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# Antibiotic Profile and Molecular Characterization of Typhoidal Salmonellosis among Abattoir Workers in the Southern Region of Nigeria

**Idongesit Ata Udom[1], Effiong Effiong Owowo[1], Lydia Etuk Udofia[2]**

[1]Department of Microbiology, Akwa Ibom State University (AKSU), Uyo, Nigeria
[2]Department of Zoology, Akwa Ibom State University (AKSU), Uyo, Nigeria
Email: Chaliblaze4014@gmail.com, etangunoowowo@aksu.edu.ng

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## Abstract

Typhoid fever remains endemic in most developing countries with large scale transmission through contaminated food and drinking water. Since 2000s, animal and their products have been found to be a common food source for Typhoidal infections. A total of 90 blood specimens, 45 samples each were collected from abattoir and non-abattoir workers in Akwa Ibom State and were screened for *Salmonella* species using standard cultural and serological techniques. The overall prevalence rate was 12 (13.3%). *Salmonella* species were distributed in all the three senatorial districts of the State. Percentage distribution of *Salmonella* isolates was 3 (3.3%) for Uyo, 4 (4.4%) for Eket and 5 (5.6%) were isolated from Ikot Ekpene districts. Out of 90 human subjects screened, 12 (13.3%) were positive and 5 (5.6%) were isolated from non-abattoir while 7 (7.7%) were isolated from abattoir workers. In this study, serological significant titre of ≥160 of Typhoid fever cases was confirmed by blood culture. According to sero-grouping and source of sample collection 4.4% *S. typhi* was isolated from abattoir workers followed by *S. paratyphi* A (3.3%) while *S. typhimurium* and *S. gallinarum* (2.2%) and *S. enteritidis* (1.1%). Increase prevalence of *Salmonella* serovars such as *S. gallinarium, S. typhi, S. Typhimurium* and *S. enteritidis* with regular consumption of slaughter meat and other product without proper disinfection and appropriate boiling, represent a serious public health risk in Akwa Ibom State. Demographic data obtained in this study showed that majority of the abattoir workers across the three districts were within the ages of 21 to 30 (33%), 39% were none graduate while 30.4% were secondary school leavers. Fever was recorded as the highest clinical signs and symptoms followed by headache, abdominal cramps,

diarrhoea and vomiting. Generally, all the isolates identified as *Salmonella* were tested for their susceptibility to antimicrobial agents. The results showed that Chloramphenicol, Ciprofloxacin and Cefuroxine were 100% sensitive to *S. gallinarum, S. paratyphi* A, 93% susceptible to *S. typhi* and 80% to *S. typhimurium and S. enteritidis.* Whereas all isolates were 100% resistant to Septrin, Tetracycline and Ampicillin antibacterial agents used. The emergence of multiple drugs resistant *Salmonella* from abattoir workers shows that the continuous use of drugs in animal husbandry as growth promoters should be re-examined. Other factors as sources of *Salmonella* contamination in slaughter markets can be minimized by good hygienic practices and biosecurity measures.

## Keywords

*Salmonella*, Abattoir, Slaughter, Antibiotics Profile

## 1. Introduction

Salmonella is ubiquitous organism, a bacterium found in animals, humans and environment [1]. This genus of salmonella belongs to the family of Enterobacteriaceae, which is a largely heterogeneous group in nature. They are gram-negative rods, having thin layer of peptidoglycan or lacking it completely and whose natural habitat is the gastro-intestinal tract of humans and animals [2]. They are also flagellated for motility and facultatively anaerobic [3]. Their reproduction is through binary fission. Across the globe, it has been reported that *Salmonella* has been one of the reported causes of food-borne pathogens from far times to present [1]. Worldwide Salmonellosis continues to be a major public health problem. As a result of the cost of investigation and monitoring, management, treatment and prevention of the illness, the economy is impacted upon negatively. Estimates have shown that the annual cost of the illness and quality adjusted life-year (QALY) loss in the United States of America caused by 14 out of 31 major food borne pathogens based on their incidence, estimates of foodborne illness in the United States of America accounts for 95% illnesses and hospitalization and 98% of deaths due to identifiable pathogens [3].

Out of 2600 different serovars and not less than 50 serogroups present in the genus *Salmonella* described in the Kauffmann white scheme, majority of the serovars been identified are responsible for infections in human and animals across the globe. *Salmonella* is further subdivided into two major groups with according to their pathogenesis. First group of serovars is host specific and its disease caused is systemic but if there is no disease, it colonises the host's intestine poorly and scarcely gets involved in human food poisoning. Serovars in this group include *Salmonella gallinarum* and *Salmonella pullorum* in poultry, *Salmonella dublin* in cattle and *Salmonella typhi* and *almonella paratyphi* A, B and C in man [4]. *Salmonella typhi* and *Salmonella paratyphi* are called typhoidal

salmonellosis. It is also called enteric fevers causing fatal diseases of human known as typhoidal fever and paratyphoidal fever respectively [4].

