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Phenotypic and Genotypic Identification of Vancomycin Resistant *Enterococci* from Different Sources

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Abstract

Enterococci are reservoirs for transmission of the most clinically important antimicrobial resistances such as vancomycin resistance. Therefore, this work aimed to determine the occurrence of enterococci and their respective vancomycine resistance genes (*van*A and *van*B) from different sources. Two hundred and twenty-four samples from chickens, turkey, fish and human urine, as well as, two types of human food including milk (raw and milk from mastitic animals) and sausage were tested for isolation of *Enterococcus* species. The isolates were identified morphologically and biochemically using catalase test**,** sodium chloride tolerance and growth at pH 9.6 and 10- 45˚C. The vancomycin resistance profile of the isolates was verified by both disc diffusion and agar dilution methods. The genotypic enterococcal identification at both genus and species levels and their vancomycine resistance genes were also ascertained using PCR amplification of the respective genes for 28 isolates. Enterococci isolation rate was 70% of the examined samples with a higher percentage of vancomycine resistance (53.5%) and the minimum inhibitory concentrations (MICs) ranged from 16 to 512 µg/mL. Molecular identification of 28 enterococcal isolates revealed the dominance of *E. faecalis* (42.8%) and clarified a higher proportion of *van*A (78.5%) and *van*B (67.8%) genes. In conclusion, administration of the antimicrobials mainly vancomycin may be considered as a pronounced stress factor in the veterinary and human practices. In addition, VRE can act as a reservoir for vancomycin resistance.

Keywords: Antimicrobial susceptibility, Vancomycin resistance, Enterococci

Introduction

Enterococci are Gram- positive cocci that present in different sources such as soil, food, water and a wide variety of living animals because of their ability to grow and survive under harsh conditions. Their major habitat is the gastrointestinal tract (GIT) of humans and other animals where they make up a significant portion of the normal gut flora [1]. Some strains are also important opportunistic pathogens responsible for serious human diseases and nosocomial infections [2,3]. Enterococci in food might survive intestinal passage and the frequent isolation of antibiotic resistant Enterococci from fermented food products implies a risk for the transmission of resistance genes to the human gut microbiota [4]. Such transmission might result in an increase of the prevalence and lateral transfer of antibiotic resistance genes, thereby constituting an impairment of human health.

Unfortunately, bacteria became rapidly resistant to several classes of clinically relevant antibiotics, hampering effective treatment [5] However, the uncontrolled use of antibiotics in therapeutics and as growth promoters in animal husbandry has led to increasing the prevalence of antibiotic resistant bacteria worldwide [6,7]. Spontaneous mutations and the acquisition of antibiotic resistance genes by horizontal gene transfer (HGT) contribute further to the spread of antibiotic resistant bacteria [8]. Particularly in the hospital environment, the use of antibiotics leads to the selection of resistant organisms, resulting in difficulties in the treatment of nosocomial infections [3,9]. The most worrisome resistance trait to emerge in

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enterococci is the resistance to vancomycin since the first report of vancomycin resistant enterococci (VRE) in France which was shown to be plasmid mediated and transferable [10].

The most common phenotype of *van*A resistance is associated with acquired, inducible, high-level resistance to both vancomycin (MIC>32 mg/L) and teicoplanin (MIC>16 mg/L), and is carried on transposon (Tn1546) that is transferable to other susceptible enterococci by conjugation. Several acquired glycopeptide resistant phenotypes have been characterized since then, including vanB and less common vanD, vanE and vanG types. The vanB phenotype, which is chromosomally mediated, inducible and transferable by conjugation, facilitated inducible resistance to vancomycin but not to teicoplanin [11]. Vancomycin resistant enterococci in the presence of inducer like vancomycin generate precursors with different terminals (D-Ala-D-lac, or D-Ala-D-Ser), which have low affinity to vancomycin and thus can continue in large part to be used to synthesize cell wall (Ala denotes alanyl or alanine and lactate for VanA, VanB, and VanD types of resistance and serine for VanE and VanC types) [12, 13]. This shift results in a reduced affinity for vancomycin by 1000 and seven times respectively [14]. Enterococci are traditionally treated with a combination of cell wall active antimicrobials such as β-lactams or glycopeptides (e.g. ampicillin or vancomycin respectively), and aminoglycosides (e.g. gentamicin and streptomycin). However, the increased rates of β-lactam and glycopeptide resistance in *E. faecium* and aminoglycoside resistance in both *E. faecium* and *E. faecalis* have called for the use of other and perhaps less efficient drugs [15]. In conclusion, enterococci may be implicated in the transfer of vancomycine resistance to different veterinary and human pathogens mainly *S. aureus* that represent great hazards in treatment failure.

