ISSN (Online): 2091-2609
DOI Prefix: 10.3126/ijasbt

International Journal of Applied Sciences and Biotechnology (IJASBT)

Research Article

Brief Survey of Antibiogram Profile of Commercially Available Probiotic Preparations in City Ranchi-India

Anil Kumar*[1], Preeti Swarupa[2], Menka Kumari[1], Renuka Kumari[1]

[1]Department of Life Sciences, School of Natural Sciences, Central University of Jharkhand, Cheri-Manatu, Kamre, Kanke, Ranchi – 835222, Jharkhand, India.
[2]Department of Microbiology, Patna Women's College, Patna 800001, India.

Article Information

Received: 25 March 2026
Revised version received: 22 May 2026
Accepted: 24 May 2026
Published: 29 June 2026

Cite this article as:
A. Kumar et al. (2026) Int. J. Appl. Sci. Biotechnol. Vol 14(2): 65-77. DOI: 10.3126/ijasbt.v14i2.95089

***Corresponding author**
Anil Kumar,
Department of Life Sciences, School of Natural Sciences, Central University of Jharkhand, Ranchi, Jharkhand, India.
Email: anil.kumar@cuj.ac.in / kumaranilvb@rediffmail.com

Peer reviewed under authority of IJASBT

Abstract

Probiotic microorganisms are frequently utilized in pharmaceutical and food formulations due to their beneficial effects on human health and gut microbiota. However, the unforeseen emergence of resistance to antibiotics among such probiotic microbes invites attention for the careful evaluation of the safety and therapeutic applicability of these probiotic microbes. The present study investigated the antibiogram profile of such isolates procured from locally available pharmaceutical probiotic formulations. Antibiotic susceptibility test revealed considerable variation among the isolates six. All six isolates exhibited complete sensitivity towards gentamicin and showed predominant sensitivity to amikacin, vancomycin, ampicillin-sulbactam, chloramphenicol, amoxiclav, and ampicillin. Intermediate resistance was recorded against ciprofloxacin, tetracycline, nalidixic acid, azithromycin, and kanamycin. Certain isolates revealed resistance to clindamycin, ceftazidime, erythromycin, and penicillin. The study highlights that probiotic strains possessing intrinsic/non-transferable resistance may provide advantages during concurrent antibiotic therapy by maintaining gut microbial balance. The occurrence of multidrug resistance, particularly on mobile genetic elements, among some isolates raises biosafety concerns regarding the possible dissemination of resistance. Besides such resistant microbes may, sometimes, compromise the efficacy of therapeutic antibiotics. Overall, this investigation emphasizes that antibiotic resistance profiling should be considered a critical criterion in the development of probiotic formulations. A comprehensive evaluation of resistance mechanisms, transmissibility, and biosafety parameters will ensure the safe, effective, and sustainable utilization of probiotics in food, pharmaceutical and clinical setups.

Keywords: Probiotics; Antibiogram; Gut; Antibiotic resistance.

Introduction

Probiotics

Probiotics are "live microorganisms that when administrated in an adequate amount confer a health benefit to the host" as explained by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) However, such strains used in food matrix should generally be regarded as safe (GRAS) (Berebon *et al*., 2019; Sreeja & Prajapati, 2013). Lactic acid bacteria (LAB) consist most part of probiotic strains, other commonly used probiotic strains include *Lactobacillus acidophilus, L. casei, L. lactis, L. helviticus, L. salivarius, L. rhamnosus, L. fermentum, Streptococcus thermophilus, Enterococcus faecium, E .faecalis, Bifidobacterium*

bifidum, B. breve, B. longum, and Saccharomyces boulardii (Sharma *et al*., 2013). A good probiotic must be able to adhere to cells, reduce or exclude pathogenic adherence, must have the ability to survive, reproduce and produce acids, hydrogen peroxide, and bacteriocins that inhibit pathogen growth, being able to be non-invasive, non-carcinogenic, and non-pathogenic while being safe, and being able to generate a normal balanced flora by coaggregation (Iannitti & Palmieri, 2010).

Probiotics are bacteria and yeasts that can repair and recolonize the digestive tracts microflora symbiosis and are considered 'Functional foods'(Iannitti & Palmieri, 2010). According to FDA, probiotics are defined as 'live biotherapeutics' which refers to the product containing whole, live microorganisms with an intended therapeutic or preventive effect in humans, regardless of the route of administration (Sreeja & Prajapati, 2013). The majority of probiotic products are made up of a combination of probiotic strains and come in powder, capsules, pills, drops, chewing gums, lozenges, straws, stick packs, bottle caps, and other forms (Iannitti & Palmieri, 2010). Characteristics of probiotic strains as biological drugs should be aiding of proper functioning of the body, the strain should be able to transit through the digestive system alive and have the ability to implant itself in the intestine, able to supply a precise dosage form and content, potency in the formulation in the terms of its capacity to produce a specific result, and stability in the matrix over a course of its stipulated shelf life (Sreeja & Prajapati, 2013).

In the human body, the gastrointestinal tract contains about 100 trillion bacteria that are crucial for our health. The intestinal environment is highly influenced by the balance of this microbial flora, and it is a critical component to maintain intestinal homeostasis (Sharma *et al*., 2013). A potential probiotic must exhibit excellent tolerability to bile tolerance and high concentration of bile components of the host. Thus, survivability of probiotics within the gastrointestinal tract (GIT) depends largely on their bile salt tolerance (Berebon *et al*., 2019)

Cheese, ice-cream, frozen yoghurt, chocolates with probiotics and a variety of other probiotic dairy and non-dairy products are now commercially accessible Categorization of probiotics have been done in different countries as natural health product in Canada, dietary supplement, drugs, medical food, live biotherapeutic agent, biological agent in USA, functional food in Japan, China, Malaysia, and India, biotherapeutic/pharmaceuticals in European countries etc. (Sharma *et al*., 2013)

The term 'probiotic' derives from the Latin word 'pro' and the Greek word 'bios' meaning for life, the concept was first introduced by Elie Metchnikoff a Russian Nobel laureate in the year 1907 (Iannitti & Palmieri, 2010). Later German scientist Werner Kollath in the year 1953 labeled probiotic as 'active substances that are essential for a healthy development of life'(Gasbarrini *et al*., 2016). In the year 1965, Lilly and Stillwell coined the phrase for probiotics as 'substances released by one organism that encourage the growth of another'(Sreeja & Prajapati, 2013). More specifically, in the year 1992 Fuller defined probiotics as a live microbial feed additive that benefits the host animal by enhancing its gut microbial balance (Gasbarrini *et al*., 2016).

