The Effect of Enteocin-Producing Enterococci Isolated from Stool on the Growth of Some Other Bacteria

سما سميع أفرام، آسو أحمد محمد نور
جامعة كركوك/كلية العلوم / قسم علوم الحياة
Asma Sami' Afram, Aso Ahmed Muhammed Noor
UNIVERSITY OF KIRKUK/ COLLEGE OF SCIENCE/ DEPARTMENT OF BIOLOGY

ABSTRACT

Enterococci are Gram-positive bacteria present inside human and animal, where comprise, beside other bacteria, the normal flora of different parts of their body, and can be found in the environment.

The research involves studying of the action of 19 enterocin-producing isolates of *Enterococcus faecalis,* isolated from stool of healthy persons collected in test tubes containing SF broth, which is a differential and selective media for enterococci.

The isolates were grown on BEA which is also differential for the genus. Further diagnosis was made, declaring that all the isolates (100%) belong to *E. faecalis* species not to *E. faecium*.

The activity of selected samples against other target bacteria was tested. The target bacteria were: *Acinetobacter baumannii, Bacillus cereus,* non-enterocin-producing *Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus* and *Staphylococcus epidermidis,* and different rates of activity of each sample were observed against each of the target bacteria. Totally, the most rate of activity was towards *Staphylococcus epidermidis,* and the least was towards *Bacillus cereus.*

Introduction

The Genus Enterococcus

The term "entérocoque" was first used by Thiercelin in a paper from France published in 1899 (1). The same year, MacCallum and Hastings characterised a similar organism, recently known to be *Enterococcus faecalis*, from a fatal case of endocarditis, thus providing a first detailed description of its pathogenic characteristics (2). However,it was preferred to know this newly found bacteria as *Streptococcus faecalis,* as they were seen that they were so characteristic of the human intestine that this term may justly be applied to it (1).

In the year 1937 Sherman proposed a separation of streptococci into four groups: pyogenic, viridans, lactic, and enterococcus, based on hemolytic reaction, group carbohydrate antigens, and phenotypic tests (3). He used the term 'enterococcus' to comprise streptococci of faecal origin which have reduction action, resist heat, tolerate bile, and ferment mannitol (4).

Based on difference in morphology, biological, cultural and serological peculiarities, Kalina proposed that *Streptococcus faecalis* and *Streptococcus faecium* should be transferred to the genus "*Enterococcus*" (5). Later, distinction between the enterococci and *Streptococcus bovis*, *Streptococcus equinus*, and other streptococci by comparative biochemical studies was demonstrated (6), and immunological studies confirmed this (7). Nucleic acid studies, in particular, DNA–rRNA homology studies and comparative oligonucleotide cataloguing of 16s rRNA have confirmed that *Streptococcus faecalis* and *Streptococcus faecium* are only distantly related to *Streptococcus bovis* and *Streptococcus equinus* (8). This work was cited in Bergey's Manual of 1984 and considered to support the creation of an entirely new genus to encompass the enterococcal group of organisms (1).

The genus belongs to the family Enterococcaceae (9), it is a member of human normal microbiota inhabiting lumen (10) and may colonise skin as a member of the

transient skin flora (11). Enterococci are primarily of faecal origin and can be human pathogens, and have the potential for causing Health Associated Infections, so they are the most common pathogens found in the blood after *Staphylococcus* spp. (12).

As well as clinical specimens, Enterococci can be isolated from food-stuffs (13), different animals, plants (14), untreated waters (15) and soil (14). This distribution may be explained by their persistence and resistance to growth-inhibiting factors such as acidity, salt, drying, heat and chemical agents (16).

Enterococci are indicators of the presence of faecal material in water, and, therefore, of the possible presence of pathogenic viruses, bacteria, and protozoa (17).

Classification of the Genus *Enterococcus*

The taxonomy of *Enterococcus* spp. was established based upon molecular tools, such as: polymerase chain reaction (PCR), nucleotide sequencing, restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), pulsed-field gel electrophoresis (PFGE), denaturing gradient gel electrophoresis (DGGE) (18).

