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Effect of Cell Free Culture Filtrate of Probiotic Isolates on the Growth of Pathogenic Isolates

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Abstract: *The emergence of drug resistant strain is a recurrent problem in clinical practice. The incidence of infections due to organisms resistant to β -lactam agents due to production of various enzymes has increased in recent years. The enteric flora comprise large number of cells in the human body and are capable of eliciting immune responses while also protecting against microbial pathogens. Probiotics have very high potential in terms of their antimicrobial activity against antibiotic-resistant enteric pathogens. They have been shown to have wide range of health beneficiary properties. The presence and dominance of Lactobacillus in the vagina is associated with a reduced risk of bacterial vaginosis and urinary tract infections. The primary objective of this work is to study antibiotic resistant pattern of probiotic isolates against β lactam group of drugs. Further study involves effectiveness of cell free culture filtrate of probiotic isolates against β lactamase* producing bacteria. *Antimicrobial activity is one of the most important selection criteria for probiotics. Bacteriocin and other inhibitory compound might be produced by probiotics has potent action against pathogens. So it can be taken as a supportive supplement along with antibiotics.*
Keywords: *β- lactam drugs, cell free culture filtrate, pathogens, Probiotic isolates*

I. Introduction

The rising trend of multidrug resistance in microbes is a serious problem worldwide. In the last two decades, antibiotic resistance is an emerging problem throughout the world [1,2] mainly in both outpatients as well as hospitalized patients due to the extensive use and misuse of antibiotics. Conjugational transmission of antibiotic resistance genes across bacterial species and genera has amplified the problem of antibiotic resistance in pathogens.

The negative effect of consuming antibiotics has already been a concern among many families. Antibiotics are designed to kill germs and disease causing organisms, however, antibiotics also kill the good bacteria in gut. Excess alcohols, diseases, exposure of toxic substances, and stress have also the same effect. In those conditions, the good bacteria in the body will decrease, and at the end will allow harmful bacteria to attack the health. [3]

This has lead to the search for new, safe and effective antimicrobial agents from alternative resources. Alternatives to antibiotics are currently receiving greater attention from the scientific community and providers of human medicine. The scientific community actively continues to seek out alternatives to traditional antibiotics to identify equal- or superior performing agents. Probiotics have very high potential in terms of their antimicrobial activity against antibiotic-resistant enteric pathogens.

Probiotics are: "Live microorganisms which when administered in adequate amounts confer a health benefit on the host". Today, specific health effects of probiotics are being investigated and documented including alleviation of chronic intestinal inflammatory diseases[4], prevention and treatment of pathogen-induced diarrhoea, and urogenital infections.[5]

Probiotic microorganisms do not act exclusively in the large intestine via affecting the intestinal flora. They also affect other organs, either by modulating immunological parameters, intestinal permeability, and bacterial translocation or by providing bioactive or otherwise regulatory metabolites. Therefore, broader definitions have been suggested, that is, by Schrezenmeir and de Vrese [6], by the International Life Sciences Institute (ILSI) Europe, according to which probiotics have been defined as "a viable microbial food supplement which beneficially influences the health of the host". [7]

The probiotics being enteric microorganism do not have any parasitic effect on human beings. Probiotic microbes exert beneficial effect via a wide array of actions. These include resistance to colonization, production of antimicrobial substances, inhibition of pathogen adhesion, degradation of toxins, stimulation of local and peripheral immunity, stimulation of brush border enzyme activity, stimulation of secretory- IgA, and prevention of microbial translocation. Because of these varied actions, unlikely pathogens will develop resistance against probiotic agents. Colonization resistance is the ability of the normal flora to protect itself against unnecessary colonization of enteric pathogens in the GI tract. Colonization resistance is achieved by complex interactions between the different resident bacteria of the mucosal microflora. [8]

The inhibitory chemical substance, bacteriocin, is defined as protein antibiotics of the type of colicin, that is, a substance characterized by its lethal biosynthesis, its killing activity of predominant intraspecies, and its adsorption to specific receptors on the surface of bacteriocin-sensitive cells. [9]

Probiotics have a known antimicrobial effect. They are very potent against pathogens. There are several proposed mechanisms for the antimicrobial action of the probiotics. Bacteriocins, organic acids, hydrogen peroxide, diacetyl, acidophilin acidolin, lactocidin, lactobrevin and other inhibitory chemicals are released by the probiotics. [10,11] These organic acids not only lower the pH thereby affecting the growth of pathogens but also are toxic to microbes. Besides producing antimicrobial toxins probiotics have ability to adhere to cells, reducing pathogenic bacteria adherence hence causing pathogen exclusion. Probiotics interact with epithelial cells and has immunomodulatory effect. Many *Lactobacilli*, *streptococcus* and *saccharomyces* species have been reported to found safe for the prevention and treatment of various infectious diseases. [12,13]

