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Prevalence of *Salmonella* Bloodstream Infection and Antimicrobial Resistance in Lagos, Nigeria.

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ABSTRACT

Backgrounds: The burden of bacteremia in febrile cases is still poorly understood in Nigeria as in many sub-Saharan African countries due to diagnostic limitations. This study aimed to determine the prevalence of *Salmonella* bloodstream infections and antimicrobial resistance patterns of bacterial isolates recovered from febrile patients in Lagos, Nigeria.

Materials & Methods: A total of 300 blood samples were collected from febrile patients attending four medical centers in Lagos during August 2020 to July 2021. Clinical isolates were identified using API 20E kit. qPCR was used to detect *Salmonella* isolates in positive blood culture samples using a specific primer set. All isolates were subjected to antimicrobial susceptibility tests using standard procedures.

Findings: Totally, 55 bacterial isolates belonging to six bacterial genera were identified, including *Salmonella* (n=4, 7.27%), *Klebsiella* species (n=23, 41.82%), *Escherichia coli* (n=6, 10.91%), *Proteus* species (n=13, 23.64%), *Serratia* species (n=7, 12.73%), and *Citrobacter* species (n=2, 3.64%). In this study, the detection rate of *Salmonella* isolates in positive blood culture samples using qPCR and *inv*A gene primer set was 100%. *Salmonella* isolates were %100 resistant to ceftazidime, cefotaxime, and doripenem. Multidrug resistance (MDR) was observed in *Salmonella* and other bacterial isolates.

Conclusion: In this study, qPCR using the *inv*A primer set was found to be highly specific for *Salmonella* detection. All the bloodstream bacterial pathogens in this study were MDR; thus, there is a need for continuous evaluation of antibiotics in medical settings. Further molecular studies on these bacterial isolates is essential.

Keywords: *Salmonella,* Bacteremia, qPCR, Antibiogram, Resistance, Febrile.

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Introduction

Salmonella diseases (salmonelloses) continue to be a global problem. This pathogen is one of the primary causes of community-acquired bloodstream infections in sub-Saharan Africa and other low- and middle-income countries [1, 2]. *Salmonella* infections in humans vary in severity based on the serotype involved and the human host's health status. *Salmonella* infections are more common in children under five years of age, the elderly, and those with immunosuppression than in healthy persons [3]. All *Salmonella enterica* serovars, including typhoidal (Typhi and Paratyphi) and non-typhoidal (other *S. enterica* serovars), have the ability to penetrate, multiply, and survive in human host cells, resulting in disease states that could lead to death [4]. *Salmonella* ability to infect host cells is encoded by genes located on *Salmonella* pathogenicity island 1 (SPI-1), one of which is the invasion gene (*inv*A) [5]. The *inv*A gene contains sequences that are unique to *Salmonella* isolates and therefore is considered as a useful polymerase chain reaction (PCR) target with diagnostic applications [6]. Typhoid fever (TF) caused by *S. enterica* serovar Typhi is a febrile infection that is acute and life-threatening. It is characterized by fever, which is usually accompanied by chills, nausea, diffuse abdominal pain, rash, anorexia, and diarrhea or constipation; the disease sometimes appears with symptoms similar to other febrile illnesses, such as septicemia, leptospirosis, and *Streptococcus pneumoniae* infections [7-10]. The case fatality rate for untreated typhoid fever had been reported to be 10–30%, which may be reduced to 1–4% with proper treatment [7]. The disease remains a public health concern in many countries, especially in those with poor sanitation and hygiene [8]. About 21 million new TF cases and over 216,000 TF-associated deaths are reported annually worldwide. The fecal-oral route is the main mode of TF transmission. Non-typhoidal *Salmonella* (NTS) strains are the most common foodborne pathogens identified in poultry meat, eggs, and dairy products. Fruits, vegetables, food products, and animal sources are the main vehicles involved in NTS transmission [11]. *S. enterica* serovar Typhimurium and *S. enterica* serovar Enteritidis are associated with invasive non-typhoidal *Salmonella* illness (iNTS); however, other serovars have been identified in febrile patients and pyrexia cases of unknown origin (PUO) [12, 13]. About 2.1–6.5 million cases of iNTS disease are reported annually. On the other hand, iNTS is prevalent in sub-Saharan Africa and other developing countries, accounting for 25% of all deaths, and patients with severe anemia, malaria, malnutrition, and HIV infection account for most cases [14-16]. Several bacteria that cause bacteremia, aside from *S. enterica* subspecies, have been identified with different geographical distributions [1, 17-21]. Infection with these organisms leads to longer hospital stays, higher healthcare costs, and higher mortality rates [21, 22]. Bacteremia is a major bloodstream infection (BSI), which remains as one of the most important causes of morbidity and mortality worldwide [21, 23]. In sub-Saharan Africa, bacteremia accounts for 53% of mortality rate in children [24]. Antibiotics are required for *Salmonella* bloodstream infection and other types of bacteremia. The use of wrong antibiotics and drug abuse practices, among other factors, increase mortality and morbidity rates in developing countries [25, 26]. Ampicillin, tetracycline, and cotrimoxazole are the first-line antibiotics used to treat bacteremia. Infections caused by multidrug-resistant (MDR) strains are more severe than infections caused by susceptible strains [27]. Due to the advent of MDR strains, fluoroquinolone and extended-spectrum cephalosporin became the first-line antibiotics for the treatment of *Salmonella* infections in the

