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Antibiotics and heavy metals resistance patterns of *Enterococcus faecalis* and *faecium bacteria* isolated from the human and the livestock sources

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Abstract

Background: *Enterococci* have emerged as a major cause of nosocomial infections and within this group, *Enterococcus faecalis* and *Enterococcus faecium* cause the majority of human and livestock *enterococcal* infections. In this article, we tried to determine antibiotics and metals resistance patterns of *E. faecalis* and *E. faecium* strains.

Methods: One hundred sixty different strains of *E. faecalis* and *E. faecium* were collected from livestock sewage and the human fecal waste during 15 months. Then bacterial antibiotics sensitivity tests were carried out using the Agar disc diffusion method.

Results: Generally, 100% of *E. faecalis* strains separated from human and livestock sources (i.e. sheep) showed penicillin (P)/ kanamycin (K)/ nitrofurantoin (N)/ loracarbef (L)/ Ciprofloxacin (Cc)/ ampicillin (AN)/ nalidixic acid (NA)/ sulfamethoxazole (S) antibiotics resistance patterns. In addition, 55% of isolated *E. faecium* showed P/S/AN/NA antibiotics resistance patterns. Each strain showed a resistance to at least two aminoglycoside antibiotics. However, *E. faecalis* strains from human and the livestock sources showed 94% and 100% of resistance to nitrofurantoin, respectively. The effects of different metal concentrations was evaluated in both strains. The agar dilution method was applied in this stage. Hg at 0.05 mmol/L of minimum inhibitory concentration (MIC) showed toxicity to both the human and livestock *Enterococcus* strains. Cadmium at 1 mmol/L and 0.5 mmol/L concentrations had the most toxicity to *E. faecalis* and *E. faecium* strains, respectively. Obviously, toxicity to bacteria is less than other metals. As a result, Zn/Ni/Cu/Co resistance pattern is suggested for both strains. Finally, antibiotics and heavy metals resistance patterns were monitored simultaneously.

Conclusion: Almost all *E. faecalis* strains isolated from humans and livestock showed antibiotics and heavy metals resistance patterns of P/K/L/Cc/S/AN/NA/Zn/Cu/Co simultaneously. Moreover, 55% *of E. faecium* strains showed similar antibiotics and heavy metals resistance patterns of P/S/AN/NA/Zn/Ni/Cu/Co.

Keywords: *E. faecalis*, Heavy metals, Antibiotics, Resistance

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Introduction

Enterococci species is gram positive, catalase enzyme negative, oxidase enzyme negative, non-spore forming and facultative anaerobic cocci with strains found in humans. The strains can be separated from animal, environmental human and livestock sources (1). Enterococci are a part of the conventional microorganism of humans and animals. They have been long recognized as necessary human pathogens and are getting progressively thus. The genus *Enterococcus* includes 17 species, though only a handful can cause clinical infections in humans. They have emerged as a serious cause of nosocomial infections and at intervals, this cluster, *Enterococcus faecalis* and *Enterococcus faecium* cause the bulk of human and therefore livestock enterococcal infections (2). These infections are also systematic together with tract, abdominal, wound infections, bacteremia and carditis. Since *E. faecalis* and *E. faecium* strains are capable of living under varied environmental conditions (such as temperature extremes and also the presence of digestive fluid salts) and since they acquire resistance to multiple antibiotics, these microorganism have become a significant cause of unhealthiness (3). *E. faecalis* and *E. faecium* are the most prevalent species cultured from humans, accounting for more than 90% of clinical isolates. Other enterococcal species known to cause human infection include *Enterococcus avium*, *Enterococcus gallinarum*, *Enterococcus casseliflavus*, *Enterococcus durans*, *Enterococcus raffinosus*, and *Enterococcus mundtii*.

E. faecium is responsible for most vancomycin-resistant enterococci (VRE) infections. This can mean serious health problems, which include the lack of available antibiotics therapy for VRE infections since most VRE strains are resistant to multiple antibiotics besides vancomycin (e.g. aminoglycosides and ampicillin (4). Enterococci vary in both intrinsic and acquired resistance and are resistant to antibiotics thus making them vital medical building pathogens. In and of itself, *enterococci* are resistant to beta-lactam antibiotics as they contain penicillin-binding proteins (PBPs); thus, they are ready to synthesize parts of the cell-wall (5). In addition, it is known that contamination of surface waters has important effects on life dynamics and the ecology of their surroundings (6).