Abattoirs in Nigeria are sources of infection and pollution by the bacteria *Salmonella*, as it attracts domestic animals, carnivorous animals and rodents. This owes to the inadequacy in slaughtering and disposal facilities. Improper processing of meat consumed by the majority of Nigerian people and people in Nigeria poses serious challenges in public health. This study will help to isolate, identify and characterize typhoidal Salmonellosis *i.e. Salmonella typhi* and *Salmonella paratyphi*, the bacteria organisms responsible for the contamination and colonization of man's gastrointestinal tract and cause of typhoid and paratyphoid fever in man.

### 1.1. Aim of the Study

This study is aim at evaluating and characterizing the typhoidal salmonellae isolated from samples collected from abattoir workers in the South-South of Nigeria.

### 1.2. Specific Objectives

- To determine the prevalence rate of Typhoidal Salmonellae among Abattoir workers in Akwa Ibom State.
- To identify the different serotype of Salmonellae through molecular characterization.
- To determine the antimicrobial profile of the salmonellae isolates.

## 2. Materials and Methods

### 2.1. The Study Design

The study is a descriptive cross-sectional study.

### 2.2. Study Area

The study was conducted in the three senatorial zones in Akwa Ibom State. Blood and stool would be collected from Abattoir and non-Abattoir workers in each senatorial zone: Ikot Ekpene (IK), Eket (EK), and Uyo (UY), Akwa Ibom State. Akwa Ibom State is located on longitude 8˚30' and latitude 5˚30' in the Southern region of Nigeria; it covers an area of 455 km$^2$. The major occupation of the people living in Akwa Ibom is business and farming (**Figure 1**).

### 2.3. Preliminary Investigation

The chosen Slaughters were visited to obtain official permission to conduct the study and to discuss the feasibility of the study and to seek the cooperation of the Managers or owners and workers.

### 2.4. Ethical Approval

Ethical clearance and approval was obtained from the department of Research

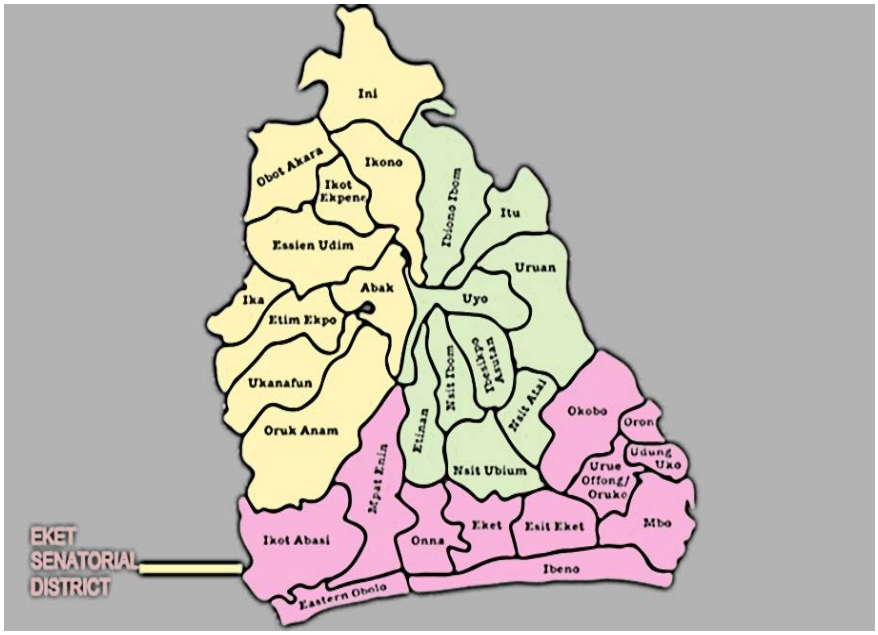


**Figure 1.** Map showing the three senatorial districts in Akwa Ibom State [5].

and Education Ethical Committee of the State Ministry of Health, to collect specimens from both Government and Private owned abattoir facilities within the study area.

## 2.5. Inclusion and Exclusion

Subject that was included in the study are between 14 years to 55 years old working as slaughter staff or owners in the study area. For the purpose of analysis, subjects were divided into three groups:

**Group 1**: consists of subject with or without symptoms of typhoid fever working in slaughters in the study area.

**Group 2**: consists of subjects with symptoms of fever due to illnesses other than typhoid fever. Market handlers of meat products were not included in the study due to possible cross contamination of salmonella from other market products. The sampling was admitted from January to June 2022 (6 months period).

