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Lactic Acid Bacteria and Antibiotics: A Comparative Study of Their Antibacterial Activities

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Abstract: The antibacterial effects of isolates from fermentedcheese whey (wara) and antibiotics against diarrheal pathogens were investigated. One hundred and twenty-three fecal samples were collected from diarrheic children aged three years and below from two hospitals in Ibadan over a period of 5 months.Thesamples were processed using standard microbiologicalmethods while the antagonistic effect of the isolated lactic acid bacteria and the conventional antibiotics were determined using agar welland disc diffusion method respectively. A total of 116 fecal samples were positive for bacterial growth with three genera of bacteria isolated viz: *Escherichia coli* (97%) *Klebsiella*species (2%) and *Morganella morgannii* (1%). The microorganisms isolated from the cheese whey included *Lactobacillus* sp. and *Pediococcu*s sp.Results of the antagonistic effects of whey isolates against the diarrheic isolates revealed that all the whey isolates showed varying inhitionagainst the diarrhiec isolates. The highest inhibition zones (18 and 15mm) were recorded against *Morganellamorganii for Pediococcus spp. and Lactobacillus spp.*The inhibition was compared favourably with that obtained when conventional antibiotics were tested against the diarrheic isolates.It could be said that the whey isolates produce bioactive constituents against diarrheal pathogens.

Key words: Diarrhea · Children · Cheese whey Lactic Acid Bacteria · Antibiotics · Sensitivity

INRODUCTION

Diarrhea is a disease condition which involves an abnormal stooling usually with blood and mucus. Diarrhea is defined loosely as the passage of abnormal liquid or unformed stools at an increased frequency [1]. Globally, diarrhea is one of the most important health hazardand is the leading cause of morbidity and mortality in children especially in developing countries [2], particularly in areas where access to immediate treatment is absent. Despite widespread use of oral rehydration therapies (ORT) and an increased understanding of the pathogenesis of diarrhea, in developing countries, 2•5 million children still die from these illnesses every year [3]. Cheese whey is a product of cheese making and is considered as a waste watery portion that separate from the curd during conventional cheese making [4]. Cheese itself is soft, whitish in color containing 70.5% moisture, 39% fat, 37.1% protein and 2.5% carbohydrate [4]. The microflora of cheese whey that has been exploited so far is Lactic acid bacteria [5]. Lactic acid bacteria and their metabolites have been shown to play an important role in improving microbiological quality and shelf life of many fermented food products and provide a good example of biopreservation [6]. Their food preservative potential relies mainly on the accumulation of organic acids and the acidification of the substrate [7]. A great number of strains of lactic acid bacteria (LAB) produce bacteriocins; ribosomally synthesized peptides that exhibit antagonistic activity against more distantly related species [8, 9] like *Listeramonocytogenes* [10].

The application of antagonistic compounds by lactobacilli are not limited to food preservation, antimicrobials of LAB have been employed successfully to prevent the formation of biogenic amines [11], to inhibit pathogens causing mastitis [12] and to inhibit enteropathogens in the small intestines of animals [13]. Some of the inhibitory components produced by lactic

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acid bacteria have been intensively studied by application in food preservation [14]. *Lactococcuslactis* produce nisin, a bacteriocin that has received particular attention because of its large inhibitory effect against a wide variety of Gram positive organisms [15]. Among some of its desirable properties as a food preservative are: non toxicity, produced naturally by *L. lactis* strains, heat stable and has excellent storage stability, destroyed by digestive enzymes, does not contribute to off-odours or off-flavours and has a narrow spectrum of antimicrobial activity [16].

It is usually very difficult to treat diarrhea, because majority of the antibiotics used in the treatment of diarrhea are now becoming ineffective due to microbial resistance. Also, antibiotics when used may induce diarrhea (antibiotic induced diarrhea) [5] and the disruption of the natural intestinal microflora in both acute and chronic diarrhea, resulting in complex interactions possibly aggravating the frequency of self-limiting conditions [17] although, with most patients diarrhea is self-limiting and can be treated with rehydration and other supportive therapy without the need for antimicrobials and microbiological investigations [18]. The use of bio-therapeutic agents is presently one of the avenues being exploited for the possible treatment of diarrhea [5]. These bio-therapeutic agents are called probiotics (microbial food supplements with beneficial effect on consumers). Hence, this study was designed to determine the bacterialorganismsresponsible for diarrhea in children and their sensitivity pattern to lactic acid bacteria isolated from cheese whey and conventional antibiotics.

MATERIALS AND METHODS

Collection of Samples: One hundred and twenty- three (54 from male and 69 from female) diarrheic fecal samples were collected from children aged 3 years and below from University CollegeHospital (UCH), Ibadan,Oyo State, over a period of 5 months. Patients from whom samples were collected had clinical signs of diarrhea as diagnosed by the consultant doctors but those that had commenced treatments were excluded from the study.