Modern classification of the genus *Enterococcus* is presented in Table 1.

Table 1: Modern Classification of the Genus *Enterococcus*

Bacteria	Domain

Firmicutes	Phylum
Bacilli	Class
Lactobacillales	Order
Enterococcaceae	Family
Enterococcus	Genus

Enterococcus spp. of Medical Importance

Enterococcus have been recognised as being potentially pathogenic for humans since the turn of the last century, two species: *E. faecalis* and *E. faecium* are the causative agent of many serious infections to human (19).

Although *E. faecalis* and *E. faecium* account for approximately 90% of the nosocomial enterococcal infections (20), some other non-faecalis and non-faecium enterococci species: *E. casseliflavus, E. gallinarum, E. avium, E. hirae, E. raffinosus, E. cecorum, E. durans* and *E. canintestini* have been isolated from clinical specimens (21).

Diseases caused by Enterococcus sp.

The two species: *E. faecalis* and *E. faecium* cause many diseases for human including periodontitis, peri-implantitis, pharyngitis, otitis, mastoiditis, meningitis, endocarditis, urogenital tract infections and even septic conditions (22).

Bacteriocins

All living organisms make antimicrobial proteins (AMPs) (23), and when these AMPs are synthesised by bacteria, they are known as 'bacteriocins' (24). The first bacteriocin was discovered by the Belgian microbiologist André Gratia from *E. coli* in the year 1925 (25).

Bacteriocins, which are ribosomally synthesised antimicrobial peptides, are active against a wide range of pathogens (26).

Since Lactic Acid Bacteria (LAB), to which *Enterococcus* spp. belong, have been utilised as starter cultures for the production of a diverse dairy products for thousands of years and are Generally Regarded As Safe (GRAS) by WHO and the US Food and Drug Administration (27), their bacteriocins are described as natural inhibitors, in regard to the long history of safe use of LAB in food industry (28). The last three decades can be regarded as "the golden era" for the discovery of novel bacteriocins produced by LAB (29).

Classification of Bacteriocins of LAB

Bacteriocins are very heterogeneous compounds in nature; they do have certain characteristics in common which can be used for classification. An approach is used to classify the bacteriocins of lactic acid bacteria (LAB), it divides these peptides into Class I peptides, Class II peptides, and Bacteriolysins (30). This classification is based on genetic and biochemical characteristics such as molecular size, chemical structure, physical properties and mode of action (31). The classification is of the bacteriosins of LAB is abbreviated in Figure 1.

Class I Bacteriocins

Class I bacteriocins are comprised of ribosomally synthesised, post-translationally modified bacteriocins (RiPPs) (30). This class includes Lantibiotics, Sactibiotics and TOMMs.

1. Lantibiotics

Lantibiotics (lanthionine-containing antibiotics) are small peptides (19–38 amino acids in length) that possess the eponymous lanthionine (Lan) or β-methyllanthionine (β-MeLan) residues (32), which are unusual amino acids (33). Lantibiotics have been

classified into four classes, class I, II, III and IV, according to the biosynthetic enzymes participate in the synthesis of their Lan and MeLan residues (34).

2. Sactibiotics

They resemble lantibiotics (35) but differ by incorporation of sulphur to α-carbon linkages (36)

3. TOMMs

Thiazole/Oxazole-Modified Microcins (TOMMs) are ribosomally produced peptides with posttranslationally installed heterocycles derived from serine, cysteine and threonine residues (37).

Class II Bacteriocins

Class II bacteriocins contain peptides with minor modifications (e.g., disulfide bridge or circularisation) or without modified residues (38). They do not require enzymes for their maturation other than a transporter and/or a leader peptidase (39). This class is divided further into four subclasses:

Subclass IIa (anti-Listeria pediocin-like bacteriocins)

They are narrow-spectrum bacteriocins (40). All the class IIa bacteriocins are reported to be active against *Listeria*, while only part of the bacteriocins from other classes are antilisterial (41).

Subclass IIb (two-peptide bacteriocins)

These are two-peptide bacteriocins, whose activity depends on the synergy between two different peptides (42).

