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Original article

Evaluation of *in vitro* bactericidal activity of human serum against *Salmonella typhi* in relation to sero groups

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ABSTRACT

Background: Series of more than 35 proteins facilitated a major role in fighting the foreign invaders in human body and other warm blooded animals, those named complement system. **Methods**: Total of 147 human serum were collected from asymptomatic volunteers by venipuncture, their sero groups were determined by reverse blood grouping method. Strain of *Salmonella typhi* (*S. Typhi)* were collected from Wudil general hospital and identified molecularly at center of biotechnological research of Bayero University, Kano. Serum bactericidal assay was done at microbiology laboratory of Wudil general hospital. Data obtained was subjected to two way ANOVA and considered significant at $p \leq 0.05$. **Results:** Complement dependent pathways shows a more than 50% kills, in which both A, AB, B and O kill percent were 74.98%, 67.87%, 78.53% and 78.60% respectively, however, no statistical significant difference. While the non-complement dependent pathways revealed kills percentage of 59.27%, 57.86%, 59.21% and 58.60% for A, AB, B and O sero groups, also no significant statistical difference. Moreover, in comparing the complement and non-complement dependent pathways, data analysis shows a significant difference with p values of *0.001*, *0.003*, *0.006* and 0.0008 for A, AB, B and O sero groups respectively. **Conclusion**: We concluded that, ABO sero-groups and complement system plays an important role as infection determinants, where AB sero-group have more chances to *S. Typhi* infection than other sero-groupings. This study suggests that the effect of many host genetic parameters on treatment of microorganisms needs to be further studied.

Introduction

The bactericidal effect of serum is an essential innate immune mechanism of the host that provides protection against harmful bacteria [1]. The defensive capacity of antibody and complement proteins in the serum is referred to as complement-mediated bactericidal activity or serum bactericidal activity and is strong-minded via an in vitro technique known as serum bactericidal assay (SBA) [2].

The system known as complement consists of more than 35 plasma and cell surface proteins that function to help protect an organism from pathogens [3]. The complement system is fairly well preserved throughout most of the phylum Chordata, and many of the components are closely related and may have arisen through gene duplications [4-6]. Unlike antibodies, complement proteins are not precise for particular immunogens and do not advance in affinity or increase in concentration with repeated

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exposure and, as such, are defined as components of innate immunity [7].

The ability of cell-free human serum to kill pathogenic microbes was exposed at the end of the 19th century. It was observed that bacteria are killed by lysis and that this lytic ability of serum was improved with prior immunization with the target bacteria [8]. When heated, serum was able to stick but not lyse the bacteria with which the host had been immunized. Lytic ability was reestablished upon mixing heated serum with fresh serum, even if the additional serum was from a nonimmunized animal [9].

Since the finding of the ABO blood group with corresponding sero-groupings, there has been an ongoing curiosity in the possible role of blood groups in infectious disease. Many blood groups are receptors for toxins, parasites, and bacteria, where they can enable colonization or invasion or evade host clearance mechanisms. Blood groups can also serve as untruthful receptors, avoiding binding to target tissue. Finally, microbes can stimulate antibodies against blood group antigens, including ABO.

The current study examined the ability of human humoral immune system in combating *Salmonella Typhi (S.typhi)* infection with emphasis on different sero groups.