Food and medications are currently regulated in India by the PFA (Prevention of Food Alteration Act) and the FDA respectively. Foods for special dietary purposes, functional foods nutraceuticals, and health supplements are defined by the Food Safety and Standards Acts of 2005 (FSSA) (Sharma *et al*., 2013). The majority of scholarly literature has implemented the definition of probiotics which has been acknowledged by the International Scientific Association for Probiotics and Prebiotics (ISAPP) (Fijan, 2014). A probiotic's minimal effective dose is approximately 10^8 – 10^9 cells per day. However, there is no evidence-based agreement on the best bacterial pro-dose concentration (Di Cerbo and Palmieri, 2015).

Antibiotics are antimicrobial agents that are produced naturally by bacteria, fungi, or synthetically (Muzikowski, 1995). Antibiotics are among the most significant medications currently available in contemporary medicine, allowing for the treatment of infections that will be otherwise fatal. However, their widespread use has resulted in an increase in antibiotic resistance (Ouwehand *et al*., 2016). Antibiotics have major modes of action that makes them efficient against pathogenic bacteria such as by inhibiting cell wall synthesis, protein synthesis, nucleic acid production, and metabolic pathway, and by disorganizing the cell wall. The fundamental issue with antibiotic use is that bacteria have the ability to adapt, and hence can develop resistance to antibiotic by several biochemical aspects such as antibiotic inactivation, targ*et al*teration, changes in efflux pump and outer membrane (OM) permeability, and by passing the target as well as genetic issues such as mutation and horizontal gene transfer (Naderi *et al*., 2014).

Table 1: Class of antibiotics and their mechanism of action inferred from 'A bacterial reporter panel for the detection and classification of antibiotic substances' by (Melamed *et al*., 2012)

Class of Antibiotics	Mechanism of action
Tetracycline	Protein synthesis inhibitor
Sulfonamide	Folic acid metabolism inhibitor
β - lactams	Cell wall synthesis inhibitor
Quinolones	DNA gyrase inhibitor
Phenicol	Protein synthesis inhibitor
Polymyxins	Cytoplasmic membrane disruptor

In a bacterial community, antibiotic resistance can arise from two sources: endogenous gene mutation or exogenous

resistance gene acquisition (Liu et al., 2009). Natural (intrinsic or induced), acquired, and mutational resistance are the three origin types of resistance (Ashraf & Shah, 2011). Natural resistance may be intrinsic, or induced, whereas all of the primary ways that bacteria acquire genetic material, including those that gives resistance, are feasible such as, transformation, transposition, and conjugation (Reygaert, 2018). According to the panel on additives and products or substances used in animal feed-FEEDAP (2008), Strains with acquired resistance as a result of external resistance gene acquisition are not suitable for use as animal feed additives (Ashraf and Shah, 2011). Four primary forms of resistance mechanisms to antibiotics that have been established by bacteria are, (i) Efflux pumps, which effectively excrete antibiotics from the cell. (ii) Inactivation of antibiotics occurs when the antibiotic substance's action is directly hampered by hydrolysis, functional group conversion, etc. (iii) Target by-pass, by overproducing the target molecule and developing other routes to get around the targeted enzymes, by structural changes in the cell wall, and prevention of the antibiotic to bind to its target. (iv) Target modification, is achieved by altering the antibiotic targets themselves (Uluseker et al., 2021). The mechanism of antibiotic action and resistance is being shown in Fig.1.

Probiotics are a series of *Lactic acid bacteria, Bifidobacterial, Enterococci, Propionibacterium,* and even Yeasts. They are available in the form of capsules, powders, fortified, yoghurts, yoghurt like items, and milk on the market (Di Cerbo and Palmieri, 2015). There were some known probiotic strain sources, and their first manifested action shown in Table 2. Fermented foods including beer, bread, wine, kefir, kumis, and cheese have been utilized for nutritional and medicinal purposes for a long time before probiotic microbes were discovered (Ozen and Dinleyici, 2015). Consumption of probiotic cells through food products are currently the most popular method for the intake of functional food (Saxelin, 2008). Probiotic products are now broadly acknowledged and well-liked by vast number of individuals as reflected by a predicted rise in economic value of more than 10 % from 2009 to 2014 (Wong *et al*., 2015). The probiotic market has been quickly expanding; in 2015, the global probiotic market was estimated at $36.6 billion USD, and it is predicted to increase at a 7% compound annual growth rate (CAGR) from 2016-2023, with Asia-Pacific accounting for more than 40% of the global share (Kesavelu *et al*., 2020).

The various mechanism by which probiotics exert their beneficial effects on the host, such as immune modulation, stimulation of gut microbiota, stimulation of digestive enzymes, pathogen displacement production of bioactive compounds etc, are shown in Fig.2 (Kwoji *et al*., 2021).

