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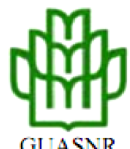


# Molecular Determinants of Virulence and Antimicrobial Resistance among *Enterococcus Species* Isolated from Chickens

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**Abstract**

Enterococci cause meat and environmental contamination during slaughter time. In this study, virulence and antimicrobial resistance (AMR) characteristics of enterococci isolated from chickens were determined. A total of 107 cloacal swabs of chickens were inoculated onto Slanetz and Bartley agar and incubated at 37°C for 24-48 h. Gram staining, catalase, and hemolytic tests were done. AMR was determined using the disc diffusion technique against twelve antimicrobials. Molecular detection of AMR genes: *blaZ*, *aphA,* *aacA-aphD*, *ermB*, *tetL*, *tetM*, and *vanC*, and virulence factors*:* $agrB_{Efs}$, $efaA_{Efs}$, *esp, gelE*, and *hyl* were done on selected isolates using PCR. Ninety-five isolates were *Enterococcus* species. The isolates showed resistance to tetracycline, cefoxitin, amoxicillin, and imipenem and possessed *tetL*, *tetM*, *ermB*, *aphA*, *vanC*, *aaca-aphD* resistance and *gelE*, *agrBef*, *efaAfs*, *espfs* and *hyl* virulence genes. This is the first detection of AMR and virulence genes in multi-drug resistant enterococci among chickens in the locality. These enterococci could constitute a reservoir of virulence and resistance properties which are of animal and public health concern.

## Introduction

*Enterococcus species* are commensals that inhabit the gastrointestinal tracts (GIT) of animals and humans and they play a role in digestion and other metabolic activities in their host (Hanchi *et al.,* 2018). Although some *Enterococcus* species are beneficial microbe, most are opportunistic pathogens that may cause nosocomial infections in humans (Rowland *et al.,* 2018). These microorganisms can be transferred horizontally through contaminated materials resulting in disease conditions such as wound infections, bacteremia, urogenital infections, septicemia, and endocarditis (Dolka *et al.,* 2019). They can penetrate the intestinal epithelium and initiate extraintestinal infections (Ramos *et al.,* 2019).

*Enterococcus spp* is known to be resistant to most antibiotics. Their resistance to antibiotics can be acquired through genetic transfer and become a reservoir of virulence and resistance properties (Munita and Arias, 2016).

The genetic flexibility of the organism aids in its ability to harbor these genes which can be transmitted to other organisms in a multi-culture environment (Chajęcka-Wierzchowska *et al.,* 2017). These organisms show resistance to cephalosporins, beta-lactams, and aminoglycosides and can acquire resistance to gentamicin, macrolides, tetracycline, streptogramin, and glycopeptides (Zalipour *et al.,* 2019); and some strains are multidrug-resistant (Farman *et al.,* 2019). Resistance to these classes of antibiotics makes treatment of enterococcal infections and infections caused by other enteric bacteria like *E. coli, Klebsiella*, and *Salmonella* which make up the intestinal microbiota very difficult (Farman *et al.,* 2019).

There are many virulence factors associated with enterococci, these include their ability to inhabit the GIT and/or invade and attach to the intestinal epithelial cells (Ahmed and Baptiste, 2018). This facilitates the assembling of bacteria for effective transmission of the conjugative plasmids from the donor to the recipient, biofilm formation, and adherence to abiotic surfaces. In both humans and animals, enterococci have been shown to act on hyaluronic acid and increase bacterial

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invasion. Virulence genes such as *asa1* (aggregation substance), *gelE,* (gelatinase) *cylA* (cytolysin), *esp* (enterococcal surface protein), *hyl* (hyaluronidase), *ace* (collagen binding protein), and *efaA* (endocarditis antigen) are very common determinant factors of enterococcal virulence (Madsen *et al.,* 2017). Researchers have shown that *Enterococcus* strains that have antibiotics resistance genes and can reveal virulence determinants cause more serious infections when compared to strains without virulence determinants (Chajęcka-Wierzchowska *et al.,* 2017). Their pathogenesis involves colonization, adhesion, and invasion of tissues which brings about resistance through defensive mechanisms (Flores-Mireles *et al.,* 2015). For a microorganism to be pathogenic, it has to harbor and express resistance and virulence attributes, and their molecular determinants (Heidari *et al.,* 2016).

Although there is documented no safety margin with respect to enterococci in chicken meat, reports have shown that transfer of antimicrobial resistant strains of enterococci to human may be possible (Conwell *et al.,* 2017), since chicken retail meat have been reported to harbor antibiotic-resistant enterococci strains (Manson *et al.,* 2019). Genetic transfer of resistance through plasmids or transposons and also through mutations in the chromosome causes setbacks in the control of infections (Munita and Arias, 2016). Dissemination of resistant genes such as tetM, ermB, and aphA-III in enterococci was reported to be associated with mobile genetic elements e.g.; transposons Tn916/Tn1545, Tn917/Tn551, and Tn5397 (Hegstad *et al.,* 2010) which could occur in E. faecalis through induction of plasmid transmission by production of pheromones (Hirt *et al.,* 2018).

Since it has been established that there is a relationship between the distribution and circulation of resistance genes in chicken production and the use of antimicrobials for growth and therapeutic interventions which is still very much in practice in the study area, there is a need for monitoring the antimicrobial resistance in chicken for the safety of the consumers and the environment (Manyi-Loh *et al.,* 2018). The prevalence of enterococci in the microflora of animals means that meat and environmental contamination during processing is highly possible. Therefore, screening chickens for virulence and resistance attributes that can constitute a threat to animal and public health should be a routine practice (Hasan *et al.,* 2018). This study was therefore carried out to ascertain the antimicrobial resistance characteristics and the presence of virulence and resistance genes in *Enterococcus spp* isolated from chickens.

## Materials and Methods

One hundred and seven cloacal swab samples were randomly collected from chickens (layers and broilers) from 11 poultry farms in Nsukka, Nigeria. Fifty-seven layers and 50 broilers were sampled. The layers were 24 weeks old while the broilers were 10 weeks old. All the farms were operating deep-litter systems. All the samples were collected from living and healthy chickens. The cloacal swab samples were inoculated onto a selective medium (Slanetz and Bartley agar) and blood agar supplemented with 5% sheep blood incubated at 37 ºC for 24 to 48 h. The enterococci isolates were identified based on their colony morphology, Gram's staining, the presence and type of hemolysis, and catalase production (Devriese *et al.* 1993). Samples that were Gram's-positive diplococci, catalase-negative, and hemolytic were presumed to be enterococci.

