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Antimicrobial Resistance Genes Associated with Enterococci from Poultry in Egypt, First Reporting of *mec*A in *Enterococcus* from Poultry Source

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Abstract | Little information available for the Egyptian setting. 117 cloacal swabs were collected from diseased poultry located in 6 districts in Egypt. Isolates were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The antibiotic susceptibility testing of *Enterococcus faecium* and *Enterococcus gallinarum* isolates against 22 antibiotics was performed with the MICRONAUT system for Gram-positive bacteria. The presence of 12 resistance-associated genes (*tet*K, *tet*L, *tet*M, *tet*S, *tet*O, *erm*(B), *msr*C, *aac*6-*aph*2, *van*A, *van*B, *van*C1 and *mec*A) was investigated by PCR. Thirty one Enterococcus isolates have been detected, *Enterococcus faecium* (n=22) and *Enterococcus gallinarum* (n=9) strains were isolated. All *Enterococcus faecium* and *Enterococcus gallinarum* isolates were multidrug-resistant. More than 15% of *Enterococcus faecium* isolates were resistant to vancomycin. Resistance rates to other antibiotics ranged between 31.8% for teicoplanin to 86.4% for penicillin G, respectively. *Enterococcus gallinarum* isolates showed a resistance rate of 100% to cefoxitin, ceftaroline, clindamycin, daptomycin, erythromycin, gentamicin and oxacillin. The resistance genes most often found were *msr*C, *erm*(B), *aac*6-*aph*2 and *mec*A. The *van*B gene was identified in two *Enterococcus faecium* isolates from which one was resistant to vancomycin whereas *van*C1 was detected in 6 *Enterococcus faecium* and *Enterococcus gallinarum* isolates. Tetracycline resistance-associated genes (*tet*K, *tet*L, *tet*M, *tet*O and *tet*S) were found in 25 isolates whereas *mec*A was detected in nine isolates. The study is the first report for *mec*A gene in *Enterococcus*. The development of resistance to antibiotics in poultry production needs special attention and investigation. The usage of antimicrobials in Egypt should follow WHO and OIE recommendations.

Keywords | *Enterococcus*, VRE, Antibiotic resistance, *mec*A, Egypt

INTRODUCTION

Poultry has been reported to carry increasingly antibiotic-resistant bacteria in its digestive tract. In the past, enterococci of the gut of humans and animals were considered to be common and harmless with little or no clinical relevance (Fisher and Phillips, 2009; Marshall and Levy, 2011). Meanwhile, they are the second and third most important etiologic agents of urinary tract infections

and nosocomial bacteremia in humans, respectively. In intensive care units enterococci may be found in 14.3% of patients (Papadimitriou-Olivgeris et al., 2014). The most common species isolated from clinical samples of humans are *Enterococcus* (*E.*) *faecalis* and *E. faecium* (Panesso et al., 2008).

One of the main reasons that enterococci can survive in the hospital environment is their intrinsic resistance to some

antibiotics and their ability to acquire resistance. Previous studies have shown that the prevalence of antimicrobial resistance and virulence factors of human and animal *Enterococcus* isolates may vary according to geographical origin and antimicrobial regime (Kwon et al., 2012; Liu et al., 2013).

Vancomycin-resistant *E. faecium* is the second leading pathogen of the priority list of antimicrobial resistance (priority pathogens) published recently by WHO that are a major threat to public health (WHO, 2017).

In poultry, enterococci are frequently isolated in cases of endocarditis, arthritis, amyloidosis and various disorders (Kense and Landman, 2011). Enterococci can also cause food intoxication through production of biogenic amines (Asadian et al., 2016).

Consumption of antimicrobials is an important risk factor for colonization with multidrug-resistant enterococci because of the suppression of the competitive indigenous microbiota of the gastrointestinal tract. The increased number of gut enterococci frequently results in bloodstream infections (Ubeda et al., 2010). Enterococci are known to easily acquire and transfer virulence and antimicrobial resistance determinants via uptake of mobile genetic elements of commensals and pathogens alike (Oliveira et al., 2016).

Several studies have been conducted to determine the prevalence, antimicrobial and virulence mechanisms of enterococci isolated from broilers in different countries (Ngbede et al., 2017; Nowakiewicz et al., 2017). However, little information is available for the Egyptian setting. Therefore, this study investigates presence and antimicrobial resistance of *Enterococcus* species in poultry flocks.

MATERIALS AND METHODS

SAMPLE COLLECTION AND CULTIVATION OF ENTEROCOCCI

Between January 2017 and November 2017, a total of 117 cloacal swabs were collected aseptically from diseased poultry with gastrointestinal disorders of 38 poultry farms (layers chicken, broilers, turkeys and ducks) located in 6 districts of Egypt (Qulyobia, Sharkia, Dakahlia, Damietta, Monofia and Gharbia governorates). The swabs were placed into micro-tubes containing sterilized phosphate-buffered saline. They were transported to the laboratory and immediately streaked out onto blood agar containing 5% sheep blood and incubated at 37.7°C for 48 h (Ulger et al., 2009).

