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Study of virulence genes in vancomycin resistant *Enterococci* (vre) from animals and human clinical isolates

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Abstract

With *Enterococcus* species in the leading cause of nosocomial infections and resistance to an array of antibiotics, this study focused to determine the frequency and distribution of vancomycin-resistant Enterococci, the presence of virulence genes and to determine the relative nucleotide sequence relatedness among isolates using 16S rRNA sequence. A random sampling of 120 fecal samples of cattle, poultry, and piggery, and human clinical isolates was analyzed. Standard bacteriological methods were employed in the isolation and characterization of isolates and the disk diffusion method was used in determining their antibiotic resistance profiles. Results showed *Enterococcus* species in cattle at 100%, followed by clinical isolates at 80%. Vancomycin resistance was observed at high rates in *Enterococcus* species from human clinical isolates and cattle isolates at 90% and 80% respectively. Multiple antibiotic-resistant isolates yielded twelve resistance profiles and 16S rDNA sequences identified *E. faecalis, E. durans, E. mundtii,* and *Enterococcus* sp. Isolates from cattle samples were the most probable source of clinical isolates at 78% homology of conserved regions with the clinical isolates. Virulence determinant genes *Asa1* was recorded at66.6%, *Cyl* at 16.6% and *GelE* at 8.3% among the isolates. This study established farm animals as possible reservoirs of VRE isolates to man. Hence, healthy and professional practices among animal farmers with antibiotic usage, as well as hygienic and preventive measures among hospital workers are here recommended.

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Introduction

Enterococcus species are a diverse group of Gram-positive, facultatively anaerobic bacteria able to adapt to harsh temperature, pH, hyper-osmolarity and prolonged desiccation conditions (Moraes *et al.* 2012; Ali *et al.*, 2014; Lebreton *et al.*, 2014). they were initially classified as Group D Streptococci due to the presence of group D cell wall antigen (Teixeira and Merquior, 2013) but with further Molecular DNA studies, Enterococcus was classified in its genus (Byappanahalli *et al.*, 2012). It is a common isolate in the intestines of most invertebrates, supporting intestinal, microbial homeostasis, stimulating immune modulation to prevent infections with pathogenic bacteria and viruses (Kondoh *et al.*, 2012).

In decades past, *Enterococcus* pathogens have been reported as a major cause of nosocomial infections in various tissues, the urinary tract, respiratory tract, peritoneum, and bloodstream (Bonten and Willems, 2012). *Enterococcus faecalis and Enterococcus faecium,* the most prevalent species cultured from humans, account for more than 90% of clinical enterococcal isolates (Fisher and Phillips, 2009). Their infections are difficult to treat because of their intrinsic and acquired resistance to many antibiotics such as ampicillin and vancomycin (Van Harten *et al.*, 2017).

Vancomycin-Resistant Enterococci (VRE) especially has emerged as a major cause of outbreaks of nosocomial infections, which with their extensive resistance to a plethora of other antibiotics have attracted more and more attention in recent years (Flokas *et al.*, 2017). Factors such as their propensity and inherent ability to acquire resistance to antimicrobials, putative virulence traits, biofilm-forming, and horizontal transfer of antimicrobial resistance and virulence determinants to other bacteria are reasons their infections are could be life-threatening (Werner, 2012). Most VRE is known to belong to the species *E. faecium,* a major agent in hospital-acquired infections (Flokas *et al.*, 2017). Enterococci comprise a widespread bacterial group of diverse species and are present in a variety of surfaces and fecal origins, which are important opportunistic pathogens causing life-threatening infections in hospitals (Liliana *et al.*, 2014). The emergence of multidrug-resistance isolates, particularly to vancomycin, erythromycin and streptomycin have become a major cause of concern for the infectious diseases community (Liliana *et al.*, 2014).

This study aimed at detecting vancomycin resistance and virulence traits in Enterococci of human and animal origin with the view of identifying the possible source(s) of clinical infections in man.

Materials and methods

Sample collection and analysis

One hundred and twenty fecal samples (120) were collected from four different sources between September and December 2018.

The collected samples were made up of Cattle (30), Poultry (30), Piggery (30) and Human clinical isolates (30). Human clinical isolates were obtained from the Microbiology laboratory Ekiti State Teaching Hospital, (EKSUTH) Ado-Ekiti. Animal samples were collected from AfeBabalola University Farm, Ado-Ekiti and Abattoir, Iworoko road Ado-Ekiti, Ekiti State, Nigeria. Samples were collected by obtaining fresh feces from large intestines of selected animals after slaughtering (Diego *et al.*, 2016). Samples were also analyzed based on color, consistency, presence of blood, mucus, or pus.

Isolation of enterococci

Samples were processed within two hours of collection, by serial dilution and inoculated on sterile plates of BileEsculin agar (Oxoid CM0888), incubated aerobically at 37oCfor 24hours.

The plates were observed for luxuriant colonies with characteristic dark coloration in agar (a characteristic of enterococci during esculin hydrolysis) (Meyer and Schonfeld, 1926). These colonies were subcultured on nutrient agar for purity before further biochemical characterization (Chuard and Reller, 1998). Isolates were stored on nutrient agar slants as stock.

