Antimicrobial Agents and *Clostridium difficile* in Acute Enteric Disease: Epidemiological Data from Sweden, 1980–1982

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The carrier rate of *Clostridium difficile* in an adult Swedish population was found to be 11 (1.9%) of 594. All isolates were toxigenic in vitro, but no healthy individual harbored free cytotoxin in stool. Of 398 patients with acute diarrhea not associated with antibiotic use, cytotoxin was found in stool filtrates of four (1%). In 4,793 patients with antibiotic-associated diarrhea from all parts of Sweden during 1980–1982, *C. difficile* cytotoxin was demonstrated in 873 (18%). The tissue culture assay was found to be more specific than cultivation for the bacterium. By weighted analysis, in the age group >70 years more women than men were infected. In the age group 21–50 years there was an even greater preponderance of infection in women than in men. Cephalosporins and lincosamides were 10–70 times more often implicated in *C. difficile* colitis than were narrow-spectrum penicillins.

Toxin-producing *Clostridium difficile* is a common bacterial isolate from patients with antibiotic-associated diarrhea (AAD) and colitis (AAC). The bacterium seems to overgrow the normal bowel flora because of changes in the intestinal tract caused by antibiotics or other agents influencing the intestinal ecology. It is still uncertain whether the organism is endogenous or acquired mainly from exogenous sources before disease [1]. In children less than two years old *C. difficile* and its cytotoxin are commonly found without association to intestinal disease [2, 3], and both bacterium and cytotoxin have been demonstrated in adults with no signs of diarrhea or colitis [4].

Colitis due to *C. difficile* has been associated with most antimicrobial agents—in particular, amoxicillin, cephalosporins, and lincosamides [5]. However, data are scarce regarding the actual risks related to the consumption of various antibiotics in a population.

The aim of this investigation was to study the occurrence of *C. difficile* in patients from all parts of Sweden with AAD/AAC in comparison to the occurrence in a general population and in patients with acute diarrhea unrelated to antibiotics. The occurrence of other bacterial enteropathogens in patients with *C. difficile*-associated colitis was also investigated. The distribution of age and sex among patients with *C. difficile* colitis was analyzed, as was the relation between antibiotics most commonly implicated in *C. difficile* colitis and their general use in the Swedish population.

**Patients and Methods**

*General population.* Five hundred ninety-four healthy adults 12–83 years of age were selected for this study (table 1). They all had normal bowel habits and were not treated with any antimicrobial agents within six weeks before sampling. Schoolchildren from four different schools in Stockholm (17 boys and 20 girls), men newly drafted for military service (*n* = 29), and women just beginning nursing school (*n* = 30) constituted the age group 12–20 years. Students and administrative staff at the Karolinska Institute or the National Bacteriological Laboratory (Stockholm) not involved in any laboratory work and healthy individuals sampled according to the law of provisions (staff of restaurants, foodstores, etc.) made up the age group 21–40 years (124 men and 108 women) and the age group 41–60 years (68 men...
### Table 1. Presence of *C. difficile* and cytotoxin in different populations by age and sex.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Median age</td>
</tr>
<tr>
<td>Population</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP</td>
<td>272</td>
<td>37</td>
</tr>
<tr>
<td>AD</td>
<td>183</td>
<td>34</td>
</tr>
<tr>
<td>AAD</td>
<td>2,120</td>
<td>54</td>
</tr>
<tr>
<td><em>C. difficile</em> in stool</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP</td>
<td>6 (2.2)</td>
<td>57</td>
</tr>
<tr>
<td>AD</td>
<td>4 (2.2)</td>
<td>36</td>
</tr>
<tr>
<td>AAD</td>
<td>NC</td>
<td>. . .</td>
</tr>
<tr>
<td>Cytotoxin in stool</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP</td>
<td>0</td>
<td>. . .</td>
</tr>
<tr>
<td>AD</td>
<td>2 (1.1)</td>
<td>. . .</td>
</tr>
<tr>
<td>AAD</td>
<td>332 (15.7)</td>
<td>66</td>
</tr>
</tbody>
</table>

**NOTE.** Ages are given in years. Numbers in parentheses are percentages. GP = general population; AD = acute diarrhea not related to antibiotic use; and NC = not calculated.

* Five patients had recently been abroad.
† Four patients also had other enteropathogens.
‡ One patient also had *Campylobacter*.

and 90 women). Persons >61 years of age (34 men and 74 women) were previous employees of the National Bacteriological Laboratory and persons living in a home for elderly persons.

