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ANTIBIOTICS RESISTANCE PROFILE AND *BLA*-TEM GENES IN *Salmonella* SEROTYPES ASSOCIATED WITH TYPHOIDAL SALMONELLOSIS IN SELECTED HOSPITALS IN KATSINA STATE, NIGERIA

Kamilu, Isah[1] and *Mukhtar, G.L.[2]
[1]Department of General studies, Kankia Iro School of Health Technology, Kankia, Katsina State, Nigeria.
[2]Department of Microbiology, Umaru Musa Yar'adua University, P.M.B 2218 Katsina, Katsina State, Nigeria
Corresponding author: *Mukhtar G.L, mukhtar.gambo@umyu.edu.ng

ABSTRACT
Typhoidal salmonellosis is common illness and remains a serious public health problem in many regions of the world especially in developing countries, where it became endemic. About 80% of deaths due to Salmonella serovars are found in Africa. Recently multi-drug resistant (MDR) strains of Salmonellae have emerged apparently due to the extensive use of antimicrobial agents in clinical and veterinary practices. The present research work was carried out to evaluate the antibiotic resistance profile and detect beta lactamase genes present in Salmonella serotypes associated with enteric fevers in selected hospitals in Katsina State, Nigeria. A total of 300 blood samples were collected from febrile patients, followed by isolation, biochemical and serological characterization of recovered isolates. Moreover, the isolates were evaluated for antibiotic susceptibility using the Kirby-Bauer disc diffusion method. The presence of β-lactamase (bla-TEM) resistance genes was evaluated by polymerase chain reaction. Biochemical characterization revealed the presence of Salmonella Typhi and Salmonella paratyphi A from 14 samples only. Result of serotyping (slide agglutination) of the isolates indicate that nine (09) were Salmonella Typhi serotypes belonging to D serogroup while five (05) were Salmonella Paratyphi A belonging to the A serogroup. The antibiogram pattern revealed that all the Salmonella enterica serovar Typhi were 100% resistance to Sulfomethaxazole- trimethoprim, Chloramphenicol, Cephalexin, Ofloxacin, Gentamicin, and Penicillin G and followed by 88.9% resistance to Ampicillin. The PCR analysis confirmed the presence of bla-TEM gene in eight (8) serovars of Salmonella enterica serovar Typhi only. Findings of the study have indicated a high prevalence of antimicrobial resistance among Salmonella serovars especially the Salmonella enterica serovar Typhi. This implies a great threat to public health, therefore appropriate measures and new guidelines should be established to address the rational use of antibiotics and to prevent their abuse.
Keywords: Salmonella, serotyping, antimicrobial resistance, bla-TEM, PCR

INTRODUCTION

The genus *Salmonella* are Gram negative, non-sporing rods, and do not ferment lactose. They are facultative anaerobic rods and belong to the family Enterobacteriaceae. All Salmonella species are motile, with the exception of *Salmonella pullorun* and *Salmonella gallinarum* (Graham 2009). There are about 2500 Salmonella serovars discovered, the majority of which belong to *Salmonella enterica* subsp. *enterica*, which causes the majority of Salmonella infections in humans (Youssef *et al.*, 2021).

Salmonella infections are endemic in Nigeria. Literatures on human *Salmonella enterica* serovars in Nigeria had documented about Fifty-three (53) *Salmonella* serotypes. Out of these, 39 serotypes were associated with *Salmonella*-bacteraemia and 31 serotypes with *Salmonella*-gastroenteritis. *Salmonella typhi* remains the commonest serotype causing bacteraemia and gastroenteritis. While *S. typhimurium,* was mostly implicated in invasive non-typhoidal salmonellosis, and followed *S. enteritidis* among others (Akinyemi *et al.*, 2021).

Salmonella spp. is spread mostly through the fecal-oral route, which involves contaminated food or water. Salmonella outbreaks are typically linked to contaminated water and the consumption of contaminated animal-based foods such as fish, poultry, meat, and milk (Akinyemi *et al*., 2018).

