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MOLECULAR CHARACTERIZATION OF ANTIBIOTICS RESISTANCE GENES OF ENTEROCOCCI ISOLATED FROM RAW MILK IN ASSIUT CITY

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ABSTRACT

Enterococcus species are Gram-positive cocci that are characterized by being catalase-negative, facultative anaerobic bacteria, and non-spore forming. *Enteroccoi* is often isolated from environmental and animal sources and inhabits the human gastrointestinal tract. *Enterococcus* species, which gets its resources from the dairy industry, animals, and people, is one of the most abundant lactic acid bacteria in raw milk. The aim of the present study is to detect antibiotic resistance of *Enterococci* isolated from raw milk by phenotypic and genotypic methods. Vitek 2 Compact System was used to identify the samples and assess their antimicrobial susceptibility. Following that, drug resistance genes (*ermB*, *aph (3')-IIIa*, and *TetM*) and one virulence gene (*esp*) were molecularly detected by PCR. Twenty isolates of *Enterococci* were phenotypically identified by routine laboratory examination and Vitek2. High rates of antibiotic resistance were found to erythromycin and tetracycline with percentages of 65% and 35%, respectively. The presence of *tetM* and *ermB* in milk isolates was found to be 100%, similarly. No detection of *aph (3')IIIa* was found in milk isolates. *Esp* was detected only in 5% of isolated samples. The prevalence of *Enterococci* among studied samples was 11.8% (20/170). The fact that *Enterococci* were resistant to erythromycin and tetracycline in our study was noteworthy since these bacteria could potentially be transmitted to people by drinking milk that has not been properly treated.

***Key words**: Enterococcoi*, Vitek2, Resistant genes, PCR

INTRODUCTION

Enterococci were first discovered in human flora in 1899, but it wasn't known until the end of the nineteenth century when two different teams Thiercelin, and MacCallum & Hastings, described human enterococcal infections in detail (Thiercelin, 1899; MacCallum and Hastings, 1899).

Enterococcus are Gram-positive cocci which are spherical or ovoid and are lactic acid bacteria (LAB). They vary in length (0.6–2.0 μm) and width (0.6-2.5 μm). They characteristically form short chains and cluster together (Langella, 2018). The range

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of body temperatures between 35 and 37°C is ideal for the proliferation of *Enterococcus* species in both humans and animals (Ch'ng *et al.,* 2019).

Additionally, *Enterococci* are resistant to drying and can survive in unfavorable settings like those seen in a hospital environment (Ch'ng *et al.,* 2019). Lactic acid bacteria are found in raw bovine milk's core, mesophilic/ psychrotrophic microbiota (Quigley *et al.,* 2013).

Faecal contamination does not seem to be a major factor in *Enterococci* entry into the dairy production chain, in spite of their connection to the intestinal microbiotas of humans and dairy animals. The main source of raw milk contamination comes from the milking machine (Kagkli *et al.,* 2007).

Mastitis can potentially be a source of *Enterococci* that are multi-drug resistant (Wu *et al.,* 2016). Seasonal variations in enterococcal variety in raw milk may also be influenced by the time of year (McAuley *et al.,* 2015).

Contagious mastitis is primarily caused by *Streptococci*. One of the environmental causes of mastitis is *Enterococci*. *Enterococci* can be transmitted from the inflamed udder to humans is another matter of concern. (Różańska *et al.,* 2019).

Both the host's immune system's resistance to infection and teat contact with the enterococcal bacterium can result in mastitis. Bovine mastitis can be caused by *E. faecium* and *E. faecalis* (Montironi *et al.,* 2020).

Different species of Enterococci are isolated from the raw milk of cows, goats, and sheep. Strains of *Enterococcus faecalis, E. faecium, E. durans, E. hirae, E. saccharominimum,* and *E. italicus* have been isolated. (Bouymajane *et al.,* 2018).

Several *Enterococci* virulence factors, mainly in *E. faecalis* and *E. faecium*, have been discovered (Homayouni *et al.,* 2018). The enterococcal virulence factors reported so far have been grouped into two categories: surface proteins that encourage colonisation of the host and the secreted chemicals that cause tissue damage to the host (Chajęcka-Wierzchowska *et al.,* 2017).