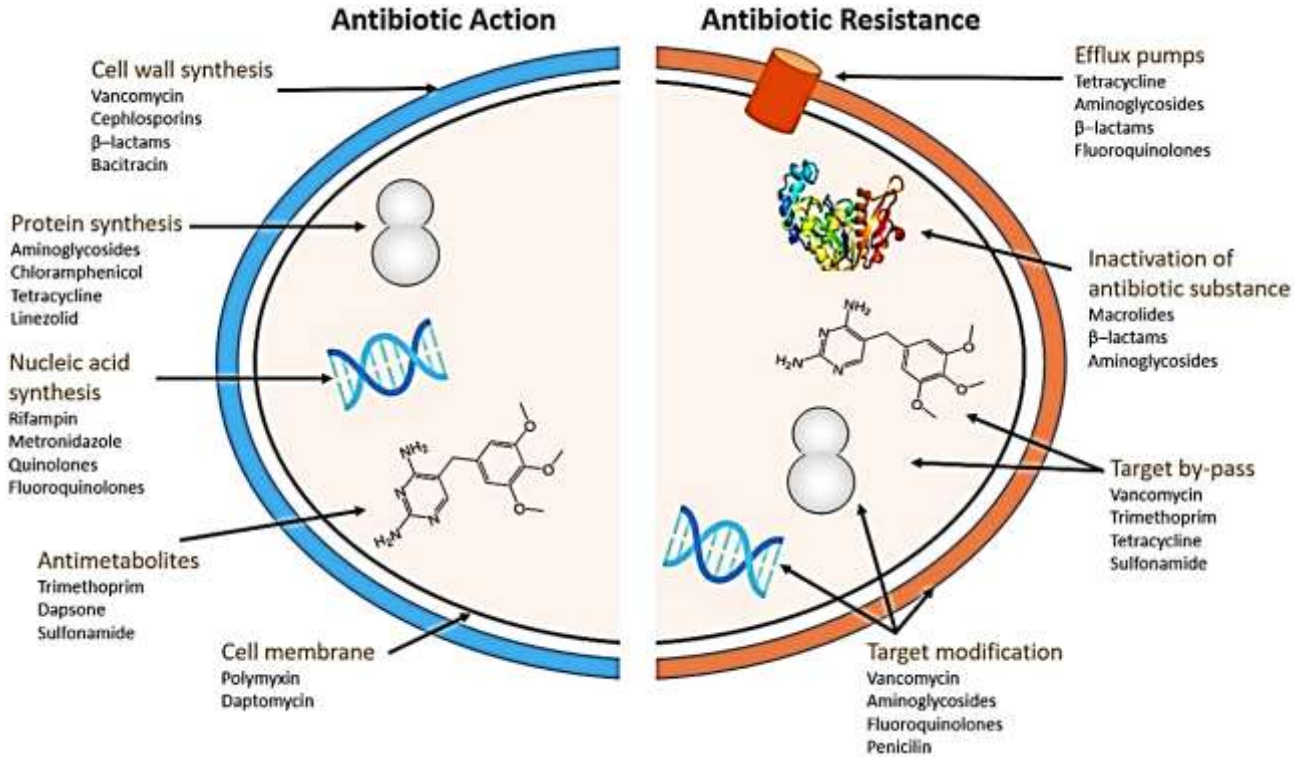

Fig.1: Diagram showing Antibiotic action and their resistance, the figure has been taken from 'A review on occurrence and spread of antibiotics resistance in wastewater and in wastewater treatment plants; Mechanism and perspective' by (Uluseker *et al*., 2021)

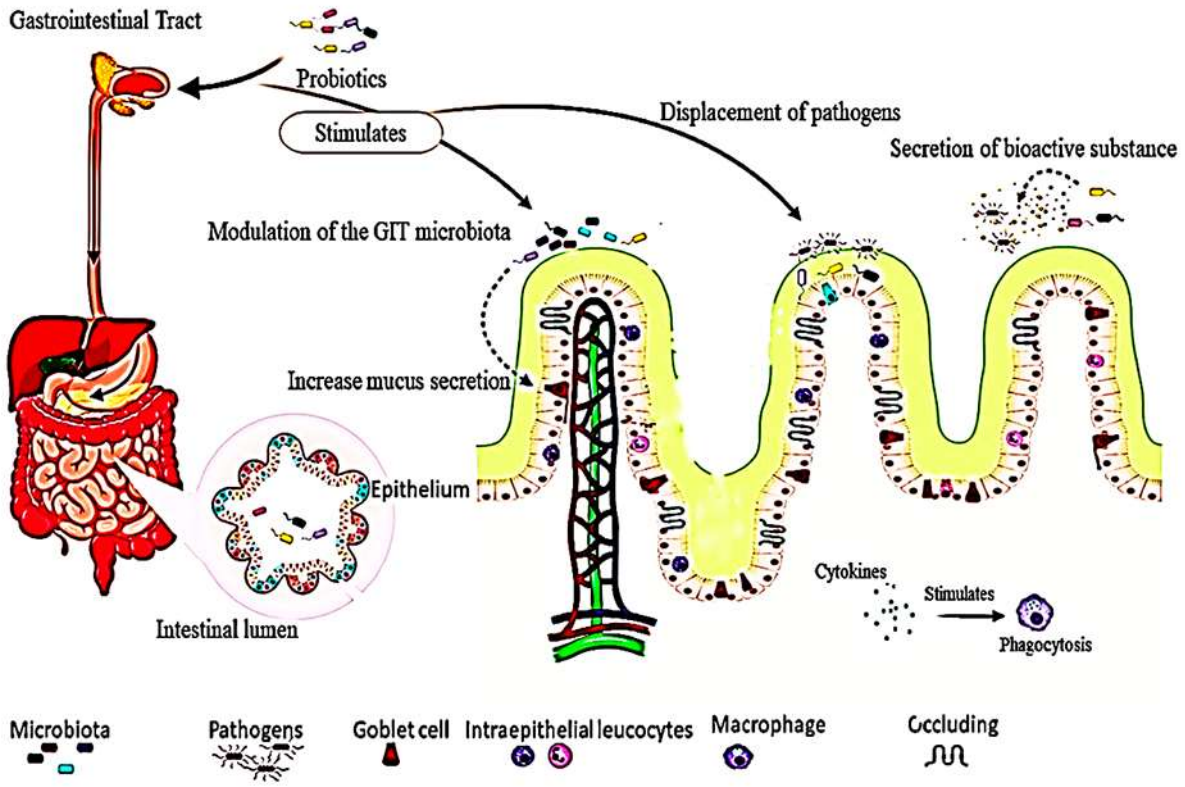

Fig. 2: Diagrammatic representation of mechanism of action of probiotic in intestine adopted from the review 'Multi strains probiotics: Synergy among isolates enhances biological activities' by (Kwoji *et al*., 2021)

Table 2: Sources of some probiotic strains and their first manifested action inferred.

