Microbial Pathogenesis 128 (2019) 195–205

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Poultry as a vector for emerging multidrug resistant *Enterococcus* spp.: First report of vancomycin (*van*) and the chloramphenicol–florfenicol (*cat-fex-cfr*) resistance genes from pigeon and duck faeces

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ARTICLE INFO

Keywords:
Enterococcus species
Biofilm formation
Vancomycin resistance genes
Chloramphenicol–florfenicol resistance genes
Pigeon
Duck
Chicken

ABSTRACT

Although commonly regarded as human and animal intestinal tract commensals, *Enterococcus* spp. have emerged as important nosocomial pathogens due to their intrinsic or acquired resistance to a number of antibiotics. Poultry has been suggested to be a reservoir for antibiotic resistance that may aggravate the problem of transmission of enterococci infections. Between January and December 2016, 106 *Enterococcus* spp. were isolated from a total of three poultry species. The collection included isolates recovered from chickens (n = 30), ducks (n = 35) and pigeons (n = 41). All enterococci isolates were screened for their ability to form biofilm. The antibiotic susceptibility was determined against 13 antibiotics using the disc diffusion method. The presence of the eight resistance genes, *vanA, vanB, vanC, catA, catB, fexA, fexB* and *cfr* was determined by PCR. All 106 isolates were resistant to clindamycin, whereas majority of isolates (> 90%) were resistant to erythromycin, oxytetracycline, doxycycline, gentamycin, ciprofloxacin, norfloxacin, and vancomycin. All isolates produced biofilms and were classified as extensive drug-resistant. $MAR_{indices}$ for all isolates was determined to be > 0.8, indicating that they have been recovered from high risk contamination sources. The *cfr* resistance gene was not detected in any of the 106 enterococci isolates, whereas the chloramphenicol resistance genes *catA* and *catB* were found in 18.9% (20/106) of the isolates. Interestingly, *fex*A 11.9% (15/106), *fex*B 8.7% (11/106), *vanA* 18.9% (20/106), *vanB* 25.5% (27/106), and *vanC* 33% (35/106) genes were also determined in our study. The present study highlights the emergence of a linezolid sensitive-vancomycin resistant enterococci, which lacks the *cfr* gene reporting also for the first time the detection of *van, fex and cat* -genes in *Enterococcus* species recovered from chickens, ducks and pigeons in Egypt suggesting that poultry species could be potential vectors for transmission of multidrug resistant enterococci posing a public health risk.

1. Introduction

Enterococcus spp. are normal inhabitants of human and animal intestinal tract microbiome [1–4]. Due to the release of large amounts of enterococci (10^5-10^7/g per stool) with faeces, the environmental contamination with these bacteria is profound [5]. As a result, enterococci can be found in different ecological niches, including soil, water, animal and fermented food and vegetables [6]. As a result, *Enterococcus* spp. have become a potentially high risk zoonotic pathogen that can cause critical public health problems and major worldwide threat [7].

People enjoy raising baby chicks and keeping backyard poultry (chicks, chickens, ducks, ducklings, geese, and turkeys) can be fun, educational and a great experience and to become very popular in the USA. Yet, the federal Centers for Disease Control and Prevention warned against outbreaks of infections linked to contact with chickens and ducks in backyard flocks or even handling baby birds displayed at

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https://doi.org/10.1016/j.micpath.2019.01.006
Received 28 September 2018; Received in revised form 2 January 2019; Accepted 4 January 2019
Available online 05 January 2019
0882-4010/

Table 1
Classification of antibiotics categorized as critically important in human and veterinary medicine.

Antibiotic	Disc concentration	Antimicrobial class	Medical importance	Prioritization criterion
Vancomycin	30 μg	Glycopeptides	Highest Priority Critically Important Antimicrobials	C1, C2, P1, P2, P3
Gentamycin	20 μg, 120 μg	Aminoglycosides	High Priority Critically Important Antimicrobials	C1, C2, P2, P3
Florfenicol	30 μg	Amphenicols	Highly Important Antimicrobials	C2, (P1, P2, P3, NA)
Chloramphenicol	15 μg	Amphenicols	Highly Important Antimicrobials	C2, (P1, P2, P3, NA)
Ciprofloxacin	10 μg	Quinolones and fluoroquinolones	Highest Priority Critically Important Antimicrobials	C1, C2, P1, P2, P3
Norfloxacin	10 μg	Quinolones and fluoroquinolones	Highest Priority Critically Important Antimicrobials	C1, C2 P1, P2, P3
Ampicillin	10 μg	Penicillins	High Priority Critically Important Antimicrobials	C1, C2, P2, P3
Erythromycin	20 μg	Macrolides and ketolides	Highest Priority Critically Important Antimicrobials	C1, C2, P1, P2, P3
Linezolid	30 μg	Oxazolidinones	High Priority Critically Important Antimicrobials	C1, C2, P1
Oxytetracyclin	20 μg	Tetracyclines	Highly Important Antimicrobials	C1, (P1, P2, P3, NA)
Doxycycline	20 μg	Tetracyclines	Highly Important Antimicrobials	C1, (P1, P2, P3, NA)
Clindamycin	20 μg	Lincosamides	Highly Important Antimicrobials	C2, (P1, P2, P3, NA)

Prioritization criterion 1 (P1): High absolute number of people, or high proportion of use in patients with serious infections in health care settings affected by bacterial diseases for which the antimicrobial class is the sole or one of few alternatives for treating serious infections in humans. **Prioritization criterion 2 (P2):** High frequency of use of the antimicrobial class for any indication in human medicine, or high proportion of use in patients with serious infections in health care settings, because use may favor selection of resistance in both settings. **Prioritization criterion 3 (P3):** The antimicrobial class is used to treat infections in people for whom there is evidence of transmission of resistant bacteria (e.g., non-typhoidal *Salmonella* and *Campylobacter* spp.) or resistance genes (high for *E. coli* and *Enterococcus* spp.) from non-human sources.
NA: not available.
Criterion 1 (C1): The antimicrobial class is the sole, or one of limited available therapies, to treat serious bacterial infections in people. **Criterion 2 (C2):** The antimicrobial class is used to treat infections in people caused by either: (1) bacteria that may be transmitted to humans from non-human sources, or (2) bacteria that may acquire resistance genes from non-human sources.
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stores [8,9]. The close contact with poultry with the public is presented in Egyptian folklore dating back to Ancient Egypt [50] and they have been an important constituent in the Egyptian cuisine since 2500 BC [10]. Keeping a backyard poultry is a popular practice in many Egyptian households which could hypothetically be a predisposing emerging cause for environmental contamination with MDR bacteria due to the tendency of hosting pigeons, chickens and ducklings as pet animals in the Egyptian homes.

