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Original Research Article

Product Activity of Lactic acid Bacteria Isolated from Samples of New Born from Women and Children Hospital in Ramadi, Iraq

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Abstract

Twelve infants aged below six months were sampled from Women and Children Hospital of Ramadi, Iraq during the period April the 1st 2021 till October the 1st 2022. Infants were divided into two main groups; breast and bottle milk fed. Each group comprise three males and three females. Samples of feces were collected and lactobacillus specie were extracted. The extract was used once alone and another as a mixture with each of Amoxicillin, Amikacin and Cefixime antibiotics on petri dishes cultured with Staphylococcus, Klabsella and Psedudomonas bacteria. Diameter of inhibition was measure and resulted data were subjected to statistical analysis. The results of the statistical analysis showed that lactobacillus specie extract with the Cefixime has significantly highest mean diameter of inhibition compared to all other types of treatments. Infants who were on breast milk fed showed significantly better respond to the treatment than their counter part in the bottle milk fed group. Klabsella was found to respond weakly under almost all of the applied treatments.

Keywords: LAB, lactobacillus specie, lactic acid bacteria, extract, antibiotics, breast fed, bottle fed, product activity, infants.

INTRODUCTION

The usage of Lactic Acid Bacteria (LAB) in the food sector is widespread. LAB have several uses in biotechnology, medicine delivery, and food ingredient manufacturing beyond their traditional role as dairy starters. The bacteria are Gram-positive rods or cocci that are facultative anaerobes due to their lack of catalase and oxidase. Milk, fruits, flowers, vegetables, dirt, feces, sewage water, etc. are all said to contain them (Lamont *et al*., 2017). Infants' guts are populated by lactic acid bacteria, some of which are supplied by human breast milk, the gold standard in infant nutrition (Kim *et al*., 2020). Breast milk's nutritious composition and diverse microbiota, which is unique to each mother, play a crucial impact in baby development (AS *et al.*, 2020). This rich microflora forms a foundation for the early evolution of gut microbes in infants. The origin of many species found in human milk is a matter of some debate. It is the endogenous transfer from the mother's gastrointestinal tract to the mammary gland, as described by (Martín *et al*., 2004) Infant feeding methods, drug exposure, and delivery setting are only few of the other variables that might affect the composition of the microflora in the infant gut (AS *et al*., 2020). Babies' immunity and metabolism are both influenced by the microbiome of their gut (Bharadia *et al*., 2020). This study sought to extract, identify, and characterize lactic acid bacteria from the feces of exclusively breast-fed and bottle-fed infants.

Lactic acid is primarily created by a class of gram-positive bacteria called Lactic Acid Bacteria (LAB), and it is a byproduct of the fermentation of carbohydrates. They have a coccioid or rod-shaped structure and do not produce spores or catalase (Fachrial and Harmileni, 2018). Diacetyl, organic acids, hydrogen peroxide, and bactericidal proteins are just few of the byproducts of lactate fermentation by LAB (Fachrial and Harmileni, 2017). Bacteria belonging to the genera Carnobacterium, Aerococcus, Enterococcus, Lactococcus, Lactobacillus, Leuconostoc, Weissella, Oenococcus, Pediococcus, Tetragenococcus, Streptococcus, and Vagococcus are all considered part of the LAB (Lase *et al*., 2021). Moreover, human use of Lactobacillus has a positive impact on health. This type of bacterium typically grows (adhesion) and makes colonies in the human intestine and is now frequently isolated; such strains are called probiotics (S. *et al*., 2013). Pathogenic bacteria can colonize the gut wall, but probiotics help fight them off and stop that from happening (Syukur *et al*., 2015). Bacteria must meet specific criteria in order to be considered a probiotic, including being risk-free, acid- and bile-resistant, colony-forming, antimicrobial, and viable (Sun *et al*., 2014). Numerous scientific studies have demonstrated the health benefits of probiotics, including their ability to inhibit the development of pathogenic bacteria, decrease the incidence of colon cancer, boost the immune response, and lower blood cholesterol. They can also cure a wide range of illnesses, including diarrhoea, asthma, and high blood pressure (Lase *et al*., 2021).

Most metabolic, nutritional, protective, and immunological activities in newborns are mediated by the gut flora. Common anaerobic bacteria associated with newborn gut microbiota include Bifidobacterium, Lactobacillus, and Enterococci (Sun *et al*., 2014). Breast milk is highly nourishing and promotes the growth of beneficial gut flora in infants (Lase *et al*., 2021). In order to ensure a healthy start in life, it is advised that infants be breastfed exclusively for the first six months. Breast milk contains a wide variety of bioactive compounds, including oligosaccharides, carbohydrates, fatty acids, nucleotides, cytokines, immunoglobulins, immune cells, lactoferrin, lysozyme, and others that modulate the immune system (Lase *et al*., 2021). Breast milk is bolstered by all of these chemicals, making it better able to protect infants from a wide range of health problems, including inflammatory bowel disease (IBD), diabetes, and obesity, and gastrointestinal and respiratory infections (Fernandez *et al*., 2013). Breast milk contains beneficial intestinal bacteria such as Lactobacillus and Bifidobacterium that can be passed on to a nursing newborn. Although antibiotics are the antithesis of probiotics, antimicrobial resistance has led to increased mortality and morbidity in many nations recently (Gómez-Ríos and Ramírez-Malule, 2019).

