

Isolation and identification of *Enterococcus faecium* from Meat products with special reference to some virulence and antibiotics resistance factors

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Abstract

Enterococcus faecium is one of the most superbug pathogen which cause many diseases in human which include wound infection and endocarditis. also, this microorganism show high resistant to common antibiotics which used in hospital especially Vancomycin so the purpose of this study is detection *E.Faecium* from meat products which can be achieved by isolation and identification of *E.faecium* and detection some virulence gene and antibiotic resistance gene by PCR. Biochemical and molecular studies were performed on seventeen unidentified gram-stain positive, catalase and oxidase negative, Enterococcus-like organisms recovered from hundred meat products samples. The result of Vitek-2 compact system identified the suspected isolates as *E. faecium*. Antibiotic susceptibility testing of the isolates indicated resistance to most antibiotics, including penicillin, ampicillin, amoxicillin and vancomycin. The molecular analysis indicated the presence of virulence genes such as *hyl* (30%), *ace* (30%) and *cylA* (90%), and antimicrobial resistance genes as followed *vanA* gene (50%) and *blaZ* gene (90%). In conclusion meat products considered a good source of infection by vancomycin resistant Enterococcus *Faecium* (VRE) which have potential risk on human being so hygienic measurement are required during meat processing.

Keywords:

Antimicrobial resistance genes, *Enterococcus faecium*, Meat products, Virulence genes.

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Introduction

Meat products, including sausages, burgers and kofta are the most common shortfall nutrients in the world and consider an alternative source of protein with all essential amino acids, iron, zinc, selenium, and B vitamins, in the diet. Using of meat products have increased due to modernization and changes in people's daily life and lifestyle (Kheyri et al., 2014; and Klurfeld, 2015) Meat products may be easily infected by different microorganisms, due to unsuitable conditions of transportation and storage, leading to the growth of spoilage and pathogenic microbes, reducing the meat quality and harm the public health (Gomes et al., 2008).

Enterococcus species normally found as commensal bacteria in the digestive tract of man, Animals and birds also are found in the environment in water, soil and food of animal origin, When the commensal relationship with the host is interrupted, enterococci, may become opportunistic pathogens and cause invasive diseases (Jettet al., 1994; and Aseel and Hind, 2018). The genus *Enterococcus* included more than 20 species. The most common species were, *Enterococcus faecalis* and *Enterococcus faecium* which are among the leading causes of several human infections, including bacteremia, septicemia, endocarditis, urinary tract infections, wound infections, neonatal sepsis and meningitis (Giraffa, 2003; and Hossein and Mohammad, 2014).

E. faecium is associated with several virulence factors including cytolysin encoding by (*cyl* gene) is one of the best characterized enterococci virulence factors. It is a bacteriocin-type exotoxin, which shows bactericidal properties towards Gram-negative bacteria and toxic properties (B hemolysis) towards erythrocytes, leukocytes and macrophages, Hyaluronidase (*hyl*) This enzyme plays a role in abolishing mucopolysaccharides of the connective tissue and cartilage and,

subsequently, in spreading bacteria and facilitate conjugative transfer of plasmids between cells. Also other virulence factors as Gelatinase (*GeIE*) this enzyme is capable of hydrolyzing gelatin and Accessory colonization factors (*ace*) plays an important role in colonization by binding to proteins of the extracellular matrix, it also shares in binding type I and IV collagen (Wioleta et al., 2016).

Enterococci are intrinsically more resistant than many bacteria to antimicrobial agents commonly used in hospitals as B-lactams, tetracycline, erythromycin, in *E. faecium* the main cause for the resistance to b-lactams is a mutations in the high-molecular weight class B penicillin binding protein 5 (PBP5) cause further reduced susceptibility to ampicillin (Fontana et al., 1994).

The extensively use of vancomycin in hospitals likely contributed to the emergence and dramatic increase of vancomycin resistant enterococci (VRE) over the past 20 years (Gowan et al., 2006). Enterococcal resistance to vancomycin is of critical apprehension due to their importance in the treatment of multi-drug resistant strains. The mechanism of enterococcal resistance to vancomycin includes intervention with cell wall synthesis by interacting with D-alanyl-D-alanine group of the peptidoglycan chains (Walsh, 2000). The presence of *vanA* gene in isolates show a high degree of resistance to vancomycin and is mainly associated *E. faecium* (Hegstad et al., 2010).

