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Characterization of Vancomycin Resistant Enterococci Isolated from Retail Poultry Meat

Sara El-Oraby, Amal Awad*, Gamal Younis

Department of Bacteriology, Immunology and Mycology, Faculty of Veterinary Medicine, Mansoura University, 35516 Mansoura, Egypt.

***Correspondence**
Corresponding author: Amal Awad
E-mail address: amalabdo@mans.edu.eg

Abstract

Antimicrobial-resistant bacteria in poultry meat threaten the public health. The present study was designed to detect the presence of *Enterococcus* species in poultry meat collected from Mansoura retail outlets with characterization of vancomycin-resistant enterococci (VRE). Thus, poultry meat samples (breast and thigh) were collected from separate grocery stores around Mansoura city, Egypt. By conventional and molecular methods, out of the total examined samples (n=120), 44 *E. fecalis* isolates have been recovered with a percentage of 36.66%. All strains were assessed for their antimicrobial susceptibility using disc diffusion method, the highest rate of resistance (100%; 44/44) was displayed to ceftazidime, streptomycin, gentamycin, and clindamycin, while, a resistance rate of 36.3%, 40.9%, 68.1% and 77.2% were displayed against ciprofloxacin, penicillin, erythromycin and vancomycin respectively. Interestingly, all isolates exhibited multidrug resistance (MDR; Resistance to three or more antimicrobial class). Screening vancomycin -resistant strains (n= 34) by PCR for the presence of vancomycin resistance genes, *van*A was identified in 47.1% *van*B in 33.4%, *van*C1 in 14.7% and *van*C-2 C-3 in 5.9%. By testing the ability of the isolated strains for the biofilm production by Congo Red Method, 31.8 % of the tested isolates were tested positive. Hence, standard manufacturing procedures and adequate hygiene conditions must be integrated into all phases of poultry meat preparation, production and consuming, and public knowledge should be improved.

KEYWORDS
Enterococcus, Poultry meat, VRE, Biofilm production

INTRODUCTION

Enterococcus faecalis, previously categorized as a component of the group D *Streptococcus* system, is a Gram-positive bacterium that is known to inhabit the gastrointestinal tracts of humans as a commensal organism (de Almeida *et al*., 2018). About 54 distinct species of enterococci have been reported, with *Enterococcus* faecalis (*E. faecalis*) and *Enterococcus faecium* (*E. faecium*) accounting for the majority of clinical isolates (Alatrouny *et al*., 2020). *E. faecalis* is a commensal bacterium that is commonly present in the microbiota of healthy individuals. However, it has the potential to induce a range of infections in humans, including endocarditis and sepsis, urinary tract infections (UTIs), meningitis, and other related conditions (Ayobami *et al*., 2020; Kim *et al*., 2020).

Enterococci are widely acknowledged as noteworthy hospital pathogens even after a span of over a century (Aly *et al*., 2008; Weiner *et al*., 2016). *Enterococcus* bacteria, particularly *E. faecalis* and *E. faecium*, are responsible for various infections in hospitalized patients such as urinary tract infections, bacteremia, intra-abdominal infections, and endocarditis. (Fiore *et al*., 2019). The prevalence of Enterococci as a nosocomial pathogen has increased over time, according to recent data, it has been found that between 2011 and 2014, it constituted 14% of hospital-acquired infections in the United States. This represents a notable increase from the 11% reported in 2007 (Aly *et al*., 2008; Weiner *et al*., 2016). Besides nosocomial infections, enterococci are responsible for causing 5-20% of cases of endocarditis acquired within the community (Pericàs *et al*., 2020).

The genus in challenging is distinguished by its ability to withstand a wide spectrum of antimicrobial agents. The species in particular displays inherent as well as acquired resistance to antimicrobial substances (Costa *et al*., 2022). Horizontal transmission of acquired resistance genetic determinants can occur both within and between genera (de Niederhäusern *et al*., 2011). Enterococci have the potential to acquire resistance to various antibiotics such as tetracycline, erythromycin, glycopeptides, and aminoglycosides, leading to the emergence of vancomycin-resistant enterococci (VRE) (Cetinkaya *et al*., 2000; Trinh and Lee, 2021).

