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Clinical Screening for Bacteriocinogenic *Enterococcus faecalis* Isolated from Intensive Care Unit Inpatient in Egypt

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Abstract

A bacterium originally isolated from stool sample from ICU inpatient was biochemically and molecularly characterized and identified as *Enterococcus faecalis* OS6. This strain showed the ability to exert antimicrobial activities against some members of Gram-positive bacteria as *Lactobacilli* and *Enterococci*. However, no activities were detected against all tested Gram-negative and fungal indicator strains. Diminishing of antimicrobial activities upon heat and proteinase K treatments confirmed the proteinaceous nature of the recorded activity. Therefore, strain OS6 was extensively screened for the presence of 10 common bacteriocin structural genes where genes of Enterolysin A and Cytolysin were detected and confirmed by further gene sequencing. Further characterization of strain OS6 showed several virulence determinants including gelatinase, haemolysin, bile salt hydrolase and multiple antibiotic resistance traits towards 17 out of 31 different antibiotics. Most of these 17 antibiotics belonged to Cephalosporins, Aminoglycosides, Lincomycins, Polypeptides, Quinolones, Rifamycins and Sulfonamides classes. To the best of our knowledge, this is the first attempt to report production of bacteriocins (mainly Enterolysin A) in between pathogenic *E. faecalis* isolated from human clinical specimen in Egypt.

Keywords: Bacteriocin; Enterolysin A; *Enterococcus faecalis*; Virulence

Introduction

Although *Enterococci* are natural inhabitants in gastrointestinal tract of humans [1], they are now considered as one of the major nosocomial pathogens and account for the majority of bacteremia cases in Intensive Care Units (ICUs) [2]. Additionally, *Enterococcus faecalis* (*E. faecalis*) typically display more virulence factors contributing to pathogenesis than many other bacteria [3]. The dramatic increase in the frequency of virulence traits present within *E. faecalis* is likely due to its capability to readily uptake plasmids and other genetic elements containing virulence genes [4]. Hence, *Enterococci* have displayed individual and multiple drug resistance towards many commonly used antibiotics. This inherent resistance has allowed *Enterococci* to survive in the hospital environment [5].

The emergence of extensive antibiotics resistant bacteria increased the demands for digging out new sources of antimicrobial agents. Especially bacteriocins have recently grasped considerable attention due to their potential applications in food industry as natural preservatives and in clinical trials to overcome the emergence of multi-resistant bacterial strains towards commercial synthetic antibiotics [6,7]. The production of bacteriocins has been reported to have a widespread occurrence among *Enterococci*, especially, *E. faecalis* [3].

In general, previous screening approaches for bacteriocinogenic bacteria were conventionally focused on their presence in environmental samples rather than clinical ones [3,8,9]. For instance, several studies have reported isolation of bacteriocinogenic *E. faecalis* from different resources; such as raw milk, cheese, vegetables, porcine intestinal tract, dry fermented sausage, grass silage, feces of minipigs and rumen [9-11]. On the other hand, studies reporting isolation of pathogenic *E. faecalis* from clinical samples collected from humans with relevant antimicrobial activities still rare [12]. However, the increased demands for new sources of antimicrobials necessitate the deep search in other non conventional resources.

In these perspectives, the present study reports the first attempt to identify and characterize a bacteriocinogenic *E. faecalis* isolated from stool sample collected from ICU inpatient in Egypt. The isolated *E. faecalis* was fully characterized and screened for its antimicrobial spectrum, its bacteriocin type and its ability to express some virulence and antibiotic resistance traits for the purpose of determining its safety for further possible direct or indirect uses in various applications.

Materials and Methods

Indicator strains and culture conditions

The indicator microbial strains used in this study and their culture conditions are presented in table 1. Maintenance of these strains were carried out in 40% glycerol and stored at –80°C.

Isolation and identification of relevant *Enterococci* with antimicrobial activities

Previously, 11 stool samples were collected from ICU inpatients at Beni-Suef and Kasr El-Aini Hospitals, Egypt. These samples were screened to isolate relevant Enterococcus bacteria with antimicrobial activities according to Novicki et al. [13]. Among these isolates, the strain showed the most remarkable antimicrobial activities was selected

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and subjected to full identification. Preliminary identification was carried out, morphologically using Gram stain and biochemically using streaking technique on bile esculin agar (Oxoid). Further confirmation was conducted using L-pyrrolidonyl-β-naphthylamide test (PYR test) according to [14].