Recently many studies have been carried out on environmental *Enterococcus*. Iranian researchers in 2014 evaluated antibiotic and heavy metal resistance patterns in *enterococcal* species cultured from waters of 9 recreational areas in Iran (7). Researchers in 2011 identified that *E. faecalis* bacteria are resistant to heavy metals and antibiotics in surface waters of the Mololoa River in Tepic, Nayarit, Mexico. The results of their study showed that prevalence increased resistance to metals and antibiotics in *Enterococcus* spp. (8). In this study, antibiotics and heavy metals resistance patterns of *E. faecalis* and faecium bacteria isolated from the human and livestock sources are evaluated.

Methods

The measurement of bacteria strains sensitivity to antibiotics

In this study, the sensitivity test of two strains, *E. faecalis* and *E. faecium* to antibiotics isolated from human and livestock samples were carried out. The antibiogram disks were provided by MAST Company. To determine bacterial sensitivity to antibiotics, Kirby-Bauer laboratory diagnostic method was used (9). Antibiogram disks were used to determine sensitivity to *E. faecalis* and *E. faecium* by Disk diffusion method (Figures 1 and 2).

Determination of maximum growth time (exponential growth) of the isolated strains

This work was carried out based on spectrophotometry. First the wavelength of the device was adjusted according to the chart prepared for Muller Hinton medium (Blank). In this case, it is equal to 400 nm. Secondly, decrease some of culture medium containing bacteria once every 30 minutes (2 ml bacterial suspensions with 0.5 McFarland turbidity in 40 cc Muller Hinton broth medium) while the container is shaken by an incubator shaker at 37°C and 150 rpm speed (Figures 3, 4 and 5) (10).

Determination of toxicity to heavy metals

Bacteria resistance to heavy metals was determined by means of Agar-dilution method in Muller Hinton Agar plates at different concentrations (mmol/liter) of metals as follows: 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, 0, 0.5, 0.2, 0.1, 0.05, 0.01, and 0.005. The plates were dried at 37°C for 30 minutes, inoculated by 10 µ of bacteria. Metal salts used in this study include zinc sulfate, copper sulfate, cobalt chloride,

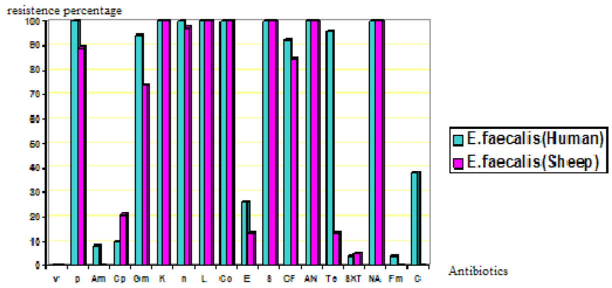

Figure 1. Comparing the percentages of antibiotics resistance of the human and livestock *E. faecalis.*

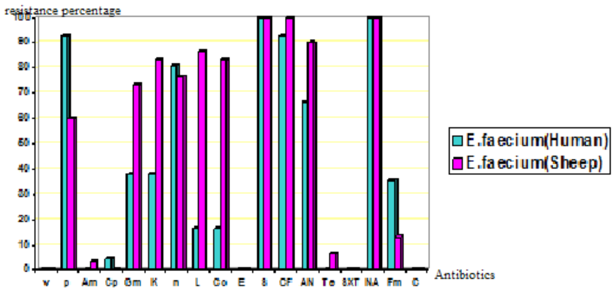

Figure 2. Comparing the percentages of antibiotics resistance of the human and livestock *E. faecium.*

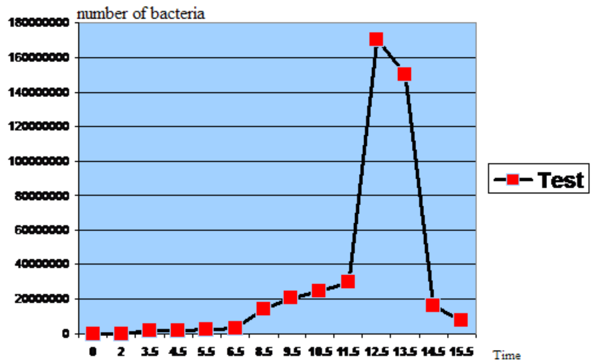

Figure 3. The growth curve of *E. faecalis* strains in the blank medium.

