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Full Length Research Paper

The antibiogram and role of antibody in prophylaxis of albino rats against diarrhoea caused by *Escherichia coli* O157:H7

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The antibiogram and role of antibody in prophylaxis to *Escherichia coli* O157:H7 infection in albino rats was investigated in this study. 500 stool samples were collected from apparently healthy individuals, out of which 80 were positive for the organism. The sensitivity patterns of the isolate to conventional antibiotics were investigated. Moreover, the role of antibodies to actively or passively protect albino rats against *E. coli* O157:H7 infection was also carried out. The antibody used for the passive protection test was raised in rabbits immunized with the organism. The isolates were sensitive to the test antibiotics in an increasing order as follows: septrin (0%), ampicillin (0%), nalidixic acid (2.5%), augmentin (5%), ciproflox (5%), peflacine (16.25%), tarivid (16.25%), ceporex (16.25%), streptomycin (33.7%) and gentamycin (81.25%). All the isolates displayed multiple resistance to all the antibiotics used. Three (3.75%) of these were resistant to 3 of the antibiotics, 2(2.5%) to 4 of the antibiotics, 25 (31.25%) to 5 of the antibiotics, 24 (30.0%) to 6 antibiotics, 20 (25.0%) to 7 of the antibiotics, 4 (5.0%) to 8 of the antibiotics, 0 (0.0%) to 9 antibiotics, and 2 (2.5%) to all the 10 antibiotics used. For the immunization assay, after the primary immunization of the animals, the antibody titre of the animals averaged 1:85.3 for rabbits and 1:128 for rats. These values increased after the administration of the booster dose to 1:2048 for rabbits and 1:512 for rats. In the protection assay, only active immunization with live cells of *E. coli* O157:H7 protected the rats from infection, passive immunization did not protect them from infection despite using antibody of high titre value (1:2048) against the infection in albino rats. This study showed that *E. coli* O157:H7 isolated from apparently healthy individuals displayed multiple resistance to all the antibiotics. However, in case of an outbreak, gentamycin can be used for treating infection caused by the pathogen. For prophylaxis, however, active immunization with live cells of *E. coli* O157:H7 is recommended.

Key words: *Escherichia coli* O157:H7, albino rats, antibiotics, immunization.

INTRODUCTION

Escherichia coli O157:H7 is known to cause foodborne illness with foods and water as its vehicles of transmission. It is an enterohemorrhagic strain of the bacterium *E. coli*. Infection often leads to hemorrhagic diarrhoea, and occasionally to kidney failure, especially in young children and the elderly (Karch et al., 2005). Since the outbreak in 1982, *E. coli* O157:H7 diarrhoeal illness has been reported from multiple locations in South Africa, Central Africa Republic, Kenya, Gabon, Nigeria and Ivory Coast (Okeke et al., 2000). This organism is well known to occur frequently in United States and Great Britain.

There are currently long term studies going on in Walkerton, Ontario, looking at the long term effects of *E. coli* O157:H7 after approximately 2500 people were infected through the municipal water system in May, 2000 (Wikipedia, 2009). There is no evidence that antibiotics improve the course of disease, and treatment with some antibiotics might precipitate kidney complication

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Table 1. Percentage of isolates that were sensitive to different antibiotics.

Antibiotic	No. of isolates that were sensitive	Sensitivity (%)
Tarivid	13	16.25
Peflacine	13	16.25
Ciproflox	4	5
Augmentin	4	5
Gentamycin	65	81.25
Streptomycin	27	33.75
Ceporex	13	16.25
Nalidixic acid	2	2.5
Septrin	0	0
Ampicillin	0	0

according to Walterpiel et al. (1992). There is therefore the need to curtail the spread of the infection caused by this organism. This study is therefore designed to investigate the possibility of prophylactic measure against the infection using live cells or corresponding antibodies so that in case of epidemiological outbreak, it will be easy to contain the infection.

MATERIALS AND METHODS

Five hundred stool samples were collected from apparently healthy individuals in Ondo State. The samples were immediately brought to the laboratory and cultured on eosin methylene blue agar and those positive for *E. coli* were subcultured on sorbitol MacConkey agar for identification of *E. coli* O157:H7. The antibiotic sensitivity pattern of the organism was carried out according to the method of Cheesbrough (2000) with slight modification.

