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Antagonistic Activities of Pharmaceutical Probiotics and Prebiotic from Fermented Food Products against Selective Human Urinary Pathogen

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KEYWORDS

Antagonistic-effect, Chemical-Indicators, Efficacy test, Probiotics, Susceptibility test.

ABSTRACT:

Introduction: Pharmaceutical probiotics are widely used for the treatment of gut-associated infections. Probiotics are prescribed along with antibiotics and in many cases instead of antibiotics. The beneficial effects of using probiotics have already been established through various research studies. But a pharmaceutical processed combination of different species of gut acting normal flora as a probiotic supplement and its toxin may result from pathogenesis in gut health.

Objective: Previous research explained low-grade side effects resulting from excessive use of probiotics. The present study was carried out to observe the effect of different doses of probiotics and their antagonistic reflex against specifically identified pathogen *Klebsiella* sp.

Method: The basic concept was triggered for isolations and condign of purified probiotics from food samples to decorate the proportional impact by an indicator pathogen determined from human urine samples. The outfit progress and results were modified by in-vitro analysis for a new medicinal approach against urinary tract pathogens. The mode of action was subjected to assemble their consecutive analysis.

Result: Implementation of additional content of the pharmaceutical product was used apart from the probiotic dosages. The expected target dose and assessments on the effects were clarified after the implementation of screened probiotics along with antibiotics.

Conclusion: The outcome inferred that, if any patient took probiotic along with long courses of antibiotics, it becomes dead in downstream blood. The secrets are the toxin triggered by accessed antibiotic doses, and the killer motif of probiotic acts against itself to prohibit the medicinal action in the gut.

1. Introduction

Probiotics are live microorganisms intended to have health benefits regarding public health significance. Pharmaceutical products are sold as probiotics per practitioner suggestions except for common foods (yogurt, curd), dietary supplements, that aren't used orally. In modern science established methodology is following the implementation of probiotics as beneficial bacteria and other microorganisms to kill germs and its toxin properly [5, 3]. The intestinal normal flora is helping to digest food, eliminate disease-causing harmful microorganisms, and produce antioxidants to synthesize vitamins [6, 9, and 12]. Normal gut flora and microorganisms in probiotic products are the same as or similar to naturally survive on their physical state in

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our bodies [2]. Previous researchers have studied probiotics to augment their solution regarding antibiotic drug resistance along with a variety of health problems. Their basic research is improvised on digestive disorders such as diarrhoea caused by infections and antibiotic-associated diarrhoea [10, 15, and 27]. Newly updated research is now implemented on irritable bowel syndrome [7], inflammatory bowel disease [28], atopic dermatitis [5], and allergic rhinitis [8] by using supplementary probiotics instead of an antibiotic. Now paediatrics treatment is replaced by probiotics on simple doses treating tooth decay, periodontal disease, and other oral health problems [4]. There is preliminary evidence that probiotic supplements along with antibiotic treatment help to prevent viral diarrhoea [11, 14], urinary tract infection [29], and irritable bowel syndrome [13]. The basic implementation based on probiotics and its induced toxin needs to be learned for UTIs. Applied research may be the solution to implement probiotics on infectious pathogens for in-vitro analysis from a collected sample of patients to diagnose the active role for UTI instead of antibiotics and NSAIDs.

