

## ORIGINAL ARTICLE

## *Klebsiella oxytoca* as a Causative Organism of Antibiotic-Associated Hemorrhagic Colitis

Christoph Högenauer, M.D., Cord Langner, M.D., Eckhard Beubler, Ph.D., Irmgard T. Lippe, Ph.D., Rudolf Schicho, Ph.D., Gregor Gorkiewicz, M.D., Robert Krause, M.D., Nikolas Gerstgrasser, M.D., Guenter J. Krejs, M.D., and Thomas A. Hinterleitner, M.D.

## ABSTRACT

From the Department of Internal Medicine (C.H., R.K., N.G., G.J.K., T.A.H.), the Institute of Pathology (C.L., G.G.), and the Institute of Experimental and Clinical Pharmacology (E.B., I.T.L., R.S.), Medical University of Graz, Graz, Austria. Address reprint requests to Dr. Högenauer at the Department of Internal Medicine, Medical University of Graz, Auenbruggerplatz 15, A-8036 Graz, Austria, or at christoph.hoegenauer@meduni-graz.at.

N Engl J Med 2006;355:2418-26.  
Copyright © 2006 Massachusetts Medical Society.

**BACKGROUND**

Antibiotic-associated hemorrhagic colitis is a distinct form of antibiotic-associated colitis in which *Clostridium difficile* is absent. Although the cause is not known, previous reports have suggested a role of *Klebsiella oxytoca*.

**METHODS**

We studied 22 consecutive patients who had suspected antibiotic-associated colitis and who were negative for *C. difficile*. Patients underwent diagnostic colonoscopy, and among those who received a diagnosis of antibiotic-associated hemorrhagic colitis, stool samples were cultured for *K. oxytoca*. We isolated *K. oxytoca* strains and tested them for cytotoxin production using a tissue-culture assay. In addition, we also cultured stool samples obtained from 385 healthy subjects for *K. oxytoca*. An in vivo animal model for antibiotic-associated hemorrhagic colitis was established with the use of Sprague-Dawley rats.

**RESULTS**

Of the 22 patients, 6 had findings on colonoscopy that were consistent with the diagnosis of antibiotic-associated hemorrhagic colitis, and 5 of these 6 patients had positive cultures for *K. oxytoca*. No other common enteric pathogens were found in the five patients. Before the onset of colitis, all five were receiving penicillins, and two were also taking nonsteroidal antiinflammatory drugs (NSAIDs). All isolated *K. oxytoca* strains produced cytotoxin. *K. oxytoca* was found in 1.6% of the healthy subjects. In the animal model, *K. oxytoca* was found only in the colon of rats that received amoxicillin–clavulanate in addition to being inoculated with *K. oxytoca*. In these rats, infection with *K. oxytoca* induced a right-sided hemorrhagic colitis that was not observed in uninfected animals that received amoxicillin–clavulanate, indomethacin (an NSAID), or both.

**CONCLUSIONS**

Our fulfillment of Koch's postulates for cytotoxin-producing *K. oxytoca* suggests that it is the causative organism in at least some cases of antibiotic-associated hemorrhagic colitis. Infection with *K. oxytoca* should be considered in patients with antibiotic-associated colitis who are negative for *C. difficile*.

**C**OLITIS IS A WELL-KNOWN COMPLICATION of treatment with antibiotic agents. The disease is generally thought to be caused by overgrowth of toxin-producing *Clostridium difficile* in the colon.<sup>1</sup> Antibiotic-associated hemorrhagic colitis is a distinct form of antibiotic-associated colitis in which *C. difficile* is absent.<sup>2</sup> This form of colitis, first described by Toffler et al.,<sup>3</sup> is usually observed during therapy with penicillins. Antibiotic-associated hemorrhagic colitis has also been reported after antibiotic therapy with quinolones and cephalosporins.<sup>4,5</sup> In contrast to antibiotic-associated colitis induced by *C. difficile*, antibiotic-associated hemorrhagic colitis is thought to resolve spontaneously after cessation of antibiotic therapy.<sup>3,4,6</sup>

The cause of antibiotic-associated hemorrhagic colitis is unknown. Several mechanisms have been suggested, including allergic reaction,<sup>3</sup> mucosal ischemia,<sup>7</sup> and infection with *Klebsiella oxytoca*.<sup>2,6</sup> This gram-negative bacterium has been found in patients with antibiotic-associated hemorrhagic colitis, but it is also found in stools of healthy subjects.<sup>2</sup> *K. oxytoca* strains isolated from patients with antibiotic-associated hemorrhagic colitis and half of the strains isolated from healthy subjects have been shown to produce a cytotoxin that is capable of inducing cell death in various epithelial-cell cultures.<sup>2,8-10</sup> In contrast to *C. difficile*-induced antibiotic-associated colitis,<sup>11</sup> however, there is no in vivo animal model for antibiotic-associated hemorrhagic colitis. Thus, it is uncertain whether *K. oxytoca* causes antibiotic-associated hemorrhagic colitis, since Koch's postulates have not been fulfilled.

We describe five patients with antibiotic-associated hemorrhagic colitis. A *K. oxytoca* strain isolated from one of these patients was used in an animal model of antibiotic-associated hemorrhagic colitis, which allowed us to fulfill Koch's postulates.

