# THE USE OF PROBIOTICS (*Lactobacillus*) IN THE MANAGEMENT OF GASTROENTERITIS IN CHILDREN

KANU, Ugochi Jennifer and NWOYE, Onyedikachi Robinson
Department of Science Laboratory Technology Federal Polytechnic Oko, Anambra State
ugochikanu03@gmail.com 08061146842

**Abstract**

The aim of this work was to use *Lactobacillus* as a probiotic in the prevention of gastroenteritis in children. *Staphylococcus aureus, Escherichia coli, Salmonella sp* and *Klebsiella sp* were isolated from the different stool samples and identified using cultural characteristics and biochemical tests. *Lactobacillus sp* was also isolated from yoghurt and identified too. Antimicrobial activity was carried out using disc diffusion method and the zones of inhibition were measured. The cell free supernatants of *Lactobacillus sp* used as antimicrobial agent inhibited *Staphylococcus aureus, Salmonella sp, Klebsiellasp* with zones of inhibition of 11mm, 12mm, 10mm respectively while *Escherichia coli* was not inhibited. The control also had zones of inhibition of 20mm, 29mm, 25mm and 20mm on *Staphylococcus aureus, Salmonella sp, Klebsiella sp* and *Escherichia coli* respectively. The intake of probiotics is therefore recommended in the prevention of gastroenteritis in children.

**Keywords:** Probiotics, Lactobacillus, Gastroenteritis, Antimicrobial, Cell-free supernants

## Introduction

Probiotics are live microorganisms promoted with claims that they provide health benefits when consumed, generally by improving or restoring the gut flora. Probiotics are considered generally safe to consume, but may cause bacteria-host interactions and unwanted side effects in rare cases. There is some evidence that probiotics are beneficial for some conditions, but there is little evidence for many of the health benefits claimed for them (Doron and Snydman, 2015). Probiotics are adjuncts in health promoting food for humans and also used as therapeutic, prophylactic and growth supplements in animal production and human health (Amund, 2016). More importantly, the emergence of antibiotic resistance in enteric pathogenic bacteria has facilitated the prophylactic and therapeutic uses of probiotics. (Giorgieva *et al.*, 2015).

Gastroenteritis is usually an acute and life threatening disease, resulting from loss of fluid and electrolytes particularly sodium ion (Na+) and water from the body, which requires specific anti-infective therapy (Nwachukwu *et al*., 2013). In adults, norovirus and campylobacter are common causes. Eating improperly prepared food, drinking contaminated water or close contact with a person who is infected can spread the disease.

Treatment and prevention of infectious gastroenteritis are not limited to antibiotics

therapy since indiscriminate use fosters the emergence of drug-resistant organisms. Consequently, the European Society for Paediatric Gastroenterology, Hepatology and Nutrition and the European Society of Paediatric Infectious Diseases guidelines made a strong recommendation for the use of probiotics for the management of acute gastroenteritis (Ciccarelli *et al*., 2013).

*Lactobacillus* is one of a number of probiotics considered to be biological therapeutics and host immune-modulating biological that are generally recognized as safe (GRAS). Recent studies demonstrated several antimicrobial mechanisms of *Lactobacillus* such as nutrient competition, production of inhibitory compounds, immune-stimulation and competition for binding sites. In addition, Lactobacillus can produce lactic acid, acetic acid, formic acid and other acids to reduce intestinal pH, which may be the most important mechanism. These bacteria can also secrete certain antimicrobial molecules, such as ethanol, fatty acid, hydrogen peroxide and bacteriocins to exert the antimicrobial activity (Giorgieva *et al.*, 2015). Through these mechanisms, Lactobacillus has demonstrated its ability to inhibit several bacterial pathogens (McFarland, 2015).

The aim of this work is to use *Lactobacillus sp* as a probiotics in the management of gastroenteritis in children.

## Materials and Methods

### Sample Collection

Stool samples were collected from children in Oko using different sterile swab sticks and then were transported immediately to the laboratory, for analysis. The yoghurt sample was purchased from the Eke Oko market, Anambra State.

### Media and Media Preparation

The media used were MacConkey agar, Mannitol salt agar, deManRogosa Sharpe agar (MRS), Eosine Methylene blue agar, Salmonella Shigella agar, Mueller Hinton agar, Nutrient agar and Nutrient broth. They were prepared according to manufacturer's instructions.

### Isolation of the test microorganisms

### Isolation of the *Lactobacillus sp* from Yoghurt sample

deManRogosa Sharpe (MRS) agar was selected for the isolation of *Lactobacillus* sp. A loopful of the yogurt samples was streaked on the sterile plate by streaking method, under aseptic conditions. They were incubated at 37°C for 24hours. After the incubation, colonies were sub cultured on the MRS agar plate at 37$^{o}$C for 24 hours for the isolation of pure colonies. Then the pure colonies were aseptically stored at 4°C in MRS agar slants.

### Isolation of the Microorganisms Associated with Gastroentritis

Stool samples were streaked on different agar plates (Mannitol salt agar, Salmonella Shigella agar, MacConkey agar a d Eosine methylene blue agar) as collected using sawp stick. They were incubated for 24 hours at 37°C. They were identified after 24hours using Microscopic examination and biochemical tests.

**Identification of the test Microorganisms**

The isolates were identified according to their morphological, cultural and biochemical characteristics.