Enterococcus species exhibit innate resistance to a range of antibiotics, encompassing low-level aminoglycosides, cephalosporins, clindamycin, lincosamides, nalidixic acid, and penicillins, among others (Niu and Li, 2019; Abutaleb and Seleem, 2020). The observation of resistance to a diverse range of antibiotics, including but not limited to high-level aminoglycosides, chloramphenicol, clindamycin, erythromycin, fluoroquinolones, penicillins, tetracycline, and vancomycin, has been documented (Marothi *et al*., 2005). Vancomycin, an antibiotic that is regulated by the *van* gene, is frequently utilized as the principal therapeutic approach for infections resulting from methicillin-resistant *Staphylococcus aureus* (MRSA). The development of vancomycin-resistant en-

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terococci (VRE) presents a substantial menace to public health owing to the potential gravity of the related infections (El-Mahdy *et al*., 2018; Fiore *et al*., 2019).

The emergence of bacteria resistant to several antibiotic treatments is a major public health threat (Ferri *et al*., 2017). Vancomycin, a glycopeptide antibiotic, was formerly effective against Gram-positive bacteria, producing life-threatening infections. Antimicrobials are used in people for treatment and in animals for infection control and growth promotion, and there is growing evidence that this practice might select resistant genes and contribute to the formation of antibiotic-resistant strains (Monger *et al*., 2021). Food-borne pathogens, opportunistic pathogens, and commensal microorganisms are all engaged in this scenario. Antibiotic resistance is spreading rapidly in this latter, and they serve as a reservoir of resistance genes for infections (Baker *et al*., 2018).

Some strains of enterococci are naturally resistant to antibiotics such semisynthetic penicillin, aminoglycosides, vancomycin, lincosamides, polymyxines, and streptogramins (Gołaś-Prądzyńska and Rola, 2021). Several additional antibiotics are similarly susceptible to acquired resistance in enterococci due to the presence of resistant characteristics carried by plasmids, integrons, and transposons (Sarathy *et al*., 2020). Since its approval for human use, glycopeptides antibiotics, primarily vancomycin, have been the standard treatment for enterococcal infections. Vancomycin was widely used in therapeutic settings, however this led to a rapid rise in the prevalence of vancomycin resistance (Kirst *et al*., 1998). This widespread use in healthcare settings, together with the use of growth promoters in cattle, may contribute to the emergence of drug-resistant strains. In 1986, for instance, the Norwegian government gave the green light to the use of avoparcin, a glycopeptide analogue, to speed up the development of livestock intended for human consumption (Borgen *et al*., 2000).

Herein this study aimed to isolate and identify enterococci from poultry meat, investigate the occurrence of vancomycin-resistant enterococci (VRE) from the isolated enterococci and its antimicrobial resistance profiles with molecular characterization of vancomycin genes (*van*A, *van*B, *van*C1/2, *van*D, *van*E, *van*G) in theses isolates and also determine the biofilm production capability of isolated strains.

MATERIALS AND METHODS

Ethical approval

This study was ethically approved by Research Ethics Committee of the Faculty of Veterinary Medicine, Mansoura University, Egypt (Code No: M/30).

Sampling

Totally, 120 samples of retail poultry meat (breast and thigh) were collected from April to August 2022 from separate grocery stores in Mansoura city, Egypt. Samples were transported to the Bacteriology, Mycology and Immunology Department Laboratory, Faculty of Veterinary Medicine, Mansoura University, Mansoura separately in plastic bags labeled, and placed into a cooler with ice pack under aseptic conditions for further investigations.