Material and Methods

Isolates were recovered from clinical stool sample through culture, Gram reaction and conventional biochemical tests at laboratory unit of Wudil general hospital, Wudil, Kano, according to the method described by American Public Health Association, [10]. Also molecular identification was done at center of biotechnological research of Bayero University, Kano. Genomic DNA extraction was based on the method described Norgen Biotech (CANADA). Extracted DNA samples were quantified on agarose gel electrophoresis to determine DNA size and assess the RNA contamination. Polymerase chain reaction amplification was carried out using-storm thermal cycler. Products of the amplification were electrophoresed and visualized under UV illumination in Gel Documentation system 2000 (Biorad, Hercules CA, USA) and stored as TIFF file format. Sizes of the amplicons were estimated in comparison with 50bp DNA ladder (CLEAVER SCIENTIFIC LTD, UK). 1.5kb band of DNA was excised from gel and purified using gel elution kit (Sigma-Aldrich, USA) based on the manufacturers protocol. Sequencing re- actions were carried out with a BigDye Terminator cycle sequencing kit (Applied Biosystems, USA), standard universal primer forward (8f ') and reverse (1542r') primer and sequenced by using ABI Prism 3100 genetic analyzer (Applied Biosystems, USA). The sequences thus obtained were assembled and edited using Clone Man- ager Version 5 (http://www.scied.com/pr_cmbas.htm). Database search was carried out for similar nucleotide sequences with the BLAST search of Non-reductant (NR) database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Partial length 16S rRNA gene sequences of strains closely related to the isolate were retrieved from National Center of Biotechnology Information (NCBI) for further analysis. Inoculum was standardized by adjusting turbidity of bacterial suspension to 0.5 McFarland standard.

Serum samples were harvested from one hundred and forty seven (147) asymptomatic individual's blood samples in Wudil town of Wudil local government area, Kano, according to the methods described by Lachmann [11] with their consent approval in accordance with underlined medical ethical consideration. While reverse grouping of the serum was done as described by Dhurba [12].

Method of Owhe-Ureghe and Okoh [13] and Heesterbeek et al. [14] were followed by slight modification for SBA. 0.25ml of fresh serum were place in three (3) set of test tubes, the first tube was subjected to thermal inactivation by the heating off at 56^{O}C for 30 minutes in a rotary water bath after which was allowed to cooled at room temperature, second tube was treated with equal amount of EDTA, all the three tubes were then inoculated with 0.25ml of the test isolates and incubated in rotary water bath at 37^{O}C for 60 minutes. Mixture was then poured into a sterile Petri dish containing a molten nutrients agar, shaken gently, allowed to solidified, inverted and incubated at 37^{O}C for 24 hours. After which colonies formed were counted and recorded.

Bacterial species were considered sensitive, if more than 50% killing was obtained by modifying the formula described by Vincent and Helen [15];

$$\boldsymbol{Equation(1)}.. \ \boldsymbol{Kill}\ (\%) = \frac{\boldsymbol{No.of\ cfu\ mL^{-1}\ (H) - No.\ of\ cfu\ mL^{-1}(F)}}{\boldsymbol{No.of\ cfu\ mL^{-1}\ (H)}}\ \boldsymbol{x}\ \frac{\mathbf{100}}{\mathbf{1}}$$

$$\textbf{Equation}(2)..\textbf{Kill}\ (\%) = \frac{\textbf{No. of cfu mL}^{-1}\ (\textbf{H}) - \textbf{No. of cfu mL}^{-1}(\textbf{E})}{\textbf{No. of cfu mL}^{-1}\ (\textbf{H})} x\ \frac{100}{1}$$

H – Heat Inactivated Sera, F – Fresh Sera and E – EDTA Treated Sera.

Equation (1) revealed the percentage of complement dependent pathway while equation (2) revealed the non-complement pathway.

Results was subjected to two way analysis of variances to compare the bactericidal activity of different serum groups.

Results

The ABO sero-grouping of all the participants in this study, sero-group AB has the highest participants of 52(35.37%) in which 17(11.56%) were children, 23(15.65%) and 12(8.16%) were adults and elders respectively. More so, B sero-group followed with 37(25.16%) participants of that 13(8.84%) were children, 15(10.2%) were adults and 9(6.12%) were elders. However, both A and O sero-groups has 29(19.73%) participants in which 13(8.84%) were children, 11(7.48%) were adults and 5(3.4%) were elders for the A sero-group while, 9(6.12%) were children, 16(10.88%) and 4(2.72%) were adults and elders respectively **(Figure 1 & Table 1)**.

The study also found that 52(35.38) were children having an age range of (1-12) years, Adults were 65(44.22%) with age range of (13 – 40) years and 30(20.40%) were elders having >40 years of age shown in **table (2)**.