The origin of cultured products dates back to the dawn of civilization mentioned in the holy sculptures. Climatic conditions for developing natural efficient microbes have favoured the development of many of the traditional soured milk or cultured dairy products such as kefir, koumiss, and curd [16]. These products are still widely consumed, had often been used therapeutically before the existence of bacteria was recognized [19]. At the beginning of the 20th century, the main functions of gut flora were completely unknown. IlyaIlyich Metchnikoff (1908), winner of the Nobel Prize in Medicine, has linked the health and longevity to the ingestion of bacteria present in yogurt. The narrated topic was about the constitution of the human body presented several disharmonies inherited from primitive mammals, such as body hair, wisdom teeth, stomach, vermiform appendix, caecum, and large intestine. The bacteria that involved in yogurt fermentation such as *Lactobacillus bulgaricus*, and *Streptococcus thermophilus*, suppress the putrefactive-type fermentations of the intestinal flora and that consumption. These yogurts played a role in maintaining health and attributed to the long life of Bulgarian peasants to their intake of yogurt containing *Lactobacillus* sp., [20]. In particular, reported methods were established for the large intestine based on the studies of usefulness to mammals in managing rough food composed of bulky vegetables. Moreover, it is the site of dangerous intestinal putrefaction processes which can be opposed by introducing *Lactobacillus* sp. into the body, displacing toxin-producing bacteria, promoting health, and prolonging life [17]. Tissier's (1906) discovery of *Bifidobacteria* sp. in breastfed infants also played a key role in establishing the concept that specific bacteria take part in maintaining health researchers reported clinical benefits from modulating the flora in infants with intestinal infections [18]. At the time, many others were sceptical about the concept of bacterial therapy and questioned in particular whether the yogurt bacteria (*L. bulgaricus*). The reported gut-acting bacteria were able to survive intestinal transit, colonize, and convey benefits [23]. In early 1920, *L. acidophilus* milk was documented to have therapeutic effects, in particular, a settling effect on digestion [22]. It was believed that colonization and growth of these microorganisms in the gut were essential for their efficacy, and therefore, the use of intestinal isolates was advocated. Shirota (1930) focused his research on selecting the strains of intestinal bacteria that could survive passage through the gut and on the use of such strains to develop fermented milk for distribution. *L. acidophilus* was the basis for the establishment of the Yakult Honsha Company. Only at the end of the century, becomes clear that intestinal microflora had several functions, including metabolic and protective ones [24]. Metabolic functions are primarily characterized by the fermentation of non-digestible dietary residue and endogenous mucus, savings of energy as short-chain fatty acids, production of vitamin K, and absorption of ions. Tropic functions are based on the control of epithelial cell proliferation and differentiation, and the development and homeostasis of the immune system. Finally, protective functions are connected with the barrier effect and protection against pathogens [21]. Thus, the normal practice is to make a product with both starter organisms, *e.g. S. thermophilus*and, *L. delbrueckii.*, *Bulgaricus* sp., and one or more species of Probio [23]. The guidelines that stipulate what is required for a product to be called a probiotic were published (FAO, 2002) [25]. The required strains were designated individually, appropriately, and retained a viable count at the end of their shelf life in

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the designated product formulation that confers a proven clinical end-point. The probiotic definition requires an assessment of this constitutes an important part of their characterization for human use [29]. Increasing antibiotic restoration has been an alarming situation throughout the world (WHO, 2006). It is about time to lock beyond antibiotics. Probiotic has been used to explain the mode of action along with the treatment of subjected antibiotics under their outfit progress. So it will be signified perspectives attribution for respective doctors and practitioners to carry out their treatment for implementation on additional content of the pharmaceutical product used apart from the probiotic dosages [30]. Finally completion of studies based on expected target dose and assessments on the effects after implementation incorporation with antibiotics against urinary pathogens.

2. Objectives

The objectives of the study were stood for isolation and identification of probiotics from pharmaceutical products,

Screening of desired organisms from Human Urine samples, and

Administration of different doses of microorganisms from pharmaceutical processed, fermented food, and extraneous sources as probiotics studying the antagonistic activity against isolated *Klebsiella* sp. of the urinary tract.

Duration of research: One year three months (February 2017- June 2018).

3. Methods

The study aimed to observe the antagonistic action of pharmaceutical probiotics on *Klebsiella* sp. assembled samples from human urine.

3.1 Sample Collection:

Pharmaceutical probiotics were collected from the medicinal market. (Sample-SP)

Curd samples were prepared in the laboratory of Assam Down Town University. (Sample-SC)

1% yeast extract was taken from agar to culture for desirable colonies in the media. (Sample-SY)

1% yeast toxin + 95% ethanol (5 fold dilutions, SYE31-SYE36) have newly been experimented with as samples to observe outcomes or antagonist activities against the urinary pathogen.

The identification of the microorganisms was a sequential process, which included a series of different types of experiments. In the present study, the following sequence of experiments was carried out for the presumptive identification of the isolates.

3.2 Colony counting (McFarland *et al.*)

Clinical data confirming the effectiveness of probiotics in preventing tentative diagnosis (TD) is relatively limited. Nevertheless, the prophylaxis of TD constitutes one of the largest markets for probiotics in Europe. McFarland *et al.* performed a meta-analysis of studies and found that probiotics significantly prevented TD (RR ¼ 0.85, 0.001). The yeast *S. boulardii*, and a mixture of *Lactobacillus acidophilus, Bifidobacterium bifidum* appeared to have the greatest efficacy. No practice guidelines for the use of probiotics in the prophylaxis of TD are currently available.