Antimicrobial susceptibility tests of the enterococci isolates were determined by the disc diffusion technique on Mueller Hinton agar (CLSI., 2018). Eleven antibiotics (Oxoid) were used and they include: amoxicillin (30 µg), ampicillin (10µg), ciprofloxacin (5 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), imipenem (10µg), sulphamethoxazole/trimethoprim (25 µg), gentamycin (30 µg), tetracycline (30 µg), vancomycin (5 µg) and streptomycin (10 µg). The antibiotics were classified as susceptible or resistant according to the criteria of the Clinical and Laboratory Standards Institute (CLSI., 2018).

### DNA extraction and antibiotic resistance gene detection

Overnight cultures of presumptive enterococci were used for DNA extraction as previously described (Cancilla *et al,* 1992). Strains were inoculated into 10 mL Nutrient broth (HiMedia, India). They were put in the incubator for 24 h at 37˚C. One milliliter of the culture was transferred into a microcentrifuge tube and centrifuged at 16,800 ×*g* for 2 min. The resultant cell pellet from the centrifugation was suspended in 200 µL of Tris-EDTA buffer (pH 7.2) and 30 µL of lysozyme (2000U/µL) and mixed by gentle rocking. The mixture was incubated at 37˚C for 1 h. Then, 33 µL of 10% SDS (v/v) was added and incubated at 62˚C for 30 min. Three hundred microlitres of phenol: chloroform: isoamyl alcohol at the ratio of 25:24:1 was added and mixed very well using a vortex mixer for 10 sec, and centrifuged at 16,800 ×*g* for 1min. The top filtrate was recovered and transferred to a new centrifuge tube, then 1/10 volume of 3 M sodium acetate was added to it and mixed very well by inverting the tube. Two volumes of ethanol (100%) were added to the mixture and incubated on ice for 5 min. The solution was centrifuged at 16,800 ×*g* for 5 min and the supernatant recovered. One milliliter of 70% ethanol was used to wash the DNA pellet and centrifuged at

16,800 × *g* for 1min and air-dried for 10 min. The DNA was resuspended in 100 µL of Tris-EDTA buffer (pH 8.0).

**Polymerase chain reaction (PCR)**

PCR detection of antibiotic-resistant genes using DNA from the selected isolates of *Enterococcus* species was carried out. They were tested for the presence of *blaZ, aacA-aphD, aphA, ermB, tetL, tetM* and *vanC* genes, that code for penicillin, aminoglycoside (gentamycin), erythromycin, tetracycline, and vancomycin resistance respectively. Table I shows the primers used and they were synthesized from Xcleris (India). The PCR volume was similar in all the processes. A 25 µL was used that contained 12.5 µL EmeraldAmp® GT PCR Master Mix (Takara Clontech, Japan), 10 pmol/µL of forward and reverse primers of every gene, 0.01 µg-0.2 µg template and sterilized nuclease-free water that made the total volume was used. All the processes were done using a Mastercycler Gradient Thermocycler (Applied Biosystems, USA). The detection of *tetL*, and *tetM*, (*ermB*, *aacA-aphD, and aphA)* was carried out in a Multiplex PCR assay under cycling conditions as described by Zehra *et al.,* (2017). The amplification conditions were denaturation of DNA at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30 sec,annealing at 55°C for 30 sec, extension at 72°C for 30 sec, and final extension at 72°C for 4 minand hold at 4°C. Amplification of *blaZ* gene was done at denaturation of DNA at 94°C for 45 sec, 30 cycles of denaturation at 94°C for 20 sec, annealing at 55°C for 15 sec, extension at 70°C for 15 sec, and extension at 72°C for 2 min and hold at 4°C (Zehra *et al.,* 2017). For *vanC* gene, the amplification mixture consists of the following: 12.5 µL master mix EmeraldAmp® GT PCR Master Mix, 2 µM of each primer, 0.1 µg template DNA, and amplification conditions were initial denaturation at 98°C for 2 min, followed by 35 cycles each of denaturation at 98°C for 10 seco,, annealing at 50°C for 1 min, extension at 72°C for 60 secand final extension at 72°C for 5 min (Saha *et al.,* 2008).

**Table 1.** The primers used in PCR for the detection of resistance and virulence genes in *Enterococcus* isolates

| Genes [1] | Primer Sequence (5' - 3') | Product size (bp) | Annealing temperature (˚C) | cycles |
|---|---|---|---|---|
| *tetM* | GTG GAC AAA GGT ACA ACG AG<br>CGG TAA AGT TCG TCA CAC AC | 406 | 55 | 35 |
| *tetL* | TCG TTA GCG TGC TGT CAT TC<br>GTA TCC CAC CAA TGT AGC CG | 267 | 55 | 35 |
| *ermB* | AGT AAC GGT ACT TAA ATT GTT TAC<br>GAA AAG GTA CTC AAC CAA ATA | 639 | 55 | 35 |
| *aacA-aphD* | CAG AGC CTT GGG AAG ATG AAG<br>CCT CGT GTA ATT CAT GTT CTG GC | 348 | 55 | 35 |
| *aphA* | ATG GGC TCG CGA TAA TGT C<br>CTC ACC GAG GCA GTT CCA T | 630 | 55 | 35 |
| *vanC* | GAA AGA CAA CAG GAA GAC CGC<br>ATC GCA TCA CAA GCA CCA ATC | 796 | 55 | 35 |
| $agrB_{Efs}$ | TTT ATT GGT ATG CGC CAC AA<br>CAT CAG ACC TTG GAT GAC GA | 173 | 52 | 35 |
| $efaA_{Efs}$ | GAC AGA CCC TCA CGA ATA<br>AGT TCA TCA TGC TGT AGT A | 704 | 52 | 35 |
| $esp_{fs}$ | TTG CTA ATG CTA GTC CAC GAC C<br>GCG TCA ACA CTT GCA TTG CCG AA | 955 | 52 | 35 |
| *gelE* | AGT TCA TGT CTA TTT TCT TCA C<br>CTT CAT TAT TTA CAC GTT TG | 403 | 52 | 35 |
| *hyl* | GAG TAG AGG AAT ATC TTA GC<br>AGG CTC CAA TTC TGT | 662 | 52 | 35 |

[1] *tetM*= encoding tetracycline resistance protein; *tetL*= encoding tetracycline resistance leader peptide; *ermB*= *tetL* macrolide-lincosamide-streptogramin B resistance protein; *aacA-aphD*= encoding aminoglycoside resistance protein; *aphA*= encoding Class B acid phosphatase; *vanC*= encoding vancomycin C-type resistance protein; $agrB_{Efs}$= encoding putative *agrB-like protein* gene of *E. faecalis*; $efaA_{Efs}$= encoding *Enterococcus faecalis* endocarditis antigen; $esp_{fs}$= encoding enterococcal surface protein; *gelE*= encoding gelatinase; *hyl*= encoding hyaluronidase.