## METHODS

### PATIENTS AND CONTROLS

We studied 22 consecutive patients who had antibiotic-associated colitis but were negative for *C. difficile*. They underwent diagnostic colonoscopy between March 2001 and April 2004. The diagnosis of antibiotic-associated hemorrhagic colitis was made on the basis of the clinical history (use of

antibiotics before the onset of diarrhea) and findings on endoscopy that are typical of segmental hemorrhagic colitis. Stool samples were examined for *C. difficile*, campylobacter, salmonella, yersinia, shigella, *Escherichia coli* O157, and *C. difficile* toxin A. In addition, we examined stool specimens from patients with antibiotic-associated hemorrhagic colitis for *K. oxytoca* using MacConkey agar plates, and bacterial identification was performed with the use of the API 20E test (bioMérieux). To determine the prevalence of *K. oxytoca* in normal bowel flora, we tested stool samples from 385 healthy subjects (139 employees and students of the Medical University of Graz and 246 recruits from the Austrian army) for *K. oxytoca*. The mean age of all healthy subjects was 24 years (range, 18 to 61). All experiments in humans were approved by the local institutional review board or the Austrian Ministry of Defense. Written informed consent was obtained from all patients and controls.

### CYTOTOXIN ASSAY AND ANTIBIOTIC SUSCEPTIBILITY

We analyzed *K. oxytoca* isolates from patients for the production of cytotoxin using a previously established cell-culture assay.<sup>2,8</sup> We used as a negative control two strains of *K. oxytoca* (American Type Culture Collection [ATCC] 13182 and 8724) that had previously been shown to be nontoxic.<sup>8-10</sup> The cytotoxin-producing strain of *K. oxytoca*, MH 43-1, served as a positive control.<sup>8</sup>

To obtain a culture supernatant from *K. oxytoca*, tryptic soy broth was inoculated with a single bacterial colony and incubated at 37°C, with centrifugation at 150 rpm, for 20 hours. Bacterial cultures were then centrifuged at 20,000×g for 10 minutes at 4°C, and the supernatant was filtered through a membrane filter with a pore diameter of 0.2 μm (Iwaki).

Cytotoxin production was assessed with the use of monolayers of HEP-2 cells (ATCC CCL-23) in 96-well tissue-culture plates. After removal of the culture medium, 100 μl of a 1:1 dilution of the filtered supernatant from the culture of *K. oxytoca* strains with Dulbecco's phosphate-buffered saline was added to the wells. The plates were incubated in a 5% carbon dioxide atmosphere at 37°C for 48 hours and were examined under a microscope. Cytotoxicity was defined as cell rounding and cell death, as described previously.<sup>2,8,10</sup> Antibiotic susceptibility testing of isolated *K. oxy-*

**Table 1.** Characteristics of the Five Patients with Antibiotic-Associated Hemorrhagic Colitis (AAHC) Who Were Positive for *K. oxytoca*.\*

Patient No.	Age yr	Sex	Antibiotic Triggering AAHC	Indication for Antibiotic Therapy	Additional Therapy before Onset of AAHC	Time from Start of Antibiotic Therapy to Onset of AAHC Symptoms days	Segments of Colon Affected	Time to Recovery days	Therapy before Diagnosis
1	36	M	Amoxicillin-clavulanate	Sinusitis	NA	7	Ascending, transverse, and descending colon	3	Symptomatic
2	28	F	Amoxicillin-clavulanate	Tonsillitis	NA	4	Ascending and sigmoid colon	4	Empirical (metronidazole)
3	63	F	Amoxicillin and metronidazole	Eradication of <i>Helicobacter pylori</i>	NSAID	5	Cecum, ascending colon	3	Symptomatic
4	37	F	Amoxicillin-clavulanate	Tonsillitis	NSAID	3	Ascending and transverse colon	4	Symptomatic
5	53	M	Amoxicillin and clarithromycin	Eradication of <i>H. pylori</i>	None	4	Cecum, ascending colon	7	Empirical (metronidazole)

\* Recovery was defined as the resolution of all symptoms (diarrhea, abdominal pain, fever) after discontinuation of antibiotics and NSAIDs. Symptomatic treatment included discontinuation of antibiotics and administration of intravenous fluid and spasmolytic agents. NA indicates that no information was available about additional therapy.

*toca* strains was performed with the use of Etest (AB Biodisk).

**EXPERIMENTS IN ANIMALS**

For in vivo experiments in the animal model, we used female Sprague-Dawley rats that weighed 180 to 200 g and the *K. oxytoca* strain AHC (antibiotic hemorrhagic colitis) 1, isolated from the stool of Patient 1. Six groups of rats were studied: group 1 (11 rats) received amoxicillin-clavulanate and inoculation with *K. oxytoca* AHC 1; group 2 (8 rats), amoxicillin-clavulanate only; group 3 (10 rats), inoculation with *K. oxytoca* AHC 1 only; group 4 (18 rats), amoxicillin-clavulanate, indomethacin, and inoculation with *K. oxytoca* AHC 1; group 5 (11 rats), amoxicillin-clavulanate and indomethacin only; and group 6 (12 rats), indomethacin only. The experiments lasted for 5 days. Amoxicillin-clavulanate (Augmentin, GlaxoSmith-Kline) was given intraperitoneally twice daily on days 1 through 5 at a dose of 20 mg, and indomethacin (Liometacin, Chiesi Farmaceutici) was given subcutaneously once daily on days 3 and 4 at a dose of 1 mg. *K. oxytoca* AHC 1 was inoculated by delivering 1 ml of a 12-hour culture containing about 4×10<sup>9</sup> colony-forming units (CFU) per milliliter through an orogastric stomach probe on days 2 through 4 in groups 1, 3, and 4; control groups 2, 5, and 6 did not receive *K. oxytoca*.

