Detection of some virulence factors of *Enterococcus faecalis* isolated from raw milk by Multiplex PCR

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Abstract

Since milk is a high nutrition food , it is extremely liable to enterococcal contamination , as this widely distributed in the environment . This study evaluated 40 raw milk samples (10 samples for each cows , sheep , goats and buffalo) for enteroccal contamination . Raw milk samples were collected from different farms in Diwaniyah city /Al-Qadisiya governorate between May and July 2012. This objective of this study was numerate the number of enterococci in the raw milk samples . Also to find out the out the distribution of major virulence factors *asa1 , gelE , cylA , esp* and *hyl* genes by multiplex PCR among *Enterococcus faecalis* isolates and the production of gelatinase and hemolytic activity were also determined . The antibiotics susceptibity of the strains was determined using the disc diffusion method . The results of the current study showed that the raw milk had a total viable count in the range from $1*10^2 – 34*10^6$ CFU/ml and all raw milk samples were positive for the presence of enterococci . Significant difference was not exist between the types of raw milk samples . twenty strains of *E. faecalis* were isolated from 40 raw milk samples . These strains were identified by conventional biochemical tests and the HiStrep system . Tn addition , *E. faecalis* specific 16S rDNA gene (amplicon size 290 bp) primers were included as a control when the biochemical identification was doubtful . Multiplex PCR for virulence factors showed that *E. faecalis* isolates carried one or more virulence encoding genes . The *asa1* gene was the predominant (75%) of the *E. faecalis* strains investigated followed by *hyl* gene (70%) . The *esp* , *gelE* and *cylA* genes were detected with percentages of 65% , 50% and 25% respectively . Antibiotics susceptibility testing showed that of the total 20 isolates 12(60%) were resistant to erythromycin and 9(45%) to ampicillin . Lower antibiotics resistance was seen with gentamycin , tetracycline , norfloxacin , penicillin and rifampicin (20% , 15% , 10% , 15% and 10% respectively) . None of the isolates was found to be resistant to be chloramphenicol .

Introduction:

Milk is a complex biological fluid and by its nature, a good growth medium for many microorganisms. Because of the specific production it is impossible to avoid contamination of milk with microorganisms therefore the microbial content of milk is a major feature in determining is quality (31). Microorganisms may contaminate milk from the animals udder , barn , milk collection materials , poor storage conditions, various ingredients added to dairy product and dairy farm workers (19).

Microbial load in fresh milk is although very low less than 10^{-3} CFU/L but this level may increase up to 100 fold if this milk is stored for many days of normal temperature (32) . Preventing the growth of contaminating microorganisms in milk involves limiting contamination levels , cooling immediately after milking and maintenance of cold storage temperature (18).

Enterococci are represent normal components of the raw milk microbiota. There no standards set for the minimum and maximum count of enterococci because they are not normally counted in microbiological analysis . A study of the levels of enterococci in raw cows milk from 10 New Zealand farm in 1997 revealed an enterococcal minimum a count of $< 10^1$ and maximum of $1.2* 10^4$ CFU/ml , though 95% of the samples of the same study had less than $1.9* 10^3$ CFU/ml (22). While enterococcal counts in goats milks can reach higher levels : log levels of $1.7* 10^4$ CFU/ml have been reported in goats milk in Czech Republic (26).

For many years *Enterococcus* species were believed to be harmless to human and considered unimportant medically (15). Recently enterococci have become one of the most common nosocomial pathogens , with patients having a high mortality rate of up to 61% (12). The genus *Enterococcus* consists of gram-positive , catalase-negative , non-spore forming , facultative anaerobic bacteria and that can occur both as single cocci and in chains (14).

They are commensal microorganisms that colonize in the gastrointestinal tract of humans and animals and are also found in several different food source such as milk and cheese (5). These bacteria are able to survive extreme environments , such as 6.5% NaCl , pH of 9.6 , high heat as well as being able to grow and survive under other harsh environmental conditions (3). Different *Enterococcus* species are found in raw milk by *Enterococcus faecalis* and *Enterococcus faecium* remain the species of the greatest importance (17).