Further identification up to species level was conducted using API 50 CHL (BioMe´rieux, Marcy l'Etoile, France) according to manufacturer's instructions and anaerobiosis was obtained by overlaying the inoculated strips with sterile paraffin oil and then incubated at 30°C. Observations were recorded after 24 and 48 h.

DNA isolation and 16s rDNA sequencing

Genomic DNA was isolated according to [15]. The obtained DNA pellets were re-suspended in 50 µl TE buffer and stored at 4°C. Amplification of 16s rDNA was done using specific primers (Table 2). PCR was carried out in 50 µl reaction volume in sterile 200 µl PCR tube. The PCR reaction mixture consisted of 500 ng genomic DNA, 10 mM dNTPs mixture, 1 µl (20 uM of each primer), 2.5 units of Phusion DNA polymerase enzyme (Finnzymes) and 10 µl 5X reaction buffer. The PCR conditions include template denaturation step at 94°C (3 min.), followed by 34 cycles of denaturing at 94°C (30 sec.), annealing at 56°C (30 sec.), extension at 72°C (60 sec.), and followed by completion of DNA synthesis at 72°C (5 min.).

The amplified gene was purified using QIAquick PCR purification kit (QIAGEN, Germany) and subjected to gel electrophoresis using 1% (w/v) agarose gel with reference to 1 kbp DNA ladder (Fermentas, Finland) [16]. After determining the concentration of amplified gene using NanoDrop-ND1000-Spectrophotometer (Thermo Scientific, Wilmington, USA), the DNA was sequenced using ABI Prism BigDye terminator sequencing ready reaction kit version 3.1 and analyzed with ABI Prism 3100 generic analyzer according to manufacturer's instructions. The obtained sequence was assembled by BioEdit software v. 7.0.9.0 and CLC sequence analyzer program, then homology search was done using BLAST search at NCBI website.

Determination of antimicrobial spectrum of *E. faecalis* OS6

Spot on lawn assay was used to detect antimicrobial activity towards 129 different bacterial strains (Table 1) according to De-Vuyst et al. [17]. Briefly, overnight grown colonies of *E. faecalis* OS6 were overlaid with 5 ml soft agar containing 70 µl of indicator strain and incubated overnight. The antimicrobial activities due to bacteriocin were observed as clear inhibition zones around colonies.

Effect of heat and proteinase k enzyme

The antimicrobial activity was assessed after both heat and proteinase K treatments. Thermal stability was tested by heating the cell free culture supernatant at 100°C for 30 min. The antimicrobial activity was determined in comparison to the non-treated cell free culture supernatant quantitatively by microtiter plate assay [18] using *Lactobacillus sakei* as an indicator strain. One Bacteriocin Unit (BU) was defined as the amount of bacteriocin causing 50% growth inhibition of the indicator strain.

Sensitivity towards proteinase K enzyme (Sigma) at a final concentration of 1 mg/ml was determined by both microtiter plate assay [18] and modified spot on lawn technique. For the later one, 1 µl proteinase K was added on the vicinity of *E. faecalis* colony and the plate was then incubated for 1 h at 37°C. Finally, the plate was overlaid

Indicator strains	Growth medium	Growth temp	Tested strains	Inhibited strains	Activity [a]
Aspergillus niger	SDA	30°C	1	0	0
Bacillus subtilis	BHI	37°C	2	1	10
Candida albicans	SDA	30°C	4	0	0
Escherichia coli	BHI	37°C	20	0	0
Enterococcus avium	GM17	37°C	2	2	14
Enterococcus cassiliflavus	GM17	37°C	2	2	15
Enterococcus faecalis	GM17	37°C	56	0	0
Enterococcus faecium	GM17	37°C	10	10	14
Klebsiella spp.	BHI	37°C	11	0	0
Lactobacillus plantarum	MRS	37°C	1	1	9
Lactobacillus sakei	MRS	30°C	1	1	17
Listeria innocua	BHI	37°C	1	0	0
Listeria monocytogenes	BHI	37°C	1	0	0
Mycobacterium pheli	BHI	37°C	1	0	0
Proteus vulgaris	BHI	37°C	6	0	0
Pseudomonas aeruginosa	BHI	37°C	5	0	0
Salmonella typhi	BHI	37°C	1	0	0
Sarcina lutea	BHI	37°C	1	0	0
Staphylococcus aureus	BHI	37°C	3	0	0

[a]Activity, expressed the average size of the inhibitory zones in mm.

