

African Journal of Biotechnology Vol. 3 (7), pp. 366-369, July 2004
Available online at <http://www.academicjournals.org/AJB>
ISSN 1684-5315 © 2004 Academic Journals

Full Length Research Paper

Sporadic diarrhoea due to *Clostridium perfringens* in children aged five years and below

M. O. Efuntoye* and A. I. Adetosoye

Department of Veterinary Microbiology and Parasitology, University of Ibadan, Ibadan, Nigeria.

Accepted 25 July 2004

In a study to identify the clostridial species associated with sporadic diarrhoea in children aged 5 years and below, clostridia species were isolated from 57 (3%) of 1761 diarrhoeic faecal samples studied. Fifty-one of the clostridial isolates were identified as *Clostridium perfringens* while the other six isolates were identified as *C. bifermentans*. Forty-seven were *C. perfringens* type A, 4 were *C. perfringens* type C. Forty-one of *C. perfringens* type A and 2 of the *C. perfringens* type C were enterotoxigenic. From this investigation, it was observed that *C. perfringens* types A and C were involved in cases of sporadic diarrhoea affecting children aged 5 years and below in Ibadan, Nigeria.

Key words: Sporadic diarrhoea, *Clostridium perfringens*, children.

INTRODUCTION

Diarrhoeal disease continues to be a health problem among young children all over the world especially in the developing countries. Several bacteria have been recognised to play a causal role in diarrhoeal diseases, including *Salmonella* spp., *Shigella* spp., serotypes of enterotoxigenic, verotoxigenic and enteropathogenic strains of *Escherichia coli*, *Vibrio* spp. and *Campylobacter* spp.

In the past two decades enterotoxigenic strains of *Clostridium perfringens* have also been identified as an important aetiological agent of diarrhoea (Boriello et al., 1985; Samuel et al., 1991; Brett et al., 1992; Mpamugo et al., 1995). *C. perfringens* has been isolated from diarrhoeal cases. Most of the investigations carried out have been on outbreak and have involved adults (Van Loon et al., 1990). The results from investigations carried out so far had not given an accurate assessment of the relative importance of *C. perfringens* in sporadic diarrhoea involving children.

This study characterises the clostridia species isolated from diarrhoeic children aged 5 years and below.

MATERIALS AND METHODS

Stool specimens were collected from 1761 children aged 5 years and below with isolated incidents of acute diarrhoeal illness without antibiotic and food association and from 450 age-matched control children without diarrhoea from five hospitals located in Ibadan. The faecal samples were collected in sterile disposable universal bottles (Sayag, France). The samples were transported immediately on ice in an air-tight container to the laboratory where they were bacteriologically studied according to standard methods (Barrow and Feltham, 1993).

Isolation procedure for clostridial species

For total viable count, approximately 1 g of each faecal sample was inoculated into 10 ml Brain Heart infusion (BHI) broth (Difco) in a screw-capped bottle. This was shaken vigorously to homogenise, after which 10-fold dilutions were prepared in appropriate amount of BHI broth and left for a few minutes to allow the debris to sediment. A 0.1 ml of the supernatant was inoculated in duplicate on sheep blood agar (Blood agar base No. 2, Oxoid cm271) supplemented with 100 µg/ml neomycin sulphate. For each dilution 0.1 ml was seeded on to Egg Yolk Medium (EYM) prepared by adding 20 ml of egg yolk suspension to BHI agar. All the inoculated plates were incubated at 37°C in an anaerobic jar using the Gas Pak system for 24 h. After overnight incubation, the total viable count was done by counting the colonies in each dilution series using the Miles and Misra technique (Miles and Misra, 1938.). The EYM plates were also examined for detection of lecithinase, lipase and proteolytic enzyme production (Barrow and Feltham, 1993).

*Corresponding author. Present address: Department of Biological Sciences, Olabisi Onabanjo University, P.M.B. 2002, Ago-Iwoye, Nigeria. Tel: 810901 ext 456. Cell phone: 0722861176. Email: mosolatoye@yahoo.com.

