Role of *Klebsiella oxytoca* in Antibiotic-Associated Diarrhea

Ines Zollner-Schwetz,[1] **Christoph Högenauer,**[2] **Martina Joainig,**[2] **Paul Weberhofer,**[2] **Gregor Gorkiewicz,**[3] **Thomas Valentin,**[1] **Thomas A Hinterleitner,**[2] **and Robert Krause**[1]

[1]Section of Infectious Diseases, Division of Pulmonology, Department of Internal Medicine, [2]Division of Gastroenterology and Hepatology, Department of Internal Medicine, and [3]Institute of Pathology, Medical University of Graz, Graz, Austria

Background. *Klebsiella oxytoca* was recently shown to be the causative agent of antibiotic-associated hemorrhagic colitis. Because it is unclear whether *K. oxytoca* also causes nonhemorrhagic antibiotic-associated diarrhea, our study investigated a possible association between *K. oxytoca* and that disorder.

Methods. A total of 371 consecutive patients were recruited into 4 study groups: (1) group A^+D^+ (patients who received antibiotics and experienced diarrhea; $n = 107$), (2) group A^+D^- (patients who received antibiotics but did not experience diarrhea; $n = 93$), (3) group A^-D^+ (patients who experienced acute-onset diarrhea but did not receive antibiotics; $n = 60$), and (4) group A^-D^- (patients without diarrhea who did not receive antibiotics; $n = 111$). Stool samples were plated on MacConkey agar and *K. oxytoca* was identified using a standard test kit. *Clostridium difficile* was detected by a toxin A/B antigen test. *K. oxytoca* strains were tested for cytotoxicity with use of cell-culture assays.

Results. In 15 of 371 stool samples, *K. oxytoca* strains were isolated during the study period. There was no significant difference in the distribution of *K. oxytoca* among the 4 study groups. Six of the 15 strains were found to be toxin producing. Three of the toxin-producing strains caused antibiotic-associated hemorrhagic colitis. No case of nonhemorrhagic antibiotic-associated diarrhea due to toxin-producing *K. oxytoca* was detected.

Conclusion. *K. oxytoca* is not the causative agent of nonhemorrhagic antibiotic-associated diarrhea. This is in contrast to the distinct clinical entity of antibiotic-associated hemorrhagic colitis. Testing for *K. oxytoca* is therefore only warranted for patients who experience bloody diarrhea during antibiotic therapy.

Antibiotic-associated diarrhea frequently occurs during antibiotic treatment. In severe cases, it can be a significant cause of morbidity and mortality, especially in elderly hospitalized patients. The mechanisms by which antibiotics lead to antibiotic-associated diarrhea are diverse, ranging from pharmacologic effects on gut motility to disturbance of the function of the indigenous intestinal flora and overgrowth by pathogenic microorganisms [1]. *Clostridium difficile* is the most common pathogenic microorganism to cause antibiotic-associated diarrhea, although it accounts for only 10%–20% of all cases [2]. The clinical spectrum of *C. difficile*–

associated disease ranges from diarrhea without colitis to antibiotic-associated pseudomembranous colitis and fulminant colitis [2].

Antibiotic-associated hemorrhagic colitis (AAHC) is a distinct form of antibiotic-associated diarrhea, in which no *C. difficile* is detected. AAHC is characterized by sudden onset of bloody diarrhea during antibiotic therapy, often in combination with severe abdominal cramps [3]. On endoscopy, the key macroscopic feature of AAHC is segmental distribution of mucosal hemorrhage and mucosal edema localized predominantly in the right colon. Recently, toxin-producing *Klebsiella oxytoca* was identified as the causative agent of this disease entity [3]. It has also been suggested that *K. oxytoca* is associated with severe colitis without preceding antibiotic therapy [4, 5] and the aggravation of Crohn disease [6]. Whether *K. oxytoca* plays a role in the development of nonhemorrhagic antibiotic-associated diarrhea has not been investigated.

The aim of our study was to elucidate the pathogenetic relevance of *K. oxytoca* in adult patients with

antibiotic-associated diarrhea and to establish whether routine testing for *K. oxytoca* is warranted in these patients. Stool specimens were cultured to isolate *K. oxytoca* from patients who had antibiotic-associated diarrhea and from control subjects. In addition, *K. oxytoca* isolates were tested for cytotoxin production.

PATIENTS AND METHODS

Patient selection. Study patients were recruited in a 280-bed department of internal medicine at a university hospital from November 2006 through February 2008. Patients were included only once during their hospital stay and always provided written informed consent. The protocol was approved by the ethics committee of the Medical University of Graz (Graz, Austria).