**Patients with acute diarrhea.** During the period January-July 1983 we prospectively investigated 398 patients admitted to the Department of Infectious Diseases at Roslagstull Hospital (Stockholm) with acute diarrhea unrelated to recent antibiotic use (table 1). Stool specimens from all patients were submitted to bacteriologic (*Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, and enterotoxigenic *Escherichia coli*), virological (rotavirus and adenovirus), and protozoal (*Entamoeba histolytica* and *Giardia lamblia*) examination. Cultivations and toxin testing for *C. difficile* were performed in parallel without prior freezing.

**Patients with AAD/AAC.** From 1980 until 1982 fecal specimens from 4,793 patients with diarrhea were investigated for the presence of *C. difficile* cytotoxin (table 1). Samples were submitted from all parts of Sweden. From these patients, *C. difficile* cytotoxin in stool was recorded in 873 patients (table 1). These patients are referred to as patients with *C. difficile* colitis. The incidence (I) is calculated as follows: \( I = N_c / ([N_0 + N_t] / 2) \), where \( N_c \) is the number of cases, \( N_0 \) is the number of inhabitants at the beginning of the study period, and \( N_t \) is the number of inhabitants at the end of the study period. The observed age- and sex-specific incidence rates were obtained by letting \( N_c \) represent the number of cases in a specific age and sex group and \( N_0 \) and \( N_t \) the number of inhabitants in the corresponding group. The mean annual incidence was obtained by dividing the rate by 3. Thus the following formula was obtained: \( I = (\text{no. of cases in 1980-1982} \times 2) / ([\text{no. of inhabitants in December 1979} + \text{no. of inhabitants in December 1982}] \times 3) \). The known association of antibiotic use both in patients with AAD/AAC and in patients with verified *C. difficile* colitis was \( \sim 60\% \). At least another \( 30\% \) of the patients had consumed an unspecified antibiotic before disease.

**Handling of stool specimens.** Stool specimens from healthy individuals were sent to the laboratory in glass tubes without diluents and immediately plated by using a cotton swab onto a preduced agar medium containing egg yolk, cefoxitin, cycloserine, and fructose [6]. Specimens were not diluted because growth of bacteria other than *C. difficile* after plating of undiluted feces was relatively unusual. The specimens were plated within 48 hr after sampling (without prior freezing) and stored at 4°C. Agar plates were then incubated for 48 hr anaerobically in GasPak® jars (BBL Microbiology Systems, Cockeysville, Md). Identification of *C. difficile* was based on colony morphology, gas liquid chromatography, and biochemical tests [7].
An initial 10-fold dilution of stool specimen was investigated for *C. difficile* cytotoxin in a tissue culture assay (human embryonic intestinal cells; Flow Laboratories, Irvine, Scotland) [8]. A positive effect was in all cases neutralizable with antisera to *Clostridium sordellii* (National Institutes of Health, Bethesda, Md) and *C. difficile* (Department of Bacteriology, National Bacteriological Laboratory, Stockholm).

Specimens from patients with AAD were initially analyzed for the presence of cytotoxin, and if positive they were further analyzed for growth of *C. difficile*. Specimens were stored at 4°C until study. Patients' specimens that were negative for cytotoxin were not cultivated because of the low isolation rate (2%) from such samples [8]. Cultivation for *C. difficile* in parallel with cytotoxin testing of specimens from these patients has previously shown that isolation of the bacterium was less frequent than demonstration of the cytotoxin [8].

During a two-month period in 1983, 108 stool samples yielding *C. difficile* cytotoxin in tissue culture assay were routinely analyzed for other bacterial enteropathogens such as *Shigella*, *Salmonella*, *Yersinia*, or *Campylobacter*.

**Antibiotics.** Since 1978 the prescription of medicines in Swedish outpatient care has been monitored. Prescription copies are collected from a continuous random sample of physicians, and data are presented in yearly issues from the National Corporation of Swedish Pharmacies (Department of Drug Information, Stockholm). Furthermore, the total sales of various drugs (both inpatient and outpatient) are presented as number of defined daily doses (DDD) per 1,000 inhabitants per day. This number gives the proportion of the population that theoretically is being treated with a particular drug. The DDD is based on the assumed average dose per 24-hr period when the drug is used for its main indication.

The main groups of antibiotics investigated in this study were (1) narrow-spectrum penicillins, including penicillin G, penicillin V, and azidocillin; (2) β-lactamase-stable penicillins, including dicloxacillin, cloxacillin, and fluclaxacillin; (3) broad-spectrum penicillins, including ampicillin, amoxicillin, pivampicillin, carbenicillin, and bacampicillin; (4) cephalosporins, including cephalothin, cephaloridine, cephalaxin, cefazolin, cephradine, cefadroxil, cefaclor, and cefuroxime (cefotaxin was included in this group); and (5) lincosamides, including clindamycin and linecomycin.