This work aimed to determine the occurrence of *Enterococcus* species in different sources and their antimicrobial susceptibilities, as well as the genotypic identification of their vancomycin resistant genes. This may be used for further examination of their conjugative transfer abilities within the same and different genus.

Material and Methods

Isolation and identification of enterococci

Two hundred and twenty-four samples from different sources (chickens, turkey, fish, humans and food products) were collected. Crop and intestinal content of chickens and turkey as well as fish intestinal content samples were collected and prepared as previously described [16]. While the samples from liver, ovary, meat or muscles of both chickens and turkey were prepared according to Peter *et al.* [17]. Human urine samples from outpatient clinic, Zagazig University Hospitals were collected and prepared as previously mentioned [18]. Finally, raw and mastitis cattle milk samples were collected under complete aseptic conditions from Sharkia Governorate, Egypt as recommended by National mastitis council (NMC) [19].

A loopfull of the prepared samples was plated on the surface of the selective media of Slanetz and Bartley agar medium as well as bile Esculin Agar (BEA) medium and incubated at 37˚C for 24 h [20]. Presumptive colonies were morphologically identified by Gram stain and biochemically examined by catalase and sodium chloride tolerance tests [20,21]. Their growth at pH 9.6 and 10-45˚C was also determined [22,23].

Antimicrobial susceptibility testing

All the Enterococcal isolates were tested for their susceptibilities to different antimicrobial agents including ampicillin (10 µg), cefotaxime (30 µg), erythromycin (15 µg), doxycyclin (30 µg), nitrofurnation (300 µg), fusdic acid (10 µg), gentamicin (10 and 120 µg), rifampin (15 µg),) and vancomycin (30 µg) (Oxoid, Hampshire, England, UK) using disc diffusion method [24]. Zone size interpretation of antimicrobial agents was according to Clinical Laboratory Standards Institute (CLSI) M100-S24 [25]. Moreover, MIC of vancomycin against the enterococcal isolates was carried out by vancomycin agar dilution susceptibility test [26], using bile esculine azid medium supplemented with different concentrations (1024, 512, 256, 128, 64, 32 and 8 µg/mL) of vancomycin.

Table 1: Oligonucleotide primer sequences used in identification of enterococci, virulence and vancomycin resistance genes by PCR assays

Specificity	Target	Primer sequences (5'-3')	PCR product size (bp)
Enterococcus Genus	16Sr RNA	**F** ATCAGAGGGGGATAACACTT **R** ACTCTCATCCTTGTTCTTCTC	337
E. faecalis	16Sr RNA	**F** ATCAAGTACAGTTAGTCTTTATTAG **R** ACGATTCAAAGCTAACTGAATCAGT	941
E. faecium	16Sr RNA	**F** TTGAGGCAGACCAGATTGACG **R** TATGACAGCGACTCCGATTCC	658
E. casseliflavus	16Sr RNA	**F** TCCTGAATTAGGTGAAAAAAC **R** GCTAGTTTACCGTCTTTAACG	288
E. gallinarum	16Sr RNA	**F** TTACTTGCTGATTTTGATTCG **R** TGAATTCTTCTTTGAAATCAG	173
Vancomycin Resistant genes	vanA	**F** CATGAATAGAATAAAAGTTGCAATA **R** CCCCTTTAACGCTAATACGATCAA	1,030
	vanB	**F** GTGACAAACCGGAGGCGAGGA **R** CCGCCATCCTCCTGCAAAAAA	433

Molecular identification

Polymerase chain reactions were done to confirm the conventional methods of isolation and identification of genus *Enterococcus* and to detect the Enterococcal species as well as vancomycin resistance associated genes (*van*A and *van*B) among the obtained VRE isolates using seven pairs of primer sets (Table 1). Extraction of DNA from the isolates was performed by QIAamp DNA mini Kit following the manufacturers' instructions. Cycling conditions of the primer sequences during PCR were at primary denaturation of 94°C/10min and for 35 cycles at (secondary denaturation of 94°C/45 sec, annealing of 50°C/45sec and extension of 72°C/45sec) then final extension at 72°C/7min for genes of; 16S rRNA of genus Enterococcus [27]; 16S rRNA of *E. faecium* and *E. faecalis*; finally *van*A and *van*B [28] while for 30 cycles at (95°C/30 sec, 55°C/1 min and 72°C/1 min) after primary denaturation of 95°C/4 min and then final extension at 72°C/7 min for 16srRNA gene of *E. gallinarum* and *E. casseliflavus* [29] using Emerald Amp GT PCR Mastermix (Takara) kit.

