

Seroprevalence of Enteric Fever among Blood Donors in Khartoum State – Sudan

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Abstract

Typhoid fever is a systemic illness with a significant morbidity and mortality in developing countries. Poor sanitation, overcrowding, low standard of living, lack of medical facilities, and indiscriminate use of antibiotics lead to endemicity of typhoid fever and multi-resistant strains of Salmonella typhi in developing countries.

The aim of this study was to determine seroprevalence of Salmonella typhi [O] Ag and Salmonella para typhi B [O] Ag. among blood Donors in Khartoum State in Sudan.

A descriptive cross-sectional study was carried out during the period from September to December 2014. A total of five hundred samples were collected from healthy blood donors attending Central Blood Bank, Khartoum, Sudan.

The diagnosis of salmonellosis in this study was based on slide method and Standard Agglutination Test (SAT) to determine the titer of Salmonella typhi [O] Ag and Salmonella para typhi B [O] Ag.

Males were 461(92.2%) and females were 39(7.8%). The positive sera for Salmonella typhi [O] Ag for Widal test by slide method were 172(34.4%) and the negative sera were 328(65.6%). The positive sera for Salmonella para typhi B [O] Ag for Widal test by slide

method were 158(31.6%) and the negative sera were 342(68.4%). The titration results of salmonellosis among blood donors for *Salmonella typhi* [O] Ag (significant, doubtful, insignificant, and negative) were 118(23.6%), 51(10.2%), 4(0.8%), 327(65.4%) respectively. The titration result among blood donors for *Salmonella para typhi* B (significant, doubtful, insignificant, and negative) by tube method were (84(16.8%), 69(13.8%), 7(1.4%) 340(68%)) respectively.

Keywords: Seroprevalence of Enteric Fever, Blood Donors, Khartoum State, Sudan

INTRODUCTION

Typhoid fever is an acute systemic infectious disease seen only in humans, is a classic example of enteric fever caused by *Salmonella enteric serovar typhi*. The classic presentation includes fever, malaise, diffuse abdominal pain, and diarrhea. Untreated, typhoid fever is a grueling illness that may progress to delirium, obtundation, intestinal hemorrhage, bowel perforation, and death within one month of onset (Evans and Brachman, 1989).

Given that salmonellosis is endemic in our region, there is a potential risk of *Salmonella* spp. being transmitted via blood transfusion. Indeed, unscreened units of blood which harbor live *Salmonella* organisms or endotoxin could cause severe, possibly fatal, post-transfusion reactions (Corales and Schmitt, 2002).

Bacterial contamination of blood components is an infrequent complication of transfusion. However, if it does occur, the potential for fulminant sepsis in the recipient is associated with high mortality. It can result from contamination during venipuncture or if an asymptomatic donor is bacteremic at the time of donation. Symptoms occur during or shortly after transfusion of the contaminated unit and include high fever, rigors, erythema, and cardiovascular collapse (Kopko and Holland, 2001).

RBCs are stored at 4°C. This makes contamination with Gram-negative bacteria such as *Yersinia enterocolitica* and *Pseudomonas* species more likely as they proliferate rapidly at this temperature. Gram-positive bacteria such as *Staphylococcus epidermidis*,

Staphylococcus aureus and *Bacillus* species proliferate more readily at room temperature and so are more commonly seen as platelet contaminants (Kopko and Holland, 2001).

MATERIALS AND METHODS

Study design

Cross-sectional descriptive study was carried out in the period from September to December 2014 in the Central Blood Bank at Khartoum state, Sudan.

A written consent was obtained from healthy blood donors after being informed about the nature of the study.

Data collection

After explaining the purpose of the study, data were collected from volunteers including age, sex and history of previous blood donation. Blood samples were collected in sterile plain container.

Laboratory work

Sample preparation

The collected blood specimens were transported on ice to the laboratory, centrifuged at 3000 r.p.m for five minutes and serum was separated and stored at -20°C until tested.

