Research Article

LABs Responsible for Enhancing Antibiotic Susceptibility Pattern and Gene Transference

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ABSTRACT: Probiotics are gram positive organisms considered beneficial for combating with various pathogens. Most of them are antibiotic resistant, giving an idea that they may be carrier of r-plasmids. Our aim of the study was to determine the proportion of LABs that associate with r-gene transference as well as in boosting up antibiotic susceptibility range. For that we isolated probiotic cultures of *Streptococcus, Lactobacillus*, and *Leuconostoc* from milk sample, and checked the susceptibility pattern of isolated probiotics and pathogens (test organisms) against carbepenem. Results showed all LABs are resistant, while most of the pure test organisms were sensitive, after that these pathogens were treated with Labs coated disc that was prepared by the Kirby Bauer method. These LAB treated organisms were again checked with carbepenem to check the susceptibility pattern, this whole protocol was carried out on Muller Hinton Agar (MHA) plate. According to our results test organisms treated with *Streptococcus* and *Lactobacillus* species occurred with a 20% and 1% of resistivity, while organisms treated with Leuconostoc species enhanced the sensitivity 66% and streptococcus 1%, whereas Lactobacillus did not show any change in antibiotic spectrum.

Keywords: Probiotics, kirby bauer method, carbepenem, susceptibility pattern.

INTRODUCTION

Probiotics group of gram positive live as a single or colonized form; tend to play essential role in improving men's immunity by maintaining the normal flora. These organisms produce secondary metabolites that carry antimicrobial activity against many of the pathogenic organisms [1], use in food and in aquaculture [2]. These gram positive bacteria are categorized as LAB [1], they are usually used as starter culture in production of dairy items. LABs are categorized in different groups [3]. These can be identified on the basis of pH motility, microscopy, catalase and oxidase production, NaCl and bile tolerance [15, 4, 29]. These organisms play role in reducing the lactose intolerance and cholesterol level, antitumor activity and activation of immune system [5]. The taxonomic tool for their identification is fructose-6-phosphate phosphoketolase (F6PPK) [5, 6]. The identification of these can be possible at molecular level by PCR. The backbone of *streptococcus* bacteria is joined together by ether linkages which separates it from other bacterial species, virulent *streptococcus* species due to absence of surface proteins (lipoproteins) [7]. Exopolysaccharide genes eps E, eps G, eps I play an important role in the production of fat reducing cheddar cheese and other dairy products [7, 8]. They boost up human immunity, present as intestinal flora (increase digestion) [10, 14] used in replacement of chemotherapy [11] and also in the treatment of Antibiotic associated diarrhea [12, 13-15]. Now days, it has gained popularity among researchers because in S. thermophillus, the genome is shorter than most, 1.8Mb [9].

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PROBIOTICS AND GENE – ACQUISI-TION

Today, probiotics have gained a lot of consideration and can be used as a standby for antibiotic growth promoter. [16, 17]. The difference in dosage is due to variation in persons age, sex and environmental factors [18, 19]. These are beneficial against intestinal pathogens. Most use is *lactobacillus* and *streptococcus* [25]. In efficient case of diarrhea [20, 25, 28]. Probiotics blocks the receptor sites antagonistically [21]. Firstly, viral replication is inhibited by the limiting nutrients to overcome diarrhea [22]. Secondly, they also produce antibodies, releasing cytokines and initiate cell mediated immunity that boost up the immunity [22]. Third mechanism is they inhibit motility of these pathogens by releasing cytokines and nitric oxide [23]. Manner A [24], shows in his in vitro study that lactobacilli produces such substances that have the capability to inhibit virions [24].

Probiotics in Developing Resistance

Resistance to antibiotic is the most controversial topic now a day. Most of the pathogen gets resistance genes. Mammalian Intestinal flora provides a most favorable environment for

gene transference between many bacterial species. No association of gene transfer identifies among dissimilar genus (Fungi-Bactria), fungi itself are able to produce medicinal components as a secondary metabolite [26].