Patients were surveyed during clinical rounds for the presence of antibiotic-associated diarrhea, diarrhea, or antibiotic treatment in the absence of diarrhea. Antibiotic treatment was defined as the receipt of any oral or intravenous antibiotic in therapeutic doses for ⩾1 day, as described elsewhere [7]. Antibiotic-associated diarrhea was defined as ⩾3 mushy or watery stools per day during or as long as 2 months after antibiotic treatment [8]. A total of 371 patients who met the inclusion criteria were recruited into 4 study groups: (1) 107 consecutive patients who received antibiotic treatment and experienced diarrhea (A^+D^+), (2) 93 patients who received antibiotic treatment for at least 3 days but did not experience diarrhea (A^+D^-), (3) 60 patients who did not receive antibiotic treatment but experienced acute onset diarrhea (A^-D^+), and (4) 111 patients who did not receive antibiotics and did not experience diarrhea (A^-D^-). Patients who experienced bloody diarrhea were evaluated by endoscopy at the attending clinician's discretion.

Patients receiving antibiotic treatment because of suspected acute bacterial diarrhea (i.e., diarrhea due to *Salmonella, Shigella, Campylobacter,* or *Yersinia* species) were excluded. In addition, patients who met ⩾1 of the following criteria were excluded from the study: inflammatory bowel disease, lactose intolerance, short bowel syndrome, hematooncologic disease, receipt of chemotherapy within the previous 2 months, abdominal surgery during the past 2 months, or hospitalization in the intensive care unit during the previous 30 days. Demographic, clinical, and laboratory data were extracted from charts and computerized databases. The diagnosis of AAHC was made on the basis of the clinical history (use of antibiotics before the onset of bloody diarrhea) and findings on endoscopy and histology that were typical of segmental hemorrhagic colitis, according to definitions published elsewhere [3].

Microbiological analysis. Stool samples were plated on MacConkey agar plates (bioMérieux). *Klebsiella* species were identified using the API 20E test (bioMérieux). Stool specimens were tested for *C. difficile* toxins A and B with use of a rapid detection assay (Immunocard Toxins A & B; Meridian

Bioscience). Stool samples from patients in group A^+D^+ were also cultured for intestinal pathogens (specifically, *Salmonella, Shigella, Yersinia,* and *Campylobacter* species) on selective agars. Stool samples of patients in group A^-D^+ were cultured for *Salmonella, Shigella, Yersinia,* and *Campylobacter* species at the clinician's discretion. Stool samples and bacterial isolates were stored at $-70°C$.

Quantitative cultures were performed as described elsewhere [7] from stool specimens positive for *K. oxytoca* that had been stored at $-70°$. In brief, the stool samples were diluted 1:10 with saline, stirred in a stool homogenizer and again diluted 1:1000 and 1:10,000 with saline. One hundred microliters of each dilution was transferred onto a MacConkey agar plate and plated evenly with a sterile glass swab. After 24 h of incubation at 37°C, suspected colonies were identified using API 20E, were counted, and colony forming units (CFU) per mL of stool sample were calculated.

Cytotoxin assays. All strains that were identified as *K. oxytoca* by the API 20E test were tested for the production of cytotoxin with use of cell-culture assays. Cytotoxic effects were assessed by microscopic examination (cell rounding and cell death) and colorimetric investigation (MTT tetrazolium method), as described elsewhere [3, 9, 10].

Statistical analysis. Statistical analysis was performed using Cramer's V test and Mann-Whitney test, where appropriate, with use of SPSS software, version 9.0 (SPSS). A P value of <.05 was considered to indicate statistical significance.

RESULTS

Group A^+D^+. In group A^+D^+ ($n = 107$), 89 patients experienced nonbloody diarrhea (figure 1). *K. oxytoca* was not detected in the stool specimens obtained from any of these 89 patients. Eighteen patients in group A^+D^+ experienced bloody diarrhea. *K. oxytoca* was recovered from the stool specimens
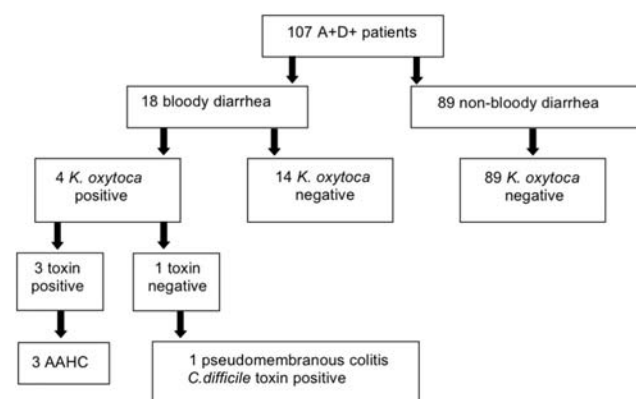

Figure 1. Patients in group A^+D^+ ($n = 107$), organized according to their symptoms and positive test results for the presence of *Klebsiella oxytoca* and its toxin.