Antibiotic susceptibility testing

A sterile wire loop was used to pick one well-isolated colony of the bacterial isolate and inoculated in peptone broth for 18hours at 37°C. The overnight culture was adjusted to 0.5 McFarland turbidity standards (Cattoir*et al.,* 2013). Antimicrobial susceptibility test was carried out on Mueller Hinton agar using disk diffusion according to the Clinical and Laboratory Standards Institute (CLSI 2016) guidelines. The following antibiotics were used vancomycin (30µg), ceftazidime (30µg), gentamicin (10µg), cefuroxime (30µg), ceftriaxone (30µg), erythromycin (5µg), cloxacillin (5µg), ofloxacin (5µg) and augmentin (30µg) (AbtekBiologicals Ltd, UK). The standard inoculum was streaked evenly to the surface of a 150mm diameter Mueller-Hinton agar plate using a sterile swab stick. After 3minutes, sterile forceps were used to place the commercially-prepared, fixed concentration, multi antibiotic disc as well as the single vancomycin disc on the inoculated agar surface. Plates were incubated for 24–48 hours at 37°C before the determination of results. The zones of growth inhibition around each of the antibiotic disks were measured to the nearest millimeter using a meter rule. The zone diameters of each drug were interpreted using the criteria published by the CLSI (2016) Results was recorded as Resistant (R) or sensitive (S) (Cattoir*et al.,* 2013).

Molecular analyses

A total of 12 isolates made up of 3 each from human clinical isolates, poultry, cattle and Piggery were selected based on their antibiotic resistance phenotypes for 3 virulence determinant genes; gelatinase (*gel*A), aggregation substance (*asa*1), cytolysin *(cyl*A), as well as confirmation of the bacterial identity using 16S rDNA sequence by Polymerase Chain Reaction (Olawale*et al.,* 2015). This was done by coupling PCR to the DNA sequencing analysis of 16S rDNA genes (Wang *et al*., 1992; Manzano*et al*., 2000). DNA was extracted using the ZymoBIOMICS™ DNA MiniprepKit (CA, USA) and the procedure was carried out according to manufacturers' guidelines. DNA concentration was quantified using a NanoDrop 1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, United States). The A260/A280 absorbance ratio was used to determine undesired contaminations. To evaluate the quality and intactness of the extracted DNA, gel electrophoresis was used. The extracted DNA (5µl) was loaded on 1.5% agarose gel (Invitrogen, California, United States), which contained ethidium bromide (1µg/ml) for DNA staining. For image acquisitions, a G:Box™ gel documentation system (Syngene, Cambridge, United Kingdom) was used (Olawale*et al.,* 2015).

PCR cycling parameters

Polymerase Chain Reaction (PCR) was performed using 27F (5'- AGAGTTTGATCMTGGCTCAG – 3') and 1525R (5'- AAGGAGGTGWTCCARCCGCA -3') universal primers and PCR protocols were performed as described by Bubert*et al.* (1992).PCR for the amplification of the 16S rDNA procedure was at 94°C for 5mins for initial denaturation, followed by 36 cycles of denaturation at 94°C for 30seconds, annealing at 56°C for 30seconds and elongation at 72°C for 45seconds followed by a final elongation step at 72°C for 7 minutes and hold temperature at 10°C. Amplified fragments were visualized on SafeView-stained 1.5% agarose electrophoresis gels using Hyper ladder 1 DNA marker. The amplicons were subjected to sequencing reactions using BigDye Terminator v3.1 Cycle Sequencing Kit. The products were loaded unto 3130xl Genetic Analyzer (Applied Biosystems) to generate the molecular sequences (Naser *et al.*, 2005). PCR for the virulence genes was at annealing temperatures of 50°C for *gelE* and 55°C for *cylA* and *asa1* (Naser *et al.,* 2005). The PCR thermal cycler used was the GeneAmp PCR system 9700 (Applied Biosystems™). Sequences were edited using the Bioedit6 software and identified using the basic local alignment search tool (BLAST) at >95% identity on NCBI database (*www.blast.ncbi.nlm.nih.gov)*.

Three designated virulence specific primer pairs (forward and reverse) (Olawale*et al.,* 2015) were used on the genomic DNA of twelve isolates to determine the presence of virulence genes. Primer sequences are detailed in Table 1.

Data analysis

Data presented in this study were subjected to statistical analyses using MEGA 7 software; Alignment of sequences (Pairwise and Multiple) was done with gap opening and extension penalty at 15 and 6.66 respectively; percentage relationships of samples from different isolates in relations to the conserved and variable regions of DNA sequences**;** and the use of Neighbor-joining statistical method at 1000 bootstrap replications for the phylogenetic analysis.

Results and discussion

Results of Bacterial Analyses

A total of one hundred and twenty fecal (120) samples from four different sources were collected for this study made up of the poultry (30), cattle (30), piggery (30) and human clinical isolates (30). The result of bacteria isolation and characterization showed 94(78.3%) growth of *Enterococcus* species. Table 2 shows the distribution of *Enterococcus* isolated from different sources in the course of this study. The positive samples showed Cattle 30(100%) with highest percentage growth, poultry 24(80%), piggery 20(67%) and from human clinical isolates collected, 20(67%) was confirmed *Enterococcus* spp. Figure 6 indicates positive esculin hydrolysis from *Enterococcus* species with a visible change of color of the media to dark brown due to the reaction of esculetin and ferric ions and pure cultures of the test organism on nutrient agar after 24 hours of incubation at 37^{o}C.

Table 1. Primers selected for detection of virulence determinants among twelve Enterococcistrains.