**Detection of Virulence genes**

PCR was performed for detecting five of the genes encoding virulence factors which include the following: enterococcal surface protein (*esp*), gelatinase (*gelE*), *Enterococcus faecalis* endocarditis antigen ($efaA_{Efs}$), putative *agrB-like protein* gene of *E. faecalis* ($agrB_{Efs}$) and hyaluronidase (*hyl*). Primers were synthesized as previously described and were as follows: *esp, hyl, gelE,* $agrB_{Efs}$ (Vankerckhoven *et al.* 2004), and $efaA_{Efs}$ (Dupre *et al.,* 2003). One percent agarose gel electrophoresis was used to separate the bands in 1x TAE buffer and

stained with Red Safe dye (CinnaGen Co., Tehran, Iran) and visualized under transillumination.

## Results

### The isolation rate of *Enterococcus* species from chicken

*Enterococcus* species were isolated from 95 (88.8%) of the 107 cloacal swab samples. Forty (46.7%) isolates were from broilers while 45 (42.1%) isolates were from layers. They were all catalase negative. Three (3.2%) isolates were hemolytic (beta-hemolysis) while 92 (96.8%) isolates were non-hemolytic on blood agar.

### Antibiotic resistance profile of *Enterococcus* isolates

Most of the *Enterococcus* isolates were resistant to tetracycline 71 (94.7%) while they showed the least resistance to amoxicillin 14 (18.7%) and imipenem 12 (16%) (Table 2).

**Table 2.** Antibacterial resistance profile of *Enterococcus* species isolated from chickens

| Antimicrobial agent | No of isolates (%) Resistant | No of isolates (%) Intermediate | No of isolates (%) Susceptible |
|---|---|---|---|
| Tetracycline | 71 (94.6) | 2(2.7) | 2(2.7) |
| Streptomycin | 61 (81.3) | 3(4) | 11(14.7) |
| Gentamicin | 50(66.7) | 4(5.3) | 21(28) |
| Sulphamethoxazole/ Trimethoprim | 42(56) | 2(2.7) | 31(41.3) |
| Ampicillin | 41(54.7) | 0 (0) | 34(45.3 ) |
| Ceftriaxone | 36(48) | 27(36) | 12(16) |
| Chloramphenicol | 32(42.7) | 5(6.6) | 38(50.7) |
| Vancomycin | 23(31.1) | 25(33.7) | 26(35.1) |
| Ciprofloxacin | 19(25.3) | 9(12) | 47(62.7) |
| Amoxicillin | 14(18.7) | 0 (0) | 61(81.3) |
| Imipenem | 12(16) | 3(4) | 60(80) |

Isolates from broilers exhibited resistance to most of the antibiotics when compared with those isolated from layers. The *Enterococcus* strains isolated from layers did not show resistance to amoxicillin and imipenem (Figure 1).

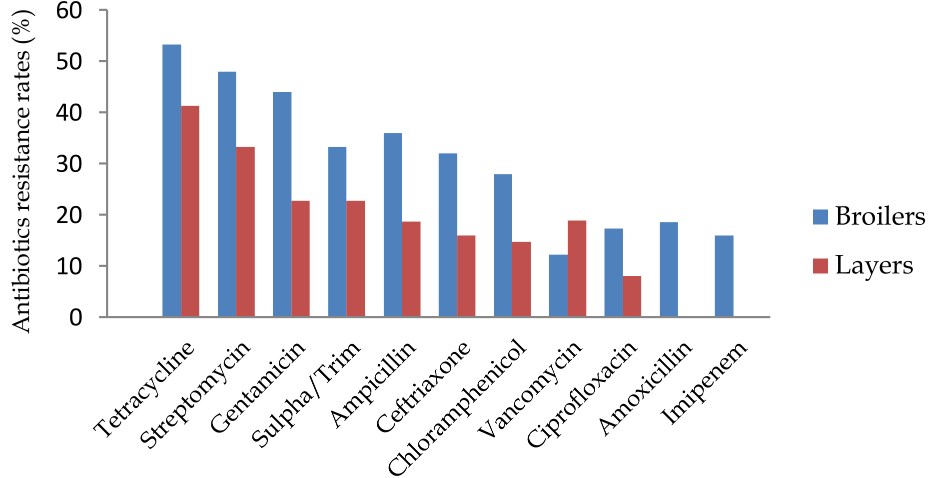


**Figure 1.** Antibiotic resistance rates (%) of *Enterococcus* species isolated from broilers and layers

The *Enterococcus* isolates showed 52 resistance patterns. TE-S and TE-S-AMP were the most predominant pattern with frequency of 4 each. Out of 72 isolates investigated, 1 (1.4%) was resistant to all the 12 antibiotics used, 3 (4.2%) were resistant to one antibiotic each, while 68 (94.7%) were resistant to 3 to 11 antibiotics used in this study (Table 3).

### Occurrence and distribution of antibiotic resistance genes

Out of 30 enterococcal isolates selected and tested for resistance genes, *tetL, tetM*, *ermB*, *aphA*, *vanC*, and *aaca-aphD* were present in 28 (93.3%), 20 (66.7%), 18 (60.0%), 115 (36.7%), 7 (23.3%) and 2 (6.7%) respectively. The *Enterococcus* strains tested harbored 6 resistance genes out of 7 genes tested. *blaZ* gene was not detected among the isolates. The resistance gene pattern of the *Enterococcus* strains is presented in Table 4. Eight patterns were observed, with *tetL- tetM* being the predominant combination. The number of resistance genes per strain ranged from one to five.

