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# Liver Enzymes Assay and Haematological Parameters of Bacteria Infected Guinea Pigs Fed with Probiotics

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**ABSTRACT**- Some *Lactobacillus* species (*L. acidophilus, L. casei* and *L. plantarum*) were isolated from locally fermented products (ogi, fura de Nunu and wara) and their effect on microbial infections caused by some pathogenic bacteria (*E.coli, K. pneumoniae, Pseudomonas aeruginosa* and *Staphyloccoccus aureus*) isolated from urine and high vaginal swab samples were studied using standard micriobiological methods.Fifiteen (15) healthy guinea pigs used for the study were divided into three (3) groups of five (5) guinea pigs each and placed in three (3) different cages. The pigs were initially fed for two (2) weeks (acclimatization period) with conventional feeds before administering the treatment. *Lactobacillus* species were introduced into the guinea pigs in cage 2 after the acclimatization period. Subsequently, the guinea pigs in cages 1 and 2 were orally infected with all the clinical bacteria pathogens while the guinea pigs in cage 3 which served as control were left with no microbial treatment. Ten (10) days after treatment, the packed cell volume (PCV), haemoglobin concentration (HBC), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity level were determined. Striking differences were observed from guinea pigs in the different cages. The effectiveness of Lactobacilli (probiotics) was evident when the guinea pigs in cages 1 and 2 were compared. The guinea pigs in cage 1 which were infected with pathogens but no probiotics had lower blood level (mean PCV= 24.8%) and inferior liver condition (mean ALT=58.18µl; mean AST=51.91µl). Higher blood level (Mean PCV=45%) and superior liver conditions (Mean ALT=9.51µl; mean AST=9.7µl) were obtained for guinea pigs in cage 2 which were infected with the same pathogens and fed with probiotics. The control (cage 3) had the highest PCV level and best liver conditions (mean PCV=46.6%, means ALT= 7.65µl; mean AST=11.83µl).Th .This might be attributed to the fact that they were not infected with pathogenic organisms. *Lactobacillus* species administered are promising probiotics against the tested bacterial pathogens.

**Keywords:** *Lactobacillus* species, Guinea pig, Bacteria pathogen, Enzymes assay, Haematological Parameters, Probiotics

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## INTRODUCTION

Urinary tract infection (UTI) is caused by the presence of pathogenic microbes and is a major health problem worldwide. This infection is usually caused by enteric bacteria foremost; *Escherichia coli* which occasionally colonize the vagina introitus.

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Women with recurrent UTI run a 4-5 fold increase risk of vaginal colonization of *E. coli*; other incriminating pathogens include; *Staphylococcus aureus* and other members of the family Enterobacteriae [1]. About four times as many women get UTI as men. Women have a shorter urethra, which makes it easier for bacteria to reach the bladder. Also, the opening of a woman's urethra is near the vagina and anus where the bacteria live. Normally, bacteria that enter the urinary tract are quickly removed by the body before they cause symptoms. But sometimes bacteria overcome the body's natural defenses and cause infections [2]. The most predominant normal flora of the vagina at puberty and before menopause is *Lactobacillus*.

Frequent and recurrent infections of the urinary tract are a common problem in women of all ages. The use of antibiotics is the principal mode of treatment for UTI

 

infections, however it is not ideal to be continually taking antibiotic drugs to treat and/ or prevent urinary tract infections: these drugs can have side effects on the liver cells thus damaging the cells of the liver leading to elevated liver enzymes; alanine transferase (ALT) and Aspartate transferase (AST) and overtime weaken one's immune system by disturbing the balance of bacteria in the intestine [3].

Elevated liver enzymes such as alanine transferase (ALT) and Aspartate transferase (AST) indicate inflammation or damage to cells in the liver which causes the enzymes to leak in the blood stream. Normal levels of ALT ranges from 7-56 units per litres of peripheral blood while that of AST ranges from 5-40 units per litres of peripheral blood [4].

The liver plays important roles in the defense against systemic infections. Two mechanisms are involved in this action. First, the kupffer cells in the liver plays a key role in the hepatic detoxification of bacteria endotoxin or their lipopolysaccharides. Secondly, the liver removes bacteria from circulations which occur mainly through the reticuloendothelia system of the liver. However, because of the liver's defense mechanism, it is important to maintain liver functions during infection such as UTI [4].

This research work was carried out to determine the probiotic effects of lactobacilli isolated from local fermented foods like, Fura de nunu, ogi and wara on three groups of Guinea pigs infected with clinical isolate of *E.coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus* from urine and high vaginal swab samples collected from pregnant women visiting ISTH in Irrua.

## MATERIALS AND METHODS

### Collection of Samples

This study was carried out between the months of June-August, 2014. Clinical samples (n=50) comprising early morning mid-stream urine (MSU) and High vaginal swabs (HVS) were collected from pregnant women attending Irrua Specialist Teaching Hospital, Edo State, Nigeria. All samples were collected under aseptic condition using standard procedures and immediately taken to the laboratory for analysis.

