

Antibiotic Susceptibility Pattern of Lactobacillus spp Isolated from yogurt samples collected from three different places of Lahore.

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Abstract— Lactobacillus is a gram positive, catalase negative, oxidase negative, endospore forming, rod shaped bacteria products. Aim of this study is to know antibiotic susceptibility and resistance of lactobacillus. For this purpose lactobacillus was isolated from a common yogurt sample. Lactobacillus was isolated then identified through gram staining and other biochemical tests. Antibiotic discs in this study (Penicillin, Streptomycin and Norfloxacin) were applied to the isolated colony of lactobacillus. Three samples were collected from different locations of Lahore. The samples collected contained, Milk sample, Yogurt sample and cheese sample. After observing the results it was concluded that Norfloxacin has the higher activity against lactobacillus than that of streptomycin and penicillin. Streptomycin has an intermediate activity, while penicillin has the lowest activity.

Key words—antibiotic, Elliker medium, lactobacillus ,resistance, spread plate, susceptibility,yogurt

INTRODUCTION

LACTOBACILLUS is a common bacteria that is responsible for the formation of yogurt and other dairy products. Yogurt, Cheese, pickles, wines and cider etc are the gifts of lactobacillus. In milk and its products lactobacilli are present naturally. Lactobacilli can also be added artificially in order to produce health benefit for consumer. Selected strains of lactobacilli are used as adjuncts e.g. cow's milk cheeses or cheddar cheese.

Some disadvantages are associated with the culture used as adjuncts, such as off flavor crumbled textures, high acidity etc. The selection of a culture is important and to know its antibiotic resistance is very important. Apart from all those benefits that are obtained from lactobacillus, it is recently discovered, that in presence of some predisposing factors, lactobacillus may cause some type of infections in human. It is declared that lactobacilli act as promoter of gastrointestinal and female urogenital tract infections. This direct extra care in order to select lactobacilli in microbial adjuncts.

Lactobacillemia is one of the therapeutic difficulty. In eradicating lactobacillus from the infected patients, especially from the endocardium. Although properly treated, still the serum antibiotic concentration exceeds the minimal inhibitory concentration (MIC). Lactobacillus is

one of the bacteria that is used as probiotic. Lactobacillus can be a host of antibiotic resistance genes. These genes can be transferred to pathogenic bacteria, so before launching any starter culture it is very necessary to verify that a single bacterial isolate do not contain transferrable genes. To know this it is very necessary to verify that a single bacterial isolate do not contain transferrable genes. To know this it is necessary to know the antibiotic susceptibility of the bacterium. In this case it is lactobacillus. Horizontal gene transfer is becoming one of the core issues in lactobacillus studies. There are many strains of lactobacillus which were regarded, generally recognized as safe (GRAS), become vectors of genes that are resistant for some antibiotics. As lactobacillus is usually consumed in high quantities and close contact with other bacteria in the human gastro intestinal tract (GIT), provides good conditions for the genes that can be horizontally transferred. Various studies have been performed on the susceptibility and the development of resistance of lactobacilli to penicillin and other antibiotics.

Bacterial resistance to antimicrobial agents is a major global public health problem, affecting not only human and veterinary medicine (Ammoret al. 2008) but also food production. The food chain is becoming a possible way of dissemination of antibiotic resistance among bacterial populations of animals and humans (Witte 2000). Many species of lactobacilli, previously generally recognized as

safe (GRAS), may become vectors of antibiotic resistance genes.

These bacteria are usually consumed in high quantities and close contact with other bacteria in the human gastrointestinal tract provides perfect conditions for horizontal transfer of conjugative plasmids and transposons with genes encoding resistance to antimicrobial agents (Mathur& Singh 2005; Jacobsen et al. 2007; Ammoret al. 2008; Nawaz et al. 2011). The absence of the acquired antimicrobial resistance has become an important criterion for evaluating the safety of lactobacilli used as starter cultures or probiotics (Mayrhofer et al.,2008).

Although the minimum inhibitory concentrations (MIC) are defined for clinically important microorganisms, internationally valid MICs for lactobacilli have not been determined yet. To distinguish the strains with the acquired antimicrobial resistance from the susceptible ones, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) of the European Food Safety Authority (EFSA) defined the microbiological breakpoints used in the assessment of bacterial resistance to antibiotics of human or veterinary importance. The breakpoint data were derived from the published body of research and from national and European monitoring programs (EFSA 2008).