Beneficial microbes, or probiotics, are those that, when given to a host in sufficient proportions, have a positive influence on the host's health (FAO/WHO, 2002). The majority of probiotics consist of bacteria very similar to those normally found in the digestive systems of humans and animals. Lactic acid bacteria are the most prevalent type of probiotic found in meals, notably in dairy products (Sirichotinun *et al*., 2018). Gram-positive LAB are either cocci or bacilli; they lack motility; they don't produce spores or catalase; and they live in either anaerobic (facultative) or aerobic (strict) conditions. Studies on various LAB species, including Lactobacillus, Bifidobacterium, and Enterococcus, have shown a cholesterol-lowering impact, both in vitro and in vivo (Guo *et al*., 2016). Cholesterol-lowering processes include the assimilation of cholesterol during LAB development and the indirect suppression of bile acid re-absorption mediated by the bacterial bile salt hydrolase (BSH) enzyme (Sirichotinun *et al*., 2018).

Key criteria for the selection of probiotic candidates include adherence to intestinal tissues and colonization in the human gastrointestinal tract, as well as tolerance in an acidic gastric environment and high concentrations of bile salts in the small intestine (Gabriel *et al*., 2017).

MATERIALS AND METHODS

After obtaining signed, written agreement from the parents of 12 newborns younger than 1 year old, the Women and Children Hospital in Ramadi, Anbar Province, Iraq, collected samples for analysis. Baby poop samples were sent to the biology lab at Alnbar University in Iraq to check for LAB colonization.

Sampling and LAB Isolation

Stool samples from infants who were otherwise healthy were analyzed to see if any LAB were present. Twelve infants' feces were collected for analysis; six were breastfed and six were bottle-fed. In addition, there are 6 females and 6 males in the sample. There were six girls and six boys among the infants in each set. The newborn infants who were found to be healthy were picked at random. DeMan, Rogosa, and Sharpe agar (MRS agar, HiMedia Laboratories Limited, Mumbai, India) was used to study the presence of LAB in the stool of healthy newborn newborns (AS *et al*., 2020). Following serial dilution of stool samples and incubation in anaerobic conditions for 24-48 hours at 37 degrees Celsius, LAB were found on the MRS medium. To obtain a pure strain, an isolated one was subcultured in MRS agar, which has a low detectable number of colonies, in order to identify colonies with identical morphological and bacterial biochemical features.

Extraction of cruds isolates

One milliliter of activated isolates were added to one hundred milliliters of MRS broth and left to ferment at room temperature for a full 24 hours. To remove cells and obtain the metabolites leaching, a sterile beaker was placed in the refrigerator and centrifuged at 10,000 rpm for 10 minutes, at which point the metabolites were slowly precipitated until saturation with ammonium sulfate (80%), after which the precipitate was stirred at high speed in the refrigerator until it settled at the bottom of the beaker (Vijay Simha *et al*., 2012).

Cryogenic centrifugation was used to collect the precipitate, which was then placed in sterile vials. This study's primary focus, the action of lactobacillus extract, was measured by measuring the diameter at which it inhibited bacterial growth in petri dishes containing Staphylococcus, Klebsiella, and Pseudomonas cultures. Among the drugs studied, Amoxicillin, Amikacin, and Cefixime all had their diameters of inhibition calculated. Furthermore, the isolate was tested twice, once when treated with just the extract and once when combined with each of the aforementioned antibiotics. There are a total of seven possible therapies here, including Amoxicillin, Amikacin, Cefixime, Extract, Extract + Amoxicillin, Extract + Amikacin, and Extract + Cefixime. Bacteria were cultivated and exposed to each antibiotic; the diameter of inhibition was then recorded. Three separate applications of each therapy were performed to eliminate any potential for bias.

RESULTS

Table 1 represents means diameter of inhibition for the combinations of antibiotics and/or extract with respect to the types of used bacteria.