**Production of the antimicrobial from *Lactobacillus sp***

Three hundred (300) ml of deManRogosa Sharpe agar (Mrs) broth were inoculated with 1% v/v of the overnight cultures of the identified isolates and incubated at 37°C for 48houres anaerobically in triplicates. After incubation, cells were separated by centrifugation at 5000rpms for 30mins (using Galenkamp centrifuge, England). The cell free supernatants were filtered using 0.45 um pore size membrane filter to obtain the antimicrobial agents present in cell-free supernatants. according to Ciccarelli *et al*.,( 2013).

**Antimicrobial Activity of Antimicrobial produced from *Lactobacillus* sp isolated against Pathogens Responsible for Gastroenteritis in Children**

The paper disc diffusion method was adopted to determine the effect of probiotics (*Lactobacillus sp*) in the management of gastroenteritis in children. Mueller Hinton agar was prepared and distributed into different sterile petri dishes. The bacterial strains were grown in a nutrient agar broth which was used for the bacterial susceptibility test. The broth culture were grown for 24 hours and serially diluted in the same broth (sterilized at 121°C for 15 minutes).

Different sterile swab stick was used to inoculate the test organisms by dipping it in the diluted culture and streaking on all the surface of the agar plates. Different sterile paper disc about 6mm diameter were seeded with the cell free supernatants of *Lactobacillus sp* and were allowed to dry for some minutes. They were placed on the surface of inoculated agar plates using sterile forceps. The plates were then incubated for 24 hours at 37$^{o}$C. After incubation, the diameter zones of the inhibition were measured using millimeter rule.

**Results**

**Table 1: The cultural characteristics, gram stain and biochemical reaction of the test organisms**

| Cultural characteristics | Morphology | Gram staining | Catalase | VogesProskauer | Indole | Methyl red | Sulphide | Urease | Citrate | Motility | Probable organism |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Greenish metallic sheen on Eosin methylene blueagar | Rod | - | + | - | + | + | - | - | - | + | *Escherichia coli* |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Yellow colonies with yellow zones on mannitol salt Agar | Cocci | + | + | + | - | + | - | + | + | - | *Staphylococcus aureus* |
| The colonies appear large, Mucoid and pink to red on MacConkey Agar | Rod | - | + | + | - | - | - | + | + | - | *Klebsiella sp* |
| Black centered colonies on Salmonella-shigella Agar | Rod | - | + | - | - | + | + | - | - | + | *Salmonella sp* |

**Key:** + = positive, - = Negative

**Table 2: The cultural characteristics, gram stain and biochemical reaction of the test organism (*Lactobacillus sp*).**

| Cultural characteristics | Catalase | Glucose | Mannose | Sucrose | Galactose | Mannitol | Xylose | Sorbitol | Rafinole | Maltase | Lactose | Arabinose | Fructose | Motility | Gas | Growth | Probable organism |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rod | - | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | *Lactobacillus sp* |

**Table 3: Antimicrobial activity of the antimicrobial substance produced from *Lactobacillus sp* on the test organisms**

| Zones of inhibition in millimeter (mm) | | | |
|---|---|---|---|
| Test Organisms (cellfree supernatants) | (Ciprofloxacin) | *Lactobacillus sp* | Control |
| *Staphylococcus aureus* | | 11 | 20 |
| *Salmonellasp* | 10 | 25 | |
| *Escherichia coli* | | Nil | 20 |
| *Klebsiellasp* | 12 | 29 | |

**Discussion**

The study was carried out to evaluate the use of *Lactobacillus* as a probiotic in the prevention of gastroenteritis in children. The organisms associated with gastroenteritis were isolated from the stool samples were identified by their morphology and biochemical tests. The organisms isolated were *Staphylococcus aureus*, *Salmonella sp*, *Escherichia coli* and *Klebsiella sp* as shown in table 1. The probiotic which is *Lactobacillus sp* was isolated from yoghurt and also identified as shown in table 2.

Table 3 shows the diameter of zone of inhibition of the *Lactobacillus sp* on the test organisms. The antimicrobial substance produced from *Lactobacillus sp* inhibited *Staphylococcus aureus*, *Salmonella* sp, *Klebsiella sp* with zones of inhibition of 11mm, 10mm and 12mm respectively except *Escherichia coli*. The result obtained in this study is in agreement with Reid *et al* (2007) who carried out a study on use of *Lactobacillus sp* as probiotics in treatment of urinary tract infection caused by some pathogenic organism and discovered that *Lactobacillus* had inhibitory activity on some pathogenic organisms such as *Klebsiella sp*, *Pseudomonas sp* and *Salmonella sp*. The antimicrobial substance produced from *Lactobacillus sp* had no effect on *Escherichiacoli* and this does not agree with the work of Reid *et al* (2006) who in their work found it active.

Consequently, interest in probiotics as alternative options to manage infections with *Klebsiella sp* has emerged. Few studies have investigated the effect of probiotics on the in-vitro growth of *Klebsiella sp* and all with promising results. This present study investigated the use of *Lactobacillus* as a probiotic against *Klebsiella sp* and the result showed that the Lactobacillus had inhibitory effect on the organism and this agrees with the work of Boris and Barbes (2010).

**Conclusion**

Based on the results of the present study, *Lactobacillus sp* successfully inhibited the growth of *Staphylococcus aureus*, *Salmonella sp* and *Klebsiella sp* but *Escherichia coli* was not. Therefore, the intake of food containing *Lactobacillus* is advised in the treatment of gastroenteritis in children since majority of the pathogenic bacteria responsible for the disease were inhibited by *Lactobacillu*s in this study.

**Recommendation**

In view of the result, probiotics should be taken in the treatment of gastroenteritis in children. Also, multi strain probiotics is recommended if strong in-vitro growth inhibition of a broad range of pathogens is desired.

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