PHYSIOLOGICAL CHANGES IN PROBIOTICS (*Lactobacillus* spp) FED INFECTED GUINEA PIGS

Okwu, G. I.[1], Obiazi, H.A.K[2] , Amaobi, C. B. [3], Amengialue, O. O.[4] and *Akpe, A.R.[5]

[1, 3,5]Department of Microbiology, Faculty of Natural Sciences, Ambrose Alli University, P.M.B. 14, Ekpoma, Edo State, Nigeria
[2]Department of Medical Laboratory Sciences, Ambrose Alli University, P.M.B. 14, Ekpoma, Edo State, Nigeria
[4]Department of Biological Sciences, College of Natural and Applied Sciences, Wellspring University, Benin City, Edo State, Nigeria
*** Correspondence** author: Email: lordromis@yahoo.co.uk

ABSTRACT

In this study *Lactobacillus* species (*L. acidophilus, L. casei* and *L. plantarum*) isolated from Nigerian locally fermented food products (ogi, fura de Nunu and wara) were fed to guinea pigs infected with clinical isolates of *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* to determine their on certain physiological parameters. Thebacteria were isolated and identified using standard microbiological methods.Fifteen healthy guinea pigs divided into three groups of five guinea pigs each and placed in three different cages were used for the study. The pigs were initially fed for two weeks (acclimatization period) with conventional feeds before administering the treatment. *Lactobacillus* species (probiotics) 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. Results obtained indicated striking differences from guinea pigs in the different cages. The effectiveness of *Lactobacillus* spp (probiotics) was evident when the guinea pigs in cages 1 and 2 were compared. The guinea pigs in cage 1 had higher % mean weight loss (24.9%), higher

temperature rise (5.46%) and blood stained urine while pigs in cage 2 had lower % mean weight loss (3.4%), lower temperature rise (2.77%) and whitish/milky urine. Values for cage 3 were: mean weight loss (3.9%), mean temperature rise (2.67%),and whitish/milky urine. Generally, the control indicated the highest body weight and lowest body temperature. 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.

Key words: Probiotics, pathogens, physiological parameters, Guinea pigs

{**Citation:** Okwu, G. I., Obiazi, H.A.K, Amaobi, C. B., Amengialue, O. O., Akpe, A.R. Physiological changes in probiotics (*Lactobacillus spp*) fed infected guinea pigs. American Journal of Research Communication, 2016, 4(4): 85-96} www.usa-journals.com, ISSN: 2325-4076.

INTRODUCTION

Lactobacillus is a Gram positive, facultative anaerobic or microaerophilic, rod-shaped bacterium with most of its species converting lactose and other sugar to lactic acid. In human they are present in the vagina and in the gastrointestinal tract where they make up a small portion of the gut micro flora. Generally lactobacilli are more usually resistant to acidic condition than other lactic acid bacteria, being able to grow at pH values as low as 4.0. This enables them to continue to grow during natural lactic acid fermentation when the pH has dropped too low for other lactic acid bacteria to survive. Thus they are responsible for the final stages of many lactic acid fermentations (James, 2005). They are known to maintain the ecological equilibrium of the

intestinal tract by protecting against pathogenic microorganisms. The physiology of the urogenital tract may change during natural history of humans and animals, with an accompanying modification of the normal microbial flora (Vintini*etal*., 2004). Recent studies have shown a correlation between loss and disruption of the normal genital micro flora in particular *Lactobacillus* species and an increased incidence of genital infections.The normal vaginal flora of healthy females can competitively block the in vitro attachment of pathogenic bacteria (Chaim*et. al*., 1997). Pre-clinical and clinical reports have focused on *Lactobacillus* strains for the prevention of human urogenital infections (Asahara*et. al*., 2001). They have not been found to be associated with disease and for over 100 years have been regarded as non-pathogenic member of the intestinal and urogenital micro flora (Hiller, 1993). The therapeutic properties and the production of antibiotic-like products by lactic acid bacteria have increased rapidly and this has revitalized the theory of prolongation of life when fermented foods are consumed.Certain species of Lactic acid producing bacteria have been promoted as probiotics.An important benefit of probiotics is capacitytocurtail or prevent infectious diseases (Wagner *et. al.,* 1997b; Colodner*et. al.,* 2003; Reid *et. al.,* 2003, 2005).