Table 1: Means and standard deviations of diameter of inhibition according to the type of treatment at each type of bacteria

Treatment	Bacteria					
	Staphylococcus		Klebsella		Pseudomonas	
	diameter		diameter		Diameter	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
AX	1.57	0.41	0.59	0.54	1.16	0.39
AK	1.69	0.35	0.66	0.61	1.06	0.40
CFM	1.70	0.46	0.92	0.90	1.42	0.73
Extract	1.66	1.69	0.88	0.82	1.46	0.52
Extract+AX	1.67	1.84	0.66	0.71	1.60	0.54
Extract+AK	2.15	1.10	0.85	0.84	1.41	0.44
Extract+CFM	2.49	0.95	1.19	1.04	2.11	0.69

AX: Amoxicillin, AK: Amikacin, CFM: Cefixime

Extract + CFM is found to have the highest mean diameter of inhibition and when compared to other types of treatments it showed a remarkable difference as presented in Figure 1.

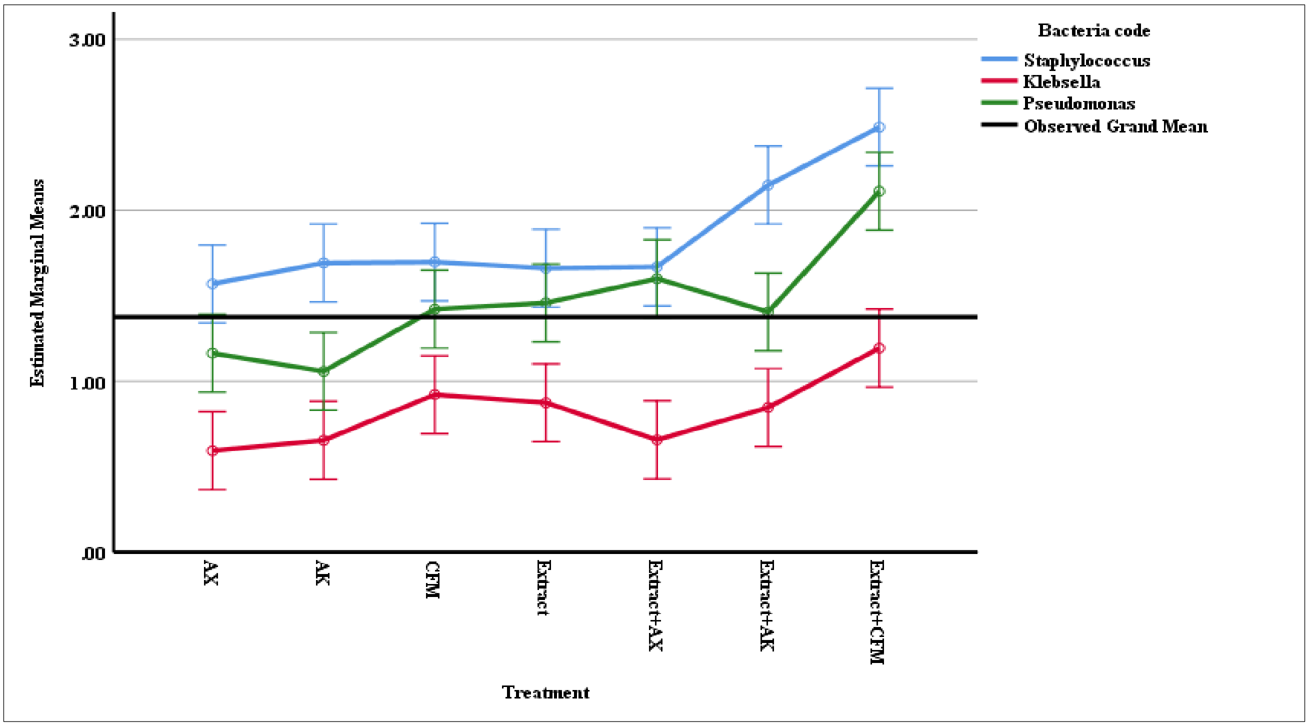

Figure 1: Means diameter of inhibition with respect to the type of treatment for each type of the used bacteria

Table 2 shows the results of multiple comparison tests of means diameter of inhibition as found at each treatment type. The minus sign in the table refers to negative means difference whereas the positive sign refers to the positive means difference. Treatments on the rows with minus/plus sign at each column indicated that means of treatment at a given row is significantly lower/higher than that at a certain column of comparison cell. Empty cells of the table indicating non-significant means differences. According to the information of the table, the mixture of the lactobacillus specie with the cefixime accounted for significantly higher mean diameter of inhibition compared to all other treatment types.

Table 2: Matrix of multiple comparison tests as obtained by the use of LSD method

	AX	AK	CFM	EX	EX+AX	EX+AK	EX+CFM
AX			-	-	-	-	-
AK			-	-		-	-
CFM	+	+					-
EX	+	+					-
EX+AX	+						-
EX+AK	+	+					-
EX+CFM	+	+	+	+	+	+	
AX: Amoxicillin, AK: Amikacin, CFM: Cefixime, EX: Extract							

Table 3 shows the means diameter of inhibition for each treatment with respect to the type of feeding and gender. It can be realized that samples from breast feeding shows relatively higher means compared to their counter parts from bottle feeding groups. With regard to gender groups, males are found to have higher means diameter inhibition than females.

