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Review Article

Antimicrobial Effects of Probiotic *Lactobacillus* and *Bifidobacterium* Species: A Review

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Abstract

The probiotics are live and specific non-pathological microorganisms. Most probiotics are part of the normal flora of the human intestine. Probiotic microorganisms are divided into three groups: bacteria, fungi and yeast. When probiotics are used in humans or animals, have beneficial effects on host health. The physiological effects associated with the consumption of probiotics include; increase bowel movement, intestinal microbial flora reconstruction after antibiotic and therapy, lowering blood cholesterol, stimulating the immune system, inhibiting bacterial infections, eliminating carcinogenic substances, improving calcium absorption and reducing the activity of fecal enzymes, production of some digestive enzymes and vitamins, and antibacterial materials such as organic acids, bacteriocins, hydrogen peroxide, diethyl acetaldehyde, lactoperoxidase system, and lactones. Some of these microorganisms are selective strains of *Lactobacillus* and *Bifidobacterium* bacteria, although strains of *Enterococcus*, *Streptococcus*, and *Escherichia coli* are also used for this purpose. Probiotic bacteria, by maintaining the natural microflora of the intestine and controlling pathogenic microorganisms, reduce the risk of food borne illness.

Keywords: Antimicrobial; Probiotic; *Lactobacillus*; *Bifidobacterium*

Introduction

Probiotics are beneficial microorganisms that live in or on the body human. They are a potential source of biological and immunological protection that protects the host from exposure to pathogenic bacteria.

Now days, the modern lifestyles have negatively affected the intestinal microbiota and disrupt the microbial balance, so it needs significant external support. The mechanisms of probiotic's influence on host processes are likely to be varied and researchers are still trying to figure out these mechanisms. Several direct and indirect mechanisms underlying the beneficial effects of probiotics against pathogens include: co-aggregation with pathogens [1], consuming nutrients needed by pathogens [2], production of bacteriocins, hydrogen peroxide, lactic acid, and other metabolites [3], inhibition of epithelial and mucosal adherence and promoting immune activities [4,5].

Repeated antibiotic use or misuse causes antibiotic resistance and detrimental effects on normal microbial flora. Great potential for prophylaxis and treatment of a range of microbial infections, make probiotics an effective remedy for this problem and encourage us to use them as alternatives for antibiotics [6,7]. Many types of bacteria and fungi are classified as probiotics, but *Lactobacillus* and *Bifidobacterium spp*. are the most common probiotic strains. This study aimed to investigate and represent the wide range of the antimicrobial activities of the *Lactobacillus* and *Bifidobacterium* species as two potential probiotic strains.

Methods

A literature review of varied databases such as PubMed, Web of Science, Science Direct, and other references like Google Scholar (a web search engine) was conducted. All articles accessible when searched with probiotics, *Lactobacilli* and *Bifidobacterium spp.* keywords were recruited for this literature review. The findings were recorded by using the following steps: (i) Different results and individual assays for antimicrobial activities of common probiotic strains were briefly explained and finally, (ii) Tables representing a wide range of antimicrobial activities of different *Lactobacilli* and *Bifidobacterium spp*. were designed.

Chosen Articles to Show Recent Results of Varied Antimicrobial Assays of Probiotics

The antagonistic activity of one microorganism against another microorganism can be determined by using varied antimicrobial assessments. According to the health benefits of probiotic microorganisms, researchers are always trying to investigate the advantageous features of isolated colonies from different dairy products. For example, Ołdak et al. [8] displayed multiple antibacterial activities of *Lactobacillus Plantarum* isolated from two kinds of specific cheeses from Poland by using a well agar diffusion method. In the method, aseptic stocks of specific Poland cheeses were prepared by using Ringer's solution. Serial dilutions were made and spread on De Man, Rogosa, and Sharpe, MRS agar. Colonies from resultant growth were identified by using phenotypic, biochemical, PCR, and 16S rDNA sequencing. After appropriate culturation and incubation of bacterial cultures, antibacterial activities of Whole Bacteria Culture

