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Bactericidal Effect of Normal Human Serum of Various Blood Groups Against *Yersinia* species

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Abstract: A total of 150 sera samples from volunteers (30 belonging to each of the A, B, O blood group) with 120 of them without history of diarrhoea infection (asymptomatic) and 30 with diarrhoea (symptomatic) were collected using standard technique. Bactericidal activities of the sera were tested against local isolates of *Yersinia pseudotuberculosis*, *Y. enterocolitica* 0:3, *Y. enterocolitica* 0:8, *Y. kristensenii* 0:11, 23, *Y. intermedia* 0:52, 53 and *Y. intermedia*-like bacteria 0:52, 53, using micro titre plate technique. The *Y. enterocolitica* 0:3 (89.15%) was found to be the most sensitive to the bactericidal effect of sera of blood group A individuals, while *Y. kristensenii* 0:11, 23 (45.58%), was the least susceptible. For group B sera, *Y. pseudotuberculosis* was the most sensitive (97.26%), while *Y. intermedia* 0:52, 53 (48.26%) was the least sensitive. *Yersinia enterocolitica* 0:3 (90.04%) was the most sensitive to blood group AB sera, while *Y. enterocolitica* 0:8 (52.77%) was the least sensitive. For sera of blood group O, only *Y. pseudotuberculosis* (56.77%) and *Y. enterocolitica* 0:3 (64.85) were sensitive, while other *Yersinia* species were resistant. The results of this study suggest that individuals with blood group O whose sera caused a relatively lower bactericidal effect will be more susceptible to Yersiniosis than individuals with other blood groups (A, B and AB). The result further suggests that circulating antibodies and/or lymphocytes induced by diarrhoeic organisms could assist in the elimination of *Yersinia* species from the blood of individuals suffering from *Yersinia bacteraemia*, a finding which is of both epidemiological and clinical significance.

Key words: Blood group, bactericidal effect, Yersiniosis

INTRODUCTION

Acute gastroenteritis has been known to be the most common infection associated with *Yersinia enterocolitica* and related species for several decades (WHO, 1974), however other human infections ranging from mild enterocolitis, mesenteric lymphadenitis, arthritis and severe septicaemia has increasingly been documented in recent times. Children and persons whose host defence mechanisms are compromised appear to be the groups at risk for septicaemia (Agbonlahor, 1983; Punsalong *et al*., 1987).

Virulence of *Yersinia* sp. in human has been associated with a number of *in vivo* and *in vitro* bacterial characteristics, including deep organ infiltration in orally inoculated mice, (Bakour *et al*., 1985), dependence on calcium for growth (Fletcher *et al*., 1988), ability to absorb a haemin type dye, Congo Red (Prpic *et al*., 1983). Each characteristic has some reliability in identifying organism with potential virulence in human, but none considers the human response to the organism (Prpic *et al*., 1985). Pathogenic bacterial strains are resistant to serum complement, penetrate human epithelial cells (Hela cells) or guinea pig conjunctivae, lethal to mice and demonstrate cytotoxicity (Cornelis *et al*., 1987). Some of these characteristics are mediated by plasmids with weights of 41 to 42 kDa for *Yersinia* strains (Portnoy *et al*., 1981; Kay *et al*., 1982).

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Bactericidal activity of human serum which is complement dependent is considered an important host defence mechanism against invasive diseases caused by Gram-negative bacteria (Vosti and Randal, 1970; Rice *et al*., 1980; Benge, 1988; David, 1988; Obi and Coker, 1989b). Consequently, the ability of Gram negative bacteria to invade the systemic circulation from mucosal sites is related to resistance of bactericidal activity existing in normal human serum (Schoolnik *et al*., 1976; David, 1988). Opsonisation of microorganisms by the C and B component of the complement system may prepare them for phagocytosis. However, surface bound C3b whether deposited by classical or alternative pathway activation, also serves as the C5 convertase which cleaves C5 and results in assembly of the membrane attack complex, C5b-9, with resultant lysis of bacteria in the absence of phagocytic cells (Duguid *et al*., 1985; Jawetz *et al*., 1998). The low occurrence of *Campylobacter jejuni* (Blaser *et al*., 1985; Obi and Coker, 1989b) and their higher occurrence in immuno-compromised patient have been suggested to be related to the sensitivity of these organism to serum bactericidal activity.