Figures 2 and 3 showed the variability of the means diameter of inhibition with respect to the different types of treatments at each feeding and gender groups.

No doubt, figures 2.1 and 2.2 showed that means diameter of inhibition for the breast feeding group are remarkable higher than that of the bottle feeding group particularly for the extract and the mixture of extract with each of the three antibiotics previously stated.

Figures 3.1 and 3.2 showed the means diameter of inhibition with respect to gender groups at each type of treatments and bacteria. The figure revealed that both gender groups respond almost similarly for the three antibiotics applied alone on petri dishes, while means diameter of inhibition were increased for the extract and the mixture of extract with the antibiotics. And once again the extract with the cefixime showed the higher means diameter of inhibition.

Table 3: Means diameter of inhibition with respect to the types of treatment at each feeding and gender groups

Treatment	**Feeding**							
	Breast				**Bottle**			
	Gender				**Gender**			
	Male		**Female**		**Male**		**Female**	
	Mean	**Standard Deviation**	**Mean**	**Standard Deviation**	**Mean**	**Standard Deviation**	**Mean**	**Standard Deviation**
AX	1.31	0.19	1.42	0.56	0.82	0.61	0.88	0.70
AK	1.44	0.48	1.34	0.44	0.94	0.74	0.82	0.61
CFM	1.79	0.31	1.90	0.44	0.79	0.74	0.91	0.80
Extract	1.70	0.30	1.59	1.33	0.45	0.65	1.58	1.50
Extract+AX	1.61	0.37	1.51	1.56	0.51	0.74	1.61	1.58
Extract+AK	1.59	0.25	1.98	1.29	1.01	0.73	1.29	1.10
Extract+CFM	2.19	0.35	2.46	1.00	1.35	0.98	1.73	1.30

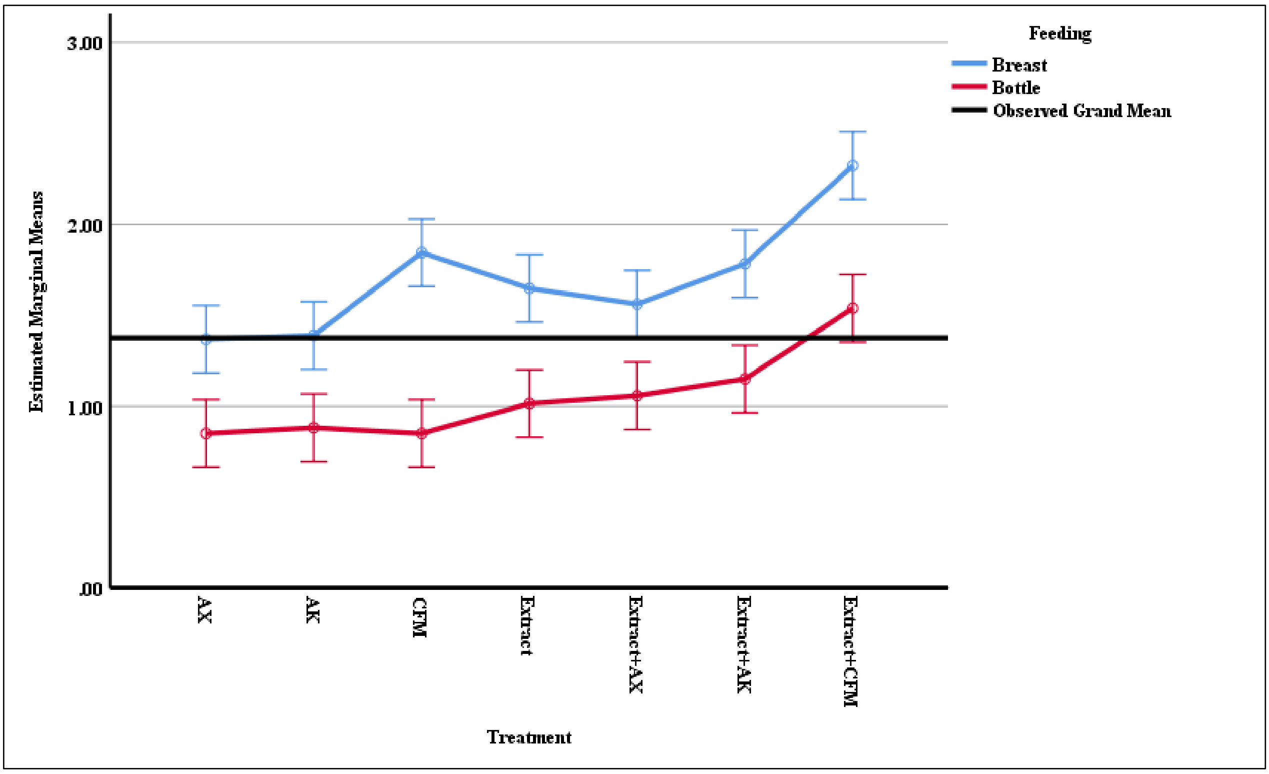

Figure 2.1: Means diameter of inhibition according to the types of treatment and feeding method