## 2.6. Sample Size

The equation below will be used to determine sample size:

$$N = pq/(E/Z)^2,$$

where; $p$ = prevalence of previous studies = 37.4% [6]

$q = 100 - p = 100 - 37.4 = 62.6$

$E$ = allowable error = 5%

$Z$ = standard normal distribution at 95% Cl, = 1.96

$N$ = number of sample to be collected

Therefore, when the values are substituted

$N = 37.4 \times 62.6/(5/1.96)^2 = \underline{2341.2/(2.55)^2 = 2341.2/(6.5).}$

$N = 360.2$ samples. The sample size is a minimum of 360 samples.

However, in course of this research work, a total of 90 blood samples were obtained and 90 questionnaires designed for personal data information of the subjects (abattoir workers and non-workers) was delivered to the subjects in the aforementioned senatorial zone in Akwa Ibom State, thirty (30) each from the three senatorial districts, giving a total of 90 samples.

### 2.7. Sampling Methods

A total of 90 blood samples were collected from patients presenting with clinical and non-clinical symptoms of enteric fever and from abattoir workers from the three zones. Abattoir workers and non-workers with fever or no fever regardless of the duration would have their 5 mls of venous blood taken aseptically with sterile vacutainer for serological analysis and culture with date and time of collections boldly written. The samples were transported to the laboratory with minimum delay to avoid death of enteric pathogens [7]. Blood was inoculated into Thioglycollate broth sub-cultured onto Deoxycholate Citrate Agar (DCA) and *Salmonella-Shigella* Agar (SSA).

### 2.8. Demographic Data Collection

Demographic data collection will be obtained with the use of Questionnaires. Some of the information required from the subjects; include age, gender, marital status, occupation, and educational level, source of drinking water and clinical signs and symptoms.

### 2.9. Serological Analysis of Blood Samples Using Widal Test

The blood specimen was analyzed using Widal test kits (standard tube test method) for detection of the presence of *Salmonella* antibodies, among the abattoir and non-abattoir workers.

### 2.10. Procedures for Widal Identification Test

Ten (10) test tubes were placed in a rack; 4 ml of saline would be added to tube 1 and 1 ml to tube 2 - 10.

One (1) ml of serum was added to tube 1 and mix to dilute the serum 1 in 5. That is 1 ml of serum plus 4 ml of saline, giving 5 ml volume.

One (1 ml) of serum-saline solution was transferred to tube 2. This dilutes the serum 1 in 10. The procedures would be repeated to tube 10. To obtain serum dilutions of 1 in 5, 1 in 10, 1 in 20, 1 in 40, 1 in 80, 1 in 160, 1 in 360, 1 in 640, 1 in 1280, and 1 in 2560. Using a fresh pipette, and starting from the highest dilution, 0.5 ml was transferred from each test-tube into a corresponding agglutination tube rack.

Point five (0.5 ml) of antigen were added to each tube, the addition of an equal quantity of antigen dilutes the serum again, the final serum dilution being

1 in 10 in the first tube, 1 in 20 in the second tube. The dilutions were repeated for other tubes.

To another agglutination tube 0.5 ml of saline and 0.5 ml of antigen was added. This tube serves as a control to show if the antigen is self-agglutinable. The agglutination rack was placed in the water-bath and water level adjusted until it covers one third of the tube. The tubes were incubated for at least 2 h and result read.

The Widal test significant if TO antigen titer is more than or equal to 1:160 in an active infection, or if TH antigen titer is more than or equal 1:160 in past infection or in immunized persons [8] [9].

### 2.11. Isolation and Identification of Salmonella Specie in Blood Specimens

The *Salmonella* species were isolated and identified in blood samples by the following methods;

### 2.12. Culture of the Blood Specimens

The blood specimens (widal positive and negative) were inoculated into thioglycollate broth and sub-cultured onto Deoxycholate Citrate Agar (DCA) and *Salmonella-Shigella* Agar (SSA) and incubated aerobically at 35˚C - 37˚C for 24 hours for the isolation of *Salmonella* species [7].

### 2.13. Cultural Characteristics of Salmonella Specie on Selective Media

Cultural characteristics of Salmonella species on Deoxycholate citrate agar (DCA) and Salmonella - Shigella agar (SSA) was used for the presumptive identification of *Salmonella* isolates [8]. *Salmonella* species are non-lactose fermenting, pale coloured colonies, with black centres (hydrogen sulphide ($H_2S$)-producing *Salmonella*) in the media [9]. The presumptive isolates were purified and stored on slants for future characterization.