On day 5 of the experiment, the animals were killed, and their intestines removed for histologic examination. Stool samples were obtained from the cecum and the distal colon for bacterial culture and testing for *C. difficile* toxin A. Two investigators, who were unaware of the treatment assignments, evaluated the bowel specimens for histologic changes, using an adapted scoring system<sup>12</sup> for four histologic features: mucosal inflammation, epithelial alteration (defined as loss of goblet cells, anisonucleosis, and increased rates of mitosis and apoptosis), mucosal hemorrhage, and erosion. Possible scores for mucosal inflammation were as follows: 0, none; 1, infiltration of the mucosal stroma by neutrophils; 2, infiltration of the epithelial layer by neutrophils; and 3, formation of crypt abscess. Possible scores for epithelial alteration and erosion were as follows: 0, none; 1, mild; 2, moderate; and 3, severe. Possible scores for mucosal hemorrhage were as follows: 0, none; 1, mild, focal; 2, moderate, focal; and 3, diffuse. For comparison, colonic biopsy samples obtained from patients with antibiotic-associated hemor-

rhagic colitis were evaluated according to the same scoring system. The experiments in the rats conformed to animal-use regulations and were approved by the Austrian Ministry of Education, Science, and Culture.

**STATISTICAL ANALYSIS**

Statistical analysis was performed with the use of two-way analysis of variance on the ranks for the histologic score and the variables of *K. oxytoca* in stool from the rats (presence or absence) and the administration of indomethacin (a nonsteroidal antiinflammatory drug [NSAID]) (presence or absence), since those data were not normally distributed. Multiple comparisons were performed with the use of the Student–Newman–Keuls test, and a P value of less than 0.05 was considered to indicate statistical significance.

**RESULTS**

**CLINICAL DATA**

Of the 22 patients evaluated, 6 received a diagnosis of antibiotic-associated hemorrhagic colitis, and *K. oxytoca* was isolated from the stool in 5 of these 6 patients (Table 1). *K. oxytoca* was found in the stools of 6 of the 385 healthy subjects (1.6%); none of these subjects reported gastrointestinal symptoms such as diarrhea.

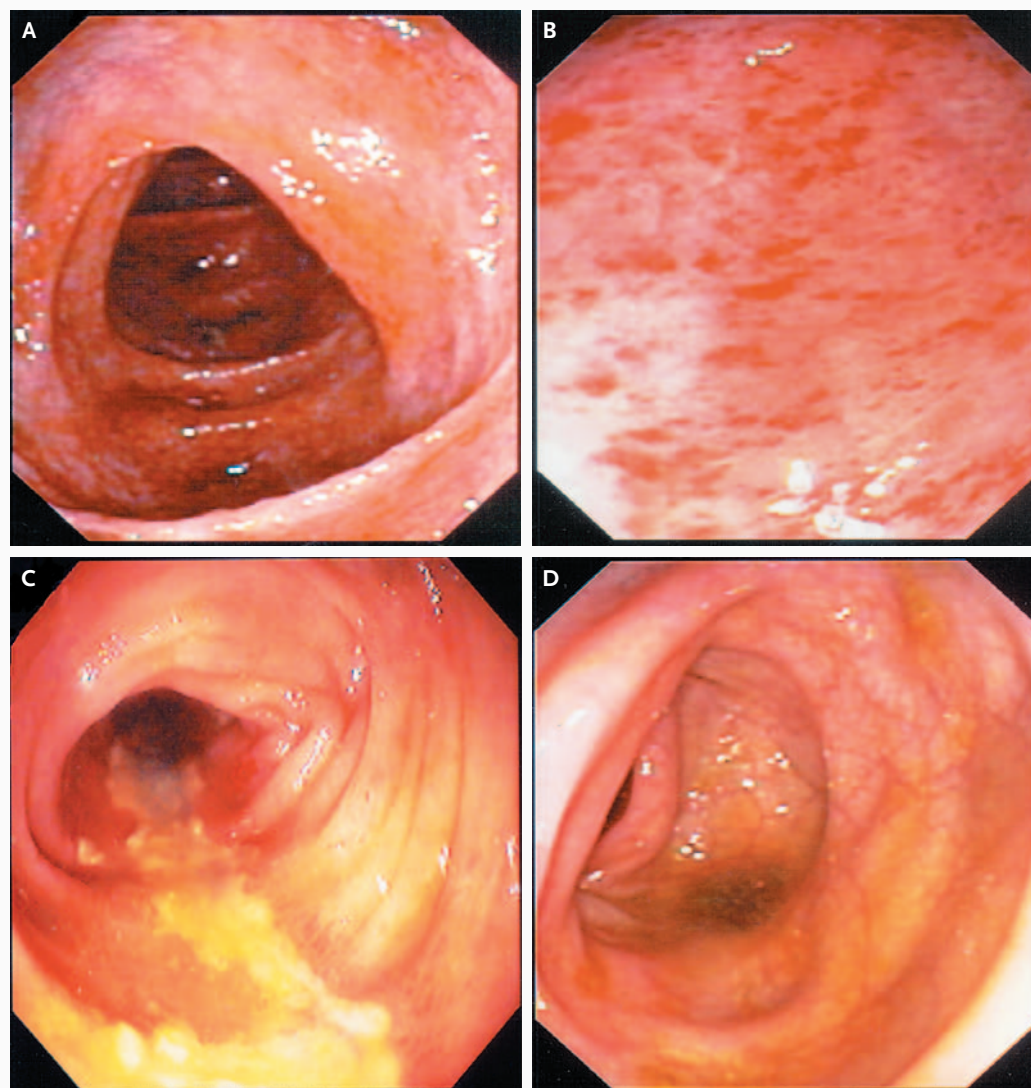
All stool samples obtained from patients with antibiotic-associated hemorrhagic colitis were negative for *C. difficile*, campylobacter, salmonella, yersinia, shigella, and *E. coli* O157. The antibiotic susceptibility of isolated strains is shown in Table 2. All five patients were receiving penicillin derivatives as outpatients when bloody diarrhea developed. Two of the five patients who were positive for *K. oxytoca* had been taking NSAIDs in addition to antibiotics. Bloody diarrhea and abdominal cramps occurred suddenly after 3 to 7 days of antibiotic treatment. All five patients had leukocytosis (mean leukocyte count, 16,500 per cubic millimeter; range, 11,700 to 20,200; normal value, less than 11,300) and elevated C-reactive protein concentrations (mean, 63 mg per liter; range, 16 to 192; normal value, less than 9).