The misuse of antibiotics in different ecological niches, including veterinary medicine and agriculture [11,12] led to consider these agents as important pollutants [13–16]. It has been evidenced that some enterococci, including *E. faecalis* and *E. faecium* possess efficient genetic exchange mechanisms and hence represent a major hub facilitating dissemination of antibiotic resistance genes [17,18].

Vancomycin resistant enterococci have been reported worldwide [19] and phenicols (chloramphenicol-florfenicol resistance) designated *fexA* (exporter florfenicol efflux gene) are one of the classes that *cfr* gene confers resistance to Ref. [20]. The carriage of *cfr* gene in clinical enterococci isolates was first reported in 2010 [21–23], however little is known about the global distribution of *cfr* positive enterococci isolates with a specific emphasis to their correlation with florfenicol resistance genes.

Reports of multidrug resistant (MDR) enterococci from different ecological niches [24–26] are global public health concern [27]. Studies examining enterococci pathogens from poultry have been reported worldwide, but little is known about the situation in Egypt. Consequently, the aim of this study was to provide insights into the prevalence of *Enterococcus* spp. within poultry species in Egypt and evaluate the propensity of antibiotic resistance in these isolates. We also report the molecular characterization of vancomycin (*van*) and the chloramphenicol–florfenicol (*cat-fex-cfr*) resistance genes in enterococci isolated from pigeon and duck faeces.

2. Materials and methods

2.1. Study area

Backyard chickens were sampled one time by cloacal swab during 2016 from thre family backyard chicken farms. In addition, backyard raised ducks were similarly sampled during the same period from three other family backyard duck raising farms. The pigeons were captured from their Pigeon lofts raised in poor neighborhoods across the city of Cairo and sampling was conducted as previously outlined.

2.2. Enterococcus strains and culture conditions

In total 106 *Enterococcus* isolates were examined in this study. The collection included isolates recovered from the faeces of various poultry species, including: pigeons (n = 41), ducks (n = 35) and chicken (n = 30). All 106 enterococci isolates were grown aerobically in brain heart infusion (BHI) broth for 24 h at 37 °C.

2.3. Isolation and conventional identification of enterococci isolates

Faecal samples (10 g) were homogenized in 90 ml buffered peptone water (Merck, Germany), incubated overnight at 37 °C and streaked on selective plates containing Slanetz-Bartley agar (Merck, Germany). One or two colonies with typical enterococcal morphology, were selected per sample. To identify possible streptococci, isolates were sub-cultured on Kennel Fecal (KF)-*Streptococcus* agar (Difco Laboratories, Detroit, MI, USA) as described by Teixeira et al. [28] and the American Public Health Association (APHA) [29]. Colonies that yielded typical enterococci characteristics were further identified by conventional methods, including Gram staining, catalase test, bile-esculin hydrolysis, pyrrolidonyl arylamidase (PYR) test and growth in brain-heart infusion broth containing 6.5% NaCl. Isolates that were identified as PYR-positive were screened for vancomycin resistance by disc diffusion test using a vancomycin disc (30 μg) (BBL/Difco, Sparks, Md.). Those that showed vancomycin resistance were selected for identification and antimicrobial susceptibility testing. All isolates were kept in BHI broth with 15% glycerol at -27°C until further analysis.

2.4. Antimicrobial drug assay

All enterococci isolates were screened for susceptibility against 13 antibiotics by disc diffusion method and assigned as sensitive, intermediate and resistant according to the recommendations of The Clinical and Laboratory Standard Institute (CLSI) [30]. The antibiotics used for

the susceptibility testing are considered by the WHO as '**the most important drugs**' still in use in healthcare settings (Table 1) [31]. This included: **Penicillin:** ampicillin (10 μg); **Phenicols:** chloramphenicol (30 μg) and florfenicol (30 μg); **Fluoroquinolones:** ciprofloxacin (5 μg) and norfloxacin (10 μg); **Lincosamide:** clindamycin (2 μg); **Aminoglycosides:** gentamycin (10 μg and 120 μg); **Oxazolidinone:** linezolid (30 μg); **Macrolide:** erythromycin (15 μg); **Tetracyclines:** oxyetracycline (30 μg) and doxycycline (30 μg); **Glycopeptide:** vancomycin (30 μg). Isolates that were resistant to gentamicin 10 μg by disc diffusion test, were further examined for their resistance to higher concentrations of gentamicin (120 μg/ml) accoding to the recommendations of the CLSI [30]. *E. faecalis* ATCC 29212 and *E. faecium* ATCC 19434 were used as positive controls.

The MAR_{index} was calculated to compare the resistance level of isolates across different sample types using the following equation [32]: MAR_{index} = A/BxC (A = number of antibiotics to which isolates were resistant; B = number of antibiotics that isolates were exposed to; C = number of isolates per sample).

2.5. Classification of acquired resistance

To classify the isolates by the expression of acquired resistance, previously standardized international terminology [33] was used. Isolates that were (i) nonsusceptible to ≥1 agent in ≥3 classes of antibiotics were defined as multidrug resistant (MDR). Those that were (ii) nonsusceptible to ≥1 agent in all but ≤2 classes of antibiotics were defined as extensive drug-resistance (XDR). Isolates that were (iii) resistant to all classes of antibiotics were defined as PanDrug resistant (PDR) enterococci.

2.6. Determination of virulence factors

2.6.1. Protease activity (casein hydrolysis)

All enterococci isolates were tested for protease activity. 106 enterococci isolates were inoculated on BHI agar supplemented with 5% (w/v) skimmed milk powder and examined for zones of inhibition after overnight growth [34]. *E. faecalis* (ATCC29212) was used as a positive control.

2.6.2. Detection of proteinase (gelatinase) activity

A three percent gelatin medium was used to detect proteinase activity [35]. Researchers incubated enterococci for 48 h at 37 °C after which they observed gelatinase activity as a transparent halo within the colonies. This was made possible when the plate was filled with Frazier solution. The positive control for this was *Staphylococcus aureus* (ATCC25923).