**Statistical methods.** The χ² analysis was performed and the regression coefficient was calculated according to standard methods.

**Results**

**General population.** The overall carrier rate of *C. difficile* among healthy individuals irrespective of age was ~2% (table 1). All isolates of the organism produced the typical cytotoxin in vitro. Cytotoxin was, however, not present in free form in any of the 594 stool filtrates tested in the tissue culture assay. There was no significant difference in the carrier rate of *C. difficile* between males and females or between the age groups. There seemed to be a slightly increased appearance of *C. difficile* in persons between 40 and 60 years of age. It is interesting that most of the positive individuals in this age group had recently traveled to southern Europe but denied any change of bowel habits.

**Patients with acute diarrhea.** *C. difficile* or cytotoxin was found in stool from 12 (3%) of 398 patients with acute diarrhea (table 1). In five cases other enteropathogens were isolated as well—namely, *Campylobacter*, *Shigella*, *Salmonella*, rotavirus, and adenovirus. Thus the occurrence of toxin-producing *C. difficile* as the only pathogen in 7 (1.8%) of 398 patients with acute diarrhea did not exceed that of the normal population (2%). However, four subjects (1.0%) had free cytotoxin in fecal filtrates, which may reflect a true occurrence of

![Figure 1. Observed mean annual incidence of patients with *C. difficile* cytotoxin in stool in Sweden during 1980-1982 by age and sex (males [■] and females [■]).](image-url)
C. difficile colitis in patients with acute diarrhea without prior antibiotic treatment.

Patients with AAD/AAC. The proportion of patients with AAD/AAC with a positive cytotoxin test was >18% during 1980–1982 (table 1). Cultivation of stool specimens with a positive toxin assay during 1981–1982 yielded C. difficile bacteria in at most, 70% of the patients investigated.

Age and sex distribution of patients with C. difficile colitis. Figure 1 shows the observed age- and sex-specific mean incidence rates of patients with cytotoxin in stool samples during 1980–1982. Most of the patients were in the older age groups; 63% of the patients were >60 years of age, but all age groups were represented. The finding of cytotoxin in stool specimens of young children with AAD may not correlate with disease because of the normally high incidence of cytotoxin found in that population [2, 3], but this finding was not further analyzed in this study. However, after infancy the proportion of patients with cytotoxin in stool specimens among those with AAD/AAC increased with age (r = .94; P < .001; figure 2).

In total, 541 females and 332 males were regarded as having C. difficile colitis (table 1). In the age group 21–50 years there was a strikingly increased preponderance of women (140 women and 62 men) among patients with C. difficile colitis (figure 1). This finding was consistent for each of the three years studied. Considering all patients investigated in these age groups, females were 1.8 times more often positive for cytotoxin than were males (P < .001; 95% confidence limits, 1.3–2.5; figure 2). Furthermore, a

![Figure 2.](image)

Table 2. Antibiotics most commonly associated with C. difficile colitis, 1980–1982.

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Single treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillins</td>
<td>50 (36)</td>
<td>47 (30)</td>
<td>62 (30)</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>27 (20)</td>
<td>30 (19)</td>
<td>37 (18)</td>
</tr>
<tr>
<td>Lincosamides</td>
<td>14 (10)</td>
<td>11 (7)</td>
<td>16 (8)</td>
</tr>
<tr>
<td>Other</td>
<td>12 (9)</td>
<td>15 (10)</td>
<td>18 (9)</td>
</tr>
<tr>
<td>Multitreatment</td>
<td>35 (25)</td>
<td>52 (34)</td>
<td>77 (37)</td>
</tr>
</tbody>
</table>

A weighed analysis of numbers of patients in the age group >70 years showed that C. difficile cytotoxin was more often found in women (ratio, 1.33; P < .01).

None of the 108 patients with C. difficile colitis examined for other possible causes of diarrhea harbored any other enteropathogenic bacteria.

Antibiotics. Most antibiotics, in combination or alone, were associated with subsequent overgrowth of C. difficile and/or demonstration of cytotoxin in stool filtrates. In single-drug treatment during 1980–1982 three groups of antibiotics were especially associated with overgrowth of C. difficile (table 2). Penicillins, cephalosporins, and lincosamides, in that order, were the groups of agents used most often, together representing 85%–90% of the cases with a single-drug treatment. However, many cases were associated with use of more than one antimicrobial agent. Among the narrow-spectrum penicillins, penicillin V was most often associated, as was clindamycin among the lincosamides. Cephalexin and cefuroxime were the most often recognized cephalosporins. Ceftazidime, moxalactam, cefoperazone, and ceftriaxone were not licensed in Sweden during the observation time.