3.3 Microbiological assessments

Pharmaceutical probiotics were isolated using a standard protocol. Pharmaceutical samples were collected aseptically then we examined the several colony characteristics in different agar and selective medium. Different dilutions of the bacterial strains were isolated from pharmaceutical probiotics and screened for antagonistic effects against *Klebsiella* sp. using the well diffusion method. Bacterial strains from a fermented food product, curd were isolated and screened for antagonistic effect against *Klebsiella* sp. using the well diffusion method. Yeast sample *Saccharomyces cerevisiae* was isolated and different dilutions of the isolates were screened for several dilutions. Each three-milliliter diluted sample (5 and 10 fold) was selected to measure optical density (SP, SC, SY, and SYE) and counted for the colony of probiotic (SP and SC) in McFarland scale. Then one loop colony of *Klebsiella* sp was transferred to each diluted sample and kept for 24 hrs incubation. The rest of the fourteen samples were subjected to different screening methods on different agar mediums, biochemical broth, slant, and artificial media. Among them, nine samples were

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selected by observing the absence of the growth of *Klebsiella* sp. and recorded by the screening of antagonistic effect.

3.4 Antimicrobial susceptibility test

The procedure was followed by preparing plates with Muller Hinton Agar (M173) for using the Kirby Baur method for rapidly growing aerobic organisms. For fastidious organisms, the agar is supplemented with 5% sterile defibrinated blood. For fungal culture, we used Muller Hinton Agar + 2% glucose + 0.5 mg / ml Methylene blue dye (GMB), the medium in the plates should be sterile and have a depth of about 4mm.

3.5 Molecular diagnosis

Real-time PCR was supposed to perform to detect and enumerate bacterial colonies especially isolated pathogens from human urine samples. Real-time PCR hybridization probe-based assay was used to detect *Klebsiella* sp. by 16S rRNA gene (Forwarded and reverse primer) for the rapid identification directly from MacConkey and CLED agar plates. Clarified cross-reactivity was observed with other *Klebsiella* sp., due to specific studies on antagonistic sensitivity against food and pharmaceutical probiotics. The molecular experiment was demonstrated for following accurate specificity that compared to the results of conventional biochemical characterization and automated analysis (VITEK 2).

4. Results

Expected research is the newly implemented probiotics, referred in studying preferably antagonistic action against urinary tract and gut infection. The protocol was stood for to experiment on excessive doses of probiotic against urinary pathogens along with implementations of antibiotics sensitivity. The implemented work has resulted in the death of normal micro flora in the gut and its sensitized toxin acted itself against normal gut flora resulting in antagonistic action. The screening of probiotics according to their doses and pharmaceutical preparation comes out for isolating and identifying the categorized samples (*Streptococcus faecalis* T-110^{TM} JPC 30 million, *Clostridium butyricum* TO-A^{TM} 2 million, *Bacillus mesentericus* TO-A^{TM} JPC 1million, *Lactobacillus sporogenes* 50 million)

4.1.1 Screening

In the present study, pharmaceutical samples were collected aseptically then examined the several colony characteristics in different agar and selective medium. Screening of routine observation was determined and assured that the provided bacteria and spore-forming bacteria were available in samples. Long laboratory work was completed after following and determining the basic probiotic from supplied pharmaceutical samples, and cultured into different agar media preparing for the conventional biochemical test for identification. To distinguish the different criteria between Pharmaceutical and fermentative probiotics, homemade curd samples were selected under their isolated probiotic microorganisms. We followed the studies of morphological, cultural, and biochemical characteristics of isolates from pharmaceutical samples following standard description of 'Bergey's Manual of Determinative Bacteriology'-8th Ed. The results were closely related to the genus *Streptococcus*, *Clostridium*, and *Lactobacillus* sp. The curd-derived isolates from diluted samples were also found closely related with the genus *Streptococcus lactis.*

4.1.2 Screening of test pathogen from urine samples

Ten urine samples from SRL Diagnostic were selected from ten patients. Previous research stood for minute affection regarding excessive use to observe the outcomes resulting from different doses of probiotic supplement against selected human urinary pathogens. The implemented desires were selected for microbes as a probiotic supplement to study the finding on urinary tract infections.