Probiotics have very high potential in terms of their antimicrobial activity against antibiotic-resistant enteric pathogens. Hence, the strategic use of probiotics may be beneficial to curb the growing phenomenon of antibiotic resistance. This work focuses on the use and potential of probiotics to prevent infections. Probiotics, though recently popular, have been an integral part of the human diet for centuries. All the civilizations from ancient times have documented the benefits of curd in the human diet. In this study 6 highly drug resistant pathogens were selected to evaluate the activity of cell free culture filtrate of 29 probiotic isolates on the growth of pathogens.

II. Materials and Methods

2.1 Sample Collection:

Probiotic strains were isolated from specimens like vaginal swab, stool sample and curd/ yogurt samples by using enrichment techniques using De Man Rogasa & Sharpe (MRS) medium -MRS broth and MRS Agar. Organisms were identified on the basis of morphology, Gram staining, culture characteristics and standard biochemical tests according to Bergey's manual.

Pathogens were collected from clinical samples like urine and pus obtained from hospitals. Pathogenic organisms were grown in Nutrient agar, MacConkey agar and Blood agar to get pure culture. Organisms were identified by standard biochemical tests using Hi 25 Enterobacteriaceae identification kit for species conformation.

2.2 Antibiotic Assay:

Antibiotic sensitivity test for probiotic strains were performed to analyse the tolerance of probiotic isolates in presence of β –lactam antibiotics by Kirby Bauer Method and zone of inhibition was measured and compared against control.

Antibiotic susceptibility of pathogens was determined by Kirby Bauer disc diffusion method according to Clinical Laboratory Standard Institute (CLSI) guidelines on Muller- Hinton agar. Various generations of β –lactam antibiotics were chosen for assay to check the resistance towards β –lactam drugs.

Various generation of β -lactam group of drugs were chosen for screening the β-lactam drug resistance. These includes

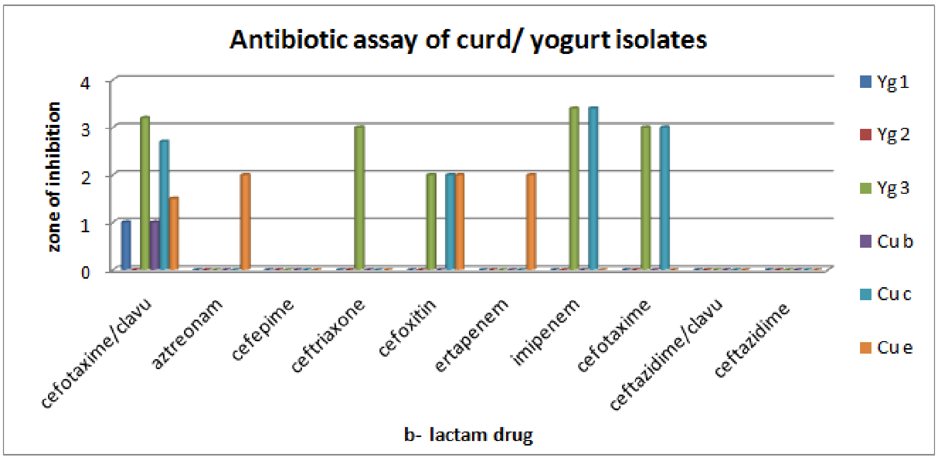
2nd generation- cefoxitin,
3rd generation- ceftriaxone, cefotaxime, ceftazidime
4th generation- cefepime,
Carbapenems - ertapenem & imipenem,
Aztreonam,
β-lactam/ β -lactamase inhibitor- cefotaxime & clavulanic acid, ceftazidime & clavulanic acid

2.3 Antibacterial Activity of Cell Free Culture Filtrate of Probiotic Isolates:

Cell free culture filtrate of probiotic isolates was prepared by centrifugation of overnight culture of isolated strain in MRS broth and incubated for 48 hrs. at 150 rpm at 37°C. After incubation, the whole broth was centrifuged at 10,000×g for 15 min and the cell-free supernatant was used as crude bacteriocin supernatant was collected to study antibacterial activity against selected pathogens by well diffusion assay procedure. Zone of growth inhibition was measured.