Biochemical and serologic tests can be used to identify four serotypes of salmonellae that cause enteric fever in the clinical laboratory. These include *Salmonella paratyphi A* (serogroup A), *Salmonella paratyphi B* (serogroup B), *Salmonella choleraesuis* (serogroup C1), and Salmonella Typhi (serogroup D). Because of their clinical importance, these serotypes are routinely monitored. This enables public health officials track and assess Salmonella infection epidemiology (Geo, *et al*., 2013).

Chloramphenicol, Ampicillin, Tetracycline, third-generation Cephalosporin, Gentamycin, Quinolones, and Trimethoprim/Sulfamethoxazole are the antibiotics most commonly used in Nigeria to treat typhoid and other Salmonella-related infections (El-Sayed *et al.,* 2012, Akinyemi, *et al.,* 2015). Multi-drug resistant (MDR) strains of Salmonellae have emerged apparently due to the extensive use of antimicrobial agents in humans and veterinary practices (Miriagou *et al.,* 2006).

Enteric salmonellosis remains a serious public health problem in many developing countries, and the emergence of multidrug resistant Salmonella serovars significantly hinders the success of antibiotic therapy. Therefore precise characterization of the serovars and their susceptibility to antibiotics in use is needed. Hence the aim of the study was to isolate and characterize Salmonellae into different serotypes, to evaluate antibiotic resistance profile and detect the β-lactamase resistant gene among Salmonella serotypes from patients with enteric fevers in selected hospitals in Katsina State, Nigeria.

MATERIALS AND METHODS

Isolation of *Salmonella* species

A total of 300 blood samples were collected from febrile patients, who were presumptively diagnosed for enteric fevers from three hospitals in Katsina State, Nigeria (General hospital Katsina, General hospital Daura and General hospital Baure). The samples were processed via broth enrichment method using thioglycollate broth, followed by subculturing on Deoxycollate Citrate Agar (Oxoid U. K). Cultures were incubated over night at 37°C and colonies growing on the plate were further sub cultured onto Bismuth Sulfide Agar and incubated overnight at 37°C. Colonies growing on the Bismuth Sulfide Agar plates that exhibit morphological and biochemical properties typical of *Salmonella* spp were selected for Gram staining and biochemical tests Cheesbrough (2006).

Biochemical characterization and identification

Salmonella species isolated were subjected to Gram staining and various biochemical tests including motility test, urease, indole, methyl-red, vogues proskauer, citrate, triple sugar iron agar tests as described by Cheesbrough (2006).

Serological Identification

Bacterial isolates that were previously identified as Salmonella were characterize into different serotypes based on the Kauffman–White classification scheme. The Salmonella isolates were subjected to serological identification using slide agglutination test as described by Abdullahi, (2010). A drop of physiological saline was placed on two separate sections of a glass slides. By using a sterile wire loop, a portion of growth from the surface of TSI was removed, mixed and emulsified in each drop of physiological saline on the slide. A small drop of Vi antiserum (specific for detection of *Salmonella* Typhi serotypes belonging to D serogroup) was added to the first suspension. The second suspension served as control. The suspension and antiserum were mixed and then the slide was tilted back and forth to observe for agglutination. The procedure was repeated however using the O antiserum for the detection of *Salmonella* Paratyphi A belonging to the A serogroup.

Antibiogram studies

Antibiotic susceptibility test was carried out using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar plates. All the Salmonella serovars were screened against seven antibiotics namely; Ofloxacin (5µg) Sulfomethaxazole-trimethoprim (25µg), Chloramphenicol (30µg), Cephalexin (30µg), Gentamicin (10µg), Ampicillin (10µg) and Penicillin G (10µg) Oxoid, UK. After 24 hours of inoculation at 37^{o}C, zones of inhibition were measured and recorded in millimeters (mm). *Escherichia coli* ATCC 25922 was used as control. Inhibition zones were interpreted according to the guidelines established by the National Committee for Clinical Laboratory Standards (CLSI, 2014).

