimicrobial disc used (CLSI, 2016).

Molecular analysis

A total of 15 representatives of *E. faecalis* isolates made up of 7 from Poultry droppings, 5 from Abattoir and 3 from Clinical specimens respectively were selected for presence of virulence determinant genes that encodes the following genes; gelatinase (*gelA*), aggregation substance (*asa1*), Cytolysin *cylA*, enterococcal surface protein (*esp*) and Collagen-binding protein (*ace*) as well as confirmation of the bacteria identity using 16S-primer by polymerase chain reaction. The selection was based on their frequency of antibiotic resistance phenotypes.

Genomic DNA Extraction

DNA extraction was carried out according to the method of Thottappilly et al. (1999) and Olawale et al. (2015). A 60 g of the selected bacterial cells were suspended in 200 µl of water and added to ZR bashing beads lysis tubes: 800 µl of lysis buffer was added to the mixture. It was mixed briefly by vortexing and left to stand at room temperature for 5 min.

The mixture was transferred to a spin column in a collection tube and centrifuged at 10,000 rcf for one minute. The spin column was transferred to a new collection tube, 400 µl of DNA pre-wash buffer was added to the spin column and centrifuged at 10,000 rcf for one minute, 500 µl of g-DNA wash buffer was added to the spin column and centrifuged at 10,000 rcf for one minute. The spin column was transferred to a clean micro centrifuge tube; 100 µl DNA Elution buffer was added to the spin column, incubated for 2-5 min at room temperature and then centrifuged at top speed for 20 s to elute the DNA. The elute dDNA was stored at ≤-20°C for further use.

Molecular determination of *E. faecalis* virulence pattern by polymerase chain reaction and gel electrophoresis

Five designated bacteria virulence specific primer pairs (one forward and one reverse) were used on the genomic DNA of fifteen (15) bacterial isolates, one positive and negative control DNA samples. *E. faecalis* strains were first propagated using a modified procedure of Onasanya et al. (2003). A 200 µl amount of *E. faecalis* strain was transferred into 75 ml of nutrient broth in a 250 ml conical flask and kept under constant shaking at 37°C for 24 h. The bacterial cells were removed by centrifugation, washed with 0.1mM Tris-EDTA and kept at -20°C for DNA extraction.

Genomic DNA extraction was performed according to Thottappilly et al. (1999) with some modification. Briefly, 0.3 g of washed bacterial cell was suspended in 200 µl of 2xCTAB buffer (50 mM Tris, pH 8.0; 0.7 mM NaCl; 10 mM EDTA; 2% hexadecyltrimethyl ammonium bromide; 0.1% 2-mercaptoethanol), followed by the addition of 100 µl of 20% sodium dodecyl sulfate (SDS) and incubated at 65°C for 20 min. DNA was purified by two extractions with an equal volume of chloroform and precipitated with 20°C absolute ethanol. After washing with 70% ethanol, the DNA was dried and re-suspended in 200 µl of sterile distilled water. DNA concentration was measured using DU-65UV spectrophotometer (Beckman Instruments Inc., Fullerton CA, USA) at 260 nm.

DNA degradation was checked by electrophoresis on a 1% agarose gel in 1 x TAE (45mM Tris-acetate, 1mM EDTA, pH 8.0). Quantitative Polymerase chain reaction (QPCR) was performed according to Creti et al. (2004) with some modifications. Briefly, oligonucleotide primers (Custom Primer Service, Life Technologies, and Gaithersburg, MD, USA) were used (Table 1). Two concentrations of each DNA preparation (24 and 96 ng per reaction) were used to test reproducibility and eliminate sporadic amplification products. Of the 6 primers used only three gave reproducible amplification products and were used, to amplify the DNA from all the 15 selected *E. faecalis* strains. Amplification was performed in 20 µl reaction mixture consisting of 4 µl genomic DNA, 2 µl 10X reaction buffer (Promega), 1 µl 25 mM dNTPs (dATP, dCTP, dGTP, and dTTP), 1 µl 2 mM primer, 1.6 µl 25 mM MgCl2 and 0.2 µl of 5 U/µl Taq polymerase (Boehringer, Germany), 2 µl 5% Tween-20, and 8.2 µl sterile distilled water.

The reaction mixture was overlaid with 50 µl of mineral oil to prevent evaporation. Amplification was performed in a thermowellmicrotiter plate (Costa Corporation) using a Perkin Elmer programmable Thermal Controller model 9600. The cycling program was 1 cycle of 94°C for 3 min followed by 32 cycles of 94°C for 20 s for denaturation, 54°C for 20 s for annealing of primer and 72°C for 40 s for extension; and a final extension at 72°C for 7 min. The amplification products were resolved by electrophoresis in a 1.4% agarose gel using TAE buffer (45 mMTris-acetate, 1 mM EDTA, pH 8.0) at 100 V for 2 h. A 1 kb ladder (Life Technologies, Gaithersburg, MD, USA) was included as a molecular size marker. Gels were visualized by staining with 0.5 g/ml ethidium bromide solution and banding patterns were photographed over UV light using a red filter.