Furthermore, the susceptibility testing of the isolates to those sero-groupings, *S. Typhi* shows sensitivity (of more than 50% kills) to complement dependent bactericidal activity in all the serum from the 4 sero-groupings with different average killing percentage revealing 74.98%, 77.88%, 78.93% and 78.60% for sero-groupings of A, AB, B and O respectively while in complement independent bactericidal activity only 15 participant's serum with sero-grouping of A shows an activity (>50% kills) against *S. Typhi* with average killing percentage of 59.27%, 27 participant's serum sero-grouping of AB shows 59.86% average killing percentage, 24 and 19 participants with sero-grouping of B and O shows an average killing percentage of 59.21% and 58.59% respectively. Fresh and EDTA treated sera shows a significant difference with p values of 0.001, 0.003, 0.006 and 0.0008 for A, AB, B and O sero groups **(Figure 2)**.

Figure 3a,b&c shows the relative increases of the human serum bactericidal activity against the *S. typhi* with addition of ages among the participants. In all cases of children, adult and elder categorization the activity accelerated up to an age of 43 years were the activity started to decelerate with addition of years.

Figure 1. ABO sero grouping of the participants.

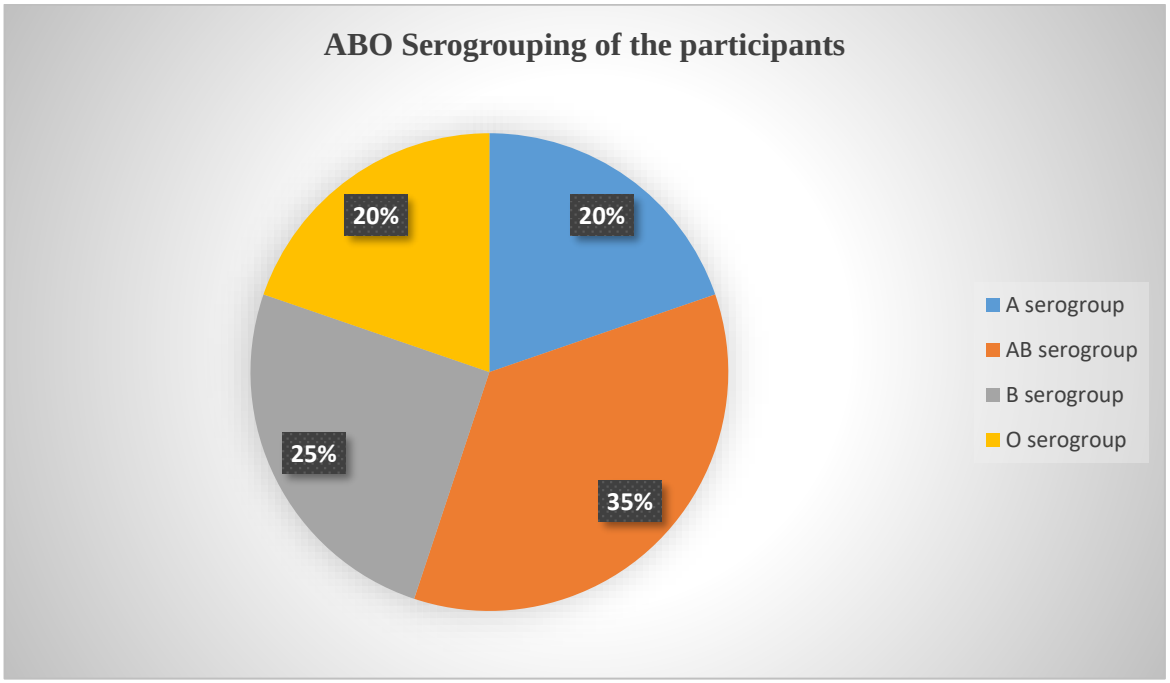
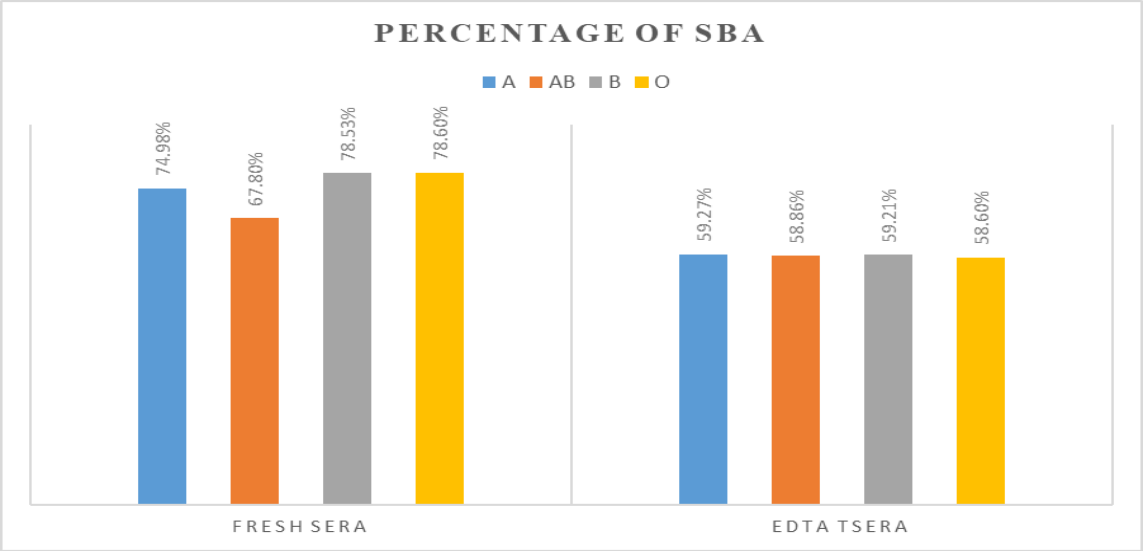