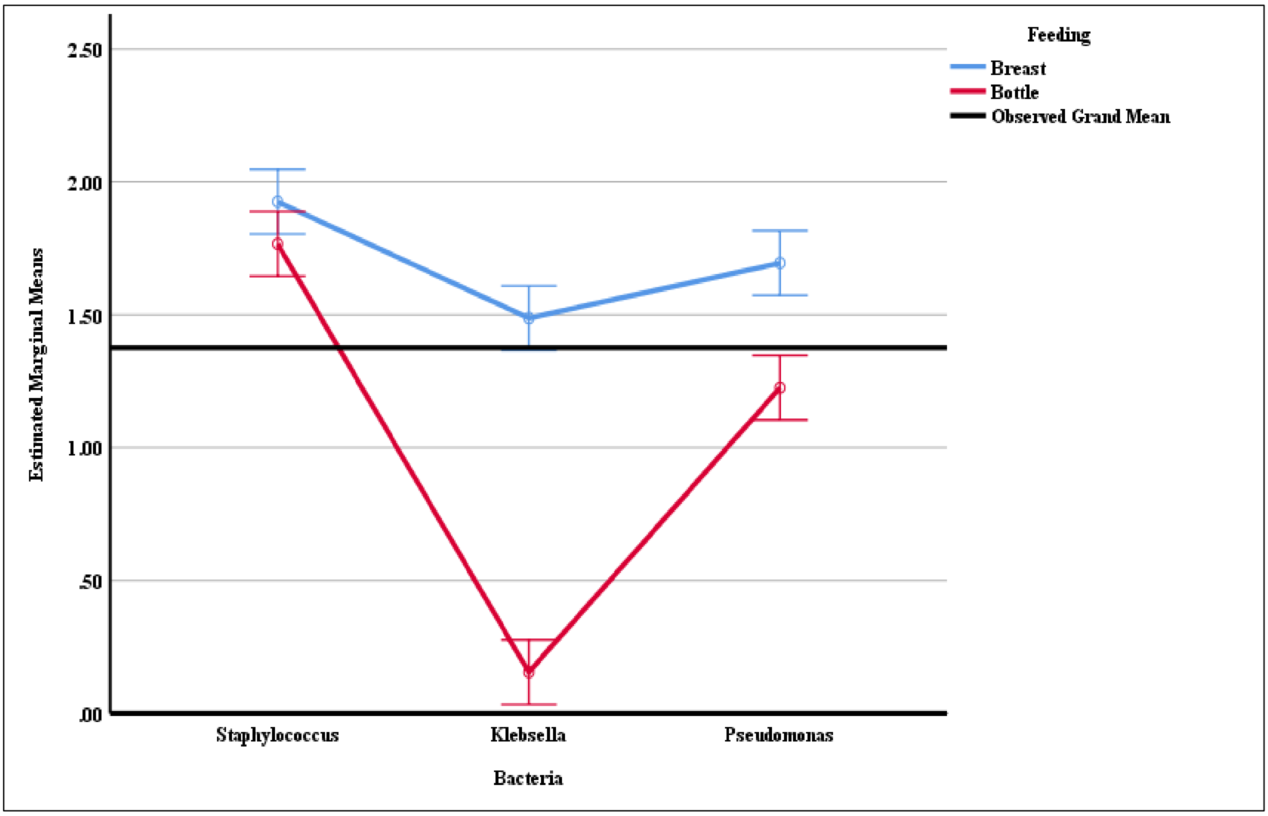

Figure 2.2: Means diameter of inhibition according to the types of bacteria and feeding method

