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Factors Related to Colonization with *Oxalobacter formigenes* in U.S. Adults

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Abstract

Goals: To elucidate the determinants of *Oxalobacter formigenes* colonization in humans.
Background: *O. formigenes* is a gram-negative anaerobic bacterium that colonizes the colon of a substantial proportion of the normal population and metabolizes dietary and endogenous oxalate. The bacterium has been associated with a large reduction in the odds of recurrent calcium oxalate kidney stones. Subjects were 240 healthy individuals from Massachusetts and North Carolina. *O. formigenes* was detected by culture of fecal swabs. Information on factors of interest was obtained by telephone interviews and self-administered questionnaires.
Study Results: The overall prevalence of *O. formigenes* was 38%. Use of specific antibiotics previously thought to affect the bacterium was significantly related to colonization, with prevalences of 17%, 27%, and 36%, for those who had used these drugs <1, 1–5, and >5 years ago, compared with 55% in nonusers. There were no significant associations with demographic factors, nutrient intake, or medical history, although the prevalence appeared to increase somewhat with increasing oxalate consumption.
Conclusions: Some antibiotics markedly affect colonization with *O. formigenes*. Although no other factor was identified as having a material influence on the prevalence of the bacterium, there is much to learn about how an individual acquires the organism and which factors affect persistence of colonization.

Introduction

In recent years there has been increasing interest in exploring the probiotic potential of intestinal microbiota.[1,2] A promising example is *Oxalobacter formigenes*, a gram-negative, obligately anaerobic bacterium that inhabits the mammalian colon.[3] The genome has been fully sequenced by the Broad Institute in Boston (www.broad.mit.edu/annotation/genome/oxalobacter_group). *O. formigenes* is unique in that dietary and endogenous oxalate are its sole energy sources. Calcium oxalate comprises the majority of kidney stones,[4] and it has been hypothesized that the bacterium lowers the risk of developing these stones by degrading oxalate in the colon and hence reducing its excretion in the urine. In a recent study conducted by our group, *O. formigenes* was associated with a 70% reduction in the odds of a recurrence of calcium oxalate stones.[5] Overall, the incidence of renal stones in the United States is about 2/1000[6–9] annually; ~7% of American women and 13% of American men, respectively, will experience a renal stone over the course of a lifetime.[10] Thus, the public health implications of this relationship are potentially large.

O. formigenes was first isolated and described in the 1980s by Allison et al[3,11] Thus far, there has been little research focusing on the natural history of this bacterium in human populations. Although it appears that a large proportion of normal individuals are colonized, there is substantial variation in the reported prevalence in adults. The estimate from our study was ~40% of healthy adult subjects from Massachusetts and North Carolina[5]; findings from several small studies conducted in various countries ranged from 46% to 77%.[12–21] Little is known about when and how individuals become colonized or the persistence of the bacterium over time. The only known factors that reduce colonization are some antibiotics (there have been a few reports in the literature,[22,23] but much of the information is unpublished) and bile salts (based on animal studies[24]). There are also limited clinical data suggesting that the prevalence of *O. formigenes* is substantially reduced in various malabsorptive states and in cystic fibrosis,[11,14,21] which may be due to excessive antibiotic use in the latter population. Here we report findings from an evaluation of the determinants of *O. formigenes* colonization based on an analysis of the control subjects with no history of renal stones from our study in Massachusetts and North Carolina.

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Materials and Methods

The data were collected from 2004 to 2006, to address the hypothesis that the presence of *O. formigenes* in the colon reduces predisposition to the formation of kidney stones; the main findings have been published.[5] The study protocol was approved by the Institutional Review Boards of the four institutions where patients were identified and by the Institutional Review Board of the Boston University Medical Campus. Written informed consent was obtained from all participating subjects.

The original study included 247 patients aged 18–69 years with recurrent episodes of calcium oxalate kidney stones. One age, sex, and region-matched control was enrolled for each case, selected from spouses, unrelated housemates, or friends nominated by other cases or ineligible stone formers (e.g., with another stone type); volunteer male controls were also enrolled. A control nominated by a particular case was not matched to that case. There were 259 initially enrolled controls.

Fecal swabs were collected by all subjects from stools passed into paper collection devices placed in the subject's toilet. The swabs were then placed in Protocult tubes and mailed in prepaid envelopes via an overnight courier. The swabs were tested for *O. formigenes* using culture[3] and polymerase chain reaction (PCR).[25] The median elapsed time from stool collection to culturing was 1 day, with a range of 0–6 days. Fewer than 1% of stool samples did not provide sufficient material to culture. Specimens were cultured in selective liquid oxalate–containing medium for 10 days. The medium was then tested for the presence of oxalate by the addition of calcium chloride. Although culture does not identify the organism directly, it demonstrates that oxalate is being degraded in the stool, and the culture medium is selective for *O. formigenes*.[3] PCR, which can directly identify the bacterium, proved to be insensitive as a primary test, but in a subset of participants, it was conducted on the positive culture supernatant: 96% were positive by PCR.[5] We therefore concluded that the culture provided an adequate identification of *O. formigenes* colonization.