2.6.3. Hemagglutination test

The test was carried out based on Patidar and his co-researchers' description with little alterations [36]. Incubation of enterococci was done at 37 °C for 24 h on Columbia agar that contained a supplement of 10% sheep blood. A loopful of bacteria was picked up with a plastic loop which was then mixed gently inside a glass slide with 25 μl of 3% erythrocyte suspension obtained from chicken and sheep and a buffered saline solution of phosphate (pH 7.4). After mixing the suspensions, the slides were gently rotated for a period of about 30 s. During this period, haemagglutination was either recorded as no agglutination, agglutination (+) or strong agglutination (++).

2.6.4. Cytolysin (haemolysin) activity assay

Enterococci isolates' cytolysin activity was determined using brain heart infusion agar and 5% horse blood as a supplement. Agar plates were incubated at temperatures of 37 °C for 24 h. The cytolytic activity was recorded as β-hemolysis: clear inhibition zone formation; α-haemolysis: greenish and partial haemolysis zone detection and γ-haemolysis: denoting zero haemolytic activity [37]. The positive control used in this test was *Staphylococcus aureus* (ATCC25923).

2.6.5. Detection of cytotoxic activity

American Type Culture Collection was used to provide vero cell line which were then grown inside the 24-well plates as aforementioned [61]. Dulbecco's modified Eagle's medium with a supplement of 4 mML-glutamine and 15% fetal bovine serum were used to grow Vero cells (Sigma-Aldrich, St. Louis, USA). Seeding of the cells was done at 2×10^3 Verocells in each well and incubated with 5% CO_2 at 37 °C for 24 h. Isolates of enterococci were left to grow overnight inside the brain heart infusion broth after which they were diluted in a medium in which cell lines were incubated. Each well that contained Vero cells was added with culture suspension amounting to 1 ml with 106 bacteria. The adhered bacteria in every cell were counted so as to estimate adhesion. This was done at 37 °C for 2 h after incubation and the product stained with May-Grunwald-Giemsa stain [38].

2.6.6. Biofilm production

2.6.6.1. Congo-red agar biofilm assay. The Congo red agar (CRA) [39] was used to assess the ability of isolates to form biofilm. The plates were incubated for 24 and 48 h at 35 °C under aerobic and microaerophilic conditions. For each isolate the experiment was carried out in triplicate.

2.6.6.2. Microtiter plate assay. The biofilm formation ability on abiotic surfaces was evaluated as earlier on described [40]. Isolates of enterococci were left to grow in BHI broth with 0·25% glucose supplement at a temperature of 37 °C for overnight after which dilution of the culture was done at a ratio of 1:20 in a fresh BHI broth with 0·25% glucose supplement at 37 °C. Every well in the 96-well polystyrene microtitre plate was then inoculated with the suspension (200 μl) and incubated at 37 °C for 24 h. PBS was then used to wash the wells, dried and stained for 15 min using 1% crystal violet. This was followed by rinsing of the wells and solubilization of the crystal violet in 200 μl ethanol/acetone at 80:20 v/v. A microplate reader was used to determine biofilm formation at A_{450nm} and classified based on the level of their score i.e. isolates with a score value of over 0.2 were said to be high biofilm producers whereas those with less than 0.081 were said to be low biofilm producers. The OD_{450nm} values that fall between 0.081 and 0.2 were classified as moderate biofilm producers. Every test was conducted thrice and in triplicate. To determine the level of biofilm formation, BF = AB-CW was applied where BF = biofilm formation, AB = stained attached bacteria OD_{450nm} whereas CW = stained control well OD_{450nm} with a bacteria-free medium. Analysis was done in triplicate to the 16 wells in every isolate for each assay. In this assay, negative controls were those wells containing a sterile BHI with *Staphylococcus epidermidis* ATCC 35984 being representing the positive control.

2.7. Determination of the antibiotic resistance genes by PCR

DNA extracts were prepared by boiling as described previously [41]. Vancomycin [*van* (A, B, C)], chloramphenicol [*cat* (A, B)] and the chloramphenicol–florfenicol resistance [*fex* (A, B), *cfr*] genes were tested by *m*-PCR for all enterococcal isolates using primers and PCR conditions as described previously (Table 2). The reactions were performed in a total volume of 50 μl, including 3 μl template DNA, 1 μl (1 pmol/μl) of each primer, 25 μl Master Mix and 4 μl RNase-free water. PCR thermal cycling conditions were 94 °C for 3 min, 30 cycles 94 °C for 1 min, 54 °C for 1 min, 72 °C for 1 min, 72 °C for 7 min. 1·2% agarose gels were used for electrophoresis. Vancomycin-resistant *E. faecium* BM4147 and *E. faecium* UAA522 were used as positive controls for *vanA* and *vanB* genotypes respectively. *E. faecium* ATCC 19434 was used as a negative control.

Table 2
Primers used for determination of *van (A, B, C), cat (A, B), fex (A, B) abd cfr* genes.

Gene designation	Oligonucleotide sequences (5′-3′)	PCR product size (bp)	References
Van A	5-GGGAAAACGACAATTGC-3 5-GTACAATGCGGCCGTTA-3	732	42
Van B	5-ACGGAATGGGAAGCCGA-3 5-TGCACCCGATTTCGTTC-3	647	
Van $C_{1,2}$	5-ATGGATTGGTAYTKGTAT-3 5-TAGCGGGAGTGMCYMGTAA-3	815/827	
Cat A	5-GGATATGAAATTTATCCCTC-3 5-CAATCATCTACCCTATGAAT-3	486	43
Cat B	5-TGAACACCTGGAACCGCAGAG-3 5- GCCATAGTAAACACCGGAGCA-3	462	44
Fex A	5-GCGATTGGAGTTCCGATT-3 5-GCCAACGAAGGGAGATAAGAT-3	795	
Fex B	5-GCAAGTTAGTTGGGTCGTAAG-3 5-AAGCGATACCTATCCCTAAAC-3	974	
Cfr	5- GTGAAGCTCTAGCCAACCGTC-3 5- GCAGCGTCAATATCAATCCC-3	746	

K = G or T; M = A or C; Y= C or T.

3. Results

3.1. Identification and prevalence of Enterococcus spp.

A total of 106 enterococci isolates from varierty of poultry species were recovered in this study, including chickens (n = 30), ducks (n = 35) and pigeons (n = 41). Of 106 enterococci isolates *E. faecium* (50%) was the most predominant species, followed by *E. avium* and *E. raffinosus* (13.2% each respectively), *E. faecalis* (10.4%), *E. durans* (7.5%) and *E. gallinarum* (5.7%) (Table 3). *E. faecium* and *E. raffinosus* were isolated from all three species of poultry with the following proportions: chickens 36.8% and 7.9%; pigeons 56.1% and 7.3% and ducks 39% and 19.5% respectively, whereas *E. gallinarum* (14.6%) was isolated from pigeons only.