Subclass IIc (circular bacteriocins)

Circular bacteriocins are synthesised as linear precursor peptides that contain a leader peptide (2 to 35 amino acid residues) bound to a propeptide (58 to 70 amino acid residues) (43). They are characterised by the head-to-tail cyclization of their backbone (44).

Circular bacteriocins are now considered possible alternatives to therapeutic antibiotics due to the exceptional stability granted by their circular structure (43).

Subclass IId (non-pediocin-like bacteriocins)

They are non-pediocin, linear, one-peptide, and leaderless bacteriocins (31). This subclass of bacteriocins show significant divergence in their primary structures and modes of action (45)

Bacteriolysins

This class was formerly known as class III bacteriocins. Bacteriolysins have large heat stable antimicrobial proteins. These are mainly non-bacteriocin lytic proteins with a domain type structure, e.g. lysostaphin, helveticin J. (46).

Differences between Bacteriocins and Antibiotics

There is some ambiguity in the literature between bacteriocins and classical antibiotics, so, the difference between the two is clarified in the following points:

1. while bacteriocins are ribosomally synthesised, antibiotics are synthesised by unique enzymatic systems (47)

2. most bacteriocins are narrow-spectrum in killing or inhibition (48), targeting closely related bacteria (49), but antibiotics are generally broader in target specificity (47). Only a small number of bacteriocins are known to be broad spectrum (50).

3. each bacteriocin has its own dedicated immunity protein, the gene coding its synthesis is linked to the gene of the bacteriocin, while genetic determinants for antibiotic resistance are not linked to the genes encoding synthesis of antibiotics, and each is expressed independently (47).

4. Bacteriocin production usually occurs in the growth phase (51), while antibiotics, which are secondary metabolites, produced in the stationary phase (52).

Enterocins

Enterocins (were also known formerly as: enterococcins, D streptococcins or D streptocins (53)), produced by *Enterococcus faecalis, Enterococcus faecium* and other Enterococci, are a group of safe and ribosomally synthesised antimicrobial peptide bacteriocins (54) notified for the first time by Ebbe Kjems while he was studying inhibition of some test bacteria that some of the enterococcal strains produced inhibitory substances, concluding that the substances might be similar to the colicins produced by *E. coli*, previously known (55). Another study represented a survey of bacteriocin production by a wide variety of well-characterised strains, and showed that more than one different bacteriocins are produced by various strains of enterococci, and certain of these bacteriocins can act on other lactic acid bacteria as well as on some bacterial genera more distantly related (56).

In a study fulfilled in 1969, the researchers also suggested the production of more than one type of enterocin by a single strain, as they noticed that products varied considerably in their response to heat, chloroform, ether and different enzymes (57).

J. Krämer and H. Brandis reported, in research performed in 1975, the purification of two enterocins produced by *Enterococcus faecium* and some of their properties. They named them: Enterocin EIA and Enterocin EIB (58).

Materials and Method

Sample Collection

Ninety seven (97) stool samples that were collected in the period November 11, 2017, until May 27, 2018, from healthy people were examined and diagnosed through cultural and microscopical characteristics of bacterial cells until genus, and then, species level. The specimens collected were stools of inpatient visitors of the Laboratory of General Health in Erbil, who were ordered to check to investigate if they have infections that may affect people they serve. Figure 2 represents distribution of the the people samples were taken according to age and sex.

Isolation

The samples were inoculated into tubes containing SF broth. Change of the colour of the medium and its turbidity declare growth of the target bacteria. Eighty two percent (80 out of the 97 samples) yielded growth of presumptive Enterococci.

The isolated microorganisms were identified depending on the microscopic examination, morphological examination and biochemical tests, including API system and using VITEK 2 system.

Microscopic Characterisation

Microscopical investigation of the presumptive bacterial colonies cultured on Blood Agar plates revealed coccal bacterial cells, Gram-positive, non-spore-former cells, arranged singly, in pairs and in short chains (59) (see Figure 3).

