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Original Research Article

Multi-drug Resistant *Enterococcus faecalis* isolated from animal and human sources

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ABSTRACT

This work was planned to investigate the prevalence of antimicrobial resistance of *Enterococcus faecalis* isolated from animal and human sources. Ten isolates of *E. faecalis* recovered from urinary tract infections in humans, as well as, ten isolates of *E. faecalis* were recovered from diarrheic dairy cattle studied for their antimicrobial sensitivity to 7 different antibacterial agents. Antimicrobial sensitivity pattern proved that most isolates were resistant to most of the tested antimicrobial agents. All isolates of human *E. faecalis* were 100 % resistant to rifamycin, gentamicin and penicillin G. Resistance to amikacin, ciprofloxacin, levofloxacin and vancomycin was 80.0%, 90.0%, 90.0% and 70.0% respectively. However animal *E. faecalis* were completely (100%) resistant to penicillin G and ciprofloxacin. Resistance to gentamicin, amikacin, levofloxacin, rifamycin and vancomycin was 70.0%, 40.0%, 20.0%, 20.0% and 0.0% respectively. PCR was applied on MDR for detection of aminoglycosides resistance genes. All human *E. faecalis* isolates were negative for aph(2'')-Ia, aph(2'')-Ib, aph(2'')-Ic and aph(2'')-Id. 40.0% of isolates were proved to harbour aph(3'')-IIIa and 10.0% (one isolate) harboured ant(4'')-Ia. However all animal *E. faecalis* isolates were negative for aph(2'')-Ib, aph(2'')-Ic, aph(2'')-Id. Two isolates (20.0%) harboured aph(2'')-Ia and ant(4'')-Ia and four isolates (40.0%) harboured aph(3'')-IIIa. In conclusion, the increased antibiotic resistance of *E. faecalis* isolated from animal and human sources complicate treatment decisions and increase public health hazard.

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1. Introduction

Enterococci are a part of the microflora of both human and animals with the most common prevalence of *E. faecium* and *E. faecalis* in the human gastrointestinal tract and *E. faecium* in production animals (Klein, 2003). There is a lack of assessment and systematic reporting of adverse events in probiotic intervention studies (Hempel et al., 2011).

Although microorganisms used as probiotics in animal feed are generally safe, some of the bacterial species pose risks mainly by transmission of antibiotic resistance to pathogenic microbes, or production of enterotoxins (Anadon et al., 2006).

In spite of several examples of beneficial effects of *Enterococcus* probiotics in animals and humans and a long history of safe use, these bacteria have been associated with several infections and the presence of transferable antibiotic resistance determinants (Franz et al., 2003; 2011).

Enterococcus species, particularly *E. faecalis* and *E. faecium* are associated with community and hospital acquired infections, and were amongst the most prevalent causes of hospital acquired infections (Spera and Farber., 1992).

Several virulence factors from *Enterococcus* have been identified and are associated with either colonization, invasion or production of pathological lesions (Franz et al., 2011).

The emergence of multi- drug resistant pathogens is now one of the greatest threats to public health (Sengupta et al., 2013). Imprudent use of antibiotics is believed to be the major cause of wide spread antibiotic resistance (Davies and Davies, 2010). Antibiotic resistance genes are generally present in plasmids, transposons and integrons of bacteria and can transfer from one bacterium to another by mechanisms of horizontal gene transfer (Carattoli, 2001).

The GIT of animals contain a complex microbial ecosystem with diverse and large numbers of microorganisms. Proximity of bacteria to each other in complex microbial ecosystem like the

intestine can favor the transfer of genetic material, including antibiotic resistance genes from non-pathogenic to pathogenic microorganisms (Aarts and Margolles, 2015). This work was achieved to detect the prevalence of aminoglycosides resistance associated genes in multi-drug resistant isolates of *E. faecalis* recovered from human and animal sources.

2. Material and Methods

2.1. Bacterial strains:-

Ten isolates of *E. faecalis* recovered from urinary tract infections in humans, as well as, ten isolates of *E. faecalis* were recovered from diarrheic dairy cattle were completely identified according to (Collee et al., 1996; and Quinn et al., 2002).

2.2. Antimicrobial resistance profile (CLSI, 2016):-

All isolates were studied for their antimicrobial sensitivity to 7 different antibacterial agents, namely; amikacin (AK), levofloxacin (LEV) , gentamicin (CN), penicillin G (PEN), ciprofloxacin (CIP), vancomycin (VAN) and Rifamycin (RD) using disc diffusion method.

2.3. Polymerase Chain Reaction (PCR), (Vakulenko et al., 2003):-

It was applied for detection of antibiotic resistance genes using the primers presented in table (1).

Table (1): List of primers used in the study.