Widal test Slide method:

The sera were then subjected to the Widal test by the slide agglutination method as per the manufacturer's instructions. Briefly, 50 µl of antigen was placed upon the slide provided in the kit followed by addition of 50 µl of serum. The slide was rocked gently for mixing. Since observation of agglutination in the form of visible clumps may have observer's bias, the result of the agglutination reaction was scored as 0 (no agglutination), 1+ (25%agglutination), 2+ (50% agglutination), 3+ (75% agglutination) or 4+ (100% agglutination). The sample was labeled as positive if the serum exhibited $\geq 2+$ or 50% agglutination (Olopoenia and King, 2000).

Standard Agglutination Test (SAT)

All the samples were subjected to the tube agglutination test to find out exact titers of antibodies, the test was done as follows:

Series of serum dilutions were made for each antigen to be tested, including tubes with 0.5 ml saline for control of each antigen to be used.

Perfectly clean and dry test tubes were made and prepared dilutions beginning with 1:10 and doubling through 1:320.

The prepared tubes were incubated at 37°C for 24 hours and observed for the agglutination reaction within the tubes (Cheesbrough, 2006). Each serial dilution had been interpreted as the follow: 1/20, 1/40, 1/80, 1/160 and 1/320; negative, insignificant, doubtful, significant and significant, respectively. Positive and negative control sera were run in parallel with each performed batch. Duplicates of each tested serum were used to assure that the antigens used in the test were sensitive as well as specific (Cheesbrough, 2000). In this study, the cut-off titer for positive cases was taken to be 1:160.

RESULTS

As shown in Fig. 1, the positive sera for widal test for *Salmonella typhi* O Ag by slide method were 172(34.4%) and the negative sera were 328(65.6%).

The positive sera for widal test for *Salmonella para typhi* B by slide method were 158(31.6%) and negative sera were 342(68.4%) as shown in Fig .2.

The titration result of salmonellosis among blood donors for *Salmonella typhi* O by tube method (significant, doubtful, insignificant and negative) were 118(23.6%), 51(10.2%), 4(0.8%), 327(65.4%) respectively. The titration result of salmonellosis among blood donors for *Salmonella para typhi* B by tube method (significant, doubtful, insignificant and negative) were 84(16.8%), 69(13.8%), 7(1.4%) 340(68%) respectively as shown in table 1.

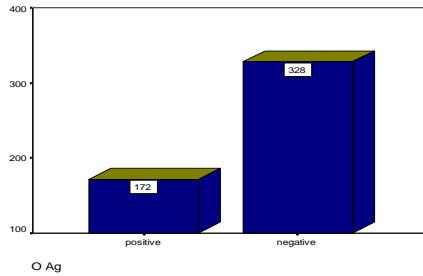


Fig.1. The positive and negative sera of Widal test for Salmonella typhi O Ag by slide method

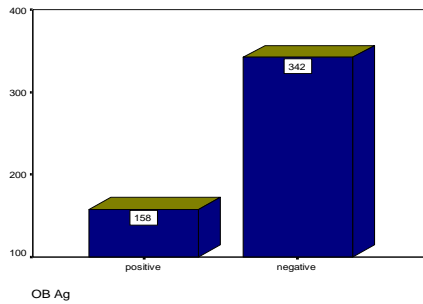


Fig.2. The positive and negative sera of Widal test for Salmonella para typhi B OAg by slide method

Table 4. The Widal test results for Salmonella typhi OAg by serial dilution in tube method:

| Result | Frequency | Percent% |
|---------------|------------|-------------|
| Significant | 118 | 23.6% |
| Doubtful | 51 | 10.2% |
| Insignificant | 4 | 0.8% |
| Negative | 327 | 65.4% |
| Total | 500 | 100% |

Table 5. The Widal test results for Salmonella para typhi B OAg by serial dilution in tube method:

| Result | Frequency | Percent% |
|---------------|------------|-------------|
| Significant | 84 | 16.8% |
| Doubtful | 69 | 13.8% |
| Insignificant | 7 | 1.4% |
| Negative | 340 | 68% |
| Total | 100 | 100% |

DISCUSSION

Although the preferred laboratory procedure for identification of *Salmonella enterica* is culture, most clinics and hospitals in developing countries do not have ready access to this method. Widal agglutination tests are widely used in many developing countries, including Sudan, as an alternative laboratory procedure for diagnosis of enteric fever. This study was aimed at assessing the prevalence of *Salmonella* antibodies among donors in the Central Blood Bank in Khartoum state in Sudan, and to assess the implications that this may have for transfusion safety in the country.

