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O. BADEMOSI and D.D.O. OYEBOLA

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Clostridium difficile in the normal adult faecal flora

V. O. ROTIMI AND D. AKINDUTIRE

Department of Medical Microbiology and Parasitology, College of Medicine, PMB 12003, Lagos, Nigeria

Summary

One hundred and seventy-seven out of 324 routine non-diarrhoeal faecal specimens investigated for the presence of *Clostridium difficile* were culture-positive. Twelve of the faecal specimens obtained from thirty-six normal healthy volunteers yielded *Cl. difficile* prior to oral administration of clindamycin. Thereafter all the volunteers excreted the organism from the second day to the fifth day during clindamycin administration. Three months after stoppage of oral clindamycin sixteen of the thirty-six volunteers still excreted *Cl. difficile*. Peak or trough serum levels of clindamycin had no relationship with the density of growth. None of the volunteers developed any *Cl. difficile* associated diseases.

Résumé

Des 324 prélèvements habituels d'excréments (sans diarrhée) dans lesquels on cherchait *Clostridium difficile*, cent soixante dix-sept avaient une culture positive. Avant l'administration orale de clindamycin, *Cl. difficile* était trouvé dans douze des prélèvements, obtenus de trente-six volontaires en bonne santé. Du deuxième au cinquième jour suivant, pendant l'administration de clindamycin, tous les volontaires ont excrété l'organisme de leur corps. Trois mois après, lorsque toute administration de clindamycin avait cessé, seize des trente-six volontaires continuaient d'excréter *Cl. difficile*. Le niveau maximum ou minimum du sérum dans le clindamycin n'avait aucun rapport avec la densité de croissance. Les volontaires n'ont développé aucune maladie associée avec *Cl. difficile*.

Introduction

The role played by *Clostridium difficile* in a wide range of intestinal problems from mere carrier existence to pseudo-membranous colitis (PMC) and antibiotic-associated diarrhoea is well established (Larson & Price, 1977; Bartlett *et al.*, 1978a, b; George, Symonds & Dimock, 1978; Larson *et al.*, 1978; George *et al.*, 1978; Bartlett, 1979). Practically any antibiotic can cause PMC but clindamycin and lincomycin are the common antibiotics implicated in the disease (Bartlett *et al.*, 1978; Bartlett, 1981; Borriello & Larson, 1981; Botton, Sherrif & Read, 1980). The causal organism, *Cl. difficile*, is an established member of the neonatal gut flora but not of adult gut flora (Larson *et al.*, 1978; George *et al.*, 1978; Rotimi & Duerden, 1981). *Clostridium* spp. are reported to be more prominent as members of the gut flora than the *Bacteroides* spp. in Africans who are on predominantly carbohydrate and fibrous diets (Drasar *et al.*, 1973). This study was undertaken to investigate the occurrence of *Cl. difficile* in routine non-diarrhoeal stool specimens to provide baseline data for its involvement in diarrhoeal diseases in Africans. Specimens obtained, after oral doses of clindamycin, from healthy normal adult Nigerians, were also studied for comparison.

Materials and methods

Specimens

Unselected consecutive 324 faecal specimens submitted to our hospital routine parasitology laboratory, as single specimens, from adult Nigerians aged 15–62 years were examined. Their records showed no previous complaints of diarrhoea in the last 6 months. Another set of stool specimens were obtained from thirty-six healthy, normal adult Nigerian volunteers aged 24–41 years, with no previous history of recent antibiotic intake or episodes of diarrhoea. Thirty-six sets of faecal specimens were

collected daily from these volunteers for 5 days after oral doses of 150 mg clindamycin sulphate were given every 6 h for 5 days. Thirty-six sets of pre-dose and post-dose stool specimens were also collected a day before the start of clindamycin administration and on fifth, tenth, twentieth days and 3 months after stopping the clindamycin dose.