---

of 4 of these 18 patients. On endoscopy, AAHC was diagnosed macroscopically in 3 of 18 patients who experienced bloody diarrhea. The diagnosis of AAHC was confirmed by histology in all cases, as described elsewhere [3]. Toxin-producing *K. oxytoca* was recovered from stool specimens of all 3 patients with AAHC, whereas *C. difficile* toxin testing results were negative for all 3 patients (figure 1). One patient who had bloody diarrhea in group A^+D^+ harbored *K. oxytoca,* which had negative toxin results. Quantitative stool sample cultures revealed a low-level carrier status of *K. oxytoca,* with <10 CFU/mL. *C. difficile* toxin was also detected in the stool of this patient, and colonoscopy revealed pseudomembranous colitis. Demographic and clinical data of all patients are summarized in table 1.

Klebsiella *species cultures.* The results of cultures for *Klebsiella* species are shown in table 2. A total of 15 *K. oxytoca* strains were isolated during the study period. There was no significant difference in the distribution of *K. oxytoca* and other *Klebsiella* species (*Klebsiella pneumoniae* and *Klebsiella terrigena*) among the 4 groups. The inclusion of AAHC cases had no impact on the analysis.

Quantitative *Klebsiella* species cultures were performed using stool samples from which *K. oxytoca* had been isolated. One of 15 stool samples (from a patient in group A^-D^+) could not be tested because there was an insufficient volume of sample. The results of quantitative cultures are shown in table 3. The quantity of *K. oxytoca* in quantitative cultures was significantly higher among patients with AAHC than that among all other groups combined ($P = .038$). For this analysis, values of <10 CFU/mL (see table 3) were substituted with 9 CFU/mL, to assume the highest possible amount of *K. oxytoca* <10.

Toxin production in *K. oxytoca* isolates is presented in table 3. Of the 4 *K. oxytoca* isolates from patients in group A^+D^+, 3 produced toxin. All 3 were recovered from patients who had AAHC. The remaining *K. oxytoca* isolates from patients in group A^+D^+ did not produce toxin.

Other intestinal pathogens. The distribution of *C. difficile* toxin was significantly different across the 4 groups, with the highest rates of *C. difficile* isolation among patients in group A^+D^+ (table 2). In this group, 11% of patients had positive test results for *C. difficile* toxin in their stool specimen. In group A^+D^+, *Salmonella, Shigella, Yersinia,* and *Campylobacter* species were not detected in any stool samples. In group A^-D^+ ($n = 60$), *Salmonella* species were detected in the stool samples of 4 patients, whereas 3 were positive for *Campylobacter* species. In 37 patients of this group, no diarrheal pathogen was detected. Sixteen patients in this group were not tested for bacterial pathogens other than *K. oxytoca* and *C. difficile.*

DISCUSSION

K. oxytoca was recently found to be the causative agent of AAHC, a distinct form of antibiotic-associated diarrhea in which *C. difficile* is absent [3]. Whether *K. oxytoca* plays a role in nonhemorrhagic antibiotic-associated diarrhea had not been investigated until now. In our study, there was no significant difference in the distribution of *K. oxytoca* species in the 4

Table 1. Demographic characteristics of patients.

Characteristic	A^+D^+ ($n = 107$)	A^+D^- ($n = 93$)	A^-D^+ ($n = 60$)	A^-D^- ($n = 111$)
Age, mean years ± SEM	68.3 ± 1.6	67.1 ± 1.7	62.4 ± 2.8	62.9 ± 1.3
Sex				
Female	48 (45)	45 (48)	38 (63)	51 (46)
Male	59 (55)	48 (52)	22 (37)	60 (54)
Location of infection				
Respiratory tract	48 (45)	49 (53)	…	…
Urogenital tract	15 (14)	9 (10)	…	…
Skin or soft tissue	16 (15)	7 (8)	…	…
Other	25 (23)	24 (26)	…	…
Unknown	3 (3)	2 (2)	…	…
Antibiotics received[a]				
β-lactams	42 (39)	40 (43)	…	…
Fluoroquinolones	49 (46)	27 (29)	…	…
Others	10 (9)	4 (4)	…	…
≥2 Agents	6 (6)	20 (22)	…	…

NOTE. Data are no. (%) of patients, unless otherwise indicated. Group A^+D^+, patients who received antibiotics and experienced diarrhea; group A^+D^-, patients who received antibiotics but did not experience diarrhea; group A^-D^+, patients who experienced acute-onset diarrhea but did not receive antibiotics; group A^-D^-, patients without diarrhea who did not receive antibiotics.