Cultural Characterization

Blood Agar

Enterococcus spp. appear as smooth, cream or white colonies with entire edges. After 24 hours of incubation, colonies were different in their ability to haemolyse human blood contained in blood agar: some isolates were nonhemolytic, others were alpha hemolytic, and some isolates showed beta haemolysis), as it is shown in the Table 2.

Table 2: Types of Haemolysis shown by the isolates of the study

Type of Haemolysis	Number of isolates
Non-haemolysis	٥٧
Alpha haemolysis	١٥
Beta haemolysis	٨
Total	٨٠

Bile Esculin Agar (BEA)

Bile esculin agar (BEA) is a selective growth media for *Enterococcus* species due to the presence of 4% bile salt in the agar (60).

The enterococci appear as small translucent colonies surrounded by black halo. Enterococci can hydrolyze esculin in the presence of bile salts and the product reacts with the iron that presents in the medium, turning its colour into black.

MacConkey Agar

Enterococci give tiny deep pink colonies, because they are lactose fermenters (61).

This medium contains bile salts that inhibit the growth of Gram-positive bacteria other than enterococci (62), due to the absence of blood as well as the presence of

high concentration of bile (63) and crystal violet in the medium which are inhibitory to them. *Enterococcus* sp. growth on such a media was observed in our test.

Mannitol Salt Agar

Enterococcus faecalis grows on this medium as small colonies with fermentation of mannitol, that is utilised to produce lactic acid, lowering the pH and turning the colour of the medium into yellow.

This medium contains a very high salt concentration (7.5%) (64) , so most bacteria are not able to grow on the agar (65), but *Enterococcus faecalis* can do (66).

Using sterile loops, all the sample bacteria were streaked on mannitol salt agar (MSA) plates. Plates were incubated at 37°C for 24 hours and growth was observed for 91% of the samples (88 out of 97) and for further 24 hours of incubation all the samples performed growth. Yellow areas appeared around bacterial growth, revealing fermentation of the sugar mannitol and production of organic acids that lower the pH of the medium and change of the indicator's colour, phenol red, from red (67).

Biochemical Tests

The samples were inoculated into tubes containing 5 mL of SF broth. This medium is selective for the detection and differentiation of enterococci from other cocci

in diagnosis (68). The medium contains peptone (as a source of nitrogen) (69) and dextrose (as a source of carbon) (70) that supply the nutrients required for the growth of enterococci, and sodium azide that exhibits a bacteriostatic effect on Gram-negative bacteria through its inhibitory action on enzymes in the electron transport system (71), and the indicator bromcresol purple serves as a pH indicator. The colour of the medium turns yellow when dextrose is fermented to lactic acid by the enterococci. Inocula from all the tubes were streaked on Brain-Heart Infusion Agar plates. After incubation and growth of the colonies, samples were taken and examined with biochemical tests.

Esculin Hydrolysis Test

The medium was streaked by bacteria obtained from Brain-Heart Infusion Agar. The bile-esculin test is widely used to differentiate enterococci (72) (Figure 4).

Test for Salt Tolerance

They also were able to grow on Brain-Heart Infusion agar containing 6.5% NaCl. The medium turns from purple to yellow as the pH drops (73). A positive result was noticed as a narrow area of the medium turned yellow during the first 24 hours of incubation, but the area grew larger through the next 24 hours.

Enterococci also show growth in Brin-Heart Infusion with pH 9.6.

All these characteristics separate them to some extent from the other Gram-positive, catalase-negative, facultatively anaerobic cocci (74).

Methylene Blue Reduction Test

The isolates showed ability of reduction of methylene blue dye (0.1%) in skim milk broth medium. This test was done using MBM medium. A tube containing the medium was inoculated with the sample bacteria, vortexed and incubated at 37°C. Another tube containing the medium too, but was not inoculated, was used as a control. After 2 hours of incubation, the medium inoculated with the bacteria turned white and the other stayed blue.

Sugar Fermentation Test

Inocula from 24 hours culture of two samples were applied to tubes containing Phenol Red Base Broth to which different sugars were added. After 24 hours incubation in 37ºC the results for selected isolates are listed in Table 3.