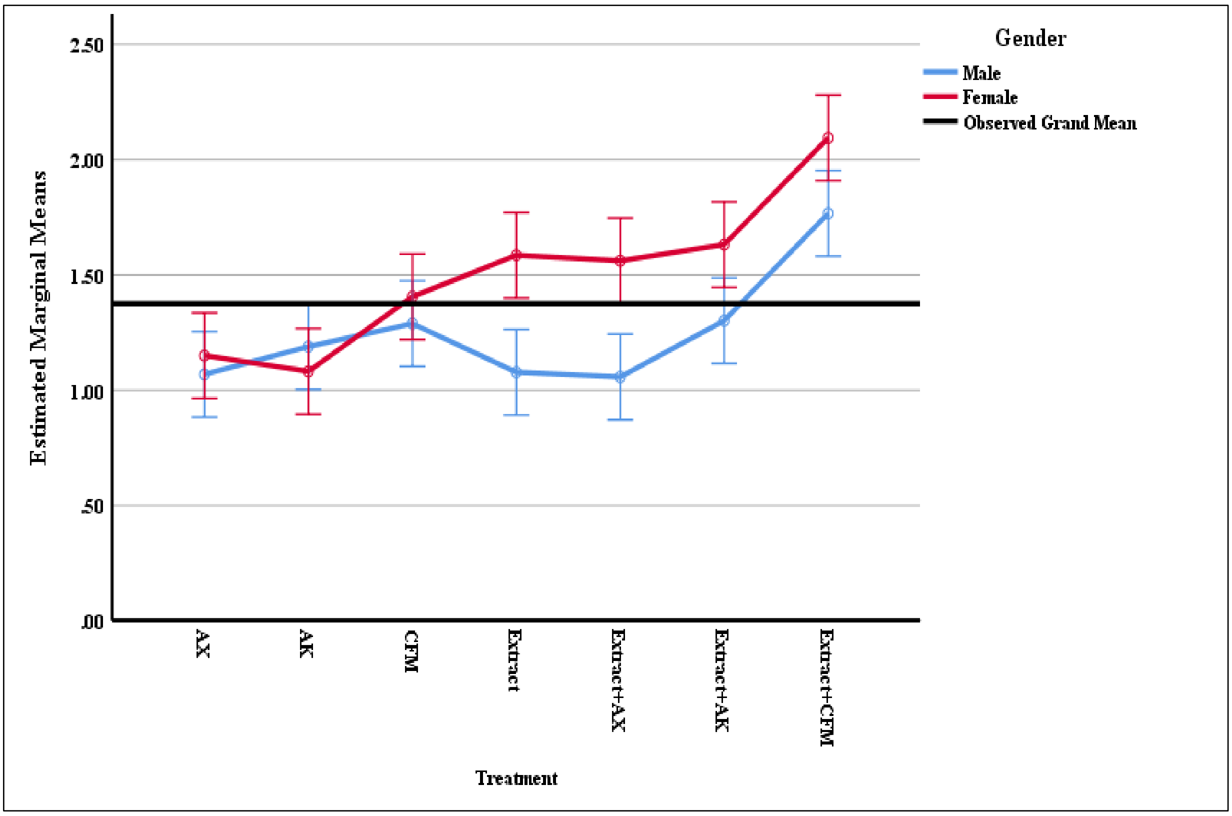

Figure 3.1: Means diameter of inhibition according to the types of treatment and gender groups