3.2. Phenotypic virulence traits

In addition to the phenotypic and genotypic antimicrobial resistance assays, a variety of phenotypic virulence assays were performed. The results of haemolytic activity, gelatine hydrolysis, casein hydrolysis, haemagglutination and Vero-cell cytotoxicity of the 106 enterococci isolated from chicken, duck and pigeon species were analyzed in this study (Table 4). All 106 enterococcal isolates recovered in this study possessed a minimum two (100% haemolysis capability and Vero-cell cytotoxicity) of the five virulence factors. We observed three haemolytic reactions in all enterococci isolates: β-haemolysis (28.6%); α-haemolysis (4%); and γ-haemolysis (68.3%). Thirty three (31.1%) of 106 isolates produced gelatinase; 56 (52.8%) were able to hydrolyze casein, and 70 (66.0%) haemagglutinated sheep blood, but only 22 isolates (20.8%) haemagglutinated chicken blood.

3.3. Biofilm formation

To assess the between-strain variability of biofilm formation in the polystyrene microplate plate assay, $biofilm_{index}$ was calculated for all enterococci isolates. The highest mean $biofilm_{index}$ was detected in 41 of enterococci isolated from pigeons, in particular in *E. gallinarum* species (0.360–2.493; $\bar{x}$0.6934). Nevertheless, all isolates were classified as strong biofilm producers, including those isolated from ducks (0.417–1.395; $\bar{x}$0.6148) and chickens (0.340–1.260; $\bar{x}$0.6111) (Table 4).

3.4. Phenotypic antimicrobial resistance patterns

All enterococci were tested for their susceptibility to 13 antibiotics representing 10 different classes (Table 5). High level of antibiotic resistance was detected in all enterococci isolates evaluated. All 106 isolates were resistant to clindamycin (100%), followed by erythromycin (99.1%), gentamycin low level aminoglycoside resistance (LLAR) (99.1%), ciprofloxacin (99.1%), oxytetracycline (98.1%), norfloxacin 96.2%), vancomycin (95.3%), doxycycline (93.4%), florfenicol (78.3%) and chloramphenicol (76.4%) (Table 5). Interestingly, only 12 out of 106 enterococci isolates (11.3%) were resistant to the clinically important antibiotic linezolid. The 12 enterococci isolates that were resistant linezolid, were also resistant to 9–11 antibiotics, including vancomycin, with the exception of 3/30 *E. faecium* isolates (1/14 isolated from chickens and 2/16 isolated from ducks). In addition, these *E. faecium* isolates were intermediate-resistant to linezolid and fully resistant all 12 other antibiotics. The 106 isolates were resistant to four antibiotics ampicillin (77/106), gentamycin (HLAR) (82/106), florfenicol (83/106) and chloramphenicol (81/106) at a rate of > 70%. In addition, 72.6% of enterococci isolates were resistant to ampicillin, an important antibiotic still in use for the treatment of enterococcal infection (Table 1, Table 5).

In addition to this, all 106 isolates were resistant to at least four of 13 antibiotics tested, representing 3–9 different classes. A combination of 10 different antibiotic resistance patterns were observed for all enterococci isolates (Table 6). Moreover, all isolates (100%) were classified as extensive drug resistant (XDR) bacteria.

Some resistance pattern differences were evident between species, including those that had lower frequency of resistance in *E. faecalis* compared to *E. faecium*. The resistance patterns in *E. faecium* did not

Table 3
Distribution of *Enterococcus* species recovered from a variety of poultry species.

Enterococcus spp.	Poultry species (n = isolates/n = samples, %)			
	Chickens	Pigeons	Ducks	Total (n = isolates/n = samples, %)
E. faecium	14/38	23/41	16/41	53/106
	36.8%	56.1%	39%	50.0%
E. avium	13/38	0/41	1/41	14/106
	34.2%	0%	2.4%	13.2%
E. raffinosus	3/38	3/41	8/41	14/106
	7.9%	7.3%	19.5%	13.2%
E. gallinarum	0/38	6/41	0/41	6/106
	0%	14.6%	0%	5.7%
E. durans	0/38	8/41	0/41	8/106
	0%	19.5%	0%	7.5%
E. faecalis	0/38	1/41	10/41	11/106
	0%	2.4%	24.4%	10.4%
Total (n = isolates/n = samples, %)	30/38	41/41	35/41	106/106
	78.9%	100%	85.4%	100%

Table 4
Potential phenotypic virulence factors of enterococcal isolates.

Source	Species	n = of examined isolates	Haemolysis (α-β- or γ)		Gelatine hydrolysis		Casein hydrolysis		Haemagglutination				Vero-cell cytotoxicity		Biofilm$_{index}$ (Range)
									Sheep blood		Chicken blood				
			n =	%	n =	%	n =	%	n =	%	n =	%	n =	%	
Chicken	*E. avium*	13	13	100.0	6	46.2	5	38.5	7	53.8	4	30.8	13	100.0	0.340–1.011
	E. raffinosus	3	3	100.0	2	66.7	3	100	1	33.3	0	0.0	3	100.0	0.459–0.695
	E. faecium	14	14	100.0	7	50.0	8	57.1	12	85.7	5	35.7	14	100.0	0.409–1.260
	Total	**30**	**30**	**100.0**	**15**	**50.0**	**16**	**53.3**	**20**	**66.7**	**9**	**30.0**	**30**	**100.0**	
Pigeon	*E. raffinosus*	3	3	100.0	1	33.3	2	66.7	2	66.7	1	33.3	3	100.0	0.464–1.150
	E. faecium	23	23	100.0	11	47.8	12	52.2	20	87.0	9	39.1	23	100.0	0.360–1.128
	E. gallinarum	6	6	100.0	2	33.3	3	50	4	66.7	2	33.3	6	100.0	0.478–2.493
	E. faecalis	1	1	100.0	0	0.0	0	0	0	0.0	0	0.0	1	100.0	0.443
	E. durans	8	8	100.0	0	0.0	8	100	8	100.0	3	37.5	8	100.0	0.401–0.647
	Total	**41**	**41**	**100.0**	**14**	**34.1**	**25**	**61**	**34**	**82.9**	**15**	**36.6**	**41**	**100.0**	
Ducks	*E. avium*	1	1	100.0	0	0.0	0	0	0	0.0	0	0.0	1	100.0	0.501
	E. raffinosus	8	8	100.0	2	25.0	3	37.5	3	37.5	1	12.5	8	100.0	0.402–1.084
	E. faecium	16	16	100.0	1	6.3	5	31.3	10	62.5	5	31.3	16	100.0	0.424–0.818
	E. faecalis	10	10	100.0	1	10.0	7	70.0	3	30.0	1	10.0	10	100.0	0.417–1.395
	Total	**35**	**35**	**100.0**	**4**	**11.4**	**15**	**42.9**	**16**	**45.7**	**7**	**20.0**	**35**	**100.0**	
Total		**106**	**106**	**100.0**	**33**	**31.1**	**56**	**52.8**	**70**	**66.0**	**22**	**20.8**	**106**	**100.0**	

