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Effect of *Salmonella enterica* ser. Typhi on the gut population of *Lactobacillus* spp. among typhoid patients in Ondo State, Nigeria

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ABSTRACT

Aims: Aberrant gut microbiota has been linked to frequent exposure to enteric pathogens, a condition capable of causing various adverse effects on human health. In this study, we looked into how the typhoid fever condition might affect the lactobacillus population in the human gut.
Methodology and results: A total of 424 faecal samples were collected from consented participants, who included 191 patients and 233 apparently healthy individuals. *Lactobacilli* and *Salmonella enterica* ser. Typhi (*S*. Typhi) was isolated from samples cultured on de Man Rogosa and Sharpe agar (MRS) and xylose lysine deoxycholate agar (XLD), respectively. The overall prevalence of *S.* Typhi was 104(24.5%), of which 83(43.5%) were patients and 21(9.0%) were healthy controls. *Lactobacillus* spp. load in patients was significantly lower among the febrile, *S*. Typhi-positive patients with an average population of 5.5 ± 0.96 $\log_{10}$ cfu/g compared with the febrile, but *S*. Typhi-negative patients with 6.41 ± $\log_{10}$ cfu/g and the apparently healthy respondents with 7.34 ± 1.1 $\log_{10}$ cfu/g. All the randomly selected *S*. Typhi strains obtained from both groups of respondents were sensitive to tetracycline and trimethoprim but resistant to chloramphenicol. Resistance to ciprofloxacin (18.2%) and ofloxacin (9.1%) was observed among the strains isolated from the febrile typhoid patients.
Conclusion, significance and impact of study: This study has demonstrated an association between the population of *Lactobacillus* spp. and the presence of *S*. Typhi in the human gut. In order to ensure the recovery of beneficial bacteria during and after the treatment of infections, it is crucial to promote critical research into new treatment methods.

Keywords: Lactobacillus spp., microbiota, *Salmonella* Typhi, typhoid fever

INTRODUCTION

The gut microbiota is regarded as the most varied and abundant microbial assemblage, interacting with the host in a variety of ways and so impacting the host's health and well-being (Senghor *et al.*, 2018). Lactobacilli have recently been identified as dominant taxa in the normal microbiota, with the greatest abundance in the small bowel (Duar *et al*., 2017). Its beneficial effect on human gut health is undoubtedly one of the most extensively researched topics in probiotic research. It has been established that *Lactobacillus* probiotics help prevent infection by enhancing the production of proteins related to the intestinal barrier and mucus, secreting anti-microbial agents like short-chain fatty acids (SCFA), bacteriocins and hydrogen peroxide. These anti-microbial agents are known to either stop the growth of pathogens or kill them by regulating the host's immune response to pathogens, preventing pathogen adhesion and competing with them for binding sites (Dempsey and Corr, 2022). Homeostasis within the host may be disturbed by dysbiosis, an altered intestinal microbiota state. This has been associated with numerous detrimental effects on human health as well as long-term effects that have the potential to cause a wide range of diseases, including inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), diabetes mellitus, obesity and colorectal cancer (Humphrey, 2009; Baothman *et al.*, 2016; Bhattarai *et al*., 2017). Frequent exposure to enteric pathogens like *Salmonella enterica* serovar Typhi has been linked to aberrant gut microbiota (Humphrey, 2009). According to a recent study by Haak *et al.* (2020), patients with typhoid fever had significantly different patterns of compositional and functional disruption of the gut microbiota when compared to patients with non-typhoidal febrile illness and healthy local controls. In healthy individuals, a balanced relationship with the microbes, with no resulting disease has been reported