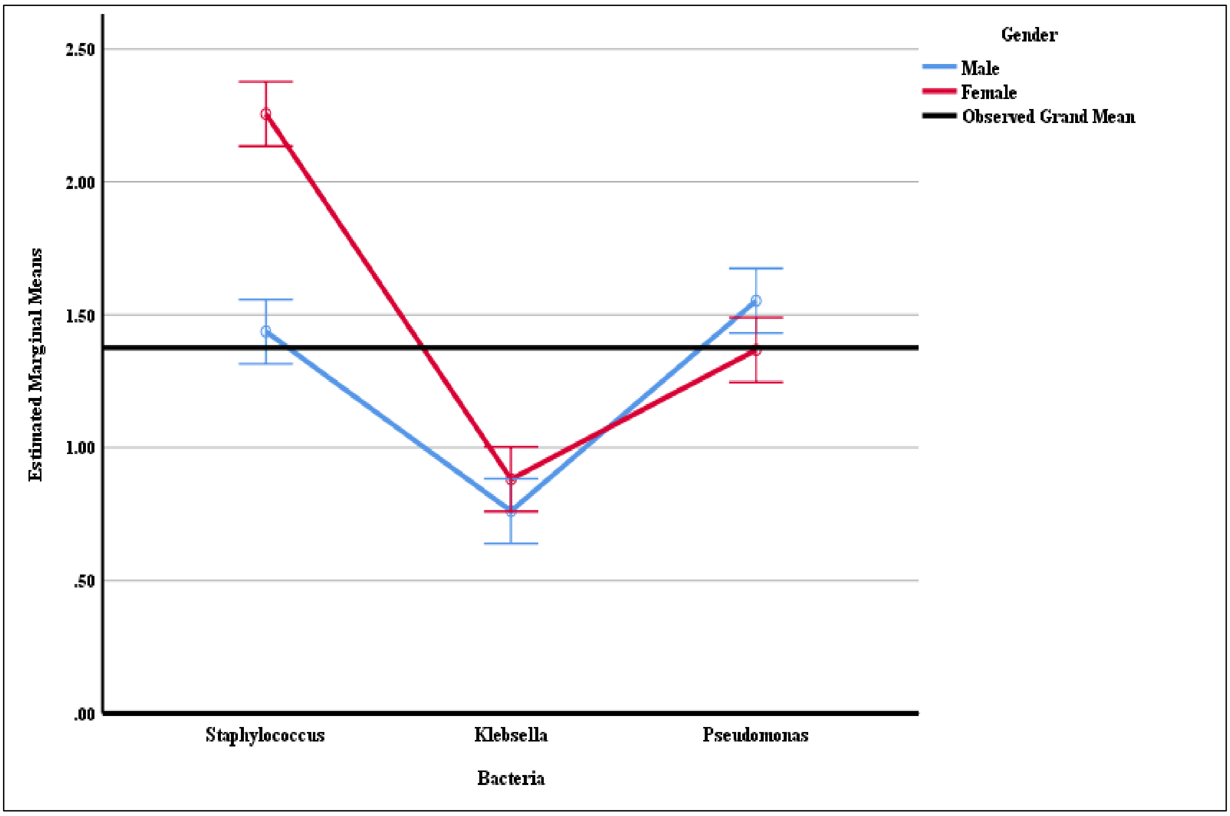

Figure 3.2: Means diameter of inhibition according to the types of bacteria and gender groups

Males and females that are on breast feeding showed almost the same respond to the combination of treatments, while males and females who are on bottle feed showed lower response to the combination of treatment in addition to their different response according to their gender groups which revealed females were respond better than males in this group.

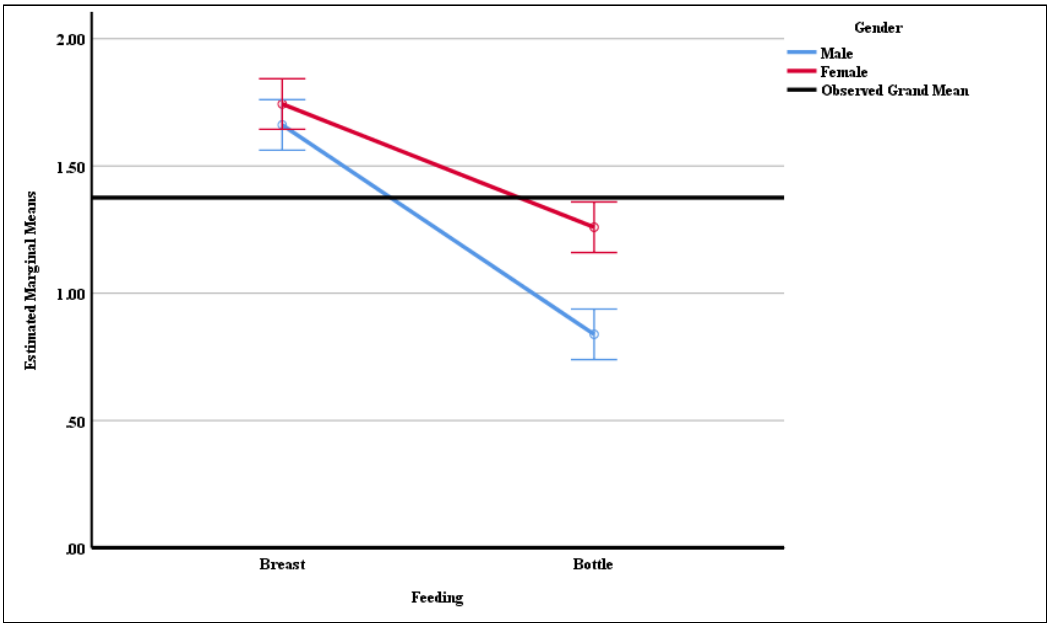

Figure 4: Means diameter of inhibition according to feeding and gender groups

The balanced design was used to analyze the data obtained from the underwent experiment. The analysis of variance table (Table 4) obtained showed that replicates is not significant which ensure unbiased results of the experiment. Moreover, the main factors of feeding, gender, treatment and bacteria were all appeared to have significant influence on the diameter of inhibition as a response variable to this experiment.

Table 4: Analysis of variance table as obtained by the use of balanced design

Source	DF	SS	MS	F	P
Replicate	2	0.534	0.2669	0.44	0.647
Feeding	1	80.7	80.6997	131.93	0
Gender	1	11.938	11.9378	19.52	0
Treatment	6	48.833	8.1388	13.31	0
Bacteria	2	135.062	67.5312	110.4	0
Error	743	454.485	0.6117		
Total	755	731.552			

Replicates found to show insignificant effect when the interaction of it with each of the bacteria and treatment considered as obtained by the use of general linear model and presented on Tables 5 and 6.

Table 5 shows the results of the analysis of variance for the diameter of inhibition as a response variable, while table 6 shows the validity of the model by examining the sum of squares for the model against the sum of squares for the residuals.