The virulence factors that encourage adhesion (all surface proteins) include the aggregation substance (AS), the collagen binding proteins Accessory colonisation factor (ACE in *E. faecalis* and ACm in *E. faecium*), endocarditis specific antigen (*Efa A*) and enterococcal surface protein (*Esp*) (Chajęcka-Wierzchowska *et al.,* 2017).

Gelatinase (gelE), hyaluronidase (Hyl), and cytolysin (Cyl) are categorized as members of the class of secreted toxins with negative effects on the host's tissues. (Chajęcka-Wierzchowska *et al.,* 2017).

The resistance of *Enterococci* to some antibiotic kinds is inherent, while resistance to others has been acquired. It is also important to note that *E. faecalis* and *E. faecium* make up the majority of the resistant *Enterococci*. (Růžičková *et al.,* 2020).

The most important antibiotics against which *Enterococci* express resistance are penicillin, aminoglycosides, cephalosporines, strepto-gramins, and lincosamides. Glycopeptides (vancomycin), macrolides, tetracyclines, linezolid, and chloramphenicol are among the drugs that have acquired resistance (Liu *et al.,* 2012).

The *ermB* gene, which is found in several transposons and plasmids in species of the *Enterococcus*, *Streptococcus*, *Clostridium*, and *Staphylococcus* genera, is the most widely distributed gene that confers resistance to macrolides in *Enterococci* (Torres *et al.,* 2018).

Tetracycline resistance can also develop through a mechanism that prevents the drug from binding to the ribosome. Tetracycline

activity is prevented by resistance genes like *tetM* and others on the ribosome. (Blair *et al.*, 2015).

On mobile elements, resistance genes for AG-modifying enzymes are frequently discovered. The development of AG-modifying enzymes such as acetyl-transferases (AACs), phosphotransferases (APHs), and nucleotidyl transferases (ANTs) is the most frequent route of resistance to AGs in clinical isolates (Potron *et al.*, 2015).

MATERIALS AND METHODS

Ethical statement. According to the World Medical Association's code of ethics (Declaration of Helsinki), the study was approved by the ethical committee of Assiut University's faculty of medicine. The number is 17101901.

Collection of samples: 170 raw milk samples were collected from Assiut University farm, farms from rural areas and milk laboratories in Assiut City. Samples were collected under sterile conditions.

Preparation of raw milk samples: For isolation of *Enterococci* from milk, the samples were centrifuged for 10 minutes at 5000 rpm with the supernatant being discarded afterward. A loopful from prepared sediments was streaked onto the surface of the blood agar plate and then incubated at 37°C for 18-24 hrs. (Abd El Tawab *et al.*, 2019).

Culture on solid media:

- **Blood agar**: *Enterococci* emerged as non-hemolytic smooth, white-colored colonies with clean edges (Murray, 1999).

- **Bile esculin agar:** Esculin hydrolysis was positive, resulting in blackening of the medium around the growth and good, luxuriant growth **(**Devhare *et al.*, 2021).

Biochemical Tests:

- **Catalase test (Fine Gold and Martin, 1982):** A sterile loop was used to transfer a loopful of isolated colonies on the surface of a dry, clean glass slide. The colony on the slide was then treated with a drop of 3% hydrogen peroxide. Gas bubble evolution in the colonies showed positive results. *Enterococci* isolated were catalase negative (Klein, 2003).

- **Identification of *Enterococci* was done by using the vitek2 compact system using a Gram-positive identification card (Kim *et al.*, 2023):** Gram-positive identification card contained 43 different biochemical test**s** applied on isolates from raw milk samples.

Antimicrobial sensitivity test performed by Vitek 2 compact system:

Vitek 2 Compact (BioMerieux Inc., France) was used to determine minimum inhibitory concentrations (MICs) for the following antibiotics; β-lactams (Ampicillin and Benzylpeniciilin), Aminoglycoside (High level gentamicin and High level strepto-mycin), Fluroquinolones (Ciprofloxacin and Levofloxacin), Macrolides (Erythromycin), Streptogramin (Quinupristin/Dalfopristin), Oxazolidinones (Linezolid), Nitrofurans (Nitrofurantoin), Glycopeptide (Vanco-mycin), Tetracycline (Tetracycline) and Glycylcycline (Tigecycline) (Sengupta *et al.*, 2023).