**Table 3.** Resistance pattern exhibited by *Enterococcus species* isolated from chickens

| Resistant pattern | No of isolates (%) |
|---|---|
| AMP | 1(1.39) |
| TE | 1(1.39) |
| VA | 1(1.39) |
| TE- CN | 2(2.78) |
| TE-S | 4(5.56) |
| S-CN | 1(1.39) |
| TE-CN-VA | 1(1.39) |
| TE-S-AMP | 4(5.56) |
| TE-CRO-CN | 2(2.78) |
| TE-S-CN | 1(1.39) |
| TE-CN-CIP | 1(1.39) |
| TE-S-SXT | 1(1.39) |
| TE-S-AMC | 1(1.39) |
| S-CN-SXT-CIP | 1(1.39) |
| TE-S-CN-SXT | 1(1.39) |
| TE-S-CN-VA | 1(1.39) |
| TE-S-CRO-CN | 1(1.39) |
| TE-S-CRO-CN-SXT | 1(1.39) |
| TE-S-CN-SXT-VA | 1(1.39) |
| TE-S-C-CN-SXT | 3(4.17) |
| TE-CRO-CN-SXT-VA | 1(1.39) |
| TE-AMP-CRO-SXT-CIP | 1(1.39) |
| TE-S-AMP-CRO-VA | 1(1.39) |
| TE-S-AMP-CRO-C | 2(2.78) |
| TE-S-CRO-SXT-VA | 1(1.39) |
| TE-CN-SXT-VA-CIP | 1(1.39) |
| TE-S-AMP-CRO-CN | 1(1.39) |
| TE-S-AMP-CRO-CIP | 1(1.39) |
| TE-S-AMP-C-SXT | 1(1.39) |
| TE-S-C-CN-SXT-CIP | 1(1.39) |
| TE-S-C-CN-SXT-VA | 3(4.17) |
| TE-AMP-CRO-CN-SXT-VA | 1(1.39) |
| TE-S-AMP-CRO-C-CN | 2(2.78) |
| TE-S-AMP-CRO-C-VA | 1(1.39) |
| TE-S-AMP-C-SXT-CIP | 1(1.39) |
| TE-S-CRO-C-CN-SXT | 2(2.78) |
| TE-S-AMP-C-CN-SXT | 1(1.39) |
| TE-S-AMP-CRO-CIP-AMC-IMP | 1(1.39) |
| TE-S-AMP-C-CN-SXT-VA | 1(1.39) |
| TE-S-AMP-C-CN-SXT-AMC | 1(1.39) |
| TE-S-AMP-CRO-C-CN-SXT | 1(1.39) |
| TE-S-AMP-CRO-C-CN-SXT-CIP | 2(2.78) |
| TE-S-AMP-CRO-C-CN-SXT-VA | 2(2.78) |
| TE-S-AMP-CRO-C-SXT-AMC-IMP | 1(1.39) |
| TE-S-AMP-CRO-CN-SXT-CIP-AMC-IMP | 2(2.78) |
| TE-S-AMP-CRO-CN-CIP-VA-AMC-IMP | 2(2.78) |
| TE-S-AMP-CRO-C-CN-SXT-CIP-VA | 1(1.39) |
| TE-S-AMP-CRO-C-CN-SXT-AMC-IMP | 1(1.39) |
| TE-S-AMP-CRO-CN-SXT-CIP-VA-AMC-IMP | 2(2.78) |
| TE-S-AMP-CRO-C-CN-SXT-VA-AMC-IMP | 1(1.39) |
| TE-S-AMP-CRO-C-CN-SXT-CIP-AMC-IMP | 1(1.39) |
| TE-S-AMP-CR0-C-CN-SXT-CIP-VA-AMC-IMP | 1(1.39) |
| Total | 72 |

Amoxicillin (AMC), Ampicillin (AMP), Ciprofloxacin (CIP), Ceftriaxone (CRO), Chloramphenicol (C), Imipenem (IMP), Sulphamethoxazole/Trimethoprim (SXT), Gentamycin (CN), Tetracycline (TE), Vancomycin (VA) And Streptomycin (S).

**Table 4**. Resistance gene pattern of the selected *Enterococcus species* isolated from chickens

| Resistance gene pattern | No of isolates (%) |
|---|---|
| *aaca-aphD – aphA – ermB – tetL – tetM* | 1(7.7) |
| *aphA – ermB – tetL – tetM* | 1(7.7) |
| *aphA – ermB – tetL – tetM – vanC* | 2(15.4) |
| *aphA – ermB – tetM* | 1(7.7) |
| *ermB – tetL – tetM – vanC* | 1(7.7) |
| *ermB – tetL* | 2(15.4) |
| *tetL – tetM* | 3(23.1) |
| *tetL* | 2(15.4) |
| Total | 13 |

**Occurrence and distribution of virulence genes**

The five virulence genes tested were detected among the isolates. Out of the 30 selected isolates tested for virulence genes, 18 (60%) isolates haboured virulence genes as follows: *gelE* (15), *agrBef* (14), *efaAfs* (12), *hyl* (4) and *espfs* (3) genes. Six patterns were observed, with *agrBef - gelE– efaAfs – hyl* and *gelE* as predominant patterns. There were one to four virulence genes per strain observed (Table 5).

**Table 5.** Virulence gene pattern of the selected *Enterococcus species* isolated from chickens

| Virulence gene pattern | No of isolates (%) |
|---|---|
| *agrBef – gelE -* | 1(14.3) |
| *agrBef - gelE – efaAfs* | 1(14.3) |
| *agrBef - gelE – efaAfs– hyl* | 1(14.3) |
| *agrBef - gelE – efaAfs – espfs* | 1(14.3) |
| *agrBef – efaAfs* | 1(14.3) |
| *gelE* | 2(28.6) |
| Total | **7** |

**Discussion**

Enterococci in poultry are mostly recovered from their faeces (Ali *et al.,* 2013). The 88.8% isolation rate of *Enterococcus* species shows that the organism is highly present in the gastrointestinal tract of chickens in the study area and could constitute a source of environmental contamination. This was greater than that reported by Amaechi and Nwankwo (2015) among chickens in Nigeria and also greater than that reported by some researchers in other parts of the world (Sanlibaba *et al.,* 2018). The results showed that broiler farms were more contaminated with enterococci than the layer farms and the variation in the isolation rate in this study may be related to differences in the rate of contamination of farm environments and the chickens themselves. This high isolation rate observed in this study indicates that the *Enterococcus* species is endemic among chickens in the area. The chickens may have acquired these bacteria through contamination of their food and water by faecal droppings. The high occurrence of enterococci in chickens could be a means of infection to human through meat or occupational hazards for farmers and poultry workers which could become a potential public health concern (Sørensen *et al.,* 2001).

The result of antibacterial testing revealed that all of the enterococci were resistant to one or more antibacterial agents tested. The high incidence of antibiotic-resistant enterococci to more than one drug is of great public health concern (Tian *et al.,* 2019). In this study 5 classes of antibiotics were used, and a higher percentage of the isolates tested were resistant to 3 or more classes of antibiotics which show that they are multidrug resistant. These results are in line with the reports of Top *et al* (2007), who found that *Enterococcus* species showed resistance to more than one antimicrobial. Their report of the organism being resistant also to disinfectants and alcohols make the presence of the organisms in the area a public health concern. Enterococci are known to be naturally resistant to many antibiotics commonly used for the treatment of several bacterial infections. These acquire by their potential to obtain and transmit genes responsible for resistance through plasmids and transposons (Munita and Arias, 2016). Statistics of resistance to antimicrobials by enterococci isolated from food animals have shown that there is a relationship between the antimicrobial used in animal production and the resistant strains in animals and animal products (Kimera *et al.,* 2020).