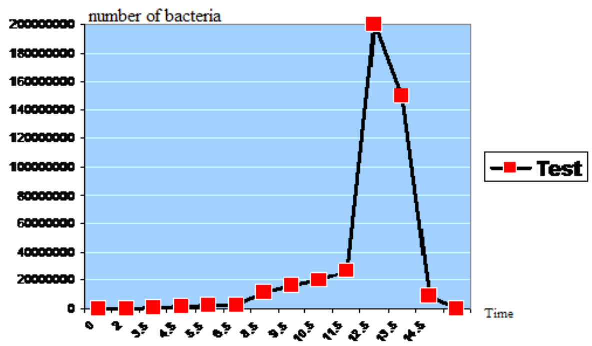

Figure 4. The growth curve of *E. faecium* strains in the blank medium

cadmium chloride, nickel sulfate and mercuric chloride, which were provided by Merck Company (Figure 6) (11). After preparing the Muller Hinton Agar plates containing different concentrations of metals, the suspension of certain bacterial strains in exponential growth phase were

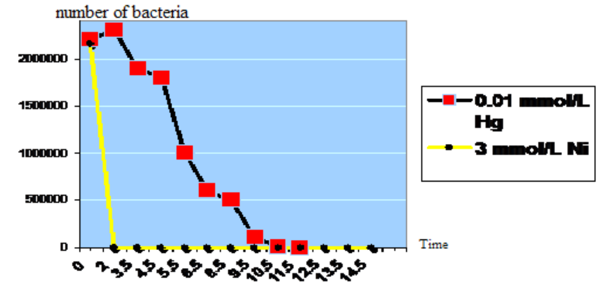

Figure 5. The growth curve of *E. faecium* strains in the medium with 0.01 mmol/L Hg and 3 mmol/L Ni.

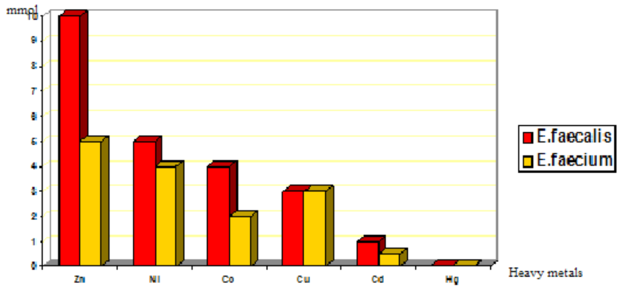

Figure 6. Comparing a metal MIC of the human strains and the livestock ones.

added in this stage and 10 UI drops numbered at certain intervals with regard to the number of strains and then they were placed on the medium containing metals (12). The plates were then incubated for 18 hours at 35°C. Finally, microbial biomass growth rate and minimum inhibitory concentration (MIC) of metals were controlled and recorded, respectively. In this case, the acceptable concentrations of metal ions were used to determine metals resistance.

Results

The determination of bacteria sensitivity related to antibiotics and the resistance pattern

The number and percentage of sensitive, semi-sensitive *E. faecalis* and *E. faecium* resistant spices are shown in Figures 1 and 2.

All species of *E. faecalis* and *E. faecium* that were isolated from the two sources were sensitive to vancomycin. This test plays a crucial role in the initial identification of *Enterococcus*. In this study, no resistant strains occurred. However, all strains of the two species have shown high resistance to penicillin. *E. faecalis* isolated from human and livestock sources showed 100% and 89.2% resistance. Moreover, *E. faecium* species isolated from the human and livestock sources showed 92.8% and 60% resistance to penicillin. On the other hand, the resistance of both species to ampicillin was equal to zero. This point is very important because *Enterococcus* strains sensitivity to ampicillin has been rarely reported.