Colonoscopy revealed segmental hemorrhagic colitis (Fig. 1) with rectal sparing in all cases. Colitis was predominantly localized in the right colon. Findings on endoscopy were mucosal edema and mucosal hemorrhage in all five patients, with erosion or longitudinal ulceration in two pa-

**Table 2. Characteristics of *K. oxytoca* Isolates.\***

Isolate	Patient No.	Antibiotic Triggering AAHC	Cytotoxin	ESBL	Ampicillin	Amoxicillin–Clavulanate	Clarithromycin $\mu\text{g/ml}$	Cefotaxime	Ciprofloxacin
AHC 1	1	Amoxicillin–clavulanate	+	+	>256	24.0†	32	3.000†	0.023
AHC 2	2	Amoxicillin–clavulanate	+	-	96	1.5	48	0.047	0.016
AHC 4	3	Amoxicillin and metronidazole	+	-	>256	1.5	64	0.032	0.12
AHC 6	4	Amoxicillin–clavulanate	+	-	>256	2.0	48	0.047	0.016
AHC 7	5	Amoxicillin and clarithromycin	+	-	>256	6.0	>256	0.064	1
Positive control (MH 43-1)		None	+	-	8	1.0	64	0.032	0.012
Negative control ATCC 13182		None	-	-	>256	1.5	48	0.047	0.012
ATCC 8724		None	-	-	32	1.0	128	0.016	0.008

\* The ranges for susceptibility and resistance to antibiotics, expressed as minimum inhibitory concentrations (MIC), were as follows: for amoxicillin (represented by ampicillin, which has a similar spectrum) and amoxicillin–clavulanate, 8  $\mu\text{g}$  per milliliter or less and 32  $\mu\text{g}$  per milliliter or more, respectively; for cefotaxime, 8  $\mu\text{g}$  per milliliter or less and 64  $\mu\text{g}$  per milliliter or more; and for ciprofloxacin, 1  $\mu\text{g}$  per milliliter or less and 4  $\mu\text{g}$  per milliliter or more. Values in between these ranges were considered to indicate intermediate susceptibility. No reference concentrations are available for the susceptibility or resistance of the Enterobacteriaceae to clarithromycin. Bacteria producing extended-spectrum beta-lactamase (ESBL) were identified with the use of Etest ESBL (AB Biodisk), a test of the MIC of ceftazidime alone in a given sample as compared with that of ceftazidime plus clavulanate (2  $\mu\text{g}$  per milliliter). The positive control, from a patient with antibiotic-associated hemorrhagic colitis (AAHC), has previously been shown to produce a cytotoxin.<sup>8</sup> The *K. oxytoca* strains we used as negative controls, American Type Culture Collection (ATCC) 13182 and 8724, have previously been shown to be nontoxicogenic.<sup>8-10</sup> Plus signs denote present, and minus signs absent. † Owing to the ESBL-positive status of the AHC 1 isolate, these concentrations were interpreted as representing resistance to the antibiotic, according to standards of the Clinical and Laboratory Standards Institute.



**Figure 1.** Colonoscopic Images in Two Patients with Antibiotic-Associated Hemorrhagic Colitis.

Panel A shows mucosal edema and hemorrhage in the transverse colon in Patient 4; Panel B shows a close-up of mucosal hemorrhage in the same patient. Panel C shows longitudinal ulceration in the sigmoid colon in Patient 2; Panel D shows rectal sparing in the same patient.

tients. Pseudomembranes were not observed in any patients. Histologic characteristics were consistent with the presence of hemorrhagic colitis.

When antibiotics were discontinued, all five patients recovered completely. Two patients were empirically treated with metronidazole before the diagnosis was established. The mean time to complete clinical remission after discontinuation of antibiotics and NSAIDs was 4 days.

During the observation period of 38 months (March 2001 through April 2004), we diagnosed

71 cases of infection with salmonella, 54 cases of infection with campylobacter, 2 cases of infection with shigella, and 121 cases of infection with *C. difficile* in addition to the 5 cases of antibiotic-associated hemorrhagic colitis induced by *K. oxytoca* at our hospital, which admits approximately 10,000 patients per year. There were no cases of infection with yersinia or enterohemorrhagic *E. coli*. The mean age of patients infected with *C. difficile* was 63 years (range, 20 to 94). Most cases of infection with *C. difficile* (68%) occurred during

hospitalization, and 78% of infected patients had undergone antibiotic therapy.

#### CYTOTOXIN PRODUCTION BY *K. OXYTOCA*

The supernatant from all cultures of *K. oxytoca* strains from patients with antibiotic-associated hemorrhagic colitis, as well as *K. oxytoca* MH 43-1, had a cytotoxic effect on monolayers of HEp-2 cells, as indicated by cell rounding and cell death (Table 2). In contrast, the supernatant from cultures of the negative control strains (ATCC 13182 and ATCC 8724) was not cytotoxic.