PCR detection of antibiotic resistance genes

i. DNA Extraction

The DNA was extracted according to method described by Turkyilmaz *et al*. (2009). Colonies of Salmonella from overnight culture were transferred to a 1.5ml tube containing 100ul sterilize lysis solution (1ml MTris_HCl, 22.5µl 1 GEPAL-Sigma, 22.5µL Tween 20-biorad 220µl protease K (10mg/ml,8.8µl DiH_2O). The tubes were vortexed and incubated for 10 min at 60-95^oC for 10 mins and cooled at 4^oC. The lysate was centrifuge for 5mins, Phenol:chloroform(1:1) treatment was followed with the clear supernatant. The DNA was precipitated with equal volume of chilled isopropylalcohol and DNA pellet dissolved in 100 µL of TE buffer.

ii. PCR amplification of *bla*-TEM genes and gel electrophoresis

PCR amplification was carried out using master-mix kit containing the Taq (*Thermos aquaticus*) DNA polymerase, PCR buffer,dNTP (dATP, dTTP, dGTP, dCTP) and $MgCl_2$. A 25 µl reaction mixture which consists of DNA template, master-mix and primers was prepared. The specific primer sets used in the PCR assay were; CATTTCCGTGTCGCCCTTAT (F) and TCCATAGTTGCCTGACTCCC (R) for the *bla*-TEM gene with 793 bp in size as described by Turkyilmaz *et al.* (2009).

After amplification, PCR product was separated by electrophoresis on 1.5% agarose gel stained with ethidium bromide. A 100bp ladder was used as molecular marker.

RESULTS

In this study, a total of 14 (4.7%) Salmonella isolates were obtained from the 300 blood samples following culture and biochemical characterization from febrile patients from the selected hospitals who were presumptively diagnosed for enteric fevers (Table 1).

Serological characterization of the recovered Salmonella isolates show that 9 (3.0%) were *Salmonella* Typhi serotypes belonging to D serogroup while 5 (1.7%) of the isolates were *Salmonella* Paratyphi A belonging to the A serogroup while (Table 2).

Table 1: Prevalence of *Salmonella* Species among Patients with Enteric Fevers Attending Some Selected Hospitals in Katsina State, Nigeria

Hospitals	No. of samples examined	No. of Salmonella isolates recovered
General hospital Katsina	120	3 (1.0%)
General hospital Baure	100	7 (2.3%)
General hospital Daura	80	4 (1.3%)
Total	**300**	**14 (4.6%)**

Table 2: Serological Characterizations of the *Salmonella* Isolates

Isolates codes	Serological profile			
	Vi Antiserum	O Antiserum	Serogroup/serovar	Prevalence
001	+	-	Serogroup D/Salmonella Typhi	
002	+	-	Serogroup D/Salmonella Typhi	
003	+	-	Serogroup D/Salmonella Typhi	
004	+	-	Serogroup D/Salmonella Typhi	*Salmonella* Typhi
005	+	-	Serogroup D/Salmonella Typhi	**9 (3.0%)**
006	+	-	Serogroup D/Salmonella Typhi	
007	+	-	Serogroup D/Salmonella Typhi	
008	+	-	Serogroup D/Salmonella Typhi	
009	+	-	Serogroup D/Salmonella Typhi	
010	-	+	Serogroup A/Salmonella paratyphi	
011	-	+	Serogroup A/Salmonella paratyphi	*Salmonella paratyphi*
012	-	+	Serogroup A/Salmonella paratyphi	**5 (1.7%)**
013	-	+	Serogroup A/Salmonella paratyphi	
014	-	+	Serogroup A/Salmonella paratyphi	

The antibiotic susceptibility test carried out showed that all the Salmonella enterica serovar Typhi exhibit 100% resistance to Sulfomethaxazole- trimethoprim, Chloramphenicol, Cephalex, Ofloxacin, Gentamicin, and Penicillin G and followed by 88.9% resistance to Ampicillin. However, the *Salmonella paratyphi* A serovar showed 100% resistance to Penicillin G only, and was 100% susceptible to Sulfomethaxazole- trimethoprim, Chloramphenicol, Cephalexin, Ofloxacin, Gentamicin and ampicillin (Figure 1).