RESULTS

The results of bacteria isolation and characterization showed 80(53.3%) yielded growth of *E. faecalis,* 70(46.7%) yielded growth of other organisms. Table 2 shows the distribution of *E. faecalis* isolated from various sources of samples studied, poultry droppings 47 (94.0%), abattoir 21 (42.0%) while clinical samples yielded the lowest growth of 12 (24.0%).

Multiple resistance patterns were obtained for *E. faecalis* isolated in poultry droppings (Figure 1). The highest resistance was obtained for antibiotics erythromycin (87.2%), while the lowest resistance was seen in ofloxacin with (44.7%). However, the isolates showed some level of susceptibility to ofloxacin (55.3%), augmentin (38.3%), ceftazidime (36.0%), ceferoxime (31.9%), cloxacillin (27.7%), ceftriaxone (23.4%), gentamycin (21.3%) and erythromycin (12.8%).

Table 1. PCR Primers selected for the detection of virulence genes in 15 samples of *E. faecalis* strains.

Target Gene	Sequence (5'- 3')	Position (bp)	Product Size (bp)
Esp	TTGCTAATGCTAGTCCACGACC	1217	932
	GCGTCAACACTTGCATTGCCGA	2149	
asal	CCAGCCAACTATGGCGGAATC	3122	529
	CCTGTCGCAAGATCGACTGTA	3651	
Ace	GGAATGACCGAGAACGATGGC	160	616
	GCTTGATGTTGGCCTGCTTCCG	776	

Table 2. Distribution of *E. faecalis* isolated from different sample sources.

Sample Source	Number collected	Number of *E. faecalis* isolates (%)	Other isolates (%)
Poultry	50	47 (94.0)	3 (6.0)
Abattoir	50	21 (42.0)	29 (58.0)
Clinical	50	12 (24.0)	38 (76.0)

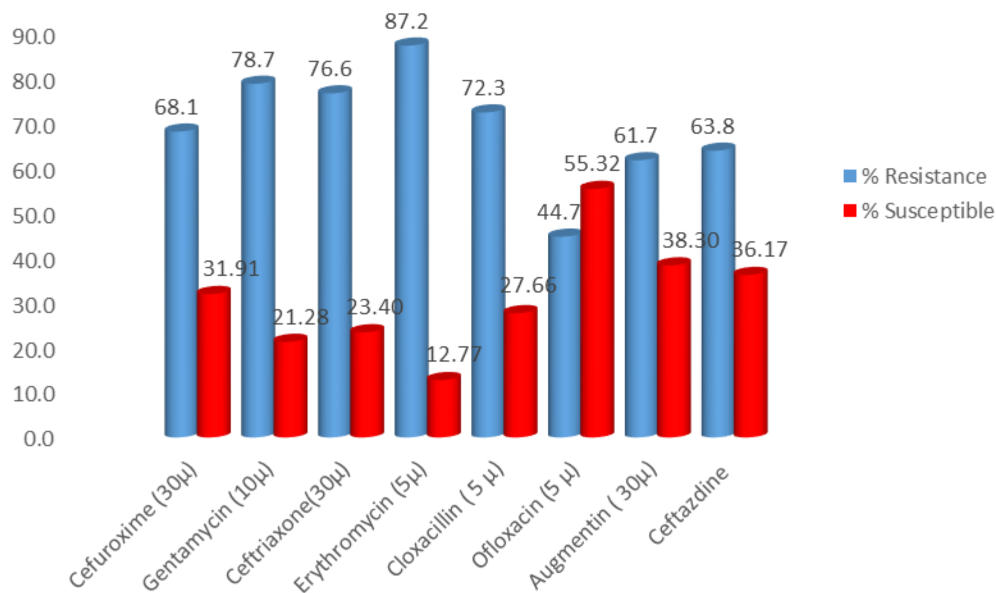

Figure 1. Antibiotics resistance pattern to poultry isolates.

The results of resistance pattern presented in Figure 2 shows antibiotics multiple resistance pattern with high frequencies to the following; augmentin (100.0%), erythromycin (100.0%), cefuroxime (100.0%), cloxacillin (90.5%) and ceftazidime (81.0%). Minimal susceptibility was seen in ofloxacin 14 (66.7%), gentamycin 13 (61.9%), ceftriaxone 9 (42.9%), ceftazidime (19.0) and lowest with cloxacillin (9.5%).