Phylogeny of Antibiotic Resistance Genes

In past horizontal gene transfer is difficult experimentally in taxonomically different group of bacteria. It totally depends on the environmental conditions. The phylogenetic approach was applied to analyze the metamorphic record of antibiotic resistance genes, encoding ribosomal protection proteins which play important role as substitute of elongation factors and confer resistance to tetracyclines [27].

MATERIALS AND METHODS

Isolation of Probiotic Organisms

In this study isolation of Probiotics was done by camel milk. Milk sample was swabbed on De Man, Rogosa and Sharpe (MRS) agar prepared medium purchased from Merk. Plate was incubated at 37°C for 48-72 hours to analyze the colony morphology [21, 22]. Gram staining was performed for microscopic analysis. It was sub cultured further to get pure colonies by inoculating single colony on MRS agar for lactobacillus spp, MRS supplemented with L-cystien 0.05% for the *streptococcus* (fat lowering bacteria) [23, 24] incubate at 37°C for 24 and 48 hours respectively, colonies were inoculated on Heart Infusion agar supplemented with 5% glucose (HIAG) and Heart Infusion Agar supplemented with 5% Sucrose, 0.5% Glucose, and 0.02% Sodium azide (HIAS) colonies obtained from HIAS were inoculated on Mayeux Sandine Elliker agar (MSE) prepared by adding (Tryptone 10g/l, Gelatine 2.5g/l, Yeast extract 5g/l, Sucrose 100g/l, Glucose 5g/l, Sodium Azide 75mg/l, Sodium citrate 1g/l, Agar 15g/l) which is elective medium for *Leuconostoc* species and incubated at 30°C for 3 days. Spot Tests of all the LAB were determined on the basis of motility, catalase, and oxidase by picking up colonies from their respective medium plates. Motility of isolated cultures was determined by cavity slide technique, leuconostoc was confirmed by growing in 6.9% NaCl. Further, it was confirmed by microscopy. We collected various pathogenic organisms from different diagnostic laboratories like *Shigella Brunetti*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Escherichia coli*, *Listeria monocytogenes*, *Micrococcus luteus* and *Salmonella*. These were treated with a synthetic drug, Imipenem, (belong to the group of Carbepenem drug, this was used because most organisms are resistant to this drug). Probiotics were also treated with Imipenem to check the presence of resistivity genes, these were compared with the results of Lab treated organisms and Bacteriocin treated organisms. Bacteriocin was obtained by isolating the organism from respective mediums and inoculated in MRS broth (for *lactobacillus* species), MRS broth supplemented with L-cysteine (*Streptococcus thermophillus*), MRS with 6.5% NaCl (for Leuconostoc). All the broth cultures were further tested for antibacterial activity by agar well disk diffusion method, in which LAB grows them and inhibits test organisms. All tubes were incubated at 37°C for 48-72 hours while *Leuconostoc* at 30°C for 72 hours (as these organisms produce bacteriocin in late log phase). All the tubes was centrifuged at 6000 RPM for 20 minutes, transparent supernatant were separated out by the help of sterile syringe passing it through the 0.2NM filter assembly to avoid any of the bacterial contamination to collect in screw caped test tube and refrigerate at -20°C. Sensitivity of human pathogen was checked out by following disk diffusion method as given by Kirby-Bauer Method on nutrient and MHA agar. Zones were considerd sensitive if break-point is greater than 8mm. All the organisms were checked for gene acquisition, after treating with bacteriocin, if there was any phylogenetic change observed. For this we took LAB treated test organisms and pure isolates; checked susceptibility pattern against synthetic drug that is imipenem by the Kirby-Bauer method. Observed susceptibility patterns of both LAB treated and pure isolates. This method is performed for all LABs that are isolated from the Raw Dromedary Camels Milk. If there is a decrease in sensitivity pattern as compared to the standard result of Imipenem this indicates Resistivity gene transference is accomplished [26, 27, 29].