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(Bäckhed *et al.*, 2005). *Salmonella enterica* infection remains a major public health concern worldwide, contributing to the economic burden of both industrialised and underdeveloped countries through the costs associated with surveillance, prevention and treatment of the disease (Crump *et al.*, 2004). Serovars of *S. enterica* cause infections in a diverse range of hosts. In humans, *Salmonella* is responsible for a broad range of clinical presentations, from gastroenteritis to invasion of normally sterile compartments such as the bloodstream or brain. Two serovars, *S.* Typhi and *Salmonella* Paratyphi A, are particularly associated with both human-restricted and invasive diseases (Ashton *et al.*, 2017). It is an acute and invasive infection of the gastrointestinal system and causes a devastating burden in many low- and middle-income countries with significant morbidity and mortality (Crump *et al.*, 2003; Wain *et al.*, 2015). *Salmonella* is among the common food-borne pathogens predominantly found in poultry, eggs, dairy products and vegetables, acquired directly or indirectly from human or animal faeces (Galgallo *et al.*, 2018). Chloramphenicol was the first antibiotic discovered to be efficacious in enteric fever and was the standard therapy for many years. However, some nations have abandoned its usage due to the discovery of plasmid-mediated resistance (Butler, 2011). The presence and frequency of antibiotic-resistant *Salmonella* in humans, animals and food is well established and poses a significant challenge to clinical care of typhoid fever, particularly in resource-limited nations such as Nigeria (O'Brien, 2002). It has been established that gut microbiota may play a significant role in protection against enteric pathogens (Pickard *et al.*, 2017). Despite the evidence of a direct correlation between typhoid fever and the dysbiosis of the gut microbiome, there appears to be a significant knowledge gap regarding the connection between typhoid fever infection and the human gut *Lactobacilli.* Given the significant function *Lactobacilli* serve in the human gut, it is critical to research the effects that *S*. Typhi infection may have on it to develop effective therapeutic strategies based on manipulating the gut microbiota, mainly probiotics to ensure overall bowel health, hence this study.

MATERIALS AND METHODS

Study area and design

A hospital and community-based cross-sectional study was carried out at five (5) different hospitals and the immediate environment, of which three (3) were located within the state capital and the other 2 were located within two different rural settings. Between 2016 and 2019, 450 human faecal samples were collected from volunteers, including 243 apparently healthy individuals and 207 patients. Volunteers for this study range in age from infants to toddlers, teenagers to adults and they come from a variety of socioeconomic backgrounds. There were 450 people in total who participated. Samples were obtained from each apparently healthy volunteer based on informed consent. Clinical patient samples were obtained from each hospital's laboratory unit. Fresh faecal specimens were collected from adults and children using sterile screw-capped containers and transported to the microbiology research laboratory of Elizade University within 2 h of collection.

Questionnaire administration

Four hundred and fifty (450) multiple-choice structured questionnaires were administered to the apparently healthy participants and patients suspected to have typhoid fever at each selected hospital's laboratory. Questionnaires sought information based on socio-demographics such as gender, age groups and marital status, as well as information that reflected on their lifestyle and medical history with regard to typhoid fever. Typhoid fever cases were defined in this study as patients presenting with a fever lasting for more than 3-5 days with one or more of the following symptoms: fever, persistent headache, diffuse abdominal pain, diarrhea, vomiting, loss of appetite, malaise, constipation, bloating, fatigue, chills, etc.

Ethical approval

Approval for the study was obtained from the Ondo State Ministry of Health and permission to collect samples from patients was obtained from the management of the hospitals used. Confidentiality was maintained in accordance with standard medical practice.

Exclusion and inclusion criteria

All patients with typhoid fever signs and symptoms, including fever with a temperature greater than 37.5 °C, abdominal pain or discomfort, headache, constipation or diarrhea, and who provided written informed consent, were included. Those on antibiotic treatment were excluded. Faecal samples of the apparently healthy participants who have been on antibiotics and/or yogurt (less than 3 months prior to sample collection) were also excluded.

Isolation, characterisation and determination of bacterial load of *Lactobacillus* spp.

Rogosa Sharpe agar (*Lactobacillus* spp. selecting agar, pH 5.4 ± 0.2, Oxoid, CM0627, UK) was used for selective isolation and enumeration of *Lactobacillus* spp., while de Man, Rogosa and Sharpe agar (pH 6.5 ± 0.2, Himedia GM641, India) was used as stock and growth media for further analysis. One gram of the faecal sample obtained from individual volunteers was weighed and collected in a 30 mL sterile universal bottle and 9 mL of sterile normal saline solution (0.85% NaCl) was added and then thoroughly homogenised using the vortex machine. The homogenised samples were serially diluted into tubes containing 9 mL of normal solution from 10^{-1} to 10^{-6}. Thereafter, 100 µL from each of the diluted suspensions

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(10^{-1} to 10^{-6}) were separately inoculated on triplet plates of Rogosa agar media (*Lactobacillus* selecting agar, pH 5.4 ± 0.2, Oxoid, CM0627) and spread using a sterile spreader. The inoculated plates were incubated under anaerobic conditions using an anaerobic gas jar containing gaspaks (*AnaeroGen*™ 3.5L, Thermoscientific, AM0035, UK) at 37 °C for 72 h. After 72 h of incubation, plates containing between 25 and 250 colony-forming units were selected, and the representative colonies, which differ in terms of their morphotype (varying in size, colour and shape) were counted. According to Hartley *et al.* (1977), ten colonies based on their differences in size and shape were randomly picked and subcultured on MRS agar (de Man Rogosa Sharpe, pH 6.2 ± 0.2, Oxoid, CM0359) and further purified for identification. The pure cultures were characterised using Gram stain, cell morphology and catalase reaction according to standard procedures. The purified cultures were activated by subculturing twice in MRS broth before use.