Results

Isolation and identification of enterococci

Enterococci isolates were recovered with an isolation rate of 70%. All the isolates were identified by conventional methods. All isolates yielded pink colonies on Slantez Bartley medium, with a narrow whitish border, while on BEA medium, they showed black colored colonies. Enterococci appeared as Gram positive, none spore forming, non-capsulated, diplococci or short chains, being somewhat elongated, catalase negative, tolerated 6.5% NaCl in brain heart infusion broth, grew at 9.6 pH and variable degrees of temperature (10 – 45˚C). The distribution of 157 enterococci isolates were 14 (100%) from turkey, 85 (78.7%) from chickens, 27 (62.7%) from different types of food products, 14 (56 %) from fish and 17 (50 %) from human urine.

Antimicrobial susceptibility testing of bacterial isolates

Enterococcal isolates revealed the highest susceptibility against vancomycin (84.7%) followed by gentamicin 120 (77%), ampicillin (73.8%), cefotaxime (63%), nitrofurnation (62.4%), doxycycline (35%) and ciprofloxacin (29.9%). On the other hand, the resistance percentages of the isolates to rifampin was 85.3%, followed by erythromycin (80.2%), cefotaxime (77%), doxycycline (45.8%), gentamicin 10 (42%) and ciprofloxacin (37.5%). Multidrug resistance (MDR) was defined as resistance of bacterial isolates to ≥ 3 antimicrobial agents and was recorded as 90.4% in 142 isolates Table (2).

Table 2: Antimicrobial susceptibility profile of Enterococcal isolates against 10 antimicrobial agents

Antibiotics	Broilers			Turkey			Milk			Sausage			Fish			Urine			Total		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
VA	70	9	6	9	5	0	17	1	0	9	0	0	11	3	0	17	0	0	133	18	6
CN10	22	32	31	2	3	9	10	3	5	4	3	2	0	3	11	6	3	8	44	47	66
CN120	57	13	15	10	2	2	18	0	0	9	0	0	12	-	2	15	2	0	121	17	19
AM	67	0	18	12	0	2	5	1	12	9	0	0	14	0	0	9	0	8	116	1	40
E	0	8	77	0	0	14	0	8	10	3	0	6	-	3	11	0	9	8	3	29	126
CIP	26	31	28	3	6	5	8	1	9	3	6	0	2	3	9	9	6	2	47	61	59
F	51	21	13	5	8	1	11	6	1	8	0	1	6	3	5	17	0	0	98	38	21
DO	27	20	38	3	5	6	8	1	9	9	0	0	5	2	7	3	2	12	55	30	72
CEF	1	29	55	0	0	14	0	3	15	0	3	6	0	0	14	0	0	17	1	35	121
RA	5	8	72	0	2	12	0	5	13	3	0	6	0	0	14	0	0	17	8	15	134

S: sensitive, I: intermediate, R: resistant, VA: Vancomycin, CN120: Gentamicin120, CN10: Gentamicin, AM: Ampicillin, CEF: Cefotaxime , F: Fitrofurnation, DO: Doxycycline, CIP: Ciprofloxacin, , RA: Rifampin, E: Erythromycin.

Moreover, vancomycin agar dilution test explored VRE as 84/157 (53.5%) that produced a black complex even in the presence of vancomycin concentrations such as 8, 16, 32, 64, 128, 256 and 512 µg/mL, where 59 isolates of them showed intermediate resistance to VA at MIC 8 µg/mL [9 isolates from chickens (6) and milk (3)]; 50 isolates [from chickens (21), turkey (3), milk (9), fish (3) and human samples (14)] at MIC 16 µg/mL and 23 isolates at MIC 32 µg/mL comprising 21 isolates from chickens and 2 from turkey. Moreover, 2 isolates from chickens expressed high level of resistance at MIC 512 µg/mL. Finally, 73 isolates showed no growth at all vancomycin concentrations.