Susceptibility or resistance of individuals to microbial infections has been related to different blood groups (Athreya and Coriell, 1967; Agbonlahor *et al*., 1993). Athreya and Coriell (1967) reported that, individuals with group B blood are highly susceptible to the malaria parasite. The sensitivity or resistance of *Yersinia* sp. to the natural bactericidal activities of normal human sera from different blood groups have not been elucidated, even with the increasing awareness of the pathogenic nature of this group of organisms.

This study therefore examines the natural bactericidal activities of normal human sera from various blood groups against local isolates of *Yersinia* sp.

MATERIALS AND METHODS

Preparation of Bacterial Suspension for Use in Serum Bactericidal Assay

The *Yersinia* sp. used for this investigation were obtained from Prof. D.E. Agbonlahor's Laboratory, Department of Microbiology, Ambrose Alli University Ekpoma, Edo State, Nigeria and kept at refrigeration temperature (+4°C) at the microbiology laboratory Delta State University, Abraka, Nigeria. These were; *Yersinia pseudotuberculosi*, *Y. enterocolitica* 0:3, *Y. enterocolitica* 0:8, *Y. krestensenii* 0:11, 23, *Y. intermedia* 0:52, 53 and *Y. intermedia*-like bacteria 0:52, 53. All the *Yersinia* sp. *were* resuscitated and re-characterized to confirm their identity. The criteria previously used by Agbonlahor *et al*. (1983) and Agbonlahor (1986) and adopted by Owhe-ureghe *et al*. (2002) were employed. These involved testing for Gram reactions, motility (at 25 and 37°C), auto-agglutination, sugar fermentation, urease production, citrate utilization, oxidase reaction and invasive property (Sereny, 1955; Agbonlahor *et al*., 1983; Cowan, 1985; Agbonlahor, 1986).

The test organisms were grown on MacChonkey Agar (MCA) plates for 18-24 h. The cells were harvested into tubes containing sterile normal saline. These were, respectively centrifuged at 2000 x g for 30 min. These and the cells were washed thrice and pellets re-suspended in sterile normal saline to yield a cell count of approximately 1.49×10^{8} cfu mL^{-1} as previously described (Nwokolo *et al*., 1998). To determine the colony-forming unit per milliliter (cfu mL^{-1}) of the test organisms, 1 mL of the re-suspended cells was serially diluted and pour plated onto sterile MCA plates and incubated at room temperature for 18-24 h.

Fresh Human Serum

Blood samples from 150 volunteers who were staff and students of Delta State University, Abraka, Nigeria were collected using venous puncture. One hundred and twenty of these volunteers had no history of diarrhoea infection and were regarded as asymptomatic. The other volunteers (30) had a history of diarrhoea and were regarded as symptomatic. These blood samples were collected into samples bottle and transported to microbiology laboratory Delta State University, Abraka, Nigeria and

allowed to clot for 3 h at room temperature. The clotted blood samples were centrifuged at 2000 x g for 20 min and the sera separated into sterile bijou bottles for use. Heat inactivated sera used as control was prepared by heating the fresh human sera at 56°C for 30 min in a water bath as previously described (Obi and Coker, 1989b).

Serum Sensitivity Assay

The modified method of David (1988) using the U-bottom microtitre plates was employed in this investigation. This comprises of dispensing 0.25 mL of fresh bacterial suspension into triplicate wells and 0.25 mL of normal human serum was added to the first well. The same amount of heat inactivated serum was added to the second well. The third well contain only *Yersinia* sp. in each test case. Thereafter 0.25 mL samples were taken from each well (after 60 min incubation in a water both) and plated on sterile MacConkey agar medium. The plates were then incubated at room temperature for 24-48 h and the colony counts obtained.

Determination of Complement Role

To verify complement dependence on serum bactericidal activity against *Yersinia* sp. sera were inactivated at 56°C for 30 min. Roles of complements pathways were assessed by adding 0.25 mL-EDTA to pooled normal human serum as previously described by Blaser *et al*. (1985) and Obi and Coker (1989a).