#### EXPERIMENTS IN ANIMALS

Treatment with amoxicillin–clavulanate reduced the total number of bacteria and changed the bacterial spectrum in rat stool. The most prominent finding was the disappearance of lactobacillus after treatment. *Klebsiella* and *C. difficile* and its toxin A were not detected in the stool of rats before or after administration of amoxicillin–clavulanate alone (data not shown).

*K. oxytoca* AHC 1 colonized the colon of rats receiving amoxicillin–clavulanate in addition to *K. oxytoca* (groups 1 and 4). The average colonization rate was  $7 \times 10^9$  CFU per gram of stool, with no difference between samples taken from the proximal colon and those from the distal colon. Isolated *K. oxytoca* strains had a pattern of biochemical and antibiotic resistance (the pattern of production of extended-spectrum beta-lactamase) that was identical to that of the inoculated AHC 1 strain. Group 3, inoculated with *K. oxytoca* but not treated with antibiotics, had no colonization.

We observed colitis (predominantly in the cecum) in rats that were inoculated with *K. oxytoca* AHC 1 and given amoxicillin–clavulanate (group 1), but not in the control groups receiving either amoxicillin–clavulanate only (group 2) or inoculation with *K. oxytoca* AHC 1 but no antibiotics (group 3) (Fig. 2). The left colon and small bowel were not affected in any treatment group. Major histologic findings were mucosal inflammation, mainly with infiltration of the lamina propria by neutrophils, and epithelial damage. In some rats, a few neutrophils entered the epithelial layer, and there were no crypt abscesses. Epithelial alteration included the loss of goblet cells, marked anisonucleosis, and increased rates of mitosis and apoptosis. Patchy mucosal hemorrhage was a less prominent finding.

The administration of indomethacin in addition to amoxicillin–clavulanate and the inoculation of *K. oxytoca* AHC 1 (group 4) produced a more severe colitis than that seen in group 1, but this difference was not significant (Fig. 2). Colonic erosion was significantly more severe in rats that received indomethacin than in those that did not, and treatment with indomethacin alone (group 6) caused erosion without colitis or mucosal hemorrhage. Indomethacin did not induce colonic damage when amoxicillin–clavulanate was also administered (group 5).

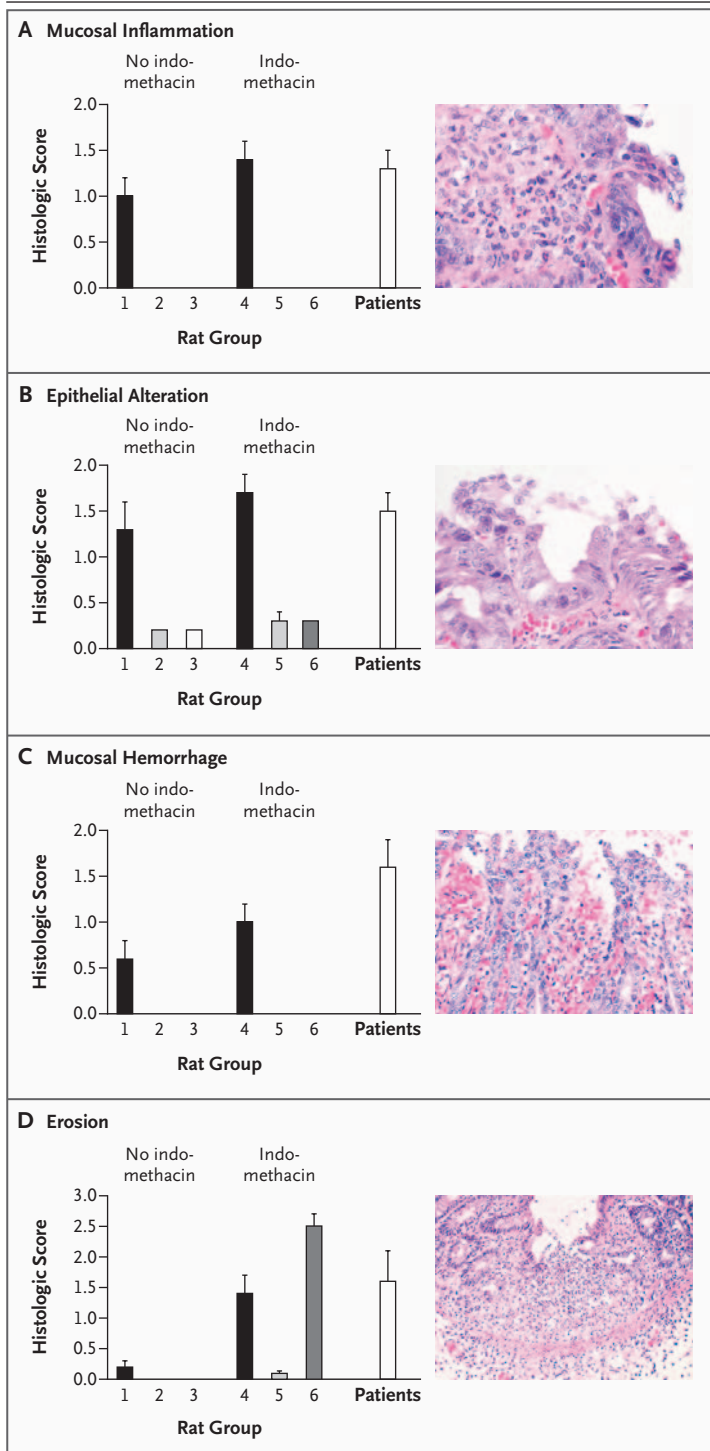
Histologic specimens obtained from rats with colitis resembled those from patients with antibiotic-associated hemorrhagic colitis (Fig. 3), and the histologic scores were similar (Fig. 2).