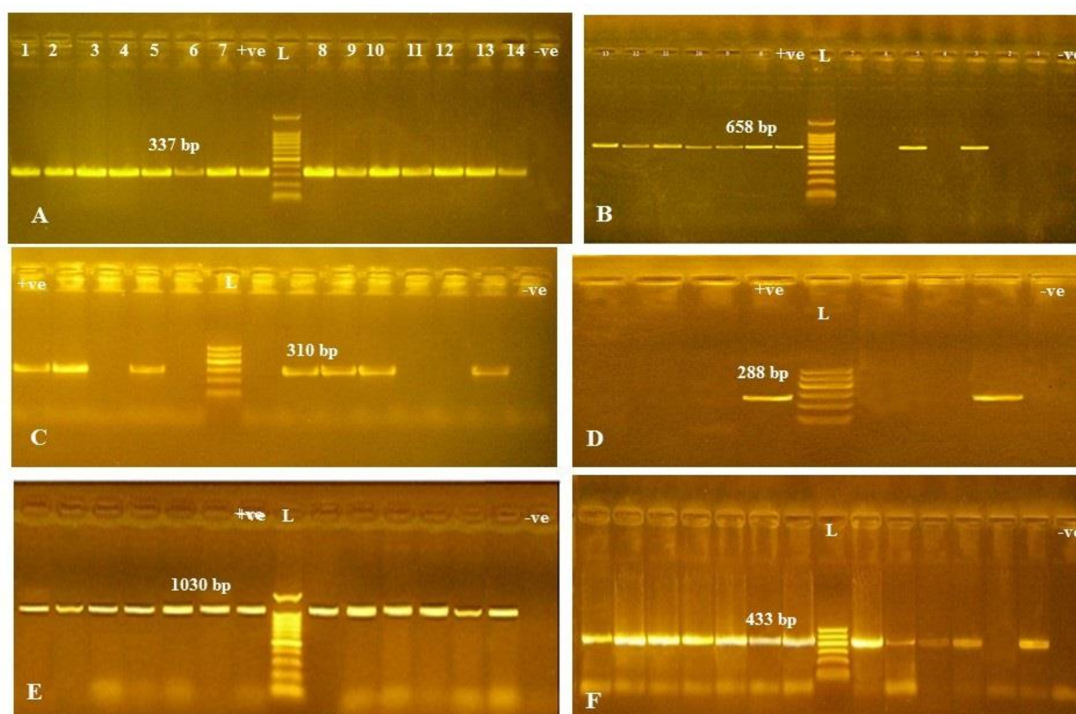

Figure 1: Agarose gel electrophoresis of PCR products for Enterococci identification; L: 100 bp Ladder, +ve: Positive control, -ve: Negative control, Positive samples show amplicons of specific size (A: 16s rRNA gene specific to genus *Enterococci* producing 337 bp, B: *E. faecium*-specific 658 bp DNA fragment, C: *E. faecalis*-specific 310 bp DNA fragment, D: *E. casseliflavus* specific 288 bp DNA fragment, E: Amplified 1030 bp products of *van*A gene, F: Amplified 433 bp products of *van*B gene).

Molecular identification

Twenty-eight representative isolates were confirmed as enterococci by the amplification of 16s rRNA gene with an amplicon size of 337 bp of (Figure 1 and Table 3). Three different *Enterococcus* spp. of various origins were identified. The most prevalent one was *E. faecalis* (42.8%) which was more frequent in chicken samples, followed by 11 *E. faecium* (39.2%). Finally, only 3.5% of the examined isolates were classified as *E. casseliflavus*. On the other side, *E. gallinarum* was not detected at all and the remaining four isolates were not detected at species level (Figure 1 and Table 3).

Vancomycin resistance associated genes (*van*A and *van*B) of 28 Enterococcal isolates were detected with a high percentage. Out of the examined isoates, 22 and 19 harbored *van*A (78.5%) and *van*B (67.8%) genes, respectively (Figure 1 and Table 3). The mentioned data confirm the high Enterococcal distribution and high risk of antibiotic resistance (MRD was 85.7%) among most of the isolation sources (Table 3).