All strains of both spices showed remarkable sensitivity to cotrimoxazole. Also high resistance to lincomycin and clindamycin in *Enterococcus* strains was observed.

However, *Enterococcus* strains of humans showed very low resistance. Resistance to nitrofurantoin, chloramphenicol, tetracycline, erythromycin, and ciprofloxacin was relatively low. Nevertheless, approximately 100% of human *E. faecalis* strains showed resistance to tetracycline.

The sensitivity to heavy metals - comparing the resistive patterns with the growth curves

Bacteria sensitivity to heavy metals and the growth of bacterial strains were determined in terms of Agar dilution method. The amounts of metal MIC of strains are shown in Figure 6 and curves.

The effectiveness of different concentrations of heavy metals on the growth of human and the livestock *Enterococcus* in terms of agar dilution method are shown in Figure 4.

As a result, the amounts of metal toxicity of both species are as follows:

E. faecalis: Hg> Cd> Cu> Co> Ni> Zn

E. faecium: Hg> Cd> Co> Cu> Ni> Zn

The relationship between antibiotic and metal resistance patterns:

As is observed *E. faecalis* and *E. faecium* strains showed resistance to multiple antibiotics. In addition, it recognized that these species are considerably resistant to four kinds of metals: cobalt, nickel, zinc, and copper.

All *E. faecalis* strains with P/K/N/L/Cc/S/AN/NA antibiotic and metal resistance patterns have Cu^{2+}/Mi^{2+}/Cu^{2+}/Co^{2+}/Zn^{+} patterns as well.

E. faecium strains metal resistance patterns were similar to *E. faecalis* strains. However, their antibiotics resistance patterns varied. human and the livestock sources showed 94% and 100% of resistance to Nitrofurantoin, respectively.

Nevertheless, *Enterococcus* strains from humans showed 40%, 64%, 70% resistance to GM, AN/NA, respectively.

The results show that all *E. faecalis* strains from human and/or livestock sources have simultaneously antibiotic and metal patterns thus: P/K/N/L/Cc/S/AN/NA/Zn^{2+}/Ni^{2+}/Cu^{2+}/C.

Almost 50 and 56% of *E. faecium* strains from human and livestock sources have simultaneously antibiotic and metal patterns of P/S/AN/NA/Zn/Ni/Cu/Co, respectively

Discussion

Determination of *Enterococcal* spp. from human and livestock sources may help to clarify their ecological characteristics (13). The research study on the coasts of the Caspian Sea, shows that the most predominant species are *E. faecalis* and *E. faecium* which is consistent with our study on animal and human sources (13). According to the study performed by de Oliveira and Pinhata, the most resistance to antibiotics was seen in *E. faecalis* and *E. faecium* species as the highest frequency of resistance observed against streptomycin and erythromycin in water samples and against erythromycin and tetracycline in sand samples (14). In the study of Issazadeh et al, all samples were found to be resistant to penicillin (7). In this study, each strain showed a resistance to at least two aminoglycoside antibiotics. However, *E. faecalis* strains from human and livestock sources showed 94% and 100% resistance to nitrofurantoin, respectively. Bacteria were isolated from the environment and livestock samples resistant to heavy metals. Kimiran-Erdem et al showed that 93% of isolates were resistant to Fe and Zn and 85% of them were resistant to Cr (15). In the study of Kermanshahi et al, the most resistance wasto Zn that the high MIC in 2 of the 3 industrial slopes were evaluated as 24 mMol thus belonging to *Coryneform* while *E. faecalis*, and the least amounts evaluated as 8 and 12 mMol belonged to *Citrobacter* and some Bacillus species, respectively (16). In this study, high resistance heavy metal in *E. faecalis* and *E. faecium* refer to Zn and low resistance heavy metal refer to Hg. Finally, the toxicity to mercury is higher than other metals.

Conclusion

According to the observed resistance to heavy metals and antibiotics and comparison with other studies, the bacteria can be spread in the environment and animal resources, therefore the control of this bacteria is very important in environmental health.

Acknowledgments

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Ethical issues

We certify that all data collected during the study is presented in this manuscript and no data from the study has been or will be published separately.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors were involved in study design, data collection, and article approval.

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