differ from those observed in *E. raffinosus*, *E. durans*, *E. avium* and *E. gallinarum*. However, this may be due to low number of samples tested. To evaluate the predominant patterns of resistance in enterococci isolates recovered from poultry, the MAR $_{index}$ was calculated. The MAR $_{index}$ range ($\bar{x}$) for chicken was 0.31–0.92 (0.78), for pigeons 0.38–0.92 (0.71) and for ducks 0.15–0.92 (0.69).

3.5. Low and high level aminoglycoside resistance

Interestingly, low level aminoglycoside resistance (LLAR) was detected in 99.1% (105/106) of enterococci isolates, whereas high level aminoglycoside resistance (HLAR) was detected in relatively low number of isolates 77.4% (82/106) (Table 5). The *E. faecium* isolates recovered from chickens (13/13, 9/13), pigeons (23/23, 17/23) and ducks (15/16, 14/16) showed higher resistance to gentamycin (LLAR and HLAR respectively) than the *E. faecalis* isolated from ducks (10/10, 4/10).

3.6. Comparison between poultry species

No single isolate was fully susceptible to all 13 antibiotics tested. The antimicrobial resistance patterns as seen in Table 6, show that in chickens and ducks the antibiotics were allocated by seven patterns each, however, were represented by 5–9 classes in chickens and 4–9 classes in ducks. The least number of antibiotic dispersion pattern was in the pigeons that was observed by five different patterns constituting 6–9 classes.

3.7. Genotypic antimicrobial resistance patterns and their combinations

All 106 enterococci were screened for the presence of antimicrobial resistance genes, including vancomycin *van* and the chloramphenicol–florfenicol *cat-fex-cfr* genes (Table 7). The *cfr* resistance gene was not detected in any of the 106 enterococci isolates, whereas the chloramphenicol resistance genes *catA* and *catB* were found in 18.9% (20/106) of enterococci isolates. Interestingly, the recently reported genes *fex*A and *fex*B were detected in 11.9% (15/106) and 8.7% (11/106) isolates respectively. Moreover, *vanA, vanB,* and *vanC* were detected in 18.9% (20/106), 25.5% (27/106), and 33% (35/106) isolates respectively (Table 7).

The presentce of combined resistance genes in 106 enterococci isolates were not consistant (Table 8). The highest combinations were detected in the three poultry species: chickens (*vanA,C, catA* in two isolates), (*vanB,C, catA* in three isolates) and (*vanA,C, catA, fexB* in one isolate); ducks: (*vanB,C, catB* in four isolates) and (*vanB, catA, fexA* in two isoltes) and pigeons (*catA, fexA,B* in one isolate) and (*vanA, catA, fexB* in one isolate).

4. Discussion

Enterococci form a very important section of intestinal microbiome in pigeons chickens and ducks [45–47] with the ability to result in human opportunistic diseases or transmit antibiotic resistant determinants thus putting the public into a great risk. As antibiotic resistance is not restricted to *E. faecalis* and *E. faecium* but it also occurs in other species of enterococci therefore, antibiotic resistance monitoring and screening should not be restricted to these two species of enterococci [48].

In this study we identified a number of enterococci species recovered from chickens, ducks and pigeons known to cause human infections [7,27]. It is important to emphasise that, *E. avium* and *E. gallinarum*, initially portrayed from chickens, were not considered as a part of intestinal microbiome of poultry [2], yet in the present study *E. avium* was isolated from chickens and ducks (34.2% and 2.4% respectively), leaving only *E. gallinarum* to confront the above hypothesis. The inconsistence among the species was attributed by previous researchers [49,50] to be due to geographic disparities and the effect of supplemented feed on the intestinal enterococcal microflora.

The prominent features of enterococcal virulence traits are haemolytic and gelatinase activities [51,52]. Our results revealed that 18.7% of the isolates exhibited haemolytic activity, due to the presence of the pore-forming exotoxin cytolysin expressing that these strains were highly virulent yet, it should be emphasized that the presence of cytolysin is not always associated with haemolysis on the blood agar and does not necessarily mean that the rest of the isolates (19.3%), that did not express haemolysis, are not virulent [53]. It is interesting to point out that the phenotypic virulence factor, gelatinase activity, among *faecalis* and *E. faecium* isolates have also been found in isolates of *E. casseliflavus*, *E. durans*, *E. hirae*, *E. avium*, *E. cecorum*, *E. gallinarum*, *E. malodoratus* and *E. faffinosus* and *E. mundtii*. The lack of phenotypic virulence activity of some of the *Enterococcus* isolates may be explained by Refs. [54–57], i) low levels or down regulation of gene expression, ii) an inactive gene product, iii) environmental factors, iv) genes for cytolysin and gelatinase may be silent. In addition it should be emphasized that, under *in vivo* conditions, the genes are expressed only due to the presence of undetected gene mutations or to the fact that detection

Table 5
Determination of phenotypic antibiotic resistance profiles of enterococci isolates recovered from poultry samples.