Different method used for identification of *Enterococcus* spp. as automated systems VITEK II analyses which proven to be an accurate technique to differentiate *E. faecium* (Marwa et al., 2016), In the last few years, to obtain more rapid identification, molecular methods such as polymerase chain reaction (PCR) has been used for the identification of *Enterococcus* species (Fang et al., 2012).

The aim of the present study was isolation and identification of *E. faecium* from meat products by using VITEK2 system, also detection of virulence genes (*cyl A*, *hyl* and *ace*) and antimicrobial resistance genes (*vanA* and *BlaZ*) by PCR.

Materials and methods

1. Samples collection:

A total of 100 random meat products samples (kofta, hamburger) were collected aseptically from different retail supermarkets in Sohag city during the period between March to September 2019, all samples are collected in sterile bags and brought to the laboratory in an insulated icebox and were examined directly.

2. Preparation of samples:

Twenty-five grams from each collected meat products sample was inoculated in 225 ml Tryptic Soya broth and incubated at 37°C for 24 h (Benson et al., 2016)

3. Isolation and identification of *Enterococcus* spp.

A loopful from overnight broth was streaked onto the Bile Esculin Azide agar (oxiod) and incubated at 37°C for 24 h (Benson et al., 2016). Morphological examination of the suspected colonies was done by using Gram staining and biochemical identification of isolates on the base of catalase activity, oxidase test, carbohydrate fermentation (mannitol and sucrose) and voges-proskauer reaction (Albert and Anicetr, 1999).

4. Identification of *Enterococcus* species by using VITEK II system:

A few isolated colonies (5–10) from fresh overnight pure culture were resuspended in 3.0 mL of sterile saline

solution. The turbidity was adjusted to 0.5 McFarland standard.

Identification cards (bioMérieux, France) were inoculated with the suspended microorganism by an integrated vacuum apparatus, and test tubes containing the bacterial suspension were placed into a special rack while the identification cards were positioned in the next slot. Cards were sealed and inserted into the VITEK 2 reader-incubator, and then subjected to fluorescence measurement every 15 min. Results were obtained automatically and interpreted by the computerized database (Yomna et al., 2017).

5. Antibiotic susceptibility testing for *E. faecium* isolates

The susceptibility of isolates was tested against ten different antimicrobials such as: penicillin (P, 10 units), ampicillin (AM, 10 µg), amoxicillin (OX, 20 mg), erythromycin (E, 15 µg), tetracycline (TE, 30 µg), vancomycin (VA, 30 µg), chloramphenicol (C, 30 µg), ciprofloxacin (CIP, 5 µg), streptomycin (ST, 300 µg) and gentamycin (CN, 120 µg). Using Kirby-bauer disk diffusion method. Briefly antibiotic susceptibility testing a loopful of cultured isolates was inoculated into 2 ml TSB and incubated at 37°C for 4 hours, the concentrates of actively growing cultures was then adjusted to a .5 McFarland standard, then a sterile cotton swab was dipped it in the suspension. Press it against the inside of the suspension tube to remove excess liquid. Then, apply it to an agar plate. The swab should be swabbed across the plate, moving from side to side all the way from the top to the bottom of the plate before rotating the plate. The plate should be swabbed in three directions across the plate. The antibiotic discs were gently applied to ensure their contact with the inoculated TSA surface, and incubated at 37°C. The plates were observed after 18-

24h and the zones of inhibition were measured by antibiotic susceptibility scale. The zone diameter for different antimicrobial agents was explained into susceptible, intermediate and resistant categories as in the interpretation table according to (CLSI, 2018).

6. Detection of virulence and antimicrobial resistance genes in *E. faecium* by PCR:

6.1. DNA extraction

The refreshment of suspected isolates was done in Brain heart infusion broth (oxoid, CM1135B) at 35 °C for 24h. DNA was extracted from 5 ml of incubated broth for each isolate by QIAamp DNA Mini kit (Qiagen, Germany, GmbH) (Catalogue no.51304) according to the manufacture instructions.