Target Gene	Sequence (5'- 3')		Position (bp)	Product Size (bp)
GelE	ACCCCGTATCATTGGTTT	F	762	405
	ACGCATTGCTTTTCCATC	R	1163	
CylA	GACTCGGGGATTGATAGGC	F	6656	688
	GCTGCTAAAGCTGCGCTTAC	R	7344	
AsaI	CCAGCCAACTATGGCGGAATC	F	3122	529
	CCTGTCGCAAGATCGACTGTA	R	3651	

Antibiotic Test Results

The percentage antibiotic resistance of cattle isolates presented in Fig. 1 shows multiple resistance patterns with high resistance in the following antibiotics: Erythromycin (93.3%), cloxacillin (93.3%), vancomycin (80%) and augmentin (60%). Ofloxacin was observed as the most suitable antibiotic in-vitro for cattle isolates with a high potencyat 100%. Multiple resistance patterns recorded are ERY- CXC- VAN (23%), ERY- CXC- AUG- VAN (50%) and ERY- CXC- AUG(27%). Fig. 2 shows the multiple resistance frequency in piggery isolates; the most potent antibiotic was ofloxacin at (100%) and the lowest was ceftazidime at (10%). Two resistance patterns were recorded: CAZ- CRX- CTR- CXC (10%) and CAZ (15%).

Table 2. Distribution of *Enterococcus* isolated**.**

Sample source	No. of isolates n=30	%
Cattle	30	100
Piggery	20	67
Clinical isolates	20	67
Poultry	24	80
Total	94	

Fig. 3 shows the percentage resistance for poultry isolates to cloxacillin (79%) followed by erythromycin (67%), ceftazidine (63.5%) and vancomycin (41%). However, the most potent antibiotic to poultry isolates was Ofloxacin (63%) and the weakest was erythromycin at (17%). Three resistant patterns were recorded from this sample source to include: ERY- CXC- AUG- VAN (21%), CAZ- CRX- ERY- CXC- AUG

(8%) and CAZ- CRX- ERY- CXC- AUG –VAN (13%). In Fig. 4, human clinical isolates were resistant to vancomycin at (90%) having the highest frequency followed by ceftazidine (80%), cefuroxime (80%), cloxacillin (80%), erythromycin (75%), augmentin (80%), ofloxacin (30%), gentamycin (25%) and ceftriaxone (0%). Ceftriaxone was recorded as the most effective amongst all the antibiotics used at (75%). Four resistance patterns were recorded in human clinical isolates which include: CAZ- CRX- ERY- CXC-AUG- VAN(15%), CAZ- CRX- ERY- CXC- OFL- VAN (10%), CAZ- CRX- GEN- ERY- CXC- AUG- VAN (10%) and CAZ (15%).

Table 3. Antibiotic resistance profile of bacterial isolates.

Sample source	Resistance profile	%
Cattle	ERY-CXC-VAN	23
	ERY-CXC-AUG-VAN	50
	ERY-CXC-AUG	27
Piggery	CAZ- CRX- CTR- CXC	10
	CAZ	15
Poultry	ERY-CXC-AUG- VAN	2
	CAZ- CRX- ERY-CXC-AUG	8
	CAZ- CRX- ERY-CXC-AUG –VAN	13
	CAZ- CRX- ERY- CXC-AUG- VAN	15
Clinical isolates	CAZ- CRX- ERY- CXC- OFL- VAN	10
	CAZ- CRX- GEN- ERY- CXC- AUG- VAN	10
	CAZ	15

KEY: CAZ- Ceftazidime CRX-Cefuroxime GEN-Gentamycin CTR- Ceftriaxone ERY-Erythromycin CXC-Cloxacillin OFL- Ofloxacin AUG-Augmentin VAN- Vancomycin.

Results of Molecular Analyses

Table 3 presents the antibiotic resistance patterns of the isolates from different sample sources while DNA bands of representative isolates visualized on safe view-stained 1.5% agarose electrophoresis gel are presented in Fig. 7. Figure 8 presents the bands of the 16S rDNA gene of 12 isolates with an amplicon size of about 1500bp using the hyper ladder 1 DNA ladder. Table 4 shows the BLAST hits of representative isolates after sequences were edited.

Table 4. BLAST hits of representative isolates.

Sample	Isolate name	Accession no.	Identity %
C1	*Enterococcus durans*strain CAU9886	MF098119.1	96.90
C2	*Enterococcus durans*strain CAU9886	MF098119.1	97.17
C3	*Enterococcus durans*strain CAU9886	MF098119.1	97.55
H1	*Enterococcus* sp. strain CAU6869	MF428647.1	92.33
H2	*Enterococcus durans*strain CAU9886	MF098119.1	99.44
H3	*Enterococcus durans*strain CAU9886	MF098119.1	99.53
S1	*Enterococcus mundtii*	HQ419189.1	96.75
S2	*Enterococcus faecalis*strain CAU6617	MF108410.1	97.03
S3	*Enterococcus durans*strain CAU9886	MF098119.1	98.91
P1	*Enterococcus durans*strain CAU6590	MF108374.1	99.27
P2	*Enterococcus durans*strain CAU9886	MF098119.1	98.28
P3	*Enterococcus* sp. strain LABC	MH734729.1	87.44

KEY: C1-C3: Cattle; S1-S3: Piggery; H1-H3: clinical; and P1-P3: Poultry isolates.

Figures 9, 10 and 11 show the results of the PCR detection of virulence genes in *Enterococcus* spp: Aggregation substance (*Asa*1), which was present in 8 isolates at (66.6%), Cytolysin (*Cyl*1) in 2 isolates at (16.6%) and Gelatinase (*Gel*E) in 1 isolate at (8.3%). This is further detailed in Table 5.

Table 5. Distribution of Virulence genes in selected isolates.

Isolates	Sample source	*Asa1* gene	*Cyl*Agene	*Gel*Egene
C1	Cattle	+	-	-
C2	Cattle	+	-	-
C3	Cattle	+	-	-
H1	Clinical	+	+	-
H2	Clinical	-	-	-
H3	Clinical	+	-	-
S1	Piggery	-	-	-
S2	Piggery	+	-	-
S3	Piggery	-	+	-
P1	Poultry	-	-	+
P2	Poultry	+	-	-
P3	Poultry	+	-	-

From the evolutionary analysis using MEGA7 software, it was inferred that cattle samples were the most probable source of infection in humans with 78% conserved region of 16S rDNA gene (Figure 5).