Enterococcus spp.	Antibiotics												
	Penicillins	Lincosamide	Macrolides	Tetracyclines		Aminoglycosides		Fluoroquinolones		Oxazolidinones	Phenicols	Phenicols	Glycopeptides
	Ampicillin (10 μg)	Clindamycin (2 μg)	Erythromycin (15 μg)	Oxytetracycline (30 μg)	Doxycycline (30 μg)	Gentamycin (10 μg) (LLAR)	Gentamycin (120 μg) (HLAR)	Ciprofloxacin (5 μg)	Norfloxacin (10 μg)	Linezolide (30 μg)	Florfenicol (30 μg)	Chloramphenicol (30 μg)	Vancomycin (30 μg)
Chickens													
E. avium	10/13	13/13	13/13	13/13	11/13	13/13	9/13	13/13	13/13	0/13	10/13	7/13	12/13
E. faecium	9/14	14/14	14/14	14/14	14/14	14/14	14/14	14/14	14/14	3/14	12/14	13/14	14/14
E. raffinosus	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	0/3	3/3	2/3	3/3
Total	22/30	30/30	30/30	30/30	28/30	30/30	26/30	30/30	30/30	3/30	25/30	22/30	29/30
	73.3%	100%	100%	100%	93.3%	100%	86.7%	100%	100%	10%	83.3%	73.3%	96.7%
Pigeons													
E. faecium	13/23	23/23	23/23	22/23	23/23	23/23	17/23	23/23	21/23	2/23	14/23	17/23	22/23
E. raffinosus	1/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	0/3	2/3	3/3	3/3
E. gallinarum	6/6	6/6	6/6	6/6	6/6	6/6	2/6	6/6	6/6	2/6	4/6	5/6	6/6
E. faecalis	0/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1	0/1	1/1	1/1
E. durans	6/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	2/8	8/8	8/8	8/8
Total	26/41	41/41	41/41	40/41	41/41	41/41	31/41	40/41	39/41	7/41	28/41	34/41	40/41
	63.4%	100%	100%	97.6%	100%	100%	75.6%	97.6%	95.1%	17.1%	68.3%	82.9%	97.6%
Ducks													
E. faecium	13/16	16/16	15/16	15/16	14/16	15/16	14/16	16/16	15/16	2/16	12/16	11/16	15/16
E. faecalis	9/10	10/10	10/10	10/10	10/10	10/10	4/10	10/10	10/10	0/10	10/10	10/10	10/10
E. raffinosus	7/8	8/8	8/8	8/8	5/8	8/8	7/8	8/8	7/8	0/8	7/8	3/8	7/8
E. avium	0/1	1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1	0/1	1/1	1/1	0/1
Total	29/35	35/35	34/35	34/35	30/35	34/35	25/35	35/35	33/35	2/35	30/35	25/35	32/35
	82.9%	100%	97.1%	97.1%	85.7%	97.1%	71.4%	100%	94.3%	5.7%	85.7%	71.4%	91.4%
Total	77/106	106/106	105/106	104/106	99/106	105/106	82/106	105/106	102/106	12/106	83/106	81/106	101/106
	72.6%	100%	99.1%	98.1%	93.4%	99.1%	77.4%	99.1%	96.2%	11.3%	78.3%	76.4%	95.3%

LLAR, Low level aminoglycoside resistance; HLAR, High level aminoglycoside resistance.

Table 6
Antimicrobial resistance profiles of *Enterococcus* spp. isolated from chickens, ducks and pigeons to various antibiotics.

Antibiotics	Number of isolates	Number of antibiotic classes
Chicken		
Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR, Ciprofloxacin, Norfloxacin	28	5
Ampicillin, Clindamycin, Erythromycin, Oxytetracycline, Gentamycin LLAR, Ciprofloxacin, Norfloxacin, Vancomycin	22	7
Ampicillin, Clindamycin, Erythromycin, Oxytetracycline, Gentamycin LLAR, Ciprofloxacin, Norfloxacin, Chloramphenicol, Vancomycin	15	8
Ampicillin, Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR, Ciprofloxacin, Norfloxacin, Florfenicol, Vancomycin	1	8
Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR and HLAR, Ciprofloxacin, Norfloxacin, Chloramphenicol, Vancomycin	1	7
Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR and HLAR, Ciprofloxacin, Norfloxacin, Florfenicol, Chloramphenicol, Vancomycin	5	7
Ampicillin, Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR and HLAR, Ciprofloxacin, Norfloxacin, Florfenicol, Vancomycin	3	8
Ampicillin, Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR and HLAR, Ciprofloxacin, Norfloxacin, Linezolide, Vancomycin	2	8
Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR and HLAR, Ciprofloxacin, Norfloxacin, Florfenicol, Chloramphenicol, Vancomycin	5	7
Ampicillin, Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR and HLAR, Ciprofloxacin, Norfloxacin, Florfenicol, Chloramphenicol, Vancomycin	13	8
Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR and HLAR, Ciprofloxacin, Norfloxacin, Linezolide, Florfenicol, Chloramphenicol, Vancomycin	1	8
Ampicillin, Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR and HLAR, Ciprofloxacin, Norfloxacin, Linezolide, Florfenicol, Chloramphenicol, Vancomycin	1	9
Pigeons		
Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR, Ciprofloxacin, Chloramphenicol	1	6
Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR, Ciprofloxacin, Norfloxacin, Florfenicol, Vancomycin	1	7
Ampicillin, Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR, Ciprofloxacin, Norfloxacin, Vancomycin	1	7
Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR and HLAR, Ciprofloxacin, Norfloxacin, Vancomycin	1	6
Clindamycin, Erythromycin, Doxycycline, Gentamycin LLAR and HLAR, Ciprofloxacin, Norfloxacin, Florfenicol, Vancomycin	1	7
Ampicillin, Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR, Ciprofloxacin, Norfloxacin, Florfenicol, Vancomycin	1	8
Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR and HLAR, Ciprofloxacin, Norfloxacin, Chloramphenicol, Vancomycin	6	7
Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR and HLAR, Ciprofloxacin, Norfloxacin, Florfenicol, Vancomycin	1	7
Ampicillin, Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR, Ciprofloxacin, Florfenicol, Chloramphenicol, Vancomycin	1	8
Ampicillin, Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR, Ciprofloxacin, Norfloxacin, Linezolide, Vancomycin	1	8
Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR and HLAR, Norfloxacin, Linezolide, Chloramphenicol, Vancomycin	1	8
Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR and HLAR, Ciprofloxacin, Norfloxacin, Florfenicol, Chloramphenicol, Vancomycin	1	7
Ampicillin, Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR, Ciprofloxacin, Norfloxacin, Linezolide, Chloramphenicol, Vancomycin	2	9
Ampicillin, Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR, Ciprofloxacin, Norfloxacin, Florfenicol, Chloramphenicol, Vancomycin	1	8
Ampicillin, Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR and HLAR, Ciprofloxacin, Norfloxacin, Florfenicol, Chloramphenicol, Vancomycin	17	8
Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR and HLAR, Ciprofloxacin, Norfloxacin, Linezolide, Florfenicol, Chloramphenicol, Vancomycin	2	8
Ampicillin, Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR, Ciprofloxacin, Norfloxacin, Linezolide, Florfenicol, Chloramphenicol, Vancomycin	1	9
Ducks		
Ampicillin, Clindamycin, Ciprofloxacin, Florfenicol, Chloramphenicol	1	4
Clindamycin, Erythromycin, Oxytetracycline, Gentamycin LLAR, Ciprofloxacin	1	5
Clindamycin, Erythromycin, Oxytetracycline, Gentamycin LLAR, Ciprofloxacin, Norfloxacin, Florfenicol, Vancomycin	1	7
Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR, Ciprofloxacin, Norfloxacin, Florfenicol, Chloramphenicol	1	6
Ampicillin, Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR and HLAR, Ciprofloxacin, Norfloxacin, Vancomycin	2	7
Ampicillin, Clindamycin, Erythromycin, Oxytetracycline, Gentamycin LLAR and HLAR, Ciprofloxacin, Norfloxacin, Florfenicol, Vancomycin	1	8
Ampicillin, Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR, Ciprofloxacin, Norfloxacin, Florfenicol, Chloramphenicol, Vancomycin	6	8
Ampicillin, Clindamycin, Erythromycin, Oxytetracycline, Gentamycin LLAR and HLAR, Ciprofloxacin, Norfloxacin, Florfenicol, Chloramphenicol, Vancomycin	2	8
Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR and HLAR, Ciprofloxacin, Norfloxacin, Florfenicol, Chloramphenicol, Vancomycin	3	7