E. faecalis is an opportunistic pathogen known to cause serious infections such as bacteraemia , septicamemia , urinary tract infections , wound infections , meningitis and endocarditis (23; 36). Also their increasing antibiotic resistance which spread rapidly among populations (1) and available antibiotics currently limits the therapeutic options (7).*E. faecalis* strains possess several putative virulence determinants , although the molecular mechanism of virulence is still not completely understood (20). *E. faecalis* may carry various genes directly or indirectly contributing to virulence (39).

Gene encoding virulence factors such as aggregation substance (*asa1*), gelatinase (*gelE*), cytolysin(*CylA*), enterococcal surface protein (*esp*), enterococcal surface adhesion (*ace*) , adhesion-associated protein(*efaA*)(also known endocarditis antigen A) and hyaloronidase (*hyl*) as secretary factors have been described in *E. faecalis* (24 ; 13).

Aggregation substance (AS) as surface protein adehesion encoded by *asa1* which is carried on a plasmid (39). Gelatinase ia a zinc-endopeptidase produced by *E. faecalis* that is capable of hydrolyzing gelatin , collagen , casein , hemoglobin and other peptides . Gelatinase encoded by the chromosomal *gelE* (40). Cytolysin is a bacterial toxin with hemolytic and bacteriolytic activity . Cytocylin genes are carried on a plasmid or are integrated into the bacterial chromosome 8).The *E. faecalis* cytolysin operon has been characterized and the regulation of cytocylin expression described (20).

The enterococcal surface protein , adhesion-associated protein and enterococcal surface adhesion , encoded by the chromosomal *esp* , *efaA* and *ace* respectively , which are associated with increased virulence , colonization and have been shown to enhance the persistence of *E. faecalis* in the urinary bladder during experimental urinary tract infection (Shanker *et al.* 2001).Recently another virulence factors, hyaluronidase was described in *E. faecalis* which is expressed by the *hyl* chromosomal gene , acts on hyaluronic acid and increases bacterial invasion (39).

The purpose of this study were to define the antibiotic resistance pattern and to investigate the presence of genes encoding several virulence factors in *E. faecalis* strains isolated from raw milk samples in Diwaniyah city- Al-Qadisiya governorate .

Materals and methods:

- Source and collection of milk samples:

This investigation was based on collection of 40 raw milk samples (10) samples for each cows , goats , buffalo and sheep) from different farms in Diwaniyah city /Al-Qadisiya governorate between May and July 2012 . The raw milk samples were collected into clean sterile bottles and transported in an ice box to the laboratory of the department Biology / College of Education / Al-Qadisiya university , where examinations of the milk samples were done after collection of samples .

- Microbiological analyses :

For the enumeration of Enterococci , one ml of each samples was diluted in peptone diluting saline to obtain the dilutions of 10^{-1}- 10^{-7} and 0.1 ml of each dilution was spread on Rapid Hi-Enterococci agar M1414 (Himedia , India). The plates were incubated for 24 hrs. at 37 C^{o} (16).Typical blue colonies with the appearance of *E. faecalis* were selected for farther study.

E. faecalis were presumptively identify by the following tests : observation of cell morphology , gram staining , catalase , growth at 15 and 45 C^{o} in the presence of 6.5% NaCl and at pH 9.6 (7). HiStrep system (Himedia , India) was used for *E. faecalis* isolates identification according to the manufacture's instructions . All isolates were kept in Brain heart infusion broth with 15% glycerol at -20 C^{o} until further analysis .

-Phenotype method for hemolytic activity and gelatinase production

The hemolytic activity of isolates was determined according to (3) on blood agar base plates containing 5% (V/V) of sheep blood . After incubating the plates at 37 C^{o} for 24 hrs. , the hemolytic reaction was recorded by the observation of hydrolysis of red blood cells and appearance of clear zone around bacterial isolates .

Gelatinase production was detected by inoculating *E. faecalis* isolates onto trypticase soya agar containing 3% gelatin . The appearance of turbid halos or zones around the colonies after incubation at 37 C^{o} for 24 hrs. was considered to be a positive indication of gelatinase production (30) .