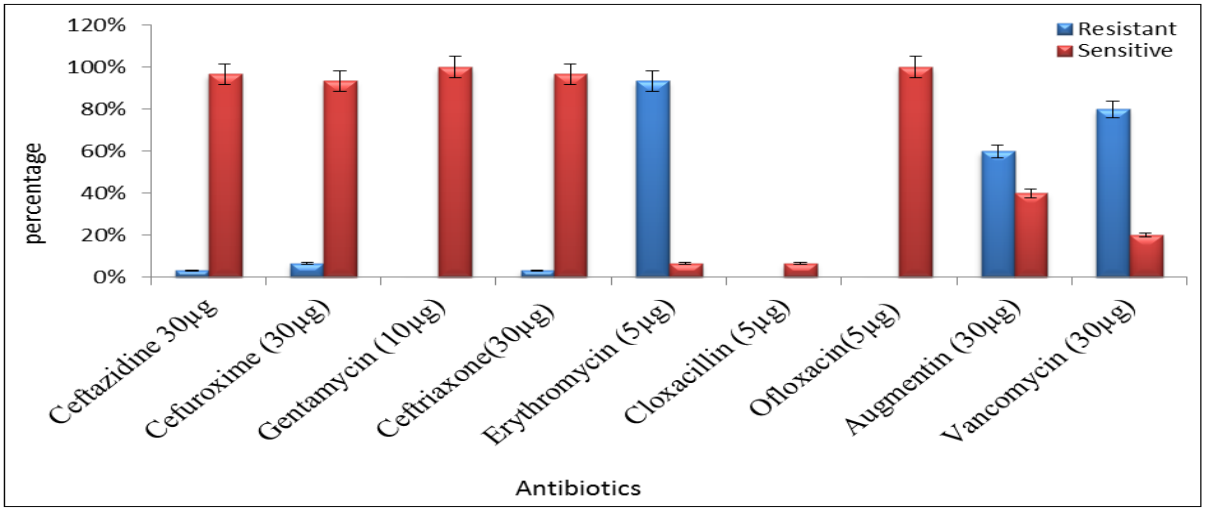

Fig. 1. Percentage antibiotic susceptibility of *Enterococcus* spp. from cattle samples to antibiotics.

Results discussion

Enterococcus species have developed from being commensal bacteria to leading pathogens that cause infections in humans and animals, having the gastrointestinal tract of mammals as their normal habitat (Knijff *et al.,* 2001).

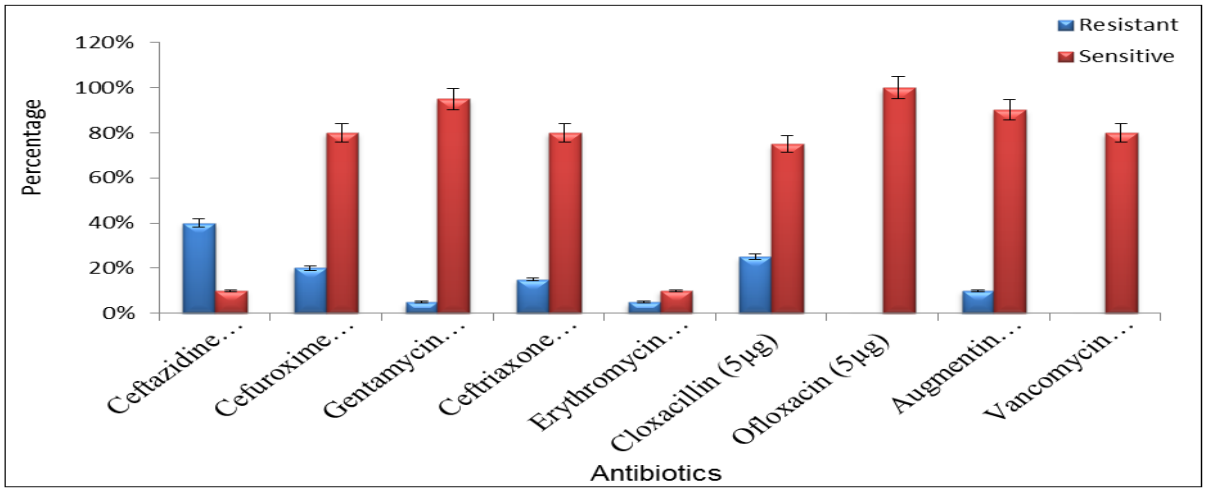

Fig. 2. Percentage antibiotic susceptibility of *Enterococcus* spp. from piggery isolates to antibiotics.

A high incidence of *Enterococcus* species was observed (78%) in this study as it is on other studies from around the world; South Africa (Iweriebor, *et al.,* 2015), Tunisia (Said *et al.,* 2017), China (Liu *et al.*, 2012), Nigeria (Anyanwu and Obetta 2015), Korea (Bang *et al.,* 2017), Turkey (Gökmen*et al.,* 2017) and Australia (Barlow *et al.*, 2017), where *Enterococcus* species are commensal organisms that inhabit the gastrointestinal tract of animals.