A category interpretation will be reported along with a MIC, according to the interpretations defined by CLSI® 2021.

Molecular detection of resistant genes and virulence genes:

The primers used for the detection of both resistant genes and virulence genes are shown in the following table (1).

Table 1: Primers used in the detection of antibiotics resistance genes and virulence genes.

Target gene	Sequence of primers	Size of amplified product (bp)	References
Aph (3')-IIIa	GGCTAAAATGAGAATATCACCGG CTTTAAAAAATCATACAGCTCGCG	523 bp	**Khani *et al.*, 2016**
***Tet* (M)**	ACAGAAAGCTTATTATATAAC TGGCGTGTCTATGATGTTCAC	170 bp	**Said & Abdelmegeed,2019**
ErmB	CCGAACACTAGGGTTGCTC ATCTGGAACATCTGTGGTATG	139 bp	**Kim *et al.*,2019**
Esp	AGATTTCATCTTTGATTCTTG AATTGATTCTTTAGCATCTGG	510 bp	**Kim *et al.*, 2019**

PCR assays: PCR was performed in a 25 µL reaction mixture containing 1 µL each of both forward and reverse specific primer pairs, 12.5 µL of PCR master mix (Thermo Fisher Scientific, United States)), 5.5 µL of nuclease-free water, and 5.0 µL of DNA template.

Two multiplex PCR were done for each of two gene sets (*ermB* and *esp*) and (*aph(3')IIIa* and *TetM*).

***ErmB* and *esp* genes:** Initial denaturation at 95°C for 5 minutes, 40 cycles each cycle consisting of denaturation at 95°C for 40 seconds, annealing at 55°C for 50 seconds, extension at 72°C for 50 seconds and final extension at 72°C for 5 minutes.

TetM* and *aph(3')-IIIa* genes*: Initial denaturation at 95°C for 5 minutes, 40 cycles each cycle consisting of denaturation at 95°C for 40 seconds, annealing at 50°C for 50 seconds, extension at 72°C for 50 seconds and final extension at 72°C for 5 minutes.

Detection of the amplified product. The resulting PCR amplicons were examined on a 1.5% agarose gel, stained with ethidium bromide, and observed for two hours under UV illumination at 80 volts.

Statistical analysis. Categorical variables were described by number and percent (N, %), All analyses were performed with the IBM SPSS 26.0 software.

RESULTS

The prevalence of *Enterococci* isolates from raw milk was 11.8%**.** The most species isolated from milk were *E. feacuim* accounting for 30%, *E.feacalis* accounting for 25%, *E. durans* 30% and *E.hiare*15%.

Antimicrobial susceptibility test: Tetracycline had the highest resistance rate with a percentage of 35%, followed by Quinuprisia/Dalfopristin with a percentage of 30%. Erythromycin resistance was observed in milk samples at a rate of 65%. *Vancomycin-resistant Enterococci* were only 10%. *Linezolid-Resistant Enterococci* was only 5% in the present study. About 10%, and 5% had resistance to benzylpenicillin and ampicillin, respectively. No resistance was detected at high levels of streptomycin, nitrofurantoin, or tigecycline as in Table (2). In milk samples, only 2 strains were found to be multidrug-resistant, and the other strains were found to be mostly sensitive, with a prevalence of 90%.

Molecular detection of antibiotics resistance genes: In milk samples isolates, *tetM and ermB* percentages were 100%, similarly. About 5% of isolates were found to carry *esp*. No detection of *aph(3')IIIa* was found as in figures (1-2). The coexistence of

4 genes in milk samples was observed as follows, with 95% of milk samples found to carry 2 genes (2 resistant genes: *ermB* and *tetM*). The rate of coexistence of three resistant genes was approximately 5% (*esp*, *ermB* and *tetM*) as shown in Table (3). In the present study, there was no detection of four genes coexistence in milk samples. About 10.5% of strains carrying two resistants were *Multidrug-Resistant Enterococci*. 35% of erythromycin-susceptible strains carried *ermB*, while 65% of erythromycin-resistant strains carried the resistant gene in strains isolated from milk samples. Milk samples contained a high rate of tetracycline-susceptible strains carrying *tetM* gene (65%) and about 35% of tetracycline-resistant strains carried the resistant gene as in Table (4)