ANTIBIOTIC SUSCEPTIBILITY TEST:

After getting the isolated strain, the antibiotics were applied to the strain.10 ug of each antibiotic (Penicillin, Streptomycin and Norfloxacin) were applied to the isolated

Table .1Recipe For Elliker Medium In1liter of Distilled Water

Serial number	Component	G/liter
1	Trypton	20
2	Yeast extract	0.5
3	sodium acetate	1.5
4	dextrose	5.0
5	ascorbic acid	0.05
6	gelatin	2.5
7	sodium chloride	4.0

Table 2. Antibiotic Suceptibilty Chart of Lactobacillus

MATERIALS AND METHODS

Lactobacillus is selectively grown on Elliker medium. The recipe for preparation of elliker broth for 1 liter of distilled water is as follows in table.1.

Prepared 100 ml Elliker broth for the isolation of lactobacillus. The broth was kept in test tubes. Common yogurt samples were taken from the local farms of Lahore. The sample was kept for one day in a closed tube in order to obtain whey.1 ml of the sample was mixed in the first test tube of the broth, and then serially diluted up to 4th test tube. Test tubes containing broth and sample were then placed in an incubator. The tubes were kept for 48 hours. Then 50 ul of the sample was spread over the elliker medium.

The spread plates were then kept in incubator for 24 hours. visible growth of lactobacilli strain was observed. the plates were further streaked in order to get the pure colony. Streaked plates were kept in incubator or 48 hours. pure colony of lactobacillus was obtained which was confirmed by applying gram staining and other biochemical tests such as catalase test ,oxidase test .

strain. The discs were placed in accordance of disc diffusion method. The plates were then kept in incubator for 48 hours to find out the results The same procedure was repeated for the rest of the two samples taken from separate locations.

Serial number ^a	Antibiotic concentration	Resistance	Intermediate	Sensitive
Sample # 1	Norfloxacin 10 ug	<12	13-16	>17
Sample # 2	Penicillin 10 ug	<11	12-21	>22
Sample # 3	Streptomycin 10ug	<12	13-17	>18

^aSample Details. (Sample # 01:from RA bazar Lahore Pakistan) (Sample #02:from bhatta chowk Lahore Pakistan) (Sample#03:from lidhar pind Lahore Pakistan)

RESULTS:

Table 3. Antibiotic Susceptibility Pattern

Sr.No	Antibiotic	Resistant	Intermediate sensitive	Sensitive
1	Norfloxacin	0	0	3
2	Penicillin	2	1	0
3	streptomycin	1	2	1

All of the three isolates from different samples were tested against the action of the three antibiotics (Norfloxacin ,Penicillin, Streptomycin).Norfloxacin was found to be most effective Against the three isolates of lactobacillus isolated from yogurt samples. All of the three isolates were sensitive to the action of Norfloxacin. Streptomycin had an intermediate activity. Two of the three strains were found to be intermediate sensitive against the action of streptomycin. While one was sensitive. Penicillin had the

lowest activity against all the three isolated strains of lactobacillus. Only one out of three was found to be sensitive against the action of penicillin.while rest of the two were found to be resistant.

The resistance of lactobacillus against the action of penicillin is considered to be due to horizontal gene transfer between bacteria and the excessive use of penicillin over the decades.

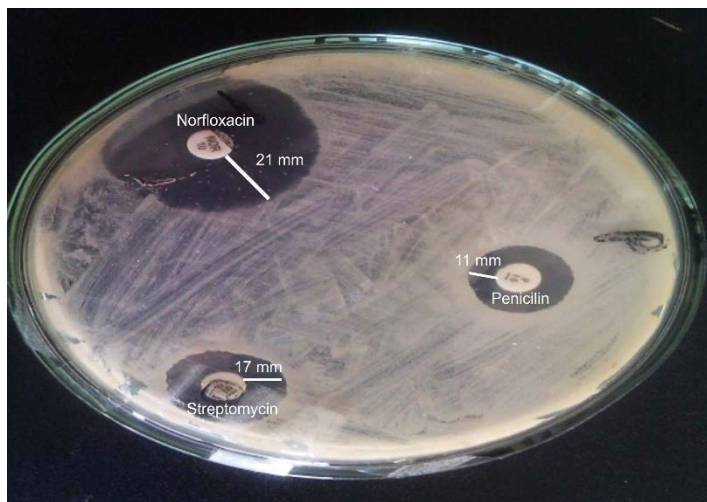


Figure 1. Zone of inhibition of Antibiotics.

Results of a single isolate.