Culture

One gram of stool specimens was mixed with 9 ml of glycerol infusion broth and one loopful (2 mm) was cultured onto a set of two selective media; cycloserine, cefoxitin, fructose, blood agar (CCFA; George *et al.*, 1979) and, a modification of CCFA, cefoxitin, neomycin, fructose, blood agar (CNFA) developed as a substitute for primary isolation of *Cl. difficile*. Each specimen was inoculated and streaked onto the media by a standard method that allowed semi-quantitative assessment of the density of growth on an arbitrary scale of 1^+–5^+ (Rotimi & Duerden, 1981). The culture plates were incubated anaerobically at 37°C for 58 h using the anaerobic generating kit system (Oxoid)

Yellow colonies on CCFA and CNFA resembling *Cl. difficile* were picked and sub-cultured onto fresh neomycin blood agar and incubated at 37°C for another 48 h. Colonial morphology, smell, Gram-stain appearance and yellowish green fluorescence on BA under u.v. light were used for the identification of isolates.

Clindamycin serum assay

About 10 ml of venous blood were collected from each of the thirty-six volunteers on the first and fifth days, approximately 5 min before the next dose and 45 min after the last dose, to determine the trough and peak clindamycin serum levels on each day. These levels were determined by standard methods (Reeves *et al.*, 1978).

Adverse reaction

Adverse side effects were watched out for in all the volunteers who were given clindamycin. Assessment of side effects included monitoring skin rash and development of diarrhoea, which was defined as loose bowel motions in excess of four times a day. The presence of cytotoxin produced by *Cl. difficile* was not investigated because of technical difficulties.

Analysis of data

Student's *t*-test was used to analyse some of the results statistically.

Results

One hundred and seventy-seven (55%) of the 324 routine faecal specimens yielded culture of *Cl. difficile* with light to moderate growths (1^+–3^+). Both CCFA and CNFA yielded the same number of positive cultures. In all, specimens from 178 males and 146 females were analysed. Eighty-seven (48%) of the 178 male specimens yielded *Cl. difficile* while ninety (62%) of the 146 female specimens were positive ($P < 0.05$) (see Table 1). The highest number of excreters were in the 21–30 years age group.

Analysis of the specimens obtained from the thirty-six volunteers showed that twelve (33.3%) were colonized by the *Cl. difficile* prior to administration of clindamycin (Table 2). By the second day all of the thirty-six volunteers were culture-positive and remained so until after day 5. On the fifth, tenth and twentieth days after stopping the clindamycin, sixteen, twenty-one and twenty-two volunteers, respectively, became culture-negative. The remaining fourteen patients, including the original twelve patients, were still culture-positive after 3 months of stopping the clindamycin administration.

The range (mean) trough and peak clindamycin serum levels were 0.45–2.9 (1.22) μg/ml and 3.8–5.9 (4.92) μg/ml on day 1, and 1.1–2.8 (1.23) and 3.8–6.4 (4.80) μg/ml on day 5, respectively (Table 3).

The semi-quantitative assessment of the density of growth of bacterial culture from the specimens obtained from the volunteers per day showed that the mean score on day 1 was 2.0^+ (range 1^+–3^+) on day 2, through days 3, and 4 to day 5, the mean scores were 2.8^+ (range 1^+–3^+), 2.8^+ (range 1^+–3^+), 2.7^+

Table 1. Colonization of adult gut by *Cl. difficile* in different age groups

	Faecal specimens	
	Number cultured	Number (%) positive
Total male	176	87 (48)
Total female	146	90 (62)
Age groups (years)		
15–20	134	57 (43)
21–30	99	84 (85)
31–40	50	23 (46)
41–50	14	10 (37)
> 50	14	3 (21)
Total	324	177 (55)

Table 2. Isolation of *Cl. difficile* from healthy volunteers after oral clindamycin

Duration of therapy	No of patients with culture-positive specimens (n = 36)
Pre-dose	12
Day 1	18
Day 2	36
Day 3	36
Day 4	36
Day 5	36
Post-cessation	
Day 5	20
Day 10	15
Day 20	14
3 months after	14*

*Included the original twelve pre-dose positives.