Sl. No.	Probiotic strains	First isolated from	First manifested action
1.	*Bifidobacterium bifidum*	From an infant stool sample	By displacement of pathogenic bacteria
2.	*Escherichia coli* Nissle DSM6601	From a healthy soldier during WWI	Prevent salmonellosis and shigellosis
3.	*Lactobacillus acidophilus Lb*	From human intestinal tract	Prevent diarrhea
4.	*Lactobacillus rhamnosus GG* (ATCC 53013)	From healthy human faeces	Improve normal colonic flora
5.	*L. rhamnosus* CNCM I-1720	From dairy starter culture	By healing peptic ulcer
6.	*Saccharomyces boulardii* CNCM I-745	From surface of lychee fruit	Prevent Cholera

(Duranti *et al*., 2021; Foster *et al*., 2011; Goldin *et al*., 1992; Ljungh and Wadström, 2001; McFarland, 2015; Wieërs *et al*., 2020)

The benefit of probiotic ranges from the improvement of intestinal health and immune system to the prevention of acute, antibiotic diarrhea (Wong *et al*., 2015). Thepast decades have seen a rise in the popularity of using probiotics to both prevent and cure a wide range of illness due to lack of effective medicines for gastrointestinal and other disorders, as well as the necessity to identify alternatives to conventional drugs like antibiotics (Quijano, 2011). Consuming probiotics is linked to a number of health advantages, including immune system stimulation, defense against nosocomial and respiratory infections as well as diarrheal diseases, lowering cholesterol, attenuation of overt immunoinflammatory disorders, and anticancer effects and these advantages are strain specific rather that species or genus specific (Quijano, 2011). Other therapeutical applications for probiotics include treatment of (i) Skin diseases and allergy, (ii) renal diseases, (iii) urinogenital infections, (iv) Diverticular diseases, (v) irritable bowel syndrome, (vi) antidiabetic activities, (vii) anti- cancer (viii) angiogenesis activity and (ix) used in surgical practices (Ashraf & Shah, 2011; Reygaert, 2018). Probiotic have found a place in pharmaceutical product

because of the above mentioned medicinal qualities (Sharma *et al*., 2013).

Lactobacilli, Streptococci, Clostridium, Coliform, and bacteroides are just a few examples of the beneficial bacteria that a healthy colon keeps in substantial balance (Amara and Shibl, 2015). In terms of present probiotic pharmacological uses, only a few well-defined strains are being clinically studied and explored for the treatment of gastrointestinal illness (irritable bowel syndrome/IBS, Ulcerative colitis, abdominal bloating), infantile colic, improving immunity vaginal diseases, cold and flu. The majority of probiotic strains are manufactured with vitamins and prebiotics, and commercially available probiotic products are made up of a mix of probiotic strains (Sreeja and Prajapati, 2013). Certain commercially available probiotics with their composition and their favorable claims are shown in Table 3.

Probiotics come in a variety of forms, and their efficacy might vary depending on whether they are single strain or multi-strain. Compared to which multi strain probiotic contain more than one probiotic strains and sometimes it include both bacteria and fungi (Kwoji *et al*., 2021). Various multi strain and single strain probiotic drug, their composition and their available form are shown in Table 4 and 5.

Table 3: Commercially available probiotics, their composition and their favorable claims inferred from a review by (Di Cerbo & Palmieri, 2015; Gasbarrini *et al*., 2016)

Product name	Manufacturer	Microorganism involved	CFU	Favorable claims
Yo plus yoghurt	Yoplait Inc	*B. animalis subsp lactis, S. thermophilus, & L. bulgaricus*	≥ 5 billion	Activity against *Helicobacter pylori* and Antioxidant activity, gut survivability etc.
Yakult cultured milk	Yakult honsha Co.	*L. casei* shirota	8 billion	Immunomodulatory activity and activity against bacteria and viruses, etc.
Ultimate probiotic formula	Swanson Health products	*B. lactis, B. longum, L. plantarum, L. acidophilus, L. casei, L. bulgaricus, L. sporogenes* and prebiotic NutraFlora	60 billion	Antitumor activity, gut survivability and Activity against IBD etc.
Good belly fruit drink	NextFoods	*L. plantarum*	20 billion	Activity against both pathogen bacteria and viruses, and oral mutans *streptococci* and *Candida albicans*
Gerber Good Start Protect Plus powdered infant milk formula	Nestle	*B. lactis*	10 billion	Adhesion to mucosal and antitumor activity
Activa yogurt	Dannon Inc	*B. animalis* and *B. regularis* with *S. thermophilus* and *L. bulgaricus*	10 billion	Activity against bacteria and viruses, activity against *Helicobacter pylori*, and cholesterol lowering activity
Gefilus juice	Valio Ltd, Helsinki Finland	*L. rhamnosus GG*	5 million	Antitumor activity, activity against toxins, antioxidants activity, and immunomodulatory activity
Kefir drinks	Lifeway foods Inc	*L. acidophilus, Lb. brevis, L. casei, L. rhamnosus, Lb subspcs, L. lactis, Enterococcus species, K. bulgaricus, K. fragilis, S. subsp., Z. rouxii* etc.	7-10 billion	Antitumor effect, cholesterol lowering effect, activity against pathogen bacteria and viruses, immunomodulatory effect, gut survivability, and activity against pancreatic.