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early 1980s [28]. There are several reports of *Salmonella* resistance to cefotaxime and ceftriaxone as well as reduced susceptibility to ciprofloxacin [2, 16, 29].

Accurate and appropriate etiological diagnosis of typhoid fever and other septicemic conditions is significant and key for implementing interventions [7]. The most common method for diagnosing typhoid fever and other septicemic conditions is the culture method, which remains as the gold standard method. However, it takes a long time (at least 3–5 days) and has low sensitivity (30–60%) [7, 8]. In developing countries, there is a need for a rapid, simple, and inexpensive method for diagnosing *S.* Typhi infection and bacterial pathogens, especially in blood, because of frequent reports of TF cases and the disease endemicity in these countries. Thus, several immunological/ serological approaches have been developed, including Widal, Typhi-Dot, Tubex6, lateral flow, and SPR8 tests based on the type of O and H surface antigens [30, 31].

In Nigeria, the Widal agglutination test is the most widely used method to diagnose typhoid fever despite major shortcomings associated with this test [10]. The efficiency of polymerase chain reaction (PCR) technique in detecting *Salmonella* isolates has been well established, and researchers have used a variety of gene-targeting probes and primer sets in this method. **Objectives:** This method has been proven to be effective for over a decade [2, 32]. Therefore, this study aimed to determine the prevalence of *Salmonella* bloodstream infections using Widal, culture, and PCR tests and antimicrobial resistance patterns of bacterial isolates recovered from febrile patients in four hospitals in Lagos, Nigeria.

Materials and Methods

Study Population, case definition, and sample collection: This study was conducted on patients attending two private hospitals (Life Fount Medical Centre and Crest Hospital) and two public referral hospitals (Lagos State University Teaching Hospital-LASUTH and Alimosho General Hospital-AGH) in Lagos State from August 2020 to July 2021. Consent to collect samples and data from the hospitals was obtained from the ethics committee of each institution before patient enrolment. Patients' consent was also sought to obtain their data. Patients' demographic information and clinical data, such as age, gender, underlying health conditions, and antibiotic resistance, were obtained from their hospital and laboratory records. All cases were assumed to be domestically acquired.

Case definition: Blood samples were collected from febrile patients with clinical symptoms of typhoid fever and/or pyrexia of unknown origin (PUO). A febrile patient was defined in this study by physicians as a patient presenting with a temperature above 37.5 °C lasting for more than 3–5 days along with one or more of the following symptoms: diarrhea, vomiting, loss of appetite, persistent headache, malaise, etc [31].

Sample size determination formula and sample collection: Sample size was determined using the following formula according to Kadam and Bhalerao (2010) [33].

$$n = \frac{Z^2 * P\,(100 - P)}{L^2}$$

Where n is the required sample size, Z is the normal distribution value of two-tailed analysis (i.e., 1.96), P is the prevalence of the disease as previously reported by Smith et al. (2012) [34] (i.e., 74%), and L is the allowable error or precision, usually 5% (i.e., 0.05).

$$\text{Therefore, } n = \frac{(1.96)2 * 74\,(100-74)}{(0.05) \times (0.05)}$$, n = 2956495,

and n≈ 300 per ten thousand [33].

Sample collection: A total of 300 blood

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samples were collected from febrile patients with some or all clinical features of typhoid fever and/or PUO. A 5 mL sterile syringe was used to collect the sample, and the sample was transferred into a sterile container containing 45 mL of brain-heart infusion (BHI) broth. The ratio of blood to broth used was 1:9 [35]. Blood samples were collected using aseptic techniques.