Table 5: Analysis of variance table for the interaction of replicates with bacteria and treatment

S.V.	SS	DF	MS	F	P
Intercept	1430.688	1	1430.688	1856.198	0.000000
Replicate	0.534	2	0.267	0.346	0.707456
Treatment	48.833	6	8.139	10.559	0.000000
Bacteria	135.062	2	67.531	87.616	0.000000
Replicate*Treatment	0.764	12	0.064	0.083	0.999986
Replicate*Bacteria	0.237	4	0.059	0.077	0.989325
Treatment*Bacteria	10.340	12	0.862	1.118	0.341853
Replicate*Treatment*Bacteria	1.644	24	0.068	0.089	1.000000
Error	534.138	693	0.771		

Table 6: Test of the coefficient of determination of the model

Test of SS Whole Model vs. SS Residual											
	Multiple - R	**Multiple - R^2**	**Adjusted - R^2**	**SS - Model**	**df - Model**	**MS - Model**	**SS - Residual**	**df - Residual**	**MS - Residual**	**F**	**p**
diameter	0.800946	0.641514	0.597237	469.3011	83	5.65423	262.2511	672	0.390255	14.48857	0

It is very important to test for the interaction of the factors used in this experiment for their effects on the diameter of inhibition. In this context, factorial experiment design was used to test for the main factors (feeding, gender, bacteria, and treatment) and their interactions. Table 7 shows the ANOVA table obtained by this design. The table shows that except the interaction of feeding by bacteria by treatment, all other main effects and interactions were significant at p-values less than 0.05.

Table 7: ANOVA table of main effects and interactions of feeding, gender, bacteria and treatment

S.V.	**DF**	**SS**	**MS**	**F**	**p-value**
Intercept	1	1430.688	1430.688	3666.037	0.000000
Feeding	1	80.700	80.700	206.787	0.000000
Gender	1	11.938	11.938	30.590	0.000000
Treatment	6	48.833	8.139	20.855	0.000000
Bacteria	2	135.062	67.531	173.044	0.000000
Feeding*Gender	1	5.418	5.418	13.883	0.000211
Feeding*Treatment	6	5.290	0.882	2.259	0.036293
Gender*Treatment	6	8.557	1.426	3.654	0.001407
Feeding*Bacteria	2	46.615	23.307	59.723	0.000000
Gender*Bacteria	2	33.384	16.692	42.772	0.000000
Treatment*Bacteria	12	10.340	0.862	2.208	0.010125
Feeding*Gender*Treatment	6	14.710	2.452	6.282	0.000002
Feeding*Gender*Bacteria	2	7.544	3.772	9.665	0.000073
Feeding*Treatment*Bacteria	12	4.579	0.382	0.978	0.468500
Gender*Treatment*Bacteria	12	28.313	2.359	6.046	0.000000
Feeding*Gender*Treatment*Bacteria	12	28.018	2.335	5.983	0.000000
Error	672	262.251	0.390		
Total	755	731.552			

DISCUSSION

One of the major criteria of probiotic strains is the ability to modulate immune responses. Immune systems are crucial; they comprise of numerous immune cells that defend the host against infection and maintain the homeostasis condition of the host. Dysfunction of the immune system due to an underlying illness, aging, mental stress, chronic diseases and cancer therapy could lead to immunological alterations resulting in inflammatory disease and damaging the immune cells (Mendes *et al.*, 2019).

Bifidobacteria and lactic acid bacteria used as probiotic contribute to the defensive function of the immune system thereby playing a crucial role in host defence mechanism through modulating, stimulating and regulating immune system through the innate and adaptive immune cell response (Bajagai *et al.*, 2016; Ding *et al.*, 2017: Mendes *et al.*, 2019).

In his research work, Abiola Oluwatosin (ABIOLA OLUWATOSIN, 2021) showed that lactobacillus specie had positive impact on the protective effect from immunological damage. This result is in agreement with what had been found by this research work as the ability of inhibition the cultured bacteria was remarkably better in the treatments comprises the extract of lactobacillus specie.

CONCLUSION

Breast milk fed infants were more likely to show protective tendency against bacteria types used in this study in comparison with their counter parts who were bottle milk fed. Males and females who were breast milk fed had almost similar ability to defend their bodies against bacteria as their means diameter of inhibition showed significant differences compared to same gender groups who were on bottle milk fed.

Extract alone as well as mixture of extract with each of the antibiotics used in the experiment of this research work showed better means diameter of inhibition compared to the types of antibiotics used alone. The contribution of lactobacillus extract to the bacterial prevention can be evaluated as significantly considerable. The mixture of lactobacillus specie with the cefixime antibiotic was found to has the significantly highest mean diameter of inhibition compared to all other treatment types used in this research work.

Klebsella was found to weakly respond to the used treatments compared to staphylococcus and pseudomonas bacteria.

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