Multiple resistance patterns of *E. faecalis* isolates in clinical samples are indicated in Figure 3; the highest resistance to antibiotics used was Erythromycin, 12 (100.0%), Gentamycin, 11 (91.7%), Cloxacillin, 11 (91.7%), Ceftriaxone, 10 (83.3%), Augmentin, 8 (66.7%), and Cefuroxime, 8 (66.7%). Isolates equally showed some level of susceptibility to some antibiotics with highest seen in Ceftazdine, 8 (66.7%) and the lowest in

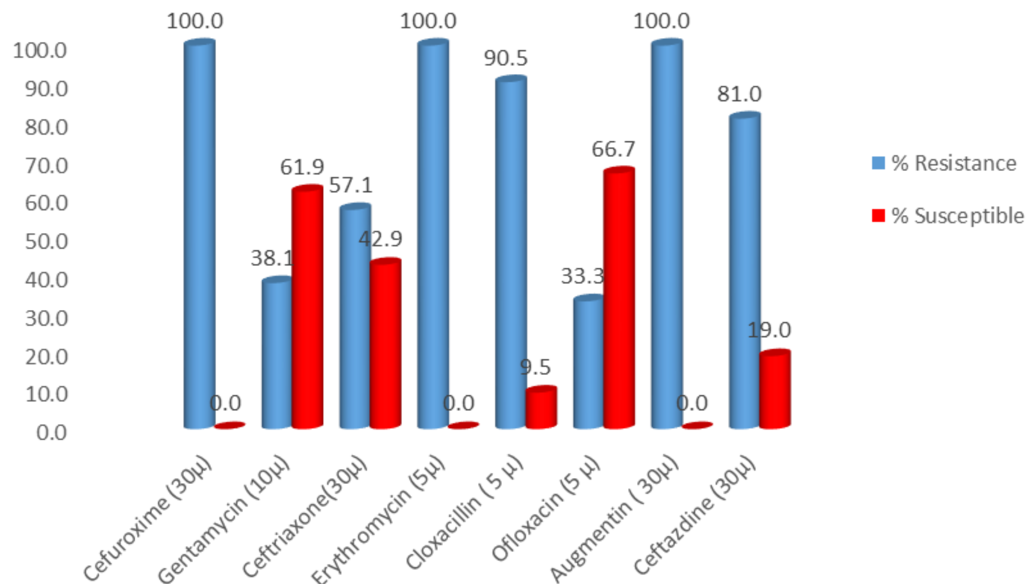

Figure 2. Antibiotics resistance pattern to abattoir isolates.

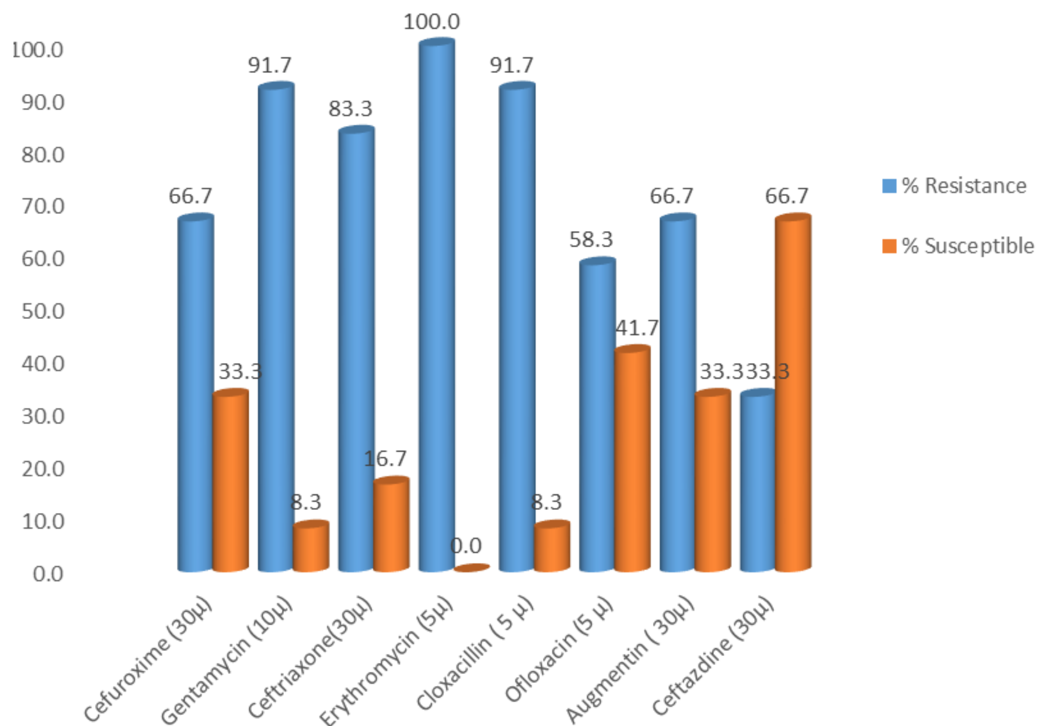

Figure 3. Antibiotics resistance pattern to clinical isolates.

Erythromycin 0 (0.0%).