Widal agglutination test: Commercially prepared colored antigens were used as *S.* Typhi O and H antigens. Cromatest antisera Widal kits containing O and H antigens of *S.* Typhi and *S.* Paratyphi A, B, and C were used to carry out slide agglutination test. Patients' sera were tested for agglutinins against each of the different *Salmonella* suspensions. This test was done according to the manufacturer's procedures and instructions stated in the manual. Positive results (reactive) were indicated by a macroscopically visible agglutination degree of 1:160. Negative results (non-reactive) were indicated by a smooth suspension with no visible agglutination as shown by negative control. An antibody titer of 1:160 or more was considered as positive [36].

Bacterial isolation: For this purpose**,** 3 mL of venous blood samples aseptically obtained from febrile patients were inoculated into bottles containing 27 mL of BHI broth (HIMEDIA, Mumbai, India). The bottles were incubated at 37 °C aerobically for 7 days. Turbid bottles were sub-cultured on blood agar, xylose lysine agar (XLD) (HIMEDIA, Mumbai, India), and MacConkey agar (MCA) (Lab M, Lancashire, UK) and incubated at 37 °C for 18-24 hours. For negative blood samples in BHI broth, sub-cultures were repeated daily for 7 consecutive days, after which the samples were finally discarded. All isolates were picked up and subjected to Gram-staining [37]. All bacterial isolates were further identified using an API 20E identification system (Institut M´erieux, Marcy l'Etoile, France). The profile code generated was used to identify all bacterial isolates in the API web database.

Antimicrobial susceptibility testing: All *Salmonella* isolates and other bacterial pathogens were investigated for their *in vitro* susceptibility to 10 commonly prescribed antibiotics in Nigeria by disk diffusion method in line with the guidelines established by the Clinical and Laboratory Standards Institute (CLSI, 2021) [38]. The following antibiotic disks were used in antibiotic susceptibility testing: ceftazidime (CAZ, 30 μg), cefotaxime (CTX, 30 μg), gentamicin (GEN, 10 μg), ciprofloxacin (CPR, 5 μg), ofloxacin (OFL, 5 μg), augmentin (AUG, 30 μg), nitrofurantoin (NIT, 300 μg), ampicillin (AMP, 10 μg), doripenem (DOR, 10 μg), and ertapenem (ETR, 10 μg) (Oxoid, Hampshire, UK). Plates containing antibiotic disks and bacterial isolates were incubated aerobically at 37 °C for 18–24 hours. The diameters of inhibition zones were measured in millimeters with a ruler and compared with the zone interpretation chart [38]. *Escherichia coli* American Type Culture Collection (ATCC) 25922 was used as a control. Multidrug resistance (MDR) phenotype was defined in this study as resistance to three or more classes of antibiotics.

DNA extraction

DNA extraction from bacterial isolates: Stock cultures of *Salmonella* isolates previously stored at 4 °C were sub-cultured on BHI culture plates. The inoculated plates were incubated at 37 °C for 18-24 hours. The DNA extraction was carried out using a QIAamp DNA mini kit (Qiagen, Stockach, Germany) according to the manufacturer's instructions. Briefly, fresh bacterial isolate (18-24 hours culture) was removed from the culture plate and suspended in 180 μL of ATL buffer. Then 20 μL of proteinase K was added, mixed, and incubated at 56 °C for 2 hours. After incubation, 200 μL of AL buffer was added and mixed by pulse-vortexing for 15 seconds. The mixture was further

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incubated at 70 °C for 10 min, then 200 μL of ethanol (96%) was added and mixed by pulse-vortexing for 15 seconds.

The mixture was carefully dispensed into labeled QIAamp mini spin columns. The column was placed in a 2 mL collection tube and centrifuged at 6000 rpm for 1 min after capping. The QIAamp mini spin column was removed and placed in another clean 2 mL collection tube, and the tube containing the filtrate was discarded. Then 500 μL of AW1 buffer was added to the QIAamp column and centrifuged at 6000 rpm for 1 min after capping. The QIAamp mini spin column was removed and placed in another clean 2 mL collection tube, and the tube containing the filtrate was discarded. Then 500 μL of AW2 buffer was added to the QIAamp mini spin column and centrifuged at full speed (20,000 rpm) for 3 min. The QIAamp mini spin column was removed and placed in a clean 1.5 mL microcentrifuge tube, and the filtrate in the collection tube was discarded. The QIAamp mini spin column was opened, and 200 μL of AE buffer was added, and the mixture was incubated at room temperature for 1 min and then centrifuged at 6000 xg for 1 min. The QIAamp mini spin column was finally discarded, and the filtrate was collected as DNA extract.