Table 3: The results of fermentation of different sugars by isolates of *Enterococcus* sp.

+	+	+	+	+	−	+	+	CE1
+	+	+	+	+	−	+	+	CE3
+	+	+	+	+	−	+	+	CE4
+	+	+	+	+	−	+	+	CE8
+	+	+	+	+	−	+	+	CE10
+	+	+	+	+	−	+	+	CE12
+	+	+	+	+	−	+	+	CE13
+	+	+	+	+	−	+	+	CE14
+	+	+	+	+	−	+	+	CE17
+	+	+	+	+	−	+	+	CE18
+	+	+	+	+	−	+	+	CE20
+	+	+	+	+	−	+	+	CE24
+	+	+	+	+	−	+	+	CE27
+	+	+	+	+	−	+	+	CE28
+	+	+	+	+	−	+	+	CE30
+	+	+	+	+	−	+	+	CE31
+	+	+	+	+	−	+	+	CE32
+	+	+	+	+	−	+	+	CE34
+	+	+	+	+	−	+	+	CE40
+	+	+	+	+	−	+	+	CE41
+	+	+	+	+	−	+	+	CE48

Hemolysin Production

This test was used to detect haemolysin production on Haemolysin Production Blood Agar. The medium streaked with *Enterococcus faecalis* samples were incubated overnight at 37°C in a carbon dioxide chamber and evaluated at 24 and 48 hours. A clear zone of β-haemolysis around the streak was considered to be a positive indication of haemolysin production (75).

Screening of enterocin-producing samples

The antimicrobial activity of the bacteriocin produced by *E. faecalis* was determined by its effect on indicator bacteria (*E. coli*). By a method described in detail later, a screening was made to differentiate enterocin-producing *Enterococcus faecalis* strains from non-producing ones. The screening was made on Mueller-Hinton Agar (76) (see Figure 6).

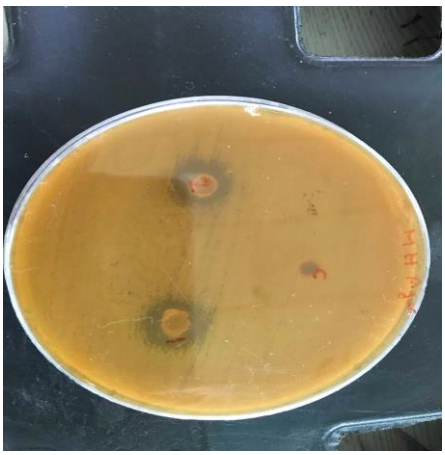

Figure 6: Enterocin-producing Enterococci Compared to Control

Assay for bacteriocin activity

Nineteen of the most apparent enterocin-producing samples were used for the next tests.

Colonies of target bacteria were suspended in normal saline (0.85% NaCl), and then the suspensions were adjusted to a turbidity equivalent to a 0.5 McFarland standard.

0.5 McFarland standard was prepared to adjust the density of culture suspensions prior to inoculation. When these standards are thoroughly shaken, the turbidity equals that of a culture containing about $1.5x10^8$ cells (77).

The suspensions were uniformly swabbed on Mueller-Hinton Agar plates and allowed to dry for 5 minutes (78).

Agar Cup-Plate Method

First, 6 mm in diameter discs of the agar were made using broad end of sterile Pasteur pipette on GYP plates. Then the *Enterococcus faecalis* samples were swabbed densely on the plates and incubated at 37°C. After 24 hours of incubation and the appearance of growth of the bacteria, the discs were transferred to Mueller-Hinton Agar plates previously swabbed with test bacteria.

Target Bacteria

Seven different bacteria were chosen to study whether *Enterococcus* is active against them or not. The bacteria are: *Acinetobacter baumannii, Bacillus cereus,* non-enterocin-producer *Enterococcus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus* and *Staphylococcus epidermidis.*

Characteristics of Target Bacteria

From the bacteria chosen, 4 are Gram-positive bacteria: *Bacillus cereus,* non-enterocin-producer *Enterococcus faecalis, Staphylococcus aureus* and *Staphylococcus epidermidis*, and the other 3 are Gram-negative: *Acinetobacter baumannii, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* From Gram-positive bacteria *Bacillus cereus* is distinguished by its ability to form spores and from Gram-negative bacteria *Klebsiella pneumonia* is distinguished by capsule formation.

