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Evaluation of Enterococcus Bacterial Contamination in Water Supplies of Iran

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ABSTRACT

Background and aims: The aim of the present study was to determine the presence of Enterococcus Bacterial Contamination in boundary Water Supplies. Materials and methods: The present study was conducted on boundary water supplies. Contamination about 50 ml was determined by taking samples from the water supply. Bacterial samples were evaluated by a microbiological technique specific for anaerobic species, used for isolation and identification of sampled strains. Results: From boundary water supplies of Iran totally 306 Enterococcus Bacterial were separated. Out of 143 clinical samples taken, 105 were from E.faecalis and 38 were from E.faecium Conclusion: Enterococcus bacterial contamination was evident in the evaluated waters and clinical samples. The contamination type was identical and some of them were resistance to antibiotics.

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INTRODUCTION

In connection with the study of composition and function of aquatic ecosystems, our aims to determine the factors affecting water quality and identifying biological ecology and other aspects of microbial communities present in the water. According to some studies, E.coli known for its many attributes are not considered core [1]. In recent years microorganisms of the genus Enterococcus with regard to resistance to most antimicrobial materials, lighting, Low temperature also influences the resistance to physical, chemical and biological increased [3]. Currently, taking account of epidemiological enterococci have special place, In addition, many studies have been conducted on pathogenic microorganisms and Finding VBNC [possess growth potential, but it cannot be cultured] can be increased, At this time Bacterial cells retained their ability to grow, but they do not come up in the culture medium [3]. The term of "enterococcus" for organisms in saline 6.5% Temperature 10 to 45 °C and PH 9.6 grown and to live at 60 °C for 30 minutes [4]. S.faecalis and S.Gallinarum Faecium in 1919 by Avrla Johnson are illustrated as a species of S.faecalis in the first picture was Sherman [4]. According to the species, S. faecalis, S.Faecium ,S.Kaslyflavvs ,S. avium, and S.Durans varieties Maluduratus Being named separately [5]. Using similar methods, the authors showed that S. Faecium, S. faecalis, S. Kaslyflavvs, S. avium, S.Durans, under the strain of S.Maluduratus Gallinarum very closely with representatives of the genus Enterococcus [5-8]. Williams and his colleagues was suggested the genus Enterococcus divided into5 groups according to gene sequence16SrRNA [9]. Among isolates 80-90 % maladies such as Enterococcus Gallinarum, Enterococcus Flavysus - , Enterococcus Kaslyflavus, E. Hirae 's somewhat important [10]. Genus Enterococcus vampire among organisms , gram-positive anaerobic are optional, Round or oval- shaped cells Pairs , short chains , mass concrete in small or isolated cells observed [10]. These organisms lack the enzymes of the cytochrome oxidase and catalase negative; some species are false positive catalase [8, 11]. Enterococci are widely distributed in nature [2, 12] and they are also part of the intestinal flora of Humans, mammals and birds [13]. Sometimes are also observed in the flora of genital tract, mouth, throat and nose [14]. A long time to survive in the soil and food and are able to grow at room temperature. The effects of physical, chemical and biological show resistance [2]. In the new hospitals have shown Enterococcus bacteria resistance because of use terms of new types of antibiotics and survival continued [15]. In Urinary tract infections, Enterococcus faecalis are the second cause of them after intestinal bacilli [16]. It is thought Enterococcus faecalis produce Cytotoxin lysis of erythrocyte and neutrophils. Synthesis of the cytotoxin Show high resistance against chemicals [17]. That is clear the Enterococcus faecalis possess proteolytic activity and hydrolysis a series of proteins, gelatin, casein, collagen, hemoglobin, etc. Bacteria will persistence Long-term in the genitourinary tract [18]. Important

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factor assessing innocuous enterococci resistant to essential antibiotics, particularly glycopeptides. Eliminate the causes of antibiotic resistance genes in the origin of the genus Enterococcus probiotics and yeast before use is necessary [19]. All the above mentioned play a major role in the development process and considering the pathogenesis of Enterococcus Special doctor's attention is captured. The first is that in the water samples and the boundary in the distribution and dynamics of bacteria Genus Enterococcus were investigated and was analyzed its effect on bacterial flora status. Our objective,To investigate the distribution of bacteria in the genus Enterococcus , identify characteristics and species And assessment of antibiotic resistance genes. The aim of the present study was to evaluate the presence of Enterococcus Bacterial Contamination in boundary Water Supplies of Iran.