***C. perfringens* spore counts**

Alcohol shock treatment was used (Koransky et al., 1978). Serial 10-fold dilutions were prepared in BHI broth and 0.1ml of each dilution was seeded on to blood agar containing 100 µg/ml neomycin sulphate. The plates were incubated anaerobically at 37°C for 24 h.

Isolation of heat-resistant of *C. perfringens*

A small portion (approx. 1 g) of the faecal sample was added to Cooked Meat Medium (CMM). This was heated at 100°C for 1 h in a water bath, cooled under tap water and incubated at 37°C for 24 h. The enrichment culture was then plated on to blood agar containing neomycin sulphate and incubated anaerobically at 37°C for 24 h. The presence of growth of *C. perfringens* on blood agar containing neomycin sulphate was taken as evidence that the *C. perfringens* was of the heat-resistant strain.

Identification procedure

The plates were bacteriologically examined according to Barrow and Feltham (1993). After incubation, the anaerobic jar was placed in a refrigerator at 4°C for 30 min to allow full development of haemolysis. Smears were made from typical colonies and Gram-stained. Colonies of Gram positive rods presumptive for clostridial species were subcultured onto freshly prepared plain sheep blood agar and incubated anaerobically. Representative colonies were inoculated respectively into CMM and incubated for 12 h. Isolates were identified according to the methods described by Sutter et al. (1984) employing tests including catalase, indole, stormy fermentation of milk, reverse CAMP test, phosphatase test, motility, egg yolk reaction, gelatin liquefaction, nitrate reduction, sulphate reduction, urease hydrolysis, oxidase test, fermentation of carbohydrates and Nagler's reaction.

Toxin-typing of *C. perfringens* Isolates

The *C. perfringens* strains were toxin-typed according to the method described by Sterne and Batty (1975) using neutralising antisera (Wellcome, England).

Enterotoxin analysis

In this investigation, the "PET-RPLA" kit (Oxoid, UK) that relied on rapid reversed latex agglutination to detect enterotoxin in *C. perfringens* was used. The procedure of assay was as described by the manufacturer.

RESULTS

Clostridial species were isolated from 57 (3%) of the 1761 diarrhoeic faecal samples. Out of 57 isolates recognised as clostridial species, 51 (89.47%) were identified as *C. perfringens* while the remaining six (10.53%) were identified as *C. bifermentans*. All isolates identified as *C. perfringens* gave typical reactions in tests such as Gram staining, motility, lactose fermentation, gelatin liquefaction, nitrate reduction, inositol fermentation

and reverse CAMP test. Majority fermented sucrose (49), fructose (50), maltose (49), trehalose (49), mannose (50) while none fermented mannitol, raffinose arabinose, cellobiose and xylose. 41 isolates were β-haemolytic while 49 isolates were positive for phosphatase production. Of the symptomatic children < 1 year old with stool cultures positive for *C. perfringens*, 9 were male and 6 were female and among toddlers between 1 and 5 years of age, 19 were male and 17 were female. The incidence of *C. perfringens* was in decreasing order as the age increased. The duplicate cfu count average ranged from 1.9×10^5 to 2.84×10^6 colony forming unit (cfu) per gram of diarrhoeic faeces. Children between the age of 3 and 5 years consisting of 8 males and 5 females particularly showed high carriage rate of *C. perfringens* compared with other age group (Table 1). In most cases, the counts on egg yolk agar was slightly higher than those on blood agar with neomycin shown in Table 2, although the differences were not quite significant since there was only a difference of 2 or 3 colonies at most. The faecal samples of age-matched control children attending hospital but without diarrhoea (450) in this study had counts of *C. perfringens* not exceeding 10^2 cfu/g of faeces with the alcohol shock treatment.

Twenty-four strains were heat-sensitive while 27 strains were heat-resistant. There was no significant difference ($P < 0.05$) in the prevalence of heat-sensitive and heat-resistant strains in the children (Table 2).