IDENTIFICATION BY MALDI-TOF MS

Isolates were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bizzini et al., 2010). Briefly, bacteria from overnight cultures were suspended in 300 μl of bi-distilled water and mixed with 900 μl of ethanol (96% vol/vol; Carl Roth GmbH, Karlsruhe, Germany) for protein precipitation. After centrifugation for 5 min at 10,000 x g, the supernatant was removed and the pellet was re-suspended in 50 μl of 70% (vol/vol) formic acid (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Fifty microliters of acetonitrile (Carl Roth GmbH) were added, mixed and centrifuged for 5 min at 10,000 x g. One and a half microliter of the supernatant was transferred onto a MTP 384 Target Plate Polished Steel TF (Bruker Daltonik GmbH, Bremen, Germany). After air-drying the material was overlaid with 2 μl of a saturated solution of α-cyano-4-hydroxycinnamic acid (Sigma-Aldrich Chemie GmbH) in a mix of 50% acetonitrile and 2.5% trifluoroacetic acid (Sigma-Aldrich Chemie GmbH). After air-drying spectra were acquired with an Ultraflex instrument (Bruker Daltonik GmbH). The instrument was calibrated with the IVD Bacterial Test Standard (Bruker Daltonik GmbH). Analysis was carried out with the Biotyper 3.1 software (Bruker Daltonik GmbH). Interpretation of results was performed according to the manufacturer's recommendation: score of ≥ 2.3 represented reliable species level identification; score 2.0–2.29, probable species level identification; score 1.7–1.9, probable genus level identification, and score ≤ 1.7 was considered an unreliable identification (Lüthje et al., 2017).

ANTIBIOTIC SUSCEPTIBILITY TESTING

The antibiotic susceptibility testing of all isolates was performed with the MICRONAUT system for Gram-positive bacteria using commercial 96-well microtiter plates (Merlin, Bornheim, Germany) according to the manufacturer´s recommendations. This system allowed the determination of minimum inhibitory concentrations (MICs) of 22 antimicrobial agents including ampicillin (β-lactam), cefoxitin (β-lactam; cephamycin), ceftaroline (cephalosporin 5th generation), clindamycin (lincosamide), daptomycin (cyclic lipopeptide), erythromycin (macrolide), erythromycin/clindamycin, fosfomycin (epoxide antibiotic), fusidic acid (steroide antibiotic), gentamicin (aminoglycoside), linezolid (oxazolidinone), moxifloxacin (fluorochinolone 4th generation), mupirocin, oxacillin (β-lactam), penicillin G (β-lactam), rifampicin (ansamycine), synercid (streptogramine), teicoplanin (glycopeptide), tigecycline (glycylcycline), trimethoprim/sulphamethoxazole (trimethoxybenzyl pyrimidine/ sulfonamide) and vancomycin (glycopeptide) in serial dilutions of the antibiotics. Bacteria grown overnight and suspended in NaCl solution (0.9%) to obtain a turbidity corresponding to a McFarland standard of 0.5 (Dr. Lange, CADAS photometer 30, Berlin, Germany). Three hundred microliters of the suspension were added to 11 ml of Mueller–Hinton broth (Oxoid Deutschland GmbH, Wesel, Germany) resulting in a concentration of

approximately 10⁶–10⁷ colony forming units (cfu)/ml. In total, 100 µl of the inoculum were put in each well. After sealing the plates, they were incubated for 18 h to 24 h at 37°C. Reading of plates was done optically. Interpretation was carried out as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2017).

DETECTION OF RESISTANCE-ASSOCIATED GENES

Genomic DNA was extracted from bacterial cultures using High Pure PCR Template Purification Kit (Roche Diagnostics, Mannheim, Germany) according to the instructions of the manufacturer. PCR amplifications of tetracycline resistance genes (*tet*K, *tet*L, *tet*M, *tet*S and *tet*O), erythromycin resistance gene (*erm*(B)), macrolide resistance gene (*msr*C), aminoglycoside resistance genes (*aac*6-*aph*2), vancomycin resistance genes (*van*A, *van*B and *van*C1) and *mec*A gene were carried out using primers given in Table 1. PCR products were analyzed by electrophoresis on 2% agarose gel following staining with ethidium bromide and visualizing under UV.

For confirmation of PCR results, PCR products for all genes used were sequenced. Briefly, the bands of amplicons were cut out after electrophoresis and purified using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) according to the manufacturer´s instructions. Cycle sequencing was done using the Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Darmstadt, Germany) according to instructions of the manufacturer. Sequencing products were analyzed with a Genetic Analyzer ABI Prism 3130 (Applied Biosystems), identification of the sequences was carried out by BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

RESULTS

ISOLATION AND IDENTIFICATION OF *ENTEROCOCCUS* SPECIES

Thirty-one *Enterococcus* isolates were isolated (Table 2). Using MALDI-TOF MS two species were identified representing 28 *E. faecium* (52.8%) and 9 *E. gallinarum* (17.0%) strains.

ANTIMICROBIAL SUSCEPTIBILITY PROFILES

Antimicrobial resistance profiles of 22 *E. faecium* and 9 *E. gallinarum* isolates could be determined (Table 3a) and (Table 3b). These 31 isolates were multidrug-resistant which means that they were resistant to at least three antimicrobial agents of different classes. All *E. faecium* isolates were resistant to daptomycin, clindamycin, erythromycin and erythromycin/clindamycin. Four *E. faecium* isolates were resistant to vancomycin. Resistance rates to other antibiotics ranged between 31.8% for teicoplanin and 86.4% for penicillin G and cefoxitin.