In this present study, Table 3 indicates the frequency of resistance of antibiotics used against isolates of *E. faecalis* from various sources; poultry isolates exhibited high resistance to 7 out of 8 antibiotics used: erythromycin (87.2%), gentamycin (78.7%), ceftriaxone (76.6%), cloxacillin (72.3%), cefuroxime (68.1%), ceftazidime (63.8%) and augmentin (61.7%). The pattern of resistance is also similar in abattoir isolates where 6 out of 8 antibiotics used show high resistance; erythromycin (100.0%), augmentin (100.0%), cefuroxime (100.0%), cloxacillin (90.5%), ceftazdine (81.0%) and ceftriaxone

Table 3. Multiple antibiotic resistance patterns to *Enterococcus faecalis* isolates from poultry droppings, abattoir, and clinical sources.

Samples	CRX	GEN	CTR	ERY	CXC	OFL	AUG	CAZ
Poultry	32 (68.1)	37 (78.7)	36 (76.6)	41 (87.2)	34 (72.3)	21 (44.7)	29 (61.7)	30 (63.8)
Abattoir	21 (100)	8 (38.1)	12 (57.1)	21 (100)	19 (90.5)	7 (33.3)	21 (100)	17 (81.0)
Clinical	8 (66.7)	11 (91.7)	10 (83.3)	12 (100)	11 (91.7)	7 (58.3)	8 (66.7)	4 (33.3)
Total	61 (76.3)	56 (72.5)	58 (72.5)	74 (92.4)	64 (80.0)	35 (43.8)	58 (72.5)	51 (63.8)

CRX , Cefuroxime (3 0µg); GEN , Gentamycin (10 µg); CTR – Ceftriaxone (30 µg); ERY , Erythromycin (5 µg); CXC , Cloxacillin (5 µg); OFL, Ofloxacin (5 µg); AUG, Augmentin (30 µg); CAZ, Ceftazdine (30 µg).

Table 4. Summary of distribution for virulence genes in *E. faecalis* isolate.

Source	Esp Gene	Asal Gene	Ace Gene
Poultry	2 (28.6%)	2 (28.6%)	2 (28.6%)
Clinical	0	0	0
Abattoir	0	3 (60.0%)	3 (60.0%)

Table 5. Distribution of Virulence Genes among selected isolates *E. faecalis* strains.

Serial number	Isolates	Sample source	*Esp* Gene	*Asal* Gene	*Ace* Gene
1	SP1A1	Poultry	-	-	-
2	SP1A2	Poultry	-	-	-
3	SP2B1	Poultry	-	+	-
4	SP3A1	Poultry	-	-	-
5	ABUAD-SPL32	Poultry	-	-	-
6	SP2A1	Poultry	+	+	+
7	PKL-41	Poultry	+	-	+
8	EKSG-3	Abattoir	-	+	+
9	EKSG-4	Abattoir	-	-	-
10	EKSG-7	Abattoir	-	-	+
11	EKSG-11	Abattoir	-	+	-
12	EKSG-20	Abattoir	-	+	+
13	EKUSTH-C18	Clinical	-	-	-
14	EKSUTH-C16	Clinical	-	-	-
15	EKSUTH-C3	Clinical	-	-	-
	Total		2	6	5
	(%)		13.3%	40.0%	33.3%

(57.0%).

The results obtained from Polymerase Chain Reaction (PCR) show the distribution of three genes virulence determinants: enterococcal surface protein (*esp*), aggregation substance *(asal*) and collagen-binding protein (*ace*) among the selected isolates. These findings are presented in Tables 4 and 5. The analyses show different prevalence of virulence genes in *E. faecalis* which range from 13.3 to 40.0%.

The PCR results showed that with P2 primer set, only isolates 2 (6 and 7) out of the fifteen isolates have *Esp* gene at specific PCR target 932 bp amplicon (Figure 4). Besides, there were other different PCR amplicons of varying molecular sizes indicating different *Esp* gene mutants. The primer P5 set revealed that *asal* gene at specific PCR target 529 bp amplicon was present in isolates 6 isolates (3, 6, 8, 9, 11 and 12) respectively (Figure 5). In addition, there were other different PCR amplicons of varying molecular sizes indicating the presence of different *asal* gene mutants. The performance

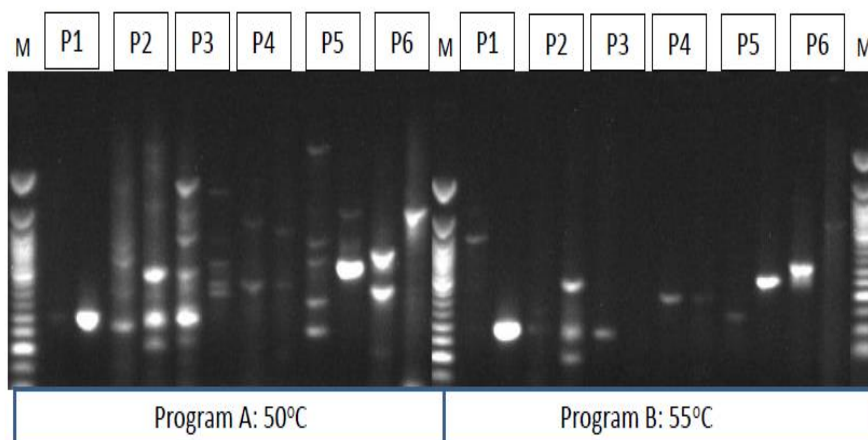

Figure 4. Results of Primer optimization.