MATERIALS AND METHODS

This study was conducted on boundary water supplies of Iran such as Aras, Karon, Sefid rivers. Bacterial samples were evaluated by means of an advanced microbiological technique specific for anaerobic species, which was used for isolation and identification of sampled strains. The main areas of research are produced water samples and samples of pathological material to isolate bacteria Enterococcus. Used media were: blood agar, BHI [agar containing brain extracts and heart], 1% sodium chloride, bile esculin agar, hydrogen peroxide, the medium containing glucose, mannitol and arginine -containing medium. In Water samples used doping method for culture and set of values produced by bacteria of the genus Enterococcus. Contamination about 50 ml was determined by taking samples from the water supply. Sampling was carried out according to the asepsis instructions described previously [1]. We across water from membrane filter with 0.45 mm mesh, for 48 hours at a temperature of 37 ° C in slanova-Borteli incubation. Bacteria in the genus Enterococcus medium produced red-pink colonies. In addition urine, feces, blood and wound fluid, peritoneal fluid, In door domestic and bone marrow biopsy and synovial fluid were taken. The sample incubation at 37°C for 30 min and then for 60 seconds were centrifuged. Peptone water containing one per cent, respectively, carried 10 times dilution. Each of them 0.1 ml was removed to the surface of Brucella agar/dilution of Cultivated. Plates in anaerobic conditions for 48 hours were taken. The medium incubation temperature was 44°C for 2 hours. Fecal origin enterococci were hydrolysing esculin. Finally, hydrolysis of esculin is produced 6,7 Dihydroxy coumarin, it is combined with iron ions diffuse black pigment in The medium creates. Evaluate of resistance to antibiotics with Discs containing antibiotics, after placing the discs, plates for 24 hours at a temperature of 37 °C Were incubation. Depending on the growth of microorganisms in the surrounding disk, bacteria were divided into three categories: Sensitive, intermediate resistant.

Results:

A total of 306 strains of the genus Enterococcus were isolated Iranian waters such as Enterococcus faecalis, Enterococcus faecium, Enterococcus avium, E. Mundtii, E. Hirae, E. Durans. E. Gallinarum is dominant and unequal distribution of the nature and characteristics depending on the location.

Table 1: Strains of the genus E.

Source	Sample locations	The dominant species
Aras	Parsabad	E. Faecium
	Larijan	E. Faecium, E. Faecalis
	Aslanduz	E. Faecium, E. Faecalis, E. Avium, E. mundtii E. Durans, E. Gallinarum, E. Hirae
	Jananloo	E. Avium, E. Faecium
Sefid	Roudbar	E. Faecalis, E. Avium, E. Faecium E. Mundtii, E. Gallinarum , E. Hirae
	Sangar	E. Mundtii, E. Hirae, E. Durans E. Faecium , E. Faecalis , E. avium
	Saramin	E. Faecium , E. Durans
Karun	Ahvaz	E. Faecium , E. Avium
	Khorramshahr	E. Faecium
	Shoshtar	E. Avium
	Masjed Soleiman	E. Faecium , E. Mundtii
	Sheibani	E. Faecalis
	Abadan	E. Faecium, E. Faecalis

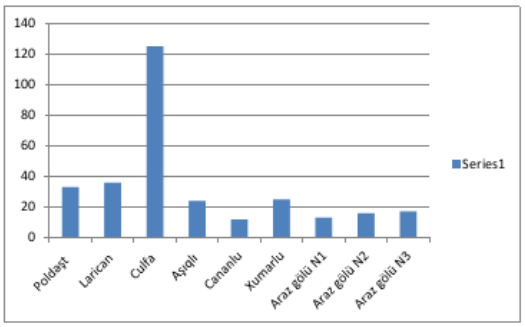


Fig. 1: Distribution of the genus Enterococcus bacteria in the water of Aras River.