### 2.14. Pure Culture Isolation and Preparation of Stock Culture

All plates were examined after incubation. Non-Lactose-fermenting colonies were picked from SSA and Deoxycholate Citrate agar and purified on nutrient agar. Stock culture of the isolates were made on nutrient agar slants and preserved in refrigeration at about 4˚C - 8˚C. Transfer onto fresh agar slopes were made at regular interval of one week. Plates showing no growth or growth of lactose fermenters were re-incubated before discarding as giving no *Salmonella* organisms [8].

### 2.15. Biochemical Characterization

Isolates would be identified using API 20 E confirmation tests. This is a standardized identification system for Enterobacteriaceae and other non-fastidious Gram negative rods which use 20 miniaturized biochemical tests and a database.

### 2.16. Principle

The API 20 E strip consists of 20 micro tubes containing dehydrated substrates. These tests are inoculated with a bacterial suspension that reconstitutes the media. During incubation, metabolism produces colour changes that are either spontaneous or revealed by the addition of reagents. The reactions are read according to the reading table and the identification is obtained by referring to the Analytical Profile index or using the identification software.

### 2.17. Antimicrobial Sensitivity

Antibacterial Sensitivity testing was prepared according to Kirby-Bauer disk diffusion method recommended by the NCCLS [10] and CLSI [11]. The inoculated plates would be allowed undisturbed on a level surface for about 5 min to allow for absorption of excess moisture. Antibiotics disc were aseptically placed on the inoculated plates and incubated at 37˚C for 18 h - 24 h. Zone of inhibition was used as index for determining sensitivity or resistance to the antimicrobial agents (Table 1).

**Confirmation of *salmonella* isolates by polymerase chain reaction.**

Primer sequence, PCR preparations and conditions used in molecular characterization and genotyping of *Salmonella enterica* obtained in the study.

| Target primer sequence | PCR preparation (25 µl) | PCR conditions | Reference(s) |
|---|---|---|---|
| *Inv*A SAL1<br>SAL2 | 12.5 µl Dream Taq mastermix,<br>8.5 µl nuclease free water, 3 µl<br>Template DNA and 0.5 µl of each<br>Primer mix. | 5 min at 94˚C prior to<br>35 cycles of 1 min at 94˚C annealing<br>Temperature at 55˚C for 1 min at 72˚C. | [12] |
| *Stn* P15<br>*Stn* M13 | 12.5 µl Dream Taq mastermix,<br>8.5 µl nuclease free water 3 µl<br>Template DNA and 0.5 µl of each<br>Primer mix. | 5 min at 94˚C prior to 35 cycles of<br>1 min at 94˚C annealing temperature<br>At 60˚C for 90 min, extension at 72˚C<br>For 1 min and a final extension of 10 min at 72˚C. | [12] |
| ERIC1<br>ERIC2 | 12.5 µl Dream Taq mastermix,<br>8.5 µl nuclease free water 3 µl<br>Template DNA and 0.5 µl of each<br>Primer mix. | 1 min at 94˚C prior to 1 min at 95˚C<br>Annealing temperature at 52˚C for<br>1 min extension at 65˚C for 8 min<br>And a final extension of 16 min at 65˚C. | [12] |
| QRDR gyrAF<br>gyrAR | 12.5 µl Dream Taq mastermix,<br>8.5 µl nuclease free water 3 µl<br>Template DNA and 0.5 µl of each<br>primer mix. | 5 min at 94˚C prior to 35 cycles of<br>1 min at 94˚C, annealing temperature<br>At 54˚C for 1 min extension at 72˚C.<br>For 1 min and a final extension of 10 min at 72˚C. | [13] |
| pCT(008)F<br>pCT(009)R | 12.5 µl Dream Taq mastermix,<br>8.5 µl nuclease free water, 3 µl<br>Template DNA and 0.5 µl each<br>Primer mix. | 5 min at 94˚C prior to 35 cycles of<br>1 min at 94˚C annealing temperature<br>At 60˚C for 1 min. extension at 72˚C<br>For 1 min and a final extension of 10 min at 72˚C. | [13] |