Isolation and identification of enterococci

A total of 10 grams of each sample were homogenized by mechanical stirring after being diluted in 90 mL of peptone water (Oxoid, Basingstoke, Hants, United Kingdom) and incubated at 37 ± 1°C for 24 ± 2 hours. Serial decimal dilutions of homogenized food were utilized to prepare aliquots that were subsequently spread onto Bile Esculin Azide (BEA) (Oxoid, Basingstoke, Hants, United Kingdom) agar plates. The plates were then incubated for a duration of 24 hours at a temperature of 35°C. Individual colonies exhibiting black pigmentation on the BEA agar were selectively retrieved from the BEA medium (Delpech *et al*., 2012). The presumptive isolates were then purified by streaking on Trypticase Soy Agar (TSA) from Oxoid Ltd, Hampshire, England, and subsequently stored at a temperature of 4°C. This procedure was conducted as a preliminary step towards conducting additional biochemical assays, such as Catalase, oxidase, gram staining, esculin hydrolysis, growth at 5% Nacl, and growth at temperatures spanning from 10-45°C (Brtkova *et al*., 2010).

Identification of enterococci

The colonies of presumptive enterococci were subjected to gram staining, and biochemical tests including, esculin hydrolysis test, growth at 5% Nacl, and growth at temperatures ranging from 10-45°C (Vandamme *et al*., 1996). The strains were subjected to genus-level identification by biochemical reactions, as outlined by Facklam *et al*. (1989).

Molecular characterization of Enterococcus

DNA extraction

The process of extracting genomic DNA was performed by boiling. A total of 3 -5 bacterial colonies were homogenized in 200 µL of deionized water. The resulting mixture was subjected to boiling at a temperature of 95°C for a duration of 15 minutes, followed by centrifugation for 3 minutes at a rate of 10,000 rpm. The liquid portion of the sample was transferred to a sterile Eppendorf tube and subsequently utilized as a source of DNA. The DNA of each isolate was preserved at a temperature of -20°C until their utilization (Ramadan *et al*., 2016). The *E. fecalis* was screened in all suspected isolates by PCR (Depardieu *et al*., 2004), using species specific primers for *E. fecalis* (Table 1). In order to generate a PCR reaction volume of 25 µL, a sterile microtube was utilized to combine 1 µL of each primer, with a total concentration of 10 pmol, and 12.5 µL of the Master Mix for each sample. Following this, the mixture was supplemented with 5.5 µL of sterile distilled water, resulting in a total volume of 20 µL. In addition, a volume of 5 µL of the extracted DNA from each isolate was utilized as a template and underwent amplification using a Thermal Cycler. The thermal cycler's cycle conditions were as follows: The experiment commenced with an initial denaturation phase at a temperature of 94°C for a duration of 5 minutes. This was succeeded by 35 cycles, each of which consisted of denaturation at 94°C for a duration of 1 minute, annealing at a temperature of 55°C for a duration of 1 minute, and elongation at a temperature of 72°C for a duration of 2 minutes. The process of final elongation was executed at a temperature of 72°C for a duration of 5 minutes. The amplified DNA fragments obtained through Polymerase Chain Reaction (PCR) were analyzed by electrophoresis on a 1% agarose gel supplemented with 0.5 mg/L of ethidium bromide. The visualization of the separated fragments was carried out using a Gel Documentation System produced by Cleaver Scientific Ltd, UK, under UV light. Antimicrobial Susceptibility Testing

The confirmed *E. fecalis* isolates were subjected to Kirby Bauer disk diffusion method to examine its susceptibility to antimicrobial agents according to the Clinical and Laboratory Standards Institute (CLSI). The antibiotic disks from Bioanalyse, Turkey were

used including vancomycin (VA; 30 µg), erythromycin (E; 15 µg), penicillin (P; 10 IU), ciprofloxacin (CIP; 5µg), clindamycin (DA; 2 µg), gentamicin (CN;10 µg), streptomycin (STR; 10 µg), and ceftazidime (CAZ; 30 µg). The findings were analyzed based on the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) (Ayandele *et al*. 2020) The term "multidrug resistance" refers to the phenomenon whereby a particular strain of microorganism exhibits resistance to three or more classes of antimicrobial agents (Basak *et al*. 2016). The Krumperman method was employed to compute a multiple antibiotic resistance index (MARI) (Joseph *et al*., 2017).