Sr.no	Unit	Zone of inhibition	Antibiotic disc
1	10 ug	17 mm	Streptomycin
2	10 ug	21 mm	Norfloxacin
3	10ug	11mm	penicillin

Discussion

An intriguing phenomenon noted in many previously cited cases of lactobacillema was the therapeutic difficulty in eradicating this organism from deep-seated infected foci, especially the endocardium, despite seemingly appropriate treatment regimens and serum antibiotic concentrations exceeding the minimal inhibitory concentration(MICs).

Many previously cited cases of lactobacillema was the therapeutic difficulty in eradicating this organism from deep-seated infected foci, especially the endocardium, despite seemingly appropriate treatment regimens and serum antibiotic concentrations exceeding the minimal inhibitory concentrations (MICs).

GEORGE W. BURNETT (1956) reported that Some strains of oral lactobacilli developed considerable resistance when grown in maximal concentrations of penicillin; others did not. On the other hand, none of the strains tested developed resistance upon prolonged growth in the initial concentration of penicillin to which they were resistant. The high resistance of the lactobacilli to penicillin was not maintained indefinitely without continued cultivation in the presence of a maximal concentration of antibiotics. The decrease in resistance of the lactobacilli to penicillin during cultivation in the absence of the antibiotic was gradual and such strains did not completely recover their former level of susceptibility to the antibiotic during the period of experimentation. The rate of antibiotic resistant bacteria has increased lately. where resistance among bacteria common to the human GI tract is of no exception. Drug resistance presents a health concerning issue, especially if resistance is pointed towards clinical antibiotics and when genetic elements carrying resistance genes are mobile and as such possibly transmitted to health harming microbes. As it is well known, LAB naturally shows both antibiotic resistance and susceptibility and since they have a long history of safe use with no indications of transfer resistance to other Kunz, Anjali N.; Noel, James M.; Fairchok, Mary P. reported that Although Lactobacillus generally is not considered a pathogen, it can cause disease in compromised hosts, including bacterial endocarditis, pleuropulmonary infections, gastrointestinal abscesses, urinary tract infection, conjunctivitis, dental caries, and endometritis (3). Lactobacillus bacteremia, presumably secondary to bacterial translocation from the gastrointestinal tract, has been reported in patient with

species, especially species of lactobacilli, leuconostoc and pediococci, they still remain leading microbes in fermentation processes and as human and animal probiotics. Besides all the properties that define a strain as a good probiotic, the antibiotic resistance and the ability of such strain to act as a donor of antibiotic resistance genes must be carefully assayed as well. Thus, combination of antibiotic and probiotic intake can give a promising advantage in treating bacterial disorders with simultaneous recovering of a weakened intestinal micro flora (Mattila-Sandholm et al.)(S. Pithva, P. Ambalam, J. M. Dave,B.R.M. Vyas)reported that Antimicrobial action of probiotic Lactobacilli may be manifested by one or combination of the following actions including competition for nutrients, adhesion and production of different antimicrobial metabolites such as organic acids, H₂O₂, bacteriocins, etc. Production of H₂O₂ by Lactobacillus spp. may be a non-specific antimicrobial defense mechanism of the normal vaginal ecosystem [16-17]. Hydrogen peroxide inhibits both Gram-positive and Gram-negative organisms.

Production of bacteriocins, recent reports have revealed that some intestinal lactobacilli and bifid bacteria produce antimicrobial substances that are active against these enteropathogens. Bacteriocins are ribosomal synthesized antimicrobial peptides and bactericidal proteinaceous molecule produced by bacteria. The term "bacteriocins" wasoriginally coined in 1953 by Jacob, specifically to define protein antibiotics of the colicin type, but it is now accepted toinclude peptide inhibitors from any bacteria [18]. Tagg in 1991 proposed the term "bacteriocins-like inhibitory substance" for designating the antimicrobial protein from Gram-positive microorganisms, to tell them apart from colicin which is produced by E. coli. Today, however, most antimicrobial peptides are named "bacteriocin',irrespective of Gram-positive or Gram-negative origin [19]. severe intestinal inflammation caused by ulcerative colitis (4). There are no published reports of bacteremia or sepsis secondary to Lactobacillus given as a probiotic agent. We report two cases of Lactobacillus GG sepsis occurring during the therapeutic use of this organism.

Lactobacillus is generally considered as a harmless bacteria. However association of lactobacillus with certain gastro intestinal diseases and the utilization of it as an adjunct suggest that its antibiotic susceptibility and resistance

should be checked. In this experiment lactobacillus was exposed to the action of three antibiotics namely, Penicillin, streptomycin, and Norfloxacin. Norfloxacin has the highest

activity streptomycin has the intermediate activity while penicillin has the lowest activity among these three antibiotics.

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