-Resistance of selected isolates to antibiotics

Antibiotic resistance of the isolated strains was tested by agar disc diffusion method on muller hinton agar with 10% of sheep blood . The following antibiotic discs (HiMedia , India) were used gentamycin , tetracycline , norfloxacin , penicillin , nitrofurantoin , chloramphenicol , erythromycin and ampicillin . After incubation 37 C^{o} for 24 hrs. Interpretation of results was according to the standards adopted from United States Committee for Standardization of Clinical Trials , 15to 20 mm zone of inhibition is sensitive , 10 to 14 mm is the medium sensitive and 10 mm below is resistant (42) .

- DNA extraction

The total genomic DNA of the *E. faecalis* was isolated using the DNA extraction and purification kit (Geneaid , Korea) according to the manufacturer

instructions . DNA preparations were then analyzed by electrophoresis in 1.5% agarose gel .

-genotype identification using 16S rDNA

Genetic identification to species level was performed by16S rDNA as described by(2) briefly , the PCR reaction mixture (Bioneer , Korea) contained 5 µl template DNA , 1.5 µl of reverse primer (10 pmol/ µl) , 1.5µl of forward primer (10 pmol/ µl) , 250µM of dNTP , 1.5 mM of $MgCl_2$, 30 mM of KCl and 1 unit of Taq DNA polymerase with PCR water added to obtain 20 µl final volume in the PCR tube . The primers used for amplification of 16S rDNA gene were described in table (1) with expected product size of 290 bp. Samples were amplified in a DNA AMP thermocycler system (TECHNE , USA) by heading for 5 min. at 95 C^o , followed by 30 cycles of 95 C^o for 60 s. , 58 C^o for 60 s. and 72 C^o for 6o s. and a final step of 72 C^o for 10 min. Gel electrophoresis was carried out by using 0.8% agarose in TBE (Tris – Borate – EDTA) . The DNA samples were loaded on the loading dye (BioBasic , Canada) and the voltage was then applied 100 v/cm after soaking in TBE buffer . The DNA was visualized using UV transilluminater afer staining the gel with ethidium bromide (10 mg/ml) for 20 min. .

-Oligonucleotide primers and Multiplex PCR :

The five Oligonucleotideprimer pairs (table 1) used to amplify the genes asa1 , *gelE* , *cylA* , *esp* and *hyl* . The expected amplicon sizes are listed in table (1) . The Oligonucleotide used in this sudy were previously reported elsewhere (39) . The Multiplex PCR assays were performed in a DNA AMP thermocycler system (TECHNE , USA) as a final volume of 20 µl total containing AccuPower Multiplex PCR premix (Bioneer , Korea) , 0.2 µM of each primer and 5 of DNA template . PCR buffer added to obtain 20 µl final volume in the PCR tube . The temperature and time conditions of the PCR program as follows : An initial activation step at 95 C^o for 15 min., during which the HotStar Taq DNA polymerase is activated , was followed by 30 cycles of denaturation 94 C^o for 1 min. , annealing 56 C^o for 1 min. and extention 72 C^o for 1 min. followed by one cycle consisting of 10 min. at 72 C^o . After amplification , 5 µl of the amplicon was added directly on 1.5% agarose without addind a loading dye to analyze the PCR products and electrophoresed for 1 hrs. at 100 v/cm in TBE buffer containing 0.05 mg of ethidium promide per liter . A 100 bp DNA ladder (Bioneer , Korea) was used as a molecular size marker.

Table 1 PCR primers and products for detection of *E. faecalis* virulence determinants

Gene	Sequence (5'-3')	Product size (bp)	References
16S rRNA	TGGCATAAGAGTGAAAGGCGC GGGGACGTTCAGTTACTAACGT	290	(2)
asa1	ASA11- GCACGCTATTACGAACTATGA ASA12- TAAGAAAGAACATCACCACGA	375	(39)
gelE	GEL 11- TATGACAATGCTTTTTGGGAT GEL 12- AGATGCACCCGAAATAATATA	213	
cylA	CYT I- ACTCGGGGATTGATAGGC CYT IIb- GCTGCTAAAGCTGCGCTT	688	
esp	ESP 14F- AGATTTCATCTTTGATTCTTGG ESP 12R- AATTGATTCTTTAGCATCTGG	510	
hyl	HYL n1- ACAGAAGAGCTGCAGGAAATG HYL n2- GACTGACGTCCAAGTTTCCAA	276	

- Statistical Analysis

The results were analysed statistically by Chi- square (X^2) test at the level of significant when P- Value ≤ 0.05 (29) .