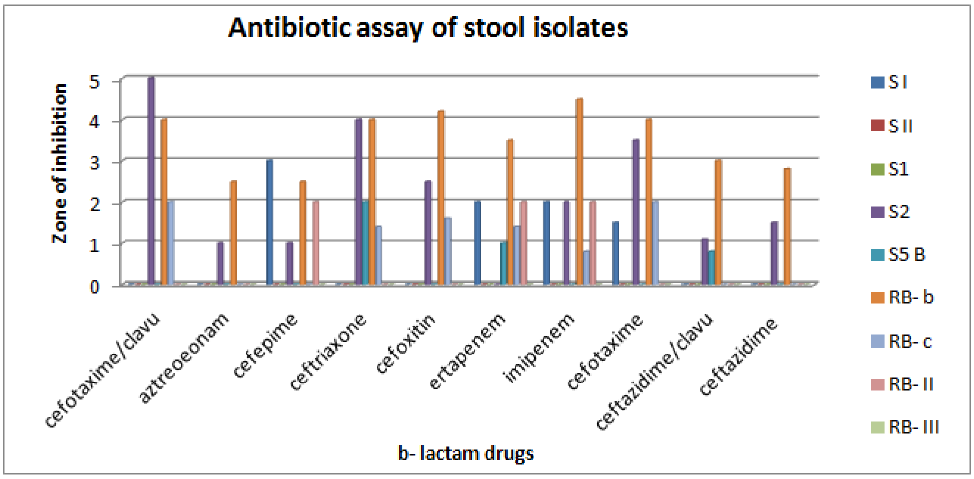
III. Result and Discussion

Total 29 probiotic strains were isolated from specimens like vaginal swab, stool sample and curd/ yogurt samples. Probiotic isolates were identified and then analyzed to know the sensitivity pattern towards β-lactam drugs. The main aim regarding this experiment was to observe that whether the probiotic isolates are able to tolerate the effect of antibiotics or not.



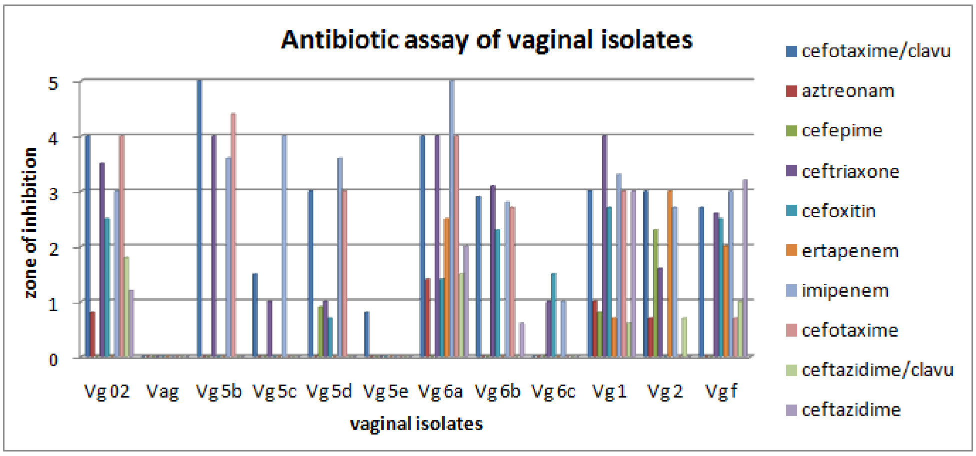
Graph: 1.1: Antibiotic assays of probiotic strains isolated from curd/ yogurt sample

According to graph 1.1 only yg3 was inhibited by few of the β -lactam drugs, rest both the samples yg1 and yg2 were completely resistant to β -lactam drugs. In case of curd isolates, cub was resistant for all generation of β -lactam drugs while cuc was sensitive towards imipenam, cefoxitin and cefotaxime. Isolate cue was also sensitive towards aztreonam, cefoxitin and ertapenam.