Table 4. Multi strain probiotic drug, their composition and their available form inferred from reviews by (Gasbarrini *et al*., 2016; Kesavelu *et al*., 2020; V. Sharma *et al*., 2021)

Probiotic drug	Probiotic strain used	Manufacturer	Available form
Eubioz	*L. acidophilus, L. rhamnosus, Bifidobacterium bifidum, B. longum, Steptococcus thermophilus, Saccharomyces boulardii*	Lupin Inc.	Powder
Bifilac	*L. sporogenes, S. faecalis, Clostridium butyricum, Bacillus mesentricus*	Tablets, Tamil Nadu, India	Capsule
Econova	*L. reuteri, L. rhamnosus*	Glenmark	Capsule
Goodlac	*L. acidophilus, L. rhamnosus, B. longum, S. boulardi*	Biomilcom	Capsule
Actigut	*L. acidophilus, L. rhamnosus, B. longum, B. bifidum, S. boulardii, S. thermophilus*	Alembic	Capsule
Lactisyn	*L. acidophilus, L. lactis, S. thermophilus, S. lactis*	Franco- Indian	Injection
Vi Bact	*S. faecalis, c. butyricum, B. mesentricus, L. sporogenes*	Unique Biotech Ltd	Sachet (powder)
Bio- K+ Probiotic	*L. acidophilus and L. casei*	Bio-K+ International Inc	Capsule
Combiflora	*L. acidophilus, Bifidobacterium longum, Bifidobacterium lactis, S. boulardii*	Medopharm Pvt Ltd	Capsule
Vibact	*Streptococcus faecalis, Clostridium butyricum, Bacillusmesentricus*	USV Private Ltd	Capsule
Reflora Z	*S. boulardii, Lactic acid bacillus*	DYOTA Numandis	Powder
VSL #3	*B. breve, B. infantis, B. longum, L. acidophilus, L. bulgaricus, L. casei, L. plantarum, and S. thermophilus*	Sigma-Tau Pharmaceuticals	Powder

Table 5: Single strain probiotic drug, their composition and available form inferred from reviews by (Kesavelu *et al*., 2020; Sharma *et al*., 2021; Sreeja and Prajapati, 2013)

Probiotic drug	Probiotic strain used	Manufacturer	Available form
Sporolac	*L. sporogenes*	Sanzyme Ltd	Capsule or powder
Darolac	*L. sporogenes*	Aristo Pharmaceuticals Pvt Ltd	Capsule
Alacforte	*L. sporogenes*	Alliance remedies	Capsule
Econorm	*S. boulardii*	BIOCODEX, France	Powder
Benegut	*B. clausii*	Abott India Ltd	Liquid
Enterogermina	*B. clausii*	Medsource Healthcare Private Ltd	Liquid
Bifilac GG	*L. rhamnosus* GG	Tablets India Limited	Powder
Regutol	*B. subtilis*	Alembic Pvt Ltd	Liquid
Cyfolac	*B. clausii*	Karnataka Antibiotics & Pharmaceuticals Ltd	Capsule
Gut pro	*B. clausii*	Synergia Life Sciences Pvt Ltd	Powder
Gnorm	*S. boulardii*	Nouveau Medicament Pvt Ltd	Powder
Vizylac	*L. sporogenes*	Unichem Private Ltd	Capsule

Due to excessive or improper use of antibiotics with probiotics often have a potential of creating a reservoir of antibioticresistant genes. Antibiotics of class Beta-lactam, macrolide, aminoglycosides, chloramphenicol, and tetracyclines are ineffective against probiotic microorganism and a significant number of research-based writing have previously reported this. Whereas recognizing antibiotic resistance from probiotic bacteria of dietary and bio-based sources has increased, reports of antibiotic resistance in dietary supplements have remained elusive (Wong *et al*., 2015). Table 6. Lists some of the well-known probiotic strains and their antibiotic resistance.

Table 6. Known probiotic strains and their resistance of antibiotics observed in reviews by (Neut *et al*., 2017; Selvin *et al*., 2020; Wong *et al*., 2015)

Probiotic strains	Antibiotic resistant to
B. bifidum	Streptomycin, Penicillin, Amoxicillin, Clindamycin, Levofloxacin, Cefixime, Oxacillin, Cefuroxime, Azithromycin, Clarithromycin, Metronidazole
B. infantis	Aztreonam
L. bulgaricus	Gentamycin
L. rhamnosus	Ciprofloxacin, Aztreonam
L. acidophilus	Streptomycin, Ampicillin, Penicillin, Oxacillin, Amoxicillin, Cefuroxime, Azithromycine, Clindamycin, Doxycycline
B. longum	Gentamycin, Ciprofloxacin, Penicillin, Oxacillin, Amoxicillin, Cefuroxime, Azithromycin, Clindamycin, Levofloxacin
L. casei	Gentamycin, Ciprofloxacin
L. sporogenes	Erythromycin, Ceftazidime
S. faecalis	Penicillin G, Erythromycin
B. mesentericus	Penicillin G, Erythromycin
S. boulardii	Erythromycin
L. salivarius	Ciprofloxacin

The potential for probiotic based beverages and drugs has increased as a result of rising health concerns and the concomitant trend towards functional food(Koirala and Anal, 2021)**.** In recent years and in years to come, significant advancements in technologies and related approaches have made it possible to make numerous strides in the prebiotic and probiotic fields. Given their modulating effects on animal immune, gut microbiota, feed intake, and productivity, probiotics and prebiotics may be used as a alternative growth promoting and health enhancing feed additives in the future (Cunningham *et al*., 2021). The above extensive literature attracted the current experimental based approach to investigate the antibiogram study of some available probiotics preparations (Brand names and related information are not disclosed to avoid legal issues) in the market within city Ranchi, Jharkhand, India.