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(WBC), Cell-Free Supernatant (CSF), and catalase treated Cell-Free Neutralized (CFN) supernatant were investigated. The probiotic cultures were centrifuged and sterilized by using 0.2 μm filters. CSFs were tested to figure out the influence of extracellular metabolites. CFNs were neutralized by 1M NaOH and hydrogen peroxide towards assessing the antibacterial activity of bacteriocins or bacteriocin-like substances. Antimicrobial effects were investigated against indicator strains, *Listeria monocytogenes, salmonella enteritidis, E. coli, Bacillus subtilis*, and *Enterococcus faecium*. In well diffusion method, Nutrient agars were inoculated with cultures of each pathogen severally (with 6 log CFU/ml concentration), and then, wells of 5.5 mm diameter were made on nutrient agars and filled with WBCs, CSFs, and CFNs. Plates were incubated at 37°C for 48 h and the zones of growth inhibition were measured. Results displayed a very powerful against *L. monocytogenes* ATCC19111, activity of WBC (18.27 mm ± 2.7 mm) and CFS (15 mm-20 mm). The smallest growth inhibition (6.71 mm ± 2.2 mm) was observed for *E. faecium*, and this matter resulted from the high tolerance of this lactic acid bacteria species to low PH and the presence of organic acids. The CFN supernatants of all *L. plantarum* strains isolated from Oscypek cheeses showed antagonistic activity towards all of the indicator pathogens. This result represents *L. plantarum* strains as a powerful bacteriocin producer.

Competing for surface receptors can inhibit pathogen adhesion and subsequent infection. One of the common techniques to evaluate the anti-adhesion property of the probiotic strains is the use of the $Caco_2$ cell line as an in vitro method. These colon carcinoma cells can differentiate spontaneously into enterocytes with a brush border layer as found in the small intestine [9]. *L. fermentum* is a potential probiotic strain that varied beneficial properties were possessed by this microorganism. This strain is widely found in dairy products and its powerful adherent ability affects pathogens colonization and enhances its ability for biofilm formation [10]. Jayashree et al. [11] used $Caco_2$ cells to assay inhibiting effect of *Lactobacillus fermentum* MTCC8711 against Methicillin-Resistant Staphylococcus Aureus (MRSA) ATCC43300. In this method, *L. fermentum* and MRSA have inoculated in MRS and BHI broth, respectively. At first, the adhesion property of *L. fermentum* and MRSA was studied separately by using in vitro adhesion assay, microscopic observation, and flow cytometry analysis. Both of them revealed potential adhesion to $Caco_2$ cells, but *L. fermentum* had a stronger ability. Anti-adhesion property was demonstrated by using inhibition of adhesion, competitive adhesion, and displacement assays. For adhesion inhibition assay, the overnight culture of *L. fermentum* with MOI of 1:100 was incubated with $Caco_2$ cells under appropriate conditions. Unattached cells were removed by washing with Phosphate-Buffered Saline (PBS). $Caco_2$ cells were secondary infected with MRSA cells with MOI of 1:10 and resultant plates were incubated for 2h at 37°C. After serial dilution, the enumeration of bacteria adhering to $Caco_2$ cells was determined by plating on MRS agar for *L. fermentum* and MSA agar for MRSA. For the competitive adhesion assay, overnight cultures of *L. fermentum* with MOI 1:100 and MRSA with MOI 1:10 were co-incubated with $Caco_2$ cells monolayer for 2 h at 37°C. After washing with PBS and Triton treatment, plates were incubated on MRS and MSA agars properly. Bacteria adhered to $Caco_2$ cells were counted and results were investigated. Finally, MRSA-infected Caco-2 cells were treated with *L. fermentum* for displacement assay. The results demonstrated *L. fermentum* as a potential probiotic strain against MRSA cells. Methods that were used in this study exhibited about 63%, 71%, and 96% reduction in the adhesion of MRSA cells in comparison with control cultures, respectively.