Table 1: The recorded antimicrobial activities of Enterolysin A against different indicator strains.

with 5 ml soft agar containing 70 µl of indicator strain (*Lactobacillus sakei*) and incubated overnight at 30°C. Diminishing of inhibition zone when compared with non-treated zone indicated the sensitivity to proteinase K.

PCR screening and sequencing of structural bacteriocins genes

The presence of genes encoding for 10 common bacteriocins in *Enterococcus* species were screened by PCR amplifications using specific primers (Table 2). Confirmatively, some available strains with known bacteriocin genes were used as positive controls (Table 2). The PCR product of interest was detected by gel electrophoresis according to Sambrook et al. [16] using 1.5% (w/v) agarose gel with reference to 100 bp DNA ladder (Fermentas, Finland). The positive gene bands on the agarose gel were cut and the DNA was extracted using QIAquick Gel Extraction Kit (QIAGEN). Final confirmations of amplified genes were carried out through genes sequencing and BLAST analysis using NCBI databases.

Determination of virulence factors and antibiotic resistance phenotypes

Haemolytic activity was determined by growing *E. faecalis* isolate onto Columbia blood agar (Oxoid) containing 5% defibrinated horse blood. Proteolytic gelatinase activity was detected by inoculating *E. faecalis* isolate onto tubes containing 10 ml 2% gelatin medium. In all cases, incubation was done at 37°C for 24 h and liquefaction of gelatin was observed after keeping tubes at 4°C for 3 h. Bile salt hydrolase activity was determined by growing *E. faecalis* isolate on MRS agar containing 0.5% thioglycholate and 0.5% sodium glychodeoxy-cholate (bile salt) and the plate was incubated at 37°C in anaerobic jar for 72 h. Formation of white precipitate around the bacterial growth indicates presence of bile salt hydrolase activity.

CLSI disc diffusion method (M02-A10) was used to determine the antibiotic resistance phenotype of *E. faecalis* against 31 different antibiotics (Table 3). Mueller-Hinton agar (Oxoid) was used and the test was proceeding as described [19]. The plates were incubated at 37°C for 20 h prior to measuring and recording zones of inhibition surrounding to the discs as described [19].

Results

Isolation and identification of *Enterococci* with relevant antimicrobial activity

Preliminary, a total of 11 bacterial strains were isolated from collected clinical specimens. These strains were screened for their capability of producing antimicrobial activities, where strain OS6 showed the highest recorded antimicrobial activity (data not shown). Hence, this strain was selected and subjected for detailed identification and characterization.

Strain OS6 showed Gram-positive reaction and diplococci

Gene	Positive control strains	Sequence 5` - 3`	Fragment (bp)	Ref.
16s rDNA	N/A[a]	TAACACATGCAAGTCGAACG	1377	LMGT[b]
		ACGGGCGGTGTGTRC		LMGT
AS-48	N/A	TTTTTGGGGTTAGCCTTGTT	191	[41]
		GCTGCAGCGAGTAAAGAAG		[41]
Bacteriocin 31	N/A	TTTGTGGCATTATTGGGATT	166	[42]
		CCATGTTGTACCCAACCATT		[42]
Enterocin A	*E. faecium* LMGT 2771	GACACAACTTATCTATGGGGGTA	155	[25]
		CTGGAATTGCTCCACCTAAA		[25]
Enterocin B	*E. faecium* LMGT 2771	TGAAACAAATTATCGGTGGAG	166	[43]
		TATACATTTGCTAACCCAGCAG		[43]
Enterocin P	*E. faecium* LMGT 2772	TTTGGTACAAAAGTTGATGCAG	153	[44]
		ATGTCCCATACCTGCCAAAC		[44]
Enterocin L50A & L50B	*E. faecium* LMGT 2763	TTGGGTGGCCTATTGTTAAA	224	[26]
		TCTATTGTCCATCCTTGTCCA		[26]
Enterocin Q	*E. faecium* LMGT 2763	TTAAAGAAAGGAGGCGGAAA	107	[45]
		TGGCAAGCATCCATATTTCA		[45]
Enterolysin A	*E. faecalis* LMGT 2333	CGCAGCTTCTAATGAGTGGT	161	[12]
		CATACACACTGCCATTTCCA		[12]
Mundticin	N/A	AACAGCAAAAGAAATGTCACAA	154	[20]
		ACCAGCTGCTCCACCAGTA		[20]
Cytolysin	*E. faecalis* LMGT 2814	TGGCGGTATTTTTACTGGAG	186	[35]
		TGAATCGCTCCATTTCTTC		[35]

[a] N/A: Not available.
[b] LMGT: Laboratory of microbial gene technology.

