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Short Communication

Production potentials of anti-*Plesiomonas shigelloides* antibody

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Out of the one hundred and fifty stool human samples screened for the prevalence of *Plesiomonas shigelloides*, only 3 (2%) were positive for the organisms. The positive isolates were used to produce *P. shigelloides* antibody in experimental animals (rabbits). Autopsy revealed marked inflammation of the liver, spleen and the kidney in the experimental animals not protected with the antibodies. This study showed that the trial rabbits were suitable for the production of antibodies against *P. shigelloides*, and further demonstrated the diagnostic and protective potentials of the antibodies.

Key words: *Plesiomonas shigelloides*, experimental animals, colloidal carbon particles, antibody and antisera.

INTRODUCTION

Plesiomonas shigelloides has been recognized recently as potential human and animal pathogen. Plesiomonads are Gram-negative, motile, non-spore forming bacilli facultative anaerobic and oxidase positive. Since 2001, *P. shigalloides* has been reported to belong to the family Enterobacteraceae (CDC, 2003). The primary reservoirs for this bacterium are fresh and estuarine water, mainly in temperature climates. This organism was isolated in 1947 by Ferguson and Henderson (1947), who named it C27. Since then, it has been variously known as *Plesiomonas shigelloides* (Basder, 1954), *Pseudomonas michignai*, *Aeromonas shigelloides* (Ewing et al., 1961), *Fergusonia shigelloides* (Sebabld and Veron, 1963) and *Vibrio shigelloides* (Heridric et al, 1971). *Plesiomonas shigelloides* normally inhabits surface waters and soil in temperate and tropical areas of the world and is not considered part of the indigenous microbiota of the human gastrointestinal tract (Arai et al., 1980).

Over the years there have been a number of reports on the isolation of *P. shigelloides* from clinical specimens (Davis et al., 1988). The increased level of awareness and seeming pathogenic roles of these organisms have recently generated interest in their study world-wide. P. shigelloides has been implicated in several large outbreak of gastro-enteritis (Rutala et al., 1982; Tsukamoto et al., 1978) and in sporadic cases of diarrhoeal illness (Reinhandt and George, 1985). Its role as gastrointestinal pathogen in human is still unclear (Herrington et al., 1987). Two features thought to play key roles in the regulation of gastrointestinal infection by enteropathogenic bacteria are gastric acidity and the presence of resident microbial flora in the lower gut. *P. shigelloides* associated diarrhea has occurred in epidemics but also in isolated cases. It has as well been implicated as an aetiological agent of food poisoning in many parts of the world, where it has been described after uncooked-shellfish consumption. *P. shigelloides* has been mentioned as a cause of traveller's diarrhoea (Holmberg et al., 1986) and has also been isolated in the immuno-compromised or persons seriously ill with cancer, blood disorders or hepatobiliary disease (Cutis et al., 1978; CDC, 2003). Tsukamoto et al. (1978) reported on the serology of *P. shigelloides* showed it to possess somatic (O) and flagella (H) antigens, which have been useful in the investigation of diarrhoeal epidemics. Early case report has demonstrated the potential pathogenicity of *P. shigelloides* as a secondary pathogen of low virulence.

P. shigelloides gastroenteritis is usually a mild self limiting disease with fever, chills, abdominal pain, nausea, diarrhoea or vomiting. Symptoms may begin 20-24 h after consumption of contaminated food or water. Diarrhoea is watery, non-mucoid and non-bloody, in severe cases, diarrhoea may be greenish-yellow, foamy and blood tinged. Duration of illness in healthy people

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may be 1 to 7days (CDC, 2003).

This paper was aimed at isolating *P. shigelloides* from stool to produce antibodies in rabbits and to determine the protective effect of the anti-antibodies against experimental infection with the organism.

MATERIALS AND METHODS

Isolation

One hundred and fifty asymptomatic and symptomatic (diarrhoeagenic) stool samples from adults and children were collected form Otibhor Okhae Teaching Hospital, Irrua and St. Camilius Hospital, Uromi. Upon collection, the samples were screened for the presence of P. shigelloides using xylose sodium deoxcycholate citrate agar, which is selective for P. shigelloides. The plates were incubated at 37°C for 24 h under anaerobic conditions. Upon incubation, the cultures were examined for the growth of P. shigelloides, which was identified using a previously described method of Alabi and Odugbemi (1990).

The Gram reaction as well as morphological and biochemical characterization were investigated using the methods of Gerhardt et al. (1994).

Production of anti-Pl. shigelloides antibody

For the study, experimental animals (rabbits) were obtained from the Rubber Research Institute, Iyanomo, Benin City and Irrua, Edo State. The experimental animals were fed on commercial diet, lemon grass and tap water regularly.