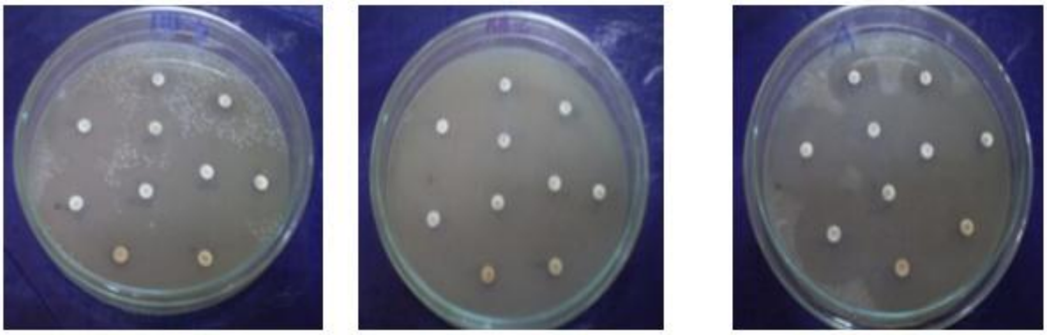
Graph: 1.2: Antibiotic assays of probiotic strains isolated from stool samples.

According to graph 1.2 Isolates SI, SII, S1, S2, S5B RB-c, RB-II and RB-III of Graph, were found to be resistant to the effect of most of the antibiotics. While RB-b was found to be sensitive to most of β- lactam group of drugs.

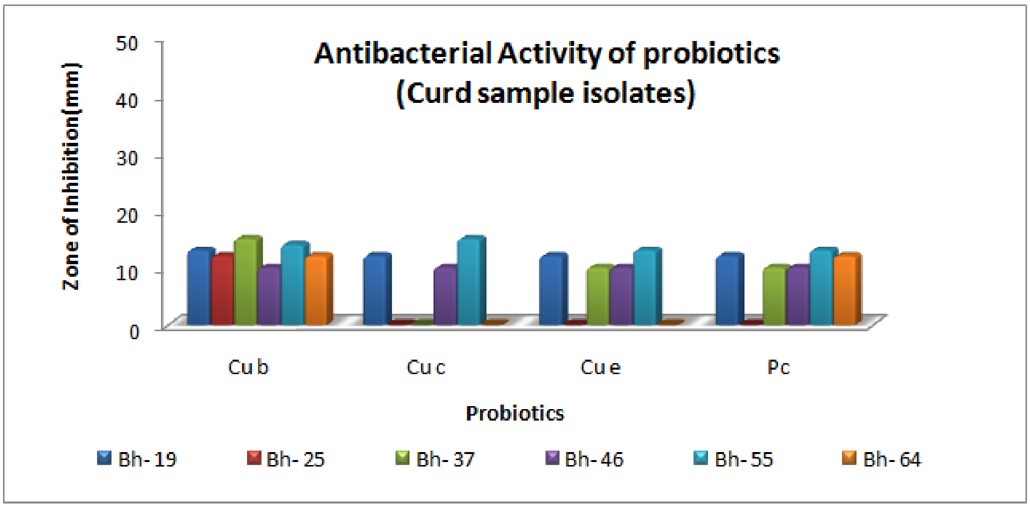


Graph: 1.3: Antibiotic assays of probiotic strains isolated from vaginal sample.

According to graph 1.3 few of vaginal isolates, vag, vg5b, vg5c, vg5d, vg6c and vg5e were found to be resistant to the effect of most of β -lactam antibiotics. In the probiotic strains, isolated from vaginal samples, aztreonam, cefepime, ceftazidime clavulanate combination were found to be having less inhibitory effect. Rest other probiotic vaginal isolates were more or less sensitive to β -lactam drugs.