Molecular identification of VRE encoding genes

The VRE genes were screened in all suspected isolates by PCR with specific primers of VRE encoding genes. Conditions of cycles in the thermal cycler were as follows: Initial denaturation started at 94°C for 4 minutes followed by 30 cycles, each cycle included denaturation at 94°C for 1 minute, annealing at 55°C for 2 minutes and elongation at 72°C for 3 minutes. Final extension was carried out at 72°C for 4 minutes. PCR products were electrophoresed in 1% agarose gel and visualized under UV light. The oligonucleotides primers sequence and amplicons size are listed in Table 1 following the referred authors listed in the Table 1.

Detection of Biofilm production by Enterococcus isolates

Biofilm production of the retrieved isolates was assessed by Congo red agar (CRA) plate assay. This methodology enables the direct observation of the colonies and the discrimination between strains that produce slime (evidenced by the presence of black colonies on the red agar) and those that do not produce slime (indicated by the presence of red-colored colonies). The strains were cultured on CRA plates, prepared by adding of Congo red (0.8 g/L) and sucrose (5g/L) (Sigma, Missouri, EUA) to 1 L of autoclaved Brain Heart Infusion Agar. The plates were subsequently incubated for 24 h at 37°C and overnight at room temperature. The appearance of black colonies with crystalline consistency indicated biofilm production (Freeman *et al*., 1989).

Table 1. Oligonucleotides primers used in this study.

Primer	Sequence (3' → 5')	Amplicons (bp)	References
VanA	GGGAAAACGACAATTGC GTACAATGCGGCCGTTA	732	Gardini *et al*. (2001)
VanB	ATGGGAAGCCGATAGTC GATTTCGTTCCTCGACC	635	Gardini *et al*. (2001)
VanC-1	GGTATCAAGGAAACCTC CTTCCGCCATCATAGCT	822	Dutka-Malen *et al*. (1995); Jánošková and Kmeť(2004)
vanC-2 C-3	CTCCTACGATTCTCTTG CGAGCAAGACCTTTAAG	439	Dutka-Malen *et al*. (1995); Jánošková and Kmeť (2004)
ddlE. faecalis	ATCAAGTACAGTTAGTCT ACGATTCAAAGCTAACTG	941	Dutka-Malen *et al*. (1995); Brtkova *et al*. (2010)
ddlE. faecium	TAGAGACATTGAATATGCC TCGAATGTGCTACAATC	550	Dutka-Malen *et al*. (1995); Brtkova *et al*. (2010)

RESULTS

Prevalence of E. fecalis isolates

In this research, 120 poultry meat samples were tested to assess the prevalence of *Enterococcus* species in the collected samples using standard culture procedures. Culture, morphological and biochemical analysis indicated that 44 isolates (36.66%) were suspected to be *E. fecalis*. (Gram positive cocci arranged in short chains, negative result with catalase and oxidase tests). The presumptive isolates were then confirmed by PCR using two pairs of primers specific to *E. fecalis* and *E. faecium* which confirmed the presence of *E. fecalis* in all tested isolates (n=44) and *E. faecium* failed to be amplified in all isolates (Figure 1).