Materials and Method

Materials

Luria Bertani (LB) agar (Hi Media, India), Luria Bertani broth (Hi Media, India), Nutrient Agar (Hi Media) used for routine purification, culture and characterization of microbes in probiotics preparation. Mueller Hinton Agar (TM Media) was used to study antibiogram profile of probiotic microbes. Vancomycin (VA^{30}), Ceftazidime (CAZ^{30}), Penicillin G (P^{10}), Ampicillin Sulbactum (AS^{10}), Amoxiclav (AMC^{30}), Amikacin (AK^{30}), Tetracycline (TE^{30}), Azithromycin (AT^{15}), Clindamycin (CD^{2}), Ampicillin (AMP^{10}), Nalidixic acid (NA^{30}), Ciprofloxacin CIP^{5}), Erythromycin (E^{15}), Kanamycin (K^{30}), Chloramphenicol (C^{30}), and Gentamicin (GEN^{10}) antibiotic discs were procured from TM Media (TITAN Biotech ltd. A-902 A, RIICO Industrial Area, Phase-III, Bhiwadi-301019, Rajasthan, India). Four single strain Probiotic Sachets (powder form) and 1 probiotic liquid suspension were purchased from the shops at Sainik market, Main Road, Ranchi. The microbial compositions in of the probiotic preparations are mentioned with specific code for the hidden brand name: A- *Lactobacillus rhamnosus*, B- *Lactobacillus rhamnosus*, C- *Saccharomyces boulardii,* D- *Saccharomyces boulardii,* EL and ES - *Bacillus clausii.*

Methods

Isolation and Purification:

All the procedures were followed for aseptic manipulation of the experiments. A solution was prepared for each powdered probiotic, except liquid probiotic in suspension form. The weight of a blank Eppendorf tube was obtained, and then the probiotic was added, weight of the probiotic was determined, sterile water was added to the tube to achieve suspension of 100 mg/ml. Inoculation and purification of culture were done by streak plate method on

fresh media plates. Routine culture of isolates was made on Luria Bertani Agar plates and were maintained at 4℃ for short time period (subculture cycle approx. 1 month). Cultures were stored in LB/NB with 20% glycerol at -20℃ for long term storage.

Characterization

Colony and Cell Morphology:

The isolated cultures colonial and morphological properties such as shape, colour, elevation, margin, transparency to light, and surface characteristics were visually inspected and visualized under Stereo microscope as well as bright field compound microscope (Kolwzan *et al*. 2011).

Gram Staining:

The test was carried out to investigate the isolates Gram's reaction and morphology. The test was performed as described by Gram's stain kit (HIMEDIA).

Antibiotic Sensitivity Test:

The antibiogram study of purified isolates was determined using the antibiotic disc diffusion technique on Mueller Hinton Agar plates (Bauer *et al*., 1996). Sterile Muller Hinton Agar plates with agar depth of 4.0 ± 0.5 mm were prepared. Purified cultures were inoculated and cultured in LB Broth for overnight at 37°C with 160 rpm shaking condition. The cultures at optical density (OD) between 0.08-0.13 at wavelength 625 nm were inoculated using a sterile cotton swab to form lawn. The antibiotic discs were placed on the surface of Muller Hinton Agar plates, with spread cultures on it, with the help of sterile applicator or forceps. The plated were incubated at 37°C for 16-20 hours or longer if needed. The diameters of the inhibitory zones were measured, and the findings were classified as sensitive (S) or Resistant (R) according to CLSI (*CLSI Publishes M100—Performance Standards for Antimicrobial Susceptibility Testing, 31st Edition*, n.d.).

Result

Isolation and Purification:

Six pure cultures were prepared from five probiotic preparations and the cultures were labelled A, B, C, D, EL and ES (Fig. 3). The characteristics of the cultured isolates are recorded in Table 7 and Fig. 4.

Fig. 3: Pure culture plates of bacterial isolates. A- *Lactobacillus rhamnosus*, B- *Lactobacillus rhamnosus*, C- *Saccharomyces boulardii,* D- *Saccharomyces boulardii,* E- (Large colony - EL and small colony - ES) - *Bacillus clausii.*

Table 7: Characteristic of the colony of bacteria from probiotics

Probiotics cultures	Colony size/cell shape	Color	Colony margin	Form	Transparency	Elevation	Grams'Stain*
A	<1mm Rod	Off white	Entire	Round	Translucent	Raised	Positive
B	<1mm Rod	Off white	Entire	Round	Translucent	Raised	Positive
C	1-2mm Oval	Off white	Entire	Round	Translucent	Raised	Positive
D	1-2 mm Oval	Off white	Entire	Round	Translucent	Raised	Positive
EL	2-3mm Long rod	Off white	Wrinkled	Undulate	Translucent	Raised	Positive
ES	1mm Long rod	Off white	wrinkled	Undulate	Translucent	Raised	Positive

- The size and characteristics of the colony were recorded at same time within 24-48 hours cultured on Luria Bertani Agar.
- The size of the colony was measured with the help of transparent 1mm grid sheet. Other observations were recorded as per standard method (Kolwzan & Adamiak, 2019).
- *C and D are Yeasts.