Results and discussion:

Enterococci naturally occur in raw milk as part of it′s microbial population , in Iraq no standards are set for enterococci either , given that these bacteria are not routinely counted in raw milk .

The results showed (table 2) , the raw milk had a total viable count in the range from $1*10^2$ – $34*10^6$ CFU/ml and all raw milk samples were positive for the presence of enterococci . There was not significant difference ($P\leq 0.05$) among the milk samples collected from different animals (cows , goats , sheep and buffalo) . These findings are similar with those of (18 ; 16 ; 26) .

Table (2): Numerate of Enterococci in raw milk samples (CFU/ml)

No. of sample	Cows	Goats	Sheep	Buffalo
Sam.1	$2*10^5$	$14*10^4$	$17*10^6$	$4*10^2$
Sam.2	$1*10^2$	$2*10^4$	$47*10^3$	$28*10^5$
Sam.3	$12*10^2$	$92*10^3$	$52*10^4$	$37*10^3$
Sam.4	$3*10^4$	$39*10^3$	$14*10^2$	$25*10^3$
Sam.5	$14*10^3$	$24*10^4$	$20*10^3$	$34*10^6$
Sam.6	$15*10^2$	$8*10^3$	$6*10^4$	$18*10^4$
Sam.7	$25*10^2$	$9*10^5$	$6*10^5$	$8*10^4$
Sam.8	$13*10^3$	$5*10^5$	$24*10^5$	$9*10^2$
Sam.9	$5*10^4$	$3*10^3$	$22*10^3$	$32*10^4$
Sam.10	$23*10^3$	$7*10^5$	$21*10^5$	$29*10^3$

Enterococci primarily inhabit the gastrointestinal tract of mammals (26) . However , recent ecologic study have demonstrated that Enterococci were spread in the environment . According to (22) enterococci come to milk primarily while grazing in the grass and secondarily from environment where milk is handled . Considering that enterococci are predominantly ubiquitous , it may be in appropriate to regard them as indicators of faecal contamination (25) because of the ability to colonize a diverse of niches , enterococci can be found in raw milk as part of its microbial population (18) .

Higher level of enterococci were considered a result of rears unclean environmental conditions ,therefore , it is likely that raw milk might be contaminated from manure , soiled bedding and soil (19) . In addition , water used for cleaning the milking equipment and washing hands has been associated with potential source of enterococci (5) , therefore, (33) suggested that prior to using detergents , it is essential that the equipment be washed with cold water to remove as much previous milk and dirt as possible followed by washing with warm water to remove fatty deposits . Afterwards , the equipment has to be washed again with warm water and stored in clean , dry and dust free area .

From the forty collected raw milk samples , a total of 20 *E. faecalis* isolates randomly picked from Rapid Hi-Enterococci agar medium (figure 1) .

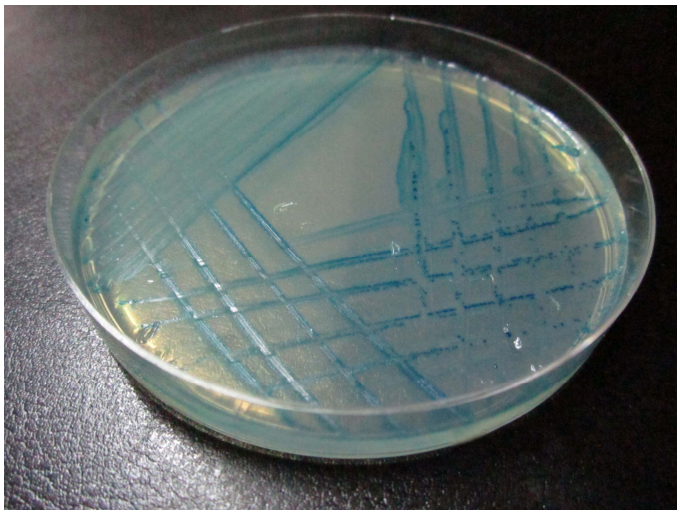

Figure (1): *E. faecalis* on Rapid Hi-Enterococci agar medium (blue color)