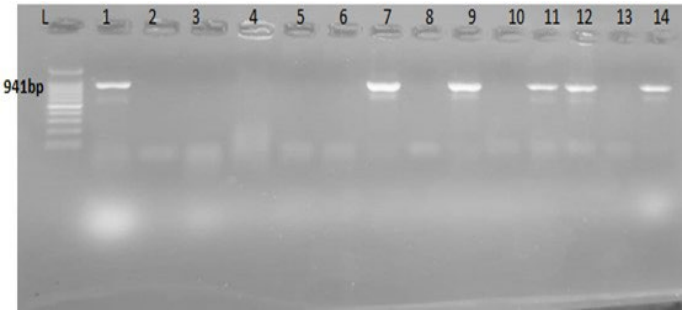

Figure 1. Agarose gel electrophoresis showing amplification of *E. fecalis* at 941bp. Lane L:100-3000 bp DNA size marker, Lane 1,7,9, 11,12,14 positive samples, Lane 2-6,8,10,13 negative samples.

Table 2. Results of Antimicrobial Susceptibility Testing.

Antibiotics	Family	Disc Code	CPD	Resistant	Sensitive
Vancomycin	Glycopeptides	VA	30	34 (77.2%)	10 (22.7%)
Erythromycin	Macrolides	E	15	30 (68.1%)	14 (31.8%)
Penicillin	β-lactam	P	10	18 (40.9%)	26 (59%)
Ciprofloxacin	Fluoroquinolone	CIP	5	16 (36.3%)	28 (63.6%)
Clindamycin	Lincomycin	DA	2	44 (100%)	00.00
Gentamycin	Aminoglycoside	GEN	10	44 (100%)	0
Streptomycin	Aminoglycoside	STR	10	44 (100%)	0
Ceftazidime	Cephalosporin	CAZ	30	44 (100%)	0

Table 3. Pattern of Antimicrobial Susceptibility Testing.

Antibiotypes	Resistance Pattern	Isolates No. (%) N=44	MAR	MAR Index
1	DA, GEN, STR, CAZ	1 (2.2%)	3	0.5
2	DA, GEN, STR, CAZ, E	2 (4.5%)	4	0.6
3	DA, GEN, STR, CAZ, CIP	2 (4.5%)	4	0.6
4	DA, GEN, STR, CAZ, VA	2 (4.5%)	4	0.6
5	DA, GEN, STR, CAZ, VA, E	2 (4.5%)	5	0.75
6	DA, GEN, STR, CAZ, VA, P	1 (2.2%)	5	0.75
7	DA, GEN, STR, CAZ, E, CIP	4 (9%)	5	0.75
8	DA, GEN, STR, CAZ, VA, CIP	4 (9%)	5	0.75
9	DA, GEN, STR, CAZ, VA, E, P	14 (31.8%)	6	0.87
10	DA, GEN, STR, CAZ, VA, E, CIP	8 (18%)	6	0.87
11	DA, GEN, STR, CAZ, VA, P,CIP	4 (9%)	6	0.87

The results of antimicrobial susceptibility testing

The antimicrobial susceptibility was conducted on all the retrieved isolates (n=44), the tested isolates and showed 100 % resistance to clindamycin, gentamycin, streptomycin, and ceftazidime. Obviously, the tested isolates exhibited a remarkable resistance to vancomycin (34/44, 77.2%), followed by erythromycin (30/44, 68.1%), penicillin (18/44, 40.9%), ciprofloxacin (16 /44, 36.3%) (Table 2). Isolates exhibiting resistance to three or more different antimicrobial classes were termed Multiple Antimicrobial Resistance (MAR). Accordance to the prior terminology, MAR was detected in all tested isolates, MDR index was ranged between 0.5-0.87, which indicates the extensive uses of these antimicrobial (Table 3).

Detection of vancomycin -resistance encoding genes in VRE isolates

By screening vancomycin- resistance isolates (n=34) for the presence of vancomycin resistance gene by PCR, *van*A, *van*B, *van*C1 and *van*C2-3 were detected in 47.1% (16/34), 32.4% (11/34), 14.7 % (5 /34) and 5.9% (2/34) respectively (Figures 2, 3, 4 and 5).

Assessment of Biofilm Formation by E. fecalis

The biofilm formed by *E. fecalis* (44 isolates) by Congo Red Method resulted in that 14 (31.8%) isolates were biofilm producer (Figure 6).

