BACTERICIDAL ACTIVITY OF HUMAN SERA AGAINST SALMONELLA TYPHI AND SALMONELLA PARATYPHI A, B, C.

E.O. IGUMBOR and D.O. OSAYANDE

ABSTRACT

Objectives: To determine the sensitivity of *Salmonella typhi* and *paratyphi* A,B,C, to normal human blood serum; and assess the role of blood groups (ABO system), Complement and Immunoglobulin in the resistance or susceptibility of *Salmonella typhi* and *Salmonella paratyphi* A,B,C infections.

Design: Cross sectional study.

Subjects: Ninety-six apparently healthy males and females volunteers, aged 18-24 years.

Main outcome measures: Resistance of *Salmonella typhi* and *Salmonella paratyphi* A,B,C infections may be blood group and immune status dependent.

Results: Blood group B was most resistant to *Salmonella typhi* and *Salmonella paratyphi* A,B,C while blood group O showed least resistance (51.9%) and (22.2%) for *Salmonella typhi* and *Salmonella paratyphi* A,B,C. There was no difference in resistance pattern when blood was pooled in respect to their group types. Age or sex of the blood donors had no effect on the bactericidal activity of the sera.

Conclusion: Blood group is an important factor in the susceptibility or resistance of an individual to *Salmonella typhi* and *paratyphi* infections. Individuals of blood group O are likely to be more susceptible to infections caused by *Salmonella typhi* and *paratyphi* A,B,C.

INTRODUCTION

Human serum is considered an important host defence mechanism against invasive diseases caused by Gram-negative bacteria(1-3). The bactericidal and bacteriolytic activities of serum have been described and the relative roles of complement and antibody established(4,5). It has been reported that there are some associations between blood groups with particular diseases and that blood groups of individuals could determine resistance or susceptibility to infectious agents(6,7).

The association of blood groups with diseases was thought to concern the relation of the antigen of the infecting organisms with those of the blood group(8). For instance, if an organism carries an antigen resembling that of blood group A, then a group A- person being unable to make an anti-A antibody would be in a more disadvantaged state than a group O-person who already had such an antibody. But the results were not the case(9). It has been reported that syphilis is markedly present in blood group- A people. The most notable feature is that in the treatment of syphilis, a positive Wasserman serological reaction of the serum is on the average much more persistent in A and B blood group persons than O-blood group person(7). Also, meningitis is marked by a considerably raised group A-frequency(9). Studies have also shown that malaria seems to be associated with blood group A individuals, but other factors, for example, glucose 6- phosphate dehydrogenase deficiency and sickle cell may be involved in resistance to malaria(7).

Previous studies have revealed that natural *Salmonella* bactericidal antibody is present in the serum of most adults, while *Salmonella* species isolated from patients with disseminated *Salmonella* infection were found to be serum resistant(10). Some workers(6,10), reported that typhoid and paratyphoid infections tend to be associated with a raised blood group-O frequency, but only in the case of paratyphoid does this reach the conventional level of significance. Thus persons with S-S haemoglobin are exceedingly susceptible to *Salmonella* infections, particularly osteomyelitis; and persons with sickle cell trait (A-S) may be more susceptible than normal individuals(10). It has been established that besides natural antibodies in the serum of apparently normal human beings, there are other factors such as antitoxins, antiviral antibodies, opsonins and antilysin, which play a role in the bactericidal activity of human serum(11,12). The complement system, which exists in the blood, is a major amplification and effectors mechanism of humoral immune responses; and it has been reported that complement
protein is essential for killing susceptible Gram-negative bacteria(13). Bactericidal antibody and terminal complements are indispensable factors for protection against diseases caused by the Gram-negative bacteria(13). In addition to complement, lysozyme, calcium (Ca++) and magnesium (Mg++) are important components of extra-cellular fluids that are required for killing Gram-negative bacteria. Calcium and magnesium ions are essential for initiation of the classical complement sequence(6). It has also been established that immunoglobulins (IgG, IgM) antibodies were able to promote killing of some gram-negative bacteria in vitro in the presence of complement(11) but immunoglobulin-M fraction was proved to be bactericidal(14).

Salmonella species have been incriminated in several disease states(15-17); and due to the high incidence of typhoid and paratyphoid fevers and the sporadic occurrence of Salmonella infections, it has become necessary to review the role of resistance factors to Salmonella species. Although reports on the baseline Salmonella antibody titre have been documented(18-20), the role played by A,B,O blood system, complement and immunoglobulins in the bactericidal activity of normal human serum against Salmonella typhi and Salmonella paratyphi A,B,C has not been documented. This study attempts to determine the sensitivity of Salmonella typhi and Salmonella paratyphi A,B,C to normal human serum and the role of complement system and immunoglobulin in bactericidal effect of blood serum.

MATERIALS AND METHODS

**Bacterial strains:** Isolates of Salmonella species used were obtained from the University of Benin Teaching Hospital. Benin City and Central Hospital Ubiaja, all in Edo State of Nigeria. These were clinical isolates from urine, blood and stool of patients who were attending clinics. Isolates were further confirmed following standard laboratory procedures as described(21-22). A standard strain of Salmonella spp. (ATCC 13076) was used as control.