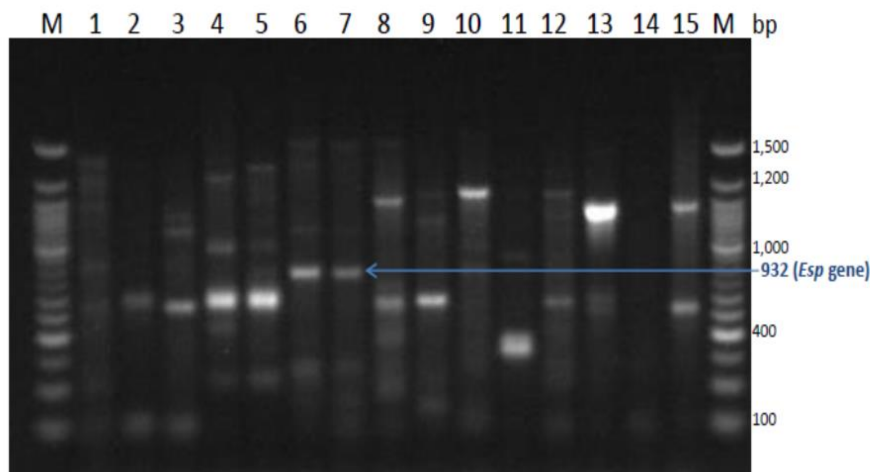

Figure 5. PCR detection of virulence determinants *Esp* gene in *E. faecalis* strains as revealed by *Esp* specific primer. Lanes M Molecular marker, *E. faecalis* strains:1SPA1A;2 SPA1A2; 3SP2B1;4 SP3A1;5 ABUAD-SPL32;6 SP2A1;7 PKL-41;8 EKSG-3;9 EKSG-4;10 EKSG-7;11EKSG-11;12EKSG-20;13 EKSG-C18; 13 EKSUTH-C18;14 EKSUTH-C16;15 EKSUTH-C3

of primer P6 set showed the presence of *Ace* gene at the specific PCR target 616 bp amplicon in isolates (6, 7, 8, 10 and 12 respectively) (Figures 6 and 7). Also, there were other different PCR amplicons of varying molecular sizes which revealed the presence of different *ace* gene mutants.

DISCUSSION

E. faecalis are ubiquitous in their occurrence, with their habitats ranging from the intestinal tract of man and variety of farm animals to different forms of food and feed. In this present study, *E. faecalis* were isolated from poultry droppings, clinical specimens from pathological cases, and from abattoir products.

Findings from this study showed that the highest number of isolates of *E. faecalis* were from poultry dropping and least in clinical samples. The pattern of *E. faecalis* distribution in this study is in agreement with the result obtained from the study carried out by Hosseni et al. (2016), where *E. faecalis* was the most common species isolated from both dairy products and meat products. Virulence of *E. faecalis* was strongly enhanced

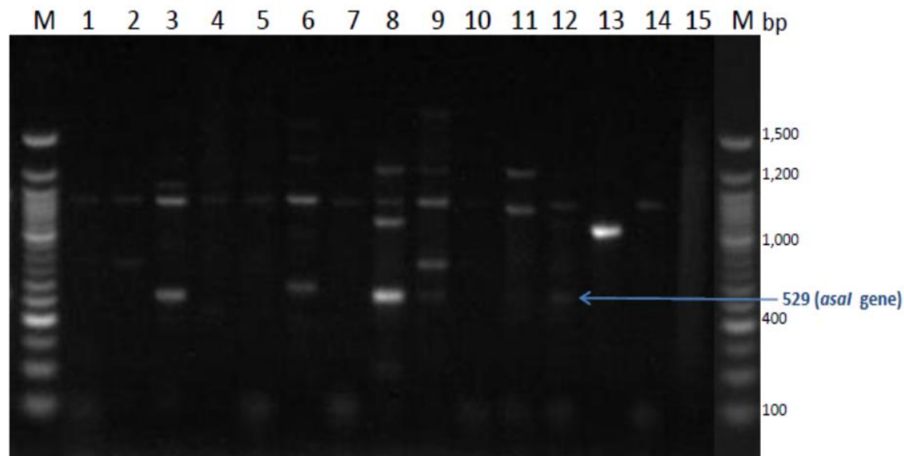

Figure 6. PCR detection of virulence determinants *asal* gene in *E. faecalis* strains as revealed by *asal* specific primer. Lanes M, Molecular marker; *E. faecalis* strains:1SPA1A; 2, SPA1A2; 3, SP2B1; 4, SP3A1; 5, ABUAD-SPL32; 6, SP2A1; 7, PKL-41;8,EKSG-3; 9, EKSG-4; 10, EKSG-7; 11, EKSG-11; 12, EKSG-20;13, EKSG-C18;13, EKSUTH-C18; 14, EKSUTH-C16; 15, EKSUTH-C3.

