Potential probiotics from faecal specimens of breastfed Nigerian infants as a therapy for bacterial gastroenteritis

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Abstract

Objective: To investigate the possibility of isolating indigenous probiotic strains from faecal specimens of breastfed infants.

Method: One hundred and sixty four *Lactobacillus* strains [*Lb. reuteri* 72 (43.9%), *Lb. casei* 40 (24.4%), *Lb. acidophilus* 35 (21.3%), *Lb. bifidus* 11 (6.71 %), *Lb. brevis* 3 (1.83 %) and *Lb. plantarum* 3 (1.83 %] isolated from fresh, faecal specimens of 57 healthy, breastfed Nigerian infants and children <12 months old were assayed for *in vitro* inhibitory potentials against reference and gastroenteritic bacterial strains, using the modified agar spot and agar well-diffusion methods.

Results: Twenty six (15.9%) of the *Lactobacillus* strains at 24 hours and 36 months of storage were inhibitory *in vitro* towards all the gastroenteritic and reference bacteria (*Bacillus cereus*, *Bacillus licheniformis*, *Citrobacter species*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella aerogenes*, *Klebsiella pneumoniae*, *Morganella morganii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*. *Salmonella enterica var.* typhi, *Salm*onella *enterica var.* paratyphi, *Salmonella enterica var.* typhimurium, *Salmonella sp.*, *Shigella dysenteriae*, *Shigella flexneri*, S*taphylococcus aureus* and *Yersinia enterocolitica*); while only 7 (4.3%) strains were inhibitory at 60 months of storage. The finally selected *Lb. reuteri* CH1 strain, which was inhibitory against all the indicator and reference bacteria, even at 60 months of storage, also had the lowest antibiotic resistance (23.3%), survived 3% bile and simulated pH conditions of 3.0-9.5.

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Conclusions: Potential gastroenteriticidal *Lactobacillus* probiotic strains with longer shelf-life can be obtained from breastfed faecal specimens of Nigerian infants, more especially children above 5 months old.

(Keywords: Breastfeeding; child health; gastroenteritis; infant mortality; *Lactobacillus*; probiotics)

Background

Gastroenteritis is still a major worldwide problem among infants and children, and one of the principal causes of infantile sickness and death in more than 85% of the world population[1.] Although antibiotic therapy has been the mainstay in the treatment of diarrhoeal cases, the onset of drug resistance threatens virtually all classes of antibacterial agents[2] and though the magnitude of the problem may vary from place to place, the problem of antibiotic resistance is quite alarming in tropical developing countries. Several workers in this country and elsewhere have also highlighted the problems of antibiotic resistance[3,4.] An alternative therapeutic approach, based on oral administration of live bacteria has therefore been under active consideration[5, 6, 7, 8.]

More than 95% of infants in Africa are currently breastfed but feeding practices are often inadequate and the importance of breast milk as a food resource in African countries is also not generally recognised[9.] Although it is important that babies are given extra food, as well as breast milk, at the right age and in sufficient amounts, to enable them grow and stay healthy, new studies on breastfeeding have also discovered or confirmed the benefits of breastfeeding to mother and child[10], while human milk has also been found to contain several antiviral and antibacterial factors[11.]

In a study with data from India, Peru and Ghana on hospitalisation for diarrhoeal and respiratory illness[12,] non-breastfed infants had a higher risk of all causes of hospitalisation when compared to infants who had

been predominantly breastfed and also had a higher diarrhoea-specific hospitalisation. Similarly, non-breastfed infants had higher risk of dying when compared to infants who had been predominantly breastfed. There have been many current attempts to prove the protective factors demonstrated in human milk, and that breastfeeding has a defensive role against various infections in infants. As earlier reported, information about the human flora has mostly been gathered from faeces, and many of the validly described gut *Lactobacillus* species have been isolated from human faeces[13]. The aim of this study is therefore to determine the possibility of isolating *Lactobacillus* species from infantile faecal flora of breastfed infants that can serve as probiotics of clinical importance, especially in infantile gastroenteritic therapy.