Some other characteristics of target bacteria are listed in Table 4 below:

Table 4: Some Common and Different Characteristics of Target Bacteria

Capsule forming	Spore forming	Shape	Gram	Bacteria
Non	Non	Bacilli	Gram-negative	*Acinetobacter baumannii*

Non	Non	Bacilli	Gram-positive	*Bacillus cereus*
Capsule-former	Non	Cocci	Gram-positive	*Enterococcus faecalis*
Non	Spore-former	Bacilli	Gram-negative	*Klebsiella pneumonia*
Non	Non	Bacilli	Gram-negative	*Peudomons aerogenosa*
Non	Non	Cocci	Gram-positive	*Staphylococcus aureus*
Non	Non	Cocci	Gram-positive	*Staphylococcus epidermidis*

Results and Discussion

An *E. coli* isolate was tested to determine the activity of the selected *Enterococcus faecalis* isolates, being used as an indicator. 85% of the samples were active against the indicator strain (68 out of the 80 isolates), from which the most active were selected for the successive tests and checking the activity against the other target bacteria.

Enterocin 96, enterocin AS-48, Enterocin E-760 are reported to have anti- *E. coli* activity (79). Enterocin AS-48 has known to have a wide inhibitory spectrum on Gram-negative bacteria. Sensitivity of these bacteria, i. e., Gram-negative bacteria, increases in incorporation with outer-membrane permeabilising treatments (80).

The diameter of activity of enterocin-producing enterococci around discs grown on was measured in millimetres (mm). The biggest value obtained from the tests was 16 mm, and the smallest was 0. Because the active substance (enterocin) was not extracted and purified, the results of diameters of zone of inhibition cannot be compared with results of antibiotics inhibition, because the latter are extracted and purified and used in defined concentrations.

It was noticed that the bacteriocin produced by the selected samples was active against the test bacteria *Acinetobacter baumannii* isolated from clinical specimen. It was perceived in a study that enterocin-A + B exhibited potential anti-bacterial, anti-biofilm activity against *Acinetobacter baumannii* and other bacteria (81).

Acietobacter baumannii is known to be one of the most successive pathogens in modern healthcare because of its surprising ability to gain antimicrobial resistance (82).

Bacillus cereus is a toxin-producer bacteria. The toxins produced by *B. cereus* can cause two types of illness: one type characterised by diarrhea and the other by vomiting and nausea (83).

Twenty one percent (21%) of the samples grown on GYP showed activity against the bacteria, while the others were inactive.

From the characterized enterocins of *Enterococcus faecalis*: Enterocin 96, enterocin AS-48 are known to have activity against *B. cereus* (79).

It has been reported that a minor fraction of the isolated enterococci from stool samples do not produce bacteriocins (84). The susceptibility of non-enterocin-producing isolate of *Enterococcus faecalis* was tested. It was noticed that all the tested samples were active against the sample in different rates [Table 5].

All the isolates grown on GYP were active against *Klebsiella pneumonia* sample in different degree.

The activity of the enterocins produced by the selected isolates of *Enterococcus faeclais* was tested against *Pseudomonas aeruginosa* also was noted (Figure 6).