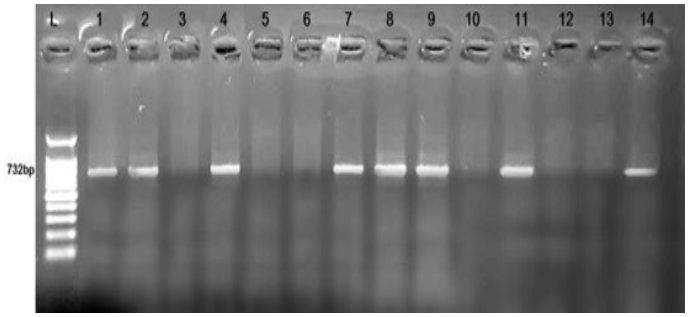

Figure 2. Amplification of *van*A at 732 bp in the vancomycin resistance *E. fecalis* strains. Lane L 100- 3000 bp DNA size marker, Lane 1,2,3,7-9,11,14 positive samples, Lane 3,5,6,10,12,13 negative samples.

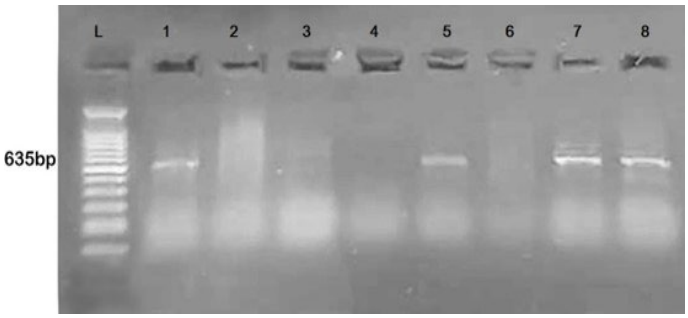

Figure 3. Amplification of *van*B at 635 bp in the vancomycin resistance *E. fecalis* strains. Lane L 100- 3000 bp DNA size marker, Lane 1,5,7,8 positive samples, Lane 2,3,4,6 negative samples.

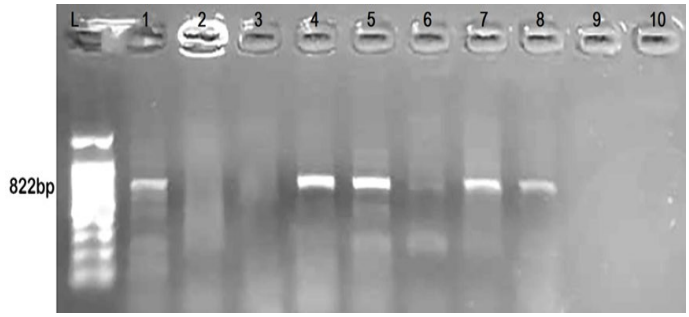

Figure 4. Amplification of *van*C1 at 822 bp in the vancomycin resistance *E. fecalis* strains. Lane L 100- 3000 bp DNA size marker, Lane 1,4,5,7, 8 positive samples, Lane 2,3,6,9,10 negative samples.

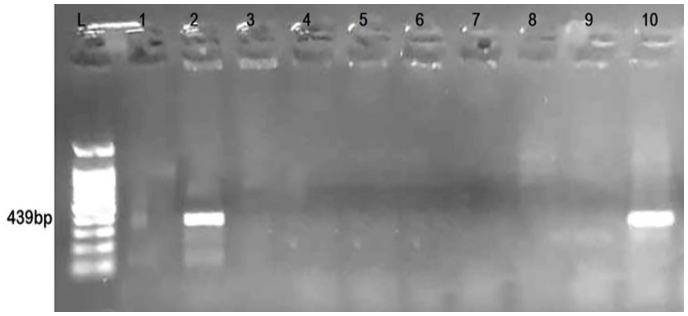

Figure 5. Amplification of *Van*C2 C3 at 439 bp in the vancomycin resistance *E. fecalis* strains. Lane L 100- 3000 bp DNA size marker, Lane 2, and 10 positive samples, Lane 1, 3-9 negative samples.