Table 1: Primer and their sequences used for the detection of antibiotic resistance-associated genes in *Enterococcus* species.

Gene	Primer sequences (5´-3´)	Expected amplicon size (bp)	Reference
*van*A	F: ATG AAT AGA ATA AAA GTT GCA ATA R: CCC CTT TAA CGC TAA TAC GAT CAA	1030	Getachew *et al.*, 2012
*van*B	F: AAG CTA TGC AAG AAG CCA TG R:CCG ACA AAA TCA TCC TC	536	Getachew *et al.*, 2012
*van*C1	F:GGA ATC AAG GAA ACC TC R:CTT CCG CCA TCA TAG CT	822	Ünal *et al.*, 2017
erm(B)	F:GAA AAG GTA CTC AAC CAA ATA R:AGT AAC GGT ACT TAA ATT GTT TAC	639	Sutcliffe *et al.*, 1996
*msr*C	F: AAG GAA TCC TTC TCT CTC CG R: GTA AAC AAA ATC GTT CCC G	342	Werner *et al.*, 2001
*tet*K	F: TCG ATA GGA ACA GCA GTA R: CAG CAG ATC CTA CTC CTT	169	Ng *et al.*, 2001
*tet*L	F: TCG TTA GCG TGC TGT CAT R: GTA TCC CAC CAA TGT AGC CG	267	Ng *et al.*, 2001
*tet*M	F: GTG GAC AAA GGT ACA ACG AG R: CGG TAA AGT TCG TCA CAC AC	406	Ng *et al.*, 2001
*tet*O	F: AACTTA GGC ATT CTG GCT CAC R: TCC CAC TGT TCC ATA TCG TCA	515	Ng *et al.*, 2001
*tet*S	F: TGG AAC GCC AGA GAG GTA TT R: ACA TAG ACA AGC CGT TGA CC	660	Aarestrup *et al.*, 2000
*aac*6-*aph*2	F: CCA AGA GCA ATA AGG GCA TA R: CAC TAT CAT AAC CAC TAC CG	219	van Asselt *et al.*, 1992
*mec*A	F: TCC AGA TTA CAA CTT CAC CAG G R: CCA CTT CAT ATC TTG TAA CG	161	Pedroso *et al.*, 2018

Table 2: *Enterococcus* species isolated from cloacal swabs of different poultry species after cultivation and MALDI-TOF MS identification.

Source	No. of samples	No. of Enterococcus positive samples (%)	No. of isolates of different Enterococcus species identified in Enterococcus positive samples (%)	
			E. faecium	E. gallinarum
Layer chickens	54	15 (27.7)	11 (73.3)	4 (26.6)
Broiler chickens	31	8 (25.8)	5 (52.5)	3 (37.5)
Turkeys	4	3 (75.0)	3 (100)	-
Ducks	28	5 (17.8)	3 (60.0)	2 (40.0)
Total	117	31 (26.4)	22 (70.9)	9 (29.0)

Table 3a: Antimicrobial resistance profiles, MIC values for each individual isolate of *Enterococcus faecium* and *Enterococcus gallinarum* isolates from Egyptian poultry.