Table (3): Resistance profile and genotypic characterization of the recovered 28 Enterococcal isolates from different sources

NO	Code	Genotypic Identification			Resistance profile	M DR	VA MIC µg/mL
		Species	vanA	vanB			
1	mm3	E.fl	+	+	AM, F, CIP, CN10, RA, E.	+	16
2	mm11	E.cassl.	+	-	AM, CEF, CIP, E.	+	16
3	mm12	E.fm.	+	+	CEF,DO, RA,	+	16
4	mm19	Other E.spp	+	+	CEF, RA,	-	16
5	ci1	E.fm	+	+	CEF, DO, RA	+	16
6	hu2	E.fl	+	+	AM, CEF, F, DO, CIP, CN10, E.	+	16
7	hu15	E.fl	+	+	CEF,DO, CN10, RA, E.	+	16
8	hu43	E.fm	+	+	CEF, CN10, RA,	+	16
9	hu54	E.fm	+	+	CEF, E.	-	16
10	hu59	E.fm	+	+	CEF, DO, CN10, RA, E.	+	16
11	ccr2-hg-	E.fm	+	+	VA, CN120, CEF, F, CIP, CN10, RA, E.	+	512
12	cr11	E.fm	+	-	CN120, CEF, F, CIP, CN10, RA, E.	+	32
13	ci23	E.fm	+	+	CN120, AM, CEF, F, CN10, RA, E.	+	32
14	hu16	E.fl	+	+	CEF, DO, CN10, RA, E.	+	16
15	mm26	Other E.spp	+	+	CEF, DO, CN10, RA, E.	+	16
16	ccr21	E.fm	+	+	VA, CEF, CN10, RA,	+	512
17	ccr10	Other E.spp	+	+	CEF, F, DO, CIP, CN10, RA, E.	+	32
18	cl1	E.fl	+	+	VA, CN10, E, F and CEF, RA.	+	32
19	ccr6	Other E.spp	+	+	CEF,DO, CIP, CN10, RA, E.	+	32
20	ccr12	E.fm	+	+	VA, CEF, DO, CIP, CN10, RA, E.	+	32
21	ccr9	E.fm	+	+	CEF, F, DO, CIP, CN10, RA, E.	+	32
22	cm6	E.fl	+	-	CN120, CEF, F, DO, CN10, RA, E.	+	32
23	ci25	E.fl	-	-	CEF, RA	-	-
24	ci27	E.fl	-	-	CEF, E.	-	-
25	ci31	E.fl	-	-	CEF, RA, E.	+	-
26	fi16	E.fl	-	-	CN10, DO , CEF and RA	+	-
27	fi38	E.fl	-	-	CN10, E, DO, CEF and RA	+	-
28	co1	E.fl	-	-	CEF, CN10, RA, E.	+	-

VA: Vancomycin, CN120: Gentamicin120, CN10: Gentamicin 10, AM: Ampicillin, CEF: Cefotaxime, F: Nitrofurnation, DO: Doxycycline, CIP: Ciprofloxacin, RA: Rifampin, E: Erythromycin. *E.fl: Enterococcus faecalis. E.fm: Enterococcus faecaium. E.cassl.: Enterococcus casseliflavus*. Other *E.*spp*.: Enterococcus* spp other than *E.fl, E.fm, E.cassl*. and E. *gallinarum.* mm: mastitis milk, ccr: chicken crop content, ci: chicken intestinal content, cl: chicken liver, co: chicken ovary, cm: chicken meat, hu: human urine, fi: fish intestinal content.

Discussion

The obtained results revealed that enterococci were widely distributed among the samples of human and food product origin (broiler, turkey, milk, meat and fish) with a high recovery rate (70%). Notably, *E. faecalis* was the predominant species recovered (42.8%), followed by *E. faecium* (39.2%), E. casseliflavus (3.5%) and only 4 isolates were not identified to species level (14.2%). Similar results of *E. faecalis* predominance but with higher percentage (49%) were reported [30]. In addition, Gangurde *et al.* [31] in South West part of Slovakia isolated *E. faecalis* (60%) in followed by *E. faecium* 32.2%.

Furthermore, Trivedi *et al.* [32] identified five different species of Enterococci including *E. faecalis* followed by *E. faecium* from foodstuffs of different. On contrary, *E. faecium* was the predominant *Enterococcus* species with the percentages of 98.4 % [33], 61% [34] and 42.9% [35]. Also, Joshua *et al.* [34] identified *E. faecalis* (29%), *E. hirae* (5.7%), *E. casseliflavus* (2.1%), *E. durans* (1.2%), *E. gallinarum* (0.7%), and *E. avium* (0.1%), in meat and only 13 isolates were not identified to species level. Herewith, the results revealed that Enterococci percentage in both meat (sausage) and milk sample are high and slightly close to each other as 64.3% and 62%, respectively. On the contrary, Krocko *et al.* [30] revealed lower levels of contamination in meat compared to milk or cheese.