DISCUSSION

The ingestion of food that is contaminated with enterococci strains that are resistant to antibiotics is regarded as a potential mode of transmission of this pathogen from animals to humans

Figure 6. The Biofilm Production by Enterococci Isolates by Congo red Method.

(Gardini *et al*., 2001). Therefore, the present study performed to explore the occurrence rate of enterococci in retail poultry meat from various locations in Mansoura city and the antimicrobial susceptibility of the retrieved isolates were also assessed using antibiotics that are significant for both human and veterinary use.

As per this study findings, 36.66% of the poultry specimens exhibited positive results for *Enterococcus* spp. A study conducted by Pesavento *et al*. (2014) reported comparable outcomes, with 28.6% of chicken meat samples tested positive for *Enterococcus* spp. Studies conducted in Tunisia (Klibi *et al*., 2013) and Greece (Gousia *et al*., 2015) have reported lower prevalence rates of *Enterococcus* spp. contamination, with percentages of 24.5% and 21.7%, respectively. Conversely, higher prevalence rates have been documented in Brazil (Fracalanzza *et al*., 2007) and Tennessee (Kilonzo-Nthenge *et al*., 2015), with 56.8% and 82.2% of *Enterococcus* spp. contamination rate, respectively. In this study, the findings revealed that *E. fecalis* was the most commonly occurring species (100%). Previous studies have also reported *E. fecalis* as a preponderant species in chicken meat samples from various regions, including Brazil (Fracalanzza *et al*., 2007), Tunisia (Klibi *et al*., 2013), and USA (Tyson *et al*., 2018). Conversely, studies conducted in Scandinavia, Spain, and Italy (de Jong *et al*., 2009), as well as Greece (Gousia *et al*., 2015), have identified *E. faecium* as the preponderant species. Different types of predominance have been associated with either geographical variations or typing methodologies (Manero and Blanch, 1999; Jackson *et al*., 2004).

According to Fard *et al*. (2019), Enterococci exhibit a high degree of growth in extreme environmental factors, including but not limited to elevated temperatures and freezing. The extensive utilization of antibiotics has led to a rapid increase in antibiotic resistance in *Enterococcus* spp. over the past few decades, as reported by Fard *et al*. (2019). The transfer of antibiotic resistance among bacteria in the intestinal microflora is a common occurrence. The presence of enterococci in food or water is strictly prohibited due to their predominant occurrence in the intestinal tracts of humans and animals. Consequently, the detection of enterococci in food is indicative of fecal contamination (Fard *et al*., 2019).

Previous research has indicated that poultry isolates exhibited comparable antibiotic resistance profiles to our isolates with respect to a similar group of antibiotics, as demonstrated in studies conducted by Ke *et al*. (1999) and Jackson *et al*. (2004). The findings of this investigation indicated that the majority of isolates exhibited resistance to the evaluated antibiotics, with vancomycin (34/44, 77.2%) demonstrating the highest level of resistance, followed by erythromycin (30/44, 68.1%), penicillin (18/44, 40.9%), and ciprofloxacin (16/44, 36.3%). In a previous study conducted in the United States between 2002 and 2014, the enterococci isolated from meats exhibited a pattern of resistance to multiple drugs, including erythromycin, gentamicin, and tetracycline (Tyson *et al*., 2018). Furlaneto-Maia *et al*. (2014) reported a substantial prevalence of enterococcal resistance to erythromycin (86.7%) and vancomycin (80.0%) in Brazil, as per their study findings. The issue of bacterial antibiotic resistance has emerged as a pressing global health concern. Presently, a significant proportion of bacteria exhibit resistance to commonly prescribed antibiotics (Furlaneto-Maia *et al*., 2014).