**Human serum and salmonella antibody screening:** Blood samples were collected from a total of ninety six adult male and female volunteers age range 18-42 years, who were not on any antibiotics for the past eight months before this study. The blood groups of individual donors were determined using commercially-available antisera (Laboratory Diagnostic Products Ltd) as previously described(3,5,18). Altogether, there were 41 females and 55 males, consisting of 20, 16, 6 and 54 of A, B, AB, and O blood groups, respectively. The blood was obtained by cardiac puncture. The blood was allowed to clot at room temperature for about three hours and the serum was separated from erythrocytes by centrifugation at 2000Xg for 15mins at 4°C. The supernatant was drawn into sterile bijou bottles. Serum was divided into two portions; the portion not used immediately was stored at -4°C in screw cap vials, while the serum portion used for the test was heat-inactivated at 56°C for 30minutes.

To determine the role of the complement system, the mixture which contained 50ul heat-inactivated serum, 50ul guinea-pig serum and 50ul final dilution of test organisms was added to 50ul 10 mMol Mg++-EGTA (Ethylene glycol tetra Acetic acid) and 10 mMol EDTA (Ethylene Diamine Tetra Acetic acid) in U-well trays. Trays were then incubated at 37°C for 60 minutes as described by Blasler et al(2).

**Determination of the role of immunoglobulin:** The role of immunoglobulin was determined using the method described by Gary et al(15). Sera were treated with 2-mercapto-ethanol by adding 50ul dilution of organism to 50ul of normal saline and 50ul of 2-mercapto-ethanol in a U-well tray and trays incubated at 37°C for 60 minutes.

**Interpretation of the bactericidal test:** Significant bactericidal activity was defined as a 50% reduction in cfu when compared with the heat inactivated serum control. This was obtained by using the formula of Skirrow(24).

\[
\text{Percent killed} = \frac{\text{No. of cfu(fresh serum)} \times 100}{\text{No. of cfu inactivated}}
\]

**Statistical analysis:** The Fisher exact test of probability for small numbers was used to test probability and the Student t-test was used to compare normally distributed population.
RESULTS

The results showed that *Salmonella typhi* and *Salmonella paratyphi A,B,C* were highly sensitive to normal human sera from blood group B. The percentage sensitivity was 93.8% for *S. typhi* and 62.5% for *S. paratyphi A, B, C* (Table 1). This was followed by blood group A, 80% and 60% for *S. typhi* and *S. paratyphi A, B, C* respectively. *Salmonella typhi* was sensitive to four (66.7%) of the six AB blood group while only two (33.3%) *S. paratyphi A, B, C* were sensitive. The results indicated that blood group O-sera recorded the least sensitivity of 51.9% and 22.2% for *S. typhi* and *S. paratyphi A, B, C* respectively. It was also shown that sera bactericidal effect is lower with *S. paratyphi* than with *S. typhi* (Figure 1).

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Total No. of donors</th>
<th><em>S. typhi</em> No. of sensitive strains</th>
<th><em>S. paratyphi A, B, C</em> No. of sensitive strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>20</td>
<td>16 (80%)</td>
<td>12 (60%)</td>
</tr>
<tr>
<td>B</td>
<td>16</td>
<td>15 (93.75%)</td>
<td>10 (62.5%)</td>
</tr>
<tr>
<td>AB</td>
<td>6</td>
<td>4 (66.7%)</td>
<td>2 (33.3%)</td>
</tr>
<tr>
<td>O</td>
<td>54</td>
<td>28 (65.9%)</td>
<td>12 (22.2%)</td>
</tr>
</tbody>
</table>

**Table 1**  
Percentage sensitivity of *S. typhi* and *S. paratyphi A, B, C* to different blood groups

When sera samples were pooled in respect to their types and experimented, the result was similar to that of individual sera. Sensitivity of *S. typhi* and *S. paratyphi* were highest for blood group B (80% and 71%) and least for blood group O (50% and 20%) respectively (Figures 2 and 3). The sex and age of subjects showed no effect on the sensitivity as result patterns were the same for pooled and individual sera. Preliminary screening of blood samples for evidence of antibody to *Salmonella* species was negative.

**Figure 2**  
Sensitivity of pooled blood groups (A, B, AB, O) against *salmonella typhi*

**Figure 3**  
Sensitivity of pooled blood groups serum against *salmonella paratyphi A, B, C*

Role of complement in bactericidal activity of serum to salmonella species: When *Salmonella typhi* and *Salmonella paratyphi A,B,C* were incubated with individual serum of blood groups, and pooled serum of same blood groups, results showed that the organisms were serum sensitive. However, when the sera were heated to inactivate the complement, the organisms became resistant with virtually no killing effect. But with the...
addition of external source of complement (Guinea pig sera), the sera regained sensitivity (Table 2). There was no significant difference between the pooled sera and individual sera (p > 0.01). When serum was treated with EDTA there was no killing effect, but treatment of serum with Mg\(^{2+}\)-EGTA caused slight bactericidal effect.