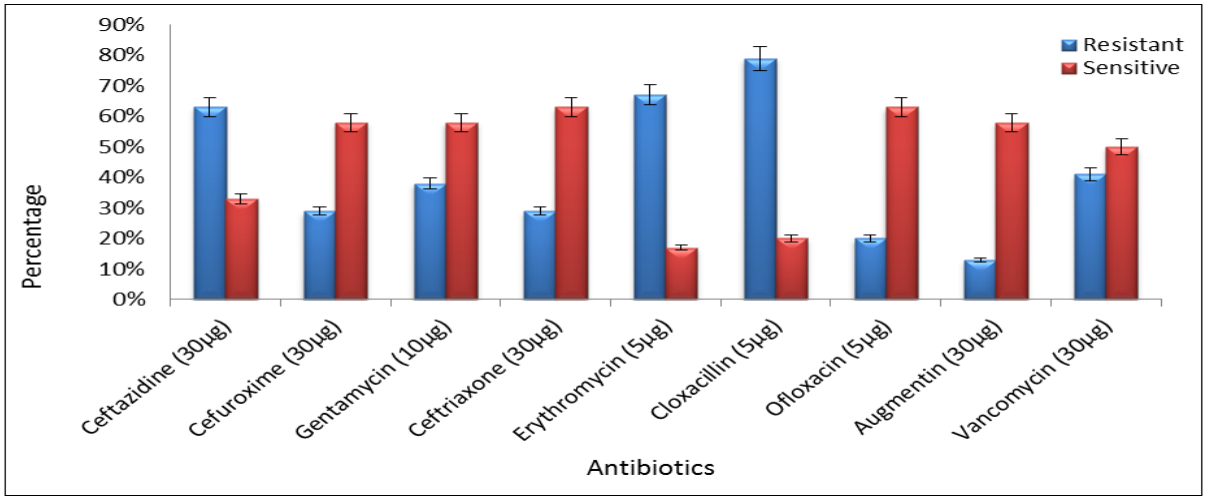

Fig. 3. Percentage antibiotic susceptibility of *Enterococcus* spp. from poultry isolates to antibiotics.

The most common species identified in this study was *E. durans*at 67% across all samples analyzed. While the clinical characteristics and treatment outcomes of species such as *E. faecalis* and *E. faecium* bacteremia are well known, those of *E. durans* bacteremia are still largely unclear. However, reports have been made of *E. durans* to have proven to lead to hematologic malignancy, and longer duration of hospital stay in bacteremia cases more than the known clinical isolates (DePerio *et al.,* 2006).

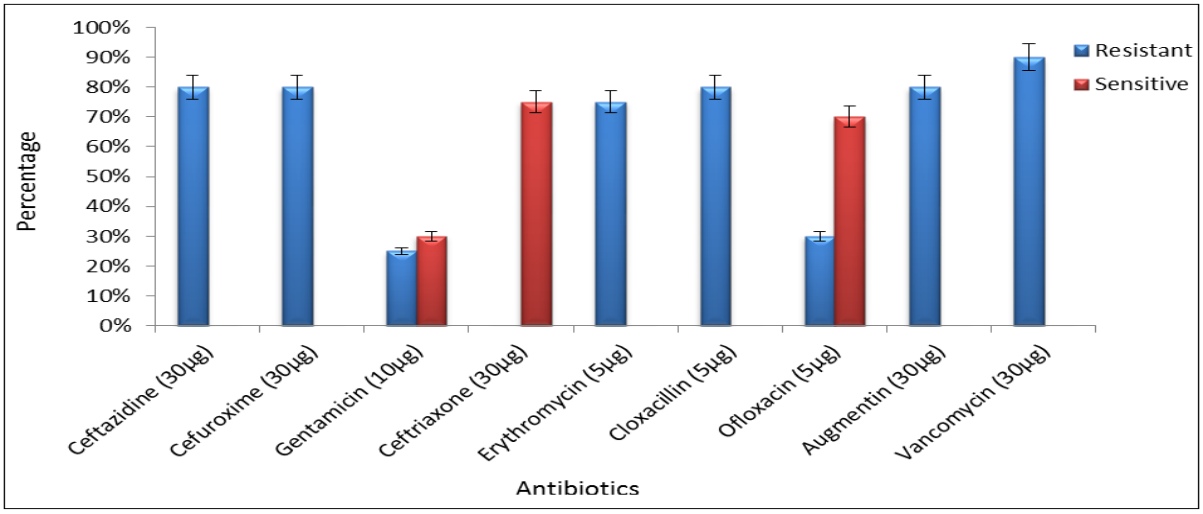

Fig. 4. Percentage antibiotic susceptibility of *Enterococcus* spp. from clinical isolates to antibiotics.

It was also reported to cause biliary and urinary tract infection and tended to cause infective endocarditis more than the clinical isolates. Most infections of *E. durans* were reported being community-acquired (Ryu*et al.,* 2019). There have been several other reports of E. durans infection in humans causing mainly endocarditis and blood access (Stepanovic*et al.,* 2004; Vijayakrishnan and Rapose, 2012; Kenzaka*et al.,* 2013; Fallavollita*et al.,* 2016; Zala and Collins, 2016).

