Laktik ve asetik asit bakterilerinin Shigella spp. Kültüründe antagonistik etkisi

Antagonistic effects of lactic and acetic acid bacteria on Shigella sp. SS10 in co-culture

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ÖZET


ANTİBİOTİK SUSCEPTIBILITY OF Shigella spp. SS10 TO OFLOXACIN, GENTAMYCIN, CEFUROXIME, CEFAZIDIME, LINCOMYCIN, OXACILLIN, CLAXICILLIN, CEFOTAXIME, CIPROFLOXACIN AND NITROFURANTOIN WAS TESTED BY DISK DIFFUSION METHOD. Shigella spp. SS10 was co-inoculated in two different experiment with 3 Lactobacillus species (Lactobacillus plantarum QN01, Lactobacillus parabuchneri SM03 and Lactobacillus fermetum SH01) and 1 Acetobacter pasteuriarun RV04 which has been previously isolated from Nigeria-produced yogurts. An 8th old Shigella spp. SS10 was introduced into an overnight culture of LAB and a fresh Shigella spp. SS10 was inoculated into overnight culture of LAB. Viable counts of pathogens at 0h and after 24h co-incubation at 37°C were observed. Results: Shigella spp. SS10 was resistant to 50% of the tested pathogens. The tested LAB effected an average of 4 log reduction in viable counts of the Shigella strain. The Acetobacter strain displayed very good inhibitory activity with a 4 log reduction in microbial load. Conclusion: Lactic and Acetic acid bacteria isolated from Nigerian yoghurt has considerable activity against Shigella spp. in co culture experiment.

INTRODUCTION

The gastrointestinal tract is subject to infections by many pathogens, which are a major cause of economic loss due to illness, suboptimal performance, and death. These infections spread by direct contact or the fecal-oral route. Enteric (gastrointestinal) and diarrheal disease is the second leading cause of death and the leading cause of malnutrition in children under five years old [1]. The main classes of agents that are responsible for diarrhea of infectious origin are enteropathogenic viruses and bacteria. Among the bacteria, Shigella, Escherichia coli, Salmonella, Campylobacter and Vibrio sp. are often recognized as the causative agents of diarrhea in children in developing countries [2]. These organisms ravage various regions of the world but commonly wreak havocs in the human gut in many developing countries where hygiene and health care systems are below par.

Shigella is a Gram-negative, facultative anaerobic, rod-shaped bacteria closely related to Salmonella, and is the causative agent of human shigellosis. It typically causes dysentery [3]. Shigella is one of the leading bacterial causes of diarrhea worldwide. Conservative estimates suggest Shigella causes about 90 million cases...
of severe dysentery [4,5] with at least 100,000 of these resulting in death each year, mostly among children in the developing world, where they are major causes of moderate-to-severe diarrhea in children under age 5 [6]. Children under the age of 11 are at the greatest risk and as few as 10-200 bacteria are capable of causing disease [7]. Because of the low infectious dose, transmission can occur via contaminated food and water or via direct person-to-person spread. In the U.S., more than 75% of cases of Shigellosis are caused by S. sonnei while S. flexneri is the most prevalent species in developing countries.

The rich and complex environment of the intestine make many pathogens thrive including Shigella spp. in large number especially due to the ever-increasing resistance of bacterial pathogens to common and previously active antibiotics. The increasing resistances even to newer antibiotics pose a greater challenge to the individual health management system of many people living in the developing nations where sources of finance are very scarce. Furthermore, many cases of diarrhea go unidentified, often resulting in inappropriate treatment. Furthermore, continual use of antibiotics may add to the burden of the gastrointestinal environment by upsetting the balance of the protective microbiota.

Lactic acid bacteria (LAB) are a group of protective microbiota in the intestine that has been proven to be effective against many intestinal pathogens especially in probiotic formulation. Also, Acetobacter is a genus of acetic acid bacteria (AAB) characterized by the ability to convert ethanol to acetic acid in the presence of oxygen. Acetic acid bacteria are Gram-negative bacteria generally isolated from a variety of natural fields such as fruits, flowers and fermented foods. They are widely used for vinegar production because of their high concentrations of acetic acid [8]. Probiotics are products or substances containing living and potentially beneficial microorganisms and are aimed at delivering the microorganisms to the gut ecosystem of humans and animals, e.g. by restoring the balance of microflora in the digestive tract [9]. Strains of LAB are the most common microbes employed as probiotics. L. acidophilus has been reported to have effect on diarrhea caused by Salmonella or Shigella. While L. casei was reported to have curative effect on infections caused by Salmonella typhimurium and E. coli [10]. However, there is scarcity of approved probiotic LAB in Nigerian market but there are LAB and AAB added to yoghurt in Nigeria for their fermentative ability but little is known about the ability of these LAB and AAB to reduce the viable population of Shigella spp. This study was, therefore, carried out to determine the inhibition of growth of clinical isolates of Shigella spp. grown in co-culture with LAB isolated from Nigerian yogurts.