DISCUSSION

The study was carried out in the Microbiology department of Jinnah University for Women, Pakistan. Different Probiotics had been isolated from the milk sample. All isolates were purified by growing in their respective supplemented medium and spot tests. *Lactobacillus* spp, *Streptococcus* spp, *Leuconostoc*, gram positive long and short rods of bacillus, cocci in chains, cocco bacillary identified under microscope. While all are oxidase and spores Negative, non motile except for *leuconostoc*, catalase negative except for *Lactobacillus* that is considered as pseudo positive [24, 26, 27]. Bacteriocin had been extracted from the late log phase stage. Antibiotics enhancing effects and gene acquisition with probiotics were observed by using synthetic drugs on pure pathogenic isolates and on LAB treated pathogenic isolates. Table **1** showes the susceptibility patterns of pure isolates *Shigella burnetti*, *Acinitobacter baumannii*, *Klebsiella pneumoniae*, *Staphylococcus au-reus*, *Proteus vulgaris*, against Imipenem, which was 25mm, 25mm, 17mm, 24mm, 28mm and 22mm respectively, while LAB treated isolates were *Eschereshia coli*, *Acinitobacter baumannii*, *Listeria monocytogenes*, *Pseudomonas aureginosa*, *Staphylococcus. aureus*, *Micrococcus leutes*, and *Klebsiella pnumoniae*, with

RESULTS:

Table 1a. Microscopic Analysis.

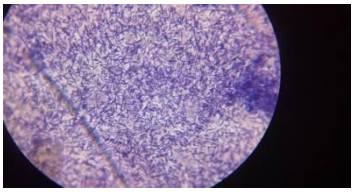
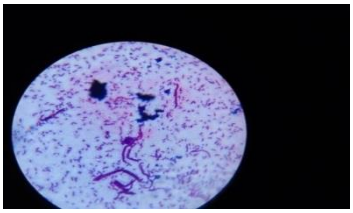
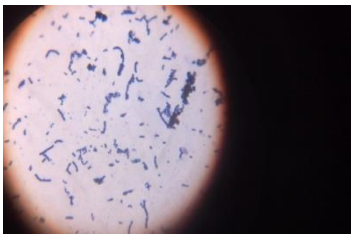
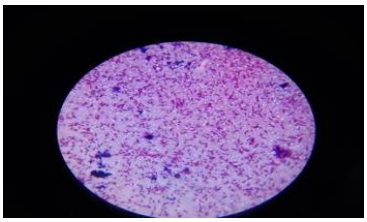
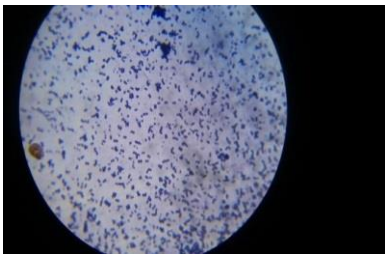
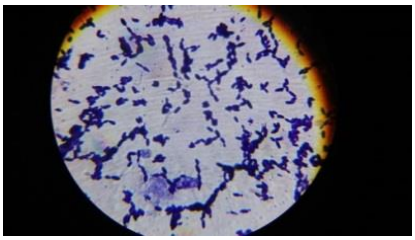
Isolated Organisms	Media	Microscopic Characteristics	Colony Morphology
Mix Culture from dromedary milk.	MRS	Gram positive, rods, cocci. Cocobacilus, scattered.	
Lactobacillus spp	MRS	Gram positive, Long and short rods of bacillus	
Streptococcus. thermophillus	MRS + L-Cystien	Cocci in chains	
Leuconostoc	MSE (6.9% NaCl)	Coccobacillary	
Pediococcus	MRS+ L-cysteine	Cocci in tetrades	
Yeast cells	MRS+ L-Cystien	Round to oval scattered cells	

Table 1b. Spot Tests for differentiation of LAB.

Isolated Organisms	Catalase	Oxidase	Motility	Spores	Gram Reaction
Lactobacillus spp	Negative (pseudo-positive)	Negative	Negative	Negative	Positive Rods
Streptococcus. thermophillus	Negative	Negative	Negative	Negative	Positive cocci
Leuconostoc	Negative	Negative	Positive	Negative	Positive coccobacillus
Pediococcus	Negative	Negative	Negative	Negative	Positive cocci
Yeast	-	Negative	Negative	-	Purple ovoid cells

Table 2. Effect of Synthetic drug on Untreated bacterial cultures.