Colloidal carbon particles in the form of Indian ink were used as a blockading agent against phagocytes. The dilution of the colloidal carbon was done as follows; 0.01 g of agar was dissolved in 100 ml saline from the mixture. Aliquots of 0.5 ml were mixed with 0.5 ml Indian ink which makes up the colloidal carbon particles, and 0.4 ml of the final suspension was used to inject the experimental animals.

Cultures of *P. shigelloides*, both local and standard strain (ATCC 14029), were inoculated onto Petri dishes containing xylose sodium deoxycholate citrate agar and incubated at 37°C for 24 h. The cultures were transferred to 9 ml phosphate butter saline (PBS) (pH 7.8), washed twice and finally resuspended in PBS at $1{\cdot}7^{10}$ cfu/ml. The experimental animals (10) weighing 3.0 to 3.5 kg were intravenously injected with increasing dose of *P. shigelloides* antibody raised against standard strain (ATCC 14029) and local strains, at 4 to 5 days intervals for four weeks. Five days after the last injection, the animals were bled and serum was separated after centrifugation, to obtain anti-*P. shigelloides antiserum.*

Passive protective test

The experimental animals (rabbits) were immunized intraperitionally. Ten of the experimental animals were injected with *P. shigelloides* and anti-*P. shigelloides* antibody, another set of 10 experimental animals were injected with P. shigelloides, colloidal carbon particles and anti-*P. shigelloides*, antibody. The third group of 10 experimental animals were injected with *P. shigelloides* and colloidal carbon particles while the fourth group of 10 experimental animals were injected only with *P. shigelloides* and finally, the last group of 10 experimental animals were injected only with colloidal carbon particles. The various inoculations were a dose each and results were obtained after a period of one week.

Table 1. Prevalence of P. shigelloides among symptomatic and asymptomatic individual screened.

Type	No examined	No and % of occurrence
Symptomatic	84	3 (3.57%)
Asymptomatic	66	0 (0%)
Total	150	3 (2%)

RESULTS

The study revealed that out of a total of 150 stools screened for the prevalence of *P. shigelloides*, only 3 (2%) were positive. All the three isolates were obtained from stools of patients with diarrhoea (Table 1). The local and standard strains (ATCC 14209) used in this study were also used to produce *P. shigelloides* antisera. Out of the 10 experimental animals injected with *P. shigelloides* and anti-*P. shigelloides* antibody only 1 (10%) died, while those injected with *P. shigelloides*, colloidal carbon particles and anti-*P. shigelloides* antibody two (20%) death were recorded. Of those injected with *P. shigelloides* and colloidal carbon particles, 7 (70%) death were recorded and 5 (5%) was recorded for those injected only with *P. shigelloides.* Finally no death was recorded for those injected with colloidal carbon particles only (Table 2).

Autopsy examination of the experimental animals (rabbits) showed inflammation of the kidney, liver and spleen. These organs were normal with control experimental animals.

DISCUSSION

*P. shigelloide*s which has been reported, as an aetiological agent of diarrhoea (Alabi and Odugbemi, 1990), is also responsible for the cause of several disease states including septicemia and colitis. In this study a total of one hundred and fifty stool samples of adult and children were screened for the prevalence of *P. shigelloides* and only 3 (2%) were positive.

It is, therefore, interesting to note that antisera against local isolates of *P. shigelloides* were successfully produced in rabbits and this is consistent with previous reports (Obi and Coke, 1988, 1989). The avidity of this antibody compares favourably with antisera using an international isolate (ATCC 14209). The titre of both the local and standard strain was $^{1}/_{64}$. The successful production of antisera against *P. shigelloides* is in harmony with the scientific self-reliance policy and the achievement of health for all in the 21st century. The death of the animals injected shows a possible lethality for *P. shigelloides* and that the lethality was enhanced by the addition of colloidal carbon particles. Colloidal carbon particles have been reported to block macrophages and macrophages are known to be important factor in the

Table 2. Passive protective effect of anti-P. shigelloides antibody in experimental animals challenged with virulent P. shigelloides.

Inoculation of experimental animals*	No. of death	% of death
A. *P. shigelloides* + anti-*P. shigelloides* antibody	1	10
B. *P. shigelloides* + colloidal carbon particles + anti-*P.shigalloides* antibody	2	20
C. *P. shigelloides* + colloidal carbon particles	7	70
D. *P. shigelloides* only	5	50
E. colloidal carbon particles	-	-

* 10 animals per group.

defence mechanism of animals (Obi and Coker, 1989). The injection of colloidal carbon particles did not result in the death of the animals, suggesting that its effect on macrophages may not be sufficient to cause death. With the injection of anti-*Plesiomonas shigelloides* with *P. shigelloides* and colloidal carbon particles, the lethality observed decreased to 20% indicating possible protective effect of anti-*P. shigelloides* antibody. This agrees with Obi and Coker (1989), who reported that rats could be passively protected against Campylobacter jejuni by an initial infection of Campylobacter anti-antibody.