Information was collected by telephone interview from all subjects, including questions on known risk factors for kidney stones, such as inflammatory bowel disease and family history of stones, and on other relevant factors such as antibiotic use. A lifetime history of use of antibiotics to which *O. formigenes* is known to be sensitive (H. Sidhu, pers. comm.) was obtained. As shown in Table 1, these included macrolides, tetracyclines, chloramphenicol, rifampin, and metronidazole (henceforth referred to as "sensitive" antibiotics), which were asked about by name. The use of other antibiotics (referred to as "nonsensitive" antibiotics) within the previous 5 years was also recorded; a lifetime history of use was not obtained.

Fluid intake and dietary history, including consumption of oxalate-containing foods, were obtained by an adaptation of the validated self-administered food frequency questionnaire developed by the Nurses Health Study.[26] Total consumption of oxalate and other dietary factors was estimated by linking the questionnaire data with a database that contained information on the contents of various nutrients in standardized portions of each food. Methodologic issues have created controversy regarding the oxalate content of foods. Recently, Holmes et al. have made progress in the reevaluation and standardization of this nutrient,[27] and their measurements were incorporated into the Nurses Health Study database.

As noted, data from this study had previously demonstrated an inverse relationship of *O. formigenes* with renal stones.[5] That analysis also examined the effect of other factors on the kidney stone/*O. formigenes* relation. The lack of research on the natural history of this bacterium directed our interest for further investigation to the identification of factors associated with colonization with *O. formigenes* in a healthy population, and for this reason the present analysis was confined to the controls. Subjects were excluded if they had taken any antibiotics within the three months before the interview ($n = 19$) because of the likelihood that such very recent use could result in an unrepresentatively low prevalence of *O. formigenes*. This left 240 subjects; the median age was 49 years and 62% were men.

O. formigenes prevalence estimates were calculated within strata of various factors. Odds ratios (OR) and 95% confidence intervals (CIs) based on unconditional logistic regression[28] were used to assess potential confounding and to provide statistical tests of apparent differences. In the logistic regression models, *O. formigenes* colonization (yes/no) was the dependent variable; independent variables included factors that were associated on a univariate basis, plus those not associated but of a priori interest. Factors included in the basic model were age, sex, region, race/ethnicity, use of sensitive antibiotics, use of any nonsensitive antibiotics, and quartiles of the average daily intake of oxalate, calcium, vitamin C, magnesium, and total calories. Trends in colonization according to nutrient intake were tested by including ordinal terms in the models, with values set to the medians of the quartiles of consumption. Although crude ORs are given for completeness, the multivariate estimates will generally be referred to in describing the results.

Results

The prevalence of *O. formigenes* among the 240 subjects was 38%. Table 2 displays the proportion colonized within strata of demographic factors. There was no linear pattern according to age; the lowest prevalence was 30% among the youngest subjects, and the highest was 47% in subjects aged 50–59 (OR, 2.5; 95% CI, 1.1–5.9). There were no significant variations in colonization according to sex, race, education, or region. In general, the multivariate ORs were reasonably similar to the unadjusted estimates. The most prominent exception was the OR for sex; in the comparison of women and men, the crude estimate was 1.2 and the multivariate OR was 1.8. The inclusion of terms for use of antibiotics in the model largely accounted for the difference in estimates.

Use of sensitive antibiotics was strongly related to colonization (Table 3): the prevalence estimates were 17%, 27%, and 36%, for those who had used these drugs <1 year ago, 1–5 years ago, and >5 years ago, respectively, compared with 55% in nonusers. The ORs were significantly below 1.0 for sensitive antibiotic use regardless of how recently this had occurred. Compared with an estimate of 42% among nonusers, the prevalence of colonization among those who had taken nonsensitive antibiotics was 22% for last use <1 year ago and 39% for last use 1–5 years ago. The ORs were 0.3 and 0.8, respectively, but the CIs included 1.0.