For identification of *E. faecalis* used standard conventional and commercial tests . Positive gram stain , growth in the presence of 6.5% NaCl , absence of catalase , growth at 15-45 C^{o} and pH 9.6 in brain heart infusion broth were observed . Hi strep. system was used for identification to species level. These bacterial isolates were further identified by partial sequencing of the gene coding for the 16S rDNA (2). The confirmation of identified isolates were *E. faecalis* (figure 2)

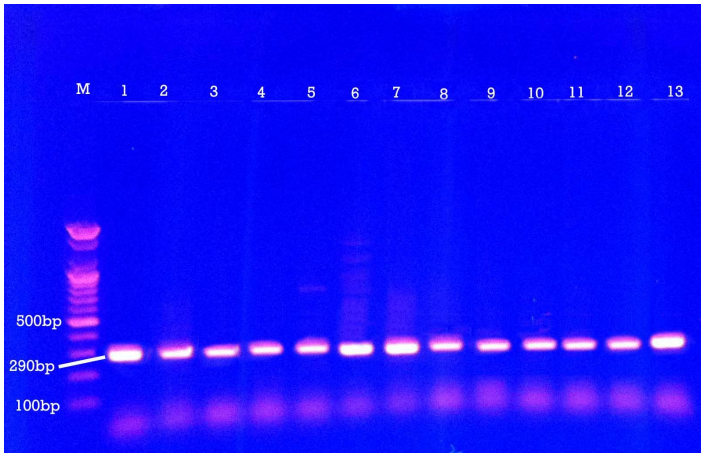

Figure (2) : Gel electrophoresis of PCR amplification products 16S rDNA (290 bp)

-Detection of virulence genes by multiplex PCR :

The multiplex PCR described is a fast , specific and reliable method which can be routinely used as an alternative to time consuming traditional tests (39). The results of the PCR amplification of the putative virulence genes tested are reported in table (3) anf figure (3). All genes were always detected in *E. faecalis* isolates. These isolates possessed between one (10%) and four (20%) determinants with the majority (55%) having three virulence factors . none of the isolates examined showed all of the virulence genes investigated in this study .

Table (3) virulence factors encoding in *E. faecalis* strains

Strains of *E.faecalis*	Phenotypic assay		Virulence genes					No.of virulence factors
	Gelatinase activity	β-hemolytic activity	*gelE*	*hyl*	*asa1*	*cylA*	*esp*	
1	+ve	-ve	+	+	+	-	-	3
2	-ve	-ve	-	+	+	-	-	2
3	-ve	+ve	-	+	-	+	+	3
4	-ve	-ve	-	+	+	-	+	3
5	-ve	-ve	-	+	-	-	-	1
6	-ve	-ve	-	-	+	-	+	2
7	+ve	-ve	+	+	+	-	+	4
8	-ve	-ve	-	+	+	-	+	3
9	+ve	-ve	+	+	+	-	-	3
10	+ve	-ve	+	+	-	-	+	3
11	-ve	-ve	-	+	+	-	+	3
12	-ve	-ve	+	+	+	-	+	4
13	+ve	-ve	+	+	+	-	+	4
14	-ve	-ve	-	+	+	+	+	4
15	+ve	+ve	+	-	-	+	+	3
16	-ve	-ve	+	-	+	-	-	2
17	+ve	-ve	+	+	+	+	-	3
18	-ve	-ve	-	-	+	-	-	1
19	-ve	-ve	-	-	+	+	+	3
20	+ve	-ve	+	-	-	-	+	2
Total (%)	8 (40%)	2 (10%)	10 (50%)	14 (70%)	15 (75%)	5 (25%)	13 (65%)	