The identification of pathogenic microbes was done based on cultural, morphological, and biochemical characteristics reading from the automated Vitek 2 compact system.

Table 1 Observational biochemical reactions recorded of *Klebsiella sp.* by automated Vitek 2 compact system

Test Organisms: *Klebsiella pneumonia ssp pneumonie*			
Test Substrate	**Text**	**Amount/well (mg)**	**McFarland 0.61**
Ala-Phe-Pro Anylamidase	APPA	0.0384	-
ADONITOL	ADO	0.1875	+
Alpha D	AGLU	0.036	-

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Glucosidase			
Glutamyl Arylamidase pNA	AGLTp	0.0324	-
Alpha Galactosidase	AGAL	0.036	+
Beta D Glucopyranosidase	BGLU	0.036	+
Beta N Acetyle glucosaminidase	BNAG	0.0408	-
Beta D Xylosidase	BXYL	0.032	+
BETA - GALACTOSIDASE	BGAL	0.036	+
Beta Alanine arylamidase pNA	BAlap	0.0174	-
Beta Glucoronidase	BGUR	0.0378	-
Citrate Sodium	CIT	0.054	+
COUMARATE	CMT	0.126	-
D Maltose	d MAL	0.3	+
D TAGTOSE	d TAG	0.3	+
D Mannitol	d MAN	0.1875	+
D TREHALOSE	d TRE	0.3	+
D Glucose	d GLU	0.3	+
D Mannose	d MNE	0.3	+
D Sorbitol	d SOR	0.1875	+
D Cellulose	d CEL	0.3	+
Ellman	ELLM	0.3	-
Glycine Arylaminidase	Gly A	0.012	-
Glu-Gly-Arg-Arylamidase	GGAA	0.0576	-
Gama Glutamyl Transferase	GGT	0.0228	+
Hydrogen Sulphide	H_2S	0.0024	-
L Lactate alkalinisation	l LATK	0.15	+
L Malate Assimilation	l MLTa	0.042	-
L ARBITOL	l ARL	0.3	-
L Histidine assimilation	l HLSa	0.087	-
L Lactate assimilation	l LATa	0.186	-
Lysine Decarboxylase	LDC	0.15	+
Lipase	LIP	0.0192	-
Malonate	MNT	0.15	-
N-acetylgalactosaminidase	NAGA	0.0306	-
O/ 0129 Resistance	O129R	0.0105	+
Ornithine Decarboxylase	ODC	0.3	-
Fermentation of Glucose	OFF	0.45	+
Phosphates	PHOS	0.0504	+
Palatinose	PLE	0.3	+
L-Pyrrolydonyl Arylamidase	PyrA	0.018	+
L-Proline Arylamidase	ProA	0.0234	-
Sacharrose	SAC	0.3	+
Succinate	SUCT	0.15	+
Tyrsine Arylamidase	Tyr A	0.0276	+
Urease	URE	0.15	+
5 Keto D Gluconate	5KG	0.3	-

The molecular assessment was conceptualized compared with the nucleotide collection of NCBI. The nucleotide was determined by similarity percentages of compared one from NCBI consists of gene alignment (GenBank+EMBL+DDBJ+PDB+Ref) sequences. The sequences were longer (60006391) than 100Mb consisting of mixed DNA and followed non-redundant identical database characters. For DNA isolation was followed by the enzymatic lysis method. The quality and quantity of genomic DNA were determined by both the spectroscopic method and agarose gel electrophoresis. Biochemical analysis and PCR-based taxonomic markers were used for species identification of the isolates. An automated Vitek-2 System (Biomerieux, France) was used for 43 biochemical tests in the case of bacterial isolates.