The highest level of resistance was observed for tetracycline. Tetracycline is a broad-spectrum antibiotic frequently used for growth promotion and treatment of chicken infection in Nigeria (Oluwasile *et al.,* 2014). The high rate of resistance to tetracycline recorded in this study may be a result of continuous use of the drug in poultry production. This result is similar to work by Ayeni *et al*. (2016) in Nigeria who reported a high rate of tetracycline resistance in *Enterococcus* species recovered from chickens and a high occurrence of resistance to

tetracycline among enterococci isolated from broilers was also reported by Cauwerts *et al.* (2007). The high occurrence of resistance to tetracycline seen in this study was in line with the high occurrence of *tetL and tetM* genes. The *tetL* gene is a large protein with 14 transmembrane domains and exhibits tetracycline resistance through active efflux (Miller *et al.,* 2014). On the other hand, *tetM* gene is often detected tetracycline resistance determinant seen in enterococci. It exhibits resistance because of the ribosome protection through large cytoplasmic proteins that resemble the elongation attributes (Cauwerts *et al.,* 2007).

In *Enterococcus*, *erm* genes are encoded by the methylation of the 23S RNA with the help of methylase enzymes, this is seen in the resistance to MLSB antibiotics by the bacteria (Miller *et al.,* 2014). In this study, *ermB* gene was seen in 8 isolates. This study did not evaluate any relationship among *tet* and *erm* genes but *ermB* gene was detected in 8 isolates and in 6 of the 8 *Enterococcus species* that possessed *tetM* gene. Wist *et al.* (2020) have recorded that tetracycline resistance is usually seen in enterococci isolated from poultry that carries *ermB* gene. It has been reported that *Enterococcus species* from chicken may harbor $MLS_B$ and tetracycline resistance genes which may be transferred to other bacteria by *trans,* a transposon, known to carry multi-resistance gene clusters (Argudín *et al.,* 2017).

The acquired mechanism of aminoglycoside resistance in enterococci include the following ribosomal target modification, transport alteration, and enzymatic modification (Miller *et al.,* 2014). High resistance rates to streptomycin and gentamicin were also observed in this study although the disc concentration was lower than recommended. This suggests that the isolates may harbor genes encoding resistance to these antibiotics. In this present study, the *aaca-aphD* and *aphA* genes were detected. These genes encode for aminoglycoside alteration and can show much resistance to aminoglycoside and to collaborate between agents that activate cell walls and aminoglycoside (Agarwal *et al.,* 2009). The *aaca-aphD* and *aphA* genes were observed in 2 and 11 isolates respectively of the 13 isolates tested. In Nigeria, Amaechi and Nwankwo (2015) recorded zero resistance to gentamicin among *Enterococcus* species isolated from chickens which are contrary to the report in this study. No reason could be given for this finding except that the use of gentamycin for the treatment of chicken infections without veterinary recommendation may be increasing in Nigeria among farmers. It has been reported that a high level of streptomycin resistance was more frequent than a high level of gentamicin resistance (Fracalanza *et al.,* 2007) as observed in this present study.

Moderate rates of resistance to ampicillin, sulfamethoxazole /trimethoprim, ceftriaxone, chloramphenicol, ciprofloxacin, and vancomycin were observed among *Enterococcus* species tested in this study. This suggests that the isolates exerted selection pressure and developed resistance against these antibacterial agents. Vancomycin resistance is of great concern since its emergence has been reported in many studies. Olawale *et al.* (2011) reported 42.9% vancomycin resistance in Ekiti state, Nigeria. Vancomycin resistance has been documented as a result of keeping vancomycin resistance genes by some enterococci (Melese *et al.,* 2020) which supports the detection *vanC* gene in 7 (23.3%) of the isolates tested in this study. Although *vanC* gene was reported to be responsible for resistance to low levels of vancomycin (Reynolds and Courvalin, 2005), Vancomycin-resistant enterococci (VRE) are responsible for difficult-to-treat infection in both humans and animals and have been reported in health facilities in most countries (Bell *et al*., 1998). The detection of *vanC* gene and vancomycin resistance in this study is therefore of both animal and public health concern.

Though moderate resistance to chloramphenicol and ciprofloxacin was recorded, Ünal *et al.* (2017) have reported high resistance to ciprofloxacin and chloramphenicol in enterococci isolated from poultry. However, the presence of enterococci that are vancomycin-resistant together with resistance to aminoglycoside needs to be given continual concern so that vancomycin resistance can be detected early to curb the spread of such multidrug-resistant *Enterococcus* species.

*Enterococcus* isolates from broilers showed higher resistance to all antibiotics than those from layers. A similar result was reported by Yoshimura *et al* (2000). This could be because in poultry production, antibiotics are commonly used in the management of infection as a growth promoter among broilers than the layers (Xu *et al.,* 2020). This has led to resistance to these antibiotics by *Enterococcus* species.

The occurrence of enterococci in food animals is a sign that there is contamination of the environment by faecal material and this poses a threat to human well-being (Mehdi *et al.,* 2018). The resistance of *Enterococcus* species in this study to antibiotics commonly used in animals and humans, and the presence of their respective genes is of great concern. This shows that *Enterococcus* can disseminate these resistant genes to other bacteria (Price *et al.,* 2019). The occurrence of *vanC* gene is an indication that the poultry farms sampled in this study could play a role in the circulation of vancomycin resistance.

The pathogenicity of *Enterococcus* is brought about by many factors which are associated with many genes. *esp* gene encodes for the virulence of *Enterococcus spp* responsible for adhesion to eukaryotic cells leading to suppression of the host

immune system (Kiruthiga *et al.,* 2020). This gene is situated in the chromosome of enterococci and it plays a role in colonization (Kiruthiga *et al.,* 2020). Its presence is associated with biofilm formation in enterococci (Azizi *et al.,* 2017). In this study, *espfs* gene specific for *E. faecalis* was detected in 3 of the *Enterococcus* isolates tested, which shows the biofilm formation capabilities. Also, *E. faecalis* with *esp* gene is multi-resistant to different antibiotics including vancomycin (Weng *et al.,* 2019). In this study, the *espfs* gene-positive isolates also harbored *vanC* gene, which suggests a relationship between the two genes.

*gelE* gene is a virulent factor encoding gelatinase (an extracellular Zn-Metallo-endopeptidase) (Del Papa *et al.,* 2007). Gelatinase binds to fibrin enhancing tissue damage in the host thereby promoting bacterial dissemination, especially in infections caused by *E. faecalis* (Ahmed and Baptiste, 2018). This protease is also involved in biofilm formation which enables enterococci to adhere in some infected areas (Del Papa *et al.,* 2007). Some researchers pointed out that there may be an absence of the gene expression even though the *gelE* determinant gene is detected (Popović *et al.,* 2018).