According to the literature, individual species of *Bifidobacterium* exhibited varied and advantageous effects in the human body as a well-known probiotic strain. Their ability to treat diarrheal symptoms and other disorders associated with the gastrointestinal tract, persuade researchers to know more about them [12,13]. Eshaghi et al. [14] investigated the antibacterial activities of *Bifidobacterium* species isolated from mother's milk and their infant stool. They used the agar-spot and agar well diffusion method. Regarding agar-spot assay, the *Bifidobacterium* isolates (*B.longum, B.breve, B.bifidum*) were cultured in MRS broth for 48 h and 3 μl of bacterial suspension (10^8 CFU/ml) was spotted on MRS agars. Plates were incubated anaerobically for 48 h at 37°C. After incubation, spots were covered with semi-solid BHI agar containing an overnight culture of pathogenic bacteria (*Shigella dysenteriae* PTCC1188, *Salmonella typhi* ATCC19430, *E. coli* ATCC4388, and a clinical sample of *Listeria monocytogenes*) with a 10^8 CFU/ml concentration. Results showed significant antibacterial effects of *B. breve* against all 4 indicator pathogens (*E. coli* (13 mm-25 mm), *Shigella dysenteriae* (12 mm-25 mm), *L. monocytogenes* (6 mm-21 mm) and *S.typhi* (9 mm-20 mm)). Further important effects include antagonistic activities of *B.bifidum* against *Shigella dysentriae* (11 mm-14 mm) and *B.longum* against *S.typhi* (7 mm-14 mm) and *E.coli* (8 mm-12 mm). In a part of the well agar diffusion method, the treatment of supernatants with proteinase K eliminated the zones of inhibition and this matter sheds light on the proteinaceous nature of the antibacterial activity.

L. acidophilus is a well-known probiotic strain that has been incorporated into fermented as well as non-fermented milk and other dairy products. This microorganism has been employed as a therapeutic agent in attempts to make better a wide range of clinical conditions in humans. Bertuccini et al. [15] investigated the antagonistic effects of *L. acidophillus* and *L. rhamnosus* on both anaerobic (*Gardnerella vaginalis* and *Atopobiumvaginae*) and aerobic (*staphylococcus aureus* and *Escherichia coli*) vaginal pathogens. *G. vaginalis* and *A. vaginae* were cultured in Trypticase Soy agar +5% sheep blood anaerobically by using the GasPak anaerobic envelope system. Aerobic pathogens, UPEC *E. coli* ATCC700928 and *S. aureus* ATCC29213 were cultured on LB broth and TSB broth respectively. The co-culture technique was conducted by incubating probiotic strains and pathogens in Defined Medium Simulating Genital Tract Secretions (DMSGTS). Different concentrations of probiotic and indicator bacteria were used and synergism of the two Lactobacillus strains was investigated too. After incubation of suspensions for different lengths of time (6 h to 48 h), 0.05 ml of the consequent suspensions were diluted and cultured on particular agars. Results showed that all of the pathogens are sensitive to the probiotic strains. *L. acidophillus* was significantly efficient *against G. vaginalis, A. vaginae,* and *S. aureus* (total inhibition of the growth after 6 h). *L. rhamnosus* showed powerful inhibitory effects against *E. coli* and *S. aureus*. Combination of the probiotic strains (10^8 CFU/ml) resulted in a considerable antagonistic activity on all 4 pathogens (totally growth inhibition after 6 h to 24 h). These kinds of *in vitro* studies have demonstrated the ability of the *Lactobacilli* as an effective probiotic in mixed forms of vaginal infections [15-18].

The antimicrobial activity of the bacteriocins produced by *L.plantarrum* ATCC8014 testes against *Staphylococcus aureus* ATCC25923, *Streptococcus sanguis* ATCC10556, and *Pseudomonas aeruginosa* ATCC90271. They used a disk agar diffusion technique to evaluate the ability of this potential bacteriocin-producing probiotic