Isolate	AMP (R≥16)	COX (R≥16)	(CFL R≥2)	CLI (R≥.5)	DPT R≥4	ERY (R≥8)	ERC (R≥4/0.5)	GEN (R≥8)	GNH R≥500	LIZ (R≥8)	MOX (R≥2)	OXA (R≥16)	PEN (R≥16)	RAM (R≥4)	SYN (R≥4)	TPL R≥32	TGC (R≥1)	T/S(R ≥4/76)	VAN R≥32
18CS0389	0	16	1	0.5	4	0.5	4/0.5	8	0	0	1	16	4	2	2	0.5	0	0	2
18CS0444	0	16	1	0.5	4	8	4/0.5	2	500	1	2	16	8	0	2	0.5	0.25	0	2
18CS0474	16	16	2	0.5	4	8	4/0.5	8	500	8	2	16	8	2	4	2	1	4/76	2
18CS0490	16	16	2	0.5	4	8	4/0.5	8	500	8	2	16	8	0	4	16	1	4/76	32
18CS0495	16	16	2	0.5	4	4	4/0.5	8	500	8	2	16	8	0	4	2 S	1	4/76	1
18CS0499	16	16	2	0.5	4	4	4/0.5	8	500	4	2	16	8	2	0	1 S	0.5	4/76	2
18CS0503	16	8	2	0.5	4	8	4/0.5	8	-	-	2	16	8	2	4	1 S	1	0.5/9.5	2
18CS0505	8	16	2	0.5	4	4	4/0.5	8	500	4	2	8	8	2	4	8	0.5	4/76	16
18CS0429	4	8	0	0.5	4	8	4/0.5	2	0	0	1	0	8	2	0	0.5	0	0.5/9.5	0.
18CS0496	8	16	2	0.5	4	8	4/0.5	8	500	2	2	16	8	1	4	2 S	1	4/76	16
18CS0428	8	16	2	0.5	4	8	4/0.5	8	128	4	2	16	8	0	4	0.5	1	4/76	2
18CS0468	4	16	2	0.5	4	8	4/0.5	8	128	2	2	16	8	2	4	1	0.1	2/38	8
18CS0463	8	16	2	0.5	4	8	4/0.5	8	125	8	2	16	8	2	4	1	1	4/76	4
18CS0477	2	16	2	0.5	4	8	4/0.5	8	500	8	2	16	8	2	4	1	1	4/76	
18CS0489	16	16	2	0.5	4	4	4/0.5	8	500	2	2	16	8	2	4	-	0.5	4/76	
18CS0391	16	16	2	0.5	4	8	4/0.5	8	500	8	2	16	8	2	4	16	1	4/76	32
18CS0425	16	16	2	0.5	4	8	4/0.5	8	500	1	2	16	8	2	4	0.5	1	4/76	0.5
18CS0471	8	16	2	0.5	4	8	4/0.5	8	0	8	2	16	8	2	4	4	1	4/76	8
18CS0501	16	16	2	0.5	4	8	4/0.5	8	500	8	2	16	8	2	4	2	1	4/76	32
18CS0512	16	0	2	0.5	4	8	4/0.5	2	0	0	0	1	8	0	4	0.12	0	0	0.25
18CS0392	8 S	16	2	0.5	4	8	4/0.5	8	128	2	2	16	8	2	2	1	0.5	1/19	4
18CS0488	16	16	2	0.5	4	8	4/0.5	8	500	2	2	16	8	2	4	0.5	1	4/76	32
18CS0507	16	16	2	0.5	4	8	4/0.5	8	500	2	2	16	8	16	4	4	1	4/76	8
18CS0385	16	16	2	0.5	4	8	4/0.5	4	0	8	0	0.25	8	2	4	0.25	0	0	0.5
18CS0387	8	16	2	0.5	4	8	4/0.5	8	500	0	2	16	4	2	2	4	0.5	4/76	8
18CS0442	16	16	2	0.5	4	8	4/0.5	8	500	4	2	16	8	2	4	4	0.5	4/76	8
18CS0382	16	16	2	0.5	4	8	4/0.5	8	500	2	2	16	8	2	4	4	1	4/76	8
18CS0498	16	16	2	0.5	4	8	4/0.5	8	0	8	2	16	8	0	4	16	1	4/76	32
18CS0508	16	16	2	0.5	4	4	4/0.5	8	500	2	2	8	0	4	16	1	1	4/76	8
18CS0422	2	16	2	0.5	4	8	4/0.5	8	128	2	2	16	8	2	4	1	0.5	4/76	2
18CS0485	2	16	2	0.5	4	8	4/0.5	8	500	2	1	8	2	2	2	1	0.5	1/19	4

AMP: ampicillin; COX: cefoxitin; CFL: ceftaroline; CLI: clindamycin; DPT: daptomycin; ERY: erythromycin; ERC: erythromycin/ clindamycin; GEN: gentamicin; GNH: gentamicin high level; LIZ: linezolid; MOX: moxifloxacin; OXA: oxacillin; PEN: penicillin G; RAM: rifampicin; SYN: synercid, TPL: teicoplanin; TGC: tigecycline; T/S: trimethoprim/sulphamethoxazole; VAN: vancomycin.

Table 3b: Antibiotic resistance of *Enterococcus faecium* and *Enterococcus gallinarum* isolates from Egyptian poultry.

Antibiotic	Class	Enterococcus faecium (n=22)				Enterococcus gallinarum (n=9)			
		S	I	R	Resistance rate (%)	S	I	R	Resistance rate (%)
Ampicillin	β-Lactam	7	2	13	59.1	4	2	3	33.3
Cefoxitin	β-Lactam; cephamycin	2	1	19	86.4	0	0	9	100
Ceftaroline	Cephalosporin 5th generation	3	1	18	81.8	0	0	9	100
Clindamycin	Lincosamide	0	0	22	100	0	0	9	100
Daptomycin	Cyclic lipopeptide	0	0	22	100	0	0	9	100
Erythromycin	Macrolide	0	0	22	100	0	0	9	100
Erythromycin/ Clindamycin	Macrolide/ Lincosamide	0	0	22	100	0	0	9	100
Gentamicin	Aminoglycosides	6	0	16	72.7	0	0	9	100
Gentamicin high level	Aminoglycosides	10	0	12	54.5	5	0	4	44.4
Linezolid	Oxazolidinone	10	4	8	36.4	6	1	2	22.2
Moxifloxacin	Fluorochinolone 4th generation	5	2	15	68.2	2	0	7	77.8
Oxacillin	β-Lactam	4	1	17	77.3	0	0	9	100
Penicillin G	β-Lactam	3	0	19	86.4	1	0	8	88.9
Rifampicin	Ansamycine	9	2	11	50.0	1	0	8	88.9
Synercid	Streptogramine	1	5	16	72.7	2	0	7	77.8
Teicoplanin	Glycopeptide	8	7	7	31.8	8	0	1	11.1
Tigecycline	Glycylcycline	6	5	11	50.0	2	4	3	33.3
Trimethoprim/ Sul-phamethoxazole	Dihdrofolatreductase/ Sulfon-amide	5	2	15	68.2	2	1	6	66.7
Vancomycin	Glycopeptide	14	4	4	18.2	8	0	1	11.1

All *E. gallinarum* isolates showed resistance to cefoxitin, ceftaroline, clindamycin, daptomycin, erythromycin, erythromycin/clindamycin, gentamicin and oxacillin. One was resistant to vancomycin.