**MATERIALS AND METHODS**

**Microorganisms**

Lactobacillus plantarum QN01, Lactobacillus parabuchneri SM03, Lactobacillus fermentum SH01 and Acetobacter pasteurianus RV04 has been previously isolated from different commercial yogurts obtained from shopping malls in Ibadan, Nigeria and identified by sequencing their 16S rRNA gene. Shigella sp. SS10 has been previously collected from an hospital in South West, Nigeria and were further characterized by its cultural, microscopic and their biochemical characteristics according to standard procedures.

**Antimicrobial susceptibility test for Shigella sp. SS10.**

The susceptibility of the Shigella sp. SS10 to different antibiotics was tested using standard antibiotic disc. A 18-hr broth culture of Shigella sp. SS10 was inoculated onto Muller Hinton agar by spread plate method. Ten different standard antibiotic disks ofloxacin, gentamycin, cefuroxime, ceftazidine, lincomycin, oxacillin, cloxacillin, cefotaxime, ciprofloxacin and nitrofurantoin were placed on the agar plates using a sterile forceps. The plates were kept on the bench for 30 min to allow diffusion of the antimicrobials before incubating at 37°C for 24 hrs. The plates were examined for clear zones of inhibition around the discs. The diameter (mm) of zone of inhibition was measured and the result interpreted by the EUCAST Clinical Breakpoint Table version 5.0 [11].

**Co-culture of LAB and Shigella sp. SS10**

Two series of experiments were performed to examine the interference of LAB with the growth of Shigella spp. SS10 by coincubating the pathogen individually with four representative LAB strains from the yogurt isolates Lactobacillus plantarum QN01, Lactobacillus parabuchneri SM03, Lactobacillus fermentum SH01 and Acetobacter pasteurianus RV04.

In the first experiment, Lactobacillus plantarum QN01 and Lactobacillus fermentum SH01 were grown in MRS broth for 24h in microaerophilic conditions while Shigella spp. SS10 was grown on Salmonella Shigella agar. From the 24h old culture, fresh Shigella spp. SS10 was grown for 8hrs and the resulting pathogen broth
Culture was centrifuged at 4000 X g for 15 minutes, the supernatant was decanted and the pellets resuspended in fresh 5ml double-strength nutrient broth with a vortex mixer. 5ml of 8h old resuspended test pathogen culture was added to 5ml of the 24h LAB culture in MRS broth, to make a 10ml co-culture mixture. The bacterial counts were made at 8hrs and 24hrs of both the pathogen monoculture and the co-culture mixture from appropriate dilutions on Salmonella-Shigella medium for viable counts of pathogens and MRS agar for LAB. The pathogen monoculture serves as control.

In the second experiment, a method described by Drago et al., [2] was used and modified in this study. Shigella spp. SS10 was inoculated into 5 ml double strength nutrient broth and then added to the overnight culture of 5ml double strength MRS culture of Lactobacillus plantarum QN01, Lactobacillus parabuchneri SM03, Lactobacillus fermentum SH01 and Acetobacter pasteurianus RV04 and incubated for 24h. Appropriate dilution of both monocultures and the mixed culture of the LAB and pathogens were evaluated at 0h by plating each LAB onto MRS agar and incubated microaerophilically at 37°C for 24hours. Monocultures and mixed cultures were plated on selective media for the respective organisms (Salmonella-Shigella agar for Shigella spp.), then incubated at 37°C for 24hours to evaluate the growth of LAB and pathogens.

RESULTS

The antimicrobial susceptibility patterns of Shigella sp. SS10 was performed and was found to be sensitive to oxolinic acid, gentamicin, ciprofloxacin, cefotaxime and nitrofurantion and resistant to ceftazidime, lincomycin, oxacillin and cloxacillin.

Lactobacillus plantarum QN01, Lactobacillus parabuchneri SM03, Lactobacillus fermentum SH01 and Acetobacter pasteurianus RV04 were examined for their antibacterial activities against Shigella sp. SS10. In the first experiment involving addition of LAB to Shigella sp. SS10 that has grown for 24h, Lactobacillus plantarum QN01 reduced Shigella sp. SS10 growth from 2.72x10⁸ at 8h to 7.54x10⁶ in a 2 log reduction while Lactobacillus fermentum SH01 reduced Shigella sp. SS10 growth from 3.8x10⁶ at 8h to < x10⁴ in a 2 log reduction (fig I).

In the second experiment involving co-cultivation of 24h LAB and fresh Shigella sp. SS10, Lactobacillus plantarum QN01 showed no log reduction in pathogen growth, Lactobacillus fermentum SH01 reduced Shigella sp. SS10 growth by 3 log reduction (fig 1), while Acetobacter pasteurianus RV04 and Lactobacillus parabuchneri SM03 reduced Shigella sp. SS10 growth by 4 log reduction respectively. (Table 1, fig 2). The lactic and acetic acid bacteria growth were not really affected by the pathogen.

Table 1. Viable counts of LAB and Shigella sp. SS10 after 24hours of co-incubation with selected LAB.