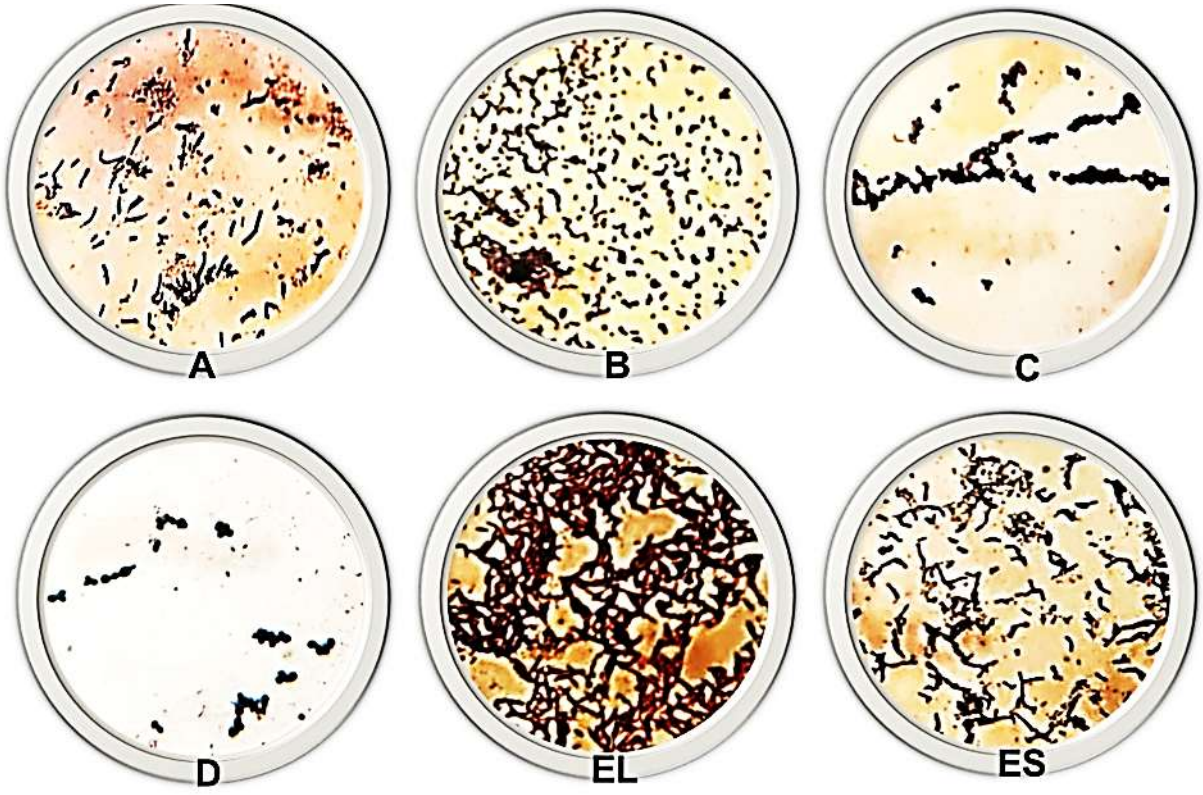

Fig. 4: Gram staining and cell morphology of culture isolates.

Antibiotic Sensitivity Test:

The activities of all 16 antibiotic discs (Fig. 5) against the 6 bacterial strains which was recorded after 16 hours are tabulated in Table 8. The interpretation of sensitivity and resistance of the cultures was inferred from the reference zone of inhibition of antibiotics (TM Media).

Fig. 5: Plates showing zone of inhibition of Antibiotic susceptibility test of Enterogermina (S) and Enterogermina (L). Here, P-Penicillin G, CD- Clindamycin, C- Chloramphenicol, AMC- Amoxiclav, AMP- Ampicillin, VA- Vancomycin, AT- Azithromycin, CAZ- Ceftazidime, E- Erythromycin, AK- Amikacin, GEN- Gentamicin, K-Kanamycin, NA- Nalidixic acid, TE- Tetracycline, A/S- Ampicillin sulbactam, CIP- Ciprofloxacin

Table 8. Zone of inhibition in mm (After 16 hours)

Antibiotic discs	A	B	C	D	EL	ES
CD^{2}	0 (R)	8 (R)	13 (R)	0 (R)	0 (R)	0 (R)
CAZ^{30}	0 (R)	14 (R)	8 (R)	16 (R)	0 (R)	0 (R)
E^{15}	9 (R)	16 (I)	13 (R)	15 (I)	7 (R)	0 (R)
P^{10}	0 (R)	19 (S)	23 (S)	21 (S)	0 (R)	0 (R)
AK^{30}	25 (S)	20 (S)	21 (S)	25 (S)	24 (S)	27 (S)
VA^{30}	21 (S)	16 (I)	20 (S)	17 (S)	18 (S)	20 (S)
AMP^{10}	11 (R)	24 (S)	28 (S)	26 (S)	9 (R)	9 (R)
AMC^{30}	11 (R)	26 (S)	30 (S)	26 (S)	10 (R)	9 (R)
CIP^{5}	30 (S)	29 (S)	25 (I)	30 (S)	26 (S)	24 (I)
TE^{30}	24 (S)	22 (S)	25 (S)	25 (S)	19 (S)	18 (S)
AS^{20}	19 (S)	26 (S)	28 (S)	28 (S)	17 (S)	16 (S)
NA^{30}	20 (S)	22 (S)	19 (S)	25 (S)	14 (I)	14 (I)
AT^{15}	9 (R)	19 (S)	12 (R)	13 (S)	0 (R)	0 (R)
K^{30}	22 (S)	16 (I)	18 (S)	21 (S)	20 (S)	20 (S)
GEN^{10}	24 (S)	24 (S)	26 (S)	27 (S)	26 (S)	24 (S)
C^{30}	10 (R)	26 (S)	25 (S)	9 (R)	8 (R)	14 (I)

Here, CD^{2}- Clindamycin (21/15-20/14), CAZ^{30}- Ceftazidime (21/18-20/17), E^{15}-Erythromycin (23/14-22/13), P^{10}-Penicillin G (15/-/14), AK^{30}-Amikacin (17/15-16/14), VA^{30}- Vancomycin (17/15-16/14), AMP^{10}-Ampicillin (17/14-16/13), AMC^{30}-Amoxiclav (18/14-17/13), CIP^{5}-Ciprofloxacin (26/22-25/21), TE^{30}-Tetracycline (15/12-14/11), AS^{20}-Ampicillin/Sulbactam (15/12-14/11), NA^{30}-Nalidixic acid (19/14-18/13), AT^{15}-Azithromycin (13/-/12), K^{30}-Kanamycin (18/14-17/13), GEN^{10}-Gentamycin (15/13-14/12), C^{30}-Chloramphenicol (18/13-17/12). Here superscript numerical represents quantity (µg/disc) of respective antibiotics. S/I/R represent Sensitive/Intermediate/Resistant to antibiotics as inferred from reference table (HIMEDIA: for *Enterobacterales/Staphylococcus/Enterococcus* spp.) as per the protocol. Zone of Inhibition was recorded at 16-24 hours.