[a] The distribution of antimicrobial agents received was statistically significantly different between patients in groups A^+D^+ and A^+D^- ($P = .001$).

Table 2. Prevalence of *Klebsiella* species and *Clostridium difficile* in stool specimens.

Organism	No. (%) of patients with an organism detected in stool specimen			
	Group A⁺D⁺ᵃ (n = 107)	Group A⁺D⁻ (n = 93)	Group A⁻D⁺ (n = 60)	Group A⁻D⁻ (n = 111)
Klebsiella oxytoca	4 (4)	1 (1)	5 (8)	5 (5)
Non-*oxytoca Klebsiella* species[b]	13 (12)	12 (13)	9 (15)	12 (11)
C. difficile	12 (11)	0 (0)	2 (3)	0 (0)

NOTE. Group A⁺D⁺, patients who received antibiotics and experienced diarrhea; group A⁺D⁻, patients who received antibiotics but did not experience diarrhea; group A⁻D⁺, patients who experienced acute-onset diarrhea but did not receive antibiotics; group A⁻D⁻, patients without diarrhea who did not receive antibiotics.

[a] Three patients who had antibiotic-associated hemorrhagic colitis are included in group A⁺D⁺.
[b] Non-*oxytoca Klebsiella* species detected were *Klebsiella pneumoniae* and *Klebsiella terrigena*.

study groups, demonstrating that *K. oxytoca* is not associated with nonhemorrhagic antibiotic-associated diarrhea. Although a total of 371 patients were investigated, this finding should be interpreted with caution because the prevalence of K. oxytoca was low in all study groups.

Eighteen patients in group A⁺D⁺ experienced bloody diarrhea. Three of these 18 patients received a diagnosis of AAHC on endoscopy, and toxin-producing *K. oxytoca* was isolated from stool samples in all 3 cases. All 3 patients in our study experienced a sudden onset of bloody diarrhea in combination with abdominal cramps during antibiotic therapy with amoxicillin-clavulanate, similar to what has been reported elsewhere [3]. Concomitant intake of nonsteroidal anti-inflammatory drugs, which has been considered to be an etiologic cofactor in AAHC [3], was verified in all 3 patients. In addition to the 3 cases of AAHC, *K. oxytoca* was isolated from the stool specimen of only 1 other patient in group A⁺D⁺. This isolate did not produce cytotoxin, and the patient received a diagnosis of *C. difficile*–related pseudomembranous colitis. Our results indicate that routine testing for *K. oxytoca* in stool samples is warranted only for patients who are experiencing bloody diarrhea during antibiotic therapy.

In our study, antibiotic-associated diarrhea was caused by *C. difficile* in 11% patients, which is consistent with other studies [2]. *Klebsiella* species were found in 15% of patients in the control group A⁻D⁻. This result is comparable to that of another study performed in a healthy Dutch population (aged 50–80 years), which detected *Klebsiella* species in 12% of stool samples [11]. *K. oxytoca* was identified in 4% of patients in our control group A⁻D⁻, which is comparable to other recently published findings [3]. Interestingly, only 1 toxin-producing *K. oxytoca* strain was found in the control group A⁻D⁻. Quantitative stool culture revealed a low concentration of this pathogen in the stool specimen. This patient was considered to be an asymptomatic carrier of toxin-producing *K. oxytoca*. We hypothesize that induction of colitis by *K. oxytoca* is associated with increased colony counts of this pathogen triggered by antibiotic therapy.

In group A⁻D⁺, 2 *K. oxytoca* isolates were found to be toxin producing. In 1 of these patients (patient 345), *K. oxytoca* was simultaneously isolated from a urine specimen during routine culture (*K. oxytoca* count, 1×10^6 CFU/mL). This isolate demonstrated no toxin production (data not shown). Because no other cause of diarrhea could be established, *K. oxytoca*—with an intestinal colony count of 2×10^5 CFU/mL—might have

Table 3. Toxin production and quantitiy of *Klebsiella oxytoca* detected in patients' stool specimens.