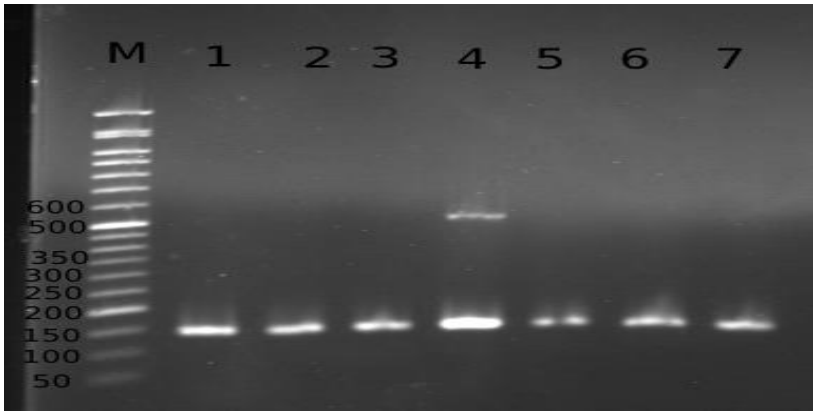

Fig.1: Agarose gel of the products of PCR to detect *ermB* (139 bp) and esp (510 bp) genes. Lane (M) shows the DNA standard ladder (50-1500 bp)
All lanes (1 to 7) show *ermB* positive isolates. Lane (4) shows *esp* positive isolate.

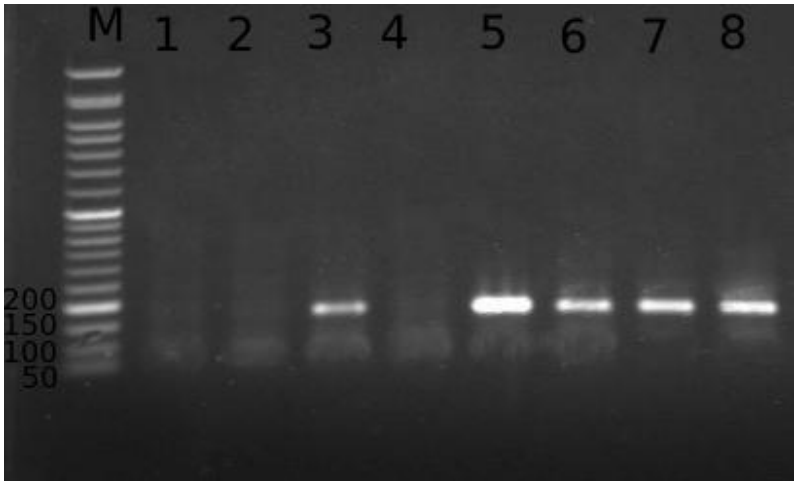

Fig.2: Agarose gel of the products of PCR to detect *tetM* (170 bp) and *aph (3')IIIa* (523 bp) genes. Lane (M) shows the DNA standard ladder (50-1500bp), and Lanes (3, 5, 6, 7, 8) show *tetM* positive isolates. No isolates were found to carry *aph (3')IIIa*.

Table 2: Antimicrobial susceptibility test of *Enterococci* isolated from milk by vitek2:

	Antimicrobial agent	Resistant		Intermediate		Sensitive		Total	
		No.	%	No.	%	No.	%	No.	%
	β – lactams:								
1	Benzylpenicillin	2	10	0	0.0	18	90.0	20	100
2	Ampicillin	1	5	0	0.0	19	95.0	20	100
	Aminoglycoside:								
3	Gentamycin High Level	1	5	0	0.0	19	95.0	20	100
4	Streptomycin High Level	0	0.0	0	0.0	20	100	20	100
	Fluroquinlones:								
5	Ciprofloxacin	1	5	0	0.0	19	95.0	20	100
6	Levofloxacin	1	5	0	0.0	19	95.0	20	100
	Macrolide:								
7	Erythromycin	4	20	9	45	7	35.0	20	100
	Streptogramin:								
8	Quinuprisia/Dalfopristin	6	30	0	0.0	14	70.0	20	100
	Oxodizolones:								
9	Linezolid	1	5	0	0.0	19	95.0	20	100
10	**Glycopeptide:**								
	Vancomycin	2	10	0	0.0	18	90.0	20	100
11	**Tetracycline:**								
	Tetracycline	7	35	0	0.0	13	65.0	20	100
12	**Glycylcycline:**								
	Tigecycline	0	0.0	0	0.0	20	100	20	100
13	**Nitrofurans:**								
	Nitrofurantoin	0	0.0	8	40	12	60.0	20	100