Real-time PCR (qPCR): Real-time PCR rotor-gene Q 2plex (Qiagen, Germany) was used to amplify and detect *Salmonella* virulence gene *invA* [39-41]. The used oligonucleotide sequences specific for the detection of *inv*A in *Salmonella* and *Citrobacter freundii* as previously used by Choudhury et al. (2016) [39], Moissenet et al. (2011) [40], and Tennant and Levine (2015) [41] are shown in Table 1. All qPCR assays were performed on a Qiagen Q 2plex real-time PCR system (Qiagen, Germany) with 36-well reaction tubes. The reaction was performed using 12.5 μL of 2x SYBR®Green Taq PCR master mix (Qiagen, Germany) dispensed into 0.2 mL PCR tubes. Then 1.5 μL of forward and reverse primers were added, and then 10.5 μL of nucleic acid-free water was added. Finally, 1.5 μL of bacterial DNA was added to the mixture and amplified using the following protocol: initial denaturation at temperature of 95 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 60 s, annealing at 64 °C for 30 s, and extension at 72 °C for 30 s. The fluorescent reporter signal was normalized against the internal reference dye (ROX) signal, and the threshold limit setting was performed in automatic mode. No template control using nucleic acid-free water (Biosystem, USA)) was included in any reac-

Table 1) Primers used in this study

Primers	Oligonucleotide Sequence	Accession Number	References
*inv*A-F	5'- ACCACGCTCTTTCGTCTGG-3'	AE006468.1	(39)
*Inv*A-R	5'- GAACTGACTACGTAGACGCTC-3'	AE006468.1	
CTX-M-3-F	5'-GGTTAAAAAATCACTGCGTC-3'	AY044436	(40)
CTX-M-3-R	5'-TTACAAACCGTCGGTGACGA-3'	AY044436	
ST-F	5’-GGAGTCGCCGTTTTTAGACA-3’	AY649698	(41)
ST-R	5’-TCCTTCAGCCAGCAGAGAAT-3’	AY649698	

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tion. Only signals generated in SYBR®Green qPCR analysis, which displayed amplification above the threshold level, were considered positive, while those signals below the threshold level were considered negative.

Findings

A total of 300 blood samples were evaluated in this study, of which 44 (14.7%) samples were positive in Widal agglutination test with a reciprocal titer of 160. The frequency distribution of positive Widal tests in different hospitals investigated is shown in Table 2. As shown in this table, more positive tests were recorded in LAGH for both H and O antigens. A total of 55 bacterial isolates were recovered from positive blood culture samples of the same 300 hospitalized febrile patients. Among which, four *Salmonella* isolates were identified, indicating a 7.27% prevalence for *Salmonella*-associated bacteremia. These four *Salmonella* isolates were previously confirmed by the API 20E kit with profile index 6504752. The proportion of other bacterial pathogens isolated from positive culture samples included: *Klebsiella pneumoniae* 41.82% (23 of 55), *Proteus* 23.64% (13 of 55), *Serratia* 12.73% (7 of 55), *E. coli* 10.91% (6 of 55), and *Citrobacter* 3.64% (2 of 55). Out of four *Salmonella* isolates obtained in this study, three isolates were obtained from public hospitals, and one isolate was obtained from a private hospital. Among bacterial pathogens isolated in this study, *K. pneumoniae* had the highest prevalence (41.82%), while *Citrobacter* had the lowest prevalence (3.64%). Similarly,

Table 2) Prevalence of bacterial isolates collected from both public and private hospitals in Lagos, Nigeria by sample source

Characters	**Public Medical Centre**	**Private Medical Centre**				**TOTAL**				**TOTAL**	
Sample source	**LASUH**	**AGH**		**LFMC**		**CH**		**CH**			
Clinical sample	Blood (%)	Blood (%)		Blood(%)		Blood (%)		Total (%)		Total (%)	
Number of samples	100	108		54		38		300(100)		300(100)	
Number of Widal positive	15	16		8		5		44(14.67)		44(14.67)	
Number of culture positive	19(34.5)	16(29.0)		11(20)		9(16.36)		55(18)		55(18)	
Samples sources		*a	*b	*a	*b	*a	*b	*a	*b	*a	*b
Salmonella isolates	*Salmonella spp.*	1	0	2	0	1	0	0	0	4	0
Other bacterial isolates	*Klebsiella spp.*	5	2	4	3	2	3	0	4	11	12
	E. coli	1	1	0	1	1	0	1	1	3	3
	Proteus spp.	3	2	2	1	1	2	0	2	6	7
	Citrobacter spp.	1	0	1	0	0	0	0	0	2	0