### Isolation and identification of bacterial pathogens

The Media used for the isolation of bacterial pathogens were MacConkey agar and Blood Agar. Pour plate technique was used for the isolation of pathogens from urine samples while for the swab samples, streak plate technique was used and the plates were incubated at $37^0$C for 24-48 hours.

### Isolation of *Lactobacilli*

Lactobacilli used as probiotic were isolated from three different local foods (Ogi, Fura de Nunu and Wara). Each of these food samples was aseptically collected in sterile containers, serially diluted and cultured onto De Mann-Rogosa-Sharpe (MRS) agar andincubated at 37ºC for 48 hours under anaerobic conditions. The isolates of *Lactobacillus* from each of the local foods were identified based on their cultural, morphological and biochemical characteristics as described in the 9th Edition of Bergey's Manual of Determinative Bacteriology.

### Preparation of experimental animal

Fifteen female guinea pigs of six weeks old were purchased from Ambrose Alli University College of Medicine, Ekpoma, Edo State. The guinea pigs were divided into three groups of five (5) each, housed in three different wooden cages (cages 1, 2 and 3). The weight of each guinea pig was recorded and they were fed with conventional diet and water for two weeks acclimatization period before the administration of treatments.

### Pre-liminary assessment for the presence of lactobacilli in the guinea pigs

A preliminary assessment for the presence of *Lactobacilli* in each of the guinea pigs was carried out from their stool samples. One (1) gram of stool sample from each guinea pig was homogenized in 9 ml of normal saline and serially diluted ($10^{-1}$-$10^{-9}$). Each of the serially diluted samples was plated onto MRS agar using the pour plate technique. Plates were incubated at 37º C for 24 hours for possible enumeration and characterization of lactobacilli.

### Administration of microbial treatments

Oral dose of 1ml (44 x $10^5$cfu/ml) of *Lactobacillus* species (*Lactobacillus acidophilus, Lactobacillus casei* and *Lactobacillus plantarum*) isolated from the fermented food samples were first administered to all guinea pigs in cage 2 only after the acclimatization two week period. Subsequently, 1ml sample of 24 hours old cultures of each of these pathogens (*E. coli, Klebsiella pneumoniae, Staphylococcus aureus,* and *Pseudomonas aeruginosa*) isolated were orally administered aseptically to all guinea pigs in cages 1 and 2 only with the aid of sterile pasture pipettes after the post-acclimatization period. There were no microbial treatments given to all guinea pigs in cage 3 which served as the experimental control.

### Assessment of experimental animal

All guinea pigs in cages 1, 2 and 3 were examined for Packed Cell Volume (PCV), Haemoglobin Concentration (HbC), Alanine Amino transferase (ALT) and Aspartate amino transferase (AST) levels within ten days observation period of post-microbial treatment. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) activities were determined using commercially available kit by Randox Manual AL100.

## RESULTS

The results obtained from assessment of packed cell volume, hemoglobin concentration, alanine

 

aminotransferase and aspartate aminotransferase of the guinea pigs are shown in Tables 1 and 2.

Table 1 showed the packed cell volume (%) and hemoglobin concentration (g/dl) of all the guinea pigs after infection; a range of 24-49% and 13.3-16.3g/dl respectively were recorded. The mean PCV (%) and HbC (g/dl) recorded were in the order, cage 3 > 2 > 1.

The alanine amino transferase (ALT) and aspartate amino transferase (AST) levels revealed a range of 3 - 88.2 µl and 5.5 - 94.8 µl respectively. Highest values (88.2 and 94.8µl) for ALT and AST respectively were recorded for pigs in cage 1 as compared with pigs in cage 2 (3and 5.5µl) for ALT and AST respectively. However, close range of values were noted with pigs in cages 2 and 3 (Table 2).

**Table 1:** Packed cell volume (PCV) and haemoglobin concentration (HbC) of guinea Pigs after 10 days of pathogens infection

| Cages | Guinea Pigs | PCV (%) | HbC (g/dl) |
|---|---|---|---|
| **1** | $A_1$ | 25 | 8.7 |
| | $B_1$ | 24 | 8.4 |
| | $C_1$ | 24 | 8.4 |
| | $D_1$ | 25 | 8.7 |
| | $E_1$ | 26 | 9.0 |
| **2** | $A_2$ | 45 | 15 |
| | $B_2$ | 43 | 14.3 |
| | $C_2$ | 47 | 15.7 |
| | $D_2$ | 45 | 13.3 |
| | $E_2$ | 45 | 15 |
| **3** | $A_3$ | 45 | 15 |
| | $B_3$ | 47 | 15.7 |
| | $C_3$ | 45 | 15 |
| | $D_3$ | 47 | 15.7 |
| | $E_3$ | 49 | 16.3 |