**Table 1.** Socio-demographic characteristics of Abbatior and non-Abbatior workers in Akwa Ibom State.

| Variables | No. examined in each district | | | Total no. examined (%) |
|---|---|---|---|---|
| | Uyo | Eket | Ikot Ekpene | |
| **Age Group** | | | | |
| 15 - 20 | 05 (1.65) | 08 (2.7) | 06 (1.98) | 19 (20.9) |
| 21 - 30 | 16 (5.28) | 13 (4.29) | 12 (3.96) | 41 (45.1) |
| 31 - 45 | 04 (1.32) | 06 (1.98) | 10 (3.30) | 20 (22.0) |
| 46 > 50 | 05 (1.65) | 03 (1.32) | 02 (0.66) | 10 (11.0) |
| Total | 30 | 30 | 30 | 90 |
| **Gender** | | | | |
| Male | 20 (66.0) | 21 (69.3) | 25 (82.5) | 66 (72.6) |
| Female | 10 (33.0) | 09 (29.7) | 05 (16.5) | 24 (26.4) |
| Total | 30 | 30 | 30 | 90 |
| **Marital Status** | | | | |
| Single | 06 (19.8) | 09 (29.7) | 04 (13.2) | 19 (20.9) |
| Married | 21 (69.3) | 20 (66.0) | 23 (75.9) | 64 (70.4) |
| Divorce | 03 (09.9) | 01 (03.3) | 03 (09.9) | 07 (07.7) |
| Total | 30 | 30 | 30 | 90 |
| **Educational Status** | | | | |
| Primary | 06 (19.8 | 05 (16.5) | 06 (19.8) | 17 (18.9) |
| Secondary | 09 (29.7) | 11 (36.3) | 09 (29.7) | 29 (31.9) |
| Tertiary | 12 (39.6) | 09 (29.7) | 14 (46.2) | 35 (38.5) |
| None | 03 (09.9) | 05 (16.5) | 01 (3.3) | 09 (09.9) |
| Total | 30 | 30 | 30 | 90 |
| **Place of Residence** | | | | |
| Rural | 13 (42.9) | 10 (33.0) | 09 (29.7) | 32 (35.2) |
| Urban | 15 (49.5) | 18 (59.4) | 14 (46.2) | 47 (51.7) |
| Semi Urban | 02 (06.6) | 02 (06.6) | 07 (23.1) | 11 (12.1) |
| Total | 30 | 30 | 30 | 90 |
| **Duration of work in Abattoir (yrs)** | | | | |
| <1 | 17 (56.1) | 14 (46.2) | 11 (36.3) | 42 (46.2) |
| 1 - 5 | 12 (39.6) | 16 (52.8) | 16 (52.8) | 44 (48.4) |
| 6 - 10 | 01 (03.3) | 00 (00.0) | 03 (09.0) | 04 (04.4) |
| 11 - 15 | 00 (0.0) | 00 (00.0) | 00 (00.0) | 00 (00.0) |

**Continued**

| | | | | |
|---|---|---|---|---|
| 16 - 20 | 00 (0.0) | 00 (00.0) | 00 (00.0) | 00 (00.0) |
| > 20 | 00 (0.0) | 00 (00.0) | 00 (00.0) | 00 (00.0) |
| Total | 30 | 30 | 30 | 90 |

## 2.18. DNA-Fingerprinting Assay to Establish Relatedness

**Principle:**

In other to establish the relatedness and genetic diversity among the *Salmonella* strains isolated DNA-Fingerprinting assay was made. The term DNA fingerprinting, or genetic fingerprinting, is applied to the scientific process whereby DNA is extracted, and used to match other extracts of DNA. DNA or genetic fingerprinting relies mainly on the principle that no two *Salmonella* strains share the same genetic code and statistically those elements of DNA that would be examined and used to obtain a match will be unique, also there is a one in sixty-four billion chance that any two unrelated bacteria would have comparable DNA: comparable DNA is DNA that has certain attributes similar to that of another person but is not identical.

## 2.19. Making DNA Fingerprints

DNA fingerprinting is a laboratory procedure that requires six steps:

**1) Isolation of DNA.**

DNA was recovered from the isolates using ZR Fungal/Bacterial DNA Mini-Prep ZRD6005 (zymo research, USA).

**2) Cutting, sizing, and sorting.**

Restriction enzymes were used to cut the DNA at specific places. Using agarose gel technique, the DNA pieces are sorted according to size by electrophoresis. The DNA pieces were separated using agarose gel.

**3) Transfer of DNA to nylon.**

The distribution of DNA pieces would be transferred to a nylon sheet by placing the sheet on the gel and soaking them overnight.

**4) Probing.**

Radioactive or coloured probes would be added to the nylon sheet to produce a pattern called the DNA fingerprint. Each probe typically sticks in only one or two specific places on the nylon sheet.

**5) DNA fingerprint.**

The final DNA fingerprint was built by using several probes (5 - 10 or more) simultaneously [14].

