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# The Diagnosis of *Salmonella typhi* Co-infection from Blood Samples of Clinically Suspected Typhoid Fever Patients at Some Hospitals in Ondo State, Nigeria

**O. E. Ajayi[1*], B. E. Boboye[1] and O. F. Olukunle[1]**

*[1]Department of Microbiology, Federal University of Technology, Akure, Nigeria.*

***Authors' contributions***

*This work was carried out in collaboration between all authors. OEA and BEB designed the study, OEA wrote the protocol and first draft of the manuscript. OFO managed the literature searches and data analysis. All authors read and approved the final manuscript.*

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## ABSTRACT

**Background:** Blood samples of clinically suspected typhoid fever patients were screened for bacterial and fungal co-infection.
**Methodology:** The study was conducted in three different hospitals in Ondo State, Nigeria viz; State Specialists' Hospital, Akure, Don Bosco Clinic, Akure and Federal Medical Centre, Owo, between February and May, 2013. Five hundred and twenty (520) blood samples collected from clinically suspected typhoid fever patients attending three hospitals in Ondo State, Nigeria were screened for the presence of *Salmonella typhi*, other bacteria and fungi using different growth media.
**Results:** Sixteen *Salmonella typhi* isolates (3.08%) were isolated and co-infection with *Escherichia coli* (31.25%), *Shigella flexneri* (12.50%), *Enterobacter aerogenes* (6.25%), *Pseudomonas aeruginosa* (6.25%), *Klebsiella pneumoniae* (6.25%), *Aspergillus flavus* (6.25%), *Aspergillus niger*

**Corresponding author: E-mail: ajayigbengaemmmanuel87@gmail.com;*

(6.25%) and *Penicillium italicum* (6.25%) was observed. There was co-infection of *Escherichia coli* with five (5) of the sixteen (16) *Salmonella typhi* isolates, *Shigella flexneri* with two (2) isolates while six of the *S. typhi* isolates had no co-infection. *Aspergillus flavus* had co-infection with two (2) *Salmonella typhi* isolates while *A. niger* and *P. italicum* had a co-infection with an *S. typhi* isolate each.

**Conclusion:** Co-infection of other microorganisms with *S. typhi* is indicative of immune compromise and other disease conditions in the typhoid fever patients.

*Keywords: Typhoid; bacterial co-infection; fungal co-infection; patients; hospitals.*

## 1. INTRODUCTION

Typhoid fever is a menace to global health security. The annual incidence of typhoid is estimated to be about 17 million cases worldwide [1]. It is an infectious disease of global distribution as reported by House et al. [2]. Typhoid fever is a systemic infection caused by *Salmonella enterica* serotype Typhi and remains an important worldwide cause of morbidity and mortality [3]. Several studies have also shown that *Salmonella typhi* bacteremia remains a major public health problem worldwide especially in developing countries [4]. Human infection with *Salmonella typhi* is mainly by the oral route through ingestion of faecal contaminated food and water, unclean hands, flies and meat from infected animals [4,5]. It is a prolonged febrile illness and continues to be a health problem in developing countries where there is poor sanitation, poor standard of personal hygiene and prevalence of contaminated foods [6]. Once the bacteria enter the person's body they multiply and spread from the intestines into the bloodstream. The microorganism can be isolated from an individual with the disease, but can also be found in individuals who are asymptomatic carriers [7]. The definitive diagnosis of typhoid fever requires the isolation of *Salmonella enterica* serotype Typhi from the patient. Cultures of blood, stool, urine, rose spots, blood mononuclear cell-platelet fraction, bone marrow, gastric and intestinal secretions can all be useful for diagnosis [8,9,10]. However, this requires laboratory equipment and technical training that are beyond the means of most primary health care facilities in the developing world. Blood culture is the recommended diagnostic method, but it is reported to be positive in only 40–80% of cases [8,9,10]. The sensitivity of blood culture varies according to the stage of illness, the volume of blood inoculated into the culture, and prior antimicrobial treatment. A low number of bacteria circulating in the blood is a crucial limitation [8]. Culture of bone marrow is more sensitive than blood but not feasible in routine practice [11] .