strain. In this method, *L.plantarum* was grown in MRS broth at 37°C for 24 h. Bacterial culture was centrifuged (7000 g for 10 min) and supernatants were adjusted to PH 6.5. The inhibitory effect of hydrogen peroxide was eliminated by using catalase enzyme (5 mg/ml) and supernatants were filtered through a 22 µm pore size filter. 100 ml of the filtrates were precipitated using ammonium sulfate. The crude precipitate was centrifuged for 20 min at 10,000 × G at 4°C. Resulting pellet was resuspended by 2 ml of 10 mM Tris-HCl pH 7.4 and concentrated by using an Amicon Ultra-4 Centrifugal Filter device with a Molecular Weight (MW) cut-off of 10 kDa. The final bacterial suspension was concentrated by freeze-drying and stored at 4°C. Disks made by 30 µg of the bacteriocins were placed onto the surface of the separated BHI agar containing the indicator pathogenic bacteria. After incubation for 24 h at 35°C, zones of inhibition exhibited powerful antimicrobial effects against *S. aureus* (18.5 mm ± 4.4 mm), *P. aeruginosa* (17.5 mm ± 2.9 mm), and *S. sanguis* (10.3 mm ± 1.7 mm). It has shown that *Lactobacillus spp*. exerts antimicrobial effects on vaginal bacterial infections by the production of lactic acid and some other metabolites [18].

The effect of probiotic strains in the treatment or prevention of nosocomial infections was investigated in many articles [19,20]. A randomized controlled trial was conducted by Barker [21] to study the efficacy of mixed probiotics (*L. acidophilus*, *L. paracasei*, and *B. lactis*) against Clostridium Difficile Infection (CDI). Randomized adult patients participated in this experiment for over 2 years. Each person received oral probiotic capsules containing the above probiotic strains (with a 1.70×10^{10} CFU/ml concentration per capsule) or a placebo, once daily for 28 days. Results showed that diarrheal outcomes, total diarrhea days (12 *vs.* 3.5), and rate of diarrhea which was calculated as the total number of diarrhea days divided by the total number of non-missing stool days (0.3 *vs.* 0.1), were notably better in patients treated with probiotic capsules compared with patients treated with placebo.

Sharma et al. [22] studied the anti-biofilm effect of bacteriocin and Exopolysaccharides (EPS) isolated from *Lactobacillus strains* (LAB) against *Pseudomonas aeruginosa*. Neonatal fecal samples from healthy breastfed infants were gathered and isolated probiotics, characterized by phenotypical features, were cultured on MRS broth. Preliminarily, antimicrobial activity of all isolated LAB supernatants and crude bacteriocins were investigated by using the well agar diffusion method. Although all of the obtained supernatants showed antibacterial activity against *P. aeruginosa*, crude bacteriocin of only 8 isolated LAB inhibited the growth of *P. aeruginosa*. Further, only 4 strains among these 8 LAB isolates exhibited EPS producing. Anti-biofilm assessment was performed using isolates that produced both EPS and bacteriocin. Pre-coating and co-incubation techniques were involved in this assessment by using flat-bottomed polystyrene 96-well microtiter plates. In the pre-coating method, each well was filled with bacteriocin and EPS and incubated for 18 h at 4°C. Plates were washed with PBS pH 7.2 to eliminate non-adhered substances. *P. aeruginosa* (10^6 CFU/ml) was added to each well and plates were incubated at 37°C for 7 days. During the incubation, cell viability in biofilm was measured by the spread plate and flow cytometry assays. In the co-incubation method, at first, *P. aeruginosa* and EPS or bacteriocin were mixed in each well and the following steps were carried out like the pre-coating experiment. Results represented that pre-coating of EPS and bacteriocin (alone or in combination) produced by one of the isolated probiotic strain, significantly reduced the number of viable cells in biofilm to 41.7%. This isolated LAB that produced both EPS and bacteriocin, and showed maximum anti-biofilm activity was characterized by using phylogenetical techniques. This potential probiotic strain was identified as the *Lactobacillus fermentum* (Table 1).