Milk from different mammalian species may contain Enterococci and, therefore, may constitute a natural source of such microorganisms for consumers. In the present study, milk samples from mastitis cow were investigated for the presence of enterococci and the identified species were *E. faecalis*, *E. faecium* (the common species), *E. casseliflavus* and only 2 isolates were not identified to the species level. Similarly, Trivedil *et al.* [32] reported that *E. faecalis* and *E. faecium* were the major species identified in dairy samples, followed by *E. casseliflavus*.

In the current study, all the enterococci isolates were tested for their susceptibility to different antimicrobial agents from several groups by disc diffusion method. The highest susceptibility obtained was to vancomycin (84.7%) followed by gentamicin$_{120}$ (77%), ampicillin (73.8%), cefotaxime (63%), nitrofurnation (62.4%), doxycycline (35%), ciprofloxacin (29.9%), gentamicin 10 (28%), rifampin (5%) and finally erythromycin (1.9%). On the other hand, the resistance percentage of isolates to rifampin was (85.3%) followed by erythromycin (80.2%). Resistance to rifampin seems to be widely spread among Enterococci and was the highest percentage between the tested antimicrobials (85.3%). This was similar to the results of Sarra *et al.* [36], while, Kročko *et al.* [30] reported that tetracycline and gentamicin resistance was the most common. Sarra *et al.* [36] found that, none of the tested isolates demonstrated resistance to ampicillin, vancomycin and gentamicin. The different data obtained previously revealed that none of the strains was resistant to vancomycin [32,35]. While, Trivedi *et al.* [32] investigated the microbial susceptibility of eight antibiotics using the disk diffusion method and indicated lower antibiotic resistance for ampicillin and gentamicin against 250 Enterococci isolated from various food-stuffs.

Most notably in this study, that the resistance of *E. faecalis* (1/12 and 2/12) and E. faecium (2/11 and 1/11) was low against vancomycin and ampicillin, respectively. The same result was obtained by Sood *et al.* [35] who stated that *E. faecalis* resistance is low against vancomycin and ampicillin but with higher levels of ampicillin resistance among *E. faecium* isolates. Erythromycin resistance in this work was 80.2%, the same as Sood *et al.* [35] study who proved that erythromycin resistance was quite high. Routine susceptibility test based on disc diffusion method was unreliable for the detection of vancomycin resistance upon primary isolation. However, the basic method in this study for detecting VRE is the incorporation of vancomycin into the esculin containing base medium (Vancomycin agar dilution test), which provides a presumptive identification at the genus level because all Enterococci hydrolyze esculin. This finding was consistent with previously reported studies [18,37].

The high percentage of VRE was detected in this study by Vancomycin agar dilution test

which revealed that 53.5% (84/157) of the isolates grew on BEA medium with variable concentrations of vancomycin, of which, 59 isolates showed intermediate resistance to VA at MIC 8-16 µg/mL; 23 isolates at MIC 32 µg/mL. Moreover, 2 isolates expressed high level of resistance at MIC 512 µg/mL and all sausage isolates were VSE. On contrary, low rate of VRE was proved as 8.4% (MIC ≥32 mg/ mL) [33] 7.31% [38] and 7% (MIC of >512µg/mL) [18]. In another study, 15 isolates were found to be VA resistant, of which 4 had MIC between 8-16 µg/mL [31].

Enterococcal antimicrobial resistance is not exclusive to the clinical arena but is also prevalent in the food industry [14]. The absence of VRE from sausage in this work was similar to of the findings of Hayes *et al.* [34] in domestic retail meats. In this study, MRD (to ≥ 3 antimicrobial) of Enterococcal isolates by disc diffusion method was considerably high as 90.4% (142/157) and was predominant in chicken isolates followed by turkey and milk, urine and fish and finally meat isolates expressing 48%, 10.5%, 10.5%, 8.6%, 8.6% and 3.8%, respectively. While, by vancomycin agar dilution test, 24 (85.7%) of 28 genotypically identified Enterococci had MDR as 10 isolates of both *E. faecium* (90%) and *E. faecalis* (83.3%). As well as 22 (78.6%) of them were VRE {11(50%) were *E. faecium*, 6 (27.3%) were *E. faecalis* and others 5 isolates were of different spp.}. Similar results were previously reported [39,40]. *E. faecium* was the predominant genotype in vancomycin resistant isolates but with a higher percentage of 83.5% [39]. Lower percentage of MDR was identified in pork and chicken samples [30].