New Zealand white rabbits and albino rats were used for the animal bioassay. These animals were purchased from local breeders in Akungba, Ondo State, Nigeria and acclimatized to the department's animal house environment for two weeks before the assay. The weight of the rabbits and the rats were determined. The infective dose of the organism was determined by orogastrically dosing the animals with increasing concentrations of the organism. For active immunization, three rabbits were each subcutaneously injected with increasing concentration of the organism at 7 days interval for three consecutive weeks, according to the method of Adebolu et al. (2010). Rats on the other hand were orogastrically dosed with an increased concentration of the organism for the same interval using a lower concentration. Seven days after the last injection, their blood were collected through venepuncture, allowed to clot and the serum was used for serological and protection assay. The immunized animals were challenged with the infective dose of the organism and were observed for symptoms of infection. Rats that were not immunized served as control. This experiment was analysed statistically using one way ANOVA (CRD).

RESULTS

Out of the 500 stool samples examined, sixteen percent were positive for *E. coli* O157:H7. Concerning antibiotic sensitivity pattern of the isolates, 81.25% were sensitive to gentamycin, 33.75% to streptomycin, 16.25% to tarivid, peflacine and ceporex, respectively. The least effective of the antibiotics was nalidixic acid (2.5%) (Table 1).

The percentage of the isolates resistant to different number of antibiotics is as follows: those resistant to only 1 antibiotic was zero, none was resistant to two, three (3.75%) were resistant to 3 antibiotics, two (2.5%) were resistant to four of the antibiotics, 25 (25.0%) to seven of the antibiotics, 4 (5.0%) to eight of the antibiotics, 0 (0.0%) to nine antibiotics, while 2 (2.5%) to all the ten antibiotics used (Table 2).

For the immunization assay, after primary immunization, the antibody titre of the animals averaged 1:85.3 for rabbits (Table 2) and 1:128 for rats (Table 3). After the administration of the first booster dose, the titre increased as shown in Table 4. The effect of immunization on the susceptibility of apparently healthy rats to infection with *E. coli* O157:H7 can be seen in Table 5, active immunization offered 100% protection, and none of the rats took ill. On the other hand, passive protection did not offer any protection; all the three rats took ill as well as the control. Changes in the physical appearance of the albino rats after infection with *E. coli* O157:H7 and after pre-treatment with antibodies to the organism prior infection can be seen in Tables 6 to 8, respectively. All the rats showed signs of illness such as physical weakness, change in faecal appearance, loss of appetite and low water intake. However, for those that were actively immunized with the live cells of the organism, none of them took ill (Table 9).

DISCUSSION

The antibiogram and role of antibody in prophylaxis of albino rats to *E. coli* O157:H7 isolated in Ondo State was carried out in this study. The result of the antibiotic susceptibility test conducted showed that this organism is resistant to all the antibiotics used except gentamycin that mediated 81.25% effectiveness against the organism. This is serious, because this means that if there is an outbreak of the infection, only gentamycin can be used for

Table 2. Relationship between the number of antibiotics and the resistant isolates of *E. coli* O157:H7 in Ondo State.

Antibiotics	No. that were resistant	Percent that was resistant
Gentamycin	0	0
Streptomycin	0	0
Septrin, ampicillin, nalidixic acid	3	3.75
Augmentin, ciproflox, peflacine and tarivid	2	2.5
Augmentin, ciproflox, nalidixic acid, ampicillin and septrin	25	31.25
Septrin, ampicillin, nalidixic acid augmentin, ciproflox and peflacine	24	30.0
Tarivid, peflacine, augmentin, nalidixic acid, ampicillin septrin and ciproflox	20	25.0
Ceporex, peflacine, tarivid, ceporex, streptomycin, nalidixic acid, septrin and ampicillin	4	5.0
Septrin, ampicillin, nalidixic acid, augmentin, ciproflox, peflacine, tarivid, ceporex	0	0
Septrin, ampicillin, nalidixic acid, augmentin, ciproflox, peflacine, tarivid, ceporex streptomycin and gentamycin	2	2.0
Total	80	100

Table 3. Antibody titres of the rabbits are determined using bacterial agglutination test (tube method).