Discussion

Probiotics are viable microorganisms that improve intestinal microbial balance. The antimicrobial activity of probiotics is regarded as one of the most fundamental effects of these advantageous strains. The exploitation of antibiosis of *Lactobacillus* and *Bifidobacterium* species is the best option for not only enhancing the microbial safety of the food products but as potential probiotics because of their antipathogenic effects and natural adaptation to the gastrointestinal environment. Efficacy of probiotics in preventing and treatment of several disorders and diseases, such as cancers, diabetes, obesity, antibiotic-associated diarrhea, allergies, and urogenital infections makes probiotics functional agents that could supply most of our nutritional and clinical supplementation requirements [116]. Unlike conventional antibiotics, probiotic use does not lead to disturbance or alteration in the composition of the population of the gut normal flora. Moreover, antibiotic resistance, Antibiotic-Associated Diarrhea (AAD), and allergic reactions to antibiotics oblige researchers to find a better substitute for antibiotics and restrict antibiotic consumption to necessary situations. Probiotic utilization, with its multiple benefits, is an effective solution for this problem.

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Table 1: Selected results of recent successful antimicrobial assays of common probiotic strains.

Probiotics	*Pathogenic bacteria*	Method(s)	Reference(s)
Lactobacillus plantarum	*P.aeruginosa - S.aureus L.monocytogenes -B.cereus*	Well agar diffusion, Microtiter plate assay	[23]
	E.coli - S.aureus	Well agar diffusion	[24]
	Y.enterocolitica - E.coli Sh.flexneri - S.aureus En.faecium	Oxford cup method, Microtiter plate assay	[25]
	L.monocytogenes - C.jejuni	Well agar diffusion	[1]
	Cl.difficile	Co-culture method	[26]
	S.typhymurium - S.aureus Sh.flexneri	Double layer agar diffusion, *In vivo* assay	[27]
	E.coli	Well, agar diffusion, Disk diffusion	[28]
	E.coli - L.monocytogenes V.parahaemolyticus S.entritidis	Anti-adhesion assay	[29]
	S.mutans - S.marcescens L.monocytogenes - E.coli S.typhymurium- Sh.flexneri K.pneumoniae	Well agar diffusion	[30]
	S.typhymurium - S.aureus L.monocytogenes - Ecoli P.aeruginosa	Micro titer plate assay	[31, 32]
	S.enterica	Well, agar diffusion, Anti-adhesion assay	[33]
	P.aeruginosa - E.coli En.faecium - S.aureus	Agar spot test, Well agar diffusion	[34]
	P.aeruginosa - S.aureus S.sanguis	Disk diffusion	[18]
	K.pneumoniae - S.aureus V.parahaemolyticus P.aeruginosa - C.sakazakii S.enterica - S.mutans	Well agar diffusion	[35]
	Campylobacter coli	Well agar diffusion	[36]
	Sh.flexneri Y.enterocolitica	Well agar diffusion	[37]
	L.monocytogenes	Oxford cup method	[38]
	E.coli - S.aureus B.subtilis - P.aeruginosa L.monocytogenes K.pneumoniae	Micro titre plate assay, Well agar diffusion	[39]
	E.coli (ETEC)	Oxford cup method, Co-culture method, Anti-adhesion assay	[40]
	S.mutans	Well, agar diffusion, Micro titer plate assay	[41]
	S.mutans	Micro titer plate assay	[42]
	S.enteritidis	Anti-adhesion assay, Micro titre plate assay	[43]
	G.vaginalis	Well, agar diffusion, Microdilution assay, Co-culture method	[44]
	A.baumanni P.aeruginosa	Well agar diffusion	[45]
	B.cereus - P.mirabilis S.enterica serovar typhi Sh.flexneri	Well agar diffusion	[46]
	C.jejuni	Anti-adhesion assay, Oxford cup method	[47]
Lactobacillus fermentum	*H.pylori*	*In vivo* assay	[48]
	Cl.difficile - Cl.perfringens	*In vivo* and *in vitro* assay	[49]
	P.aeruginosa	Well radial streak method, microtitre plate assay	[50]
	H.pylori	Spot-on-lawn method	[51]
	H.pylori	Well agar diffusion, Urease inhibition assay	[52]
	H.pylori	Micro titre plate assay	[53]
	C.jejuni - C.coli	Well agar diffusion	[36]
	S.typhimurium- Sh.flexneri	Double layer agar diffusion, *in vivo* assay	[27]
	S.aureus - Lmonocytogenes	Well agar diffusion	[54]
	Sh.flexneri - Sh.sonnei	Well agar diffusion	[55]
	Salminella and Shigella species	Viable cell overlay assay, Co-culture method	[56]
	C.jejuni	Adhesion assay, Well agar diffusion	[57]
	Sh.flexneri Y.enterocolitica - E.coli	Well agar diffusion	[38]
	S.aureus - E.coli S.typhimurium	Disk diffusion method	[58]
	G.vaginalis	Well agar diffusion, microdilution assay, Co-culture method	[44]
	A.baumanni - P.aeruginosa	Well agar diffusion	[45]
	Methicillin-resistant S.aureus(MRSA)	Anti- adhesion assay	[11]
	P.acnes - Sh.sonnei H.pylori - En.cloacae V.parahaemolyticus - E.coli L.monocytogenes -S.aureus En.faecium - S.epidermidis	Agar spot test	[59]
Lactobacillus acidophilus	*C.jejuni - L.monocytogenes*	Well agar diffusion	[1]
	Sh.sonnei - V.cholerae	Anti-adhesion assay	[60]
	P.acnes - E.coli P.aeruginosa	Anti-adhesion assay, Well agar diffusion, Anti-quorum sensing assay, Micro tire plate assay	[61]
	B.subtilis	Micro titre plate assay	[62]