6.2. PCR amplification reaction

Different primers coding for virulence genes (*hyl*, *cylA* and *ace*) and antimicrobial resistance genes (*vanA*, and *blaZ*) were used in this work (Table 1). The amplification of DNA was performed in a final volume of 25µl, composed of 6µl DNA, 12.5 µl Master mix (Emerald Amp GT PCR), 1µl for each primer and 4.5 µl of PCR grade water. The PCR reaction was done in thermal cycler (MJ Research, Inc. Watertown, MA) with the following program: initial denaturation at 94°C for 5 min followed by 35 cycles of 94°C for 30sec, 55°C for 30sec and 72°C for 30sec with a final extension at 72°C for 10 min. The amplicons size (bp) were detected by electrophoresis on 1.5% agarose gel (BioshopR, Candaine) stained with ethidium bromide, and visualized by a UV transilluminator.

Table 1: Nucleotide sequences of primers and amplicons size (bp)

Gene	Primer Sequence 5'-3'	Amplified product	Reference
<i>hyl</i>	ACAGAAGAGCTGCAGGAAATG	276 bp	Vankerckhoven et al., 2004
	GACTGACGTCCAAGTTTCCAA		
<i>cylA</i>	ACTCGGGGATTGATAGGC	688 bp	
	GCTGCTAAAGCTGCGCTT		
<i>ace</i>	GGAATGACCGAGAACGATGGC	616bp	Creti et al., 2004
	GCTTGATGTTGGCCTGCTTCCG		
<i>vanA</i>	CATGACGTATCGGTAAAATC	885 bp	Patel et al., 1997
	ACCGGGCAGRGTATTGAC		
<i>blaZ</i>	ACTTCAACACCTGCTGCTTTC	173 bp	Duran et al., 2012
	TGACCACTTTTATCAGCAACC		

Results

The results of bacteriological examination of 100 of samples (50 from each kofta and burger) revealed that 17 sample (17%) showed a tiny black colony were grown on bile esculin azide agar. this is atypical sign that these samples may be

positive for enterococcus spp. The biochemical scheme used for identifying the suspected colonies revealed positive result for Mannitol, Sucrose, Voges Proskauer and negative result for catalase activity test and oxidase test.

The highest incidence of contaminated samples was recorded in kofta 11/50 (22 %)

followed by burger 6/50 (12 %), as shown in Table 2. The phenotypic identification didn't overcome on the problem of the similarity in characters between these isolates. Therefore, it has been resorted to using VITEK II system, the result of Vitek II system cleared that 17 isolates identified as *E. faecium* with percentage 100%.

The results of antimicrobial susceptibility test cleared that most of isolates (94.1%) were resist for each penicillin, ampicillin followed by amoxicillin (88.2%) and vancomycin (58.8%). Some isolates showed intermediate resistance to erythromycin (11.8%) followed by tetracycline, gentamycin and amoxicillin (9.5% for

each). On the other hand, all the isolates were sensitive to streptomycin 100% followed by gentamycin (88.2%) and chloramphenicol (64.7%) as illustrated in Table 3. Furthermore the antibiogram cleared that our isolates revealed resistance to five patterns of antimicrobials. The most resistant pattern included penicillin amoxicillin, ampicillin and vancomycin (35.3%), followed by the pattern included penicillin, amoxicillin, ampicillin, tetracycline and chloramphenicol (29.4%), this is means that most *E. faecium* were multidrug resistance isolates (resist for more than three family of antimicrobials) and this is a problem that must be taken in consideration.

Table 2: Incidence of *E. faecium* isolated from examined samples:

Type of samples	No. of the samples	No. of positive samples for <i>E. faecium</i>	
		No.	%
Kofta samples	50	11	22%
Burger samples	50	6	12%
Total samples	100	17	17%

Table 3: Antimicrobial resistance of *E. faecium* isolates:

Antimicrobial reagents	Sensitive		intermediate		resistant	
	No.	%	No.	%	No.	%
p	1	5.9%	0	0%	16	94.1%
OX	1	5.9%	1	5.9%	15	88.2%
AM	1	5.9%	0	0%	16	94.1%
E	10	58.8%	2	11.8%	5	29.4%
TE	9	52.9%	1	5.9%	7	41.2%
CN	15	88.2%	1	5.9%	1	5.9%
VA	6	35.3%	1	5.9%	10	58.8%
C	11	64.7%	0	0%	6	35.3%
ST	17	100%	0	0%	0	0%
CIP	12	70.6%	0	0%	5	29.4%

Penicillin (P), Ampicillin (AM), Amoxicillin (OX), Erythromycin (E), Tetracycline (TE), Gentamycin (CN), Vancomycin (VA), Chloramphenicol (C), Streptomycin (ST), Ciprofloxacin (CIP).