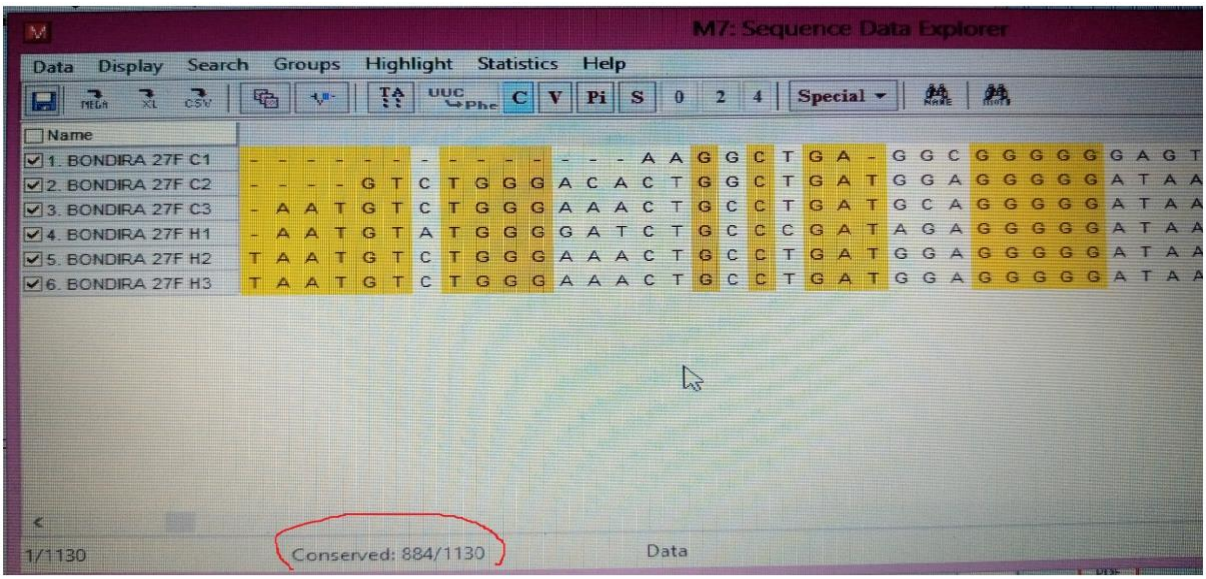

Fig. 5. Conserved regions of aligned 16S rDNA sequences of isolates of clinical and cattle origin using MEGA7 software.

Several genes, including *van*A, *van*B, *van*C, *van*D, and *van*E, contribute to resistance to vancomycin in enterococci, and most commonly, this resistance is seen in *E. faecium* and *E. faecalis*, but also has been recognized in *E. raffinosus*, *E. avium*, *E. durans*, and several other enterococcal species (CDC, 2010). Vancomycin resistance was recorded in isolates from all samples except the piggery where a low level of resistance was seen to all antibiotics. The plasticity of the enterococcal genomes allows Enterococci to respond rapidly and adapt to selective constraints by acquiring genetic determinants that increase their ability to colonize or infect the host (VanTyne and Gilmore, 2014).

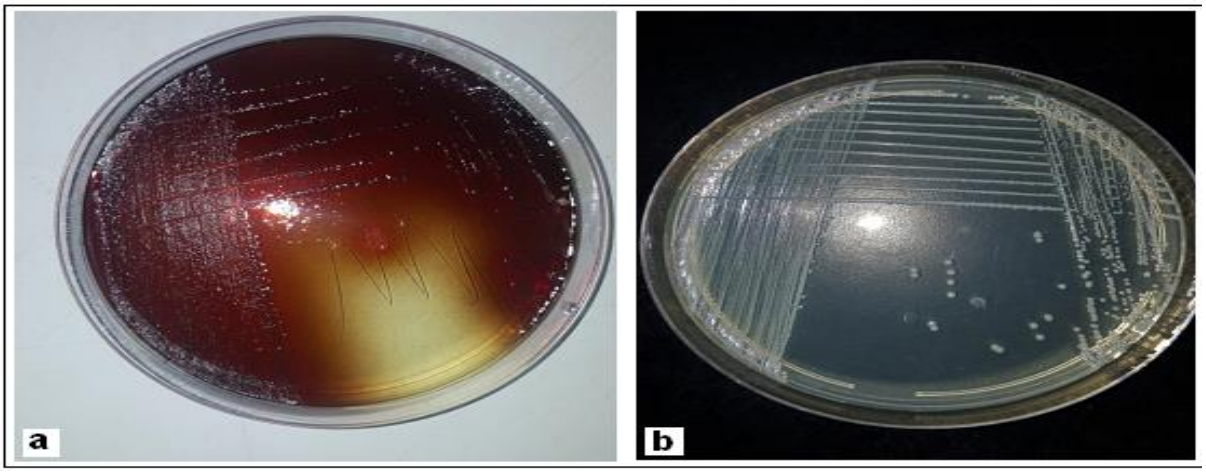

Fig. 6. (a) Esculin Hydrolysis by *Enterococcus*isolate showing dark coloration on bile esculin agar. (b) *Enterococcus*spp subculture on Nutrient agar after 24 hours of incubation at 37ºC.

Though most vancomycin-resistant Enterococci (VRE) belong to the species *E. faecium*, a major agent in hospital-acquired infections (Flokas *et al.,* 2017), this study reports vancomycin-resistant *E. durans*. There have been different reports of vancomycin resistance in E. durans, *vanA* and *vanB*as the means of resistance (Hall *et al.,* 1992; Torres *et al.,* 1994; Cercenado *et al.,* 1995, Descheemaeker*et al.,* 2000; Jenney *et al.,* 2000). However, a recent study reports *E. durans* isolates susceptible to penicillin, ampicillin, and vancomycin (Ryu *et al.,* 2019). All three virulence genes, aggregation substance (*Asa*1), Cytolysin (*CylA)*

and Gelatinase (*GelE*) were present in the study isolates, indicating them as potential pathogens if an opportunity arises. The gelatinase is an extracellular metalloprotease known to hydrolyze collagen, gelatin, and small peptides (Comerlato*et al.,* 2013), while the enterococcal cytolysin is a member of bacteriocin family known to help escape the host immune system by destroying macrophages and neutrophils. Moreover, *asa1* mediates the production of aggregation substances involved in adherence to eukaryotic cells; cell aggregation and conjugation (Upadhyaya *et al.,* 2009; Ferguson *et al.,* 2016).