## 2.20. Polymerase Chain Reaction Assay

Isolation of Plasmid DNA of Salmonellae. The resistant isolate were subjected to polymerase chain reaction plasmid DNA isolation following the protocol of *ZR Genomic DNA™* Miniprep kits:

**Protocol:**

1) To obtained cell suspensions containing less than $5 \times 10^6$ cells, salmonellae isolates were sub-cultured into prepared luria-bertani medium (LB medium) and incubated at 37˚C for 18 hours.

2) Hundred microliters (100 µl) of Luria-bertani broth culture of salmonellae was added in micro-centrifuge tube and then followed by adding 95 µl of 2× digestion buffer and 5 µl of proteinase K.

3) The mixture will be mix and the tube incubated at 55˚C for 20 minutes.

4) Seven hundred microliter (700 µl) of genomic lysis buffer was added to the tube and mix thoroughly by vortexing.

5) The mixture was transfer to a Zymo-Spin™ IIC column in a collection tube and centrifuged at 12,000 rpm for one minute.

6) Two hundred microliter (200 µl) of DNA pre-wash buffer was added to the spin column in a new collection tube and centrifuged at 12,000 rpm for one minute.

7) Four hundred microliter (400 µl) of DNA pre-wash buffer was added (for the second time) to the spin column and centrifuged at 12,000 rpm for one minute.

8) The spin column was transfer to a clean micro centrifuge tube and 80 µl DNA elution buffer to the spin column and then incubates for 5 minutes at room temperature, then finally centrifuged at top speed (*i.e.* 14,000 rpm) for 30 seconds to elute the DNA.

9) The eluted DNA was stored at −20˚C for future use for detection using PCR technique [14].

### 2.21. Agarose Gel Electrophoresis

**Protocol:** A 1% agarose gel was used to resolve the plasmid fragment. The agarose gel was prepared by combining 2% 1 g of agarose in 2 ml of Tris acetate ethylene diamine tetra acetate buffer and 90 ml distilled water in a 250 ml beaker flask and heating in a microwave for 1 minute until the agarose is dissolved. About 10 µl ethydium bromide was added to the dissolved agarose solution with swirling to mix. The gel will be poured onto a mini horizontal gel electrophoresis tank and the casting combs will be inserted. It was allowed to gel for 30 minutes. The casting combs were carefully removed after the gel had completely solidified. Electrophoresis buffer (prepared by adding 6 ml of TAE to 300 ml of distilled water) was added to the reservoir until the buffer just covered the agarose gel [15] [16].

**Loading plasmid DNA Samples:** A three microliter (3 µl) of gel tracking dye (bromophenol blue) was added to 15 µl of each sample with gentle mixing. Eighteen microliter (18 µl) of the sample was loaded onto the wells of the gel, the mini horizontal electrophoresis gel set up was covered and the electrodes connected. Electrophoresis was carryout at 100 mV for 45 minutee. At the completion of the electrophoresis, the gel was remove from the buffer and the gel was viewed under a long wave UV light box *i.e.* transilluminator. The band pattern

of the DNA fragments were then photographed with a Polaroid camera and documented using an electrophoresis gel documentation system [16]. Purified PCR products were sequenced by Sanger method (3730 ×l, Applied Biosystem). Nucleotide sequence was edited by BIOEDIT and compared with the known sequence in Genbank, using BIASTIN of the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST/).

**Genotypic Determination of diversity among Salmonella isolates using DNA Fingerprinting Assay**

All characterized *Salmonella* isolates were analysed using entero-bacterial repetitive intergenic Concensus fingerprint (ERIC-PCR) to determine their genetic relatedness [13].

**Protocol:** The PCR products (amplicons) were separated by electrophoresis on a 2% (w/v) agarose gel containing 2 µl florosafe. Electrophoresis will be ran at 100 V for 30 min for gene detection while for DNA fingerprinting electrophoresis was ran at 120 V for 45 min and visualized on an ultra violet (UV) trans illuminator gel imaging System (Bio-Rad Gel Imaging System, Bio-Rad California, USA). Bands will be photographed and bond positions were determined and compared to molecular weight markers.

## 2.22. Quality Controls

Control experiments (positive and negative) were set-up to monitor the efficiency of the media, reagents and different biochemical and serological test performed.

## 2.23. Data Analysis

Data obtained from this study were statistically analysed using Epi Info (version 7.0), program excel (Microsoft$^{R}$ Office Excel 2010, Professional Edition) and SAS software (version 9.0). Descriptive statistics were used to determine the association of various risk factors and Salmonella positivity (**Figure 2**).