Table 2: The designed PCR primers and positive control strains used in this study.

arrangements. The colonies showed shiny black appearance on bile esculin agar with characteristic odor (Figure 1). The isolated colonies were able to hydrolyze L-pyroglutamic acid betanaphthylamidein PYR discs and formed with cinnamaldehyde reagent a bright pink to cherry red color within one minute.

The fermentation profile using API 50 CHL showed that this isolate was able to ferment Glycerol, Ribose, Galactose, Glucose, Fructose, Mannose, Mannitol, Sorbitol, N-Acetyl Glucosamine, Amygdaline, Arbutine, Esculine, Salicine, Celibiose, Maltose, Saccharose, Trehalose, Melezitose, Gentibiose, Tagatose and Potassium Gluconate. Partial 16s rDNA sequencing yielded a sequence of up to 1377 bp. The homology of obtained sequences using BLAST tool at NCBI revealed that the species of *Enterococcus* isolate is closely related to *E. faecalis* with 99% maximum identity to *E. faecalis* strain JCM 5803 (accession number NR_040789.1) and the obtained sequence was submitted in NCBI GenBank database under accession number (JX536092), Therefore, the isolated strain was called *E. faecalis* OS6.

Classes	Antimicrobial agents (Concentration µg)	Sensitivity
Aminocyclitols	Apramycin (15 µg)	S
Aminoglycosides	Amikacin (30 µg)	R
	Streptomycin (10 µg)	R
	Gentamycin (10 µg)	R
	Kanamycin (30 µg)	S
Carbapenems	Meropenem (10 µg)	R
	Imipenem (10 µg)	S
Cephalosporins	Ceftriaxone (30 µg)	R
	Cefepime (30 µg)	R
	Cefoperazone/sulbactam (75/30 µg)	R
	Cefoperazone (75 µg)	R
	Cephalaxen (30 µg)	R
Chloramphenicol	Chloramphenicol (30 µg)	S
Fusidic acid	Fusidic acid (10 µg)	R
Glycopeptides	Vancomycin (30 µg)	S
Lincomycins	Clindamycin (2 µg)	R
Macrolides	Azithromycin (15 µg)	R
	Clarithromycin (15 µg)	S
	Erythromycin (15 µg)	S
Penicillins and Penicillins combinations	Piperacillin (100 µg)	S
	Ampicillin (10 µg)	S
	Penicillin (10 IU)	S
	Amoxicillin/ Clavulinic acid (20/10 µg)	S
Polypeptides	Polymyxin B (300 µg)	R
Quinolones	Lomifloxacin (10 µg)	R
	Ofloxacin (5 µg)	R
	Gatifloxacin (5 µg)	S
Rifamycins	Rifamicin (30 µg)	R
Sulfonamides	Sulfamethoxazole/trimethoprim (1.25/23.75 µg)	R
Tetracycline	Doxycycline (30 µg)	S
	Tetracycline (30 µg)	S

Table 3: The recorded antibiotic resistance and sensitivity pattern of E. faecalis OS6 against antibiotics with their corresponding tested concentrations.

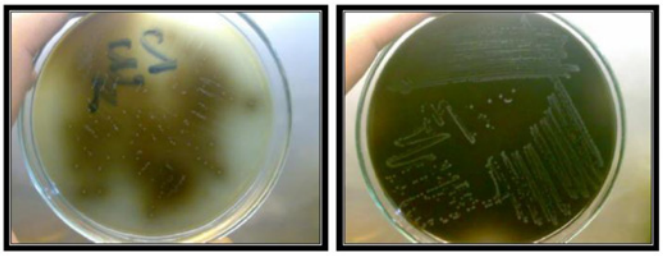

Figure 1: Picture with observed shiny black colonies of *Enterococcus*, cultivated on bile esculin agar, incubated at 37˚C.

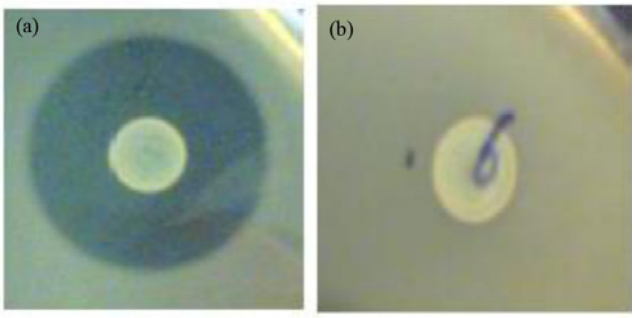

Figure 2: (a) The recorded antimicrobial activity, represented by zone of inhibition of the growth of *Lactobacillus sakei* when treated with crude Enterolysin A released from *E. faecalis* OS6. **(b)** Enterolysin A pre-treated with proteinase K enzyme.