The virulence gene *efaA* is associated with the pathogenesis of endocarditis (Ahmed and Baptiste, 2018). The two most common variants are *efaAfs* in *E. faecalis* and *efaAfm* in *E. faecium* (Stępień-Pyśniak *et al.,* 2019). This study found that 12 of the enterococci tested harbor *efaAfs* gene which shows that those isolates may be *E. faecalis*. This is a very important species that is incriminated with nosocomial infections in humans (Bhardwaj, 2019) and whose vancomycin-resistant strains have been implicated in difficult-to-treat infections.

The *hyl* gene encodes a hyaluronidase which hydrolyses hyaluronic acid that is involved in translocation (Starr and Engleberg, 2006). This gene is associated with antimicrobial-resistant genes and pilin genes found in the plasmid (Laverde Gomez *et al.,* 2011). In our study, the *hyl* gene was present in one Enterococcus isolate from sampled chicken that is *vanC*-positive strains. Gram-positive bacteria expressing hyaluronidase have been shown to affect the mucosal and/or skin surface of humans and animals causing different lesions/infections (Hynes and Walton, 2000).

Numerous authors have reported that there are many resistant genes and virulent attributes present in both pathogenic and non-pathogenic bacteria (Nowakiewicz *et al.,* 2017). In our findings, enterococci present as normal flora in chickens sampled had some virulence attributes from which pathogenic strains of enterococci could originate in humans and animals. This is because some of the pathogenic strains have been identified as antibiotic-resistant epidemic clones. This finding can be supported by the fact that *Enterococcus faecalis* isolated from human infections and those from poultry have shown close genetic makeup which may suggest a transmitting route from poultry to humans thereby strengthening the zoonotic potential of enterococci (Abat *et al.,* 2016). Fast and accurate detection of a gene is a good means of fighting against potentially resistant bacteria. The virulence attributes and detection of antibiotic-resistance gene in *Enterococcus* species would give insight into developing an effective way of combating antimicrobial resistance (Argudín *et al.,* 2017).

## Conclusion

This is the first study that shows the presence of both virulence and antibiotic resistance genes of enterococci in chickens reared in Nsukka, south-eastern Nigeria. The prevalence of *Enterococci* isolated from these chickens was high and these microorganisms were resistant to some commonly used antibiotics. These chickens can be a potential reservoir for virulence and multiple antibiotic resistance genes which could be a means of transmission to man and other animals through the meat. This can constitute a danger to the entire population through the food chain and as an occupational hazard.

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## References

Abat C, Huart M, Garcia V, Dubourg G & Raoult D. 2016. *Enterococcus faecalis* urinary-tract infections: do they have a zoonotic origin? Journal of Infection, 73: 305–313. DOI: 10.1016/j.jinf.2016.07.012

Agarwal J, Kalyan R & Singh M. 2009. High-level aminoglycoside resistance and -lactamase production in enterococci at a tertiary care hospital in India. Japanese Journal of Infectious Diseases, 62: 158–159. PMID: 19305061.

Ahmed MO & Baptiste KE. 2018. Vancomycin-Resistant Enterococci: A review of antimicrobial resistance mechanisms and perspectives of human and animal health. Microbial Drug Resistance*,* 24: 590-606. DOI: 10.1089/mdr.2017.0147

Ali SA, Hasan KA, Bin Asif H & Abbasi A. 2013. Environmental enterococci: I. Prevalence of virulence, antibiotic resistance and species

distribution in poultry and its related environment in Karachi, Pakistan. Letters in Applied Microbiology, 58: 423–432. DOI: 10.1111/lam.12208

Amaechi N & Nwankwo IU. 2015. Evaluation of prevalence and antimicrobial resistance using enterococci isolates from pigs and poultry birds in Abia state, Nigeria. International Journal of Current Microbiology and Applied Sciences, 4 (2): 825-833.

Argudín MA, Deplano A, Meghraoui A, Dodémont M, Heinrichs A, Denis O, Nonhoff C & Roisin S. 2017. Bacteria from animals as a pool of antimicrobial resistance genes. Antibiotics, 6: 12. DOI: 10.3390/antibiotics6020012

Ayeni FA, Odumosu BT, Oluseyi AE & Ruppitsch W. 2016. Identification and prevalence of tetracycline resistance in enterococci isolated from poultry in Ilishan, Ogun State, Nigeria. Journal of Pharmacy and Bioallied Sciences, 8 (1): 69–73. DOI: 10.4103/0975-7406.171729

Azizi M, Hasanvand B, Kashef M, Alvandi AH & Abiri R. 2017. Virulence factor and biofilm formation in clinical enterococcal isolates of the west of Iran. Jundishapur Journal of Microbiology, 10(7): e14379. DOI: 10.5812/jjm.14379

Bell JM, Paton JC & Turnidge J. 1998. Emergence of vancomycin-resistant enterococci in Australia: phenotypic and genotypic characteristics of isolates. Journal of Clinical Microbiology, 36: 2187-90. DOI: 10.1128/JCM.36.8.2187-2190.1998

Bhardwaj SB. 2019 *Enterococci*: An important nosocomial pathogen. DOI: 10.5772/intechopen.90550

Cancilla MR, Powell LB, Hillier AJ & Davidson BE. 1992. Rapid genomic fingerprinting of Lactococcus lastis strains by arbitrarily primed polymerase chain reaction with 32P and fluorescent labels. Applied and Environmental Microbiology, 58: 1772-1775. DOI: 10.1128/aem.58.5.1772-1775.1992

Cauwerts K, Decostere A, De Graef EM, Haesebrouck F & Pasmans F. 2007. High prevalence of tetracycline resistance in *Enterococcus* isolates from broilers carrying the *erm*(*B*) gene. Avian Pathololgy, 36: 395–399. DOI: 10.1080/03079450701589167

Chajęcka-Wierzchowska W, Zadernowska A & Łaniewska-Trokenheim L. 2017. Virulence factors of *Enterococcus* spp. presented in food. LWT- Food Science and Technology, 75: 670–676. DOI: 10.1016/j.lwt.2016.10.026

CLSI. 2018. Clinical and Laboratory Standards Institute. Performance standards of antimicrobial susceptibility testing. Twenty-second information supplement, CLSI Document 2018; M10 0-S22, Wayne. PA.