+: presence virulence factor gene +ve : positive results

- : absence virulence factor gene -ve : negative results

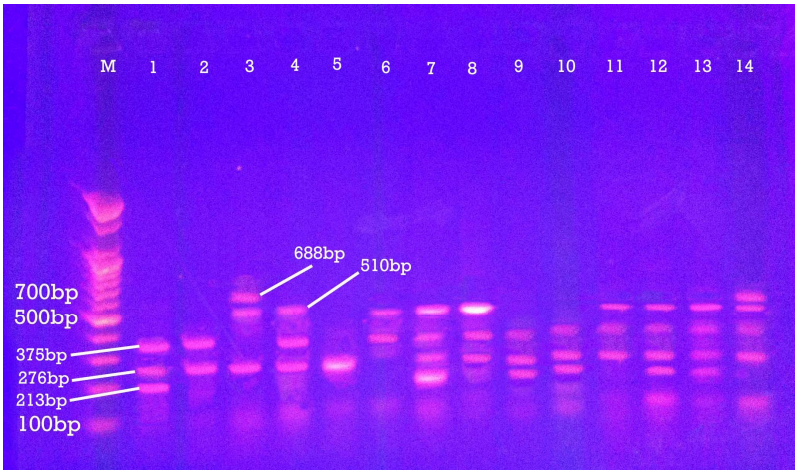

Figure (3) agarose gel electrophoresis of amplified *asa1*(375bp), *esp*(510bp), *gelE*(213bp), *cylA*(688bp) and *hyl* (276bp) by multiplex PCR . 1.2 kbp DNA ladder .

Furthermore , the results showed (table 4) . that multiplex virulence gene co-existed in the *E. faecalis* isolates . three genes were found to co-exist predominantly in the strains , generally the genotype *hyl* , *esp* and *asa1* were predominantly found in 4 (20%) of the strains study . (11) suggested that presence of determinants in combination possible synergistically activity in causes of infections .

According to the type factor , no distinct pattern could be observed but this study found that 75% of positive *asa1* strains (10).

Table (4) : Genetic linkage of virulence factors-related gene clusters among *E. faecalis* strains isolated from raw milk.

Virulence markers	No. (n=18) (%)
hyl , asa1	1(5%)
gelE, hyl,asa1	3(15%)
hyl,cylA,esp	1(5%)
hyl,asa1,esp	4(20%)
asa1,esp	1(5%)
gelE,hyl,asa1,esp	3(15%)
gel,hyl,esp	1(5%)
hyl,asa1,cylA,esp	1(5%)
gelE,cylA,esp	1(5%)
asa1,cylAesp	1(5%)
gelE,esp	1(5%)

The present study(table 3) revealed higher frequency of the *asa1* gene (coding aggregation substance) among *E. faecalis* . in agreement with other research that revealed higher incidence this gene in *E. faecalis* (11 ; 41). The results showed that *hyl* gene was found in 14 *E. faecalis* isolates (70% of 20) table 3. more often *hyl* gene was combined with the other virulence factors , alone it was present in only one strain . According to some investigation points out its role in infection (38). Up until now , the *hyl* gene seemed to be specific for *E. faecium* (39). However , in this study it

was also detected in *E. faecalis* . it can be concluded that *E. faecalis* can code for *hyl* gene . this agreement with other study by (38).

The present study revealed 65% of the *esp* gene (coding enterococcal surface protein) among *E. faecalis* isolates as for adhesion . the percentage of strains bearing esp genes was similar to that reported in other studies for *E. faecalis* (40 ; 28). Since the first study on esp gene and its role in bacterial adhesion has been published (37), several conflicting results have been reported (23) demonstrated that in vitro adherence to urinary tract catheters was independent by *esp*.

Gelatinase and hemolytic activity producing strains of *E. faecalis* have been shown to be virulent in animals models of enterococcal infection (Cosention *et al.* , 2010). The *gelE* gene (coding for gelatinase is an extracellular zinc metallo endopeptidase) was present in (10) 50% of the *E. faecalis* isolates (table 3). This results of present study are similar to that shown by (28) Londrina , Brazil .