DETECTION OF ANTIBIOTIC RESISTANCE DETERMINANTS IN ENTEROCOCCI

Detection of resistance-associated genes showed the presence of several of these genes in the 31 phenotypically resistant enterococci isolates (Table 4). The most often identified resistance genes were *msr*C responsible for macrolide resistance (n=20) and *erm* (B) associated with erythromycin resistance (n=20). The *aac*6-*aph*2 genes connected with aminoglycoside resistance and *tet*L associated with tetracycline resistance were also detected (n=17 each).

Vancomycin resistance genes were found in 14 isolates. The *van*B gene was identified in two *E. faecium* isolates from which one was resistant to vancomycin. The *van*C1 gene was detected in four *E. faecium* isolates. None of these isolates was phenotypically resistant to vancomycin. Six *E. gallinarum* isolates harbored the *van*C1 gene but only one of them was phenotypically resistant.

Genes corresponding to tetracycline resistance (*tet*K, *tet*L, *tet*M, *tet*S and *tet*O) were found in 25 isolates. The *tet*L gene

was detected in 18 isolates (13 *E. faecium* and 5 *E. gallinarum*) followed by 16 *tet*M-carrying isolates (12 *E. faecium* and 4 *E. gallinarum*). Phenotypic resistance to tetracycline was not found even in isolates which harboured three of the *tet* genes (*tet*L, *tet*M, *tet*S). *tet*K was detected in five isolates. One of them was identified in an *E. gallinarum* isolate. One *E. faecium* isolate harboured the *tet*S gene.

The *mec*A gene corresponding to β-lactam resistance was detected in seven *E. faecium* and two *E. gallinarum* by PCR. Five out of 28 *E. faecium* isolates were phenotypically resistant to β-lactam antibiotics like ampicillin, oxacillin and penicillin G. *E. gallinarum* isolates showed also phenotypically resistance to β-lactam antibiotics.

To confirm the PCR results concerning *mec*A gene amplicons were sequenced. The sequence of PCR products was identical with GenBank entry (Acc. No. MK991791).

DISCUSSION

Antimicrobial resistance in enterococci is not only of major concern in the clinical setting of hospitals. Bacteria may also affect animal health or may contaminate food of animal origin (Silva et al., 2012). The rising number of infections in humans caused by resistant bacteria that

Table 4: Genotypic and phenotypic resistance profiles of *Enterococcus faecium* and *Enterococcus gallinarum* isolated from different poultry species in Egypt.

Source	Isolate	Species	Resistance-associated gene	Phenotypic resistance
Layer chickens	18CS0389	E. faecium	*erm*(B), *tet*L, *mec*A	COX, CLI, DPT, RAM
	18CS0444	E. faecium	*erm*(B), *msr*C, *aac6-aph*2, *tet*S, *tet*L, *tet*M	COX, CLI, DPT, ERY, ERC, GNH, PEN
	18CS0474	E. faecium	*erm*(B)	AMP, COX, CFL, CLI, DPT, ERY, ERC, GEN, GNH, LIZ, MOX, OXA, PEN, RAM, SYN, TGC, T/S
	18CS0490	E. faecium	*erm*(B), *msr*C, *aac6-aph*2, *tet*L, *tet*M	AMP, COX, CFL, CLI, DPT, ERY, ERC, GEN, GNH, LIZ, MOX, OXA, PEN, SYN, TPL, TGC, T/S, VAN
	18CS0495	E. faecium	*msr*C, *van*C1, *tet*K, *tet*L, *tet*M, *mec*A	AMP, COX, CFL, CLI, DPT, ERY, ERC, GEN, GNH, LIZ, MOX, OXA, PEN, SYN, TGC, T/S
	18CS0499	E. faecium	*msr*C, *van*B, *aac6-aph*2, *tet*L, *tet*M, *mec*A	AMP, COX, CFL, CLI, DPT, ERY, ERC, GEN, GNH, MOX, OXA, PEN, SYN, T/S
	18CS0503	E. faecium	*erm*(B), *msr*C, *van*C1, *aac6-aph*2, *tet*K, *mec*A	AMP, CFL, CLI, DPT, ERY, ERC, OXA, PEN, RAM,
	18CS0505	E. faecium	*erm*(B), *msr*C, *tet*K	COX, CFL, CLI, DPT, ERY, ERC, GEN, GNH, MOX, OXA, PEN, SYN, TPL, T/S
	18CS0429	E. faecium	*erm*(B), *msr*C, *tet*L	CLI, DPT, ERY, ERC
	18CS0496	E. faecium	*erm*(B), *msr*C, *van*C1, *aac6-aph*2	COX, CFL, CLI, DPT, ERY, ERC, GEN, GNH, MOX, OXA, PEN, SYN, TGC, T/S
	18CS0428	E. faecium	*tet*K, *mec*A	COX, CLI, DPT, ERY, ERC, GEN, MOX, OXA, PEN, SYN, TGC, T/S
	18CS0468	E. gallinarum	*msr*C, *van*C1, *aac6-aph*2, *tet*M	COX, CFL, CLI, DPT, ERY, ERC, GEN, MOX, OXA, PEN, RAM. SYN
	18CS0463	E. gallinarum	*van*C1, *tet*M	COX, CFL, CLI, DPT, ERY, ERC, GEN, LIZ, MOX, OXA, PEN, RAM, SYN, T/S
	18CS0477	E. gallinarum	*erm*(B), *aac6-aph*2, *tet*L, *tet*M	COX, CFL, CLI, DPT, ERY, ERC, GEN, LIZ, MOX, OXA, PEN, RAM, SYN, TGC, T/S
	18CS0489	E. gallinarum	*msr*C, *aac6-aph*2, *tet*L	AMP, COX, CFL, CLI, DPT, ERY, ERC, GEN, GNH, MOX, OXA, PEN, RAM, SYN, T/S
Broiler chickens	18CS0391	E. faecium	*erm*(B), *msr*C, *tet*L	AMP, COX, CFL, CLI, DPT, ERY, ERC, FUS, GEN, GNH, MOX, OXA, PEN, RAM, SYN, TPL, TGC, T/S, VAN
	18CS0425	E. faecium	*erm*(B), *msr*C, *tet*L, *tet*M	AMP, COX, CFL, CLI, DPT, ERY, ERC, FUS, GEN, GNH, MOX, OXA, PEN, RAM, SYN, TGC, T/S
	18CS0471	E. faecium	*erm*(B), *msr*C, *aac6-aph*2, *tet*L, *tet*M	COX, CFL, CLI, DPT, ERY, ERC, GEN, LIZ, MOX, OXA, PEN, RAM, SYN, TPL, TGC, T/S
	18CS0501	E. faecium	*msr*C, *tet*L, *tet*M	COX, CFL, CLI, DPT, ERY, ERC, GEN, LIZ, MOX, OXA, PEN, RAM, SYN, TGC, T/S, VAN
	18CS0512	E. faecium	*msr*C	AMP, CFL, CLI, DPT, ERY, ERC, PEN
	18CS0392	E. gallinarum	*erm*(B)	COX, CFL, CLI, DPT, ERY, ERC, GEN, MOX, OXA, PEN, RAM
	18CS0488	E. gallinarum	*van*C1, *aac6-aph*2, *tet*L, *mec*A	AMP, COX, CFL, CLI, DPT, ERY, ERC, FUS, GEN, GNH, MOX, OXA, PEN, RAM, SYN, TPL, TGC, T/S, VAN
	18CS0507	E. gallinarum	*erm*(B), *van*C1, *aac6-aph*2	AMP, COX, CFL, CLI, DPT, ERY, ERC, GEN, GNH, MOX, OXA, PEN, RAM, SYN, TGC, T/S
Turkeys	18CS0385	E. faecium	*erm*(B), *msr*C, *van*C1, *aac6-aph*2, *tet*M, *mec*A	AMP, COX, CFL, CLI, DPT, ERY, ERC, LIZ, PEN, RAM, SYN
	18CS0387	E. faecium	*erm*(B), *msr*C, *aac6-aph*2, *tet*M	COX, CFL, CLI, DPT, ERY, ERC, GEN, GNH, MOX, OXA, RAM, T/S