*Key: LASUTH: Lagos State University Teaching Hospital; AGH: Alimosho General Hospital; LFMC: Life Fount Medical Centre; spp: species; CH: Crest Hospital; *a: isolates from widal positive agglutination test; *b: isolates from widal negative blood samples*

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Table 3) Comparison of culture, biochemical, and PCR identification of *Salmonella* isolates recovered from hospitalized febrile patients

S/N	I/c	Isolate	Blood Culture	Analytical Profile Index Code	*S.T* gene (*S. Typhi*)	Culture positive *Salmonella* isolate	qPCR
							InvA gene detection Cycle threshold
1	S1	*S. Typhimurium* (control)	+	6706752	-	+	31.09
2	S2	*Salmonella sp*	+	6704752	-	+	31.60
3	S3	*Salmonella sp*	+	6504752	-	+	28.06
4	S4	*Salmonella sp*	+	6504752	+ (29.81)	+	23.59
5	S5	*Salmonella sp*	+	6504552	-	+	27.15

S/n Serial Number, I/c Isolate code

Table 4) Antibiotic resistance of Salmonella and other bacterial species isolated from blood samples

Isolate	Antibiotics									
	CAZ	CRX	GEN	CPR	OFL	AUG	NIT	AMP	ETR	DOR
Salmonella N (%)	4 (100)	4 (100)	2 (50)	2 (50)	1 (25)	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)
Citrobacter N (%)	2 (100)	2 (100)	1 (50)	2 (100)	0 (0)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)
Klebsiella N (%)	23 (100)	23 (100)	15 (65)	10 (43)	5 (22)	23 (100)	23 (100)	23 (100)	23 (100)	23 (100)
E. coli N (%)	6 (100)	6 (100)	6 (100)	1 (16.67)	0 (0)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)
Proteus N (%)	13 (100)	13 (100)	0 (0)	0 (0)	0 (0)	13 (100)	13 (100)	13 (100)	13 (100)	13 (100)
Serratia N (%)	7 (100)	7 (100)	0 (0)	0 (0)	0 (0)	7 (100)	7 (100)	7 (100)	7 (100)	7 (100)

Keys: CAZ: Ceftazidime; CRX: Cefuroxime; GEN: Gentamicin; CPR: Ciprofloxacin; OFL: Ofloxacin; AUG: Amoxycillin/ Clavulanate; NIT: Nitrofurantoin; AMP: Ampicillin; ETR: Erterpenem,; DOR: Doripenem

bacterial isolates detected in Widal agglutination test-negative blood samples included 12 *Klebsiella*, seven *Proteus*, three *E. coli,* two *Serratia,* and two *Citrobacter* isolates. No *Salmonella* species were found in Widal-negative culture samples. In this study, different prevalence rates were recorded for *Salmonella* isolates based on the identification/ detection methods used; 14.7% (44 of 300) by the Widal test, 1.3% (4 of 300) by the conventional culture technique, and 1.3% (4 of 300) by the API 20E kit. The qPCR results of *Salmonella* isolates and corresponding blood samples for *inv*A gene primer sets revealed

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Table 5) Antibiotic resistance patterns of Salmonella and other bacterial pathogens isolated from blood samples

Resistance pattern	Pattern code	No of antibiotics	*S. Typhi (4)*	*Citrobacter (2)*	*Klebsiella (23)*	*E. coli (6)*	*Proteus (13)*	*Serratia (7)*
CAZ CRX GEN CPR OFL AUG NIT AMP ETR DOR	A	10	1	0	5	0	0	0
CAZ CRX GEN CPR AUG NIT AMP ETR DOR	B	9	2	1	15	1	0	0
CAZ CRX CPR AUG NIT AMP ETR DOR	C	8	2	1	10	0	0	0
CAZ CRX GEN AUG NIT AMP ETR DOR	D	8	4	1	15	6	0	0
CAZ CRX AUG NIT AMP ETR DOR	E	7	4	2	23	6	13	7