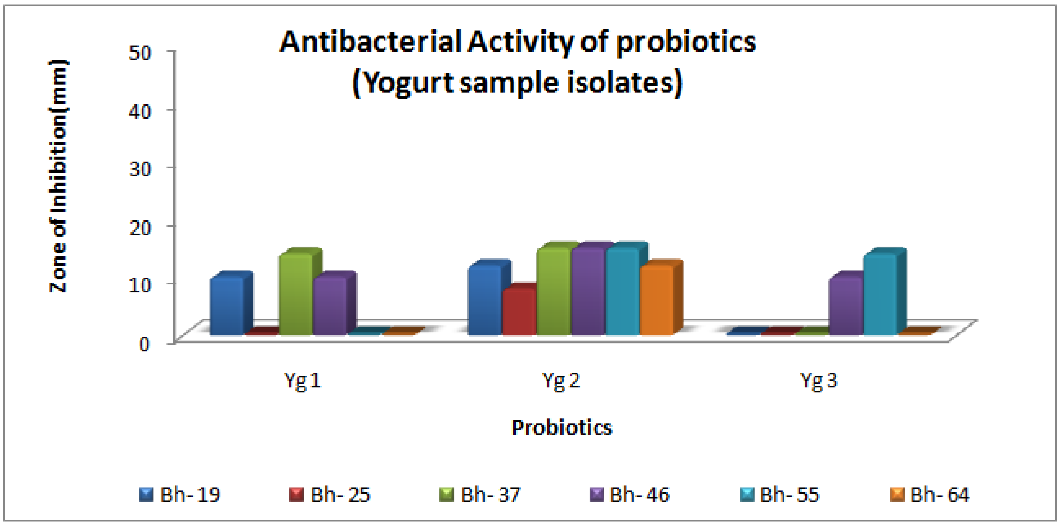


Antibiotic assay of probiotic strains



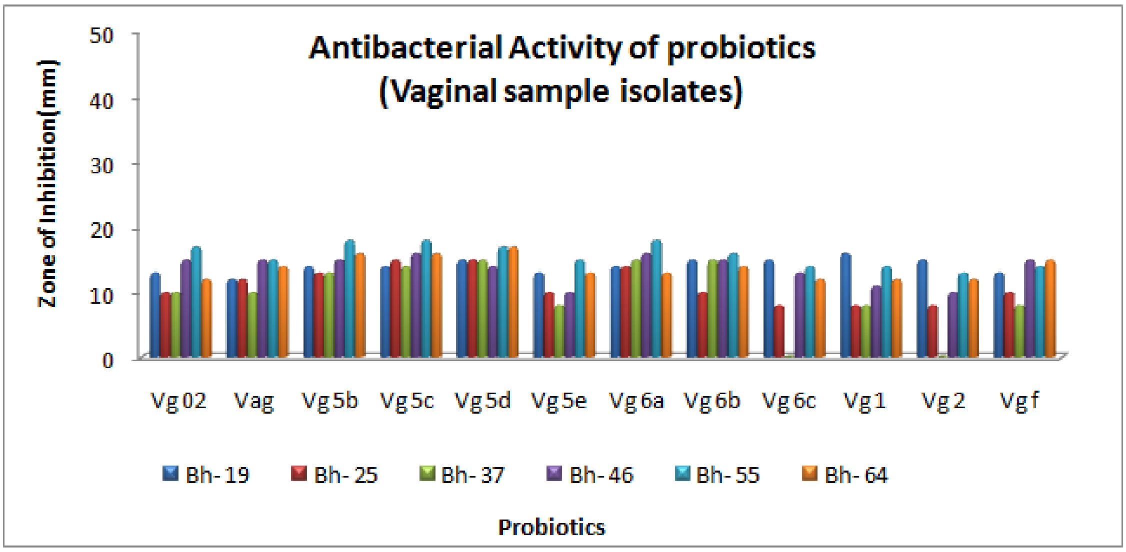
Graph: 2 .1: Antibacterial activity of cell free culture filtrate of probiotic strains isolated from curd samples on selected pathogens**.**

According to graph 2.1, except isolate Bh-25 all pathogens were showing the inhibitory effect due to cell free culture filtrate. Bh-37 and Bh-64 were not inhibited by cuc and cue.



Graph: 2.2: Antibacterial activity of cell free culture filtrate of probiotic strains isolated from yogurt samples on selected pathogens.

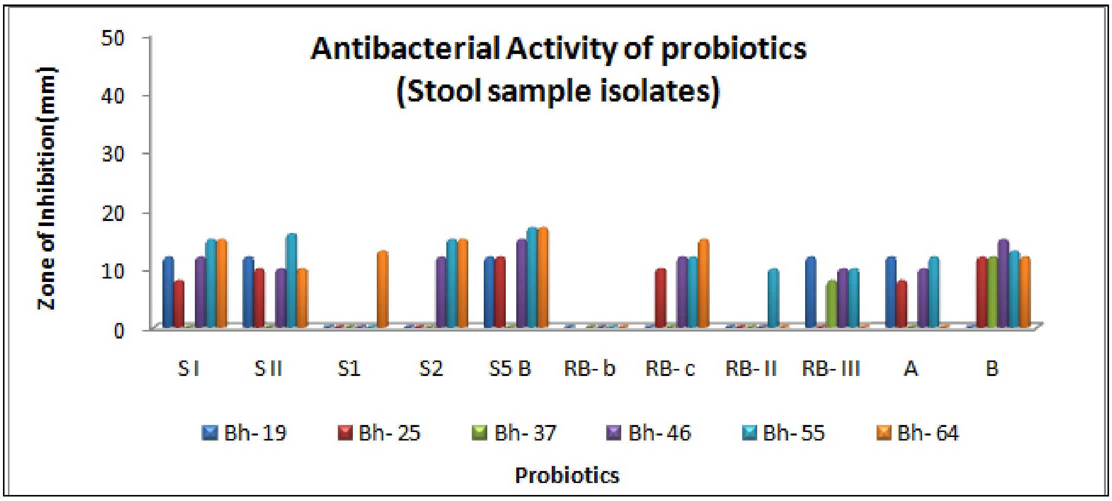
Cell free culture filtrate of isolate yg2 in graph 2.2 has shown very good antibacterial properties against all the pathogens. While yg1 Cell free culture filtrate was not able to inhibit Bh-25, Bh-55 and Bh-64. Cell free culture filtrate of yg3 was showing antibacterial effect only for Bh-46 and Bh-55.