The global dissemination of vancomycin resistance has been documented. Vancomycin is a glycopeptide antibiotic that exhibits activity against a wide range of Gram-positive bacteria, including enterococci (McNamara and Steckelberg, 2005). The phenomenon of glycopeptide resistance was initially reported in enterococci and is attributed to the presence of six *van*A-G genes (Leclercq *et al*., 1988). Enterococci can be transmitted to humans via the food chain, as well as through oral-fecal pathways. The transfer of antibiotic resistance genes to other microflora members and bacterial pathogens is facilitated by horizontal gene transfer in these bacteria (Leclercq *et al*., 1988). There is evidence of recurrent VRE prevalence in various societies (Giraffa, 2002). Furthermore, Fard *et al*. (2019) has reported elevated levels of vancomycin-resistant enterococci (VRE) in a range of food items, including meats, vegetables, fruits, salads, and cheeses, which were also included in our investigation. The current investigation revealed the presence of *van*A, *van*B, *van*C1, and *van*C2C3 genes in VRE isolates, which are accountable for the bacterial resistance to vancomycin. The *van*A and *vanc*1 genes are the most observed and dominant forms of vancomycin resistance, whereas *van*B and *van*C2C3 are less prevalent. In addition, it has been reported by various researchers (Gousia *et al*., 2015; Tripathi *et al*., 2016) that *van*A and *van*B are the most prevalent among vancomycin resistant genes. The present investigation revealed that the *van*A gene exhibited a dominant presence (47.1%) among the isolated vancomycin-resistant enterococci (VRE). The findings of this study were consistent with those of previous research conducted by Salem-Bekhit *et al*. (2012) and Daghighi *et al*. (2014) who reported the presence of the *van*A gene in 87.8% and 89.3% of VRE isolates, respectively. Furthermore, the findings of this study were comparable to those reported by Amberpet *et al*. (2016) wherein the *van*A gene was detected in all 83 VRE isolates, and Phukan *et al*. (2016) who reported the presence of the *van*A gene in 56.25% of VRE isolates.

The elevated occurrence of this gene may be attributed to the disproportionate utilization of vancomycin in the aforementioned regions (Amberpet *et al*., 2016; Phukan *et al*., 2016). Conversely, the *van*B gene was identified in our investigation, comprising 32.4% of the sample. The findings of the present study align with those of Karki *et al*. who reported the presence of *van*B gene in 17.5%8 of VRE isolates (Karki *et al*., 2012). This is consistent with the results reported by Alatrouny *et al*. (2016), in contrast to the findings of Daghighi *et al*. (2014) and Amberpet *et al*. (2016) who reported the absence of *van*B gene in all VRE isolates. However, none of the individuals mentioned possessed the name *van*A.

All the isolates that demonstrated resistance to vancomycin also displayed resistance to multiple drugs. Aforementioned observation was also documented by Hashem *et al*. (2015) whereby the vancomycin-resistant isolates exhibited resistance to multiple drugs. The current investigation revealed a high level of sensitivity for ciprofloxacin as a viable treatment alternative for VRE. The research demonstrates that the VRE isolates obtained from poultry in Mansoura city exhibit a considerable prevalence of antibiotic resistance. Given the significance of vancomycin-resistant enterococci (VRE) in the context of worldwide public health, it is imperative to ascertain the antibiotic resistance profiles of *Enterococcus* species isolates, as this is an essential component of ensuring food safety throughout the entire food production process.

CONCLUSION

This study revealed a high contamination rate of retail poultry meat with VRE which also displayed resistance to multiple drugs. Therefore, efficient control survey programs are recommended to determine the presence of enterococci in environmental sources, particularly in food, as a means of preventing the dissemination of pathogenic strains. If the presence of enterococci contamination from various origins can be confirmed and restricted, it may be possible to mitigate the increase in colonization and infection rates among hospitalized patients, as well as antibiotic resistance. It is recommended that long-term policies incorporate interna-

tional survey systems for the purpose of monitoring contamination in carcasses, foods, animals, and humans.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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