Molecular characterisation of *Lactobacillus* spp.

The DNA isolation procedure was carried out as previously described (Salehi *et al.*, 2005). The DNA isolation method used was the heat-thaw technique, where 1.5 mL of an overnight culture of each suspected *Lactobacillus* spp. grown in 10 mL MRS broth were centrifuged at 10,000 rpm for 5 min at 4 °C. The pelleted cells were resuspended in 200 µL of sterile deionised water. The resuspended bacterial cells were then boiled for 10 min, placed in the freezer for 10 min, allowed to defrost, then centrifuged at 10,000 rpm for 10 min. The supernatants were then transferred to another sterile Eppendorf tube and saved in the fridge for later use. The primer sequences used in this study were purchased from Inqaba Biotec Nigeria. It was adopted as designed and used by Dubernet *et al.* (2002) with an expected band size of approximately 250 bp (≈250 bp). The primer sequences are R16-1 (F) - 5′-CTT GTA CAC ACC GCC CGT CA-3′ for the forward primer and LbLMA1 (R) - 5′-CTC AAA ACT AAA CAA AGT TTC for the reverse primer. These primer sequences are from the 16S ribosomal RNA intergenic spacer region and correspond to the flanking terminal sequence of the 16S rRNA gene, known to be conserved among various bacteria including Lactobacilli. The reaction conditions for DNA amplification comprise an initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 sec, annealing at 55 °C for 30 sec, extension at 72 °C for 30 sec and final holding at 4 °C until use. The electrophoresis of the amplicon was carried out in 1.5% agarose gel in 1× (concentration) TAE buffer solution stained with ethidium bromide.

Isolation and identification of *S.* Typhi

Stool samples were homogenised in sterile normal saline (0.85% NaCl) and four drops of the stool suspension was added into 10 mL of selenite F broth and incubated for 18 h at 35 °C. After incubation, a loopful of the suspension was sub-cultured in xylose-lysine-deoxycholate agar (XLD) and Salmonella Shigella agar (SSA), and then incubated at 35 °C for 24 h. Identification of the *S.* Typhi was done based on the colony morphology, Gram staining and biochemical tests (catalase reaction, oxidase reaction, fermentation of sugar and alcohol, urease, indole, citrate utilisation and H_2S production) following standard bacteriological methods.

Molecular identification of *S.* Typhi

Suspected *S.* Typhi isolates were further identified using PCR method with *S.* Typhi servovar - specific primers as previously described by Massi *et al*. (2003). The primer sequence includes forward ST1 5′-ACT GCT AAA ACC ACT ACT-3′ and the reverse ST4 5′-TGG AGA CTT CGG TCG CGT AG-3′ with expected band size of 367 bp. The PCR reagents used was purchased from Inqaba Biotec. Nigeria, but produced by New England Biolab, UK. Before setting up the PCR process, the frozen reagents were thawed (except the Taq polymerase enzyme), gently spanned down and then kept on ice throughout the period of reaction preparation. The reaction mixture (50 µL) consisted of Taq polymerase, dNTPs mix, ions, Taq buffer in a 25 µL volume 1× final concentration, 1 µL each of 0.5 µM forward and reverse primers, 3 µL DNA template and nuclease-free water. The reaction condition for the DNA amplification includes initial denaturation at 95 °C for 1 min, followed by 30 cycles denaturation, annealing and extension at 95 °C for 30 sec, 45 °C for 1 min and 72 °C for 2 min, respectively, before final hold at 4 °C until use. Electrophoresis of the amplicon was carried out in 1.5% agarose gel in 1× (concentration) TAE buffer solution stained with ethidium bromide.