Group and patient number	Toxin production	Quantity (CFU/mL)
A⁺D⁺		
218	No	<10
AAHCᵃ		
19	Yes	4.0×10^6
43	Yes	2.1×10^6
300	Yes	4.6×10^6
A⁺D⁻		
342	No	5.0×10^5
A⁻D⁺		
68	Yes	1
144	No	2.0×10^4
251	No	ND
210	No	<10
345	Yes	2.0×10^5
A⁻D⁻		
67	No	1.0×10^2
198	No	<10
241	Yes	2
277	No	1.0×10^{10}
301	No	<10

NOTE. AAHC, antibiotic-associated hemorrhagic colitis; CFU, colony-forming units; group A⁺D⁺, patients who received antibiotics and experienced diarrhea; group A⁺D⁻, patients who received antibiotics but did not experience diarrhea; group A⁻D⁺, patients who experienced acute-onset diarrhea but did not receive antibiotics; group A⁻D⁻, patients without diarrhea who did not receive antibiotics; ND, not done because of insufficient volume of stool sample.

[a] Patients received a diagnosis of AAHC on the basis of their clinical history and typical findings on endoscopy and histology.

caused nonbloody diarrhea in this patient without preceding antibacterial therapy. In the second patient's stool sample, <10 CFU/mL of *K. oxytoca* was found, indicating that it was extremely unlikely that this patient's diarrhea was caused by *K. oxytoca*. In contrast, *K. oxytoca* could be detected in large quantities in all of the patients who had AAHC (mean *K. oxytoca* count in stool sample, 3.6×10^6 CFU/mL) in our study, suggesting that the administration of antibiotics alters the indigenous intestinal flora and promotes the growth of *K. oxytoca*. Large quantities of this microorganism apparently produce large amounts of cytotoxin, thus causing hemorrhagic colitis. It is currently unknown whether patients who develop AAHC are colonized with *K. oxytoca* prior to antibiotic therapy, with antibiotic therapy leading to *K. oxytoca* overgrowth with toxin production, or whether patients who develop AAHC are newly infected with *K. oxytoca*.

It has to be noted that the receipt of fluoroquinolones was more common in group A^+D^+ than in group A^+D^-. In accordance with a recent investigation [12], 4th-generation fluoroquinolones, the predominant quinolones used in our study, were obviously associated with a higher risk of antibiotic-associated diarrhea among our study population.

In conclusion, our study demonstrates that *K. oxytoca* is not associated with nonhemorrhagic antibiotic-associated diarrhea in adult patients. Routine testing for *K. oxytoca* is therefore only warranted for patients who are experiencing bloody diarrhea during antibiotic therapy.

Acknowledgments

References

1. Bartlett JG. Clinical practice: antibiotic-associated diarrhea. N Engl J Med **2002**; 346:334–9.
2. Kelly CP, Pothoulakis C, LaMont JT. *Clostridium difficile* colitis. N Engl J Med **1994**; 330:257–62.
3. Hogenauer C, Langner C, Beubler E, et al. *Klebsiella oxytoca* as a causative organism of antibiotic-associated hemorrhagic colitis. N Engl J Med **2006**; 355:2418–26.
4. 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.
5. Soussi F, Tchirikhtchian K, Ramaholimihaso F, et al. Diclofenac-induced colitis complicated by *Klebsiella oxytoca* infection [in French]. Gastroenterol Clin Biol **2001**; 25:814–6.
6. Plessier A, Cosnes J, Gendre JP, Beaugerie L. Intercurrent *Klebsiella oxytoca* colitis in a patient with Crohn's disease [in French]. Gastroenterol Clin Biol **2002**; 26:799–800.
7. Krause R, Schwab E, Bachhiesl D, et al. Role of *Candida* in antibiotic-associated diarrhea. J Infect Dis **2001**; 184:1065–9.
8. Fekety R. Guidelines for the diagnosis and management of *Clostridium difficile*–associated diarrhea and colitis: American College of Gastroenterology, Practice Parameters Committee. Am J Gastroenterol **1997**; 92:739–50.
9. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods **1983**; 65:55–63.
10. Minami J, Okabe A, Shiode J, Hayashi H. Production of a unique cytotoxin by *Klebsiella oxytoca*. Microb Pathog **1989**; 7:203–11.
11. Degener JE, Smit AC, Michel MF, Valkenburg HA, Muller L. Faecal carriage of aerobic gram-negative bacilli and drug resistance of *Escherichia coli* in different age-groups in Dutch urban communities. J Med Microbiol **1983**; 16:139–45.
12. Torres A, Garau J, Arvis P, et al. Moxifloxacin monotherapy is effective in hospitalized patients with community-acquired pneumonia: the MOTIV study—a randomized clinical trial. Clin Infect Dis **2008**; 46:1499–509.