(*continued on next page*)

Table 6 (*continued*)

Antibiotics	Number of isolates	Number of antibiotic classes
Ampicillin, Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR and HLAR, Ciprofloxacin, Norfloxacin, Chloramphenicol, Vancomycin	2	8
Ampicillin, Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR and HLAR, Ciprofloxacin, Norfloxacin, Florfenicol, Vancomycin	5	8
Ampicillin, Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR and HLAR, Ciprofloxacin, Norfloxacin, Florfenicol, Chloramphenicol, Vancomycin	9	8
Ampicillin, Clindamycin, Erythromycin, Oxytetracycline, Doxycycline, Gentamycin LLAR and HLAR, Ciprofloxacin, Norfloxacin, Linezolide, Florfenicol, Chloramphenicol, Vancomycin	2	9

LLAR, Low level aminoglycoside resistance; HLAR, High level aminoglycoside resistance.

by PCR of a single gene inside an operon, as is the case of the *cylA* gene for cytolysin production, may overlook the absence of other genes that are necessary for phenotypic expression [58].

Biofilm formation has a considerable role in enterococcal infections and antibiotic resistance [59–61]. To our knowledge this is the first report of biofilm formation by enterococci isolates recovered from the faeces of poultry which had a strong biofilm forming capability to play a key role in virulence and drug resistance [62]. This result coincides with the fact that biofilm formation results from adhesion which is an important feature of gut colonization [63]. Popović et al. [63] reported that commensal isolates from human feces exhibit higher biofilm formation ability than endocarditis isolates, suggesting that biofilm formation is not exclusively related to the pathogenesis and that it is more a result of adhesion properties, an important feature of gut colonization.

In Egypt, there is a lack of information on the degree of antimicrobial usage in poultry, medications through growth promoters included in their feed and amid remedial or preventive medicines. Another important element that has been overlooked in developing countries is the release of pharmaceutical waste containing active pharmaceutical compounds from antibiotic manufacturing plants, into the rivers or the environment, contributes to the selection of antibiotic resistant organisms posing a significant threat to public health [64,65]. The WHO released an alert that, antibiotic resistance is not limited by geographical or biological borders [66] and the contrasting published differences is governed by the antibiotics used in medical and veterinary practices such as overuse, incorrectly prescribed antibiotics, extensive use in agriculture, and a halt in the development of new antibiotics by the pharmaceutical industry [67]. In our study the MAR_{index} values for 106 isolates were greater than 0.8 (> 0.8), indicating that antibiotics were highly used in the poultry farms they have been recovered from, high–risk contaminated sources with frequent use of antibiotics, human faecal contamination [68,69], industrial emissions of pharmaceuticals [64,65].

The enterococci isolates in our study expressed high resistance to the β-lactams, lincosamines and aminoglycosides classes of antibiotics in contrast to the results outlined in the review presented by Garrido et al. [70], highlighting a public health crisis in Egypt. The resistance to erythromycin is a worrisome finding as erythromycin is an alternative antibiotic for patients who are hypersensitive to penicillins [70]. In our study, the lower levels of resistance between *E. avium* on one hand and *E. faecium* and *E. faecalis* on the other, is attributed by the minor frequency of aminoglycoside modifying enzymes in *E. avium* strains, as compared to *E. faecium* and *E. faecalis* strains [71].

The decreased level of poultry enterococcal resistance to chloramphenicol, was a consequence of the ban against the use of chloramphenicol in poultry since the end of the 20th century in the USA and Europe [72]. This regulation did not apply to Egypt, as chloramphenicol continued to be used for clinical purposes and in poultry industry, hence reflected in our findings as high levels of resistance (71.4%) to chloramphenicol among eneterococci islates recovered from the three poultry species. Also, the high levels of vancomycin resistance in our study was somewhat alarming, considering that it is the *pis aller* glycopeptide for the treatment of enterococcal infections in contrast to the situation in the EU [73]. This could be attributed to the fact that in the EU avoparcin has been baned while in Egypt it is widely used in the poultry industry.

Several researchers have highlighted the increasing threat of the *cfr* resistance gene to public health [74,75]. Zhang et al. [75], suggested that the multiresistance gene *cfr* among linezolid-resistant enterococci

Table 7
Prevalence and distribution chloramphenicol–florfenicol *cat-fex-cfr* and the vancomycin *van* resistance genes in *Enterococcus* isolates recovered from poultry.