Two toxin-types were recorded. Type A was the predominant toxin type (47 strains, 92.2%), while four isolates representing 7.8% of total *C. perfringens* isolates belonged to *C. perfringens* type C. Forty-one strains (87.2%) of *C. perfringens* type A produced enterotoxin while 2 strains (50%) of *C. perfringens* type C were also enterotoxigenic. None of the 8 *C. perfringens* isolates from control group produced enterotoxin. Other bacteria isolated from some of the remaining diarrhoeic faecal samples (164 samples) include *Salmonella* spp, *Klebsiella* spp, *Bacillus* spp, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

DISCUSSION

Most studies on *C. perfringens*-associated diarrhoeas had been conducted on the elderly aged 60 years and above, who are more vulnerable, and the investigations have been conducted during outbreaks and in association with antibiotic therapy (Stringer et al., 1980; Jackson et al., 1986). There appeared to be no statistical significance ($P < 0.05$) in the occurrence of *C. perfringens* diarrhoea in male and female children since both genders had close incidence of isolation of the organism in cases affecting them. The heat activation procedure which involves heating at 80°C for 10 min, and the alcohol

Table 1. Age distribution of children with *C. perfringens* and their faecal spore counts.

Age group (year)	No. of samples positive		
	Male	Female	Duplicate court average (cfu/g)
< 1	9	6	1.96 X 10 ⁵
1.1 – 2.0	6	7	3.0 X 10 ⁵
2.1 – 3.0	5	5	4.1 X 10 ⁵
3.1 – 4.0	5	3	1.7 X 10 ⁶
4.1 – 5.0	3	2	2.84 X 10 ⁶

Table 2. Heat sensitive/resistant *C. perfringens* strains from diarrhoeic stools of children.

Age group (year)	No. examined	Heat sensitive (%)	Heat resistant (%)
< 1	15	9(60)	6(40)
1.1 – 2.0	13	7(53.8)	6(46.2)
2.1 – 3.0	10	5(50)	5(50)
3.1 – 4.0	8	2(25)	6(75)
4.1 – 5.0	5	1(20)	4(80)

shock treatment (Koransky et al., 1978) did not reveal any appreciable difference in spore counts. Sutton (1966) reported that *C. perfringens* type A occurs in man as part of the normal intestinal flora in relatively small numbers of up to 10³ cfu/g. However, Yamagishi et al. (1976) in a related study among the elderly aged 63 to 79 years old reported persistently high count of *C. perfringens* in faeces up to 10⁷ to 10⁹ cfu/g. These reports were corroborated by the findings of Stringer et al. (1985) who investigated the faecal carriage of *C. perfringens* in adults. The increase of heat resistance with age as observed in this study could be as a result of change in immune system which allows for proliferation of heat resistant strains.

Faeces of children with sporadic diarrhoea and in which *C. perfringens* was the only bacterium detected contained counts above 10⁵ and in some cases in excess of 10⁶ cfu/g. This suggests that high spore counts are usually associated with diarrhoea in which *C. perfringens* is the causative agent.

Forty-one isolates of *C. perfringens* type A and 2 *C. perfringens* type C from diarrhoeic faecal samples were enterotoxigenic. None of the *C. perfringens* type A isolated from apparently healthy control children produced enterotoxin. These findings agreed with published reports of Samuel et al. (1991), Brett et al. (1992) and Mpamugo et al. (1995) where enterotoxigenic *C. perfringens* strains were isolated from cases of sporadic diarrhoea. That 47 (92.2%) *C. perfringens* isolates were identified as type A is not surprising since type A is primarily of human health importance, being the type usually encountered in food-borne infections.

The isolation of *C. perfringens* type C in humans is rare. However in 1987, Johnson et al. (1987) reported cases of *C. perfringens* type C in Thailand and Van Loon

et al. (1990) reported it in Australia. This is the first known report of *C. perfringens* in children with diarrhoea in Nigeria. The isolation of *C. perfringens* type C in Nigeria is significant because consumption of sweet potato, which is one of the predisposing factors, is not common in the area of this investigation (Davis, 1985). Further work on *C. perfringens* type C in diarrhoea in children is in progress to ascertain the factors that contribute to its proliferation in children's bowels.

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