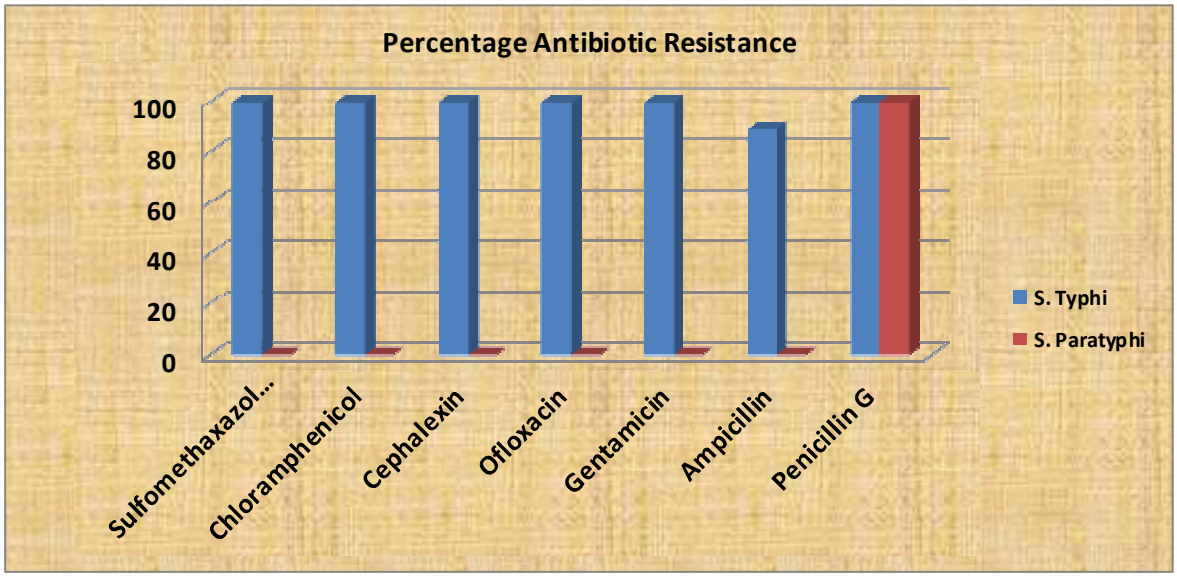

Figure 1: Antibiotic Resistance Profile of Salmonella serovars Among Patients with Enteric Fevers Attending Some Selected Hospitals in Katsina State, Nigeria.

The PCR analysis confirmed the presence of bla-TEM gene in eight (8) serovars of Salmonella enterica serovar Typhi only as shown in plate 1.