	18CS0442	E. faecium	*erm*(B), *msr*C, *van*C1, *tet*L, *tet*M, *mec*A	AMP, COX, CFL, CLI, DPT, ERY, ERC, GEN, GNH, MOX, OXA, PEN, RAM, SYN, T/S
Ducks	18CS0382	E. faecium	*aac*6-*aph*2	AMP, COX, CFL, CLI, DPT, ERY, ERC, GEN, GNH, OXA, PEN, RAM, SYN, TPL, T/S
	18CS0498	E. faecium	*msr*C, *van*B, *aac*6-*aph*2, *tet*L, *tet*M, *mec*A	AMP, COX, CFL, CLI, DPT, ERY, ERC, GEN, LIZ, MOX, OXA, PEN, SYN, TPL, TGC, T/S, VAN
	18CS0508	E. faecium	*erm*(B), *msr*C, *van*C1, *aac*6-*aph*2, *tet*K, *tet*L, *tet*M	AMP, COX, CFL, CLI, DPT, ERY, ERC, GEN, LIZ, MOX, OXA, PEN, SYN, TPL, TGC, T/S
	18CS0422	E. gallinarum	*erm*(B), *van*C1, *aac*6-*aph*2, *tet*L	COX, CFL, CLI, DPT, ERY, ERC, GEN, OXA, PEN, RAM, SYN, T/S
	18CS0485	E. gallinarum	*erm*(B), *msr*C, *van*C1, *aac*6-*aph*2, *tet*L, tetM, tetO	COX, CFL, CLI, DPT, ERY, ERC, GEN, GNH, OXA

AMP: ampicillin; COX: cefoxitin; CFL: ceftaroline; CLI: clindamycin; DPT: daptomycin; ERY: erythromycin; ERC: erythromycin/clindamycin; GEN: gentamicin; GNH: gentamicin high level; LIZ: linezolid; MOX: moxifloxacin; OXA: oxacillin; PEN: penicillin G; RAM: rifampicin; SYN: synercid, TPL: teicoplanin; TGC: tigecycline; T/S: trimethoprim/sulphamethoxazole; VAN: vancomycin.

originate from animal reservoirs is of great concern. In fact, results from previous studies showed that transfer of resistance genes from enterococci of animal origin to enterococci in human beings occurred through the food chain (Lester et al., 2006).