Table 5: Numerical Values of Degree of Activity of *Enterococcus faecalis* Samples against Target Bacteria in mm

Pseudomonas aeruginosa	*Staphylococcus epidermidis*	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Enterococcus faecalis*	*Bacillus cereus*	*Acinetobacter baumannii*	Target Bacteria / Sample Number
8	12	14	12	8	0	12	CE1
8	12	12	12	8	0	8	CE9
10	12	12	12	8	8	10	CE10
10	12	14	14	8	8	0	CE12
12	14	14	12	8	8	0	CE13
10	14	12	10	8	0	0	CE17
8	14	12	10	8	0	0	CE20
10	16	12	12	10	0	12	CE30
8	14	12	12	10	0	10	CE31
8	14	14	14	8	0	12	CE32
12	16	14	14	12	0	14	CE39
12	14	12	12	8	0	10	CE46
10	12	10	12	8	0	12	CE47
10	16	10	8	10	0	10	CE49
10	14	12	8	8	0	0	CE53
8	10	8	8	12	0	0	CE64
12	12	8	8	8	0	10	CE65
12	14	8	8	8	8	0	CE82
12	16	8	8	10	0	0	CE95

Enterocin 96, enterocin AS-48 produced by *Enterococcus faecalis* are reported to have activity against *Pseudomonas aeruginosa* (79).

The results show that *Staphylococcus aureus* isolate performs sensitivity towards all the samples grown on GYP.

Enterocin 96, enterocin AS-48, Enterocin E-760, Enterocin EJ97 and Enterocin K1 are reported to have activity against *Staphylococcus aureus* (79).

Stahylococcus epidermidis isolate showed the more susceptibility from the target bacteria towards the enterocin-producing enterococci.

The inhibition zones around cup agars and wells are due to the bacteriocins secreted by the bacteria *Enterococcus*, not to acids produced, or to bacteriophages. Test bacteria in the same plates out of the inhibition zones survived and tolerated the condition meaning that the cause is not acids. Bacteriophages are infective (85), so, if the cause was bacteriophages all the test bacteria in the plate was infected.

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الخلاصة

البكتريا الكروية المعوية من البكتريا الموجبة لصبغة كرام، والتي تتواجد في الإنسان والحيوانات، حيث تشكل، جنباً الى جنب مع بعض البكتريا الأخرى، النبيت الطبيعي في أعضاء مختلفة، كما أنها تتواجد في البيئة.

تضمنت الدراسة جمع ٩٧ عينة من البراز لأشخاص أصحاء في أنابيب أختبار تحتوي وسط SF، وهو وسط تفريقي واختياري للبكتريا الكروية المعوية.

تمت تنمية العزلات على وسط BEA الذي، هو الآخر، تفريقي للجنس. في خطوة لاحقة تمت تنمية العزلات على وسط MSA، حيث نمت العزلات على الوسط، مؤكدة أن العزلات تعود، وبنسبة ١٠٠%، الى نوع *E. faecalis* وليس الى نوع *E. faecium*، حيث لا يتمكن الأخير من النمو على الوسط وليس لديها القدرة على تخمير سكر المانيتول، بينما يمتلك نوع *E. faeclais* هذه الخاصية.

تم تأكيد التشخيص بواسطة نظام API واستخدام جهاز VITEK ٢ Compact.

استخدمت عزلة من بكتريا *E. coli* كسلالة مؤشرة على قدرة البكتريا قيد البحث على انتاج الانتيروسين. حيث تم عمل أقراص من وسط GYP نمِّيت عزلات البكتريا عليها مسبقاً وثبتت على أوساط مولر–هينتون منمّى عليها البكتريا الهدف ولوحظت مناطق تثبيط حول الأقراص، حيث كانت نسبة ٨٥% من البكتريا قادرة على انتاج الانتيروسين.

تمت تجربة قدرة العزلات المنتقاة من البكتريا الكروية المعوية على انتاج الانتيروسين ضد عدد من البكتريا المختلفة شملت كلاً من: *Acinetobacter baumannii*، *Bacillus cereus*، *Enterococcus faecalis* غير قابلة على انتاج الانتيروسين، *Klebsiella pneumoniae*، *Pseudomonas aeruginosa*، *Staphylococcus aureus* و*Staphylococcus pidermidis*. ولوحظت درجات متفاوتة من النشاط للعزلات ضد كل نوع من البكتريا الهدف. وككل، فإن درجة النشاط الأعلى لوحظ ضد بكتريا *Staphylococcus epidermidis*، فيما كانت الأدنى ضد بكتريا *Bacillus cereus*.