Table 3. Clindamycin serum levels of the volunteers

Time of measurement	Clindamycin levels (μg/ml)			
	Trough		Peak	
	range	mean	range	mean
Pre-dose	0	0	0	0
Day 1	0.45–2.9	1.22	3.8–5.9	4.92
Day 5	1.1–2.8	1.23	3.8–6.4	4.81

(range 1^+–3^+) and 2.8^+ (1^+–3^+), respectively. There was no particular relationship between the clindamycin serum levels and the density of growth.

None of the thirty-six volunteers developed any objectively measurable side effect after administration of the clindamycin. No diarrhoea or PMC was observed.

Discussion

The results of this study amply demonstrate the common occurrence of *Cl. difficile* in the faecal specimens of adult Nigerians. The *Cl. difficile* isolation rate of 55% in routine specimens and 100% in specimens after clindamycin administration showed that the organism is a very common member of the adult gut flora. This is in sharp contrast to the findings of other workers on its contribution to the normal flora of the gut of adult Caucasians in Europe and America (Drasar *et al.*, 1973; Nash *et al.*, 1982). No patient in the 'routine' group had symptoms suggestive of *Cl. difficile* infection and none of the volunteers had taken any antibiotics prior to the study. Both the initial isolation of *Cl. difficile* in 33% of the thirty-six adult volunteers (pre-dose) and the isolation rate of 100% on the second day after clindamycin administration were remarkably high. This contrasts with the general belief that this organism is not a common flora of gastrointestinal tract of adults (Botton *et al.*, 1980; Rotimi & Duerden, 1981; Nash *et al.*, 1982).

In this study the majority of the colonized subjects (85%) were in the age group 21–30 years and most of them were females. The significance of this finding is not very clear especially when there was no concomitant *Cl. difficile*-associated infection in any of them. The role of *Cl. difficile* without the production of cytotoxin therefore becomes less easily defined. Unfortunately the presence of cytotoxins in the faecal specimens was not investigated due to technical difficulties. The high isolation rate in volunteers prior to clindamycin administration further supports the results of routine specimen investigations and showed that the organism is indeed a common flora of the adult gut. The antibody levels (secretory and humoral) to *Cl. difficile* and its cytotoxin in Nigerians is currently being investigated.

The results of the study on volunteers also confirmed that oral clindamycin did induce *Cl. difficile* colonization of the adult gut. The relative increase in the density of growth between the base line semi-quantitative count and counts thereafter for 5 days indicated that the drug induced fairly heavy colonization, which was in line with the report of Kim *et al.* (1951).

It was noteworthy to find that the serum trough and peak levels of clindamycin in the volunteers had no relationship with the density of growth of *Cl. difficile*. The mean peak level in our study was higher than the reported mean peak level in the American subjects (Bartlett, 1981) but without the development of pseudomembraneous colitis (PMC) which, of course, confirms Bartlett's (1979) report that the development of PMC has no relationship to either the dose or serum levels of the incriminated drug.

Clinical evaluation of the CNFA medium which is a modification of the well-tested and routinely used CCFA, by substitution of cycloserine with high concentration of neomycin (100 μg/ml), showed that the medium was just as sensitive as the CCFA for the primary isolation of *Cl. difficile*. All *Cl. difficile* on the CNFA medium produced yellow and big colonies that fluoresced golden yellow under the u.v. light. The density of growth was also comparable. Since cycloserine is not readily available in our country but neomycin is, its substitution by the latter should make the isolation of *Cl. difficile* relatively easy in our laboratories. The medium should also be very useful for the screening of *Cl. difficile*, in the busy routine laboratory, for large scale clinical work.

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