Discussion

The present study evaluated the antibiotic susceptibility profiles of probiotic isolates recovered from commercially available pharmaceutical formulations against commonly prescribed antibiotics. The findings demonstrated that all six probiotic isolates were uniformly sensitive to gentamicin, amikacin, tetracycline, and ampicillin/sulbactam, while complete resistance was observed against clindamycin and ceftazidime. In contrast, susceptibility to the remaining antibiotics varied among isolates, with responses ranging from susceptible to intermediate or resistant. These results highlight the strain-dependent nature of antibiotic susceptibility in probiotic microorganisms and emphasize the importance of individual evaluation of probiotic strains prior to their therapeutic or industrial application. The universal susceptibility of all isolates to gentamicin, amikacin, tetracycline, and ampicillin/sulbactam suggests that these antibiotics remain highly effective against the tested probiotic strains.

Conversely, the consistent resistance of all isolates to clindamycin and ceftazidime may represent intrinsic resistance rather than acquired resistance. Intrinsic resistance is a naturally occurring characteristic encoded within the bacterial chromosome and is generally considered non-transferable. Such resistance is less likely to contribute to the dissemination of antimicrobial resistance genes through horizontal gene transfer, thereby presenting a comparatively lower biosafety concern. This distinction between intrinsic and acquired resistance is critical during probiotic selection, as strains carrying transferable resistance determinants may act as reservoirs for antimicrobial resistance genes within the gastrointestinal tract.

The variable susceptibility observed against several other antibiotics further demonstrates the heterogeneity among probiotic isolates. Differences in susceptibility profiles may arise from species-specific characteristics, strain-level genetic diversity, variations in cell wall structure, membrane permeability, antibiotic efflux systems, or mutations affecting antimicrobial targets. These findings reinforce the recommendation that antibiotic susceptibility should be evaluated individually for each probiotic strain rather than generalized at the species level.

Knowledge of antibiotic susceptibility profiles has important clinical implications, particularly when probiotics are administered alongside antibiotic therapy. Probiotic strains that possess intrinsic resistance to a

prescribed antibiotic may survive antimicrobial treatment, allowing them to maintain gut microbial balance, reduce antibiotic-associated dysbiosis, and potentially lower the incidence of antibiotic-associated diarrhea. Therefore, understanding the compatibility between probiotics and antibiotics may facilitate the rational selection of probiotic formulations with the greatest potential for synergistic therapeutic outcomes.

Despite these potential benefits, the occurrence of antibiotic resistance in probiotic strains requires careful safety assessment. International regulatory agencies recommend that probiotic strains intended for human consumption be screened for antibiotic resistance and evaluated for the presence of transferable resistance genes. The major safety concern is not the existence of resistance itself but whether resistance determinants are located on mobile genetic elements such as plasmids or transposons that can be horizontally transferred to pathogenic bacteria. Consequently, comprehensive molecular characterization is necessary to distinguish intrinsic, chromosomally encoded resistance from acquired, transmissible resistance.

Overall, the present findings indicate that probiotic isolates from locally available pharmaceutical formulations exhibit diverse antibiotic susceptibility patterns, characterized by consistent sensitivity to gentamicin, amikacin, tetracycline, and ampicillin/sulbactam, uniform resistance to clindamycin and ceftazidime, and variable responses to other antibiotics. These observations support the inclusion of antibiotic susceptibility profiling as an essential criterion during probiotic selection and quality assessment. Future investigations should incorporate molecular analyses of antibiotic resistance determinants, whole-genome sequencing, and horizontal gene transfer studies to confirm the genetic basis and transmissibility of the observed resistance phenotypes. Such approaches will contribute to the development of safer probiotic formulations while ensuring their compatibility with antimicrobial therapy and minimizing the risk of dissemination of antibiotic resistance genes.

Future Perspectives

The use of probiotics in various diseases or health problems is appealing when the medical community is looking for alternative medicines due to the introduction of new and multidrug resistant infections, however there are still a lot of concerns to be resolved before probiotics are elevated to the status of regular treatment. Probiotic research examining the impact on antibiotic use in the future should check for the presence or lack of relevant resistance genes to better grasp the potential. Future perspectives for the development of nutritional or pharmacological inventions to sustain health include modulating the microbiota using probiotics or next generation beneficial bacteria. To refine the clinical indication of certain probiotic strains, to better understand the postbiotic impact of chemicals generated by probiotic bacteria, and the parabiotic effect of inactivated bacterial cells, further clinical research is still required.

Conflict of Interest

The authors have no conflict of interest with the present publication.

Authors' Contribution

All authors contributed equally at all stages of research and preparation of the manuscript. Final form of manuscript was approved by all authors.

Funding

This work was supported by DBT-BUILDER program and Central University of Jharkhand, Cheri-Manatu, Kamre, Kanke, Ranchi, Jharkhand, India-835222.

Acknowledgments

This study was supported by DBT BUILDER program [BT/PR9028/INF/22/193/2013], work station provided by Central University of Jharkhand, Cheri-Manatu, Kamre, Kanke, Ranchi, Jharkhand, India-835222.

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