Table 3: Coexistence of resistant genes and *esp* in milk samples.

No. of genes	Genes	No. of isolates (20)	No. of *multidrug-resistant Enterococci* strains	No. of sensitive *Enterococci* strains
One gene	0	0	0	0
Two gene	*ErmB, TetM*	19 (95%)	2 (10.5%)	17 (89.5%)
Three genes	*Esp, ErmB, TetM*	1 (5%)	0	0
Four genes	*Esp, ErmB, TetM aph(3')IIIa*	0	0	0

Table 4: Relation between phenotype and genotype of antibiotics resistance genes of *Enterococci* isolated from milk samples.

Antimicrobial agent	Phenotype susceptible				Phenotype resistant			
	Genotype resistant		Genotype susceptible		Genotype resistant		Genotype susceptible	
	No.	%	No.	%	No.	%	No.	%
Erythromycin	7	35	0	0.0	13	65	0	0.0
High level streptomycin	0	0.0	20	100	0	0.0	0	0.0
Tetracycline	13	65	0	0.0	7	35	0	0.0

DISCUSSION

Enterococci are common on many plant surfaces and are naturally found in the colon of warm-blooded animals (Gorgy & Ali, 2016).

This study revealed that the prevalence of *Enterococci* in milk samples was 11.8%. This may be due to good handling of milk and good sanitation of milking. This was consistent with the percentages reported by Akinyemi *et al.* (2023) for Nigeria (12%) and Bouymajane *et al.* (2018) for Morocco (11.3%). Based on a study by Hamzah & Kadim (2018) in Iraq, the percentage of *Enterococcus* species was 31% in raw milk. According to a study done in the El-Behera governorate in Egypt, 22% of raw milk samples tested positive for *Enterococci* (Gorgy & Ali, 2016). In a comparable study conducted in Benha, Egypt, 60% of enterococcal species were present, according to Abdeltawab *et al.* (2019).

According to our study, the most species isolated from raw milk samples were *E. faecium* (30%), *E. faecalis* (25%), *E. durans* (30%), and *E. hiare* (15%). Hamzah & Kadim (2018) found that *E. feacalis* (72%), *E. durans* (3%) and *E. gallinarum* (12%) were the most common isolates from raw milk. There was no detection of the isolation of *E. hiare*. A study conducted in Morocco by Bouymajane *et al.* (2018) documented that *E. faecalis* (64.7%), *E. faecium* (17.6%), *E. durans* (11.8%), and *E. hirae* (5.9%) were the most common enterococcal isolates. Kim *et al.* (2022) detected that *E. faecalis* (48.8%) and *E. faecium* (51.2%) were only the two *Enterococcus* species that were most frequently isolated from raw milk.

Tetracycline resistance in raw milk samples was found to be high in our investigation, at 35%. The high percentage of tetracycline resistance may be due to extensive use of it due to its availability and low cost, as indicated by Krawczyk *et al.* (2021), or may be due to its usage as a growth promoter, as mentioned by Michalova and Schlegelova (2004). This was more prevalent than the South Korean study mentioned by Kim *et al.* (2022), which indicated a prevalence of 17.1%.