4.1.3 Assessment of visible and non-visible findings

Yeast (1% killed) + 95% Ethyl alcohol (SYE35 and SYE36) are used to be compilations of data on reporting antibiotic sensitivity experiments. This experimental finding was an estimated range and sequenced data approach for analyzing sensitiveness and resistance against the different antibiotic disks. Following this, the categorization of data came out under a shorter length of expression on media based on the resistance level. The percentages were estimated following resistant level of counting as Ampicillin 76.92%, Cefepime 100%, Ceftriaxone 94.73%, Cotrimoxazole 100%, Vancomycin 85.71%. Again regarding sensitivity strength, it was reported to follow on Amikacin 46.66%, Erythromycin 65%, Imipenem 57.57%, Ofloxacin

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54.54%. So in the case of *Saccharomyces* sp, the researcher introduced first-time dilution with ethanol to observe antibiotic susceptibility along with prebiotic. This methodology was applied the first time and showed the antagonistic effect observing the result on each Petri plate.

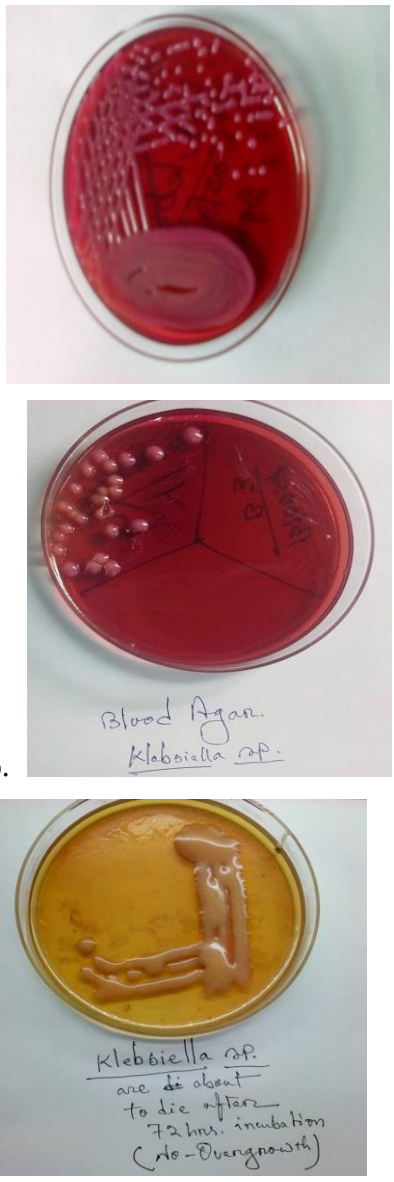

Figure 1 (a) Colonies were pinkish-red in MacConkey agar, isolated from urine samples (*Klebsiella sp*).

(b) Colonies were pinkish and translucent in blood agar (1% Sheep blood), isolated from urine samples (*Klebsiella* sp).

(c) Death of colonies has occurred without any degenerative loss of pathogenic properties on the agar media composed with yeast toxin and ethanol.

Acceptable units were selected on OD reading 0.20-0.85 and colonies were 0-3.5 mcf. Synchronization of chronological data of samples, from thirty-six, twenty-two samples were discarded and the rest of fourteen were selected for further research methodology. From this aspect the inference came out that probiotic organisms could not be able to inhibit the growth of *Klebsiella* sp. somehow mycotoxin was able to do.

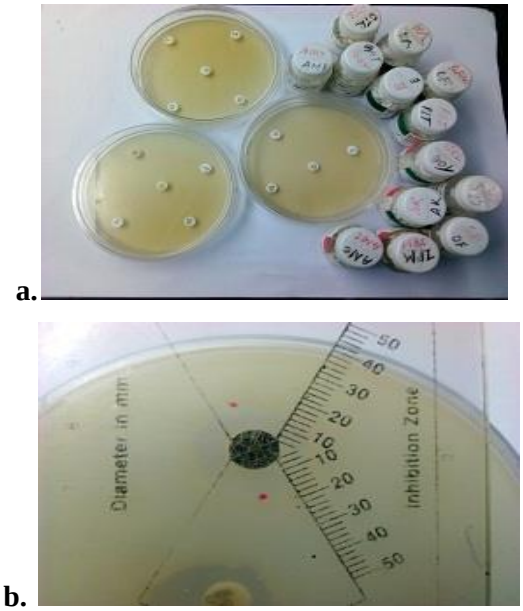

Figure 2 (a, b) samples were designed for several antibiotic disc susceptibility tests in Muller-Hinton agar media following the Kirby-Bauer disc method.