Cytolysin gene (coding hemolysin causes rapture of variety of the target membranes , including bacterial cells , erythrocytes and other mammalian cells , with a hemolytic activity on some types of blood agar (9) was found in 5 *E. faecalis* strains (25% of all) . such results didn't agree with study of (43) who wasn't found any *E. faecalis* isolates had this gene.

As the correlation between phenotypic and genotypic tests , in this study the detection of some factors by PCR did not always correlate with phenotypic expression . the gelatinase gene was found in 10 isolates in all tested species . Moreover, 2 of the gel positive isolates didn't express gelatinase activity (table 3). Two silent gene not showing phenotypic activity despite the presence of the gel gene were found in *E. faecalis* . these silent genes were also described by (38) and (10) in their studies.

The *cylA* gene was also observed only in 5 isolates in all tested species but 3 of the gel positive isolates didn't express hemolytic activity on blood agar (table 3). That mean three silent genes didn't show phenotypic activity in spite of presence of the *cylA* gene . these silent genes were described by (34) and (10). Therefore both genotypic and phenotypic assays seem necessary for a better characterization of the strain.

- Antibiotic resistance

Overuse and misuse of antimicrobial in food animals represent a public health risk as they contribute to the emergence of resistant forms of disease-causing bacteria . such as resistant bacteria can be transmitted from those food animals to humans, primarily via food . then , infections can results that are difficult to cure since the resistant bacteria don't respond to treatment with covenantal antimicrobials (7).

Antibiotic resistance showed that (100%) *E. faecalis* isolates were susceptible to chloramphenicol . whereas 12(60%) *E. faecalis* isolates were resistant to the erythromycin , 9(45%) isolates were resistant to ampicillin , 6(30%) isolates were resistant to the nitrofurantoin , 4(20%) isolates resistant to gentamycin . 3(15%) isolates were resistant to the teteacycline and pencillin and 2(10%) isolates were resistant to norfloxacin and rifampicin. Multi drug resistance was observed in 13(65%) isolates of *E. fecalis* for 2 or more antibiotics (table 5) .

In the present study of antibiotic resistance, the strains were seen to be highly sensitive to the more commonly used antibiotics (chloramphenicol , rifampicin , tetracycline , and norfloxacin (100% , 90% , 70% and 70% respectively) . Whereas resistance was observed in *E. faecalis* isolates against erythromycin , ampicillin and

nitrofurantoin (table 5) . These results agreed with the study (7) and (24) who found that the rate of resistance to ampicillin in *E. faecalis* isolates were (42% and 41%) respectively . on the other hand these results disagreed with (6) who found that the rate of resistance to ampicillin was 88.2% . The study on antibiotic resistance among *E. fecalis* revealed that although many of these strains showed resistance to one or more of the antibiotics , the majority of the isolates were still sensitive to the clinically relevant antibiotics such as chloramphenicol and rifampicin . This results agreed with the study (27) who found that rate of susceptible was high to a large number of antibiotics

Table (5) : percentage of susceptible , intermediate and resistant to 9 commonly used antibiotic among *E. faecalis* strains.