**Table 2:** Activity level of alanine aminotransferase and aspartate aminotransferase of Guinea pigs after 10 days of bacterial infection

| Cages | Guinea Pigs | ALT( µl) | AST(µl) |
|---|---|---|---|
| **1** | $A_1$ | 88.2 | 94.8 |
| | $B_1$ | 52.7 | 50.75 |
| | $C_1$ | 43 | 36 |
| | $D_1$ | 12 | 31.0 |
| | $E_1$ | 65 | 47 |
| **2** | $A_2$ | 12 | 5.5 |
| | $B_2$ | 3 | 10.6 |
| | $C_2$ | 11.4 | 13 |
| | $D_2$ | 17 | 8.7 |
| | $E_2$ | 4.16 | 10.66 |
| **3** | $A_3$ | 4.48 | 8.4 |
| | $B_3$ | 9.12 | 10.15 |
| | $C_3$ | 8.0 | 11.66 |
| | $D_3$ | 12.8 | 13.32 |
| | $E_3$ | 3.85 | 15.6 |

## DISCUSSION

The four bacterial pathogens (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus*) isolated from the high vaginal swab and urine samples in this study, had been known to be among the commonly isolated organisms in many cases of urinary tract infections [5-8]. The *Lactobacillus* species isolated from the locally fermented foods (wara, fura de nunu and ogi) were *Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactobacillus plantarum*. These organisms are frequently associated with these local food samples as they are known to be natural and spontaneous fermenters of the food samples.

Investigation of the guinea pigs for the presence of Lactobacilli at the onset of the study yielded no positive result. This finding agrees with the work of [9] who reported that lactobacilli are usually harboured only by a small percentage of mice. Although work done by [10] indicated that minimal amount of lactobacilli and neutral pH have been documented for various animals.

The infected guinea pigs in cage 1 had the least ranges of Packed Cell Volume (PCV) (24-26%) and Hemoglobin Concentration (HbC) (13.3-15g/dl). The infected guinea pigs in cage 2 that were fed with *Lactobacillus* spp. and the control guinea pigs in cage 3 had ranges of PCV and HbC of (43-47% and 13.3–15.7g/dl) and (45-49% and 15- 16.3g/dl) respectively. The least PCV and HbC recorded in cage 1 may be due to bacteraemia which may have resulted in the destruction of some of the red blood cells coupled with the absence of the lactobacilli, which would have acted as probiotics against the infectious bacterial agents. The normal range of PCV for healthy guinea pigs has been reported to be 37 - 48% [11].

The blood serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) level activity as determined showed a significant high level in infected guinea pigs in cage 1 compared to infected guinea pigs that were fed with *Lactobacillus* spp ( cage 2) and control guinea pigs in cage 3. The ALT values ranged from 12.0-88.2µl; 3.0-17µl and 3.85-12.8µl for guinea pigs in cages 1, 2, and 3 respectively. Work done by [12] reported that the normal ALT value of guinea pigs ranged between 10-25µl. Liver damage leads to an increase in the level of alanine aminotransferase (ALT) and Aspartate aminotransferase (AST). According to [13], a rise in plasma transaminase activities is a sensitive indicator of damage to cytoplasmic and/or mitochondrial membranes. These

 

enzymes are mostly contained in the liver, and when parenchymal cells of the liver are damaged, these enzymes leak into the blood. The liver enzymes ALT and AST are raised in these guinea pigs because of the fact that the toxins generated by the bacteria may have affected or damaged both the mitochondrial and cytoplasmic membranes leading to their proportional increase. According to [14], liver enzymes level are usually raised in acute hepatotoxicity but tend to decrease with prolong intoxication and infection due to liver damage. This is because prolong intoxication by the toxins from the bacteria may have contributed to liver damage which has caused massive destruction of hepatocytes leaving behind a few cells, hence a decrease in the activities of two enzymes ALT and AST. This could be the possible reason why the ALT values of guinea pigs in cage 1 was the highest as compared to those in cages 2 and 3. The lower value obtained in cages 2 could be attributed to the probiotic effects of lactobacilli administered. Furthermore, the AST values ranged from 31-94.8µl, 5.5-13µl and 8.4-15.6µl for guinea pigs in cages 1, 2 and 3 respectively. These values were found to be higher in guinea pigs in cage 1 as against those in cages 2 and 3 which fell within acceptable normal limits [4]. The *Lactobacillus* spp. administered acted as probiotics for the pigs in cage 2 thus lowering the level of liver damage in them as against the guinea pigs in cage 1. It can be summarized that liver enzyme assay can be used to determine the pathological status of infected animals.

## CONCLUSIONS

In this study bacterial pathogens (*E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus*) commonly associated with urinary tract infections were isolated from HVS and urine while natural and spontaneous *Lactobacillus* species (*Lactobacillus acidophilus, Lactobacillus casei* and *Lactobacillus plantarum*) were isolated from locally fermented foods. Bacterial infection of guinea pigs can cause reduction in PCV and HbC and rise in the level of liver enzymes-Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST). Liver enzyme assay therefore can be used to determine the pathological status of infected animals. Guinea pigs used in this study did not originally harbor *Lactobacillus* species but the administration of probiotics (*Lactobacillus* species) to them helped to mitigates the adverse effects of pathogens. Hence the use probiotics in animal feeds and human diet is strongly advocated.

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