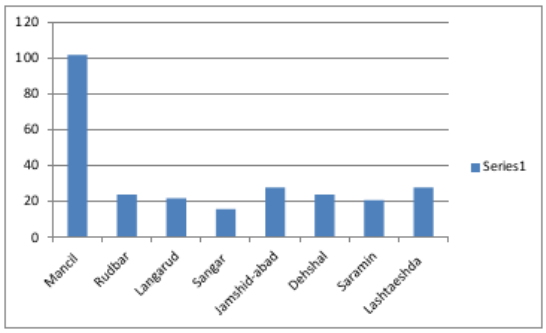


Fig. 2: Distribution of the genus Enterococcus bacteria in the water of Sefid River.

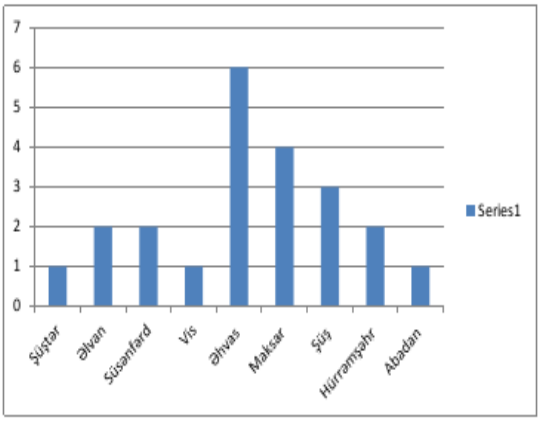


Fig. 3: Distribution of the genus Enterococcus bacteria in the water of Karun River.

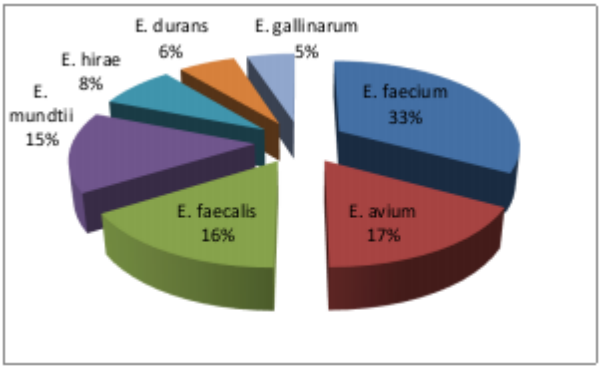


Fig. 4: Genus Enterococcus bacteria isolated from water supplies of Iran.

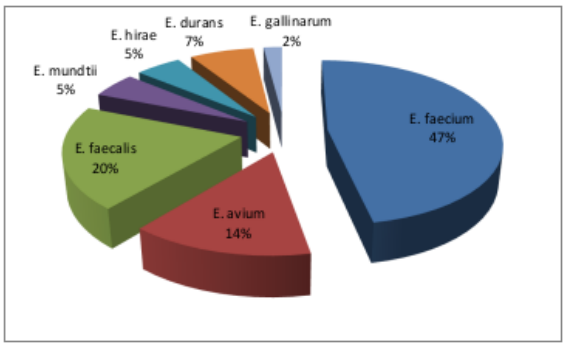


Fig. 5: Genus Enterococcus bacteria isolated from different areas of the Aras River.