Ten isolates of *E. faecium* were chosen randomly and subjected to PCR test for detection virulence genes (*hyl*, *cylA* and *ace*) and antimicrobial resistance genes (*vanA*, and *blaZ*).

The results cleared that *hyl* gene was detected in 3 isolates (30%), *cylA* gene detected in 9 isolates (90%) and *ace* gene

was detected in 3 isolates (30%) (Table 5 and Fig. 1, 2, and 3).

While antimicrobial resistance genes were detected in isolates as followed *vanA* gene were detected in 5 isolates (50%) and *blaZ* gene was detected in 9 isolates (90%), (Table 5 and Fig 5 and 6).

Table 4: Multidrug resistance (MDR) patterns of *E. faecium* isolated from meat products.

Pattern of Multidrug resistance	No. of isolates	MDR%
P, OX, AM, VA	6	35.3%
P, OX, AM, TE, C	5	29.4%
P, OX, AM, E, VA, CIP	4	23.5%
P, AM, TE, CIP, CN	1	5.9%
E, TE, C	1	5.9%
Total	17	100%

Penicillin (P), Ampicillin (AM), Amoxicillin (OX), Erythromycin (E), Tetracycline (TE), Gentamycin (CN), Vancomycin (VA), Chloramphenicol (C), Streptomycin (ST), Ciprofloxacin (CIP).

Table 5: The distribution of virulence and antimicrobial resistance genes in the tested isolates.

Isolates no.	Source	PCR				
		<i>cylA</i>	<i>hyl</i>	<i>ace</i>	<i>vanA</i>	<i>blaZ</i>
1	kofta	+ve	+ve	-ve	-ve	+ve
2	kofta	+ve	+ve	-ve	+ve	+ve
3	kofta	+ve	-ve	-ve	+ve	+ve
4	kofta	+ve	+ve	+ve	-ve	+ve
5	burger	+ve	-ve	+ve	-ve	+ve
6	burger	+ve	-ve	+ve	+ve	+ve
7	kofta	+ve	-ve	-ve	+ve	+ve
8	burger	+ve	-ve	-ve	+ve	+ve
9	burger	+ve	-ve	-ve	-ve	+ve
10	kofta	-ve	-ve	-ve	-ve	-ve

+ve = positive -ve = negative

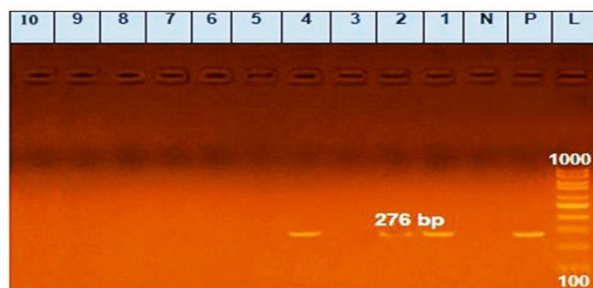


Fig. 1. Agarose gel electrophoresis of *hyl* gene at 276 bp detected in *Enterococcus faecium* isolated from Kofta (Lane 1, 2, 3, 4, 7 and 10) and Burger (lane 5, 6, 8 and 9). Lane L:100 pb ladder as molecular size DNA marker. Lane P: control positive, Lane N: control negative, Lane 1, 2 and 4: positive isolates for *hyl* gene Lanes 3 and 5 to 9, 10: negative isolates for *hyl* gene.