Samples were aseptically collected onto sterile universal bottles with indications of age, sex and time of collection and were immediately taken to the laboratory for microbiological analyses.

Fresh cheese whey samples were purchased from Fulanischeese (wara) sellers in Ibadan, Oyo state, Nigeria. The samples packed in sterile sample bottles were transported to the laboratory and analysed immediately.

Microbiological Analyses

Culturing: Fecal samples were suspended in 0.1% Nutrient broth and 0.1ml portion of the suspended fecal samples were platedonto Desoxycholate citrate agar and MacConkey agar plates by spread method as described by[5] while the cheese whey was serially diluted after fermentation for 72 hrsand 0.1 ml of each dilutions was spreadplated onto De Man Rogosa and Sharpe agar [5] and MacConkey agar. The plates were incubated at 37°C for24 hours. Representative discrete colonies were sub-cultured for identification.

Identification of Isolates from Faecal and Whey Samples: Pure cultures of the isolateswere subjected to Gram staining and biochemical tests [18]. The lactic acid bacteria isolate was characterized to genera level according to the criteria documented by [19].

Determination of Antibacterial Activity of Lactic Acid Bacteria Isolated from Diarrheal Feces: Bacterialisolates from cheese whey were inoculated into peptone water and incubated overnight at 37°C. The cultures were centrifuged at 3,000 revolutions per minutes for 20 minutes.The supernatants were sterilized by filtration using a Millipore filter (Corning Incorporated, Corning 431220, Germany). Antibacterial activity was carried out using agar well diffusion method as described previously [20].

Overnight broth culturesof the test bacteria after adjusted to match 0.5 McFarlandturbidimetricstandard were inoculated onto Mueller Hinton agar plates using flooding method [11]. Wells were made into the agar media using a sterile 6 mm cork borer. Two ml, each of the supernatant of the cheese whey isolates were introduced into each of the wells using a micropipette. The plates were incubated aerobically for 24 hours at 37°C. The diameter zones were measured in millimeter using a transparent ruler.

Antibiotic Sensitivity Testing: The agar disc diffusion technique was used for antibacterial susceptibility testing of isolates [11] using commercial antibiotics. The same method as described above was used except that antibiotics were used instead of isolates from whey. The following commercial antibiotic discs perched from (Oxoid) except one :Ceftazidime 30 µg, Ofloxacin 5µg, Pefloxacin 5µg, Levofloxacin 5µg(Biotec), Ciprofloxacin 5µg, Amikacin 30µg, Cefuroxime 30µg and cloxacillin 5µg. Statistical analysis Frequency distribution and analysis of variance (ANOVA) were used for analyzing the data while the level of significance was set at $p<0.05$.

RESULTS

Of the 123 fecal diarrheic samples cultured, 116 (94.3%) fecal samples were positive for bacterial growth with three genera of bacteriaviz*Escherichia coli, Klebsiella*species and *Morganella* were isolated and identified. *Escherichia coli* was predominant 112 (96.6%), followed by *Klebsiella* sp.3 (2.5%) and *Morganella morganni* 1 (0.9%) as shown in Table 1. *Lactobacillus*sp, *pediococcus*sp, *E coli and Proteus mirabilis*were isolated from whey.

Out of these isolates, only 10, 2 and 1 isolates of *E. coli, Klebsiella* and *M. morganmi* respectively with three standard organisms (ETEC *E. coli, Klebsiellapneumoniae* and *Staphylococcus aureus*)which served as controlwere subjected to antibacterial activity of Lactic acid bacteria from whey and antibiotics.There was nostatistical significant difference (X^2=0.0424, P>0.05) in the occurrence of bacteria between the females 64 (45%) and the male 52 (55%) and also among the different age groups (X^2=0.74, P>0.05). However,25 to 30 months age group with 31 (27%) and the 31 to 36 months age group 3(3%) had the highestandlowest occurrence respectively (Table 2).

The antibacterial activity of the lactic acid bacteria (*Lactobacillus* sp., *Pediococcus*sp.) against bacterial isolates from diarrhea stools (*Escherichia coli*, *Klebsiella*. and *Morganella morganni*) was shown in

Table 1: Frequency of bacteria isolates from diarrhea stool samples

Bacterial Isolates	n	% Frequency
Escherichia coli	112	96.6
Klebsiella pneumonia	3	2.6
Morganella morganni	1	0.9

n=number of bacterial isolates

%=percentage of bacterial isolate relative to the total number of organisms

Table 3. The two *Klebsiella*test organisms responded well to the inhibitory actions of *Pediococcus*sp *and Lactobacillus sp.* with 11 and 10 mm zones of inhibition respectively (Table 3). *Lactobacillus* sp*and Pediococcus*spwere active against *Morganella morganni* isolatewith 18mm and 15 mmzones of inhibition respectively.