In this study we used Widal serological test (tube method) by using both *Salmonella typhi* O Ag and *Salmonella typhi* para B O Ag, due to the availability of this test in routine practice applications here in Sudan. Several studies have highlighted the limitations of using the Widal serological test in the laboratory diagnosis of *Salmonellosis* (Hamze *et al.*, 1989; Olopoenia & King, 2000). Despite this, there seems to be a general consensus that the Widal test remains a valuable method for the diagnosis and control of typhoid fever in many developing countries (Hamze *et al.*, 1989; Parry *et al.*, 2000). In this study, the cut-off titer for positive cases was taken to be 1:160.

In our study, 23.6 % and 16.8% of the total donor population found to be SAT Widal-positive for *S. typhi* O Ag and *S. typhi* para B OAg, respectively. This result was higher than that reported by Nsutebu *et al.*, in Yaounde, Cameroon, who found that 10% of blood donors yielded positive result. Whereas it was lower than that (53%) recorded by Teddy *et al.*, 2010 in Nigeria, this variation in finding may be attributed to the levels of hygiene practice or the level of endemicity of salmonellosis in these countries. also, the high percent that recorded in Nigeria, may be attributed to the fact that most blood donors were commercial donors, who are known to constitute a high-risk group in the blood supply chain in Nigeria. Not all blood units which test positive for Widal antigens carry live *Salmonella* bacteria. The survival rate of *Salmonella* in blood stored in blood bank conditions (4°C–8°C) should be very low since these bacteria grow optimally at 37°C, and usually do not thrive below 8°C. Transfused fresh blood may, however, transmit live *Salmonella* bacteria. In addition, live *Salmonella* organisms at the time of phlebotomy may

release pyrogen-inducing endotoxin into the donor unit even after refrigeration has decimated the bacterial population. Endotoxin from *Salmonella* is capable of inducing immunosuppression by causing neutropenia and, consequently, leucopenia in the host (Hornick *et al.*, 1970; Dick *et al.*, 1990).

The high infection rate of *salmonellosis* observed among the blood donors highlighted the risk of this pathogenic organism in our blood supply chain, this study also confirms that salmonellosis is endemic in Sudan and that many of our blood donors may be *Salmonella* carriers.

However, for more accurate evaluation, further well-designed studies with increased number of samples. are needed.

This study also meant to attract the attention of the health institution of the hazard of *Salmonella* transfusion through the blood and to take the necessary action to curb its prevalence through testing of blood donors. Finally, this research is the first one to be conducted in a blood bank in in Sudan.

REFERENCES

1. **Cheesbrough, M. (2006)**, Medical laboratory manual for tropical countries; Cambridge university press, 2nd Ed. part 1, pp. 260-261.
2. **Corales, R. and Schmitt, S. K. (2002)**. Distribution of antibodies to *Salmonella* in the sera of blood donors in the south-western region of Nigeria. *Blood transfus*; **8** (3): 163-169.
3. **Dick HM, Wilkinson PC, Powis S. Topley and Wilson's (1990)** Principles of Bacteriology, Virology and immunity. 8th Edition. Hodder and Stoughton Ltd.; **360**
4. **Evans, A. and Brachman, P. (1998)**. Bacterial Infections of Humans. *Epidemiology and Infection journal*; **121** (3): 569-577.
5. **Hamze M, Naboulsi M & Vincent P (1998)**. Evaluation of the Widal test for diagnosing of typhoid fever in Lebanon. *Pathologie Biologie*; **46**: 613-6.
6. **Hornick RB, Greisman SE, Woodward TE, et al. (1970)** Typhoid fever: pathogenesis and immunologic control. *New Engl J Med*; **283**: 686-91, 739-46.
7. **Kopko, P. M. and Holland, P. V. (2001)**. Mechanisms of severe transfusion reactions. *Transfus Clin Biol*; **8**: 278-81.
8. **Olopoenia L. A. & King A. L. (2000)**. Widal agglutination test - 100 years later: Still plagued by controversy. *Postgrad Med J*; **76**: 80-4.
9. **Parry CM, Hien TT & Dougan G (2000)**. Typhoid fever. *New Engl J Med*; **347**: 1770-82