#### DISCUSSION

Five of six patients with antibiotic-associated hemorrhagic colitis had *K. oxytoca* in the stool, whereas stool cultures for other intestinal pathogens, including *C. difficile*, were negative. All *K. oxytoca* isolates from patients with antibiotic-associated hemorrhagic colitis produced cytotoxin on cell-culture assay.

The clinical features of antibiotic-associated hemorrhagic colitis in our patients were similar to those described previously.<sup>3,4,6,13</sup> The condition was observed mainly in young and otherwise healthy outpatients after brief treatment with penicillin derivatives, whereas *C. difficile*-associated diarrhea occurred mainly in older, hospitalized patients. The observed colitis was segmental and was mainly present in the right or transverse colon. Whereas sigmoidoscopy is thought to be sufficient for the diagnosis of antibiotic-associated colitis induced by *C. difficile*, colonoscopy is required for the diagnosis of antibiotic-associated hemorrhagic colitis, since sigmoidoscopy alone would miss many cases.

To establish a causal link between antibiotic-associated hemorrhagic colitis and toxigenic *K. oxytoca* according to Koch's postulates, we developed an in vivo animal model. In rats receiving antibiotics to suppress the physiologic colonic flora, oral inoculation of a *K. oxytoca* strain isolated from Patient 1 resulted in colonization of the colon in high concentrations and induced right-sided hemorrhagic colitis. In the absence of treatment with antibiotics, inoculation with *K. oxytoca* resulted in neither colonization of the colon nor



**Figure 2. Morphologic Changes in the Cecum of Rats, as Compared with Pathological Findings on Colonic Biopsy in Patients, According to the Histologic Score.**

Group 1 received amoxicillin–clavulanate and inoculation with *K. oxytoca* AHC 1; group 2 amoxicillin–clavulanate only; group 3 inoculation with *K. oxytoca* AHC 1 only; group 4 amoxicillin–clavulanate, indomethacin, and inoculation with *K. oxytoca* AHC 1; group 5 amoxicillin–clavulanate and indomethacin only; and group 6 indomethacin only. Photomicrographs show characteristic changes for each histologic criterion (hematoxylin and eosin). T bars indicate standard errors. There were significant differences in inflammation ( $P < 0.001$ ), epithelial alteration ( $P < 0.001$ ), and mucosal hemorrhage ( $P < 0.001$ ) in groups 1 and 4 (in which stool samples were positive for *K. oxytoca*) as compared with groups 2, 3, 5, and 6 (in which stool samples were negative for *K. oxytoca*). Erosion was significantly more severe in rats that received indomethacin than in rats that did not ( $P < 0.001$ ).

rhagic colitis have largely been fulfilled: an organism (*K. oxytoca*) was identified in most patients with a disease that is clinically well described, the organism was cultured and caused disease (right-sided colitis) in animals, and *K. oxytoca* could again be cultured from the diseased colon in the animals.

Previous data suggesting *K. oxytoca* as the cause of antibiotic-associated hemorrhagic colitis were limited to descriptive clinical observations of patients with antibiotic-associated hemorrhagic colitis and data from in vitro studies using a cytotoxin isolated from *K. oxytoca*. Several case reports and small series have demonstrated that *K. oxytoca* is present in the stool of most patients with antibiotic-associated hemorrhagic colitis.<sup>2,4-6,8,14</sup> The prevalence of *K. oxytoca* among our healthy subjects was 1.6% in Austria, as compared with 9% in France,<sup>2</sup> suggesting that the prevalence varies by geographic region. Two case reports, in which *K. oxytoca* was the presumed cause of colitis in patients who had not received antibiotic therapy,<sup>15,16</sup> deserve special attention, since they suggest that *K. oxytoca* may be pathogenic even in the absence of antibiotics under certain circumstances.

In experiments performed by two groups of researchers in Japan, *K. oxytoca* strains isolated from patients with antibiotic-associated hemorrhagic colitis were shown to be capable of producing a cytotoxin that was toxigenic in cultures of HEp-2, Vero, Chinese hamster ovary, and HeLa cells.<sup>8,10</sup> Furthermore, this cytotoxin, a low-molecular-weight organic substance, induced mucosal

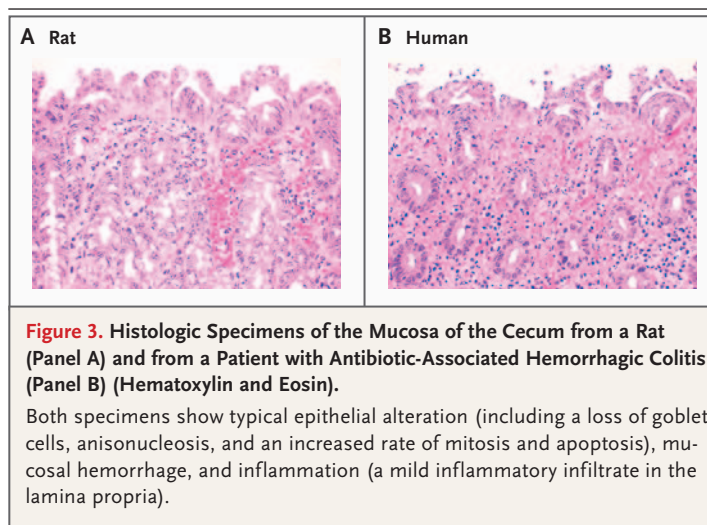
colitis in rats. The histologic features and location of colitis (the right colon) in this animal model were similar to those in patients with antibiotic-associated hemorrhagic colitis. Thus, Koch's postulates for antibiotic-associated hemor-