Rabbit No.	Reciprocal antibody titre
1	64
2	128
3	64
Average titre	85.3

Table 4. Antibody titres of rat sera against *E. coli* 0157: H7

Rat No.	Reciprocal antibody titre
1	128
2	128
3	128
Average titre	128

Table 5. Antibody titres of antisera prepared against live cells of *E. coli* 0157:H7 after secondary immunization.

Animal	Sex	Titre
Rabbits		
A	Male	1:2048
B	Male	1:2048
C	Female	1:2048
Rats		
A	Male	1:512
B	Male	1:512
C	Male	1:512

therapeutic purposes and since it cannot be administered orally, it means that only medical personnel can handle it. So, if there is an outbreak in rural communities where basic medical facilities are rare, the teeming population there will become highly susceptible to the complications of the infection. Efforts therefore should be made to make sure that the outbreak of infection is prevented.

Concerning the animal experiment, the estimated infective dose of enterohemorrhagic *E. coli* (EHEC) was 10^2 (100 cells), which is much lower than 10^8 for enterotoxigenic *E. coli* (ETEC) as recorded by Nataro and Kaper (1998), and 10^6 enteroinvasive *E. coli* (EIEC) as recorded by Dupont et al. (1971). This shows that the organism is highly pathogenic. The initial high antibody titre recorded in rats as compared with that of rabbits in Tables 2 and 3 could be due to the route of inoculation. Rabbits were inoculated subcutaneously while rats were inoculated orogastrically. A rise in antibody titre from 1:85.3 for rabbits and 1:128 for the rats to 1:2048 and 1:512 respectively is an evidence of active humoral response to infection. This is in agreement with the report of Betty et al. (2007). From the result, active protection with live cells of *E. coli* O157:H7 gave hundred percent for the rats against the infection caused by organism, but passive protection with antibody to *E. coli* O157:H7 did not protect the rats from infection caused by the organism. This shows that antibodies did not play major role in protecting infection in the active protection experiment. Other immune cells such as the macrophages, neutrophils and T-cells probably contribute to the successful protection of the rats from the infection.

CONCLUSION AND RECOMMENDATION

Gentamycin has been found as the only antibiotic that

Table 6. Effect of immunization on the susceptibility of apparently healthy rats to infection when challenged with the infective of *E. coli* 0157:H7.

Pre-treatment	Route of infection	No. of animals used	Interval after injection (h)	No. that took ill	Percent that took ill
Active protection with *E. coli* 0157:7H	Orogastrically	3	24	0	0
			48	0	0
			72	0	0
Passive protection with antibodies to antibodies to *E. coli* 0157:7H	Intraperitoneal	3	24	3	100
			48	3	100
			72	3	100
Control	Orogastrically	3	24	3	100
			48	3	100
			72	3	100

Table 7. Changes in the physical appearance and faeces of apparently healthy albino rats after infection with *E. coli* 0157:H7.

Parameter	Days after infection		
	1	2	3
Eating ability	Loss of appetite	Loss of appetite	Recovering
Water intake	Little	Little	Recovering
Agility	Very weak	Very weak	Recovering
Faecal appearance	Brown and soft	Brownish and soft	Brownish and solid

Table 8. Effect of pre-treatment with antibodies to *E. coli* 0157:H7 on the physical appearance and faeces of rats infected with *E. coli* 0157:H7 through the intraperitoneal route.

Parameter	Days after infection		
	1	2	3
Eating ability	Loss of appetite	Loss of appetite	Recovering
Water intake	Little	Little	Recovering
Agility	Very weak	Very weak	Recovering
Faecal appearance	Brown and soft	Brownish and soft	Brownish and solid

Table 9. Effect of active immunization with live cells of *E. coli* 0157:H7 on the physical appearance and faeces of rats infected with *E. coli* 0157:H7.

Parameter	Days after infection		
	1	2	3
Eating ability	Normal	Normal	Normal
Water intake	Normal	Normal	Normal
Agility	Very active	Very active	Very active
Faecal appearance	Brown and soft	Brownish and soft	Brownish and solid

mediates effectiveness against *E. coli* O157:H7 isolated from the stools of apparently healthy individuals in Ondo State. There is therefore the need to search for more effective antibiotics against the organism. To prevent

epidemiological outbreak of the infection; however, immunization with live cells of the organism should be employed.

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