Figure 2. Fresh and EDTA treated sera shows a significant difference with p values of *0.001*, *0.003*, *0.006* and *0.0008* for A, AB, B and O sero groups.



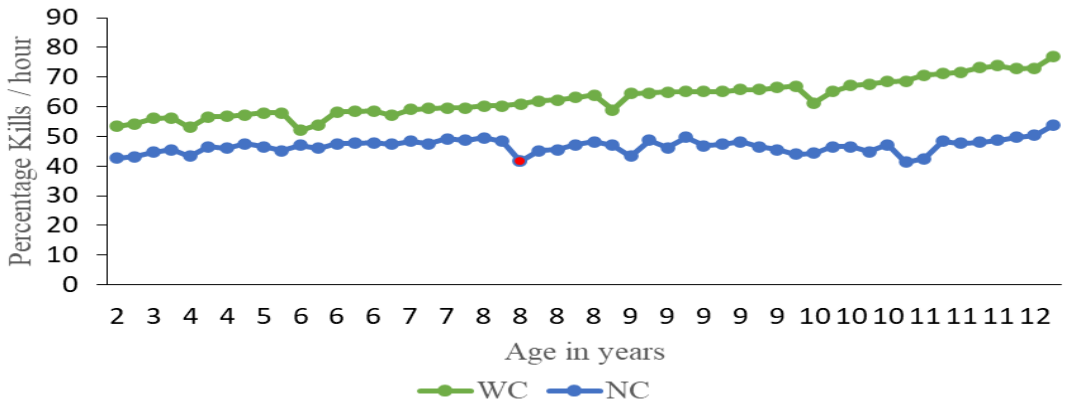


Fig.3a. Salmonella Typhi Response to Child's Serum Bactericial Activity with and without Complement Mediation