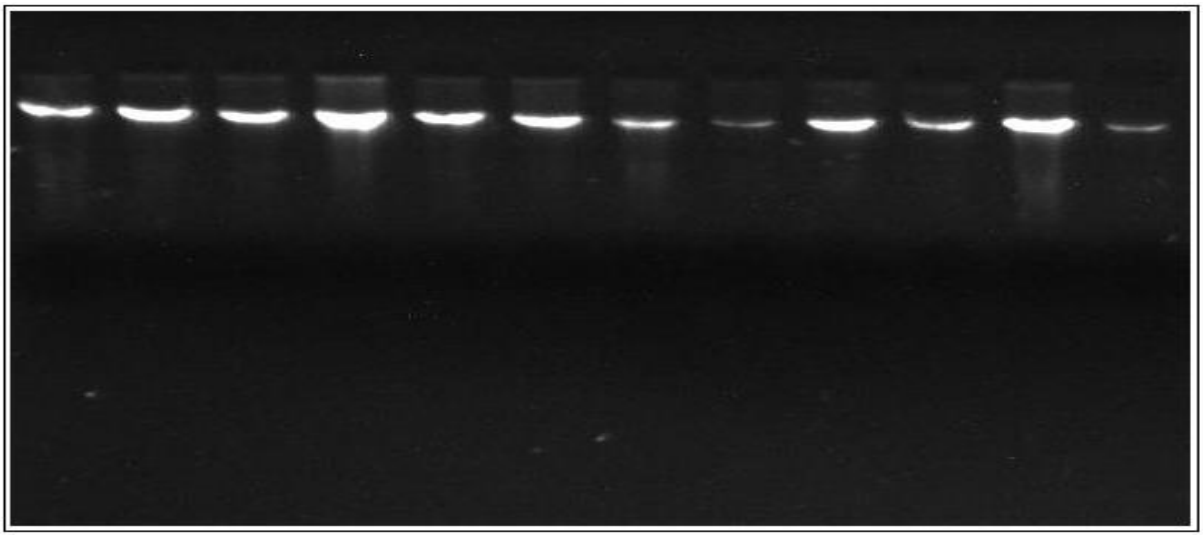

Fig. 7. DNA bands of representative isolates.
KEY: C1-C3: Cattle isolates; S1-S3: Piggery isolates; H1-H3: clinical isolates; P1-P3: Poultry isolates.

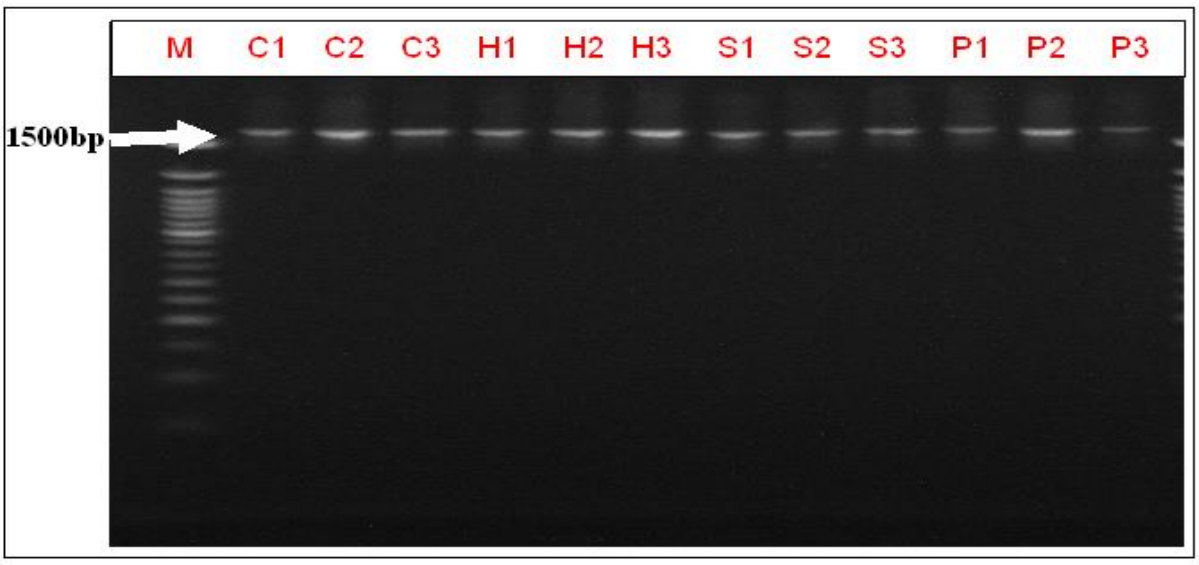

Fig. 8. 16S rRNAAmplicon of representative isolates of *Enterococcus* species.
KEY: C1-C3: Cattle isolates; S1-S3: Piggery isolates; H1-H3: clinical isolates; P1-P3: Poultry isolates.

Reports like Foka and Ateba (2019) have recorded the presence of the three genes in *E. durans* either all in one isolate or two more/less across the same species. While *Asa1* was present in isolates from all samples analyzed, *CylA* was present in isolates of clinical and piggery origin and *GelE* was present only in an isolate of poultry origin. A study in Eastern Cape Province of South Africa concluded that *Enterococcus* spp. from pigs and poultry must be treated with the highest caution because they may be reservoirs for virulence and antibiotic resistance genes (Iwerieboret *al.,* 2015). *Enterococcus mundtii* is rarely reported in

human infections and rarely known to harbor virulence genes, as the case of the isolate in this study and others but the resistance gene of *vanC* has been reported (Foka and Ateba, 2019).