Antibiotic susceptibility profile of *S.* Typhi isolates

The anti-biogram of the enteric pathogens was analysed using the Kirby-Bauer disc diffusion techniques. The procedure for the experiment was carried out as specified in the Clinical and Laboratory Standard Institute (CLSI, 2014) guideline. Mueller-Hinton agar was prepared according to the manufacturer's instruction. The following antibiotic disc purchased from Himedia (India) was used: erythromycin (15 µg), chloramphenicol (30 µg), ceftriaxone (30 µg), tetracycline (30 µg), cefuroxime (30 µg), ofloxacin (5 µg) and trimethoprim (5 µg). Suspensions of the indicator organisms' overnight culture (18 h) were prepared with turbidity equivalent to 0.5 McFarland standards in sterile normal saline solution. The bacterial suspension was aseptically inoculated using a sterile swab stick to ensure even distribution of inoculum and incubated aerobically at 37 °C for 24 h. Diameter of the zone of inhibition was measured and recorded to the nearest millimeter using a ruler. The susceptibility status was determined using the zone size interpretative chart as provided by the Clinical and Laboratory Standards Institute (2014).

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Statistical analysis

The statistical analysis was performed using the DATAtab Team (2022), an online statistics calculator. Point-biserial correlation was run to determine the relationship between abundance of *Lactobacillus* spp. and the occurrence of *S*. Typhi in both groups.

RESULTS

Socio-demographic and clinical parameters

Of the 450 stool samples collected from participants recruited, 424(94.2%) met the inclusion criteria. Of these, 191(45.1%) were *S.* Typhi suspected patients and the apparently healthy respondents were 233(54.9%). Of the 191 febrile patients, the age range was 2-84; the mean age was 26 years, and the standard deviation (SD) was 17.89. The age category 1-10 has the highest number of respondents, 55(28.8%) among the febrile patients. On the other hand, of the 233 apparently healthy persons, the mean age was 26 years, with a SD of 15.49, an age range of 2-68 years. The highest number of participants were of the age group 21-30, 59(25.3%). Out of the 191 febrile patients, 90(47.1%) were males and 101(52.9%) were females. While 137(58.8%) were males and 96(41.2%) were females among 233 healthy respondents. Among the febrile respondents, 50(26.2%) were married, 98(51.3%) were unmarried while 2(1.05%) were either divorced or widowed. Of the healthy respondents, the unmarried were 135(57.9%) and the married were 98(42.1%). The unemployed have the highest number of respondents among the febrile, 96(50.3%) and the healthy respondents, 126(54.1%). The majority of the study participants were residents in the rural community 113(59.2%) and 137(58.8%) for the febrile and the healthy respondents, respectively (Table 1).

As shown in Table 1, *S.* Typhi was detected in 83(43.5%) of the 191 febrile patients and also detected in 21(9.01%) out of the 233 apparently healthy persons. The PCR diagnosis results confirm 83(43.5%) positive cases, comparable to the results of the widal test performed on hospital patients, where 101(52.9%) of the patients tested positive. The highest prevalence of *S*. Typhi infection was observed among the febrile participants in the age group 51-100 years 12(66.7%), while *S.* Typhi detected among healthy respondents was highest among the age group 41-50. The highest prevalence of *S*. Typhi 51(26.7%) was observed in females among the febrile respondents. The married within the febrile patients was observed to have the highest prevalence of *S.* Typhi 38(76.0%). With regard to employment status, the employed showed the highest prevalence of *S.* Typhi 15(71.4%) among the febrile patients. Patients that are residents within the rural community were observed to have the highest number of infected persons 54(47.8%), while healthy participants that are residents within the rural communities were 16(11.7%). Table 2 provides information on the health status of each respondent.

Table 1: Socio-demographic characteristics and the distribution of *S*. Typhi among patients and healthy persons studied within Akure and its environs from February 2016 to April 2019 in Ondo State. Nigeria.

Variables	Patients		Healthy persons	
	Samples screened (n=191)	Positive cases (%)	Samples screened (n=233)	Positive cases (%)
Age (Years)				
1-10	55	21(38.2)	47	1(2.1)
11-20	25	10(40.0)	47	2(4.3)
21-30	39	19(48.7)	59	6(10.2)
31-40	34	12(35.3)	41	5(12.2)
41-50	20	9(45.0)	21	4(19.1)
51-100	18	12(66.7)	18	3(16.6)
Gender				
Males	90	32(35.6)	137	13(9.5)
Females	101	51(50.5)	96	8(8.3)
Marital status				
Married	74	38(76.0)	98	6(6.1)
Unmarried	115	45(45.9)	135	15(11.1)
Divorced/widowed	2	0(0.0)	0	0(0)
Employment status				
Employed	35	15(71.4)	66	6(9.1)
Unemployed	112	49(51.0)	126	6(4.8)
Self-employed	44	19(57.6)	41	9(22.0)
Residence location				
Urban	68	29(42.7)	96	5(5.2)
Rural	123	54(47.8)	137	16(11.7)

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Table 2: Clinical condition of respondents.