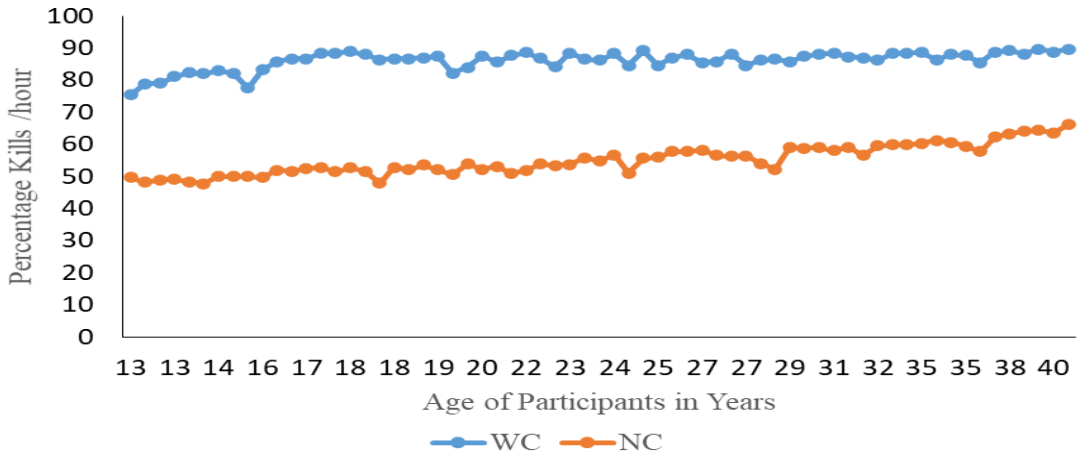


Fig.3b. Salmonella Typhi Killing Response to Adult's Serum Bactericidal Activity with and without complement mediation

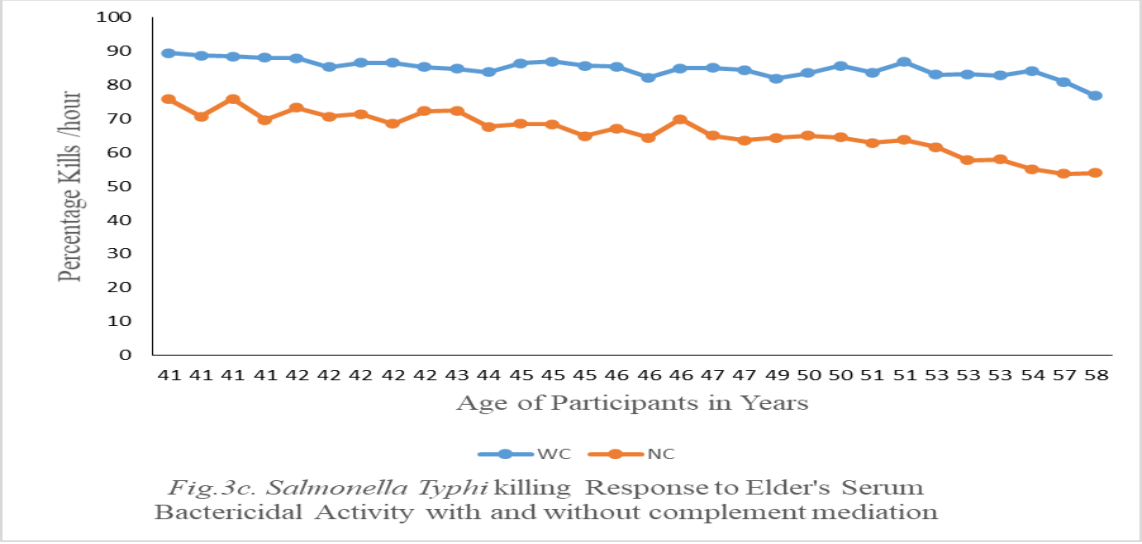


Fig.3c. Salmonella Typhi killing Response to Elder's Serum Bactericidal Activity with and without complement mediation

Table 1. ABO sero-grouping distributions among subject volunteers participate in this study.

Serogroping	Age Range			Total (%)
	Children (%)	Adults (%)	Elders (%)	
A	13(8.84)	11(7.48)	5(3.40)	29(19.73)
AB	17(11.56)	23(15.65)	12(8.16)	52(35.37)
B	13(8.84)	15(10.20)	9(6.12)	37(25.16)
O	9(6.12)	16(10.88)	4(2.72)	29(19.73)
Total	**52(35.37)**	**65(44.22)**	**30(20.41)**	**147(100)**

Keys; A, AB, B and O represent the sero-grouping of the participants,

Table 2. Age distribution of the subject volunteers participate in this study.

Parameter	Frequency (n)		
Subject categorization	**Sex (n)**		**Total (%)**
	F (%)	**M (%)**	
Children (1 – 12 years)	31 (21.09)	21(14.29)	52(35.38)
Adults (13 – 40 years)	23(15.65)	42(28.57)	65(44.22)
Elders (>40 years)	09(6.12)	21(14.28)	30(20.40)
Total	**63(42.86)**	**84(57.14)**	**147(100)**

Keys: F = Female participants, M = Male participants and (%) = percentage.