Determination of antimicrobial spectrum of *E. faecalis* OS6

E. faecalis OS6 was able to produce antimicrobial activities against some of closely related microorganisms (Table 1). Members of Gram-positive bacteria as *Lactobacillus sakei*, *Lactobacillus plantarum* and *E. faecium* were sensitive, while some other species of Gram-positive bacteria as *Listeria* species and *Staphylococcus aureus* were resistant. All tested Gram-negative bacteria were resistant. The highest recorded antimicrobial activity was shown against *Lactobacillus sakei* (Table 2 and figure 2a), so it was used as the main indicator microorganism during the rest of this study.

Effect of heat and proteinase k enzyme

The antimicrobial activity was diminished where no zone of inhibition was observed (Figure 2b) after either heat or proteinase K enzyme treatment. The recorded antimicrobial activity of the cell free culture supernatant was initially calculated to be 160 BU/ml using microtiter plate assay. This activity was dropped to almost zero BU/ml after either heat or proteinase K treatments.

PCR screening and sequencing of bacteriocin structural genes

Interestingly, PCR screening for structural genes corresponding to 10 different bacteriocin genes (Table 2) showed a PCR product at 161 bp (Figure 3a) in case of PCR reaction for detection of Enterolysin A, and another PCR product was observed at 186 bp (Figure 3b) in case of PCR reaction for detection of Cytolysin. The detected PCR

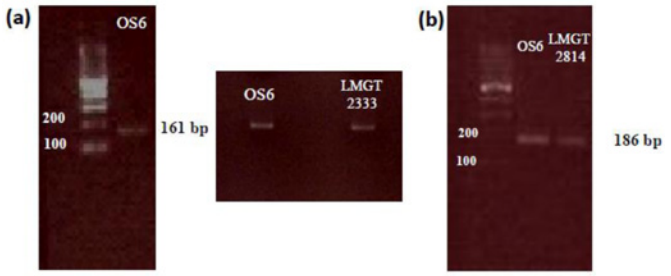

Figure 3: (a) The observed PCR products in gel electrophoresis corresponding to Enterolysin A gene of *E. faecalis* OS6 with reference to control of *E. faecalis* LMGT 2333. **(b)** Cytolysin gene of *E. faecalis* OS6 with reference to control of *E. faecalis* LMGT 2814.

products had similar sizes to these of positive control strains (Figure 3). Further confirmation of the detected PCR products was done by gene sequencing and BLAST analysis which revealed presence of exact genes sequences with 100% identity to those corresponding to Enterolysin A and Cytolysin genes.

Determination of virulence factors and antibiotic resistance phenotypes of *E. faecalis* OS6

E. faecalis OS6 showed the ability to produce several virulence determinants including; proteolytic gelatinase activity by liquefying gelatin media, bile salt hydrolase activity and β-hemolytic activity. Besides, multiple antibiotic resistance traits were detected, where *E. faecalis* OS6 showed resistance towards 17 out of 31 different antibiotics (Table 3). Most of these 17 antibiotics belonged to Cephalosporins, Aminoglycosides, Lincomycins, Polypeptides, Quinolones, Rifamycins and Sulfonamides classes (Table 3).

Discussion

A bacterium originally isolated from stool sample from ICU inpatient was characterized and identified as *E. faecalis* OS6. *Enterococcus* species was considered harmless to humans for a long time [3], however in the last decade *Enterococci* have been considered among the leading causes of nosocomial infections [20] and reported as the second most common cause of wound and urinary tract infection and the third most common cause of bacteraemia [21]. Hospitalized patients may have a greater incidence of Enterococcal infection (57%) compared with healthy individuals (39%) [3,22].

E. faecalis OS6 showed the ability to produce antimicrobial activity with inhibitory spectrum against some of closely related Gram-positive bacteria as *Lactobacillus sakei*, *Lactobacillus plantarum* and *E. faecium* while all tested Gram negative bacteria were resistant. Similarly, several previous studies [23,24] have reported that Enterococci exerting antimicrobial activities are usually less active towards Gram-negative bacteria [20,25-27].