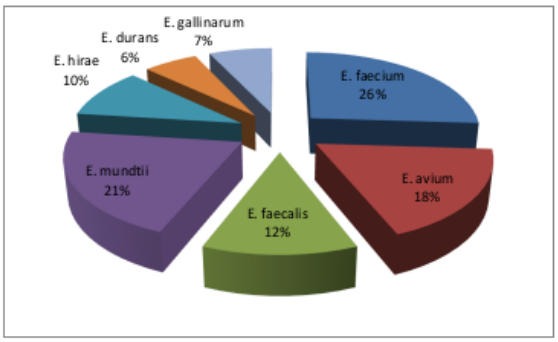


Fig. 6: Genus Enterococcus bacteria isolated from different regions of the Sefid River.

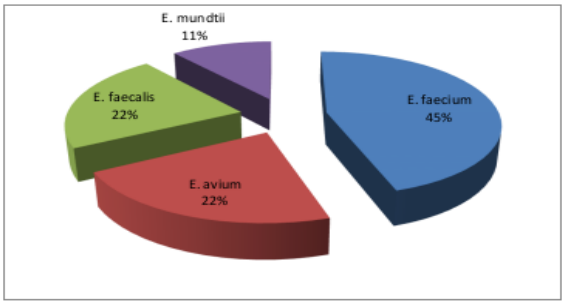


Fig. 7: Genus Enterococcus bacteria isolated from different regions of the Karun River.

Table 2: Enterococci in pathological or clinical samples.

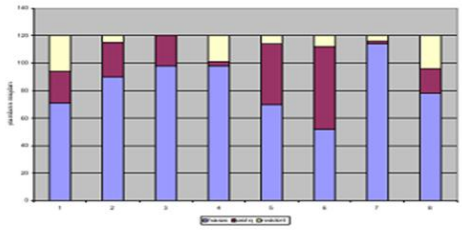
Clinical Samples	Cfu/100m			
	Enterococcus faecalis	Enterococcus faecium	Enterococcus durans	Total
Urine	37	21	-	58
Stool	21	5	-	26
Purulent fluid inside	12	4		16
Blood and plasma	18	5	-	23
Peritoneal fluid	17	3	-	20
Total	105	38	-	143
Amount [%]	73.4	26.6	-	100

Enterococcus isolates were detected in 143 clinical samples, 105 isolates of them were Enterococcus faecalis, 38 Enterococcus isolates were Faecium.

Genus Enterococcus were isolated from the wastewater of 92 isolates, 68 isolates of Enterococcus Faecium and 24 isolates were Enterococcus faecalis.

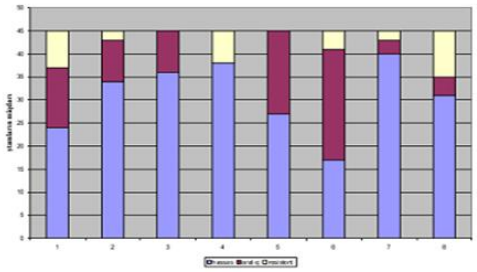
From 105 Enterococcus faecalis strains isolated from clinical samples 3.6% resistance to Vancomycin has shown, 24 Enterococcus faecalis isolated from a wastewater samples 4.2% resistance to Vancomycin.

From 105 Enterococcus faecalis strains isolated from clinical samples 36.2% resistance to Gentamicine has shown and 24 Enterococcus faecalis isolated from a wastewater samples 4.2%% resistance to Gentamicine.



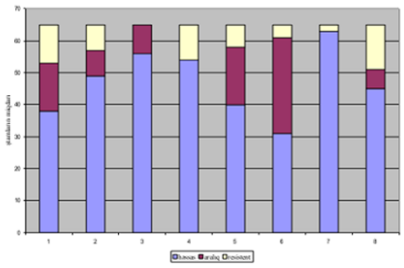
1 - streptomisin, 2 - tetrasiklin; 3 - vankomisin; 4 - benzilpenisillin; 5 - sitoprofloksin; 6 - eritromisin; 7 - gentamisin; 8 - rifampisin

Fig. 8: Degree of sensitivity to antibiotics of bacteria in the genus Enterococcus.