Source	Species	n = of examined isolates	Chloramphenicol–florfenicol resistance genes										Vancomycin resistance genes					
			*cat*A		*cat*B		*fex*A		*fex*B		*cfr*		*van*A		*van*B		*van*C	
			n =	%	n =	%	n =	%	n =	%	n =	%	n =	%	n =	%	n =	%
Chicken	***Enterococcus** avium*	13	6	46.2	2	15.4	0	0.0	1	7.7	0.0	0.0	3	23.1	2	15.4	7	53.9
	***Enterococcus** raffinosus*	3	0	0.0	1	33.3	0	0.0	0	0.0	0.0	0.0	1	33.3	1	33.3	0	0.0
	***Enterococcus** faecium*	14	5	35.7	1	7.1	1	7.1	3	21.4	0.0	0.0	3	21.4	3	21.4	8	57.1
	Total	**30**	**11**	**36.7**	**4**	**13.3**	**1**	**3.3**	**4**	**13.3**	**0**	**0.0**	**7**	**23.3**	**6**	**20.0**	**15**	**50.0**
Pigeon	***Enterococcus** raffinosus*	3	1	33.3	0	0.0	0	0.0	2	66.7	0	0.0	1	33.3	1	33.3	0	0.0
	***Enterococcus** faecium*	23	4	17.4	4	17.4	1	4.4	0	0.0	0	0.0	0	0.0	5	21.7	6	26.1
	***Enterococcus** gallinarum*	6	1	16.7	0	0.0	3	50.0	2	33.3	0	0.0	0	0.0	2	33.3	0	0.0
	***Enterococcus** faecalis*	1	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	100.0
	***Enterococcus** durans*	8	0	0.0	2	25.0	6	75.0	0	0.0	0	0.0	6	75.0	2	25.0	2	25.0
	Total	**41**	**6**	**14.6**	**7**	**17.1**	**10**	**24.4**	**4**	**9.8**	**0**	**0.0**	**7**	**17.1**	**10**	**24.4**	**9**	**22.0**
Ducks	***Enterococcus** avium*	1	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	***Enterococcus** raffinosus*	8	1	12.5	1	12.5	1	12.5	3	37.5	0	0.0	4	50.0	1	12.5	2	25.0
	***Enterococcus** faecium*	16	2	12.5	7	43.8	3	18.8	0	0.0	0	0.0	2	12.5	9	56.3	8	50.0
	***Enterococcus** faecalis*	10	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	10.0	1	10.0
	Total	**35**	**3**	**8.6**	**9**	**25.7**	**4**	**11.4**	**3**	**8.6**	**0**	**0.0**	**6**	**17.1**	**11**	**31.4**	**11**	**31.4**
Total		**106**	**20**	**18.9**	**20**	**18.9**	**15**	**11.9**	**11**	**8.7**	**0**	**0.0**	**20**	**18.9**	**27**	**25.5**	**35**	**33.0**

Table 8
Diversity of antibiotic resistance genes in *Enterococcus* spp. isolated from chickens, ducks and pigeons.

Source	Antibiotic resistance genes	Number of isolates
Chicken	*vanA*	3
	vanB	2
	vanC	2
	vanA, catB	1
	vanB, catB	1
	vanC, catB	1
	vanC, fexA	1
	vanA,C, catA	2
	vanA,C, catA, fexB	1
	vanB,C, catA	3
	vanC, catA	5
	catA, fexB	1
	fexB	2
Ducks	*vanB*	2
	vanC	4
	vanA,C	1
	vanB,C	2
	vanA, catB	3
	vanA, fexB	3
	vanB,C, catB	4
	vanB,catA,fexA	2
	catB	1
	catA, fexA	1
	catB, fexA	1
Pigeons	*vanA, fexA*	5
	vanA, catA, fexB	1
	vanB	4
	vanB,C	2
	vanB, fexB	2
	vanB,C, catB	2
	vanC	1
	vanC, catA	3
	vanC, catB	1
	catA	1
	catB	4
	catA, fexA,B	1

creates a potential risk of spreading linezolid resistance [75]. There is no single previous report has been provided on *cfr* in *Enterococcus* species recovered from chickens, ducks and pigeons. In this study we report the emergence of a linezolid sensitive-vancomycin resistant *Enterococcus* that lacks the *cfr* gene. This is a promising finding as linezolid is the last resort antibiotic for treatment of Gram-positive bacteria which is a great concern in the healthcare settings [75].

Since there were no data available on the occurrence and distribution of the florfenicol resistance genes *cfr*, *cat* and *fex* in enterococci recovered from poultry, we screened 106 *Enterococcus* isolates for the presence of these genes. The presence of more than one phenicol resistance gene in the same isolate, as detected in the present investigation, has already been described in other bacteria, including *Staphylococcus*, *Salmonella enterica* and *E. coli* [76]. This study did not detect *cfr* gene despite the fact that florfenicol and chloramphenicol resistance were identified in 85.7% and 71.4% of the isolates respectively, and this apparent failure of an intact *cfr* gene in mediating resistance was previously indicated by Liu et al. [77] which may be caused by two reasons: First, is the presence of an unknown 'silent' antibiotic resistance gene reservoir within pathogenic bacteria which could go undetected by diagnostic techniques that are entirely reliant on resistance phenotype; Second, false-positive results may be produced by surveillance studies targeting on the *cfr* gene through microarray approaches or PCR.

According to Ribeiro et al. [52], they accounted the discrepancies to: (i) Differences in resistance phenotypes can be due to nonfunctional and/or silent genes. (ii) Cases where the resistance phenotype was observed but the resistance gene failed to be amplified by PCR, can be explained by variations in the nucleotide sequence of the resistance gene, preventing primer hybridization during PCR amplification. (iii) The presence of other genes conferring resistance to the same antibiotic, (iv) The effect of temporal changes on the incidence of antibiotic resistance genes in enterococci.

5. Conclusions

This study provides insights that poultry can be a reservoir of potentially pathogenic enterococci endowed with antimicrobial resistance and could be potential vectors for transmission of MDR enterococci. The emergence of a linezolid sensitive-vancomycin resistant enterococci, which lacks the *cfr* gene and also the detection of *van, fex and cat* -genes in *Enterococcus* species recovered from chickens, ducks and pigeons informs the authorities to develop better public health policies and effective strategies to takle antibiotic resistance that is fast spreading in the Egyptian community.

Declarations

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Availability of data and materials

All data generated or analyzed throughout this research are included in this published article.

Authors' contributions

KO, JB, AO, AE, AS, MDSI and MHH were involved in the study design, execution, data analyses, manuscript writing and revisions.

Ethics approval and consent to participate

Faecal samples collected were part of standard screening by the veterinary officials and these samples became available for the study leading to this manuscript. All studies were carried out according to the Department of Poultry Diseases guidelines for research ethics in animals, reviewed, approved and supervised by the Ethical Committee of the Animal Health Research, Institute, Dokki, Ministry of Agriculture, Egypt according to the declaration of Helsinki.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

All staffs of the Department of Microbiology.

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