Characteristics	Healthy control (n=233)		Patients (n=191)	
	Healthy control (n=212)	Control, *S.* Typhi detected (n=21)	Febrile, non-typhoid (n=108)	Febrile, typhoid (n=83)
History of typhoid fever and treatment	11(5.19)	19(90.5)	94(87.0)	69(83.1)
History of non-prescription:				
Herbal (%)	63(29.7)	9(42.9)	22(20.4)	31(37.3)
Antibiotics (OTC) (%)	89(41.9)	19(100)	81(75.0)	33(39.8)
Herbs & antibiotics	34(16.0)	6(28.6)	41(37.9)	24(28.9)
Signs and symptoms:	NA	NA		
High fever (%)			108(100)	83(100)
Persistent headache (%)			108(100)	83(100)
Abdominal pain (%)			98(90.7)	67(80.7)
Diarrhea (%)			24(22.2)	8(9.6)
Loss of appetite			91(84.3)	78(93.9)
Fatigue (%)			92(85.2)	73(87.9)
Nausea (%)			101(93.5)	77(92.8)
Vomiting (%)			9(8.3)	7(8.4)

OTC = Over the counter, NA = Not applicable.

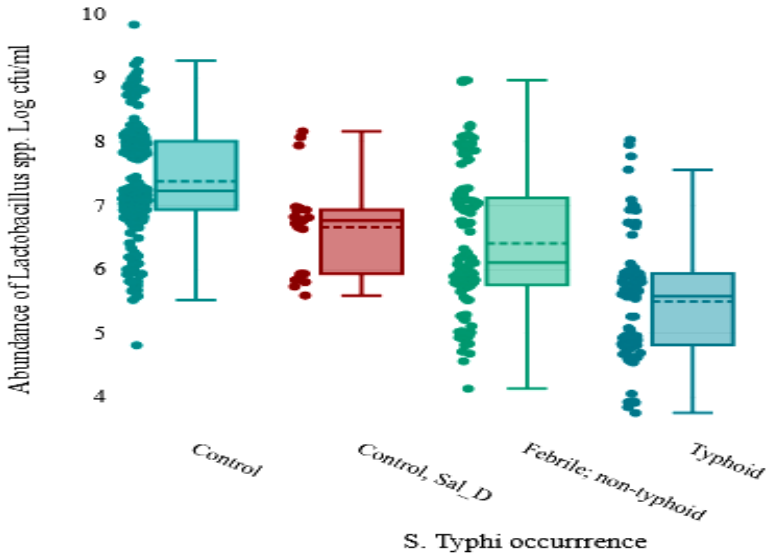

Figure 1: Box plot showing a comparison in the distribution of the level of abundance of *Lactobacillus* spp. in individuals in the different groups studied, which is represented as log cfu/ml on the y-axis. The boxes represent the interquartile range (IQR) between the first and third quartiles (25th and 75th percentiles, respectively) and the horizontal broken and continuous lines inside the box define the mean and the median, respectively.

Analysis of the effect of the occurrence of *S.* Typhi on the abundance of *Lactobacillus* spp. in patients and apparently healthy individuals studied

Figure 1 shows an overview of the abundance of *Lactobacillus* spp. in the healthy and febrile groups. Participants in the healthy group who had *S*. Typhi detected in their feces showed a significant decrease in the abundance of *Lactobacillus* spp. when compared to the control group with no occurrence of *S*. Typhi. A point-biserial correlation was run to determine the relationship between the abundance of *Lactobacillus* spp. and the occurrence of *S*. Typhi in both groups. The analysis shows a positive correlation between the abundance of *Lactobacillus* spp. and the occurrence of *S*. Typhi, which is statistically significant (p<0.001).

Table 3 contrasts the preponderance of Lactobacilli between patients with typhoid fever and healthy people across a range of socio-demographic characteristics. The outcome shows the mean colony forming unit (cfu/g) in a log of *Lactobacillus* spp. for each group across the various socio-demographic characteristics. The population level of *Lactobacillus* spp. found among the *S.* Typhi-positive respondents was all within the range of 5

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Table 3: Abundance of *Lactobacillus* spp. in faeces of typhoid fever-positive patients and healthy respondents.