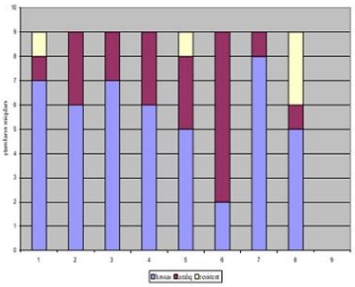
1 - streptomisin, 2 - tetrasiklin; 3 - vankomisin; 4 - benzilpenisillin; 5 - sitoprofloksin; 6 - eritromisin; 7 - gentamisin; 8 - rifampisin

Fig. 9: Degree of sensitivity to antibiotics of bacteria in the genus Enterococcus isolated from the Aras River.



1 - streptomisin, 2 - tetrasiklin; 3 - vankomisin; 4 - benzilpenisillin; 5 - sitoprofloksin; 6 - eritromisin; 7 - gentamisin; 8 - rifampisin

Fig. 10: Degree of sensitivity to antibiotics of bacteria in the genus Enterococcus isolated from the Sefid River.



1 - streptomisin, 2 - tetrasiklin; 3 - vankomisin; 4 - benzilpenisillin; 5 - sitoprofloksin; 6 - eritromisin; 7 - gentamisin; 8 - rifampisin

Fig. 11: Degree of sensitivity to antibiotics of bacteria in the genus Enterococcus isolated from the Karun River.

Table 3: Sensitivity to antibiotics of E.faecalis and E.faecium.

Antibiotics	E. faecalis	E. faecium
cl-Am-sgn-te-Gm-Na-Dx-E-ox-nit-liz-cip	49.60	49.89
V-cl-sgn-sxt-Te-Gm-Na-ox-E-Dx-liz	50.8	51.12
d-Am-sgn-Te-Gm-Na-ox-Dx-nit-cip	55.52	51.32
cl-Am-sgn-Gm-Na-Dx-E-Ox-Dx-nit-liz	51.59	42.78
d-Am-sgn-Te-Gm-Na-E-Ox-Dx-liz	53.13	48.44
d-Am-sgn-sxt-Gm-Na-Ox-Dx-nit-cip	52.78	50.56
d-sgn-sxt-Te-Gm-Na-Ox-Dx-nit-cip	58.38	48.16
d-sgn-sxt-Gm-Na-E-Ox-Dx-liz-nit	49.09	42.66
d-Am-sgn-sxt-Na-e-Ox-Dx-liz	51.45	47.02
d-sgn-sxt-te-Gm-Na-E-Dx-nit	47.17	46.51
d-Am-sgn-sxt-te-Na-E-Dx-nit-liz	45.14	42.34
d-Am-sgn-sxt-Gm-Na-E-Dx-liz	45.14	48.26
d-sgn-sxt-Na-E-nit-Ox-cip	54.84	47.60
d-sgn-sxt-Na-E-Dx-nit	50.94	40.20
d-sgn-sxt-Na-Te-Dx-nit-Ox	**62.95**	40.42
d-sgn-sxt-Gm-Na-Dx-liz-Ox-cip	54.14	46.04
d-sgn-sxt-Gm-Na-E-Ox-liz-nit	49.65	43.28
Am-sgn-sxt-Na-Ox-E-Dx-nit	57.07	51.67
d-sgn-sxt-Na-Ox-liz-cip	58.15	42.74
d-sgn-Te-Na-E-Ox-liz	60.58	40.88
d-sgn-Na-Ox-Dx-nit-cip	59.08	38.34
Na-Dx-liz-Ox-nit-cl-A-te	49.71	34.77
V-E-Am-gm-cip-Ox-sxt	40.54	**79.02**

V: vankomisin, E: eritromisin, A: amitsasin, Am: ampisilin, Gm: gentamisin, cip: tsiproflaksin, cl: xloramfenikol, Te; tetrasiklin, sgn: sinersid, Tei: teikoplanin, nit: nitroforantin, Ox: oksatsilin, sxt: trimetoprim, liz: linezolid, Na: nadiliks turşusu, Dx: doksitsiklin-sulfametaksazol

Table 4: Sensitivity to antibiotics of genus Enterococcus isolated from pathological samples.