In this study, the dominant *Enterococcus* species was *E. faecium* (70.9%) followed by *E. gallinarum* (29.0%) which is a similar to the findings of (Ünal et al., 2017) who isolated *E. faecium* (60.4%) and *E. gallinarum* (2.6%) from broiler samples. No other *Enterococcus* species were detected in this study which could be attributed to different origin and feed contamination (Butaye et al., 1999) *E. faecium* was also the most commonly isolated *Enterococcus* species from poultry cloacal swabs in Turkey (Dilik et al., 2010) In contrast, *E. avium* and *E. gallinarum* were found to be the most predominant *Enterococcus* species in pigeon and duck faeces samples in Egypt (Osman et al., 2019). Pigeon and duck faeces were collected in Cairo city and poor neighborhoods while in the presented study samples were collected in farms of six governorates outside the Egyptian metropolis. Thus, differences concerning the origin of samples, housing, feeding, breeding but also host specificity may have influenced study outcome.

E. faecium and *E. faecalis* are the most predominant enterococci species causing human infections worldwide (Billington et al., 2014; Kajihara, 2015). They are also a main cause of healthcare-associated infections (Ben Sallem, 2016). These two species have developed resistance to a wide variety of clinically important antibiotics (Ngbede et al., 2017; Ünal et al., 2017; Bertelloni et al., 2015; Kim et al., 2019). In Egypt, another often ignored but critical circumstance is the uncontrolled discharge of large amounts of pharmaceutical waste containing active compounds from antibiotic manufacturing plants into rivers and the soil environment. This practice contributes to the emergence of antibiotic-resistant organisms resulting

in considerable hazard to public health (Grenni et al., 2018). Thus, the high rates of antibiotic resistance found in this study may be caused by uncontrolled use of antibiotics for therapeutic or prophylactic purposes. It is noteworthy, that antibiotics are still used as growth promoters included in feed for poultry.

In the present study, four *E. faecium* and one *E. gallinarum* strains showed resistance to vancomycin which is in accordance with results obtained by (Ünal et al., 2017) for broiler cloacal samples in Turkey. Vancomycin resistance was also detected in 10/153 (6.5%) of *Enterococcus* isolates originated from food samples which were collected in different supermarkets and groceries in Egypt (Raafat et al., 2016). (Osman et al., 2019) found *van*B and *van*C genes in 25.5% and 33.0% in enterococci isolates from poultry in Egypt, respectively. Those findings are was comparable to results found in this study. A similar frequency of resistance (23.1%) was found in Egyptian *E. faecium* isolates from hospitals (Moemen et al., 2015).

The *van*A gene could not be detected in any of the isolates as *E. feacalis* is the common carrier for this gene, while *van*B and *van*C1 genes were found in 14 out of 31 *E. faecium* and *E. gallinarum* isolates by PCR. Although vanC are intrinsic in *E. gallinarum*, unfortunately it was not detected in 3 of *E. gallinarum* isolates. Only two of them showed phenotypic resistance, so it is evident that there exists a discrepancy between phenotypic resistance and presence of resistance-associated genes.

Vancomycin resistance in our study reached an alarming rate as it is used for the treatment of enterococcal infections in humans in Egypt in contrast to the situation in the EU (Hao et al., 2016). In contrast to the EU, where the use of avoparcin which shows chemical similarity to vancomycin is forbidden in livestock, feeding avoparcin is widely used in Egypt as growth promoter and for prevention of necrotic

enteritis in the poultry production, which may have led to an increased prevalence of vancomycin resistance in bacteria (Bager et al.,1997).

The resistance rates to antibiotics which are of high importance for treatment of humans e.g. daptomycin, linezolid and tigecycline were 100%, 36.4% and 50.0%, respectively. In contrast, (De Jong et al., 2018) detected absence or low levels of resistance to these antibiotics. These results are alarming, because multidrug-resistance in enterococci in poultry isolates is very likely to spill over to the human population via food or intensive contact in farming. Egyptian doctors admitted hospital hygiene failure and overuse of antibiotics as major causes for the high rate of antimicrobial resistance in Egypt (Saied, 2006).

The highest frequencies of resistance were found to clindamycin, daptomycin, erythromycin and erythromycin/clindamycin. A finding which is similar to that of (Osman et al., 2019) nearly all of their isolates were resistant to clindamycin and erythromycin. The described resistance rates were higher than those obtained by (Aslantas, 2019). In that study resistance to erythromycin was the most common (77.1%).

The *msr*C gene as well as the *erm*(B) gene both associated with macrolide/erythromycin resistance were found in 67.7% and 64.5% of isolates, respectively, rates that were lower than those reported by (Nowakiewicz et al., 2017; Kim et al., 2019) They identified the *erm*(B) gene in 96.0% and 98.4% of their isolates, respectively. These genes are located on mobile genetic elements like Tn917, Tn551 and Tn3871 and can be easily transferred horizontally (Shaw and Clewell, 1985; Jurado-Rabadan et al., 2014).

Resistance of enterococci to tetracycline is commonly mediated by *tet* genes (*tet*K, *tet*L, *tet*M, *tet*S and *tet*O). Some isolates carried one or more of these genes, however, phenotypic resistance to tetracycline could not be detected. In contrast to this study (Kim et al., 2019) identified *tet*L in 89.3% and *te*tM in 95.3% of isolates from chicken meat, respectively. The high resistance rates in Egypt can be explained by the frequent use of tetracycline in both, human and animal medicine for treatment of diseases but also for prophylaxis. Especially in animal and poultry production the easily affordable tetracyclines are used without microbiological identification and antibiogram of the causative pathogen i.e. cheap (Haggag et al., 2018).