Variables	Patients				Healthy persons	
	Typhoid fever		Non-typhoid fever		Samples (n=233)	log_{10} ± SD (CFU/g)
	Sample (n=83)	log_{10} ± SD (CFU/g)	Sample (n=108)	log_{10} ± SD (CFU/g)		
Age group (Years)						
1-10	21	5.11 ± 0.71	34	6.54 ± 1.12	47	7.20 ± 0.99
11-20	10	5.55 ± 1.15	15	6.53 ± 1.20	47	7.24 ± 0.89
21-30	19	5.56 ± 0.93	20	5.81 ± 1.25	59	7.41 ± 0.82
31-40	12	5.56 ± 1.09	22	6.18 ± 0.95	41	7.34 ± 0.79
41-50	9	5.93 ± 1.26	11	5.73 ± 0.80	21	7.35 ± 1.02
51-100	12	5.64 ± 0.84	6	6.56 ± 0.55	18	7.11 ± 0.91
Gender						
Males	32	5.67 ± 1.06	58	6.46 ± 1.03	137	7.25 ± 0.15
Females	51	5.39 ± 0.88	50	6.35 ± 1.18	96	7.41 ± 0.18
Marital status						
Married	38	5.55 ± 0.98	36	6.41 ± 1.03	98	7.23 ± 0.82
Unmarried	45	5.42 ± 0.95	70	6.40 ± 1.16	135	7.37 ± 0.93
Divorced/widowed	0	0	2	6.54 ± 0.48	0	0
Employment status						
Employed	15	5.96 ± 1.34	20	6.27 ± 1.12	66	7.41 ± 0.78
Unemployed	49	5.36 ± 0.84	64	6.45 ± 1.11	126	7.32 ± 0.94
Self-employed	19	5.49 ± 0.81	24	6.40 ± 1.08	41	7.13 ± 0.86
Residence location						
Urban	29	5.27 ± 0.86	39	6.70 ± 1.13	96	7.04 ± 0.85
Rural	54	5.61 ± 1.00	69	6.24 ± 1.05	137	7.49 ± 0.87

Table 4: Antibiotic susceptibility pattern of *S.* Typhi isolate obtained from patients and healthy persons studied within Owo, Akure and its environs in Ondo State, Nigeria.

Source	No. of isolates	Number of resistant isolates (%)							
		ER	CH	CIP	CTR	TE	CXM	OF	TR
Patients	11	11(100)	9(81.8)	2(18.2)	2(18.2)	0(0)	3(27.3)	1(9.1)	0(0)
Healthy persons	9	8(88.9)	9(100)	0(0)	1(11.2)	0(0)	4(44.4)	0(0)	0(0)

Key: ER = Erythromycin (15 mcg), CH = Chloramphenicol (30 mcg), CIP = Ciprofloxacin (5 mcg), CTR = Ceftriaxone (30 mcg), TE = Tetracyclin (30 mcg), CXM = Cefuroxime (30 mcg), OF = Ofloxacine (5 mcg), TR = Trimethoprim (5 mcg).

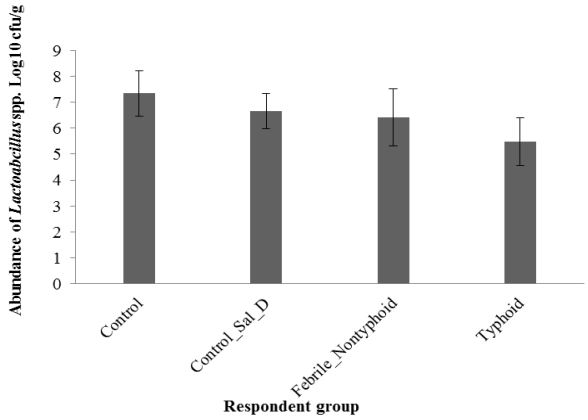

Figure 2: A graph showing the average population of *Lactobacillus* spp. among the different groups studied.

log cfu/g. Meanwhile, the population of *Lactobacillus* spp. found among the healthy respondents was within the 7-log range. When compared to the respondents among the healthy individuals, a log reduction of 2 was observed for the febrile respondents. Also, a 1 log reduction (6 log cfu/g) of *Lactobacillus* species was observed among the febrile but non-typhoidal patients when compared to the healthy persons. Figure 2 also shows the average population of *Lactobacillus* spp. across the different groups, including the control, control with faecal *S*. Typhi, febrile non-typhoid patients and the typhoid patients. This suggests a noticeable variation in *Lactobacillus* spp. abundance between the febrile patients and the apparently healthy group.