The preponderance of women over men with C. difficile colitis in the age group 21–50 years was primarily due to the treatment of women with broad-spectrum penicillins (70%) before diarrhea. This finding probably reflects treatments for urinary tract infections in this age group. The official sales statistics showed an increased prescription rate of antibiotics to women in this age group. The increased incidence rate of C. difficile colitis in younger women as compared with men may therefore be a result of an increased consumption of antibiotics among women in this age group (figure 1).

It has not been possible to determine the extent to which the patients with C. difficile colitis received
Discus

In patients with diarrhea or colitis associated with C. difficile cytotoxin in fecal filtrates, recovery of the bacterium was at most 70%. It is possible that handling of stool specimens coming from all parts of Sweden has not been optimal for this fastidious organism, a lapse resulting in a low isolation rate of C. difficile in these patients. There also remains the possibility that other clostridial strains may produce a toxin that causes a similar cytotoxic effect and is neutralized by C. difficile or C. sordelli antitoxin [9]. Repeated typing of toxin-producing clostridial species from patients with colitis [8], however, has shown these strains to be C. difficile.

The carrier rate of C. difficile in a general population >12 years of age was found to be low (2%). Although all strains were able to produce cytotoxin in vitro, no toxin was detectable in fecal filtrates from healthy persons. These data are in accordance with other reports [3, 10], which show that C. difficile is rarely found in feces from healthy individuals. However, low numbers of bacteria may not be detected with this method [6]. A taurocholate-cefoxitin-cycloserine-fructose agar medium might increase the recovery of C. difficile spores in feces, and this medium might be more appropriate for determination of the normal carrier rate of C. difficile when optimal handling of specimens is not possible [11]. In contrast to what has been reported from a healthy Japanese population [12], we were not able to show an increased carrier rate among elderly persons. The slightly increased number in the age group 41–60 years in our study was contributed to by persons who had recently been abroad. On interview they denied change of bowel habits, but the possibility remains that a change of normal bowel flora during travel predisposed to overgrowth of C. difficile [13].

Our data clearly show that individuals >60 years of age constitute the majority of persons with C. difficile colitis. This result does not seem to be clearly correlated to an increased colonization of elderly persons (table 1) or to an increased consumption of antibiotics, according to statistical data from the National Corporation of Swedish Pharmacies. Furthermore, the mean age of patients with C. difficile colitis associated with narrow-spectrum penicillins, cephalosporins, and lincosamides did not vary significantly. Therefore it is possible that other factors such as hospital care and subsequent risk of exposure to C. difficile in the hospital environment during antibiotic treatment play a role [14–16]. An increased proportion of toxin-positive elderly patients was noted (figure 2). Poor intestinal drug absorption or increased excretion of antimicrobial metabolites with the bile in elderly persons with kidney insufficiency may also be important [17], as may be the immune status of the patient [18].

The increased relative risk associated with C. difficile colitis for lincosamides as compared with other antimicrobial agents is well in accordance with the dramatic suppression of the anaerobic intestinal flora exerted by the lincosamides [19]. However, this comparison does not take into consideration un-
derlying disorders and the status of patients, which may contribute to development of C. difficile colitis.

One hundred eight patients with C. difficile colitis were negative when examined for other bacterial enteropathogens. Although only one specimen from each patient was examined, these data strongly suggest that once C. difficile cytotoxin is demonstrated in patients with AAD/AAC, other enteropathogenic bacteria are not likely to be found. In patients with acute diarrhea unrelated to previous use of antibiotics, C. difficile cytotoxin in stool samples without other bacterial enteropathogens was found in ~1% of the cases. Thus C. difficile seems to be an enteric pathogen of importance almost exclusively in association with previous use of antibiotics, although occasional reports have suggested C. difficile as the likely pathogen in what appears to be non-antibiotic-associated colitis [20, 21]. Also, in children “spontaneous” C. difficile colitis seems to occur without association with antibiotics [22].

In conclusion, our data indicate a low carrier rate of C. difficile in a healthy population. The bacteria is a rare cause of acute diarrhea except after antibiotic treatment. In addition, diagnosis of C. difficile colitis is more specific with demonstration of cytotoxin than with culture for the bacterium. When optimal handling of stool specimens is not possible, cytotoxin assay is more sensitive than simple cultivation using cefoxitin-cycloserine-fructose agar medium. Penicillins are the antibiotics most commonly associated with C. difficile colitis, whereas in comparison to their use lincosamides and cephalosporins are the drugs most often implicated.

References