Table 2 Listed antagonistic activities of probiotic against *Klebsiella* sp. in urine samples were implemented for further analysis

Urine samples	The ratio of 5ml urine and 5 ml diluted probiotic sample (Kept in 24 hrs)	Culture in agar media (24 and 48 hrs kept in incubation)		
		MacConkey (Inoculums, *Klebsiella* sp.)		
		24 hrs	48 hrs	Inference

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Sample U1	U1+ SY28	Less growth	died	Act as mild probiotic
Sample U2	U2+ SY29	Less growth	died	Act as mild probiotic
Sample U3	U3+ SY30	Less growth	died	Act as mild probiotic
Sample U4	U4 + SYE31	absent	absent	Act as probiotic
Sample U5	U5+ SYE32	absent	absent	Act as probiotic
Sample U6	U6+ SYE33	absent	absent	Act as probiotic
Sample U7	U7+ SYE34	absent	absent	Act as probiotic
Sample U8	U8+ SYE35	absent	absent	Act as probiotic
Sample U9	U9+ SYE36	absent	absent	Act as probiotic

The rest of the fourteen samples were implemented for different screening methods on applying different agar mediums, biochemical broth, slant, and artificial media. Among them, nine samples were selected in conformation absence on the growth of *Klebsiella* sp. To find out selected research outcomes for urinary pathogens, samples were designed for several antibiotic disc susceptibility tests. The common findings were as an antibiotic Gatifloxacin, Tobramycin, and Imipenem found very much susceptible to pharmaceutical probiotics and curd Probio than yeast. From this aspect, respective doctors can easily suggest fermentative curds to their patients along with antibiotic treatment except for Gatifloxacin, Tobramycin, and Imipenem. On the contrarily the *Klebsiella* sp. showed a similar effect along with other antibiotic discs. That significance augmented our research on the basic principle of developing probiotics against *Klebsiella* sp. in urine samples.

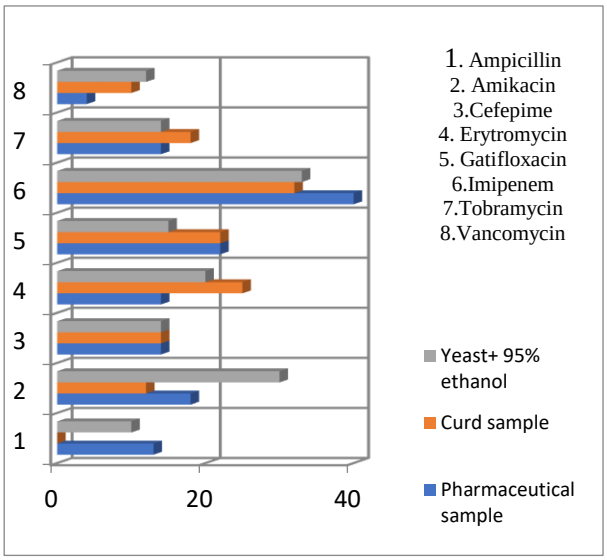

Figure 3 Comparative responses were assembled to clarify the function of the antimicrobial susceptibility test of selected samples and the antimicrobial susceptibility test reports were demonstrated by a graphical view in the range of MIC (Graphical view).

5. Discussion

Long laboratory work was completed as done before following to determine the basic probiotic from supplied curd samples and yeast extract. Here we isolated the probiotic bacteria from fermented food products following the methods of a single study (Erickson KL, and Hubbard NE, 2000). But the previous researcher compiled their works for findings on nutritional aspects whereas our research was carried out to find out the antagonistic effect of fermentative probiotics on itself and *Klebsiella* sp. The rest of the fourteen samples were implemented for different screening methods on applying different agar mediums, biochemical broth, slant, and artificial media. Among them, nine samples were selected in conformation absence on the growth of *Klebsiella* sp. For finding out research outcomes for gut health significance, samples were designed for several antibiotic disc susceptibility tests. In this graphical representation, the common findings were as an antibiotic Gatifloxacin, Tobramycin, and Imipenem found very much susceptible to pharmaceutical probiotics and curd Probio than yeast. From this aspect, respective doctors can easily suggest fermentative curd to their patient along with antibiotic treatment except for Gatifloxacin,

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Tobramycin, and Imipenem. On the contrarily the *Klebsiella* sp., showed a similar effect along with other antibiotic discs (Figure 3).