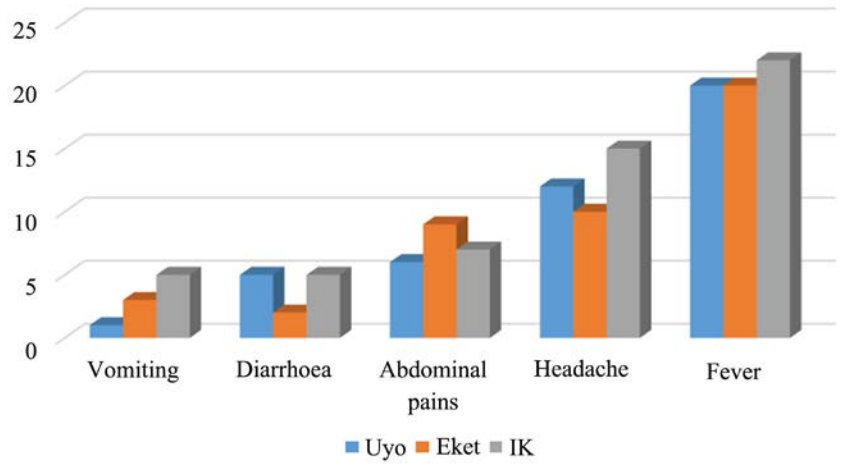


**Figure 2.** Distribution of clinical signs and symptoms of abattoir and non-abattoir workers in the three senatorial districts. Akwa Ibom State.

## 3. Discussion

This research revealed the presence of typhoidal and non-typhoidal *Salmonella* species among the abattoir and non-abattoir workers with overall isolation rate of 26.4% (**Table 2**). This finding is not surprising since *Salmonella* is reported to be animal pathogens capable of surviving and proliferating in diverse environments including gastrointestinal tract of animal [15]. This is in agreement with 10.9% reported by [17] in Jos, plateau. Widal agglutination tube test was sensitive to 26.7% (**Table 3**) specimens, at a significant titre level of 1:160.

The diagnostic utility of widal test has been limited due to low specificity and positive predictive value [18]. Studies by [19] showed 92.5% false positive results of widal titre which was associated with cross reaction of antibodies from serum of febrile patient. This is in support of the [20] report that *Salmonella* species were isolated from Slaughter workers non-slaughter workers in Egypt.

Isolation of *S. typhimurium* in slaughter workers and non-workers 2.2% (**Table 5**) is lower compared to 22.2% [21]. It supported the report by [22] that domestic animals are the major reservoir and foods of animal origin are the major vehicles of *S. typhimurium* infection in human. This agreed with the finding by [23] that the distribution of non-typhoidal *Salmonella* serovars among livestock from slaughterhouses were similar to those in human, and is higher than 6.24% as reported by [24].

In **Table 5**, *S. typhimurium* (2.2%) and *S. gallinarum* (2.2%) samples were isolated from slaughter and non-slaughter samples, this could be attributed to close contact between the abattoir workers and the domestic animals kill within the slaughter. This is in agreement with [15] reported that *S. typhimurium* and *S. enteritidis* were isolated from poultry and asymptomatic poultry workers in Akwa Ibom State. *Salmonella gallinarum* and *S. paratyphi A* were found 2 (2.2%) and 3 (3.3%) respectively from slaughtered animals. Animals can easily be infected by these organisms through contaminated water source and vertical transmission by infected breeders. *S. enteritidis* 1.0 (1.0%) was isolated from abattoir workers; this isolation may be due to the presence of infected animals, which have been reported to aid the distribution of Salmonellosis in abattoir workers, this is lower than 6.24% as reported by [25]. *S. typhi* 4 (4.4%) was isolated equally from abattoir and non-abattoir workers, due to poor sanitary conditions and unhygienic practices (**Table 4**).

The antibiogram revealed multiple drug resistant index of 0.4 - 0.9 with *Salmonella* serovars incriminated in both abattoir workers and non-workers salmonellosis. Overall results showed that all serovars were 100% resistant to Septrin, Tetracycline and Ampicillin, 71.8% to Amoxacillin, 60.7% to Gentymicin and 60% to Ceftriaxone. This could be attributed to misuse of drugs in both human and veterinary medicine, most especially for prophylaxis and as a growth promoter in domestic animals. However, susceptibility test revealed 100% of the isolates were susceptible to Ciprofloxacin, Chloramphenicol and Cefuroxine (**Table 5**).