Pediococcus sp. exhibited inhibitory zones to only seven isolates with zones of inhibition that ranged from 7 mm to 13 mm while three isolates were resistant to *pediococcus* sp (Table 3). Converse*ly, Lactobacillus* sp. wasactive against six of the ten tested *E. coli* (Table 3). *Pediococcus*sp exhibited inhibitory effects against the standards organisms which included ETEC *Escherichia coli, Staphylococcus aureus* and *Klebsiellapneumoniae,* with zones of inhibition 12mm, 13mm and 8mm respectively. *Lactobacillus* sp. on the other hand, showedreasonable activity against the standard organisms except for *Klebsiellapneumoniae.* Figure 1shows the antibacterial activity of*Lactobacillus* sp. against *Staphylococcus aureus*.

Table 2: Distribution of bacteria isolates from diarrhea stool samples based on age groups and sex

Age group (months)	No of samples examined			No of positive samples			
	Male	Female	Total	Male	Female	Total	Percentage %
1 - 6	3	2	5	3	2	5	4
7 - 12	9	11	20	8	11	19	16
13 - 18	14	17	31	13	17	30	26
19 - 24	13	18	31	13	15	28	24
25 - 30	14	18	32	14	17	31	27
31 - 36	1	3	4	1	2	3	3
Total	54	69	123	52	64	116	100

Table 3: Antibiotic sensitivity pattern of bacterial isolates of diarrhea

Bacterial isolates	N	%	CAZ	CIP	AK	PEF	CAM	LFX	OFX	OB
E.coli	10	6.9	6(60)	0(0)	3(30)	0(0)	20(20)	3(30)	0(0)	0(0)
K.pneumoniae	2	15.4	2(100)	0(0)	0(0)	0(0)	1(50)	0(0)	0(0)	0(0)
Morganella morganni	1	7.7	1(100)	0(0)	1(100)	0(0)	1(100)	0(0)	0(0)	0(0)
Total	13	(100)	9(69.2)	0(0)	4(30.8)	0(0)	4(30.8)	3(23.1)	0(0)	0(0)

Key:

N= Number of diarrhoeic bacterial organisms subjected to inhibitory effect of antibiotics

%= percentagesensitivity of diarrhoeic bacterial organisms to antibiotics

CAZ=Ceftazidime, CIP = Ciprofloxacin, AK=Amikacin, PEF= Perfloxacin, CXM= Cefuroxime, LFX= Levofloxacin, OB= Cloxacillin, OFX= Ofloxacin

Table 4: Mean Antibacterial activity of lactic acid bacteria and the conventional antibiotics

Pathogenic Bacleria	N	*Pediococcus* (X±S.D) mm	*Lactobacillus* (X±S.D)mm	*Ceftazidine* (X±S.D)mm	*Amikacin* (X±S.D)mm
Escherichia coli	10	6.90±0.95	4.00±1.75	14.2±2.10	13.6±1.60
Klebsiella sp.	2	11.50±0.71	10.00±2.00	19.00±1.80	14.00±2.00
Morganella morganni	2	18.00±0.00	15.00±0.00	20.00±2.10	21.00±6.31

N= Number of diarrhoeic bacterial organisms subjected to inhibitory effect of Lactic acid bacteria

Fig. 1:

The antibiotic effect against the isolates from stool as shown in Table 4 revealed that two out of all the antibiotics tested were effective. All the tested organisms were sensitive to Ceftazidime and amikacin with zones of inhibition that ranged from 10mm to 24 mm and 9 mm to 22 mm respectively (Table 3 and 4). The organisms however, responded moderately to the inhibitory effects of ciprofloxacin, cefuroxime and levofloxacin (Table 4). All the tested organisms were resistant to Pefloxacin, ofloxacin and cloxacillin.Comparing the inhibitory effects of lactic acid bacteria and the conventional antibiotics, it was observed that the activity shown by the two agents were comparable but to varying degree of activity.

DISCUSSION

The role of lactic acid bacteria as antibacterial agent has been documented [31]. The non significant difference in the occurrence of bacteria between the male and the female correlates with the finding of Prakason [23] in which there was no significant association between gender and enteropathogenic bacteria isolated from diarrhealinfection. The finding of this study also corroborates with that of Panigua *et al*. [24] who reported that sex has no significant impact on the occurrence of enteropathogenic bacteria.

The low frequency of diarrhea causing organisms observed in the age groups 1 to 6 months and 31 to 36 months could be attributed to the fact that in the former, children within the age range are in most cases under exclusive breastfeeding [25]. This is because failure to breast feed has been reported as one of the predisposing factors to diarrhea [22]. The latter could be as a result of the improved or more developed immune system of the children within that age range.