Furthermore, it has been reported that frequent injection with a particular antigen may stimulate two types of antibody responses, one of which is usually directed against the antigen and the other one is directed against the antibody. The anti-antibody is involved in the regulation of immunity (Klustens and Kohler, 1974). The anti-antibody exhibits immunosuppressive properties, neutralizing properties and are useful in the regulation of immune responses.

In conclusion, the diagnostic and protective potential of the antibodies will serve to provide new frontiers for the laboratory diagnosis and passive protection of animals against *P. shigelloides* infection.

REFERENCES

Alabi SA, Odugbemi T (1990). Biocheml characteristics and a simple scheme for the identification of Aeromonas spp and *Plesiomonas shigelloides*. J. Trop. Med. Hyg. 93: 166-169.

Aria T, Ikejima N, Itoh T, Sakai S, Shimada T, Sakazaki R (1980). A survey of *P. shigelloides* from aquatic environments,
domestic animals, pets and human. J. Hyg. 84: 203-211.

Bader RE (1954). Uber die herstelling enies agglutnierenden serums gapen die Rundform von Shigella sonnei mit einem stamm der Gattung Pseudomonas. Z. Hyg. 14: 450-456.

Center for Disease Control (2003). Foodborne pathogenic microorganisms and natural toxins handbook. Atlanta, Gorgia. pp. 1-4.

Cohen VL, Holyk PR, McCarthy LR, Peiffer RL (1983). Aeromonas hydrophilia and *P. shigelloides* endophthalmitis. Am. J. Ophth. 96:403-404.

Curtis AJ, Lin JH, Szabo K (1985). Overwhelming post splenectory injection with *P. shigelloides* in a patient cured of Hodpkin's disease. Am, J. Clin. Pathol. 83: 522-524.

Davis WAH, Kare JG, Garagusi VF (1978). Human Aeromonas infections; A review of the Literature and a case report of endocarditis. Medicine 57: 267-277.

Ewring W, Hugh R, Johnson J. (1961). Studies onthe Aeromonas group. U.S. Department of Health, Education and Welfare, communicable disease center,Atlanta. pp 126.

Hendrie MS, Shewan, JM, Veron M (1971). *Aeromonas shigelloides*: A proposal that it be transferred to the genus Vibrio. Int. J. Sys. Bacterial. 21: 25-27.

Herrington, DA, Izipor S, Robins-Browne RM, Tall BD, Lerine MU (1987). Invited and *in vivo* pathogenicity of *P. shigelloides.* Inf. Immunol. 25: 979-985.

Holmberg SD, Wachsmith K., Hichman Brenner FW, Blake PA, Farmer JJ (1986). Plesiomonas enteric infections in the U.S. Ann. Int. Med. 105: 690-694.

Khusten SL Kohler H (1974). Repulation of immune response by indigenous antibody against receptor proceed. Nat. Acad. Sci. 71: 5083-5087.

Nolte FS, PooleRM, Murphy GW, Clark, Ranner BJ (1988). Proctitis and fatal septicemia and cause by *P. shigelloides* in a bisexual man. J. Clin. Microbiol. 26: 388-391.

Obi CL,Coker AO (1988). Production of antisera against Campylobacter species in Lagos, Nigeria. Cent. Afr. J. Med. 34: 139-141.

Obi CL, Coker AO. (1989). Passive protection of rats against Campylobacter using Campylobacter anti-idiotypes. Nig. J. Technol. Rev. 1: 99-101.

Rutala WA, Sarubi FA, Finch CS, McCormack JN, Stenikraus, G.E. (1982). Oyster associated with outbreak of diarrhoea disease possible caused by *Plesiomonas shigelloides* Lancet. 1: 739.

Sabald M, Veron M. (1963). Teneur en bases del ADN et classification des Vibrios. Ann. Inst. Pasteur. 105: 879-910.

TsukamotoT, Kinoshita Y, Shimada T, Sakazak R. (1978). Two epidermcs of diarrheoal disease possibly caused by *P. shigelloides*. J. Hyg. 80: 275-280.

Vandepitte J, Makudu A, Gatti, F. (1974). *Plesiomonas shigelloides*: Survey and possible association with diarrhoea in Zaire. Ann. Ass. Belg. Trop. Med. 54: 503-513.