Methods

Collection of specimens and isolation of gastroenteritic isolates

Stool and vomitus specimens were obtained from gastroenteritic epidemic victims in three eastern states of Nigeria and from gastroenteritic patients at Oni Memorial Children Hospital, Ibadan, Nigeria. The stool and vomitus specimens were collected in alkaline peptone water at pH 8.6, and immediately transported to the laboratory for the preliminary microbiological analysis on molten (45^0C) nutrient agar (NA) (Oxoid, Basingstoke, England), thiosulphate citrate bile sucrose agar (TCBS) (Oxoid) agar, pH 8.2; deoxycholate citrate agar (DCA) (Oxoid), pH 7.2; MacConkey agar (Oxoid), cystein lactose electrolyte deficient agar (CLED) (LAB M, Topley House, Lancashire, England) and *Salmonella-Shigella* agar (LAB M) at pH 7.4. Representatives of each different bacterial colony types were randomly picked from the primary culture plates of each specimen and sub-cultured on sterile NA and CLED plates by the streaking method. All the pure bacterial isolates from the gastroenteritic specimens were kept in triplicates on Brain Heart Infusion (BHI) (Oxoid) agar slants as working and stock cultures. Final identification analysis was carried out at the Nigeria Institute of Medical Research (NIMR) laboratories according to standard taxonomic tools[14].

Isolation and characterisation of Lactobacillus strains

Ethical approval was granted by the Institute of Child Health and Department of Paediatrics, University College Hospital (UCH), Ibadan, Nigeria for the collection of faecal specimens from subjects at the Institute of Child Health, UCH, Ibadan, Nigeria. Subjects were also solicited by providing questionnaires to parents during their visits to post-natal clinics and at family homes. Questionnaires were completed by providing demographic information and details of feeding patterns of the child prior to collection of the stool specimen. Information sought through the questionnaires included age, sex, term of child at birth (full term, premature or post-mature), feeding patterns of the children and antibiotic therapy within 5 weeks prior to collection of fresh faecal specimens. Faecal specimens of three children who were on antibiotic therapy within the stipulated time period were excluded from analysis.

One ml volumes of overnight MRS broth cultures (de Man, Rogosa and Sharpe, Oxoid, Basingstoke, England) of the infantile faecal specimens from healthy children at pH 5.5-5.7 were separately transferred to sterile Petri dishes by the pour-plate method before incubation anaerobically in 5% CO_2 (Gas Pak Anaerobic System, Oxoid, Basingstoke, England) at 32-35^{0}C for 24-48 hours. Representatives of each different bacterial colony type were randomly picked from the primary plates of each specimen and repeatedly sub-cultured on to sterile MRS agar plates to obtain pure cultures.

Different randomly isolated *Lactobacillus* strains were phenotypically identified at genus and species level by classical tests including the analysis of cell morphology, homo/heterofermentative and biochemical characters, sugar fermentation patterns and growth at different temperatures. The *Lactobacillus* strains were examined microscopically, and initial confirmation and grouping of the lactobacilli was based on Gram's reaction, catalase reaction with hydrogen peroxide, growth at 15^{0}C and 45^{0}C in MRS and medium, gas and acid production from glucose and fermentation of lactose, sucrose, arabinose, fructose and mannitol[15].

The isolates that met the preliminary identification criteria were separately grown in replicates overnight (18–24 hours) in 10ml Rogosa broth at 35^{0}C until the weight of the cell mass obtained was 0.05–0.1g. The purity of the strains was checked, and the cells were washed twice in 0.9% sterile NaCl solution after centrifuging. The isolates were then stored at 4^{0}C in Hogness freezing buffer (3.6mM K_2HPO_4; 1.3mM KH_2PO_4; 2.0mM Na-citrate; 1.0mM $MgSO_4$; 12% glycerol) and kept frozen. Additional taxonomic studies were carried out on the purified isolates on the basis of their cultural, morphological, biochemical and physiological characteristics and

sugar fermentation patterns. Final identification analysis was carried out at the laboratories of Nigeria Institute of Medical Research (NIMR), Department of Medical Microbiology & Parasitology, University College Hospital, Ibadan and Department of Botany & Microbiology, University of Ibadan according to standard taxonomic tools [16, 17].