damage in isolated loops from rabbit ileum.<sup>17</sup> However, neither the isolated cytotoxin nor the toxin-producing strain of *K. oxytoca* (OK-1) induced inflammation or damage in the colonic mucosa of rabbits.<sup>17</sup> The cytotoxic effect was also observed by Beaugerie et al.: 14 of 17 *K. oxytoca* strains (82%) obtained from patients with antibiotic-associated hemorrhagic colitis, and 42 of 90 (47%) obtained from healthy controls, were cytotoxic to HEp-2 cells in their study.<sup>2</sup>

Histologic findings in patients with antibiotic-associated hemorrhagic colitis are similar to those in patients with other toxin-induced forms of colitis, such as colitis due to enterohemorrhagic *E. coli* or shigella.<sup>7,18,19</sup> The histologic findings in patients with these forms of colitis may resemble the findings in patients with ischemic colitis<sup>18,19</sup>; the resemblance with ischemic colitis has also been described for antibiotic-associated hemorrhagic colitis.<sup>7</sup> Furthermore, abnormalities are mainly located in the right colon both in patients with hemorrhagic colitis due to enterohemorrhagic *E. coli*<sup>18</sup> and in those with antibiotic-associated hemorrhagic colitis. Crypt abscesses, which are commonly found in patients with infectious colitis due to invasive bacteria, were absent in both humans and rats with antibiotic-associated hemorrhagic colitis in our study. We therefore assume that *K. oxytoca* induces colitis, probably by means of the cytotoxin produced by certain strains.

*K. oxytoca* strains isolated from three of our patients were resistant to the antibiotics that were administered before the onset of colitis. *K. oxytoca* isolates in two patients receiving amoxicillin-clavulanate were, however, sensitive in vitro to this beta-lactam antibiotic. Previous studies investigating the effect of orally administered amoxicillin-clavulanate on fecal microflora revealed that fecal enterobacteria that are sensitive to amoxicillin-clavulanate may persist in the intestinal microflora during antibiotic therapy.<sup>20,21</sup> This may be due to very low fecal concentrations of amoxicillin-clavulanate.<sup>20</sup> We assume that, in our study, low amoxicillin-clavulanate concentrations, especially in the right colon, were sufficient to suppress most bacteria in the intestinal microflora but were too low to inhibit the growth of *K. oxytoca*.

We suggest the following pathogenetic model for antibiotic-associated hemorrhagic colitis. Cytotoxin-producing *K. oxytoca* strains are present



at least temporarily in the colon of some people, in whom antibiotic therapy can lead to overgrowth of toxigenic *K. oxytoca* in the colon, owing to selection of this organism. Since klebsiella is nearly always resistant to penicillins because it expresses a beta-lactamase, antibiotic-associated hemorrhagic colitis is particularly seen during therapy with penicillin derivatives. Overgrowth of *K. oxytoca* in the colon finally results in high cytotoxin concentrations that induce mucosal damage.

We observed frequent use of NSAIDs in our patients with antibiotic-associated hemorrhagic colitis, as have other investigators.<sup>6,7</sup> Use of NSAIDs has also been reported to be associated with colitis and diarrhea of various causes.<sup>22-24</sup> In our experiments in rats, concomitant administration of indomethacin and inoculation of *K. oxytoca* tended to cause more severe colitis and to induce erosion. Thus, NSAIDs appear to aggravate colitis caused by potentially pathogenic bacteria in the intestinal lumen.

Our study suggests that toxigenic *K. oxytoca* should be considered in the differential diagnosis of potential intestinal pathogens. When a patient with antibiotic-associated colitis is negative for *C. difficile*, stool samples should be cultured for *K. oxytoca*. In these patients, it will probably suffice to discontinue antibiotics and NSAIDs and to begin symptomatic therapy. We consider it important to draw attention to toxigenic *K. oxytoca*, since relatively young patients may have received a misdiagnosis of ischemic colitis with spontaneous resolution in the past, and since the diagnosis of antibiotic-associated hemorrhagic coli-

tis has consequences for the care of such patients, who could be harmed by unnecessary treatment with drugs.

Supported by a grant from the Medical University of Graz.

No potential conflict of interest relevant to this article was reported.

We thank Prof. Gregor Högenauer, Prof. Friederike Turnowsky, Georg Waeg, Ph.D., and Gebhard Feierl, M.D., for their helpful

ideas and advice; Hans Hosbein, Andrea Fuchsichler, Christina Strempl, and Waltraut Eberharth for their technical help with the experiments; Babak Yazdani-Biuki, M.D., Col. Hans-Christoph Stenzel, M.D., and First Sgt. Thomas Scheucher for their help in obtaining samples from healthy subjects; Dr. T. Chida of the Department of Microbiology, Medical and Dental University, Tokyo, for kindly providing the cytotoxin-producing *K. oxytoca* strain MH 43-1 that was used as a positive control; and Eugenia Lamont, B.A., for editorial assistance.