Antibiotic susceptibility profile of selected *S.* Typhi

Table 4 shows the antibiotic susceptibility profile of the *S.* Typhi isolates. The 20 randomly selected *S*. Typhi obtained from both patients and healthy individuals showed comparable sensitivity patterns to the different antibiotics used. One hundred percent of the *S*. Typhi obtained from the patients showed resistance to erythromycin, while 81.8%, 18.2%, 27.3% and 9.1% showed resistance to chloramphenicol ciprofloxacin and

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ceftriaxone, cefuroxime and ofloxacine, respectively. Regarding the S. Typhi isolates from the healthy participants, every isolate showed chloramphenicol resistance, while 88.9%, 11.2%, 44.4% and 88.9% of isolates showed erythromycin, ceftriaxone and cefuroxime resistance, respectively. The *S*. Typhi isolates obtained from both groups of respondents were all susceptible to tetracycline and trimethoprim. All of the strains isolated from healthy individuals were ofloxacin sensitive.

DISCUSSION

The overall prevalence of S. Typhi over the 3-year period of investigation among the febrile patients sampled was 43.5% (83 of 191) in the selected hospitals. As opposed to the result obtained in this study over a 3-year period, a previous study conducted in Lagos, Abuja and Kano showed percent positivity of 7% to 18.6% (over a 23-year time period), 3.9% to 10.4% (3-year period) and 0.8% to 2.4% (4-year period) respectively (Akinyemi *et al.*, 2018). The current study, however, was based on faecal-culture isolation followed by PCR confirmation, whereas the previous study, as reported earlier, was based on blood-culture isolation followed by conventional microbiological techniques. Access to adequate diagnostic tools has been a major challenge and has been linked to underreported cases of infectious diseases such as typhoid fever in low-income countries, particularly in Sub-Saharan Africa (Wijedoru *et al.*, 2017). The true burden of typhoid infection is likely to be underestimated, in addition to the availability of adequate diagnostic tools, because most of those with the disease might not always seek treatment at hospitals. The presence of *S.* typhi among healthy people in this study could be attributed to the pathogens' failure to clear when infected in the past. Approximately 8% of the healthy participants in this study had been infected and treated for almost a year before collecting their faecal samples. Previous reports have described different periods to distinguish between temporary and chronic carriage of *S.* Typhi (Gunn *et al*., 2014). While some reports define temporary carriage as asymptomatic shedding that lasts up to 3 months, other studies set the time limit at 12 months or more (Buchwald and Blaser, 1984; Parry *et al*., 2002).

In our patients with positive typhoid cultures, high fever, persistent headaches, appetite loss, fatigue and nausea were the most prominent clinical symptoms. However, diarrhea and abdominal pain were noticeably greater in febrile non-typhoid patients, which could be attributed to other gastrointestinal diseases such as amebiasis. Our findings are consistent with earlier research, which revealed that fever, headache and loss of appetite (Table 2) were the most common symptoms among typhoid fever patients (Nsutebu *et al.*, 2003; Mitra *et al.*, 2009; Adhikari *et al.*, 2015). Even though vomiting and diarrhea have been frequently reported in other studies, the proportions of people experiencing these symptoms in our study were lower than in previous studies (Kakria *et al*., 2014; Ohanu *et al.*, 2019).