Antibiotic susceptibility testing

Agar disc-diffusion method: The gastroenteritic bacterial pathogens were assayed for their susceptibility / resistance patterns using the agar disc-diffusion method. Sterile Mueller-Hinton agar, nutrient agar, brain heart infusion agar, tryptone soy agar and MacConkey agar plates were seeded with each of the indicator (gastroenteritic) isolates, after which antibiotic discs commonly used for routine studies in the country, were aseptically placed on the seeded agar surfaces. After overnight incubation at 35^0C, zones of inhibition were recorded in mm diameter[18.] Zones less than 10 mm in diameter or absence of zones of inhibition were recorded as negative / no inhibition.

Modified agar spot-diffusion method: The antibiotic susceptibility test for the gastroenteritic bacterial species using paediatric antibiotic suspensions was determined according to the modified[19] agar spot-diffusion method of Tagg and Dajani[20.]. Sterile agar plates were seeded with the indicator organisms by streaking the entire surface of the culture plates (as indicated above), followed by separately dispensing the modified broth cultures (250µl, 500µl, 750µl, 1000µl) (soft agar) of the putative organisms (*Lactobacillus* strains) on the agar surface and incubated at 35^0C for 24-48 hours. The demonstration of antagonism depends on the release of a diffusible inhibitor early in the growth phase of the putative organisms into the assay media. Inhibitory zones surrounding the putative organisms were noted and recorded in mm diameter. Zones less than 10 mm in diameter or absence of zones of inhibition were recorded as negative / no inhibition.

Modified agar well-diffusion method: The antibiotic susceptibility test for the gastroenteritic bacterial species using paediatric antibiotic suspensions was also determined according to the modified[19] agar well-diffusion method of Tagg and Dajani[20]. Holes, 6 mm in diameter were aseptically bored out of sterile agar plates (as indicated above), which were then seeded with the indicator organisms by streaking the entire surface of the agar plates. Modified broth cultures (soft agar) of the putative organisms (250µl, 500µl, 750µl, 1000µl) were separately dispensed into

the holes and incubated at 35^0C for 24-48 hours. The demonstration of antagonism depends on the release into the assay media of a diffusible inhibitor early in the growth phase of the putative microorganisms. Inhibitory zones surrounding the putative bacteria were noted and recorded in mm diameter. Zones less than 10 mm in diameter or absence of zones of inhibition were recorded as negative / no inhibition.

The oral paediatric antibiotics used with their generics and batch numbers were: Emgyl [metronidazole] (5558fF, 6178F), Jawaclox [cloxacillin] (D4078, L4040), Evans metronidazole (3C950007, 3H950006), Barbicillin [ampicillin] (0683, 0800). Suphtim [sulfamethoxazole /trimethoprim] (SS05908, SS04805), Faplox [cloxacillin] (AC065, FG049), Barbimycin [erythromycin] (0080, 0977), Throtal [erythromycin] (TD-017, TD-12), Emtrim [septrin] (3173F, 3051H), Evans cotrimoxazole (5H802013, 4H802012), Bactrim [septrin] (LS24196, Ls24109), Primpex [cloxacillin] (03010, 05010), Mopson metronidazole (04-2234, 04-2235), Elgyl [metronidazole] (IU510, IV464) and Nichem ampicillin (A20S, A07S). The antibiotics were all manufactured in Nigeria, as indicated on their packages.

Determination of antibiotic susceptibility of the Lactobacillus strains: The most-inhibitory *Lactobacillus* strains from infantile faecal samples were screened for their susceptibility to 17 antibiotics used in clinical practice by the agar disc diffusion method on MRS agar. MRS agar plates seeded with each *Lactobacillus* culture broth were left for about 15 minutes before aseptically placing antibiotic discs on the agar surfaces and incubating the plates anaerobically at 37^0C for 18-24 hours. Zones of inhibition and the diameter of the zones were measured and recorded in millimeter diameter[21.] Zones less than 10 mm in diameter or absence of zones of inhibition were recorded as negative / no inhibition.