That significance augmented our research on the basic principle of developing probiotics against *Klebsiella* sp. in urine samples. Nine urine samples from the patient of different ages were selected from several categories the only basis on isolating *Klebsiella* sp. in SRL Diagnostics. Five ml of solution mixed up with five ml of urine and kept at room temperature for twenty-four hours. Then a loop of mixed sample transferred to culture on a different agar medium which was a suitable and supportive medium for the growth of *Klebsiella* sp. After twenty-four and forty-eight hours incubation and cultured for several times the result came as an inference that yeast toxin acted slow and mild inhibition on the growth of *Klebsiella* sp. whereas yeast suspension with 95% ethyl alcohol completely inhibits the growth of this lactose fermenting pathogen. So among the nine samples SY28, SY29, SY30 acted and were designed as mild probiotics, and SYE31, SYE32, SYE33, SYE34, SYE35, SY36 were subjected as probiotics against *Klebsiella* sp. On the contrarily, the isolated pharmaceutical and fermented food probiotics were selected for further reexamined against *Klebsiella* sp. Experimental exploration was revealed on pharmaceutical probiotics and bacterial isolates from fermented food proving the absence of an antagonistic effect on *Klebsiella* sp. whereas the yeast strain of *Saccharomyces cerevisiae* showed an antagonistic effect against *Klebsiella* sp.

So findings stood for the fact that, after having repeated doses of probiotic medicines, it does not carry any significant role for a patient due to toxicity effects on itself. In the case of gram-negative selection, K-toxin of *Klebsiella* sp. as *the* heat-labile toxin is not antagonistic itself. To find out the urinary significant Probio against *Klebsiella* sp. two types of artificial medium were implemented for observation on its growth, colony characteristics, and biochemical reaction against it (Table 2, Figure 3). Among the nine, three samples were discarded due to contaminants. And re-dilution occurred to prepare the same dilution and finally nine were selected for a urine test (Table 2). Nine urine samples from a patient of different ages were selected from several categories the only basis on isolating *Klebsiella* sp. in SRL Diagnostics (Table 1, 2). Five ml of solution mixed up with five ml of urine and kept at room temperature for twenty-four hours. Then a loop of mixed samples was transferred to culture on a different agar medium which was the suitable and supportive medium for *Klebsiella* sp. growth (Table 2, and Figure 2).

So analyzed findings of this research were:

The probiotic toxin acts antagonistically against itself, but k toxin of *Klebsiella* sp. non-antagonist by itself to it. So it is impossible to develop a killed vaccine from this pathogen.

Gram-positive spore-forming probiotic has no antagonistic effect on Gram-negative pathogens.

1% killed yeast are very much effective than 1% live yeast against UTI infection.

Prebiotic suspension with methyl alcohol acted very specifically against urinary tract pathogen *Klebsiella* sp.

Conclusion

The results of different assessments were determined by performing standard protocols in the laboratory along with the principles. The pharmaceuticals, homemade curd, 1%yeast, and 1% yeast toxin were implemented for in-vitro analysis following practitioner suggestions that the microbial safety of investigated samples depends not only on the environmental, gut health conditions but also on the personnel hygiene. Expected research was newly implemented of probiotic, studying preferably antagonistic action against urinary tract and gut infection. Our conclusive studies were provided the solution to respective doctors and practitioners carrying out their treatment against urinary tract pathogen along with probiotic and prebiotic supplements. Here researcher's conclusion is to analyze and categorize antagonistic activities of gut acting probiotic against pathogenic *Klebsiella sp*., responsible for urinary tract infection, and later to take a part of this study in molecular-based at a broad aspect of further research.

Supplementary Description

Colony morphology (form, elevation, margin, surface, color etc.) and cellular morphology of the pure culture isolates were recorded from agar plates. Bacterial and fungal isolates were also grown in enriched media for

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their colony characterization and comparison to the type species. The microscopic features of the isolates were recorded for all the isolates. Some biochemical analysis and PCR based taxonomic markers were used for species identification of the isolates. An automated Vitek-2 System (Biomerieux, France) was used for 43 biochemical tests in case of bacterial isolates.

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Data availability

Data sharing is not applicable to this article due to the advancement of our next extension on this work.

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Conflicts of interest

The authors declare that they have no conflicts of interest relevant to the manuscript that is being submitted following the Medical Diagnostic Methods.

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