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection and Culture

#### 2.1.1 Ethical clearance

Informed consent was sought in clinically suspected typhoid fever cases and approval for the study was obtained from the Ethics Committee of the Ondo State Ministry of Health and the Federal Medical Centre, Owo, Ondo State. Confidentiality was maintained in accordance with the standards of medical practice.

#### 2.1.2 Study area

Samples were collected from Federal Medical Center Owo, Don Bosco Clinic, Akure and State Specialists' Hospital, Akure, Ondo State, Nigeria. A total of five hundred and twenty (520) blood samples were collected from presumptive typhoid fever patients attending the listed hospitals.

#### 2.1.3 Blood collection

Aseptically collected venous blood sample (3ml) was obtained from five hundred and twenty (520) clinically suspected typhoid fever patients, who sought medical attention from Don Bosco Clinic, Akure, State Specialists' Hospital, Akure and Federal Medical Centre, Owo, Ondo State, Nigeria between February and May, 2013.

### 2.2 Isolation of Bacteria and Fungi from Blood Samples

Collected blood sample (3 ml) was introduced into 30 ml of sterile Brain Heart Infusion Broth (Oxoid, England) and the mixture was gently shaken to enhance homogeneity. The mixture was afterwards incubated at 37ºC for 48 hours. This served as inoculum for subsequent analyses.

#### 2.2.1 Isolation of bacteria

A loopful of the inoculum prepared above was streaked on *Salmonella Shigella* agar. Samples that were positive for the presence of *S. Typhi* were later sub-cultured on Nutrient agar, Blood agar, Potato dextrose agar, Sarbouraud dextrose agar, MacConkey agar and Malt extract agar using streak method. The streaking was carried out using an inoculating loop sterilized in hot flame. It was allowed to cool, used to take part of a grown colony from a cultured plate and streaked on the surface of a freshly prepared plate. The plates were thereafter incubated at 37ºC for 24 hours.

#### 2.2.2 Identification of bacterial isolates

The bacterial isolates were presumptively identified using their colony morphological characteristics. The appearance of each colony on the agar media and characteristics such as shape, edge, colour, elevation and texture were observed as described by Olutiola et al. [12]. The isolates were thereafter subjected to all relevant biochemical identification tests using the taxonomic scheme of Bergey's Manual of Determinative Bacteriology [13] while antiserum agglutination test was conducted to confirm the identity of the *S. typhi* isolates and also distinguish them from other salmonellae.

#### 2.2.3 Fungal isolation and identification

Each mycelium colony from a grown mixed culture was sub-cultured onto fresh Potato dextrose agar (PDA), Sarbouraud dextrose agar (SDA) and Malt extract agar (MEA) plates with the aid of a sterile inoculating needle. The inoculated plates were incubated at 25ºC for 72 hours. The fungal isolates were identified based on their cultural and microscopic features as described by Barnet and Hunter (1972) [14].

## 3. RESULTS

Sixteen (16) *Salmonella typhi* isolates representing 3.08% were recovered from the five hundred and twenty (520) cultured blood samples. Of these 16 isolates, 10 (62.5%) had co-infection with other bacteria; such as *Shigella flexneri* (2), *Enterobacter aerogenes* (1), *Escherichia coli* (5), *Klebsiella pneumoniae* (1), and *Pseudomonas aeruginosa* (1) as shown on Table 1.

Three (3) fungi (moulds) were found to co-exist with *S. typhi* in the screened blood samples. The fungi were *Aspergillus flavus* (6.25%), *Aspergillus niger* (6.25%) and *Penicillium italicum* (6.25%). *Aspergillus flavus* co-existed with two (2) out of the sixteen (16) *S. typhi* isolates while *Aspergillus niger* and *Penicillium italicum* co-existed with an *S. typhi* isolate each twelve out of the sixteen *S. typhi* isolates had no co-existence with any fungus. The result is presented in Table 2.