Keys: CAZ: Ceftazidime; CRX: Cefuroxime; GEN: Gentamicin; CPR: Ciprofloxacin; OFL: Ofloxacin; AUG: Amoxycillin/ Clavulanate; NIT: Nitrofurantoin; AMP: Ampicillin; ETR: Ertapenem; DOR: Doripenem

the presence of *inv*A in 100% of *Salmonella* isolates and their corresponding culture-positive blood samples. ST-F/R primers specific for *S.* Typhi as well as CTX-M-F/R primers for *C. freundii* were also used but not detected in this study. *Salmonella* isolates were identified in all age groups of 1-10 years, indicating that these individuals are at higher risk of *Salmonella* infection. Similar results were observed for *Klebsiella* species. *Salmonella* isolates and other bacterial pathogens were more prevalent in females than in males. The results of *in-vitro* antibiotic susceptibility testing (AST) revealed that 100% of *Salmonella* isolates were resistant to ceftazidime, cefotaxime, augmentin, nitrofurantoin, ampicillin, ertapenem, and doripenem. Also, 50% of the isolates were resistant to ciprofloxacin and gentamicin, and a low level of resistance (25%) was observed to ofloxacin. All bacterial isolates exhibited resistance to 7 out of 10 different antibiotics examined and were identified as multidrug resistant (MDR). According to the results, five different resistance profiles were observed in this study, including A) CAZ-CRX-GEN-CPR-OFL-AUG-NIT-AMP-ETR-DOR, B) CAZ-CRX-GEN-CPR-AUG-NIT-AMP-ETR-DOR, C) CAZ-CRX-CPR-AUG-NIT-AMP-ETR-DOR, D) CAZ-CRX-GEN-AUG-NIT-AMP-ETR-DOR, and E) CAZ-CRX-AUG-NIT-AMP-ETR-DOR; however, CAZ-CRX-AUG-NIT-AMP-ETR-DOR (E) was the most frequently observed resistance pattern.

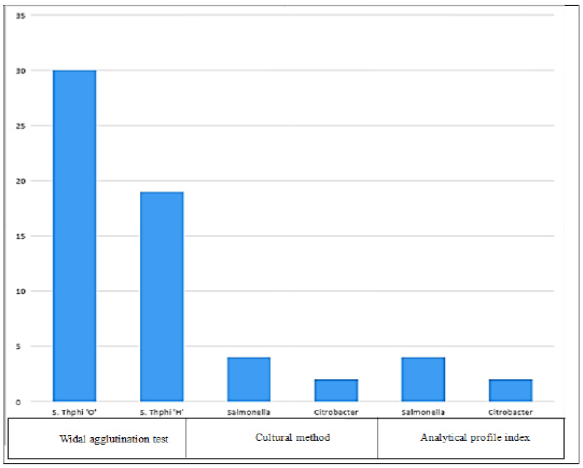

Figure 1) Comparison of three different identification methods used to identify *salmonella* and *Citrobacter*

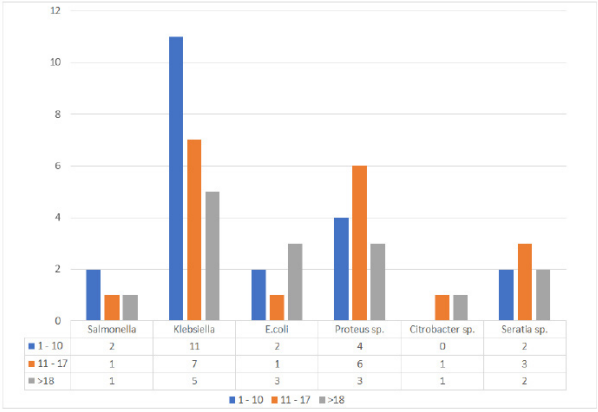

Figure 2) Prevalence of *Salmonella* and other pathogens by age in febrile patients in Lagos, Nigeria

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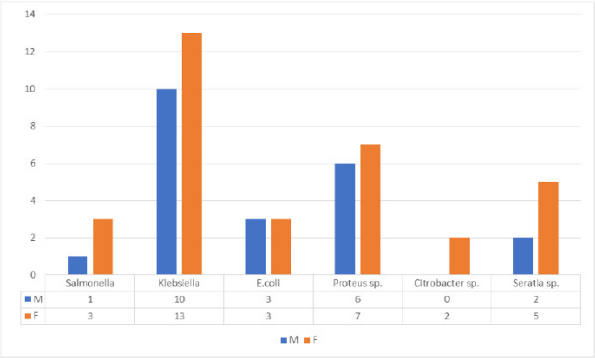

Figure 3) Prevalence of *Salmonella* and other pathogens by sex in febrile patients in Lagos, Nigeria