Conwell M, Daniels V, Naughton PJ & Dooley JSG. 2017. Interspecies transfer of vancomycin, erythromycin and tetracycline resistance among *Enterococcus* species recovered from agrarian sources. BMC Microbiology, 17: 19. DOI: 10.1186/s12866-017-0928-3

Del Papa MF, Hancock LE, Thomas VC & Perego M. 2007. Full activation of *Enterococcus faecalis* gelatinase by a C-terminal proteolytic cleavage. Journal of Bacteriology, 189 (24): 8835–8843. DOI: 10.1128/JB.01311-07

Devriese LA, Pot B & Collins MD. 1993. Phenotypic identification of the genus *Enterococcus* and differentiation of phylogenetically distinct enterococcal species and species groups. Journal of Applied Bacteriology, 75: 399–408. DOI: 10.1111/j.1365-2672.1993.tb02794.x

Dolka B, Cisek AA & Szeleszczuk P. 2019. The application of the loop-mediated isothermal amplification (LAMP) method for diagnosing Enterococcus hirae-associated endocarditis outbreaks in chickens. BMC Microbiology, 19: 48. DOI: 10.1186/s12866-019-1420-z

Dupre I, Zanetti S, Schito AM, Fadda G & Sechi LA. 2003. Incidence of virulence determinants in clinical Enterococcus faecium and Enterococcus faecalis isolates collected in Sardinia (Italy). Journal of Medical Microbiology, 52: 491-498. DOI: 10.1099/jmm.0.05038-0

Farman M, Yasir M, Al-Hindi RR, Farraj SA, Jiman-Fatani AA, Alawi M & Azhar EI. 2019. Genomic analysis of multidrug-resistant clinical *Enterococcus faecalis* isolates for antimicrobial resistance genes and virulence factors from the western region of Saudi Arabia. Antimicrobial Resistance and Infection Control, 8: 55. DOI: 10.1186/s13756-019-0508-4

Flores-Mireles AL, Walker JN, Caparon M & Hultgren SJ. 2015. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nature Reviews Microbiology, 13: 269–284. DOI: 10.1038/nrmicro3432

Fracalanza SS, Scheidegger E, Santos P, Leita P & Teizeira L. 2007. Antimicrobial resistance profiles of enterococci isolated from poultry meat and pasteurized milk in Reo De Janecro. Memórias do Instituto Oswaldo Cruz, 102: 853-859. DOI: 10.1590/S0074-02762007005000120

Hanchi H, Mottawea W, Sebei K & Hammami R. 2018. The genus *Enterococcus*: between probiotic potential and safety concerns—an update. Frontiers in Microbiology, 9: 1791. DOI: 10.3389/fmicb.2018.01791

Hasan KA, Ali SA, Rehman M, Bin-Asif H & Zahid S. 2018. The unravelled *Enterococcus faecalis* zoonotic superbugs: Emerging multiple resistant

and virulent lineages isolated from poultry environment. Zoonoses and Public Health, 65 (8): 921–935. DOI: 10.1111/zph.12512

Hegstad K, Mikalsen T, Coque TM, Werner G & Sundsfjord A. 2010. Mobile genetic elements and their contribution to the emergence of antimicrobial resistant *Enterococcus faecalis* and *Enterococcus faecium*. Clinical Microbiology and Infection, 16: 541–554. DOI: 10.1111/j.1469-0691.2010.03226.x

Heidari H, Emaneini M, Dabiri H & Jabalameli F. 2016. Virulence factors, antimicrobial resistance pattern and molecular analysis of enterococcal strains isolated from burn patients. Microbial Pathogenesis, 90: 93–97. DOI: 10.1016/j.micpath.2015.11.017

Hirt H, Greenwood-Quaintance KE, Karau MJ, Till LM, Kashyap PC, Patel R & Dunny GM. 2018. *Enterococcus faecalis* sex pheromone cCF10 enhances conjugative plasmid transfer in vivo. mBio, 9 (1): e00037-18. DOI: 10.1128/mBio.00037-18

Hynes WL & Walton SL. 2000. Hyaluronidases of Gram-positive bacteria. FEMS Microbiology Letters, 183: 201–720. DOI: 10.1111/j.1574-6968.2000.tb08958.x

Kimera ZI, Mshana SE, Rweyemamu MM, Mboera LEG & Mate MIN. 2020. Antimicrobial use and resistance in food producing animals and the environment: an African perspective. Antimicrobial Resistance and Infection Control, 9: 37. DOI: 10.1186/s13756-020-0697-x

Kiruthiga A, Padmavathy K, Shabana P, Naveenkumar V, Gnanadesikan S & Malaiyan J. 2020. Improved detection of *esp, hyl, asa1, gelE, cylA* virulence genes among clinical isolates of *Enterococci*. BMC Research Notes, 13: 170. DOI: 10.1186/s13104-020-05018-0

Laverde Gomez JA, van Schaik W, Freitas AR, Coque TM, Weaver KE, Francia MV, Witte W & Werner G. 2011. A multi-resistance mega-plasmid pLG1 bearing a $hyl_{Efm}$ genomic island in hospital *Enterococcus faecium* isolates. International Journal of Medical Microbiology, 301 (2): 165–175. DOI: 10.1016/j.ijmm.2010.08.015

Madsen KT, Skov MN, Gill S & Kemp M. 2017. Virulence factors associated with *Enterococcus faecalis* infective endocarditis: a mini review. The Open Microbiology Journal, 11: 1-11. DOI: 10.2174/1874285801711010001

Manson AL, Van Tyne D, Straub TJ, Clock S, Crupain M, Rangan U, Gilmore MS & Earl AM. 2019. Chicken meat-associated enterococci: influence of agricultural antibiotic use and connection to the clinic. Applied and Environmental Microbiology, 85: e01559-19. DOI: 10.1128/AEM.01559-19

Manyi-Loh C, Mamphweli S, Meyer E & Okoh A. 2018. Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. Molecules, 23: 795. DOI: 10.3390/molecules23040795

Mehdi Y, Letourneau-Montminy MP, Gaucher ML, Chorfi Y, Suresh G, Rouissi T, Brar SK, Cot C, Ramirez AA & Godbout S. 2018. Use of antibiotics in broiler production: Global impacts and alternatives. Animal Nutrition, 4: 170-178. DOI: 10.1016/j.aninu.2018.03.002

Melese A, Genet C & Andualem T. 2020. Prevalence of Vancomycin resistant *enterococci* (VRE) in Ethiopia: a systematic review and meta-analysis. BMC Infectious Diseases, 20: 124. DOI: 10.1186/s12879-020-4833-2

Miller WR, Munita JM & Arias CA. 2014. Mechanisms of antibiotic resistance in enterococci. Expert Review of Anti-infective Therapy, 12 (10): 1221–1236. DOI:10.1586/14787210.2014.956092

Munita JM & Arias CA. 2016. Mechanisms of antibiotic resistance. Microbiology Spectrum, 4: 2. DOI: 10.1128/microbiolspec

Nowakiewicz A, Ziólkowska G, Troscianczyk A, Zieba P & Gnat S. 2017. Determination of resistance and virulence genes in *Enterococcus faecalis* and *E. faecium* strains isolated from poultry and their genotypic characterization by ADSRRS-fingerprinting. Poultry Science, 96 (4):986-996. DOI: 10.3382/ps/pew365