Due to its influence on human health and disease, the human gastrointestinal microbiota, a complex of microorganisms, has recently drawn considerable attention. Recent studies on the relationship between *Salmonella* and the human gut microbiota showed that *Salmonella* has evolved molecular tools that enable them to compete with the gut microbiota (Rivera-Chávez *et al.*, 2016; Aljahdali *et al.*, 2020). *Salmonella enterica* for example, has been shown to effectively compete with the gut microbiota and overcome colonisation using a variety of strategies, including the expression of virulence factors and the exploitation of intestinal inflammatory processes (Aljahdali *et al.*, 2020). In this study, we observed that the febrile patients across the socio-demographic spectrum who tested positive for *S.* Typhi showed a considerable reduction in the population of *Lactobacillus* spp. (a member of the gut commensals) compared to their healthy counterparts. This finding proves that there is a direct link between the presence of *S*. Typhi and the abundance of *Lactobacillus* species. This is in tandem with a recent study by Haak *et al*. (2020), who reported that patients with typhoid fever had significantly altered patterns of compositional and functional disruption of the gut microbiota compared with healthy persons. In the same study, typhoid fever subjects were found to be significantly more colonised by other pathogens, with a significant decrease in the population of beneficial bacteria. Previous studies have observed similar patterns in a preclinical model with *S.* Typhimurium (Rivera-Chávez *et al*., 2016; Bronner *et al*., 2018). Also, the composition of the gut microbiota in pigs' cecum was found to change as a result of infections with *S*. Typhimurium, according to a recent study (Borewicz *et al*., 2015). Alteration of the intestinal microbiota by S. Typhi has been attributed to the aerobic expansion of the intestinal environment, leading to the loss of obligate anaerobes (Haak *et al*., 2020). Loss or reduction of obligate anaerobes (commensals) has been shown to increase the risk of gastrointestinal infection due to reduced colonisation resistance (Haak *et al*., 2020). Despite the gut microbiota's noble role in preventing the proliferation of bacterial pathogens in the gut, one is left wondering how *S.* Typhi is able to overcome commensal colonisation resistance. Strong evidence from earlier research suggests that *Salmonella* can "jump-start" and fuel their growth bloom during the early stages of infection by scavenging or "stealing" the hydrogen produced by the local microbiota (Maier *et al.*, 2013). The effects of typhoid fever infection on immunomodulation have also been reported to indirectly affect gut microbiota balance (Thompson *et al*., 2009). *Salmonella* has been shown to cause host inflammation, eventually reversing the bacterial population pyramid and becoming the dominant species in the intestine (Khan, 2014).

The significant differences in faecal *Lactobacillus* spp. abundance observed between febrile non-typhoid and febrile typhoid patients could be attributed to antibiotics, herbs and/or other infectious or non-infectious diseases other than enteric fever. Today, abnormalities in the gut microbiota have been associated with a wide range of

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bowel illnesses, such as inflammatory bowel diseases (IBD) like irritable bowel syndrome (IBS), colorectal cancer and other diarrheagenic infectious diseases (Li *et al*., 2021; de Vos *et al*., 2022). One of the current study's limitations is its reliance on faecal samples, which are used as a proxy for intestinal microbiota but do not accurately represent the bacterial population in the gut. According to Tang *et al*. (2020), faeces have become the sample source of choice for most bacterial microbiota studies due to their convenience and non-invasive nature.

According to the results of the questionnaire analysis, the majority of our respondents are accustomed to using over-the-counter medications like traditional antibiotics, herbs, or both. According to Ohanu *et al.* (2019), over-prescribing antibiotics for typhoid fever in developing countries with weak antibiotic policies, based on clinical suspicion and/or a single Widal test, presents a significant risk for the emergence of antibiotic resistance. Tests for antibiotic susceptibility revealed that all of the *S.* Typhi strains isolated from the patients were resistant to erythromycin and chloramphenicol, respectively. In relation to the healthy participants, chloramphenicol resistance was present in all of the strains, while erythromycin resistance was present in 88.9% of the strains. The *S.* Typhi strains obtained from both groups of respondents were sensitive to tetracycline and trimethoprim.

Meanwhile, all the selected *S*. Typhi obtained from the healthy respondents were susceptible to ciprofloxacin and ofloxacin, which have previously been reported to be effective in the treatment of MDR typhoid fever (Effa *et al.*, 2011). However, 18.2% (2 of the 11 isolates) and 9.1% (1 of the 11 isolates) of the strains obtained from the patients showed resistance to ciprofloxacin and ofloxacin, respectively. Although the sample size used for the antibiotic sensitivity analysis represents a limitation in the current study, the result reveals the potential of rising cases of quinolone-resistant *S*. Typhi in Ondo State, Nigeria. An increasing occurrence of ciprofloxacin-resistant *S*. Typhi has been reported (Lin *et al*., 2015).

CONCLUSION

Understanding the underlying mechanisms inherent in gut microbiota balances in health and disease, as well as strategies for preserving or re-establishing a healthy composition, is essential for developing therapeutic interventions that are effective. This study has therefore established a positive correlation between the abundance of *Lactobacillus* spp. and the occurrence of *S.* Typhi. Given the aforementioned evidence of the role of the microbiota in colonisation and resistance of enteric pathogens and the disruption of the *Lactobacillus* spp. abundance observed in our study, it is vital to investigate new treatment strategies that preserve this group of microorganisms during and after infection treatment.

CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

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