Another study revealed that 61% of *E. faecium* and 11% of *E. faecalis* isolates showed MDR to 17 different antibiotics including vancomycin [41]. It is worth noted that identification of vancomycin resistance via detection of both *van*A and *van*B genes by PCR in the 28 genotypically identified Enterococci isolates, proved that 22 were VRE isolates in which *van*A gene was detected in 100% (22/22) while *van*B gene present in 86.3 %(19/22) of VRE isolates. Likewise, *van*A was reported in 100% [38] and 96.5% [39] of the examined VRE isolates in previous studies.

Conclusion

In conclusion, the obtained results indicated the spread of VRE isolates which in turn highlight the urgent need to limit the uncontrolled use of antibiotics in veterinary medicine and food animals to avoid drug resistance mainly VR and consequently treatment failure.

Conflict of interest

The authors have no conflict of interest to declare.

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الملخص العربي

التعريف الظاهري والجيني لميكروب الانتيروكوكاي المقاوم للفانكومايسين في المصادر المختلفة

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تعتبر المكورات المعوية الانتيروكوكاى مصدر لنقل المقاومة ضد أهم مضادات الميكروبات المستخدمه فى المجال الاكلينيكى مثل الفانكوميسين . لذلك قد خصصت هذه الدراسه بهدف تحديد مدى انتشار أنواع من هذه المكورات المعوية المقاومة للفانكوميسين و المعزولة من مصادرمختلفة عن طريق التحديد الوراثي لها وكذلك لجينات مقاومه الفانكوميسين الخاصه بها *van*A and *van*B.) (لذلك تم تجميع ٢٢٤ عينة لعزل الانتيروكوكاي من الدجاج والرومي الاسماك وبول الانسان وكذلك نوعين من الاطعمه مثل اللبن الخام والمصاب بالتهاب الضرع والسجق والتعرف عليها مظهريا عن طريق اختبار تواجد انزيم الكتاليز و مقدرتها على تحمل تركيز عالى من ملح الطعام وكذالك النمو في وسط عالى القلويه يحتوي على الاس الهيدروجيني ٩,٦ وفي درجات حرارة مختلفة من ١٠-٤٥ درجة مئوية وايضا تم تأكيد نمط مقاومة المعزولات للمضادات البكتيرية المختلفة بطريقتي الانتشار عبر القرص و باستخدام تخفيفات مختلفة من الفانكوميسين في الاجار. كما تم اجراء التعريف الجيني لـ ٢٨ عزله من الانتيروكوكاى للكشف عن جنس الانتيروكوكاي وتحديد الصفات الوراثية للانواع المختلفة منها جينيا وكذلك تحديد جينات المقاومة للفانكوميسين بها عن طريق تقنية تفاعل انزيم البلمرة المتسلسل. وقد اسفرت النتائج في هذا البحث عن تصنيف ٧٠% من المعزولات مظهريا كميكروب الانتيروكوكاي بنسبة مقاومة عالية للفانكوميسين (٥٣,٣%). حيث تراوح التركيز الأدني لمنع نمو البكتريا (MIC) من ١٦ الي ٥١٢ ميكروجرام لكل ملي. ولقد اوضح التحديد الجزيئي لـ ٢٨ عزلة من عزلات ميكروب الانتيروكوكاي ان *E. faecalis* هي السائدة بنسبة (٤٢,٨%) ، كما اوضح ايضا ارتفاع نسبة تواجد جينات المقاومة للفانكوميسين vanA (٧٨,٥%) و vanB (٦٧,٨٪) فى هذه العزلات. وعليه تم الإستنتاج الي أن تناول المضادات الميكروبية خصوصا الفانكوميسين يمكن أن يمثل عامل مؤثر سلباً نتيجة الممارسات البيطرية والبشرية. كما تعتبر المكورات المعوية المقاومة للفانكومايسين حاملا لمقاومة الفانكومايسين.