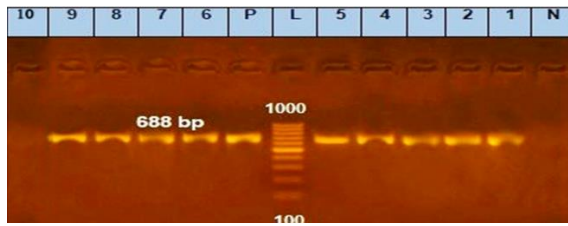


Fig. 2. Agarose gel electrophoresis of *cylA* gene at 688 bp detected in *Enterococcus faecium* isolated from Kofta (Lane 1, 2, 3, 4, 7 and 10) and Burger (lane 5, 6, 8 and 9). Lane L: 100 pb ladder as molecular size DNA marker. Lane P: control positive Lane N: control negative Lanes 1-9: positive isolates for *cylA* gene. Lane 10: negative isolate for *cylA* gene.

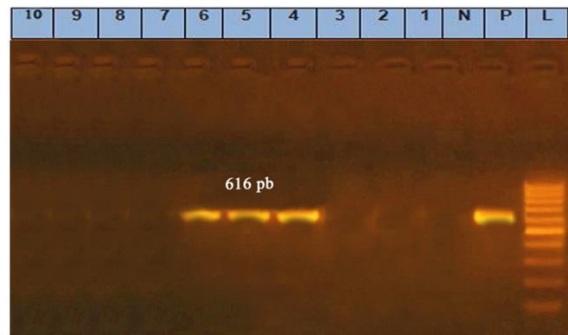


Fig. 3. Agarose gel electrophoresis of *ace* gene at 616bp detected in *Enterococcus faecium* isolated from Kofta (Lane 1-4, 7 and 10) and Burger (lane 5, 6, 8 and 9). Lane L: 100 pb ladder as molecular size DNA marker. Lane P: control positive, Lane N: control negative Lanes 4-6: positive isolates for *ace* gene. Lanes 1, 2, 3, 7, 8, 9 and 10: negative isolates for *ace* gene.

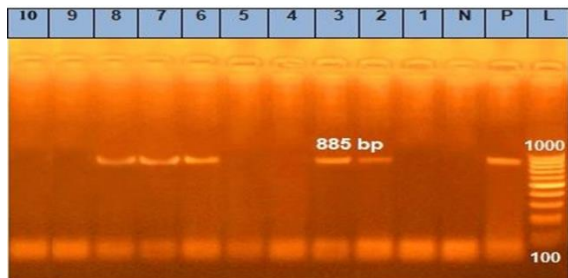


Fig. 4. Agarose gel electrophoresis of *vanA* gene at 885 bp detected in *Enterococcus faecium* isolated from

Kofta (Lane 1-4, 7 and 10) and Burger (lane 5, 6, 8 and 9). Lane L: 100pb ladder as molecular size DNA marker. Lane P: control positive Lane N: control negative Lanes 2, 3, 6, 7 and 8: positive isolates for *vanA* gene Lanes 1, 4, 5, 9 and 10: negative isolates for *vanA* gene.

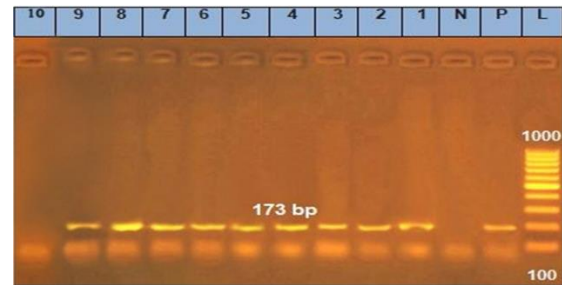


Fig. 5. Agarose gel electrophoresis of *blaz* gene at 173 bp detected in *Enterococcus faecium* isolated from Kofta (Lane 1, 2, 3, 4, 7 and 10) and Burger (lane 5, 6, 8 and 9). Lane L: 100 pb ladder as molecular size DNA marker. Lane P: control positive Lane N: control negative Lanes 1-9: positive isolates for *blaz* gene Lane 10: negative isolates for *blaz* gene

Discussion

Food is a highly perishable commodity as it easily gets spoiled by various types of organisms. Raw meat and other meat products can act as vehicles of various hazards that may have a serious impact on human health. There are various types of hazards, which may be chemical, biological or physical. Biological hazards are very important because the microorganisms or pathogens are found naturally in the environment or even on live animals (Sofos, 2014), also the identification of these pathogens should be used as a part of a threat in the microbiological analysis.