S. No.	Test Organisms	Media	Drug	Zones
1-	*Shigella burnetti*	MHA	Imipenem	25mm
2-	*Acinitobacter baumannii*	MHA	Imipenem	25mm
3-	*Klebsiella.pneumoniae*	MHA	Imipenem	17mm
4-	*Pseudomonas.aureginosa*	MHA	Imipenem	24mm
5-	*Staphylococcus.aureus*	MHA	Imipenem	28mm
6-	*Proteus vulgaris*	MHA	Imipenem	22mm
7-	*Salmonella typhi*	MHA	Imipenem	40mm

*All isolated Labs were resistant to Imipenem which indicates presence of Resistivity genes.

Table 3. Effects of Synthetic Drug on LAB treated Test organisms.

S. No.	Test Organisms (LAB Treated)	Media	Synthetic Drug Treatment	
			Drug	Zones
1-	*Eschereshia coli*	MHA	Imipenem	19mm
2-	*Acinitobacter baumannii*	MHA	Imipenem	18mm
3-	*Listeria monocytogenes*	MHA	Imipenem	27mm
4-	*Pseudomonas aureginosa*	MHA	Imipenem	22mm
5-	*Staphylococcus aureus*	MHA	Imipenem	25mm
6-	*Micrococcus leutes*	MHA	Imipenem	R
7-	MHA	Imipenem	25mm	MHA

Table 4. Screening of Bactericin by Comparing With Gene-Acquisition to Check Resistivity Pattern or Sensitivity in Combination with Synthetic Drug.

S. No.	Test Organisms (Untreated)	Zone of Prepared Disks of Streptococcus	Media
1-	*Eschereshia coli*	12mm	Nutrient Agar
2-	*Acinitobacter baumannii*	15mm	Nutrient Agar
3-	*Listeria monocytogenes*	25mm	Nutrient Agar
4-	*Pseudomonas aureginosa*	19mm (no geen pigment production)	Nutrient Agar
5-	*Staphylococcus aureus*	16mm	Nutrient Agar
6-	*Micrococcus leutes*	13mm	Nutrient Agar
7-	*Klebsiella pneumoniea*	10mm	Nutrient Agar
8-	*Proteus vulgaris*	19mm	Nutrient Agar
9-	*Shigella burnetti*	20mm	Nutrient Agar

Table 5. Effects of Effect of Imipenem on LAB (*Streptococcal*) treated pathogens for Gene-Acquisition.

S. No.	Test Organisms (Treated)	Media	Drug	Zones
1-	*Eschereshia coli*	MHA	Imipenem	20mm
2-	*Acinitobacter baumannii*	MHA	Imipenem	15mm
3-	*Listeria monocytogenes*	MHA	Imipenem	20mm
4-	*Pseudomonas aureginosa*	MHA	Imipenem	18mm (pigment production)
5-	*Staphylococcus aureus*	MHA	Imipenem	25mm
6-	*Micrococcus leutes*	MHA	Imipenem	23mm
7-	*Klebsiella pneumoniea*	MHA	Imipenem	29mm
8-	*Proteus vulgaris*	MHA	Imipenem	29mm
9-	*Shigella.burnetti*	MHA	Imipenem	25mm

Table 6a. Efficiency of extracted bacteriocin from *Lactobacillus* on untreated bacterial cultures.

S. No.	Test Organisms (Untreated)	Media	Zone of Prepared Disks of Lactobacillus
1-	*Eschereshia coli*	MHA	R
2-	*Salmonella typhi*	MHA	30mm
3-	*Pseudomonas aureginosa*	MHA	R
4-	*Shigella burnetti*	MHA	R

5-	*Staphylococcus aureus*	MHA	R
6-	*Micrococcus leutes*	MHA	R
7-	*Klebsiella pneumoniea*	MHA	R
8-	*Proteus vulgaricus*	MHA	R

Table 6b. Effect of Imipenem on LAB (*Lactobacillus*) treated pathogens for Gene-Acquisition.