Bioassay to determine in vitro inhibitory activities of Lactobacillus species from breast-fed infantile faecal specimens against gastroenteritic bacterial species: Twenty six *Lactobacillus* strains that exhibited the highest rates of antimicrobial activities against the gastroenteritic indicator (gastroenteritic) organisms within 24 hours and 36 months of storage were further screened for their antimicrobial activities against selected gastroenteritic and reference strains, using the modified agar spot and agar well-diffusion methods. Broth cultures (250, 500 750 and1000µl) of each of the *Lactobacillus* strain were separately dispensed into the agar wells in Mueller-Hinton and

brain-heart infusion agar plates already seeded with the gastroenteritic indicator bacterial species. After overnight incubation at 35^0C, inhibition observed by a clear zones extending laterally from the border of the producer isolates were noted and recorded in mm diameter[22.] Zones less than 10 mm in diameter or absence of zones of inhibition were recorded as negative / no inhibition.

Results

Gastroenteritic bacterial species isolated from epidemic patients (7 months - 7 years), hospital in-patients (5 months - 5½ years) and hospital out-patients (9 months - 4½ years) assayed for in this study were *Bacillus cereus* (4), *Bacillus licheniformis* (5), *Citrobacter sp*. (1), *Enterobacter aerogenes* (2), *Escherichia coli* (5), *Klebsiella aerogenes* (3), *Klebsiella pneumoniae* (5), *Morganella morganii* (1), *Proteus mirabilis* (3), *Proteus vulgaris* (1), *Pseudomonas aeruginosa* (1), *Salmonella enterica var. Typhi* (1), *Salm*onella *enterica var. Paratyphi* (1), *Salmonella enterica var. Typhimurium* (1), *Salmonella* sp. (1), *Shigella dysentariae* (1), *Shigella flexneri* (1), S*taphylococcus aureus* (2) and *Yersinia enterocolitica* (1).

The viable counts of the infantile faecal specimens were between 1.1 x 10^3 and 7.2 x 10^3cfu ml^{-1}. No microbial growth was recorded for faecal specimen CH001, while no lactobacilli was observed in 16 infantile faecal specimens of subjects 4-16 days old. The 164 *Lactobacillus* strains randomly isolated from faecal specimens of healthy and well-nourished neonates and infants were characterised in this study. *Lactobacillus reuteri* (43.9%), *Lactobacillus casei* (24.4%) and *Lactobacillus acidophilus* (21.3%) were the most prevalent strains, while *Lactobacillus bifidus* (6.71%), *Lactobacillus brevis* (1.83%) and *Lactobacillus plantarum* (1.83%) were also obtained. There was however, a higher recovery rate of *Lactobacillus* strains from faecal samples of older healthy infants and children (5-11 months).

The Gram-negative bacterial species from epidemic sources (n=10) exhibited very high (70-90%) to total (100%) phenotypic resistance towards amoxicillin, ampicillin, augmentin, ceftazone, ceftriazone, chloramphenicol, ciprofloxacin, cotrimoxazole, fortum, gentamicin, nalidixic acid, nitrofurantoin, norfloxacin, ofloxacin, perfloxacin and tetracycline but lowest resistance (20.0%) were displayed towards claforan, while the resistance rates among the Gram-negative hospital isolates was between 17.6% (tarivid) and 88.2% (amoxicillin, augmentin and ceftriazone). Antibiotic resistance rates were relatively lower among the Gram-negative gastroenteritic bacterial species of hospital sources. Total resistance (100%) rates were also recorded among the Gram-positive bacterial strains (epidemic sources) towards more of the antibiotics - amoxicillin, augmentin, azithromycin, cefuroxime, cephalexin, ciprofloxacin, clindamycin, cloxacillin, colistin, cotrimoxazole, erythromycin, gentamicin, norfloxacin, ofloxacin, penicillin, rocephine, septrin, streptomycin and tetracycline; while similarly higher resistance rates (75.0%) were also exhibited towards claforan and tarivid but lower resistance rates were recorded among the Gram-positive bacteria (Table 1).

The phenotypic antibiotic resistance profiles of the gastroenteritic bacterial isolates from epidemic and hospital specimens showed relatively lower resistance rates towards 30 oral paediatric antibiotic suspensions (when compared to the rates recorded for the antibiotic discs), except towards chloramphenicol, erythromycin, metronidazole and the non-antibiotic anti-gastroenteritic medications (Table 2). However, there were no differences in the antibiotic susceptibility / resistance values on the different culture media.