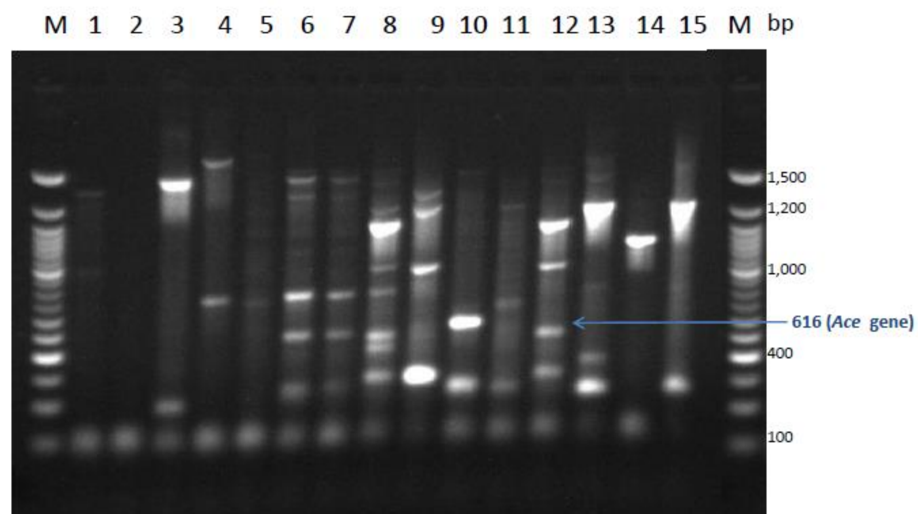

Figure 7. PCR detection of virulence determinants *ace* gene in *E. faecalis* strains as revealed by *ace* specific primer. Lanes M, Molecular marker; *E. faecalis* strains:1SPA1A; 2, SPA1A2; 3, SP2B1; 4, SP3A1; 5, ABUAD-SPL32; 6, SP2A1; 7, PKL-41;8,EKSG-3; 9, EKSG-4; 10, EKSG-7; 11, EKSG-11; 12, EKSG-20;13, EKSG-C18;13, EKSUTH-C18; 14, EKSUTH-C16; 15, EKSUTH-C3.

by their frequent resistance to commonly used antibiotics. Antibiotics which can be both intrinsic and acquired make *E. faecalis* effective opportunists in nosocomial infections. In this study, poultry isolates exhibited high resistance to Erythromycin, Gentamycin, Ceftriaxone, Cloxacillin, Cefuroxime, Ceftazidime, and Augmentin. The pattern of resistance was also similar in abattoir isolates where 6 out of 8 antibiotics used showed high resistance. These findings are in conformity with that of Trivechi et al. (2011) where high resistance pattern to erythromycin, ofloxacin, and cephalothin was reported in *E. faecalis* isolated from dairy and poultry products. Olawale et al.

(2015), also reported high resistance pattern for erythromycin, cloxacillin, augmentin, and gentamycin.

E. faecalis isolates from clinical samples in this study showed high resistance to most antibiotics used. The intrinsic resistance to many available antibiotics suggests that the treatment of an infection with *E. faecalis* could be difficult. Also, the major risk- associated with this resistance is that they are transferable, the gene coding for all of these antibiotic-resistant traits may be transferred by pheromone-mediated, conjugative, plasmids or transposons. However, emergence of resistance to common antibiotics in these isolates has compromised the clinical usefulness of several antimicrobial classes, including macrolides, amino-glycosides, and glycopeptides. The need for sourcing for other antimicrobial agents is important and critical for continued access to antimicrobials with clinical efficacy and balance of potencies against *E. faecalis* emerging pathogens (Oranusi et al., 2013).

The results obtained from PCR analysis showed the distribution of three genes virulence determinants: enterococcal surface protein (*esp*), aggregation substance *(asal*) and collagen-binding protein (*ace*) among the selected isolates. Virulence determinant, aggregation substance, and collagen-binding protein were common to isolates from poultry and abattoir. This revealed that there was a high distribution of virulence potentials among *E. faecalis* from poultry and abattoir origin.