Discussion

In Nigeria, bacteremia in febrile individuals is frequently misdiagnosed and treated as salmonellosis. In this study, out of 300 blood samples, 44(14.67%) samples were positive in Widal agglutination test with a cut-off titer of 1:160. This result is in agreement with the finding of a similar study by Farhan and colleagues (2018) [42], reporting 17(14.16%) positive Widal tests among 120 blood samples of typhoid patients in Baghdad, Iraq. Farhan and colleagues (2018) [42] reported the weak specificity of Widal serological agglutination test in the diagnosis of typhoid fever. The reason for the slight disparity between these two studies might be associated with differences in sample size, implementation techniques, interpretation, sensitivity and specificity of assays used in diagnosis, and other factors such as patients' exposure period to infectious agents and immunological responses [36, 43-46]. The culture results confirmed using the API 20NE rapid kit indicated that 55 positive samples contained six bacterial genera. This result corresponds with the finding of another study by Nahab and colleagues (2018) [47], in which the API 20E system was reported to be a rapid biochemical kit for the confirmation of culturally characterized isolates, especially organisms with close morphological and biochemical characteristics, such as members of *Enterobacteriaceae* that were identified in this study. Specifically, 55 bacterial isolates obtained in this study comprised six bacterial genera, including *Salmonella* (n=4, 7.27%), *Klebsiella* species (n=23, 41.82%), *E. coli* (n=6, 10.91%), *Proteus* species (n=13, 23.64%), *Serratia* species (n=7, 12.73%), and *Citrobacter* species (n=2, 3.64%). This result is consistent with the result of another study by Oluyege et al. (2015) [48], reporting the presence of nine different bacterial genera from the *Enterobacteriaceae* family in blood samples and *Klebsiella* species as the most prevalent (n=36, 36.66%) bacterial genus isolated. In line with this study findings, Akinyemi et al. (2005) [49] in Lagos, Nigeria reported the presence of *S.* Typhi (16.2%), *Proteus* species (14.9%), *E. coli* (12.2%), *Klebsiella* species (9.5%), and *Plasmodium* species (44.6%) in samples of 74 patients with pyrexia of unknown origin. Unfortunately, *Plasmodium* detection was not carried out in this research, which is one of the limitations of this study. Similarly, Akinyemi et al. (2021) [16] isolated other pathogens in their study with *Proteus* spp. (49.8%) as the most frequently encountered pathogen, followed by *K. pneumoniae* (19.8%), *K. oxytoca* (18.5%), and *Citrobacter* spp. (2.5%). These observations support the growing evidence that invasive *Salmonella* infections do not always present with a distinct clinical picture, and that other bacterial infections (as revealed in this study) as well as viral and protozoan infections may have similar presentations [50, 51]. In recent decades, febrile individuals in Lagos, Nigeria have been tested and treated for typhoid fever based on symptoms and Widal agglutination test without resorting to culture isolation of etiological agents. This study revealed a prevalence rate of 7.3% for *Salmonella*-associated bacteremia. This result contrasts with the finding of a similar study conducted in Lagos about 15 years ago, reporting a higher prevalence

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rate of 16.2% for *Salmonella* bacteremia [12]; also, in another study conducted less than 10 years ago, 30% of *Salmonella* isolates in Lagos were recovered from patients with persistent pyrexia [52]. In the work conducted by Akinyemi et al. (2018) [10], the range of *S*. Typhi positivity was reported to be from 7 to 18% in Lagos and from 0.8 to 2.4% in Abuja during the years 2008 to 2017. In Warri, Delta State, Nigeria, a prevalence rate of 14% was reported for *Salmonella* infections by Ehwarieme (2011) [53]. The decrease in the prevalence of *Salmonella* infections as observed in this study may be attributed to increased sanitation because *Salmonella* infections are spread via the fecal-oral route. It may also be due to the increase in public awareness by the Lagos State government on proper method of hand washing and sanitizing using hand sanitizers, which has become imperative since the coronavirus outbreak. Similarly, Popoola et al. (2019) [54] isolated other common bacteremia-associated bacteria, including NTS, *E. coli,* and *K. pneumoniae* along with other few bacterial pathogens and invasive *Salmonella* (4%) from febrile patients attending four hospitals in Ibadan. Other studies that have reported similar prevalence rates for invasive *Salmonella* in blood samples include: Begum et al. (2009) [55] in Bangladesh, Oluyege et al. (2015) [48] in Ekiti State, Nigeria, Akinyemi et al. [56-59] in Lagos, Nigeria, Devrim et al. (2008)[36] in Ankara, Turkey, etc.