Twenty six *Lactobacillus* strains from infantile faecal specimens *Lb*. *reuteri* 11 (42.3%) *Lb*. *casei* 7 (26.9%), *Lb*. *acidophilus* 3 (11.5%), *Lb*. *plantarum* 2 (7.7%), *Lb*. *bifidus* 1 (3.8%), *Lb*. *brevis* 1 (3.8%) exhibited total (100%) *in vitro* inhibition of the gastroenteritic indicator organisms between 24 hours and 36 months of storage but only 4 (15.3%) *Lb*. *reuteri*, 2 (7.7%) *Lb*. *acidophilus* and 1 (3.8%) *Lb*. *casei* strains were inhibitory towards all the target organisms at 60 months of storage. The finally selected potential probiotic candidate, *Lb*. *reuteri* CH1 had the lowest resistance (23.3%) towards the test antibiotics, survived 3% physiological bile, simulated pH (3.0-9.5), and exhibited significant spectrum of inhibitory activities against 7 reference strains and 12 selected gastroenteritic strains from children (Table 3).

Table 1
Phenotypic antibiotic resistance profiles of epidemic and hospital Gram-positive and Gram–negative gastroenteritic bacterial isolates

Gram–positive (% R)				Gram–negative (% R)			
Antibiotics [24]	Conc. (μg)	Epid. (n=4)	Hospt. (n=7)	Antibiotics [21]	Conc. (μg)	Epid. (n=10)	Hospt. (n=17)
Amoxicillin	25μg	100.0	100.0	Amoxicillin	25μg	100.0	88.2
Ampicillin	10μg	50.0	85.7	Ampicillin	25μg	100.0	52.9
Augmentin	30μg	100.0	100.0	Augmentin	30μg	100.0	88.2
Azithromycin	10μg	100.0	57.1				
				Ceftazone	20μg	100.0	52.9
				Ceftriazone	30μg	100.0	82.4
Cefuroxime	20μg	100.0	57.1				
Cephalexin	10μg	100.0	71.4				
Chloramphenicol	30μg	50.0	42.9	Chloramphenicol	25μg	70.0	23.5
Ciprofloxacin	5μg	100.0	54.5	Ciprofloxacin	10μg	90.0	29.4
Ciproxin	10μg	50.0	71.4				
Claforan	30μg	75.0	59.5	Claforan	30μg	20.0	70.6
Clindamycin	10μg	100.0	81.8				
Cloxacillin	5μg	100.0	90.9				
Colistin	10μg	100.0	100.0				
Cotrimoxazole	25μg	100.0	100.0	Cotrimoxazole	25μg	100.0	58.8
Erythromycin	5μg	100.0	85.8				
				Fortum	30μg	70.0	64.7
Gentamicin	10μg	100.0	42.9	Gentamicin	10μg	90.0	29.4
				Nalidixic acid	30μg	100.0	70.6
				Nitrofurantoin	300μg	100.0	47.1
Norfloxacin	10μg	100.0	71.4	Norfloxacin	10μg	100.0	41.2
Ofloxacin	30μg	100.0	42.9	Ofloxacin	30μg	70.0	41.7
Penicillin	15μg	100.0	85.8				
				Perfloxacin	5μg	100.0	23.5
Rocephine	30μg	100.0	42.9	Rocephine	30μg	40.0	58.8
Septrin	25μg	100.0	71.4	Septrin	25μg	40.0	23.5
Streptomycin	10μg	100.0	71.4				
Tarivid	5μg	75.0	57.1	Tarivid	5μg	50.0	17.6
Tetracycline	10μg	100.0	100.0	Tetracycline	30μg	100.0	47.1
				Zinnat	30μg	50.0	58.8

Epid. = epidemic patients, Hospt. = hospital patients

Table 2
Phenotypic antibiotic resistance profiles of gastroenteritic bacterial isolates from epidemic and hospital specimens using oral paediatric antibiotic suspensions

Antibiotics [30]	% Phenotypic antibiotic resistance		
	Epidemic bacterial isolates	Hospital bacterial isolates (Vomitus)	(Faecal)
Ampicillin-cloxacillin [8]	46.2	43.8	52.5
Cephalexin [6]	27.2	27.2	25.0
Chloramphenicol [2]	100.0	100.0	100.0
Cotrimoxazole [4]	30.6	43.8	70.0
Erythromycin [2]	81.8	100.0	100.0
Metronidazole [8]	77.1	84.4	86.1
NANT [5]	100.0	100.0	100.0