***E. faecalis* isolates** / **Antibiotic category**	**Gentamycin**	**teteacycline**	**norfloxacin**	**pencillin**	**nitrofurantoin**	**chloramphenicol**	**rifampicin**	**erythromycin**	**ampicillin**	**No. of antibiotic resistance**
1	R	R	S	S	R	S	S	S	S	3
2	S	S	R	S	S	S	R	R	S	3
3	S	S	S	S	S	S	S	I	R	1
4	R	S	S	S	R	S	S	I	R	3
5	S	I	I	S	S	S	S	S	R	1
6	S	S	I	I	S	S	R	R	S	2
7	S	S	I	S	S	S	R	R	S	2
8	S	S	S	S	R	S	S	S	R	2
9	R	S	S	I	R	S	S	R	S	3
10	S	R	S	R	S	S	S	R	S	3
11	S	S	I	S	S	S	S	R	R	2
12	S	I	S	S	I	S	S	R	S	1
13	I	S	S	R	I	S	S	R	R	3
14	I	S	S	S	R	S	S	S	S	1
15	S	S	S	S	S	S	R	R	R	3
16	I	I	S	I	I	S	S	R	S	1
17	S	S	S	I	S	S	R	S	R	2
18	S	R	S	S	S	S	S	R	S	2
19	R	S	S	R	S	S	S	S	R	3
20	S	S	R	S	R	S	S	R	S	3
Resistance (%)	4 (20)	3(15)	2(10)	3(15)	6(30)	0	2(10)	12(60)	9(45)	
Susceptible(%)	13(65)	14(70)	14(70)	13(65)	11(55)	20(100)	18(90)	6(30)	11(55)	
Intermediate(%)	3 (15)	3(15)	4 (20)	4 (20)	3(15)	0	0	2(10)	0	

*R: Resistance , S: Susceptible , I: Intermediate

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التحري عن بعض عوامل الضراوة في المكورات المعوية البرازية المعزولة من الحليب الخام باستخدام تفاعل الكوثرة المتعدد

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الخلاصة :

بما أن الحليب غذاء عالي التغذية فانه عرضة للتلوث بالمكورات المعوية والتي تنتشر بشكل واسع في البيئة . قيمت هذه الدراسة 40 عينة من الحليب الخام (10 عينات لكل من الأبقار والأغنام والماعز والجاموس) للتلوث بالمكورات المعوية . جمعت عينات الحليب الخام من مزارع مختلفة في مدينة الديوانية \ محافظة القادسية للمدة من أيار إلى تموز للعام 2012 .تضمنت الدراسة إحصاء عدد المكورات المعوية في عينات الحليب الخام ، كما تضمن موضوع هذه الدراسة الكشف عن جينات بعض عوامل الضراوة الرئيسية للمكورات المعوية البرازية وهي *asa1* و *gelE* و *cylA* و *esp* و *hyl* بواسطة تفاعل البلمرة المتعدد . تم تحديد أنتاج هذه العزلات لإنزيم الجيلاتنيز والفعالية التحللية للدم مظهريا .

أظهرت نتائج هذه الدراسة احتواء عينات الحليب على المكورات المعوية بعدد تراوح بين $1*10^2 - 34* 10^6$ وحدة حقل الخلية / مل . كما كانت جميع عينات الحليب الخام ايجابية التواجد للمكورات المعوية . لم تسجل فروق معنوية بين عينات الحليب الخام المختلفة المصدر . عزلت 20 عزلة بكتيرية اثبت عائديها للمكورات المعوية البرازية باستخدام الاختبارات الكيموحيوية التقليدي ونظام HiStrep . كما استخدمت تقنية PCR للتحري عن ألجين 16S rDNA كعامل أثبات على أن البكتريا المشخصة هي المكورات المعوية البرازية بحجم قطعة وراثية مبلمرة بلغت 290 زوج قاعدي .

اظهر تفاعل البلمرة المتعدد امتلاك المكورات المعوية البرازية لواحد او أكثر من جينات عوامل الضراوة المدروسة . كان الجين *asa1* السائد وبنسبة (75%) ، يليه الجين *hyl* وبنسبة (70%) . في حين أظهرت الجينات *esp* و *gelE* و *cylA* نسب مئوية بلغت 65% و 50% و 25% على التوالي . اظهر اختبار فحص الحساسية للمضادات الحيوية ان من مجموع 20 عزلة كانت 12 (60%) عزلة مقاومة لمضاد الاريثرومايسين و 9(45%) عزلات مقاومة للامبسلين . ظهر انخفاض واضح للمقاومة بين عزلات المكورات المعوية البرازية للمضادات الجنتامايسين و التتراسايكلين و النورفلوكساسين و البنسلين والريفامبسين وبنسب 20% ، 15% ، 10% ، 15% ، 10% على التوالي . لم تظهر أي من العزلات مقاومتها لمضاد الكلورامفينيكول اذ كانت جميعها حساسة له.