Increased frequency of bacteria isolated among the age groups 7 to 12 months, 13 to 18 months, 19 to 24 months and 25 to 30 months could be ascribed to the fact that children in these age groups in addition to breast milk as the case may be, are also fed with solid foods which would not have been properly pasteurized or had been contaminated during the process of preparation either from water, utensils, or hands of persons preparing the milk [27].

The isolation of *Escherichia coli, Klebsiella* sp. and *Morganella morganni* from the study isin contrast with the result obtained byAbdullahi *et al*. [25] in which *Salmonella* species and *Shigella*species were isolated from the samples that were collected from children. The result also disagrees with that Panigua *et al*. [24] who reported *Entamoeba histolytica/Entamoeba dispar, Salmonella typimurium, Infestis, Anatum, Newport, Giardia intestinalis* and *Shigellaspp*. (*flexneri*and *sonnei*) as isolates from the samples collected from children. Major factors responsible for the contrast in these resultscould be more of environmental, individual anatomy and level of hygiene/the type of organism carried by those that are breast fed /prepared the foods.

Escherichia coli (96.6) being the most predominant isolatesin the study is in agreement with the result ofGhosh *et al*. [28] who reported isolation of *E. coli* (57.4%) from diarrheagenic stool samples from children as predominant isolate. Thisalso is in line with the findings of Al-Jarousha *et al*. [29] who investigated the etiology of bacterial enteropathogens causing diarrhea among children and reported that Enterohemorrhagic *Escherichia coli* (8.3%) is the predominant pathogen isolated.

Klebsiella sp. being one of the bacteria isolated from the diarrheal stool samples is in contrast with the result of Abdullahi *et al*. [25] in which *Klebsiella* was not part of the isolates recovered from the stool samples of children tested. One of the factors that could be responsible for the presence of *Klebsiella* in stool could be hematogenous spread of the organisms as a result of other infections such as chest infections, wound infections and urinarytract infection [18].

The occurrence of *Morganella morganni* which was formally known as *Proteus morganni*in stool samples of children is surprising as the organism is not a common cause of diarrhea but a sporadic etiology of diarrhea in children [18]. This is also in parallel with the result obtained by Al-Jarousha *et al*. [29] in which *M. morganni* was not isolated from the research work. *M. morganni*, however, can be found in human and animal intestines, in sewage, soil, water and occasionally, it causes urinary infections and other infections which are often hospital acquired [18]. The variationmay be due to differences in the studied subjects and the environments.

The isolation of lactic acid bacteria (*Lactobacillus* sp and *Pediococus*sp) from cheese whey is in consonance with the findings of Olorunfemi *et al*. [5] who reported *Lactobacillus acidophilus* and *Pediococcus cerevisiae* as part of theisolates from cheese whey. Also, Savadogo *et al*. [30] identified *Lactobacillus fermentum* and *Pediococcus* sp. as part of the lactic acid bacteria from fermented milk.

Lactobacillus sp. and *Pediococcus* sp. inhibitory effects against the diarrhea causing bacteria and the standard organisms is in consonance with the results reported by Olorunfemi *et al*. [5] that Lactic acid bacteria has inhibitory effects against diarrhea causing organisms. In a controlled study to test the hypothesis that treatment with *Lactobacillus* improves clinical outcomes in children with infectious diarrhea, it was observed that *Lactobacillus* intake iseffective as a treatment for children with acute infectious diarrhea [31]. *Lactobacillus*species had been reported to have potential to preserve vegetarian food system and a south Indian special dosa batter [15].

The multidrug resistant pattern of *Escherichia coli*, *Klebsiella* sp. and *Morganella morganni*to all the tested quinolones and cloxacillin agrees with the reports of Karlowsky *et al*. [32] and Abdalla *et al*. [33] that could be attributed to the presence of resistant gene in these organisms [34, 35].

The factor that could be responsible for the zones of inhibition of the antibiotics exceeding that of the lactic acid bacteria tested may not be unconnected to the method of preparation of the cheese whey. In a research to compare the antimicrobial activity of locally and laboratory prepared cheese whey against some common diarrhea causing bacteria, it was observed that the laboratory prepared whey had better inhibitory effects than the locally prepared cheese whey [36].

This study hasshown that cheese whey isolates: *Lactobacillus*sp and *Pediococcus* sp. has inhibitory effects against the tested diarrheal organisms. It is therefore concluded that organisms from fermented cheese whey do have potential in combating diarrhea in children but there is a need for more research on the isolation and purification of the antimicrobial compounds present in the lactic acid bacteria in order to determine the best form of applying it *in vivo*. Also, the multidrug resistance pattern exhibited by the diarrheal isolates against the antibiotics suggests that the antimicrobial susceptibility profile of individual isolates should be used to guide treatment.

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