This studyis to investigate the effectiveness of *Lactobacillus* species (probiotics) on guinea pigs infected with clinical bacterial isolates.

MATERIALS AND METHODS

Collection of samples

A total of 50 clinical samples comprising of early morning mid-stream urine (MSU) and High vaginal swabs (HVS) were collected from pregnant women at Irrua Specialist Teaching

Hospital, Edo State, Nigeria. The mid-stream urine was collected with sterile universal containers while the High vaginal Swabs were collected with sterile swab sticks. All samples were collected under aseptic condition using standard procedures and immediately taken to the laboratory for analysis.

Isolationand identification of bacterial pathogens and lactobacilli

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 37^0C for 24-48 hours.

Lactobacilli used as probiotic were isolated from three different Nigerian local fermented 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 and incubated at 37^oC for48 hours under anaerobic conditions. All the bacterial isolates were identified based on their cultural, morphological and biochemicalcharacteristics as described in the 9^{th} 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. Theguinea pigs were divided into three groups of five guinea pigs each, housed in three different wooden cages(cages 1, 2 and 3). The weight of each guinea pig wasrecorded and they were fed with conventional diet and water for two weeks acclimatization period before the administration of treatments.

Preliminary 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 gram of stool sample from each guinea pig was homogenized in 9mls of normal saline and serially diluted. Each of the serially diluted samples was plated onto MRS agar using the pour plate technique. Plates were incubated at 37^{o} C for 24 hours for possible enumeration and characterization of lactobacilli.

Treatments with bacterial pathogens and lactobacilli

Oral dose of 1ml 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 weeks period. Subsequently,1ml(44 x 10^{5}cfu/ml) sample of24 hours cultures of each of these pathogens (*Escherichia coli, Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) isolated were orally administered to all guinea pigs in cages 1 and 2 onlywith the aid of sterile pasture pipettes after the acclimatization period. There was no microbial treatment 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 urine colour, weight change and temperature.

RESULTS

The results obtained from assessment of weights, temperature and appearance of urine of the guinea pigs are shown in Tables 1-5.

Table 1 showed the weights (g) of the individual guinea pigs at day 1 and at the end of the two weeks acclimatization period (day 14). Increase in body weight was observed in all the pigs. The

mean body weights of the guinea pigs in cages 1, 2 and 3 were 458, 482 and 479g respectively. The weight of the guinea pigs after infection with pathogens is shown in Table 2. A drastic loss in weight was recorded for guinea pigs in cages 1, 2, and 3 with a percentage mean loss of 28.95%, 7.21% and 3.92% respectively.

The mean of mean temperature (^oC) of the guinea pigs during the acclimatization period for cages 1, 2 and 3 were 37.34^oC, 37.6^oC and 37.52^oC respectively (Table 3).In Table 4 is shown the mean temperature (^oC) of guinea pigs after being infected with pathogens. The percentage rises in temperature for the guinea pigs in cages 1, 2 and 3 as calculated from their mean of mean temperatures were 5.46%, 2.77% and 2.67% respectively.

The physical appearance of urine sampleof guinea pigs after being infected with pathogens is as shown in Table 5. The urine colour of pigs in cages 2 and 3 was milkish white while that in cage 1 was slightly reddish with blood stain.

Table 1: Weights(g) of guinea pigs within two weeks of acclimatization

	Guinea Pig		
Days	**Cage 1**	**Cage 2**	**Cage 3**
0	(A_1)280	(B_1)400	(C_1)500
	(A_2)400	(B_2)390	(C_2)480
	(A_3)390	(B_3)400	(C_3)400
	(A_4)390	(B_4)400	(C_4)400
	(A_5)400	(B_5)400	(C_5)300
14	(A_1)400	(B_1)500	(C_1)510
	(A_2)500	(B_2)500	(C_2)500
	(A_3)400	(B_3)500	(C_3)480
	(A_4)490	(B_4)510	(C_4)500
	(A_5)500	(B_5)500	(C_5)405