## 4. DISCUSSION

Being a diagnosis of bacterial and fungal co-infection in clinically suspected typhoid fever

**Table 1. Bacteria that co-existed with *Salmonella typhi***

| ***Salmonella typhi* isolates** | ***Klebsiella pneumoniae*** | ***Shigella flexneri*** | ***Enterobacter aerogenes*** | ***Pseudomonas aeruginosa*** | ***E. coli*** |
|---|---|---|---|---|---|
| 1 | - | - | - | - | + |
| 2 | - | - | - | - | - |
| 3 | - | - | - | - | + |
| 4 | - | - | - | - | - |
| 5 | - | - | - | - | - |
| 6 | - | - | - | - | - |
| 7 | - | + | - | - | - |
| 8 | - | - | - | + | - |
| 9 | - | - | - | - | + |
| 10 | - | - | - | - | - |
| 11 | - | - | + | - | - |
| 12 | - | - | - | - | + |
| 13 | - | - | - | - | - |
| 14 | - | + | - | - | - |
| 15 | - | - | - | - | + |
| 16 | + | - | - | - | - |
| Rate of co-existence | 1/16 | 2/16 | 1/16 | 1/16 | 5/16 |

*Key: +: Present - : Absent*

**Table 2. Fungi that co-existed with *Salmonella typhi***

| ***Salmonella typhi* isolates** | ***Aspergillus niger*** | ***Penicilium italicum*** | ***Aspergillus flavus*** |
|---|---|---|---|
| 1 | - | - | + |
| 2 | - | - | - |
| 3 | - | - | - |
| 4 | - | - | - |
| 5 | - | - | - |
| 6 | - | - | - |
| 7 | - | - | - |
| 8 | - | - | + |
| 9 | - | - | - |
| 10 | - | + | - |
| 11 | - | - | - |
| 12 | - | - | - |
| 13 | + | - | - |
| 14 | - | - | - |
| 15 | - | - | - |
| 16 | - | - | - |
| Rate of co-existence | 1/16 | 1/16 | 2/16 |

*Key: +: Present -: Absent*

cases, it is not out of place to isolate a variety of microorganisms (both bacteria and fungi) from the patients. The percentage of bacterial isolation among patients with typhoid fever varies enormously [15]. Detection of bacteria by blood culture may be influenced by the culture medium employed, the number of circulating bacteria, the time of blood collection, the volume of blood employed for the culture, the host's immune response system as well as the intracellular character of the bacteria [15,10]. The low prevalence (3.08%) of *Salmonella typhi*, coupled with the presence of other bacteria in the blood of clinically suspected typhoid fever patients in this study, indicated that some of the patients were not actually suffering from typhoid fever and that a good number of them must have presented symptoms which are similar to those of typhoid fever. Though clinically suspected typhoid fever patients were used in the study, the authors were open-minded as to consider the possibility of other presenting disease conditions in the patients. Isolation of a number of moulds which is quite uncommon in typhoid fever infection cases typifies a high level of immune compromise giving rise to a breakdown of the defense mechanism of the patients [16]. *Salmonella typhi* causes bacteremia in critically ill typhoid fever patients [10]. Various researchers in their respective studies have reported the isolation of *S. typhi* from the blood samples of clinically suspected typhoid fever patients [17,18]. This study has revealed that other bacteria and some fungi are capable of causing co-infection in clinically suspected typhoid fever patients.

The occurrence of *Escherichia coli* co-infection in this study (31.25%) is at variance with the findings of Alfred & Edet [18] who recorded the highest occurrence with *Staphylococcus aureus* (35.90%) with *Escherichia coli* (15.40%) being the second highest in their study. *Escherichia coli* are a normal colonizer of the human gastrointestinal tract and a common source of bacteremia [13]. *S. typhi*'s co-existence with *E. aerogenes* also corroborates the findings of Alfred & Edet [18]. Bodey et al. [16] had earlier reported that *Enterobacter aerogenes* are emerging pathogens in nosocomial infections. The co-infection of the moulds with sixteen (16) *Salmonella typhi* isolates is suggestive of possible immune compromise [19]. Kontoyiannis et al. [20] opined that *Aspergillus* fungemia can be seen exclusively in patients with heamatological malignancies in late stages of invasive fungal infection. Bodey et al. [16] also reported that *Aspergillus* fungemia is rarely encountered even in the setting of disseminated disease. *Aspergillus niger* fungemia is specifically reported in immunocompromised patients [20].

## 5. CONCLUSION

*Salmonella* bacteremia is a common occurrence in critically ill typhoid fever patients. Other bacteria are capable of co-existence with *S. typhi* in typhoid fever patients depending on other

presenting disease conditions and the occurrence of certain moulds in the blood samples of the patients could be traced to opportunistic infections arising from immune compromise.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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