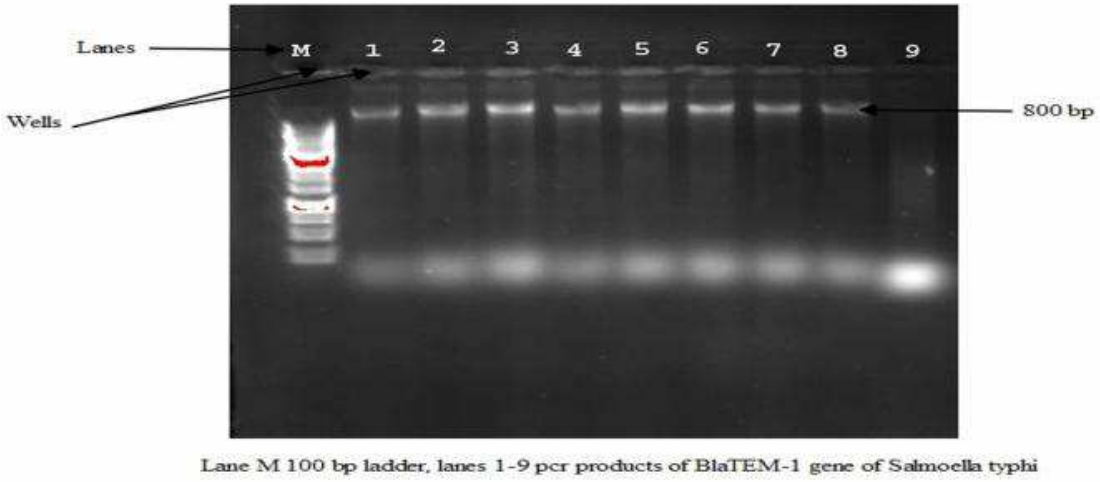

Plate 1: Agarose Gel Electrophoretogram showing Positive Bands for the blaTEM genes (793bp) of *Salmonella typhi serovar*
Key: Lane M = Marker (100-bp DNA ladder)
Lane 1-8 = PCR product of bla-TEM genes

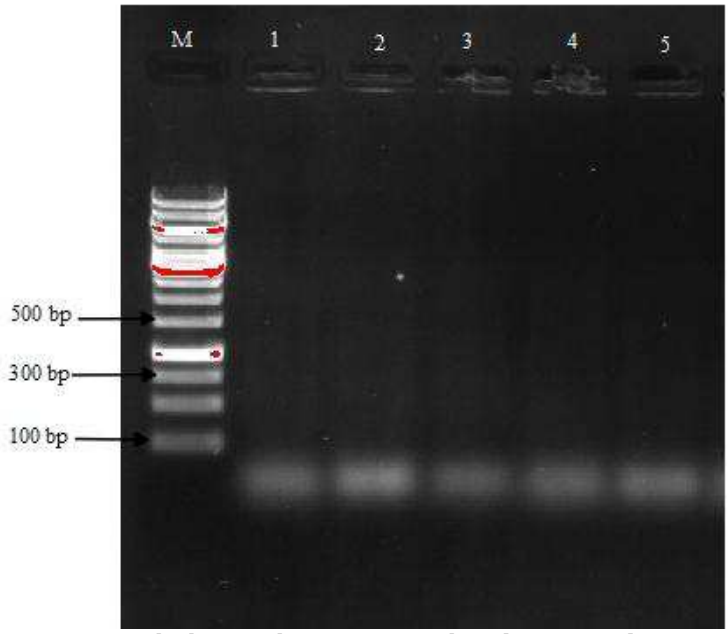

Plate 2: Agarose Gel Electrophoretogram showing Negative Bands for the blaTEM genes (793bp) of *Salmonella paratyphi serovar*
Key: Lane M = Marker (100-bp DNA ladder)
Lane 1-8 = PCR product of bla-TEM genes

DISCUSSION

Salmonella infections are endemic in developing countries of Africa. In this study, 4.7% occurrence of *Salmonella spp.* was observed among the study population. This is similar to the 5% occurrence of *Salmonella spp.* as reported by Mzungu *et al.* (2016). However, higher occurrences have been reported, Tesfahun *et al.* (2016) reported 10.8% occurrence of *Salmonella spp.* from diarrheic stools in a study conducted in Ethiopia while Kabir *et al.* (2007) reported 17% occurrence of *Salmonella spp.* from stool specimen of patients with gastroenteritis in a study conducted in Lagos, Nigeria.