S. No.	Test Organisms (Untreated)	Media	Drug	Zones
1-	*Eschereshia coli*	MHA	Imipenem	R
2-	*Salmonella typhi*	MHA	Imipenem	R
3-	*Pseudomonas aureginosa*	MHA	Imipenem	R
4-	*Shigella burnetti*	MHA	Imipenem	R
5-	*Staphylococcus aureus*	MHA	Imipenem	R
6-	*Micrococcus leutes*	MHA	Imipenem	R
7-	*Klebsiella pneumoniea*	MHA	Imipenem	R
8-	*Proteus vulgaricus*	MHA	Imipenem	R

Table 7a. Efficiency of extracted *bacteriocin* from *Leuconostoc* on untreated bacterial cultures.

S. No.	Test Organisms (Untreated)	Media	Zone of prepared Disks of Leuconostoc
1-	*Eschereshia coli*	Nutrient Agar	R
2-	*Acinitobacter baumannii*	Nutrient Agar	R
3-	*Listeria monocytogenes*	Nutrient Agar	R
4-	*Psuedomonas aureginosa*	Nutrient Agar	R
5-	*Staphylococcus.aureus*	Nutrient Agar	R
6-	*Micrococcus leutes*	Nutrient Agar	R
7-	*Klebsiella.pneumoniae*	Nutrient Agar	15mm
8-	*Proteus.vulgaricus*	Nutrient Agar	19mm
9-	*Shigella.burnetti*	Nutrient Agar	R

Table 7b. Effect of Imipenem on LAB (*Leuconostoc*) treated pathogens for Gene-Acquisition.

S. No.	Test Organisms (Treated)	Drug	Media	Zone
1-	*Eschereshia.coli*	Imipenem	MHA	32mm

2-	*Acinitobacter baumannii*	Imipenem	MHA	23mm
3-	*Listeria.monocytogenes*	Imipenem	MHA	25mm
4-	*Psuedomonas.aureginosa*	Imipenem	MHA	28mm
5-	*Staphylococcus.aureus*	Imipenem	MHA	35mm
6-	*Micrococcus leutes*	Imipenem	MHA	30mm
7-	*Klebsiella.pneumoniae*	Imipenem	MHA	32mm
8-	*Proteus.vulgaricus*	Imipenem	MHA	29mm
9-	*Shigella.burnetti*	Imipenem	MHA	27mm

19mm, 18mm, 27mm, 22mm, 25mm, R, 25mm, respectively. *Streptococcus* is playing 22% role in resistance gene transference Major organisms were *Acinitobacter baumannii* and *Pseudomonas aureginosa* that showed 1% enhanced antibiotic *susceptibility*, *Lactobacillus* is involving 1% resistance gene transference. On the other hand *Leuconostoc* is not involved in gene transference while enhancing the antibiotic susceptibility pattern about 66%. LABs are resistant to many of the antibiotics [30]. This is indication that LAB may be involved in gene transference within the pathogens. [31] Also reported that different Labs like *Lactobacillus* spp and *streptococcus species* in GIT are involved in Resistance gene transference [31]. GTF gene, is accountable for the coding of glucosyltransferase (GTF) and has the capability to produce polysaccharides from sucrose, that was obtained by means of transposons from various LAB, *e.g. Lactobacillus* and *Streptococcus* come across in fermented food [32]. According to another study [33]. It is revealed that probiotics in combination with synthetic drug are responsible for a better antibacterial activity. It requires small dose to treat a disease with any probiotic formula that is also observed in our results, most of the organisms involved are *leunonostoc* treated *Staphylococcus aureus, Shigella burnetti, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas. Aureginosa*

CONCLUSION

Nowadays, probiotics are gaining importance and are used as a stand by (growth promoter) instead of multiply resistant drug. Probiotics are given with different dosages in accordance with sex, age and environmental factors. It is specially used against intestinal, pathogens and boost up immunity level. Purpose of this study was to determine antibiotic susceptibility pattern and differentiate antibiotic resistence gene transference.

CONFLICT OF INTEREST

Declared none.

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