Discussion

Susceptibility of *S. typhi* to serum bactericidal activity especially in the complement dependent pathway shows that complement proteins plays an important roles in serum killing of *S. typhi* however, the ability of EDTA treated sera (in which both alternative and classical pathways were blocked) to kill *S. typhi* proved that the serum bactericidal activity is not solely dependent to complement proteins.

The lower bactericidal activity shown with AB sero-group compared to other sero-grouping indicated a compromised in the serum capacity of AB group to fight for *S. typhi.* The result of this study shows that human sero-group has a role to play in resistance or susceptibility to *S. typhi.* This result was in lined with report of **Owhe-Ureghe and Okoh**, that bactericidal activity of normal human serum against pathogenic microorganisms varies with sero-group of the volunteers [13]. It was also reported that the typhoid outbreak was observed among sero-grouping AB individuals while the least

infection was noticed among sero-grouping O individual [16]. Further study also described that AB sero-grouping was associated with typhoid fever, sero-grouping AB presented minimum resistance for typhoid and paratyphoid fever [17].

The possible relationship between infectious disease and ABO sero-grouping is determined by its carbohydrate on RBC surface. This assemble carbon, hydrogen and oxygen become a receptor for my infectious agents and facilitate their internalization in to the cells [17]. Sero-groupings are frequent targets in epidemiological investigations since they are genetically determined qualities with known polymorphic manifestation among individuals and populations.

The low bactericidal activity found in children is probably related to the lack of C8 and low concentration of C9 complement component as compared to adult and elder volunteers, those component of complement plays a critical role in the formation of terminal complement complex which disrupt the activity of the cell membrane, leading to the cell degradation and death [18]. Also the complement component plays a significant role in identifying pathogens [19]. The study agrees with the previous finding that reported a compromised bactericidal activity of neonate against some Gram negative isolates [5].

Several genetic, developmental, and clinical conditions can affect ABO typing, with implications for epidemiology studies. In many epithelial tissues, ABO expression is heavily dependent on the inheritance of the Secretor/FUT2 gene, which cannot always be ascertained by red cell typing alone [20]. Anti-A and anti-B are naturally occurring antibodies, arising in the absence of blood transfusion or pregnancy. Bacteria, particularly Gram-negative such salmonella found in the gastro-intestinal tract of warm blooded animals, appear to be the primary immune stimuli underlying their development [15]. However, in the cause of an immune response, pathogenic Infections become accessible via receptors of the plasma cells. Also the finding of this study were in line with that of Gondwe *et al.* who state that an individual with AB serogroupings were more likely to become infected with *S. Typhi* which contain the antibodies A and B [21].

The presence of pilli and fimbriae in most of the Gram negative bacteria including *S. Typhi* plays an important role in attaching the bacteria to the surface of host cell mucous membranes and initiate an infection [22]. The little compromised of AB serogroupings in the development of *S. typhi* infection may likely be due to the presence of A and B antigen which serve as the complementary adhesion factor of *S. typhi* as reported by **Kundera et al.** [23] and **Busch et al.** [24].

Moreover, in comparing the complement and non-complement dependent pathways, data analysis shows a significant difference with p values of *0.001*, *0.003*, *0.006* and *0.0008* for A, AB, B and O sero groups respectively. These revealed that the bactericidal activity of human serum is not absolutely complement dependent rather, an effective tasks of antibodies, which include pathogen and toxin nullification, complement activation, and opsonin raise of phagocytosis and pathogen abolition

Conclusion

Conclusively, the finding of this study support that human serum bactericidal activity against *S. typhi* is not solely complement dependent.

Moreover, ABO sero-grouping plays an important role as infection determinants, where AB sero-grouping are more vulnerable to *S. typhi* and *S. aureus* infection than other sero-groupings.

This study suggests that the effect of many host genetic parameters on treatment of microorganisms needs to be further studied.

Conflict of interest: None.

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