Among the strains isolated for our study, quinupristin/dalfopristin resistance was 30% prevalent. According to the findings of our work, *E.faecium* (16.7%) and *E. faecalis* (100%) were both resistant to quinupristin/ dalfopristin. This resembles the results found by Gołaś-Prądzyńska *et al.* (2022), who detected that all *E. faecalis* isolates were resistant to quinupristin/dalfopristin with a percentage of 100%, while among *E.faecium* 33% were resistant to this agent. The reason for the high resistance of *E.feacalis* to quinupristin/dalfopristin was the intrinsic resistance to this agent (Gołaś-Prądzyńska *et al.*, 2022). There was no detection of resistance to quinupristin/dalfopristin in *E.durans* or *E.hiare* in our study.

The percentage of erythromycin-resistant strains in the current study was 20%, while the prevalence of strains expressing intermediate resistance was 45%. Beyond disease prevention, animals are frequently fed antibiotics to promote growth or boost feed efficiency, which raises the possibility of the emergence of multi-resistant strains as Tóth *et al.* (2020) reported. In 2022, a study revealed that the prevalence of erythromycin was 34.1%. There was no detection of the presence of intermediate resistance in that study (Kim *et al.*, 2022).

Our work revealed that the percentage of vancomycin resistance was 10%. *E.faecium* was the species that resisted vancomycin in the current study. This comes in accordance with Kročko *et al.* (2011) who found that the prevalence of Vancomycin resistance was 9.9%. The results obtained in the present study were higher than those found by Kang *et al.* (2021), with a prevalence of 0.5%.

Ciprofloxacin resistance was one of the lowest antibiotic resistances in the current

study, with a prevalence of 5%. A similar study was conducted in Morocco by Bouymajane *et al.* (2018), who found the prevalence to be 5.9%. Another search done by Kang *et al.* (2021**)** found the percentage of ciprofloxacin resistance to be 1.1% which was lower than our results. There was no resistance to ciprofloxacin found in South Korea (Kim *et al.*, 2022)**.**

In the present study, the ampicillin resistance percentage was 5%. Kang *et al.* (2021) showed that 1.1% of raw milk contained ampicillin resistance. Kim *et al.* (2022) showed that isolated isolates had no detectable ampicillin resistance.

Only 5% of the *Enterococci* in the current study were found to be linezolid-resistant, which contrasts with Zarzecka *et al.* (2022) who detected that 26.9% of the *Enterococci* were linezolid-resistant.

In the current study, 90% of the *Enterococci* strains isolated were sensitive strains with resistance to one or two antibiotics. Only two strains were multidrug resistant; one was resistant to 4 groups of antibiotics (macrolides, streptogramin, glycopeptide and tetracycline), and the other was resistant to 5 groups of antibiotics (β-lactams, aminoglycosides, fluoroquinolones, glycopeptide and tetracycline). The multidrug-resistant strains isolated were found to be *E.faecium*. In contrast, Gołaś-Prądzyńska *et al.* (2022) reported that *Multidrug-Resistant Enterococci* (mostly *E.faecium* and *E.feacalis*) were resistant to at least of three groups of antibiotics which constituted 19.7%.

In the present work, the resistant genes were present in milk samples with a high prevalence rate. The *ermB* and *tetM* resistant genes were 100% detected in our study, but the *aph(3')IIIa* resistant gene was not detected in milk samples. Gołaś-Prądzyńska *et al.* (2022) mentioned that the prevalence of *tetM* in milk was 94.7%, which is almost in agreement with our data. In contrast, only a small amount of *ermB* was found in their research. In addition, Hammad *et al.* (2022) detected the prevalence of *ermB* and *tetM* in their study at 4.1% and 16.6%, respectively. Morandi *et al.* (2015) reported that the *ermB*-resistant gene was not found in their study, but the *tetM* gene was found in 53% of the raw milk samples. Based on a study by Kang *et al.* (2021), the prevalence of *tetM* and *ermB* were 36.3% and 71.4%, respectively. Also, Bag *et al.* (2022) demonstrated the prevalence of resistant genes: *tetM* and *aph(3')IIIa* were 50% and 12.5%, respectively. According to Đorđević *et al.* (2022), the prevalence of *ermB*, *tetM*, and *aph (3') IIIa* was 19.2%, 8.5%, and 17%, respectively, in Serbia.