The antimicrobial activity produced by *E. faecalis* OS6 was lost after heat and proteinase K treatments. Thermal instability and sensitivity to proteinase K enzyme confirmed the proteinaceous nature of the obtained antimicrobial activity [28]. Interestingly, when the bacteriocinogenic *E. faecalis* OS6 was screened for structural genes corresponding to 10 different bacteriocin genes, Enterolysin A and Cytolysin genes were detected and confirmed. The presence of these genes together was detected before where Hickey et al. [10] have characterized an *E. faecalis* strain with cytolysin and a heat-labile antimicrobial protein of Enterolysin A (34 kDa). Interestingly, in the present study, it was observed that the antimicrobial activity was severely decreased in liquid culture compared to the recorded activity on the agar media. Similarly, Nilsen et al. [12], have reported that the antimicrobial activity of Enterolysin A was detected only on agar media, and no antimicrobial activity was found in liquid cultures. Several strains of *E. faecalis* producing Enterolysin A were isolated previously from environmental origin such as raw milk [10] or from animal origin such as rumen [11]. Yet, studies reporting isolation of pathogenic *E. faecalis* from clinical human samples with relevant antimicrobial activity still rare [12].

It is necessary for all bacteriocinogenic *Enterococcus* strains isolated from clinical samples and have good potential for direct or indirect applications in industry, to be checked for the presence of virulence determinants and antibiotics resistance in order to manage high safe conditions [22,29-31]. Accordingly, several virulence determinants were phenotypically checked for presence of proteolytic gelatinase, bile salt hydrolase and haemolysin activities where all these virulence factors were detected in this strain. It was previously reported that *E. faecalis* strains especially those of medical origins were equipped with many virulence determinants [10,32,33]. Hemolysin activity is seen as a potent virulence factor and has been previously detected in most cases associated with *E. faecalis* isolated from clinical specimens [34,35]. A group of enzymes including gelatinase and bile salt hydrolase are involved in virulence of *Enterococcus* species [10,36]. The main role of them in Enterococcal pathogenesis is thought to be in providing nutrients to the bacteria and they also may have some functions in biofilm formation [37,38]. In general, *E. fecalis* OS6 showed multi drug resistant to 17 out of 31 different antimicrobial agents, most of these 17 antibiotics belong to Cephalosporins, aminoglycosides, Lincomycins, Polypeptides, Quinolones, Rifamycins and Sulfonamides classes. Indeed, multi drug resistances of *Enterococci* made them considered to be one of the major nosocomial pathogens [39]. On the other hand, *E. faecalis* OS6 was sensitive to tetracycline, chloramphenicol, and glycopeptides antibiotics likely that previously detected and reported [29]. Being sensitive to glycopeptides (vancomycin) is in agreement with what have been reported by Bearman and Wenzel [40] where only 2% of *E. faecalis* isolates from clinical samples were resistant to vancomycin.

Conclusion

The main aim of the present study was to grasp attention to new approach in screening antimicrobial activities in almost untapped non-conventional resources (such as pathogenic bacteria). This is among the urgent needs for the coming era where the emergence of multi drug resistant bacteria became a worldwide serious threat. To the best of our knowledge, this is the first time to report and identify Enterolysin A in between pathogenic *E. faecalis* isolated from clinical specimen collected from human in Egypt.

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References

1. Pabich WL, Fihn SD, Stamm WE, Scholes D, Boyko EJ, et al. (2003)

Prevalence and determinants of vaginal flora alterations in postmenopausal women. J Infect Dis 188: 1054-1058.

2. Peters RP, van Agtmael MA, Gierveld S, Danner SA, Groeneveld AB, et al. (2007) Quantitative detection of *Staphylococcus aureus* and *Enterococcus faecalis* DNA in blood to diagnose bacteremia in patients in the intensive care unit. J Clin Microbiol 45: 3641-3646.

3. Fisher K, Phillips C (2009) The ecology, epidemiology and virulence of Enterococcus. Microbiology 155: 1749-1757.

4. Franz CM, Muscholl-Silberhorn AB, Yousif NM, Vancanneyt M, Swings J, et al. (2001) Incidence of virulence factors and antibiotic resistance among Enterococci isolated from food. Appl Environ Microbiol 67: 4385-4389.

5. Kayser FH (2003) Safety aspects of enterococci from the medical point of view. Int J Food Microbiol 88: 255-262.

6. De-Vuyst L, Vandamme EJ (1994) Antimicrobial potential of lactic acid bacteria: 345 Bacteriocins of lactic acid bacteria. (1stedn), Blackie Academic & Professional, London, UK.