It has been reported that *ace* genes are very important virulence-associated factors (Singh et al., 2010). However, finding in this study was able to detect *ace* and *asal* genes in both poultry and abattoir strains; none of these genes should be considered definitive markers of pathogenicity in poultry *E. faecalis;* it is more likely they contribute to virulence potential of *E. faecalis*, but is independent of additional virulence factor present or a decreased disease-resistance of the host before causing infection (Marra et al., 2007).

Aggregation substance gene (asal) was also common among the poultry and abattoir strains, but not detected in clinical strains. Previous studies of the occurrence of *asal* in enterococcal isolates are contradictory; some indicated a higher prevalence of *asal* in clinical isolates compared to other sources (Waar et al., 2002), whereas other studies found a similar prevalence (Huycke and Gilmore, 1995).

The gene encoding the enterococcal surface protein, (*esp)*, has been associated with increased virulence, colonization and biofilm formation (Rahmadan and Hegedus, 2005; Latasa et al., 2006). The *esp* was the least frequently detected virulence gene in poultry isolates. This is in agreement with previous reports from studies carried out by Hammerum and Jensen (2002) and Poeta et al. (2006).

Conclusion

E. faecalis are widespread in the environment and have emerged as opportunistic pathogens, responsible for increasing the percentage of nosocomial infections. High prevalence of virulence potentials among *E. faecalis* from poultry and abattoir origin were determined in this study. The high degree of contamination of animal source analysed was, therefore, an indicator of how high the probability of colonization by these microorganisms of human intestine can be. *E. faecalis*, however, should be considered not only as potential pathogens but also as a reservoir of genes encoding for antibiotic resistance that can be transferred to other pathogenic and non-pathogenic microorganisms. Distribution of potentially virulent *E. faecalis* in this study area portends danger for reservoir of high antibiotic resistance pathogens and serious health hazards. This should necessitate adoption of stringent infection control measures. There is a need for improved hygiene practices with strict enforcement of good manufacturing practices in the study location and beyond. Further investigations such as phylogenetic analysis are required to evaluate the similarity of virulence genes on nucleotide level between poultry, abattoir and clinical isolates.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Adesida SA, Ezenta CC, Adagbada AO, Aladesokan AA, Coker AO (2017). Carriage of Multidrug Resistant Enterococcus faecium and Enterococcus Faecalis among apparently Healthy Humans. African Journal of Infectious Disease 11(2):83-89.

Barrow GI, Feltham RK, Cowan A, Stell S (2004). Manual for Identification of Medical Bacteria. Cambridge University Press 331 p.

CLSI (2016). Performance Standard for Antimicrobial Disk Susceptibility: Tests approved standard, Eleventh Edition, M02-A11-32c11.Replaces M02-A10:2a (1).

Creti R, Imperi M, Bertuccini L, Fabretti F, Orefici G, Di Rosa R (2004). Survey for virulence determinants among *Enterococcus faecalis* isolated from different sources. Journal of Medical Microbiology 53:13-20.

Diego C, Andrighetto C, Lombardi A (2016). Presence of virulence factor and antibiotics resistance in *Enterococcous* spp collected from dairy products and meat. Food Control 19(9):886-892.

Ekuma AE, Oduyebo OO, Efunshile AM, Konig B (2016). Surveillance for Vancomycin Resistant Enterococci in a Tertiary Institution in South Western Nigeria. African Journal of Infectious Disease 10(2):121-126.

Fawole MO, Oso BA (2001). Laboratory Manual of Microbiology, Spectrum Books Ltd, Ibadan 127 p.

Franz SK, Edwards JR, Courval JM, Hill H, Tenover FC, Lawton R (2011). Intensive Care Antimicrobial Resistance Epidemiology (ICARE) Project and the National Nosocomial Infections Surveillance (NNIS) System Hospitals. The effect of vancomycin and third-generation cephalosporins on prevalence of vancomycin-resistant

enterococci in 126 U.S. adult intensive care units. Annal of Internal Medicine 135:175-183.

Giraffa MF, Skinner FD (2010). Origin, evolution and phylogeny of giraffes camelopardis. Transctions of Royal Society of South Africa 58:234-241.

Hammerum AM, Jensen LB (2002). Prevalence of *Esp,* encoding the enterococcal surface protein in *E. faecalis* and *E. faecium* isolates from hospital patients, poultry, and pigs in Demark. Journal of Clinical Microbiology 40:43-96.

Hosseni SM, Zeni B, Ratyani S, Jafari R, Shambo F, Tabar ZK, Arabestani MR (2016). Presence of virulence factors and antibiotics resistance in *Enterococcus* spp collected from dairy product and meat. Der Pharmacia letters 8(4):138-145.

Huycke MM, Gilmore MS (1995). Frequency of aggregation substance and cytolysin genes among enterococcal endocarditis isolates. Plasmid 34:152-156.

Jett BD, Huycke MM, Gilmore MS (2014). Virulence of enterococci. Clinical Microbiology Review 7:462-478.