#### REFERENCES

1. Bartlett JG. Antibiotic-associated diarrhea. *N Engl J Med* 2002;346:334-9.
2. Beaugerie L, Metz M, Barbut F, et al. *Klebsiella oxytoca* as an agent of antibiotic-associated hemorrhagic colitis. *Clin Gastroenterol Hepatol* 2003;1:370-6.
3. Toffler RB, Pingound EG, Burrell MI. Acute colitis related to penicillin derivatives. *Lancet* 1978;2:707-9.
4. Koga H, Aoyagi K, Yoshimura R, Kimura Y, Iida M, Fujishima M. Can quinolones cause hemorrhagic colitis of late onset? Report of three cases. *Dis Colon Rectum* 1999;42:1502-4.
5. Bellaiche G, Le Pennec MP, Choudat L, Ley G, Slama JL. Value of rectosigmoidoscopy with bacteriological culture of colonic biopsies in the diagnosis of post-antibiotic hemorrhagic colitis related to *Klebsiella oxytoca*. *Gastroenterol Clin Biol* 1997;21:764-7. (In French.)
6. Sakurai Y, Tsuchiya H, Ikegami F, Funatomi T, Takasu S, Uchikoshi T. Acute right-sided hemorrhagic colitis associated with oral administration of ampicillin. *Dig Dis Sci* 1979;24:910-5.
7. Yonei Y, Yoshizaki Y, Tsukada N, et al. Microvascular disturbances in the colonic mucosa in antibiotic-associated hemorrhagic colitis: involvement of platelet aggregation. *J Gastroenterol Hepatol* 1996;11:681-5.
8. Higaki M, Chida T, Takano H, Nakaya R. Cytotoxic component(s) of *Klebsiella oxytoca* on HEp-2 cells. *Microbiol Immunol* 1990;34:147-51.
9. Minami J, Saito S, Yoshida T, Uemura T, Okabe A. Biological activities and chemical composition of a cytotoxin of *Klebsiella oxytoca*. *J Gen Microbiol* 1992;138:1921-7.
10. Minami J, Okabe A, Shiode J, Hayashi H. Production of a unique cytotoxin by *Klebsiella oxytoca*. *Microb Pathog* 1989;7:203-11.
11. Bartlett JG, Chang TW, Gurwith M, Gorbach SL, Onderdonk AB. Antibiotic-associated pseudomembranous colitis due to toxin-producing clostridia. *N Engl J Med* 1978;298:531-4.
12. Rath HC, Herfarth HH, Ikeda JS, et al. Normal luminal bacteria, especially *Bacteroides* species, mediate chronic colitis, gastritis and arthritis in HLA-B27/human  $\beta$ 2 microglobulin transgenic rats. *J Clin Invest* 1996;98:945-53.
13. Heer M, Sulser H, Hany A. Segmental hemorrhagic colitis following amoxicillin therapy. *Schweiz Med Wochenschr* 1989;119:733-5. (In German.)
14. Benoit R, Dorval D, Loulergue J, et al. Post-antibiotic diarrheas: role of *Klebsiella oxytoca*. *Gastroenterol Clin Biol* 1992;16:860-4. (In French.)
15. Chen J, Cachay ER, Hunt GC. *Klebsiella oxytoca*: a rare cause of severe infectious colitis: first North American case report. *Gastrointest Endosc* 2004;60:142-5.
16. Soussi F, Tchirikhtchian K, Ramaholimihaso F, et al. Diclofenac-induced colitis complicated by *Klebsiella oxytoca* infection. *Gastroenterol Clin Biol* 2001;25:814-6. (In French.)
17. Minami J, Katayama S, Matsushita O, Sakamoto H, Okabe A. Enterotoxic activity of *Klebsiella oxytoca* cytotoxin in rabbit intestinal loops. *Infect Immun* 1994;62:172-7.
18. Griffin PM, Olmstead LC, Petras RE. *Escherichia coli* O157:H7-associated colitis: a clinical and histological study of 11 cases. *Gastroenterology* 1990;99:142-9.
19. O'Loughlin EV, Robins-Browne RM. Effect of Shiga toxin and Shiga-like toxins on eukaryotic cells. *Microbes Infect* 2001;3:493-507.
20. Lode H, Von der Hoh N, Ziege S, Borner K, Nord CE. Ecological effects of linezolid versus amoxicillin/clavulanic acid on the normal intestinal microflora. *Scand J Infect Dis* 2001;33:899-903.
21. Brumfitt W, Franklin I, Grady D, Hamilton-Miller JM. Effect of amoxicillin-clavulanate and cephradine on the fecal flora of healthy volunteers not exposed to a hospital environment. *Antimicrob Agents Chemother* 1986;30:335-7.
22. Gleeson MH, Davis AJM. Non-steroidal anti-inflammatory drugs, aspirin and newly diagnosed colitis: a case-control study. *Aliment Pharmacol Ther* 2003;17:817-25.
23. Etienne I, Beaugerie L, Viboud C, Flahault A. Non-steroidal anti-inflammatory drugs as a risk factor for acute diarrhea: a case crossover study. *Gut* 2003;52:260-3.
24. Evans JM, McMahon AD, Murray FE, McDevitt DG, MacDonald TM. Non-steroidal anti-inflammatory drugs are associated with emergency admission to hospital for colitis due to inflammatory bowel disease. *Gut* 1997;40:619-22.

Copyright © 2006 Massachusetts Medical Society.

#### EARLY JOB ALERT SERVICE AVAILABLE AT THE NEJM CAREERCENTER

Register to receive weekly e-mail messages with the latest job openings that match your specialty, as well as preferred geographic region, practice setting, call schedule, and more. Visit the NEJM CareerCenter at [www.nejmjobs.org](http://www.nejmjobs.org) for more information.