	H.pylori - En.faecium E.coli - L.monocytogenes S.epidermidis	Agar spot test	[59]
	G.vaginalis - A.vaginae	Anti-adhesion assay, *In vivo* assay	[63]
	Cl.difficile	Co-culture method, *In vivo* assay	[64]
	S.typhi	Well agar diffusion, Anti-adhesion assay, Co-culture method	[65]
	S.typhimurium - E.coli Cl.difficile - B.vulgatus Cl.hystolyticum	Well agar diffusion, Anti-adhesion assay	[66]
	S.mutans	*In vitro* and *in vivo* assay	[67]
	B.cerus - B.subtilis Cl.difficile - Saureus K.pneumoniae - E.coli P.aeruginosa - V.cholerae Sh.flexneri - S.marcescens S.epidermidis - Y.pestis Y.enterocolitica S.typhimurium L.monocytogenes Methicillin-resistant S.aureus(MRSA) F.tularensis	Spot-on-lawn method	[68]
	G.vaginalis - A.vaginae S.aureus - E.coli	Co-culture method	[15]
	S.entritidis	*In vivo* assay	[69]
	S.marcescens	Micro titre plate assay	[70]
	H.pylori	Clinical assay	[71]
Lactobacillus casei	*L.monocytogenes - C.jejuni*	Well agar diffusion	[1]
	S.aureus - S.typhimurium	*In vivo* assay	[72]
	B.cereus - Sh.flexneri P.aeruginosa	Well agar diffusion	[46]
	S.aureus	Disk diffusion, Microdilution method	[73]
	S.mutans	Well agar diffusion, microtitre plate assay	[41]
	P.acnes - H.pylori L.monocytogenes - E.coli En.cloacae - S.aureus V.parahaemolyticus En.faecium - Sh.sonnei S.epidermidis	Agar spot test	[59]
	Cl.difficile	Agar spot test, well agar diffusion	[74]
	S.typhimurium - E.coli	Anti-adhesion assay, Co-culture method	[75]
	Methicillin-resistantS.aureus(MRSA)	Agar spot test, Co-culture method	[76]
	C.jejuni	Anti-adhesion assay, Oxford cup method	[47]
	S.aureus - En.faecium E.coli - L.monocytogenes Sh.sonnei - Salmonella spp.	Well agar diffusion	[77]
	H.pylori	Well agar diffusion	[78]
	H.pylori	Micro titre plate assay	[53]
	S.aureus	Micro titre plate assay, anti-adhesion assay	[79]
	S.typhimurium - S.aureus S.enteritidis - E.coli	Oxford cup method - anti-adhesion assay	[80]
	E.coli - B.cereus S.aureus - V.cholerae	Anti-adhesion assay, well agar diffusion	[81]
	Cl.difficile	Clinical assay	[82]
	S.aureus - E.coli	Well agar diffusion, Oxford cup method, micro titre plate assay	[83]
Lactobacillus rhamnosus	*E.coli - S.typhimurium*	Well agar diffusion, Micro titre plate assay	[84]
	E.coli - S.typhimurium	Well agar diffusion, Micro titre plate assay	[84]
	E.coli - L.monocytogenes S.aureus - S.typhimurium	Agar disk diffusion assay	[85]
	B.cereus - S.aureus E.coli - L.monocytogenes Sh.sonnei - En.faecalis K.pneumoniae	Deferred agar spot assay, Spot-on-the lawn assay	[86]