Klare I, Heier H, Claus H, Bohrne *G,* Martins M, Seltmann G (2005). *Enterococcus faecium* strains with vanA-mediated high-level glycopeptide resistance isolated from animal foodstuffs and faecal samples of humans in the community. Microbial Drug Resistant pp. 265-272.

Latasa CC, Solano JR, Penades Lasa I (2006). Biofilm associated proteins. C.R. Biology 329:849-857.

Marra AF, Dib-Hajj I, Lamb F, Kaczmarek WC, Shang G, Beckius AJ, Milici I, Medina Gootz TD (2007). Enterococcal virulence determinants may be involved in resistance to clinical therapy. Diagnostic Microbiology Infection Diseases 58:59-65.

Murray BE (2010).The life and times of the *Entcrococcus.* Clinical Microbial Reviews 3:46-65.

Ndubuisi JC, Olonitola OS, Olayinka, AT, Jatau ED, Iregbu KC (2017). Prevalence and antibiotics susceptibility profile of *Enterococcus* spp isolated from some hospitals in Abuja, Nigeria. African Journal of Clinical and Experimental Microbiology 18(3):154-158.

Olawale AK, Salako RJ, Olawale AO, Famurewa O (2015). Antibiotics-Resistant *Enterococcus Faecalis* isolated from canteens in Osun State, Nigeria. British Microbiology Research Journal 6(4):196-206.

Olutiola PO, Famurewa O, Sonntag HC (1991). Introduction to General Microbiology: A practical approach. Heidelberger veriagasanstalt and Druckerei, GmbH, Heidelberg.

Onasanya A, Mignouna HD, Thottappilyy G (2003). Genetic finger printing and phylogenetic diversity of isolates of *Staphylococcus aureus* from Nigeria. African Journal of Biotechnology 2:246-250.

Oranusi S, Onyeike E, Galadima M, Umoh VJ (2013). Hazard analysis critical control points of food prepared by families in Zaria, Nigeria. Nigeria Journal of Microbiology 18(1-2):346-362.

Pinheiro ET, Anderson MJ, Gomes BPFA, Drucker DB (2006). Phenotypic and genotypic identification of enterococci isolated from canals of root-filled teeth with periapical lesions. Oral Microbiology Immunology 21:137-144.

Poeta P, Costa D, Rodrigues J, Torres C (2006). Detection of genes encoding virulence factors and bacteriocins in fecal enterococci of poultry in Portugal. Avian Diseases 50:64-68.

Rahmadan AA, Hegedus E (2005). Biofilm formation and *esp* gene carriage in enterococci. Journal of Clinical Pathology 58:685-686.

Rehaiem A, Belgacem ZB, Edalatian MR, Martínez B, Rodríguez A, Manai M, Guerra NR (2014). Assessment of potential probiotic properties and multiple bacteriocin encoding-genes of the technological performing strain *Enterococcus faecium* MMRA. Food Content 37:343-350.

Sedgley C, Nagel A, Dahlen G, Reit C, Molander A (2013). Real-time quantitative polymerase chain reaction and culture analyses of *Enterococcus faecalis* in root canals. Journal of Endodology 32:173-177.

Shinde RS, Koppikar GV, Oommen S (2012). Characterization and antimicrobial susceptibility pattern of clinical isolates of Enterococci at a tertiary care hospital in Mumbai, India. Annal of Tropical Public Health 5(2):85-88.

Singh KV, Nallapardy SR, Sillanpaa J, Murray BE (2010). Importance of the collagen adhesion Ace in pathogenesis and protection against *E. faecalis* experimental endocarditis. Plos Pathologens 6(1):323-331.

Sunde PT, Olsen I, Debelian GJ, Tronstad L (2012). Microbiota of periapical lesions refractory to endodontic therapy. Journal of Endodology 28:304-310.

Trivechi K, Cupakova S, Karpiskova R (2011). Virulence factors and antibiotics resistance in enterococcus isolated from food-stuffs. Veterinarian Medicina 56(7):352-357.

Thottappilly G, Mignouna HD, Onasanya A, Abang M, Oyelakin O, Singh NK (1999). Identification and differentiations of isolates of *Collectotrichum gleosporioides* from yam by random amplified polymorphic DAN makers. African Crop Science Journal 7(2):7213-7218.

Waar K, Muscholl-Silberhorn AB, Williams RJI, Slooff HJM, Dengner JE (2002). Geno grouping and incidence of virulence factors of *enterococcus faecalis* in liver transplant patients differ from blood culture and fecal isolates. Journal of Infectious Diseases 185:1121-1127.

Yilema A, Moges F, Tadele S, Endris M, Kassu A, Abebe W, Ayalew G (2017). Isolation of enterococci, their antimicrobial susceptibility patterns and associated factors among patients attending at the University of Gondar Teaching Hospital. BMC Infectious Diseases 17:276.