Reading of Results

Bacterial species were considered sensitive, if more than 50% killing was obtained by the formula of Obi and Coker (1989a).

$$\text{Kill (\%)} = \frac{1 - \text{No. of cfu mL}^{-1}\text{(Fresh serum)}}{\text{No. of cfu mL}^{-1}\text{(Heat inactivated serum)}} \times \frac{100}{1}$$

RESULTS AND DISCUSSION

The susceptibility analysis of the test organisms against the sera of asymptomatic individuals of blood group A shows that *Y. enterocolitica* 0.3 (89.15%) was the most sensitive, while *Y. kristensenii* 0:11, 23 (45.58%) was the least sensitive. For group B sera, *Y. pseudotuberculosis* is the most sensitive (97.15%), while *Y. intermedia* 0:52, 53 is the least sensitive (48.26%). For sera of blood group AB, *Y. enterocolitica* 0:3, is the most sensitive (90.04%), while *Y. enterocolitica* 0:8 is the least sensitive (52.78%). In the case of sera of blood group O, *Y. enterocolitica* 0:3 is the most sensitive (64.85%), followed by *Y. pseudotuberculosis* (56.77%), while *Y. kristensenii* 0:11, 23S (43.05%) *Y. enterocolitica* 0:8 (32.75%) *Y. intermedia* 0:52, 53 (23.68%) and *Y. intermedia*-like bacteria 0:52, 53 (11.60%) were resistant. The ANOVA test showed that, there is no significant difference between the bactericidal effects of sera of blood groups A, B and AB against *Yersinia* sp. ($p>0.05$) but for the bactericidal effects of blood group O against *Yersinia* sp. there is no significant difference ($p<0.05$) (Table 1).

Table 1: Bactericidal activity of normal sera of blood groups (A, B, AB and O) against *Yersinia* sp.

	Killed (%)			
Yersinia strains	A	B	AB	O
Y. pseudotuberculosis	72.75±3	97.15±2	83.73±1	56.77±4
Y. enterocolitica 0:3	89.15±1	84.06±2	90.04±4	64.85±3
Y. enterocolitica 0:8	88.35±2	83.05±1	52.77±6	32.75±2
Y. kristensenii 0:11.23	45.58±3	89.26±3	65.08±2	43.05±4
Y. intermedia 0:52.53	50.86±2	48.86±1	75.56±1	23.68±5
Y. intermedia-like bacteria 0: 52. 53	61.75±2	96.27±3	82.00±2	11.60±3

Blood groups A, B, AB/*Yersinia* strains ($p>0.05$) Blood group O/*Yersinia* strain ($p>0.05$)

Table 2: Bactericidal activity of asymptomatic pooled blood group (A,B, AB and O) sera against *Yersinia* sp.

Yersinia strains	Killed (%)	Remark
Y. pseudotuberculosis	83.25±1	S^S
Y. enterocolitica 0:3	59.86±3	S^S
Y. enterocolitica 0:8	89.96±2	S^S
Y. kristensenii 0:11, 23	68.89±2	S^S
Y. intermedia 0:52, 53	89.90±1	S^S
Y. intermedia-like bacteria 0:52, 53	86.17±2	S^S

S^S = Serum sensitive

Table 3: Bactericidal activity of symptomatic blood group O sera against *Yersinia* sp.

Yersinia strains	Killed (%)	Remark
Y. pseudotuberculosis	90.35±2	S^S
Y. enterocolitica 0:3	58.83±1	S^S
Y. enterocolitica 0:8	54.09±3	S^S
Y. kristensenii 0:11, 23	86.02±2	S^S
Y. intermedia 0:52, 53	89.05±2	S^S
Y. intermedia-like bacteria 0:52, 53	73.60±1	S^S

S^S = Serum sensitive

Table 4: Bactericidal activity of EDTA treated sera of asymptomatic pooled blood group against *Yersinia* Sp.