In this study, the conventional identification of *Enterococcus* spp. as typical tiny black colonies on Bile

EsculinAzide agar and microscopic features as gram positive cocci was like Benson et al., (2016), Ashraf et al., (2019). In addition, Mohamed et al. (2018), Pinar and Esra (2018), Richard et al., (2002) and Albert and anicetr, (1999) recorded the same results of the biochemical scheme used with our isolates.

The incidence of *Enterococcus* in the examined samples of meat products were 17%. This result is nearly similar to Ozmen et al., (2010) (20%) and Channaiah and Lakshmikantha, (2009) (16.2%). Higher results were recorded by Gregory et al., (2017) (28.6%) and Wioleta et al., (2016) (39.7%). The percentage of contamination of meat products varied between studies, due to several factors, such as; unhygienic slaughtering, handling and processing conditions, workers' hands, unhygienic abattoir, or from inherent micro-flora in normal tissues of animals, air and environment. Different microbes get presented at each stage of meat processing after slaughtering, and these have a tendency to contaminate the meat (Mahendra et al., 2018).

Different methods as conventional culture and biochemical tests, as well as commercially standardized systems such as VITEK2, so far, been used in most clinical microbiology laboratories for the accurate identification of *Enterococcus* spp (Fang et al., 2012). In addition, Marwa et al., (2016) Proved that accuracies of Vitek 2 system can reach to 100%. In the current study, our isolates were needed further identification to reach to accurate spp. So, using of VITEK 2 system enable us to identify seventeen isolates as *E. faecium* with a

percentage of 100%. Several studies supported the identification of *Enterococcus* spp. by the VITEK 2 identification system (Charlene et al. 2004; Donna et al., 2016; Yomna et al., 2017 and Othman et al., 2019). Based on the results of antibiotic sensitivity test, all tested isolates were sensitive to Streptomycin and Gentamycin with percentage 100%. Our results were supported by Antonio et al., (2010), Trivedi et al., (2011), Jan et al., (2003), Suely et al., (2007) and Antonio et al., (2008).

Due to the overuse and misuse of antibiotics in agricultural, livestock breeding and human medical environments, the problem of antibiotic resistant bacteria is growing, and constitutes a real threat to public health (Kamelia et al., 2019). Many environments, such as soil, sludge, surface water and animal waste, have been proven to be important reservoirs for antibiotic resistance genes because many have been detected in these environments (Anna et al., 2019).

In this study, most of our isolates were resistant to B-lactamases (Table 3). Our results were supported by Abdulhakim et al., (2015), Heba and Eman, (2019) and Wedad et al., (2020), lower results were recorded by Mohamed and El-Sayed, (2016) (42.8 %) and Suely et al. (2007) (40%).

Also, our isolates were resistant to vancomycin with percentage 58.8%. These results were supported by Carla et al., (2011), lower results were recorded by Wedad et al., (2020) (18.2%), Heba and Eman, (2019) (18.8%) and Pesavento et al., (2014) (3.57%). This resistance to vancomycin may be back to excessive use

of avoparcin in clinical cases of both human and animal (Ribeiro et al., 2007).

Moderate resistant was recorded against erythromycin (29.4%) among our isolates, the previous results were supported by Antonio et al., (2010) (33.3%), lower results were recorded by Trivedi et al., (2011) (12%) and Jan et al., (2003) (18%), higher results were recorded by Wedad et al., (2020) (100%), Abdulhakim et al. (2015) (97.5%). Furthermore our isolates showed resistant to tetracycline (41.2) %, Nearly similar results were recorded by Mohamed and El-Sayed, (2016) (50%), lower results were gained by Antonio et al. (2008) (6.66%) and Pesavento et al., (2014) (16.4%) higher results were recorded by Abdulhakim et al., (2015) (92.5%) and Suely et al., (2007) (80%).

High resistance to chloramphenicol (35.3%) were recorded in our results in comparison to Pesavento et al., (2014) (5%). However lower rate of resistance was recorded to ciprofloxacin among our isolates when compared by Abdulhakim et al., (2015) (87.5%), Antonio et al., (2008) (66.6%). High antibiotic resistance in the study area may be a marker for differences in multiple factors such as antimicrobials use, disease control measures, or genetic mutations leading to multidrug-resistant phenotypes (Jadhav et al., 2011).