<table>
<thead>
<tr>
<th>LAB</th>
<th>CFU/ml</th>
<th>Shigella sp SS10 CFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lb. plantarum QN01</td>
<td>6.32x10⁹</td>
<td>(7.24x10⁹)</td>
</tr>
<tr>
<td>Lb. parabuchneri SM03</td>
<td>4.22x10⁹</td>
<td>(2.4x10⁹)</td>
</tr>
<tr>
<td>Lb. fermentum SH01</td>
<td>1.6x10⁹</td>
<td>(3.1x10⁹)</td>
</tr>
<tr>
<td>Acetobacter pasteurianus RV04</td>
<td>1.24x10¹⁰</td>
<td>(2.1x10¹⁰)</td>
</tr>
</tbody>
</table>

Key: Values in parentheses show the growth of controls, i.e. pure culture, under the same condition.
DISCUSSION

*Shigella* sp has been observed to be generally resistant to antibiotics and also one of the leading causes of enteric infections in developing countries. Antimicrobial susceptibility of *Shigella* spp. and *Escherichia coli*, isolated from diarrheal patients in Lagos, was reported by Iwalokun et al. [4] and found out that over 70% of the *Shigella* isolates were resistant to two or more drugs. Twenty-one distinct multidrug resistance patterns were observed in the isolates. Between 1990-2000, they reported drastic increase in resistance to drugs of choice. In this study, *Shigella* sp. SS10 was resistant to cefuroxime, cefazidine, lincomycin, oxacillin and cloxacillin. In spite of this, ciprofloxacin and ofloxacin seemed to have been the ideal alternatives and are still active against the *Shigella* and *Escherichia coli*, but not without few resistance patterns starting to emerge at this contemporary time. Bolaji et al., [12] reported the presence of antibiotic resistant bacteria in hospital waste water in Ede, Southwestern, Nigeria and that *Shigella* spp and the other organisms isolated in the study have become resistant to septrin, chloramphenicol, amoxicillin and streptomycin while they were also 90% resistant to pefloxacin, ofloxacin, 80% resistant to ciprofloxacin, 70% resistant to gentamycin. The multidrug resistance was as a result of indiscriminate use of the antibiotics among other causes. There is in-vitro bacteriologic efficacy of gentamicin and nitrofurantoin as reported previously (4, 13) to *Shigella* spp., it still holds true for the result got in this study too, over two decades.

The LAB used in this study has a lot of antimicrobial activities on *Shigella* sp. thereby drastically reducing its log count. Four yogurt isolates which include *Lactobacillus plantarum* QN01, *L. parabuchneri* SM03, *L. fermentum* SH01, and *Acetobacter pasteurianus* RV04 were used against *Shigella* sp. SS10. The *Shigella* sp. exhibited a 4-log reduction in viable count on co-incubation with the three LAB and one AAB tested after 24 h co-incubation, increased acidity has been observed to have inhibitory effects against *Shigella* spp., thereby drastically reducing their log count. The 8 h co-incubation yielded less log reduction in comparism with inoculating the pathogen into fully grown LAB. There was a drastic reduction in viable counts of the pathogen after 24 hrs co-incubation. *L. parabuchneri* SM03, *L. fermentum* SH01, and *Acetobacter pasteurianus* RV04 had an average of 3.67-log reduction in the viable counts of the *Shigella* sp. SS10. The result is in line with Beata et al., [15], who co-cultured six *Lactobacillus* strains with *Salmonella* Senftenberg, an *Enterobacteriaceae*, and noted that all the tested LAB strains inactivated the growth of the test pathogen during a 48 h of cultivation. They team reported that the co-incubation method generally gave very good in vitro activity of the co-incubated LAB against the pathogens. *L. buchneri* have been shown to be able to resist gastrointestinal conditions and have shown potential in reducing serum cholesterol which still makes the isolate healthful [16, 17]. Amin et al., [18] also isolated lactobacilli strains from fresh vegetables, and documented the remarkable antimicrobial activity exhibited by the reported lactobacilli against a panel of pathogenic bacteria such as *Escherichia coli*, *Salmonella typhi*, *Shigella dysenteriae*, *Bacillus anthracis* and *Staphylococcus aureus*.

In a similar fashion, AAB species, *Acetobacter pasteurianus* use in this study also displayed good antibacterial activities against the *Shigella* sp. Acetic acid has been shown to have good antibacterial activity against micro-organisms such as *Pseudomonas aeruginosa* [19]. The ability of AAB to inhibit microbial growth could not be much surprising since they have been found to produce appreciable quantity of acetic acid which may have inadvertently reduce the pH of the environment thereby suppressing the growth of the tested pathogen.

The growing concern about the presence of and the spread of multidrug resistant gastrointestinal species was emphasized by the findings earlier discussed thereby underscoring the need for rational application of antibiotics and other necessary interventions that will help to control the menace of antibiotic resistance [20]. The multiresistant *Shigella* sp. examined in this study have higher susceptibility to LAB and AAB relative to the antibiotics used against them. Therefore, the yoghurt from Nigeria have LAB and AAB in them with strong inhibitory effects against *Shigella* sp. in co culture experiment.

REFERENCES


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