	Group B streptococcus	Randomized controlled trial	[87]
	S.sanguinis - F.nucleatum	Anti-biofilm assay	[88]
	S.aureus	*In vivo* method	[89]
	S.typhimurium - E.coli L.monocytogenes - Sh.sonnei	Agar disk diffusion assay	[90]
	Uropathogenic E.coli S.typhimurium	Biofilm formation assay	[91]
	S.aureus- C.sakazakii L.monocytogenes En.faecalis - Salmonella spp.	Micro titre plate assay	[92]
	L.monocytogenes	Well agar diffusion	[93]
	S.pyogenes - S.snaguinis E.coli - A.baumannii E.aerogenes - S.mutans K.pneumoniae - P.aeroginosa	Double layer agar diffusion, Co-competition assay	[94]
	G.vaginalis - A.vaginae	Anti-adhesion assay, *In vivo* method	[95]
	Cl.difficile	Agar spot test, Well agar diffusion	[74]
Lactobacillus reuteri	*E.coli - S.typhi S.typhimurium*	Anti-adhesion assay	[96]
	S.mutans	Well agar diffusion, Micro titre plate assay	[41]
	S.typhi - L.monocytogenes E.coli - En.faecalis	Anti-adhesion assay	[97]
	Group B streptococcus	Randomized controlled trial	[87]
	S.mutans - T.forsythia S.gordonii	Disk diffusion assay(Kirby Bauer method)	[98]
	E.coli - B.cereus P.aeruginosa	The dual-culture overlay diffusion method	[99]
	L.monocytogenes	Well agar diffusion assay	[93]
	K.pneumoniae	Co-culture method	[100]
	H.pylori	Micro titre plate assay	[53]
	H.pylori	Pilot study	[101]
	E.coli - S.typhimurium	Micro titre plate assay	[102]
Bifidobacterium spp.	*Sh.dysenteriae - E.coli L.monocytogenes - S.typhi*	Well agar diffusion, Agar-spot test	[14]
	L.monocytogenes - E.coli C.sakazakii - C.jejuni	Well agar diffusion, Anti-adhesion assay	[1]

	E.coli - S.typhi S.typhimurium	Well agar diffusion, Anti-adhesion assay	[103]
	Cl.difficile	Co-culture method	[104]
	Cl.difficile	Co-culture method, *In vivo* assay	[105]
	K.pneumoniae	*In vivo* assay	[106]
	C.trachomatis - E.coli En.faecium - S.aureus P.mirabilis - Styphimurium Y.enterocolitica	Anti-adhesion assay, Agar-spot test	[107]
	S.enteritidis - E.coli	Well agar diffusion	[108]
	L.monocytogenes	Microdilution method	[109]
	Cl.difficile	Co-culture method, Anti toxicity test	[110]
	H.pylori	Randomized controlled trial	[111]
	S.typhimurium	*In vivo* assay	[112]
	S.typhimurium - E.coli Sh.sonnei - Sh.flexneri	Microdilution method, Anti-adhesion assay	[113]
	Multi-drug resistant E.coli	Well agar diffusion, Anti-biofilm assay	[114]
	P.acnes - S.aureus S.epidermidis	Double agar layer diffusion assay	[115]

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