NANT = non antibiotic anti-gastroenteritic medications

Table 3
Phenotypic inhibition of indicator bacterial species by the antimicrobial-producing *Lb. reuteri* CH1 at 24, 48 hours incubation at 60 months of storage using modified agar spot and agar well–diffusion methods

Indicator organisms	Source	Zones of inhibition (mm diameter)		
		24h	48h	60m
B. cereus 25S	stool	+(18.0)	+(18.0)	+(16.0)
B. licheniformis 26S	stool	+(14.0)	+(14.0)	+(14.0)
*Bacillus sp.*39S	stool	+(12.0)	+(12.0)	+(12.0)
B. subtilis NCTC 8236	Ref. strain	+(18.0)	+(18.0)	+(12.0)
*Citrobacter sp.*55S	stool	+(16.0)	+(16.0)	+(12.0)
E. coli K12	Ref. strain	+(18.0)	+(18.0)	+(14.0)
E. coli V157	Ref. strain	+(16.0)	+(16.0)	+(13.0)
E. coli NCTC 10418	Ref. strain	+(18.0)	+(18.0)	+(16.0)
E. coli NTCC 11560	Ref. strain	+(18.0)	+(18.0)	+(14.0)
Kleb. aerogenes 24S	stool	+(14.0)	+(14.0)	+(12.0)
Kleb. aerogenes 412S	stool	+(16.0)	+(16.0)	+(15.0)
Kleb. pneumoniae 29V	vomitus	+(18.0)	+(18.0)	+(13.0)
Kleb. pneumoniae 32S	stool	+(16.0)	+(16.0)	+(10.0)
Kleb. pneumoniae 57S	stool	+(14.0)	+(14.0)	+(12.0)
Pr. mirabilis 22aS	stool	+(16.0)	+(16.0)	+(12.0)
Pr. mirabilis 22bS	stool	+(14.0)	+(14.0)	+(10.0)
Ps. aeruginosa 23S	stool	+(22.0)	+(22.0)	+(20.0)
V. cholerae MA *Vibrio*	stool	+(16.0)	+(20.0)	+(16.0)
V. cholerae INABA	Ref. strain	+(18.0)	+(20.0)	+(14.0)

The values indicate the diameter of inhibition zones in mm with 250μl, 500μl, 750μl and 1000μl of the lactobacilli culture using agar spot and well–diffusion methods at 24hrs, 48hrs and 60 months.

Discussion

In this current study, no culturable colony was obtained from faecal specimen obtained from a 4-day old, low-birth weight, female neonate. As earlier suggested by Blakey *et al*[23, 25] and Sakata *et al*[24,26], the absence of bacterial growth may be due to the immaturity of the digestive organs of the subject. Similarly, no lactobacilli were isolated from the faecal specimens of infants below 20 days old, which may be in accordance with the trend in the previous report of Yoshioka *et al.* [25, 27].

Nader de Macias[26] had previously documented that *Lb. acidophilus* and *Lb. casei* were the most prevalent *Lactobacillus* species in infantile faecal samples, while Axelsson[27] also stated that *Lb. acidophilus*, *Lb. fermentum* and *Lb. salivarius* were frequently reported ingredients of the faecal flora, and *Lb.* casei, *Lb. crispatus*, *Lb. gasseri*, *Lb. plantarum*, *Lb. leichmanii* and *Lb. reuteri* were thought to be typical representatives. Isolate identification in the study of Pei-Pei *et al*[28] also indicated that the infantile faecal *Lactobacillus* species belonged to *Lb. acidophilus*, *Lb. salivarius* and *Lb. paracasei*. The observed inter-species differences in infantile faecal flora between the previous and current findings of this study, in which *Lb. reuteri* and *Lb. acidophilus* were the most prevalent, may be due to differences in diets and geographical locations. Murray[29] in carrying out studies on *Enterococcus* spp. similarly reported that *E. faecium* and *E. faecalis* in humans are dependent on geographical location. However, there was no observable difference in the groups of bacterial species isolated from clinical specimens of gastroenteritic epidemic or hospital patients in this study, in comparison to other previous studies. Most of the aetiologic agents of gastroenteritis, affecting mainly infants in developing countries are therefore, still of the genera *Bacillus*, *Citrobacter*, *Enterobacter*, *Escherichia coli*, *Klebsiella*, *Morganella*, *Proteus*, *Pseudomonas*, *Salmonella* and *Shigella*, irrespective of the source of the pathogens Pei-Pei, *et al.* [29].