Polymerase chain reaction using specific target gene primers is considered as a good method for the detection of *Salmonella* species. It is worth noting that *inv*A gene contains sequences unique to *Salmonella* isolates and is considered as a suitable PCR target with valuable diagnostic applications [6]. In this study, the detection rate of *inv*A gene in four *Salmonella* isolates was 100%. This result confirms the high specificity and sensitivity of PCR using the *inv*A gene as a target for *Salmonella* bloodstream infection diagnosis compared to conventional culture and Widal agglutination methods. In this study, four *Salmonella* isolates were identified with profile indices 6504752 and 6104512 of the API 20NE kit. This study corroborated the finding of Ammar et al. (2016) [60], who also reported 100% detection of the *inv*A gene in *Salmonella* isolates. However, some studies have reported a slightly lower detection rate for the *inv*A gene in *Salmonella* isolates from febrile patients' blood samples, such as 90.15% in Sharma and Das (2016) [61], 75% in Akinyemi et al. (2021) [2], and 50% in Kadry et al. (2019) [62]. The reason for this difference may be attributed to differences in study methods, specific nucleotide sequences, sample size, and geographical location.

The results of *in-vitro* antimicrobial susceptibility testing conducted on all *Salmonella* isolates revealed a high level of resistance (100%) to ceftazidime, cefotaxime, gentamicin, nitrofurantoin, ampicillin, ertapenem, and doripenem. Moreover, 50% of *Salmonella* isolates exhibited resistance to ciprofloxacin and gentamycin, and 25% showed resistance to ofloxacin. These results are contrary to the findings of Akinyemi et al.'s (2005) study [49], where 100% sensitivity was exhibited by invasive *Salmonella* isolates to ciprofloxacin and ofloxacin. This is because a decade and half ago, antibiotics were still strictly prescribed by clinicians and less abused due to the high cost of their procurement in Nigeria. Multidrug resistance (MDR) and reduced fluoroquinolone susceptibility is an important issue in Nigeria [2] and other West African countries such as Malawi and Tanzania [13]. Since the introduction of antibiotics over a century ago, there have been increasing reports of antibiotic resistance [29, 63]. Furthermore, the 100% resistance to cefotaxime recorded in this study is contrary to the finding of a study by Zhan et al. (2019) [64] in China, reporting low resistance of *Sal-*

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monella isolates (8.4%) to cefotaxime. The reason for this may be related to the ban of antibiotics usage in veterinary medicine and their strict usage for humans. Thus, the government should place restrictions on arbitrary purchase of antibiotics. When a patient is treated extensively with antibiotics, some resistant strains may survive and flourish because of their competitive advantage over non-resistant strains.

In this study, five different resistance patterns (A to E) were observed among six bacterial genera isolated. All bacterial pathogens exhibited the resistance pattern E. Also, some strains of *Klebsiella* species were found to exhibit all the resistance patterns (A to E). Akinyemi et al. (2021) [16] reported that *K. pneumoniae* exhibited the resistance pattern CAZ-CRX-OFL-CIP-COT which is similar to the resistance pattern E in this study. It is noteworthy that four (100%) *Salmonella* isolates exhibited the resistance patterns D and E, while two (50%) isolates exhibited the resistance patterns B and C. This result is in line with the finding of Akinyemi et al. (2021) [2], reporting the resistance pattern CAZ-CTX-GEN-CPR-OFL-AUG-NIT-AMP-ETR-DOR, similar to the resistance pattern A in this study, in three *Salmonella* isolates.

Conclusion

This study revealed that the Widal agglutination test is unreliable for the diagnosis of *Salmonella* bacteremia as other bacteria in the *Enterobacteriaceae* family mimic *Salmonella* agglutinins which form the core of Widal test kit. It was also revealed that the culture method remains as the gold standard for bacterial diagnosis, and qPCR using the *inv*A primer set was found to be highly specific for the detection of *Salmonella*. All the bloodstream bacterial pathogens in this study were MDR; thus, there is a need for continuous evaluation of these antibiotics in medical settings. Further molecular studies on these bacterial isolates is essential.

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Ethical permissions: This study was approved by the Human Research and Ethics Committee of Lagos State University Teaching Hospital with reference number LREC/06/10/1381 in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Conflicts of interests: The authors declare that there is no conflict of interest.

Authors' contributions: MOA collected blood samples and performed sample analysis. KOA conceived and designed the study, SOA and COA contributed to sample analysis and manuscript writing. ROA, WOS, and AAL contributed to the manuscript writing. MOA, KOA, and SOA performed data analysis. KOA edited the manuscript. All authors approved the final copy of the manuscript.

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