A_1– A_5 = Guinea pig in cage 1

B_1 - B_5 = Guinea pig in cage 2
C_1 – C_5= Guinea pig in cage 3

Table 2:Weights of guinea pigs after 10 days of pathogens infection

Cages	Guinea Pigs	Mean Weight (g)
1	A_1	265
	A_2	370
	A_3	251
	A_4	360
	A_5	374
2	B_1	484
	B_2	388
	B_3	480
	B_4	491
	B_5	486
3	C_1	492
	C_2	489
	C_3	458
	C_4	482
	C_5	380

Table 3: Mean temperature (^{0}C) of guinea pigs within two weeks of acclimatization

	Guinea Pig		
	Cage 1	**Cage 2**	**Cage 3**
	(A_1)37.2	(B_1)37.4	(C_1)37.9
	(A_2)37.4	(B_2)37.6	(C_2)37.4
	(A_3)37.3	(B_3)37.7	(C_3)37.7
	(A_4)37.6	(B_4)37.5	(C_4)37.2
	(A_5)37.2	(B_5)37.8	(C_5)37.4
Mean of means	**37.34**	**37.60**	**37.52**

Key:
$A_1 – A_5$ = Guinea pig in cage 1
$B_1 – B_5$ = Guinea pig in cage 2
$C_1 – C_5$ = Guinea pig in cage 3

Table 4: Temperature (^{0}C) of guinea pigs after 10 days of pathogen infection

	Guinea pigs		
	Cage 1	**Cage 2**	**Cage 3**
	(A_1)39.4	(B_1)38.8	(C_1)38.6
	(A_2)39.2	(B_2)38.6	(C_2)38.4
	(A_3)39.3	(B_3)38.5	(C_3)38.6
	($A_4$39.2	(B_4)38.6	(C_4)38.5
	(A_5)38.8	(B_5)38.7	(C_5)38.5
Mean of means	**39.38**	**38.64**	**38.52**

Table 5: Physical appearance urine of guinea pig after 10 days of pathogens Infection

Cages	Guinea Pigs	Appearance
1	A_1	Reddish
	A_2	Reddish
	A_3	Light Red
	A_4	Light Red
	A_5	Blood Stained
2	B_1	Milky
	B_2	Milky
	B_3	Milky
	B_4	Whitish
	B_5	Whitish
3	C_1	Whitish
	C_2	Milky

C_3	Milky
C_4	Milky
C_5	Milky

DISCUSSION

In this study, four bacterial pathogens were isolated from the high vaginal swab and urine samples; namely *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus.* These organisms had been known to be among the commonly isolated organisms in many cases of urinary tract infections (Khamenoh, 2005;Kucheria*et. al.,* 2005;Roos*et. al.,* 2007 and Bi *et. al.,* 2009).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 have been known to be frequently associated with these local food samples as they are known to be natural and spontaneous fermenters of the food samples.

The preliminary assessment of the guinea pigs for the presence of lactobacilli yielded no positive result. This finding agrees with the work of Meysick and Gerber, (1992) who reported that lactobacilli are usually harbored only by a small percentage of mice. Although work done by McGrory and Gerber, (1991) indicated that minimal amount of lactobacilli have been documented for various animals.

There was a significant increase in weight across all guinea pigs during the acclimatization period. The overall increase in weight is obviously as a result of feeding theguinea pigs to a point of satiation during the period. The result obtained is in agreement with that of James, (2004) which indicated normal weights of healthy guinea pigs as 400 – 900g.The observed weights loss in the guinea pigs (cage 1) after infection as presented in Table 2, indicated sign of ill health;

they may have succumbed to the bacteria infection while guinea pigs in cage 2 may have gained a protective action from the lactobacilli administered to them.

Slight differences were recorded in the mean temperature (^{O}C) amongst the guinea pigs during the two weeks acclimatization period (Table 2).The results showed thatthe mean temperature was within the standard temperature range of healthy guinea pigs which has been reported to be between 37 – 39.5^{0}C (Katherine *et. al.,* 2004).The mean of mean temperature of guinea pigs within the ten days acclimatization assessment period as shown in Table 4 indicated that guinea pigs in cage 1 had the highest mean of mean temperature of 39.38^{0}C as compared to those of cages 2 and 3 with mean of mean temperatures of 38.64 and 38.52^{0}C respectively. The highest temperature recorded for guinea pigs in cage 1 is index of infection-induced fever due to antigen/antibody interactions.There might have been slight infection in the control guinea pigs due to proximity of the cages, hence the rise in temperature.