Interestingly, 35% of erythromycin-susceptible strains carried the *ermB* resistance gene, while 65% of erythromycin-resistant strains carried *ermB* isolated from milk samples. The lack of correlation between phenotype and genotype in the ermB-resistant gene was discovered by Demirgül and Tuncer (2017) and Đorđević *et al.* (2022), and this could be due to the absence of gene expression, which is known as a "silent gene". In our study, a silent gene was also found to be present in the tetracycline-resistant gene: 35% of the tetracycline-resistant strains and 65% of the tetracycline-susceptible strains both included the *tetM*-resistant gene. The presence of the *tetM* silent gene was documented by Đorđević *et al.* (2022), who reported that one case was susceptible to tetracycline despite carrying the *tetM*-resistant gene. Interestingly, Morandi *et al.* (2015) also reported that 37% of tetracycline-susceptible strains carried a *tetM* resistance gene. Silent genes are known to exist, and their presence may be explained by inactive gene products or by low levels, or downregulation, of gene expression.

Esp, an enterococcal surface protein is connected to the growth of biofilms. Vancomycin, *esp*, and multiple antibiotic resistance are all related in *E. faecium* strains,

as has been proven by Ochoa *et al.* (2013). In the current study, 5% of the milk samples had the virulence gene (*esp*). The prevalence of the *esp* virulence gene in dairy products was determined to be 4.1% by Hammad *et al.* (2015), which is consistent with our results.

Only two strains in milk samples isolates were found multi-drug resistant. The two multi-drug resistant strains were found to carry two resistant genes (*ermB* and *tetM*).

CONCLUSION

Our present study revealed that the prevalence of *Enterococci* isolates in milk was 11.8%. Two strains were found to be *Multidrug-Resistant* were isolated in milk samples. *ErmB* and *TetM* were found in milk with high percentages. Some samples of milk that were susceptible to tetracycline and erythromycin were found to carry their specific resistant genes which is called silent gene. The fact that *Enterococci* were resistant to erythromycin and tetracycline in our study was noteworthy since these bacteria could potentially be transmitted to people by drinking milk that has not been properly treated.

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التوصيف الجزيئي للجينات المقاومة للمضادات الحيوية للمكورات المعوية المعزولة من الحليب الخام في مدينة أسيوط

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المكورات المعوية هي بكتريا موجبة الجرام تتميز بكونها بكتيريا لاهوائية اختيارية سلبية الكاتلاز، والتي تعيش عادة في القناة الهضمية للإنسان بالإضافة إلى كونها معزولة من المصادر البيئية والحيوانية. المكورات المعوية تحصل على مواردها الغذائية من الألبان والحيوانات والبشر، وهى واحدة من أكثر بكتيريا حمض اللاكتيك وفرة في الحليب الخام. هدفت هذه الدراسة إلى الكشف عن مقاومة المضادات الحيوية للمكورات المعوية المعزولة من اللبن الخام بالطرق المظهرية والجينية. تم استخدام نظام Vitek 2 المضغوط لتحديد العينات وتقييم مدى تعرضها لمضادات الميكروبات. بعد ذلك تم الكشف جزيئيًا عن جينات مقاومة الأدوية (ermB، IIIa - (3 ') aph ، و TetM) وجين ضراوة واحد (esp) بواسطة تفاعل البلمرة المتسلسل (PCR). تم التعرف ظاهريا على عشرين عزلة من المكورات المعوية عن طريق الفحص المخبري الروتيني و Vitek2. تم العثور على معدلات عالية من مقاومة المضادات الحيوية للإريثروميسين والتتراسيكلين بنسب ٦٥٪ و ٣٥٪ على التوالي. وجد tetM و ermB في عزلات اللبن بنسبة ١٠٠٪ لكل منهما. لم يتم الكشف عن IIIa (3 ′) aph في عزلات اللبن. تم الكشف عن Esp في ٥٪ من العينات المعزولة. بلغ معدل انتشار المكورات المعوية بين العينات المدروسة ١١,٨٪ (١٧٠/٢٠). و خلص البحث إلى أن المكورات المعوية مقاومة للإريثروميسين والتتراسيكلين في دراستنا جديرة بالملاحظة لأن هذه البكتيريا يمكن أن تنتقل إلى الناس عن طريق شرب الحليب الذي لم يتم علاجه بشكل صحيح.