Genes	Primers	Annealing temperature	Product size (bp)	REF
<i>aac(6')-Ie-aph(2'')-Ia</i>	CAGAGCCTGGGAAGATGAAG CCTCGTGAATTCATGTTCTGGC	55°C	348	
<i>aph(2'')-Ib</i>	CTTGGACGCTGAGATATATGAGCAC GTTTGTAGCAATTCAGAAACACCCTT	55°C	867	
<i>aph(2'')-Ic</i>	CCACAATGATAATGACTCAGTTCCC CCACAGCTTCCGATAGCAAGAG	55°C	444	Vakulenko et al. (2003)
<i>aph(2'')-Id</i>	GTGGTTTTTACAGGAATGCCATC CCCTCTTCATACCAATCCATATAACC	55°C	641	
<i>aph(3'')-IIIa</i>	GGCTAAAATGAGAATATCACCGG CTTTAAAAAATCATACAGCTCGCG	55°C	523	
<i>ant(4'')-Ia</i>	CAAAGTCTAAATCGGTAGAAGCC GGAAAGTTGACCAGACATTACGAACT	55°C	294	

2. Results

3.1. Results of antibacterial sensitivity:-

3.1.1. *Enterococcus faecalis* of human origin:

All isolates (10) were 100 % resistant to rifamycin, gentamicin and penicillin G. Resistance to amikacin, ciprofloxacin, levofloxacin and vancomycin was 80.0%, 90.0%, 90.0% and 70.0% respectively.

3.1.2. *Enterococcus faecalis* of animal origin:

All isolates (10) were completely (100%) resistant to penicillin G and ciprofloxacin. Resistance to gentamicin, amikacin, levofloxacin, rifamycin and vancomycin was 70.0%, 40.0%, 20.0%, 20.0% and 0.0% respectively.

3.2. Results of the prevalence of antimicrobial resistance genes in studied isolates:

Aminoglycosides resistance genes *aac(6'')Ie*, *aph(2'')-Ia*, *aph(2'')-Ib*, *aph(2'')-Ic*, *aph(2'')-Id*, *aph(3'')IIIa* and *ant(4'')-Ia* genes were studied for their prevalence in human and animal isolates of *E. faecalis* and the following results were obtained.

3.2.1. *E. faecalis* of human origin:

All isolates were negative for *aph(2'')-Ia*, *aph(2'')-Ib*, *aph(2'')-Ic* and *aph(2'')-Id*. 40.0% of isolates were proved to harbour *aph(3'')-IIIa* and 10.0% (one isolate) harboured *ant(4'')-Ia*.

3.2.2. *E. faecalis* of animal origin:

All isolates were negative for *aph(2'')-Ib*, *aph(2'')-Ic*, *aph(2'')-Id*. Two isolates (20.0%) harboured *aph(2'')-Ia* and *ant(4'')-Ia* and four isolates (40.0%) harboured *aph(3'')-IIIa* (Table 2).

Table (2): prevalence of aminoglycosides resistance genes of recovered *E. faecalis* isolates.

Aminoglycosides resistance genes	<i>E. faecalis</i> of human origin	<i>E. faecalis</i> of animal origin
Aph(2'')-Ia	Negative	20.0%
aph(2'')-Ib	Negative	Negative
aph(2'')-Ic	Negative	Negative
aph(2'')-Id	Negative	Negative
aph(3'')-IIIa	40.0%	40.0%
ant(4'')-Ia	10.0%	20.0%

% was calculated according to number of examined isolates. (NO. = 20)

4. Discussion

Although microorganisms used as probiotics in animal feed are relatively safe, precautions should be taken to protect animals, humans and

the environment from potentially unsafe microorganisms.

Theoretically, risks associated with the use of probiotics in animal feed are gastrointestinal or systemic infection of the animal fed the probiotic and consumers of the animal products and transfer of antibiotic resistance for probiotics to other pathogenic microorganisms (Doron and Snyderman, 2015).

The antibiotic susceptibility of an isolate is usually required for effective clinical control. Microbial resistance to antibiotics is increasing among many bacterial species partially as a result of bacterial dynamism in adapting to its environment as well as increasing use, and misuse, of existing antibiotics in human and veterinary medicine and rapidly becoming a major world health problem (Van Den Bogaard et al., 1999).

Resistance to antimicrobial agents is encoded by chromosomal and plasmid genes harboured by the bacterial organisms, these genes may be inherent or acquired via vertical or horizontal transfer mechanisms (Tenover, 2006).

In the present work, 20 MRD isolates of *E. faecalis* isolated from cases of urinary tract infection (10 human isolates) and gastrointestinal tract (10 animal isolates) were subjected to 7 different antibacterial agents and the results proved that all isolates were multi- drug resistant (MRD). These results were agreed with the results obtained by several authors such as Lukasova et al., (2003); Lombardi et al., (2008) and Barbosa et al., (2009).

In vitro antibacterial resistance to gentamicin and amikacin of the aminoglycosides group was 100% and 90.0% for human isolates respectively and 70.0% and 40.0% for animal isolates respectively. These isolates were screened for the presence of 6 different amino glycosides resistance genes and the following results were obtained, two out of 6 screened genes were the only detected genes in human isolates with a percentage of 40.0% for *aph(3'')-IIIa* and 10.0% for *ant(4'')-Ia*.

For isolates of animal origin, 3 genes were detected namely: *aph(2'')-Ia*(20.0%), *ant(4'')-Ia*(20.0%) and *aph(3'')-IIIa* (40.0%).

Antibiotic resistance genes are generally present in plasmids, transposons and integrons of bacteria and can transfer from one bacterium to another (Blair et al., 2015). The GIT of animals contain a complex microbial ecosystem with diverse and large numbers of micro-organisms and if a bacterium intended to be used as an animal probiotic is harbouring transferable antibiotic resistance genes, this could be a medium for transfer of antibiotic resistance to the environment and humans (Gonzalez-zorn and Escudero, 2012).

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