The urine sample of the guinea pigs in cages 2 and 3 showed milky to colourless appearance indicating healthy state of the pigs. The *Lactobacillus* spp administered to guinea pigs in cage 2 could have conferred some level of protections against the bacteria pathogens.Guinea pigs in cage 1 which were infected with pathogenic bacteriahad reddish and blood stained urine; indicatingnon-healthy and diseased conditions of the guinea pigs. The pathogens they were infected with may have destroyed some of the red blood cells. Also absence of *Lactobacillus* spp (probiotics) may have been a contributing factor too.

The overall result showed that guinea pigs in cage 2 which were orally fed with *Lactobacillus* spp appeared healthier than those in cage 1. Also, pigs in cages 2 and 3 exhibited comparable results in all the parameters assayed.

REFERENCES

Asahara, T., Nomoto, K., Watanuki, M., and Yokkoura, T. (2001). Antimicrobial activity of intraurethral administrated probiotics *Lactobacillus casei* in a murine model of *Escherichia coli* urinary tract infection. *Antimicrobial Agent chemotherapy*.45: 1751-1760.

Bi, X. C., Zhang, B. and Ye, Y. K. (2009).Pathogen incidence and antibiotic resistance patterns of catheter – associated urinary tract infection on children.*Journal of Chemotherapy*.(21): 661 – 665.

Chaim,W., Mazor, M. and Leieorman, J. R. (1997). The relationship between bacterial vaginosis and Preterm birth. *Gynaecology and Obstetrics*.259:51-58.

Colodner, R., Edelstein, H., Chazan, B. and Raz, R. (2003).Vaginal Colonization by orally administrated *Lactobacillus Rhamnosus*GG.*Isr Medical Association Journal*.5:767-769.

Hiller, S. l. (1993).Diagnostic microbiology of Bacterial Vaginosis. *American Journal of Obstetrics and Gynaecology*169:455 – 459.

James, M. J. (2005). Modern Food Microbiology.Fourth Edition.CBS Publishers and Distributors. New Delhi.P. 701.

Katherine, E., Quesenberery, D. and James, C. (2004).Ferrets, Rabbits and Rodents: In Clinical, Medical Surgery of Animals.Elsevier Science Britain.P. 908

Khameneh, Z. R. and Afshar, A. T. (2005).Antimicrobial susceptibility pattern of urinary tract pathogens.*Saudi Journal of Kidney Diseases and Transplant*.(20): 251 – 253.

Kucheria, R., Dasgupta, P. Sacks, S., Khan, M. and Sheerin, N. S. (2005). Urinary tract infections: New insight into a common problem. *Journal of Postgraduate Medicine*.(81): 83 – 86.

McGrory, T. and Garber, G. E. (1991). Mouse intravaginal infection with *Trichomonas vaginalis* and role of *Lactobacillus acidophilus* in sustaining infections. *Infection and Immunology*.(60):2375 – 2379.

Meysick, K. C. and Garber, G. E. (1992).Interactions between *T. vaginalis* and vaginal flora in a mouse model.*Journal of Parasitology*.(78): 157 – 160.

Reid, G., Jass, J., Sebulsky, M. T. and McCormick, J. K. (2003). Potential uses of Probiotics in Clinical practice. *Journal of Clinical Microbiology Review* 16(4):658-72.

Reid, G., Bingham, M., Wadstorm, T., Fueler, R., Anukam, K., and Katsivo, M. (2005).Probiotics for the developing world.*Journal of clinical Gastroenterology* 39*:485*-488.

Roos, D., Raksha, R., Srinivasaa, H. and Macaden, R. S. (2007).Occurrence and characterization of uropathogenic *Escherichia coli* in urinary tract infections.*Indian Journal of Medical Microbiology*.21: 102 – 107.

Vintini, E., Ocana, V., and Nader-Macis, M.(2004). Effect of Lactobacilli administration in the vaginal treat of mice: evaluation of side of side effects and local immune response to local administration of selected strains. *Methods of molecular biology.* 268: 401-410.

Wagner, R. D., Warner, T., Roberts, L., Farmer, J., and Balish, E. (1997b).Colonization of Congenitally immunodeficient mice with probiotic bacteria.*Infection and Immunology.* 65:3345-3351.