Olawale KO, Fadiora SO & Taiwo SS. 2011. Prevalence of hospital-acquired *enterococci* infections in two primary-care hospitals in Osogbo, Southwestern Nigeria. African Journal of Infectious Diseases, 5 (2): 40-46. DOI: 10.4314/ajid.v5i2.66513

Oluwasile BB, Agbaje M, Ojo OE & Dipelu MA. 2014. Antibiotic usage pattern in selected poultry farms in Ogun state. Sokoto Journal of Veterinary Science, 12 (1): 45-50. DOI: 10.4314/sokjvs.v12i1.7

Popović N, Dinić M, Tolinački M, Mihajlović S, Terzić-Vidojević A, Bojić S, Djokić J, Golić N & Veljović K. 2018. New insight into biofilm formation ability, the presence of virulence genes and probiotic potential of Enterococcus sp. dairy isolates. Frontiers in Microbiology, 9: 78. DOI: 10.3389/fmicb.2018.00078

Price VJ, McBride SW, Hullahalli K, Chatterjee A, Duerkop BA & Palmer KL. 2019. *Enterococcus faecalis* CRISPR-Cas is a robust barrier to conjugative antibiotic resistance dissemination in the murine intestine. mSphere 4: e00464-19. DOI: 10.1128/mSphere.00464-19

Ramos Y, Rocha J Hael AL, van Gestel J, Vlamakis H, Cywes-Bentley C, Cubillos-Ruiz JR, Pier GB,

Gilmore MS, Kolter R & Morales DK. 2019. PolyGlcNAc-containing exopolymers enable surface penetration by non-motile *Enterococcus faecalis*. PLoS Pathogens, 15 (2): e1007571. DOI 10.1371/journal.ppat.1007571

Reynolds PE & Courvalin P. 2005. Vancomycin resistance by synthesis of precursors terminating in D-alanyl-D-alanine, *Antimicrob Agents Chemother*, 49: 21-5. DOI: 10.1128/AAC.49.1.21-25.2005

Rowland I, Gibson G, Heinken A, Scott K, Swann J, Thiele I & Tuohy K. 2018. Gut microbiota functions: metabolism of nutrients and other food components. European Journal of Nutrition, 57: 1–24. DOI 10.1007/s00394-017-1445-8

Saha B, Singh AK, Ghosh A & Bal M. 2008. Identification and characterization of a vancomycin-resistant *Staphylococcus aureus* isolated from Kolkata (South Asia). Journal of Medical Microbiology, 57 (1):72-79. DOI: 10.1099/jmm.0.47144-0

Sanlibaba P, Tezel BU & Senturk E. 2018. Antimicrobial Resistance of Enterococcus Species Isolated from Chicken in Turkey. Korean Journal for Food Science of Animal Resources, 38 (2):391~402. DOI 10.5851/kosfa.2018.38.2.391

Sørensen TL, Blom M, Monnet DL, Frimodt-Møller N, Poulsen RL & Espersen F. 2001. Transient intestinal carriage after ingestion of antibiotic-resistant *Enterococcus faecium* from chicken and pork. New England Journal of Medicine, 345(16): 1161-1166. DOI: 10.1056/NEJMoa010692

Starr CR & Engleberg NC. 2006. Role of hyaluronidase in subcutaneous spread and growth of group A *streptococcus*. Infection and Immunity, 74 (1): 40-48. DOI: 10.1128/IAI.74.1.40-48.2006

Stępień-Pyśniak D, Hauschild T, Kosikowska U, Dec M & Urban-Chmie R. 2019. Biofilm formation capacity and presence of virulence factors among commensal *Enterococcus* spp. from wild birds. Scientific Reports, 9:11204. DOI 10.1038/s41598-019-47602-w

Tian Y, Yu H & Wang Z. 2019. Distribution of acquired antibiotic resistance genes among *Enterococcus* spp. isolated from a hospital in Baotou, China. BMC Research Notes, 12: 27. DOI 10.1186/s13104-019-4064-z

Top J, Willems R, Blok H, de Regt M, Jalink K, Troelstra A, Goorhuis B & Bonten M. 2007. Ecological replacement of *Enterococcus faecalis* by multiresistant clonal complex 17 *Enterococcus faecium*. Clinical Microbiology and Infection, 13: 316–319. DOI: 10.1111/j.1469-0691.2006.01631.x

Ünal N, Aşkar Ş & Yildirim M. 2017. Antibiotic resistance profile of *Enterococcus faecium* and *Enterococcus faecalis* isolated from broiler cloacal samples. Turkish Journal of Veterinary and Animal Sciences, 41 (2): 199–203. DOI: 10.3906/vet-1607-26

Vankerckhoven V, Van Autgaerden T, Vael C, Lammens C, Chapelle S, Rossi R, Jabes D & Goossens H. 2004. Development of a multiplex PCR for the detection of *asa1, gelE, cylA, esp* and *hyl* genes in enterococci and survey for virulence determinants among European hospital isolates of Enterococcus faecium. Journal of Clinical Microbiology, 42: 4473–4479. DOI: 10.1128/JCM.42.10.4473-4479.2004

Weng PL, Ramli R & Hamat RA. 2019. Antibiotic susceptibility patterns, biofilm formation and esp gene among clinical enterococci*:* is there any association? International Journal of Environmental Research and Public Health, 16: 3439. DOI:10.3390/ijerph16183439

Wist V, Morach M, Schneeberger M, Cernela N, Stevens MJA, Zurfluh K, Stephan R & Nüesch-Inderbinen M. 2020. Phenotypic and genotypic traits of vancomycin resistant enterococci from healthy food producing animals. Microorganisms, 8: 261. DOI: 10.3390/microorganisms8020261

Xu J, Sangthong R, Edward McNeil E, Tang R & Chongsuvivatwong V. 2020. Antibiotic use in chicken farms in northwestern China. Antimicrobial Resistance and Infection Control, 9: 10. DOI: 10.1186/s13756-019-0672-6

Yoshimura H, Ishimaru M, Endoh YS & Kojima A. 2000. Antimicrobial susceptibility of *Enterococci* species isolated from faeces of broiler and layer chickens. Letters in Applied Microbiology, 31: 427-432. DOI: 10.1046/j.1365-2672.2000.00842.x

Zalipour M, Esfahani BN & Havaei SA. 2019. Phenotypic and genotypic characterization of glycopeptide, aminoglycoside and macrolide resistance among clinical isolates of Enterococcus faecalis: a multicenter based study. BMC Research Notes, 12: 292. DOI: 10.1186/s13104-019-4339-4

Zehra A, Singh R, Kaur S & Gill JPS. 2017. Molecular characterization of antibiotic-resistant *Staphylococcus aureus* from livestock (bovine and swine). Veterinary World, 10 (6): 598-604. DOI: 10.14202/vetworld.2017.598-604