Yersinia strains	killed (%)	Remark
Y. pseudotuberculosis	74.47±1	S^S
Y. enterocolitica 0:3	83.70±2	S^S
Y. enterocolitica 0:8	96.95±2	S^S
Y. kristensenii 0:11, 23	64.73±3	S^S
Y. intermedia 0:52, 53	18.83±1	S^R
Y. intermedia-like bacteria 0:52, 53	79.21±1	S^S

S^S = Serum sensitive, S^R = Serum resistant

Table 2 showed that all the test organisms were sensitive to the sera with percentage sensitivities ranging from 89.96% for *Y. enterocolitica* 0:8 to 59.86 for *Y. enterocolitica* 0:3. All the strains were sensitive with percentages of sensitivities ranging from (54.09%) for *Y. enterocolitica* 0:8 to (90.35%) for *Y. pseudotuberculosis* (Table 3).

When the *Yersinia* sp. were incubated with pooled normal human serum treated with EDTA, which inactivated both the alternative and classical pathways, only *Y. intermedia* 0:52, 53 was resistant to the sera, while other *Yersinia* strains showed a high degree of sensitivity ranging from (64.73%), for *Y. kristensenii* 0:11, 23 to (96.95%) for *Y. enterocolitica* 0:8 (Table 4).

The results obtained from this investigation indicate that human blood groups has a role to play in the susceptibility or resistance of individuals to *Yersinia* sp. infections. This agrees with earlier reports that the sensitivity of microorganism to the bactericidal effect of normal human sera vary with the blood group of the individual (Athreya and Coriell, 1967; Agbonlahor *et al*., 1993; Ndip *et al*., 1999).

Serum resistance has been associated with virulence in several Gram negative bacteria including; *Neisseria gonorrhoeae* (Schoolnik *et al*., 1976; Eisentein and Sparling, 1977), *Serratia marcescens* (Simberkoff *et al*., 1978) *Pseudomonas aeruginosa* (Borowski and Schiller, 1983) *Vibrio vulnificus* (Johnson *et al*., 1984), *Klebsiella oxytoca* (Benge, 1988), *Aeromonas hydrophilia* (Nwokolo *et al*., 1998) and *Plesiomonas shigelloides* (Ndip *et al*., 1999).

The results suggest that individuals with blood group O (Table 1) whose sera showed a relatively lower bactericidal effects will be more susceptible to yersiniosis than individuals with other blood groups (A, B and AB). Furthermore, the results also suggest that *Y. enterocolitica* 0:8 (32.75%), *Y. kistensenii* 0:11, 23, (43.05%) *Y. intermedia* 0:52, 53, (23.68%) and *Y. intermedia*-like bacteria 0:52, 53 (11.60%) may cause yersiniosis in individuals with blood group O than *Y. pseudotuberculosis* (56.77%) and *Y. enterocolitica* 0:8 (64.85%).

Table 5: Bactericidal activity of *Yersinia* anti-antisera against *Yersinia* sp.

Yersinia strains	Killed (%)	Remark
Y. pseudotuberculosis	90.28±2	S^S
Y. enterocolitica 0:3	95.55±2	S^S
Y. enterocolitica 0:8	92.97±1	S^S
Y. kristensenii 0:11, 23	85.25±2	S^S
Y. intermedia 0:52, 53	83.17±2	S^S
Y. intermedia-like bacteria 0:52, 53	92.97±1	S^S

S^S = Serum Sensitive

Result suggests that the specificity of the bactericidal activity of the normal human sera observed when the sera of the respective blood groups were used could be annulled when sera of individuals of the different blood groups are pooled together. This agrees with the study of Nwokolo *et al*. (1998) and Ndip *et al*. (1999).

That circulating antibodies induced by diarrhoeic organisms could assist in the elimination of *Yersinia* sp. from the blood of infected individuals. This was further confirmed by the very high level of percentages of sensitivities experienced by *Yersinia* sp. when they were treated with *Yersinia* anti-antisera as shown in Table 5.

When human sera was treated with EDTA, which blocks both alternative and classical pathways of the complement system. (Blaser *et al*., 1985; Obi and Coker, 1989b) cell sensitivity (*Yersinia* sp.) was still high (Table 1) except for *Y. intermedia* 0:52, 53. This suggests that elimination of *Yersiniae bacillus* from the circulatory systems of human and animals may not be complement dependent. This study suggest further that the resistance of *Yersinia* sp. to serum bactericidal effect of some A, B, O blood groups could be an important marker for the identification of prevalent serotypes and strains responsible for Yersiniosis in this environment.

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