Fig. 9. PCR detection of *Asa1* gene *inEnterococcus* spp.
KEY: C1-C3: Cattle isolates; S1-S3: Piggery isolates; H1-H3: clinical isolates; P1-P3: Poultry isolates.

It has been associated with raw milk, plants, the intestinal tract of humans and dairy cattle (Collins *et al.*, 1986; Giraffa*et al.*, 1997; Giraffa, 2003; Espeche*et al.*, 2009), it has low GC content ranging between 38 and 39% and lacks catalase and cytochrome-C oxidase enzymes, but can contribute in carbohydrates fermentation to produce lactic acid. It produces enterocins such as Bacteriocin ST15, that are quite active against bacteria such as *Pseudomonas, Clostridium, Klebsiella, Lactobacillus*, and *Acinetobacter,* etc. (De Kwaadsteniet*et al.*, 2005; Ferreira *et al.*, 2007; Settanni*et al.*, 2008).

It was reported to be used for the prevention of mastitis in cows (Espeche*et al.*, 2009). However, a case report of endophthalmitis in a 66-year-old individual and reports of the presence of some virulent genes (such as *asa1, esp, ace, hyl,* and *efaA*) calls for caution with the isolate (Higashide*et al.,* 2005; Trivedi *et al.,* 2011).

Fig. 10. PCR detection of *CylA gene* in *Enterococcus* spp.
KEY: C1-C3: Cattle isolates; S1-S3: Piggery isolates; H1-H3: clinical isolates; P1-P3: Poultry isolates.

This study also reported that the isolates had a high incidence of the aggregation substance gene as observed in other studies (Kataoka *et al.,* 2014; Ossiprandi and Zerbini, 2015). It is of importance to note that *Enterococcus* strains can have silent virulence genes as well and that environmental signals play a vital role in gene expression, hence influencing pathogenicity (Iseppi*et al.*, 2015).

Regardless of the fact that the *gel*E gene was least prevalent, it is not indicative of the production of gelatinase. It has been suggested that other genes are associated with the expression of gelatinase (Lindenstraub*et al.*, 2011). According to Kim *et al.* (2013), it is important to note that the presence of virulent strains among *Enterococcus* isolates alone is not predictive of infection as there may be other mediators of pathogenicity that have yet to be elucidated. It has been suggested that pathogenicity is also related to the ability of virulent strains to grow in high densities in the intestinal tract and spread to other sites in the body. Host factors, such as predisposing medical conditions, immune status, and exposure to antibiotics, are also thought to play a role in the ability of Enterococci to establish infection (Mundy *et al.,* 2000).

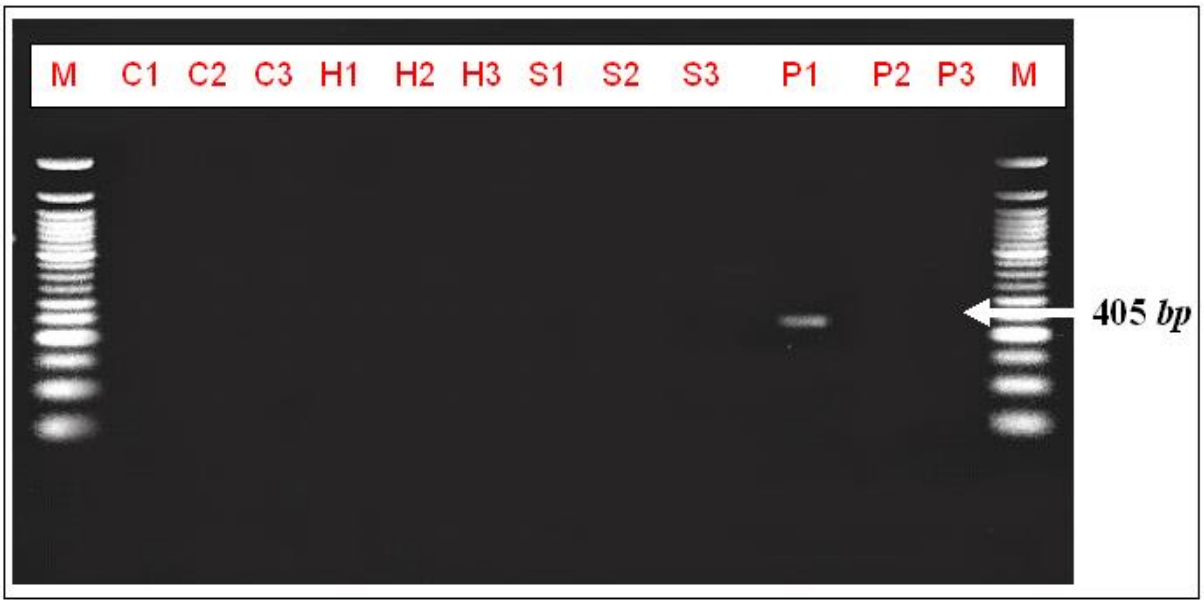

Fig. 11. PCR detection of *gelE gene* in *Enterococcus* spp.
KEY: C1-C3: Cattle isolates; S1-S3: Piggery isolates; H1-H3: clinical isolates; P1-P3: Poultry isolates.

Conserved nucleotide sequence analysis of the isolates in this study showed that isolates from cattle were closest to human clinical isolates at 79%, establishing the fact that animals act as reservoirs of most bacteria species later found in humans. According to Kataoka *et al.* (2014), animals are generally not affected by enterococcal infections; however, they act as a reservoir for pathogenic strains.

Conclusion

The results of this study showed that VREs of potential pathogenicity in humans are of animal origin, hence the need to practice safe agricultural procedures and a good level of hygiene which involves hand hygiene, contact/barrier precautions and source control.

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