**Table 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum sensitivity of all groups tested</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pooled normal human serum</td>
<td>&gt;75% killing</td>
<td>S(^r)</td>
</tr>
<tr>
<td>Pooled decomplemented normal human serum</td>
<td>&lt; 50% killing</td>
<td>S(^s)</td>
</tr>
<tr>
<td>Pooled decomplemented + complemented</td>
<td>&lt; 75% killing</td>
<td>S(^s)</td>
</tr>
<tr>
<td>Pooled normal human serum treated with EDTA</td>
<td>&lt; 50% killing</td>
<td>S(^s)</td>
</tr>
<tr>
<td>Pooled normal human serum treated with Mg(^{2+})-EGTA</td>
<td>Slight bactericidal action</td>
<td>&lt; 50%</td>
</tr>
<tr>
<td>Control</td>
<td>No killing</td>
<td>S(^r)</td>
</tr>
</tbody>
</table>

**Table 3**

<table>
<thead>
<tr>
<th>Serum tested</th>
<th>Bactericidal titre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. typhi</td>
</tr>
<tr>
<td>Individual serum</td>
<td>1024</td>
</tr>
<tr>
<td>Pooled serum</td>
<td>512</td>
</tr>
<tr>
<td>Individual serum treated with 2-mercapto-ethanol</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Pooled serum treated with 2-mercapto-ethanol</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

**Role of immunoglobulin:** Addition of 2-mercapto-ethanol reduced the killing potential of antibody. Bactericidal titre of serum dropped from 1024 and 512 to <2 for Salmonella typhi and Salmonella paratyphi, respectively (Table 3).

**DISCUSSION**

The bactericidal activity of normal human sera to Salmonella typhi and Salmonella paratyphi ABC was investigated. Results obtained revealed that the most prevalent isolates of Salmonella species in this locality are serum sensitive. Salmonella typhi was extremely sensitive to blood group A and B sera (93.8% and 80%), respectively. Salmonella paratyphi ABC, showed 62.5% and 60% sensitivity to blood group A and B sera. This result is in agreement with previous studies(3,5). These authors reported that the sensitivity or resistance of bacterial isolates to natural serum bactericidal activity could serve as an important epidemiological marker in the study of bacteria infections. In the present study, group O blood sera were least active to the Salmonella species studied, 51.9% for Salmonella typhi and 22.2% for Salmonella paratyphi. This suggests that the species, particularly Salmonella paratyphi ABC may resist natural bactericidal action of blood sera of group O individuals, which implies that individuals of blood group O could be more susceptible to infections caused by these species of Salmonella.

When serum samples were pooled with respect to their blood groups, group B sera still showed the highest percentage killing effect to the organisms. Percentage sensitivity was higher with individual serum than pooled sera. This may be due to the varied surface antigenic make up and the immunological properties that exist between individuals as suggested(6). Statistically, the difference between individual serum and pooled sera was not significant (p > 0.01). Age and sex of donors showed no effect on the reaction pattern of the sera as shown by the pooled sera test, implying that there is no age limitation to bactericidal activity of human serum. Serum concentration was graduated and used to test for sensitivity; the result pattern was the same irrespective of the dilution. This indicates absolute resistance of blood group sera to Salmonella typhi and paratyphi, A, B, C.

The results of this study indicate that antibody alone did not reduce viable count of the organisms, which implies that killing of Salmonella species by normal human serum is complement-dependent as reported by previous workers(25,26). The present study showed that treatment of serum with Mg\(^{2+}\)-EGTA, which inhibits complement activities, produced slight bactericidal action and treatment of serum with EDTA reduced killing effect, which showed that part of the killing was mediated by complement.

It was shown that presence of antibody enhances total bactericidal activity and is crucial in the in-vivo host defence against Salmonella species(27), but study has demonstrated that only IgM serum fraction has proved to be bactericidal(15). The reducing agent mercapto-ethanol inhibits IgM antibody(15). Treatment of whole serum with mercapto-ethanol to determine immunoglobulin class responsible for the bactericidal activity, showed decrease in bactericidal activity to a titre as low as <2, which shows that IgM mediates the bactericidal activity of serum. Salmonella bactericidal activity of normal human sera is a function of IgM as determined by its susceptibility to mercapto-ethanol. This result is in agreement with previous reports(10,28).

This study reveals that complement determines the serum sensitivities to Salmonella infection as decomplemented human serum resulted in virtually no killing of the organisms. Also killing of Salmonella typhi and Salmonella paratyphi A, B, C is dependent on the presence of IgM antibody. Blood group O, which constitutes the highest number in the population, is the least active blood group to Salmonella species. The study may explain why certain individuals are susceptible to Salmonella typhoid fevers than others. The results have
provided useful information in determining individuals sensitivity or resistance to infection caused by Salmonella typhi and Salmonella paratyphi A,B,C. Since it has been reported that vaccine is not adequate protective measure against Salmonella infections, strict hygienic precautions should be adapted to avoid ingestion of the organism.

REFERENCES