In vitro antimicrobial activities have been known to vary with the infant's age and according to the different pathogens more common in infants of that age[24.] Apart from a strain of *Kleb*siella *aerogenes* CD332, which was susceptible to all the test antibiotics, the gastroenteritic bacterial isolates were resistant to at least six of the 24 antibiotics tested. Multiple antibiotic resistance (MAR) rates of

between 25.0% and 95.8% among the gastroenteritic bacterial strains were also observed in this study. It is known that several antimicrobial agents have become available for use in newborns and children with suspected or proven bacterial infections[30] but the *in vitro* results of the antibiotic susceptibility of the gastroenteritic bacterial isolates to 30 oral paediatric antibiotic suspensions in this study gave relatively higher percentage resistance rates than expected in paediatric chemotherapy, most especially towards cotrimoxazole, metronidazole, erythromycin and chloramphenicol. This shows the great danger these commonly prescribed and abused antibiotics may pose in paediatric infectious conditions in the country, especially with the world-wide problems of antibiotic resistance[31,32].

In addition to the clinical and public health implications of antibiotic resistance in paediatric chemotherapy, adverse side effects of such therapy, such as allergic reactions, renal toxicity, gastric intolerance, skin rashes, anaemia, antibiotic-associated diarrhoea etc. are quite numerous and somehow very dangerous to infants and children. However, human breast milk has been reported to contain potential probiotic lactic acid bacteria; therefore, breastfeeding can be a significant source of lactic acid bacteria to the infant gut[23,33,34]. The present study highlights the fact that *Lactobacillus* strains isolated from breastfed infants can clearly inhibit all the test target organisms even at 60 months of storage. *Lb. reuteri* CH1 isolated from Nigerian breastfed infants had the lowest antibiotic resistance.

The findings of this study indicated the *in vitro* inhibitory activities of certain *Lactobacillus* strains towards gastroenteritic and reference bacterial species- *Bacillus cereus*, *Bacillus licheniformis*, *Citrobacter sp*., *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella aerogenes*, *Klebsiella pneumoniae*, *Morganella morganii*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*. *Salmonella enterica var.* Typhi, *Salm*. *enterica var*. Paratyphi, *Salmonella enterica var.* typhimurium, *Salmonella* sp., *Shigella dysenteriae*, *Shigella flexneri*, S*taphylococcus aureus* and *Yersinia enterocolitica*, even at 36 and 60 months of storage. The finally selected *Lb*. *reuteri* CH1 strain was inhibitory against all the indicator and reference bacteria, even at 60 months of storage, had the lowest antibiotic resistance (23.3%) and survived 3% bile and simulated pH conditions of 3.0-9.5, thereby having the potential properties of a probiotic.

The recovery of potential probiotic *Lactobacillus* candidates from faecal specimens of healthy breastfed Nigerian infants was confirmed by this study, indicating the beneficial significance of breastfeeding. This data can therefore, be used in promoting breast feeding in children. In addition, the low antibiotic resistance profiles of the probiotic candidates is of utmost importance in that the probiotics are not likely to participate in transference of antibiotic resistance to other bacterial flora in same ecological niche. Survival of the probiotic candidates in simulated pH conditions also infers their likely ability to survive in the gastrointestinal tracts of children, while producing inhibitory activities against gastroenteritic pathogens.

In conclusion, this study confirms that high quality, antimicrobial-producing *Lactobacillus* strains can be isolated from faecal specimens of breast-fed infants, especially older babies (5-11 months) who still receive complementary breast-feeding during weaning, thereby suggesting that nursing mothers, especially career-mothers, should not stop breast feeding of their children prematurely.

Acknowledgements

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