**Table 2.** Prevalence rate of Salmonella isolates from Abattoir and Non-workers in Akwa Ibom State, Nigeria.

| Sample Source | No. of Samples | Senatorial districts | | | Pos (%) |
|---|---|---|---|---|---|
| | | Uyo | Eket | IK. | |
| Non-workers (control) | 45 | 1 | 2 | 2 | 05 (11.0) |
| Abattoir workers | 45 | 2 | 2 | 3 | 07 (15.5) |
| Total | 90 | 3 | 4 | 5 | 12 (13.3) |

key: IK-Ikot Ekpene, No: Numbers.

**Table 3.** Distribution of Salmonella strains from Abattoir and Abattoir workers using phenotypic Characteristics in Akwa Ibom State, Nigeria.

| Source | Total | Salmonella Specie | | | | | Total % |
|---|---|---|---|---|---|---|---|
| | | *S. gal.* (n = 2) | *S. typh* (n = 2) | *S. typhi* (n = 4) | *S. ent* (n = 1) | *S. para* (n = 3) | |
| Abattoir | 45 | 1 | 1 | 2 | 1 | 2 | 7 (15.4) |
| Non-Abattoir C | 45 | 1 | 1 | 2 | 0 | 1 | 5 (11.0) |
| Total | 90 | 2 | 2 | 4 | 1 | 3 | 12 (26.4) |

Key: C = control group, n = numbers, *S. gal*: *Salmonella galinarium*, typh: typhimurium, ent: enteritidis, para.: paratyphi.

**Table 4.** Comparison of Salmonellae carrier state for abattoir and non-abattoir workers using the Widal serological tests in Akwa Ibom State, Nigeria.

| Subjects | No. of Sample | Widal Test (1 ≥ 160) | No. of Isolates |
|---|---|---|---|
| Abattoir Workers | 45 | 14 | 7 |
| Non-abattoir wkrs | 45 | 10 | 5 |
| Total | 90 | 24 | 12 |

Key: NO-Number, Wkrs-Workers.

**Table 5.** Distribution and phenotypic of multiple-drug resistance of Salmonella isolates in Akwa Ibom State, Nigeria.

| Salmonella serotype | Source | Susceptibility & Resistance | | | | | | | | | | Virulene gene | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SXT | AMC | CIP | C | CAZ | CRO | CN | TET | AMP | S | invA | stn |
| *S. galinarum* | abattoir | R | S | S | S | S | R | S | R | R | S | + | + |
| *S. galinarum* | Non-abattoir | R | S | S | S | S | R | S | R | R | S | + | + |
| *S. typhimurum* | abattoir | R | S | S | S | S | R | S | R | R | R | + | + |
| *S. typhimurum* | Non-abattoir | R | R | S | S | S | R | S | R | R | R | + | − |
| *S. enteritidis* | abattoir | R | R | S | S | S | S | R | R | R | R | − | + |
| *S. typhi* | abattoir | R | S | S | S | S | S | R | R | R | R | + | + |
| *S. typhi* | abattoir | R | S | S | S | S | S | R | R | R | R | − | + |
| *S. typhi* | Non-abattoir | R | S | S | S | S | S | S | R | R | R | + | − |

Key: R: resistant, S: susceptibility, InvA = Salmonella invasion genes, stn = Salmonella enterotoxin gene, SXT: Septrin, AMC: Amoxyl, CIP: Ciprofloxacin, C: Chloramphenicol, CAZ: Cefuroxine, CRO: Ceftriaxone, CN: Gentymicin, TET: Tetracycline, AMP: Ampicillin, S: Streptomycin.

Widal test appears to correlate poorly with blood culture results in Akwa Ibom State. It may be useful for rapidly diagnosing typhoid fever in emergencies; example during outbreaks, their performance is likely to justify deployment in routine care settings in Akwa Ibom State. Isolation of *Salmonella* by blood culture has remained the gold standard for diagnosis of enteric fever.

In this study abattoir workers have been identified as high risk vehicles for the introduction of infectious agents of public health significance. Contaminated slaughtered animals have been implicated as sources of *Salmonella* in slaughter houses, which could lead to wide scale epidemic.

Abattoir workers have been indicted as a cheaper source of carrier of *Salmonella* and a possible route of transmission to non-abattoir workers.

## 4. Recommendation

The following recommendations should prove useful to ensure proper management and control of Salmonellosis among Abattoir workers: Public awareness on health benefits on the consumption of contaminated meat and their product by Salmonellae. Strict compliance on good personal hygiene should be encouraged among abattoir workers. Biosecurity practices should be maintained in all slaughter markets. Partnership between professionals, government and stake holders in the abattoir sector should be encouraged. Continued surveillance by the researchers to determine regular antimicrobial susceptibility pattern in order to identify changes in resistance should be encouraged.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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