Although tetracycline and erythromycin are not the drugs of choice for treatment of enterococcal infections, resistance to them is still of great clinical importance because they are effective for the treatment of other bacterial infections (Arias et al., 2010). Therefore, the ease

with which enterococci can disseminate erythromycin and tetracycline resistance traits to other bacteria raises serious clinical and public health concern (Hammerum, 2012).

Resistance to aminoglycosides represented by gentamicin was detected phenotypically in 72.7% of *E. faecium* and all *E. gallinarum* isolates. This rate was higher than those reported by other researchers (Ngbede et al., 2017). High level gentamicin resistance (HLGR) is clinically important, because a combination of gentamicin with cell wall-active antibiotics (e.g. β-lactams, vancomycin) has been used for treatment of severe enterococcal infections (e.g. endocarditis). HLGR was detected in 54.5% of *E. faecium* and 44.4% of *E. gallinarum* isolates which is higher as the rate was reported by (Aslantas, 2019) with 14% HLGR isolates. Resistance to aminoglycosides is mediated by the *aac*6-*aph*2 genes and was detected in 54.8% of isolates in which all of them showed phenotypic aminoglycoside resistance. A lower rate of *aac*6-*aph*2 was reported by (Padmasini et al., 2014) who found 38.2% of clinical isolates harbouring these genes.

Resistance to β-lactam antibiotics (ampicillin, cefatoxine, oxacillin and penicillin G) for both, *E. faecium* and *E. gallinarum*, was high in general and excelled the rates given by a previous report from (Ngbede et al., 2017) who found 45.1% of resistant enterococci isolated from poultry and cattle farms in Nigeria. Resistance to β-lactam antibiotics is an important problem of therapeutic medications, especially when associated with high levels of resistance to ami-noglycosides and glycopeptides as found in our study. Multiple mutations in the active site of the *pbp*5 gene are common causes for high-level resistance instead of over-expression of PBP5 protein as shown by other authors (Hsieh et al., 2006; Jureen et al., 2004).

Penicillin-resistance detected by (Gousia et al., 2015) was lower than in this study, 41% vs. 29%. Although *mec*A is mainly reported in staphylococci (Hanssen et al., 2004; El-Kharroubi et al., 1991) has detected *mec*A homologue in *Enterococcus hirae* that led to the deduction that *mec*A also occurs in non-staphylococcal genera (Fitzgerald et al., 2003). The *mec*A gene associated with resistance to methicillin/oxacillin was detected in nearly 29% of isolates. All of them showed phenotypic resistance to one or more antibiotics of the β-lactam group. This is the first description of the *mec*A gene in *Enterococcus* species isolated from poultry and our results have been confirmed by sequencing PCR product for *mec*A gene. This gene was detected in enterococci isolated from soft cheese (Resende et al., 2018) and *E. faecalis* isolated from surface water (Kassem et al., 2008). Transfer of *mec*A gene between *Enterococcus* isolates is mainly due to acquiring of plasmid and or transposons (Tang et al., 2014).

It has to be noted that *Enterococcus* species can acquire and spread genes responsible for antimicrobial resistance at much higher rates than Gram-negative ones (Chajęcka-Wierzchowska et al., 2015).

The trimethoprim/ sulphamethoxazole resistance was in *E. faecium* and *E. gallinarum* almost similar to that reported by (Stepien-Pysniak et al., 2016). They reported a resistance rate of 88% of poultry isolates. This finding may be caused by the fact that trimethoprim/sulphonamides are used regularly for prophylaxis and treatment of poultry and animal coccidiosis.

In Egypt the high level of resistance to above mentioned antimicrobials in comparison to other countries is possibly the result of transmission of genes between isolates of animal and human origin. Enterococci and fecal-derived bacteria are often present in river water at the same time as water is contaminated with sewage from both, humans and animals (Romanis, 2013). It has been shown that river water is indeed a major source of multidrug-resistant bacteria due to pollution by waste products of agriculture, animal excreta and raw sewage (Lupo et al., 2012). Thus, spread of enterococci and other bacteria carrying antibiotic resistance genes may regularly occur via the water of the River Nile that is used for all purposes without sterilization/ decontamination e.g. drinking water for humans and animals but also irrigation purposes.

CONCLUSION

Enterococci have become high risk zoonotic pathogens which can cause life threatening health problems. Enterococci isolated from poultry, especially *E. faecium* are known to cause nosocomial diseases in humans. The characterization of those isolates will help to decision makers to decide on counter measures along the value chain and in clinical settings at the hospital. All *E. faecium* and *E. gallinarum* isolates were multidrug-resistant and may cause serious health problems. Vancomycin-resistance was found in several isolates and underlines the importance of prudent use of antibiotics. This study also considered as first report for *mec*A gene in *Enterococcus* isolates from poultry, thus provide information that enterococci from poultry are possible vectors for transmission of critical and risky resistance-associated genes.

In general, the use of antimicrobials in poultry and food production should be controlled and prudent. Use of last line agents as vancomycin in animal production should be prevented worldwide and meticulous control of levy quantifies also should be implemented in developing countries.

CONFLICT OF INTEREST
The author reports no conflicts of interest in this work.

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