7. Klaenhammer TR (1993) Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiol Rev 12: 39-85.

8. Kühn I, Iversen A, Burman LG, Olsson-Liljequist B, Franklin A, et al. (2003) Comparison of enterococcal populations in animals, humans, and the environment--a European study. Int J Food Microbiol 88: 133-145.

9. De Kwaadsteniet M, Todorov SD, Knoetze H, Dicks LM (2005) Characterization of a 3944 Da bacteriocin, produced by Enterococcus mundtii ST15, with activity against Gram-positive and Gram-negative bacteria. Int J Food Microbiol 105: 433-444.

10. Hickey RM, Twomey DP, Ross RP, Hill C (2003) Production of enterolysin A by a raw milk enterococcal isolate exhibiting multiple virulence factors. Microbiology 149: 655-664.

11. Nigutova K, Morovsky M, Pristas P, Teather RM, Holo H, et al. (2007) Production of enterolysin A by rumen Enterococcus faecalis strain and occurrence of enlA homologues among ruminal Gram-positive cocci. J Appl Microbiol 102: 563-569.

12. Nilsen T, Nes IF, Holo H (2003) Enterolysin A, a cell wall-degrading bacteriocin from Enterococcus faecalis LMG 2333. Appl Environ Microbiol 69: 2975-2984.

13. Novicki TJ, Schapiro JM, Ulness BK, Sebeste A, Busse-Johnston L, et al. (2004) Convenient selective differential broth for isolation of vancomycin-resistant enterococcus from fecal material. J Clin Microbiol 42: 1637-1640.

14. Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA (2007) Manual of Clinical Microbiology. (9thedn), American Society for Microbiology, Washington DC, USA.

15. Birri DJ, Brede DA, Nes IF (2012) Salivaricin D, a novel intrinsically trypsin-resistant lantibiotic from Streptococcus salivarius 5M6c isolated from a healthy infant. Appl Environ Microbiol 78: 402-410.

16. Sambrook J, Maniaris T, Fritsch EF (1989) Molecular Cloning: A Laboratory Manual. (2ndedn), Cold Spring Harbor Laboratory, Cold Spring Harbor.

17. De-Vuyst L, Callewaert R, Pot B (1996) Characterization of the antagonistic activity of Lactobacillus amylovorus DCE 471 and large scale isolation of its bacteriocin amylovorin L471. Syst Appl Microbiol 19: 9-20.

18. Holo H, Nilssen O, Nes IF (1991) Lactococcin A, a new bacteriocin from Lactococcus lactis subsp. cremoris: isolation and characterization of the protein and its gene. J Bacteriol 173: 3879-3887.

19. Clinical and Laboratory Standards Institute (2007) Performance standards for antimicrobial susceptibility testing: 17th informational supplement. CLSI document M100-S17, Wayne PA, USA.

20. Bennik MH, Vanloo B, Brasseur R, Gorris LG, Smid EJ (1998) A novel bacteriocin with a YGNGV motif from vegetable-associated *Enterococcus mundtii* : full characterization and interaction with target organisms. Biochim Biophys Acta 1373: 47-58.

21. de Fátima Silva Lopes M, Ribeiro T, Abrantes M, Figueiredo Marques JJ, et al. (2005) Antimicrobial resistance profiles of dairy and clinical isolates and type strains of enterococci. Int J Food Microbiol 103: 191-198.

22. Mundy LM, Sahm DF, Gilmore M (2000) Relationships between enterococcal virulence and antimicrobial resistance. Clin Microbiol Rev 13: 513-522.

23. Devriese LA, Pot B (1995) The genus *Enterococcus. In*: Wood BJB Holzapfel 395 WH (Eds), The Lactic Acid Bacteria. The Genera of Lactic Acid Bacteria 2: 327-367.

24. Giraffa G (2002) Enterococci from foods. FEMS Microbiol Rev 26: 163-171.

25. Aymerich T, Holo H, Håvarstein LS, Hugas M, Garriga M, et al. (1996) Biochemical and genetic characterization of enterocin A from *Enterococcus faecium*, a new antilisterial bacteriocin in the pediocin family of bacteriocins. Appl Environ Microbiol 62: 1676-1682.

26. Cintas LM, Casaus P, Holo H, Hernandez PE, Nes IF, et al. (1998) Enterocins L50A and L50B, two novel bacteriocins from *Enterococcus faecium* L50, are related to staphylococcal hemolysins. J Bacteriol 180: 1988-1994.