In the last few years, to decrease the possibility of misidentification and obtain more rapid identification, molecular methods such as polymerase chain reaction (PCR) has been considered as alternative approaches to the phenotypic methods (Fang et al., 2012).

In this study, ten isolates were selected, nine out of ten isolates harbored β -lactamase resistance gene (*blaz* gene) with a high percentage (90%) (Table 5 and Fig. 5). Several authors noted different percentage as; Carla et al., (2011) found the incidence of *blaz* gene was (15%) in meat samples and Fontana et al., (2009) found the incidence of *blaz* gene was (10%) in dry fermented sausages. Resistance to β -lactam antibiotics is a vital problem of therapeutic medications, Multiple mutations in the active site of the *pbp5* gene are common causes for high-level resistance as shown by other authors (Jureen et al., 2004; Rice et al., 2004 and Hsieh et al., 2006).

Furthermore, vancomycin-resistant *E. faecium* is the second leading pathogen of the priority list of antimicrobial resistance (priority pathogens) published recently by WHO that area major threat to public health (WHO, 2017). By using PCR technique, *vanA* gene we detected in 5 out of 10 isolates (50%) from each of kofta and burger samples (Table 5 and Fig 4). This result reinforced by María et al., (2009) and Carla et al., (2011) who detected *vanA* gene in 50% and (60%) of food samples and meat respectively, while Yaeghob et al., (2012) recorded a high percentage (89.5%) of *vanA* gene in *E. faecium* isolates. The current study advises that the predominant phenotype vancomycin resistance in clinical and dairy isolates does not occur by clonal dissemination of resistant strains but rather through the transfer of *van A* gene by transposable elements, like Tn1546 (Ribeiro et al., 2007).

Virulence factor is an effector molecule that enhances the ability of microorganisms

to cause disease beyond that intrinsic to the species background (Sanchez et al., 2009).

Different virulence genes were harbored by *E. faecium*, one of the most important virulence gene was Cytolysin (*Cyl*) and was one from the most characterized enterococci virulence factors that damages host cells and promotes infection. It has β -haemolytic properties which considered undesirable in foods (Fifadara et al., 2003).

Cytolysin is a bacterial toxin encoded by the cytolysin operon consisting of eight genes which may be facilitating dissemination of this virulence genes (Julia et al., 2019). In present study, *cylA* gene detected in 9 isolates out of 10 isolates (90%) from Kofta and burger (Table 5 and Fig 2). Antonio et al., (2008) and Trivedi et al., (2011) found that the *cylA* gene was detected in 26.6% and 25% of *E. faecium* isolates from food samples, while Hashem et al., (2015) found that 29% of isolates from clinical samples have *cylA* gene.

Hyaluronidase enzyme encoded by the *hyl* gene which responsible for the breakdown of hyaluronic acids, which facilitates bacterial colonization by reducing the viscosity of the extracellular matrix (Julia et al., 2019). In present study, *hyl* gene detected in 3 isolates from kofta out of 10 isolates (30%) (Table 5 and Fig. 1). Vanessa et al., (2004) and Tanya et al., (2016) found the incidence of *hyl* gene was (27%) and (27.5%) from isolates of clinical samples respectively, also Xin et al., (2020) detected *hyl* gene in 26.3% in isolates of fecal samples.

Accessory colonization factor is another surface protein with adhesive properties, it is encoded by the *ace* gene. In

present study, *ace* gene detected in 3 isolates out of 10 isolates (30%) (Table 5 and Fig 3). Luana et al., (2014) found the incidence of *ace* gene was (33.3%) in Enterococcus strains isolated from goat milk, Tanya et al., (2016) found the incidence of *ace* gene was (72.8%) in the clinical isolates, Trivedi et al., (2011) detected *ace* gene in 12% of Enterococcus isolated

from meat samples and Xin et al., (2020) found the incidence of *ace* gene was (52.8%) in isolates from fecal samples.

Conclusion

E. faecium was isolated from different meat products, also several authors suggesting a possible pathological role in human infections as a result of their possessing many virulence factors. vancomycin-resistant Enterococcus species are extremely rare and would play a role in decreasing efficacy of such a potent antibiotic. So bacteriological examination of meat products. It has become important and necessary to identify such microbes and reduce their risks.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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