Several studies have reported typhoid fever disease burden in Africa. Knowledge of the burden of disease is important in understanding the effects and trend of the disease on human health. In Egypt, Smith *et al*., (2016) reported as estimated incidence of typhoid fever of 59/100,000 persons/year. In a study conducted in Ghana, it was reported that typhoid fever was among the leading causes of outpatient illness, accounting for 0.92 % of hospital admissions. In Cameroon, a cross-sectional study was carried out to determine the prevalence of typhoid fever in 200 patients and typhoid fever was confirmed in 2.5 %. In a study that determines a population-based incidence of typhoid fever in an urban informal settlement and a rural area in Kenya, Breiman *et al.* (2012) reported very high rates of bacteraemic typhoid fever among the studied population. In Nigeria, a study of a 4-year cumulative prevalence of Salmonella infection reported a high prevalence of about 63.8 % typhoid in patients attending the General Hospital Etinan, Akwa Ibom State (Uttah, *et al.*, 2013). A study from Northwest Nigeria showed S. typhi to be the most frequently encountered, in 40.7 % of cases (Abdullahi *et al*., 2014).

Antibiotic-resistant Salmonellae is becoming a major public health concern especially in Africa; data from studies indicate an increase in the emergence of antibiotic-resistant strains. In this study, Salmonella enterica serovar Typhi exhibit 100% resistance to Sulfomethaxazole-trimethoprim, Chloramphenicol, Cephalexin, Oflaxacin, Gentamicin, and Penicillin G and followed by 88.9% resistance to Ampicillin. In a similar study, Abdullahi *et al.,* (2014) reported high resistance to Ampicillin (94.2 %), Chloramphenicol (72.8 %) and lower resistance (31.8 %) to Co-trimoxazole among Salmonella serotypes in Katsina State, Nigeria.

Multidrug resistance to the traditional first-line antimicrobial agents namely Ampicillin, Chloramphenicol and Trimethoprim–sulphamethoxazole are common among S. typhi (Smith *et al.,* 2016). This implies a major challenge to healthcare systems because effective treatment options for the disease is reduced, hence leading to complications and death.

The detection of *bla-TEM* resistance genes among the *Salmonella* Typhi *serovars* implies that these genes are most likely responsible for the phenotypic resistance observed, hence conferring resistance to the beta-lactam antibiotics- Penicillin G (100% resistance) and 88.9% resistance to Ampicillin.

CONCLUSION

The study identified high level of antimicrobial resistance among Salmonella serovars especially the Salmonella enterica serovar Typhi associated with enteric fevers among patients attending some selected hospitals in Katsina State, Nigeria This implies a great threat to public health, therefore appropriate measures and new guidelines should be established to address the rational use of antibiotics and to prevent their abuse.

REFERENCES

Abdullahi, B: Abdulfata, K, Wartu, J.R, Mzungu, I., Muhammad H.I.D and Abdulsalam, A.O. (2014): Antibiotic susceptibility pattern and characterization of clinical Salmonella serotypes in Katsina State. *African Journal of Microbiology Research*; 8:915-921

Abdullahi, M. (2010). Incidence and Antimicrobial Susceptibility Pattern of Salmonella species in Children attending some Hospitals in Kano metropolis, Kano State –Nigeria. *Bayero Journal of Pure and Applied Sciences*, 3(1): 202 - 206

Akinyemi, K.O., Iwalokun, B.A., Alafe, O.O., Mudashiru, S.A. and Fakorede, C. (2015) Bla CTX-M-1 Group Extended Spectrum Beta Lactamase-Producing Salmonella typhi from Hospitalized Patients in Lagos, Nigeria. *Infection and Drug Resistance*, 2015, 99-106. https://doi.org/10.2147/IDR.S78876

Akinyemi, K.O., Ajoseh, S.O., Iwalokun, B.A., Oyefolu, A.O.B., Fakorede, C.O., Abegunrin, R.O. and Adunmo, O. (2018). Antimicrobial Resistance and Plasmid Profiles of Salmonella enterica Serovars from Different Sources in Lagos, Nigeria. *Health,* 10, 758-772. https://doi.org/10.4236/health.2018.106058