27. De Kwaadsteniet M, Todorov SD, Knoetze H, Dicks LM (2005) Characterization of a 3944 Da bacteriocin, produced by *Enterococcus mundtii* ST15, with activity against Gram-positive and Gram-negative bacteria. Int J Food Microbiol 105: 433-444.

28. Mareková M, Lauková A, Skaugen M, Nes I (2007) Isolation and characterization of a new bacteriocin, termed enterocin M, produced by environmental isolate *Enterococcus faecium* AL41. J Ind Microbiol Biotechnol 34: 533-537.

29. Gupta H, Malik RK (2007) Incidence of virulence in bacteriocin-producing enterococcal isolates. Lait 87: 587-601.

30. Aguirre M, Collins MD (1993) Lactic acid bacteria and human clinical infection. J Appl Bacteriol 75: 95-107.

31. Hancock LE, Gilmore MS (2000) Pathogenicity of enterococci in: Gram-Positive Pathogens, (Fischetti VAedn), 251–258, American Society for Microbiology, Washington DC, USA.

32. Busani L, Del Grosso M, Paladini C, Graziani C, Pantosti A, et al. (2004) Antimicrobial susceptibility of vancomycin-susceptible and -resistant enterococci isolated in Italy from raw meat products, farm animals, and human infections. Int J Food Microbiol 97: 17-22.

33. Eaton TJ, Gasson MJ (2001) Molecular screening of Enterococcus virulence determinants and potential for genetic exchange between food and medical isolates. Appl Environ Microbiol 67: 1628-1635.

34. Booth MC, Bogie CP, Sahl HG, Siezen RJ, Hatter KL, et al. (1996) Structural analysis and proteolytic activation of *Enterococcus faecalis* cytolysin, a novel lantibiotic. Mol Microbiol 21: 1175-1184.

35. Gilmore MS, Segarra RA, Booth MC, Bogie CP, Hall LR, et al. (1994) Genetic structure of the *Enterococcus faecalis* plasmid pAD1-encoded cytolytic toxin system and its relationship to lantibiotic determinants. J Bacteriol 176: 7335-7344.

36. Semedo T, Santos MA, Lopes MF, Figueiredo Marques JJ, Barreto Crespo MT, et al. (2003) Virulence factors in food, clinical and reference Enterococci: A common trait in the genus? Syst Appl Microbiol 26: 13-22.

37. Gilmore MS, Clewell DB, Courvalin PM, Dunny GM, Murray BE, et al. (2002) Enterococci: Pathogenesis, Molecular Biology, Antibiotic Resistance, and Infection Control, The. American Society for Microbiology, Washington DC, USA.

38. Mohamed JA, Huang DB (2007) Biofilm formation by enterococci. J Med Microbiol 56: 1581-1588.

39. Klein G (2003) Taxonomy, ecology and antibiotic resistance of enterococci from food and the gastro-intestinal tract. Int J Food Microbiol 88: 123-131.

40. Bearman GML, Wenzel RP (2005) Bacteraemias: a leading cause of death. Arch Med Res 36: 646-659.

41. Martínez-Bueno M, Maqueda M, Gálvez A, Samyn B, Van Beeumen J, et al. (1994) Determination of the gene sequence and the molecular structure of the enterococcal peptide antibiotic AS-48. J Bacteriol 176: 6334-6339.

42. Tomita H, Fujimoto S, Tanimoto K, Ike Y (1996) Cloning and genetic organization of the bacteriocin 31 determinant encoded on the *Enterococcus faecalis* pheromone-responsive conjugative plasmid pYI17. J Bacteriol 178: 3585-3593.

43. Franz CM, Worobo RW, Quadri LE, Schillinger U, Holzapfel WH, et al. (1999) Atypical genetic locus associated with constitutive production of enterocin B by *Enterococcus faecium* BFE 900. Appl Environ Microbiol 65: 2170-2178.

44. Cintas LM, Casaus P, Håvarstein LS, Hernández PE, Nes IF (1997) Biochemical and genetic characterization of enterocin P, a novel sec-dependent bacteriocin from *Enterococcus faecium* P13 with a broad antimicrobial spectrum. Appl Environ Microbiol 63: 4321-4330.

45. Cintas LM, Casaus P, Herranz C, Håvarstein LS, Holo H, et al. (2000) Biochemical and genetic evidence that *Enterococcus faecium* L50 produces enterocins L50A and L50B, the sec-dependent enterocin P, and a novel bacteriocin secreted without an N-terminal extension termed enterocin Q. J Bacteriol 182: 6806-6814.