%- lə	V	E	A	Am	Gm	Cl	Te	Sgn	Tei	nit	Ox	cip	sxt	liz	Na	Dx
E. faecalis	3.6	29	40	27	36.2	20	83	100	3.3	18.6	93	44	51	5.1	94	44
E. faecium	85	84	3.7	8.3	78	18	59	6.2	48	8.2	67	80	76	0	52	34

V: vankomisin, E: eritromisin, A: amitsasin, Am: ampisilin, Gm: gentamisin, cip: tsiproflaksin, cl: xloramfenikol, Te; tetrasiklin, sgn: sinersid, Tei: teikoplanin, nit: nitroforantin, Ox: oksatsilin, sxt: trimetoprim, liz: linezolid, Na: nadiliks turşusu, Dx: doksitsiklin-sulfametaksazol

Table 5: Sensitivity to antibiotics of genus Enterococcus isolated from waste water samples

%- lə	V	E	Am	Gm	Cl	Te	sgn	Tei	Nit	Cip	Liz
E. faecalis	4.2	32	22	30	24	79	100	2	12.4	48	1
E. faecium	3	47	34	36	28	41	1.3	3.1	28	62	0.8

Discussion and Conclusions:

Enterococci is representative of macrobiotic natural human intestine and in this way the amounts of them release to the environment.In assessing the quality of surface water basins of the bacteriology and hygiene, as a health indicator is the base of existence of microorganisms in water. According to studies, E.Coli characteristics cannot be considered as the only criterion for water sanitation, for this reason, according to the Europ Union, also determine of existence of E.Coli is necessary to determine enterococci with the origin of stool. There are two principal methods for determining Entercocci levels: titration and passing the filter. The second method has a high priority because it gives an accurate count of the microorganisms present in the sample. Enterococcis grow easily on conventional environment, while the environment should contain inhibitors of other microbial growth. The investigation shows that the majority of bacteria from genus of Enterococcus in sample stations around the Poldasht and Larijan cities are in order to 33 and 36 cfu per 100 ml. The highest degree of pollution around Julfa exceeded registered with 125 cfu per 100 ml. The Enterococcus was observed in samples of around the other cities too, and their values were changeable between 12 cfu per 100 ml [Janlu] and 25 cfu per 100 ml [Khomarlu].

According to the investigation comes that high potential pathogenic microorganisms from Enterococcus genus can be seen in water samples taken from the Delta Manjil Dam and this is essential that the health and care and environmental conditions are monitored. Enterococcis are also located in different places in the prepared samples were observed ,such as Makser 4 cfu per 100 ml-Shoosh 3 cfu per 100 ml-Khoramshahr 2 cfu per 100 ml and Abadan 1 cfu per 100 ml.

Based on these results, it was clear that accumulation of organic matter and bionutrients, releasing agricultural wastes in the flow path is linked with the Karun pollution with Enterococcis. A total of 4 % of the bacteria isolated in the present study was to possess hemolytic activity. From 5 types of them are Beta-hemolytic and 5types of them are Alpha-hemolytic and the other types were induced erythrocyte hemolysis. Noteworthy among the bacteria isolated from Karun, no one has hemolytic's activity and this effect is result of the lower of developmental impact [antropogen] of surrounding area.

Genus Enterococcus were isolated from the pathological samples some of them [32] related with aac[6]-le-alpha[2]-la, and 3 genus of them related with alpha[2]-lb,3 genus related with boreaph[2]-ld and non of them related with alpha[2]-lc. Genus Enterococcus were isolated from the wastewater some of them [17] related with aac[6]-le-alpha[2]-la,and 1 genus of them related with alpha[2]- lb,2 genus related with boreaph[2]-ld and non of them related with alpha[2]-lc.

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