Akinyemi, Kabiru O, Samuel O Ajoseh1, Christopher O Fakorede (2021). A systemic review of literatures on human *Salmonella enterica* serovars in Nigeria (1999-2018). *Journal of Infection in Developing Countries*; 15(9):1222-1235. doi:10.3855/jidc.12186

Breiman, RF, Cosmas L, Njuguna H, Audi A, Olack B, Ochieng JB et al (2012). Population-based incidence of typhoid fever in an urban informal settlement and a rural area in Kenya: implications for typhoid vaccine use in Africa. *PLoS One;* 7(1):e29119

Cheesbrough M. (2006). District Laboratory Practice in Tropical Countries. Part 2, Second edition. Cambridge University press PP.182-183.

CLSI (2014). Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: 15th informational supplement M100-S15. Wayne.

El-Sayed, M.A., Tawakol, W.M., Ragab, Y.M. and Amin, M.A. (2012). Phenotypic Detection of Potential Antimicrobial Resistance among Environmental Salmonella Species. *International Journal of Microbiology Research*, 3, 38-45.

Geo. F. Brooks, Karen C. Carroll, Janet S. Butel, Stephen A. Morse, and Timothy A. Meitzner (2013). *Jawetz, Melnick, & Adelberg's Medical Microbiology*, Twenty-Sixth Edition. The McGraw-Hill Companies, Inc. USA.

Graham S.M., (2009). Non-typhoidal *Salmonella* a management challenge for children with acquired invasive disease in Tropical African countries. *Lancet,* 372; 267-269

Kabir OA, Babajide SB, Akitoye OC. (2007). Salmonellosis in Lagos, Nigeria: Incidence of *Plasmodium falciparum*-associated co-infection, patterns of antimicrobial resistance, and emergence of reduced susceptibility to fluoroquinolones. *Journal of Health Popul Nutr.*; 5(3): 351-358.

Miriagou, V., Carattoli, A. and Fanning, S. (2006) Antimicrobial Resistance Islands: Resistance Gene Clusters in Salmonella Chromosome and Plasmids. *Microbes and Infection*, 8, 1923-1930. https://doi.org/10.1016/j.micinf.2005.12.027

Mzungu I, Inabo HI, Olonitola SO, Aminu M. (2016). Antibiotic susceptibilities of *Salmonella* species prevalent among children of 0-5 years with diarrhea in Katsina State, Nigeria. *Archives of Medical and Biomedical Research*; 3(1): 39-51.

Smith, S. I., A. Seriki, and A. Ajayi (2016). Typhoidal and non-typhoidal Salmonella infections in Africa. *European Journal Clinical Microbiology and Infectious Diseases*. 35:1913–1922. DOI 10.1007/s10096-016-2760-3

Tesfahun L, Tsige K, Ketema B. (2016). Prevalence and Antimicrobial Resistance in *Salmonella* and Shigella Species Isolated from Outpatients, Jimma University Specialized Hospital, Southwest Ethiopia. *Can J Infect Dis Med Microbiol*; 42(10): 760-781.

Turkyilmaz S., Hazimoglu S., and Bozdogan B., (2009) Antimicrobial Susceptibility and Resistance Genes in Salmonella Enterica serovar Enteritidis Isolated from Turkeys. *Israel Journal of Veterinary Medicines 64(3);175-200.*

Uttah EC, Osim SE, Etta H, Ogban E, Okon NEE (2013). Four year longitudinal assessment of the prevalence of typhoid fever among those attending the General Hospital Etinan, Nigeria. *Int J Sci Res Pub;* 3(7):150–153

Youssef, R.A.; Abbas, A.M.; El-Shehawi, A.M.; Mabrouk, M.I.; Aboshanab, K.M. (2021). Serotyping and Antimicrobial Resistance Profile of Enteric Nontyphoidal Salmonella Recovered from Febrile Neutropenic Patients and Poultry in Egypt. *Antibiotics*, 10, 493. https:// doi.org/10.3390/antibiotics10050493