Graph: 2.3: Antibacterial activity of cell free culture filtrate of probiotic strains isolated from vaginal samples on selected pathogens.

All the vaginal isolates Cell free culture filtrate of graph 2.3 have shown high antibacterial activity against almost all the pathogens. Bh-37 was not inhibited by isolate vg6c and vg2. This suggests probiotics produce inhibitory chemicals which are having high activity against pathogens.

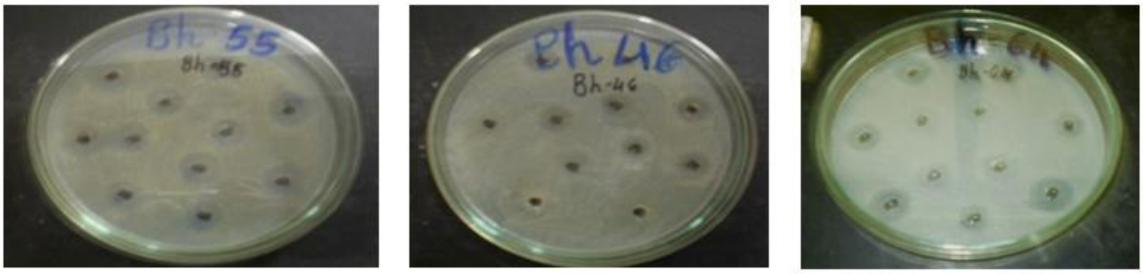
Studies of bacteriocin of probiotics on pathogens also suggest that probiotics has been found effective against *Clostridium spp., Bacteriodes spp., Enterobacteriaceae spp., Staphylococcus spp.*, and *Pseudomonas spp.* in microbiological assays. Lactocidin released by strains of *lactobacillus acidophilus* is found active against *Staphylococcus aureus* and *Pseudomonas aeruginosa.* [14]



Graph: 2.4: Antibacterial activity of cell free culture filtrate of probiotic strains isolated from stool samples on selected pathogens.

Cell free culture filtrate of stool isolates of graph 2.4 has moderate effect on pathogens. SI, SII, S5B and B isolates were showing sufficient inhibition, while some others like S1, RB-b and RB-II have poor inhibition.

There has been a study that Lactobacillus acidophilus LB release chemicals that are effective against both gram positive and gram negative microorganisms. These chemicals released in the broth were effective against *Staphylococcus aureus, Listeria spp., Salmonella typhimurium, Shigella flexneri, E. coli, Klebsiella pneumoniae, Bacillus cereus, Pseudomonas aeruginosa*, and *Enterobacter spp.* [15]



Effect of Cell free culture filtrate of various probiotic strains on pathogens

IV. Conclusion

Members of the medical community wish to protect the vulnerable inventory of disease-fighting antimicrobials currently available. The goal is to enhance the knowledge base of alternative antimicrobials currently under investigation and the protection of human health. Antibiotic susceptibility of probiotic strains were showing increase resistance of strains towards antibiotics, which were of great importance in therapeutic purpose.

CFC of probiotic isolates was also shown wide range of antibacterial property, which is a significant characteristic of probiotic strains. Though CFC of probiotic strains has variation in its spectrum of activity, some isolates were powerful inhibitor while other have moderate to poor inhibitory activity on pathogens. Antibacterial activity against 6 pathogens shows that highly sensitive includes 36.3%, moderate inhibited were 39.3% and very less sensitive or resistant includes 24.2%.

In the present situation antibiotic resistance is adversely affecting not only the patients but also the medical practitioners; the probiotics seems to be a promising and highly effective option to tackle the problem.

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