Table 1. HC-1 *Oxalobacter formigenes* Antibiotic Sensitivity Pattern

Antibiotic sensitivity		*Antibiotic resistance*			
Antibiotic	*μg/mL (or Units)*	*Antibiotic*	*μg/mL (or Units)*	*Antibiotic*	*μg/mL (or Units)*
Chloramphenicol	<1.5	Amikacin	>18	Kanamycin	>18
Colistin	<0.5	Ampicillin	>6	Lincomycin	>1.2
Doxycycline	<1.5	Amoxicillin	>18	Nalidixic acid	>18
Erythromycin	1.5	Bacitracin	>6U	Neomycin	>18
Polymyxin B	<15U	Carbenicillin	>60	Penicillin	>6U
Rifampin	3	Cefaclor	>18	Piperacillin	>60
Tetracycline	3	Cefluroxime	>18	Streptomycin	6
		Ceftazidime	>18	Sulfadiazine	>150
		Clindamycin	>1.2	Tobramycin	>6
		Ciprofloxacin	>3	Trimethoprim	>3
		Gentamycin	>6	Vancomycin	>18

Subjects could have used both sensitive and nonsensitive antibiotics during the 5-year exposure interval. To allow for overlapping use, we examined the prevalence of *O. formigenes* colonization according to five mutually exclusive categories of sensitive and nonsensitive drug use (Table 4). Users within the past 5 years were divided into three categories: sensitive plus nonsensitive, sensitive only, and nonsensitive only. Twenty-seven subjects had used both types, and only four of these were *O. formigenes* positive (15%). The prevalence was higher for subjects who used only sensitive antibiotics in the last 5 years (27%), higher still for nonsensitive only users (48%), and highest of all for those who had not taken any antibiotics (59%). The ORs for the two categories that included sensitive antibiotics were both significantly below 1.0, but not statistically different from each other; the OR for the nonsensitive only category was not significant. Users of sensitive antibiotics >5 years ago were separated into those who had also used nonsensitive antibiotics within the previous 5 years and those who had not. The results for both categories were nearly identical (prevalence estimates, 36%–38%; ORs, 0.3 for each category), indicating minimal effect from the more recent use of nonsensitive antibiotics. With one exception, the median interval since the most recent episode was higher in those colonized with *O. formigenes* than in those who were not for each of the five exposure categories; that is, the further in the past that an antibiotic had been used, the greater the likelihood of colonization.

With regard to the effects of specific antibiotics on *O. formigenes*, sufficient numbers of users were available to estimate the prevalence of colonization for two sensitive

Table 2. Prevalence of *Oxalobacter formigenes* Among 240 Control Subjects According to Demographic Factors

	O. formigenes *positive*			
Factor	*No.*	*(%)*	*Crude OR*	*MVOR (95% CI)*
Age (years)				
<40	17/56	(30)	1.0[a]	1.0[a]
40–49	26/72	(36)	1.3	1.5 (0.7–3.5)
50–59	33/70	(47)	2.1	2.5 (1.1–5.9)
60–69	18/42	(43)	1.7	1.8 (0.7–4.7)
Sex				
Male	56/148	(38)	1.0[a]	1.0[a]
Female	38/92	(41)	1.2	1.8 (0.9–3.6)
Race				
Non-Hispanic white	75/198	(38)	1.0[a]	1.0[a]
Other	19/42	(45)	1.4	1.2 (0.6–2.8)
Education (year)				
≤12	12/33	(36)	0.9	0.9 (0.3–2.3)
13–15	23/64	(36)	0.9	0.7 (0.3–1.5)
16	29/65	(45)	1.3	1.2 (0.6–2.6)
>16	30/78	(39)	1.0[a]	1.0[a]
Region				
Massachusetts	71/179	(40)	1.0[a]	1.0[a]
North Carolina	23/61	(38)	0.9	0.8 (0.4–1.6)

[a]Reference category.

CI = confidence interval; OR = odds ratio; MVOR = multivariate odds ratio.

Table 3. Prevalence of *Oxalobacter formigenes* Among 240 Control Subjects According to Antibiotic Use

	O. formigenes *positive*			
Antibiotic last use	*No.*	*(%)*	*Crude OR*	*MVOR (95% CI)*
Sensitive[a]				
None	51/92	(55)	1.0[b]	1.0[b]
<1 year	6/35	(17)	0.2	0.1 (0.05–0.4)
1–5 year	13/48	(27)	0.3	0.3 (0.1–0.6)
>5 year	24/65	(36)	0.5	0.4 (0.2–0.8)
Nonsensitive[c]				
None	67/161	(42)	1.0[b]	1.0[b]
<1 year	5/23	(22)	0.4	0.3 (0.1–1.0)
1–5 year	22/56	(39)	0.9	0.8 (0.4–1.6)

[a]Erythromycin, clarithromycin, azithromycin, tetracycline, minocycline, doxycycline, and metronidazole.

[b]Reference category.

[c]Ampicillin, amoxicillin, benzylpenicillin, dicloxacillin, penicillin NOS, cephalexin, cefadroxil, cefaclor, cefprozil, clindamycin, vancomycin, ciprofloxacin, levofloxacin, enrofloxacin, nitrofurantoin, trimethoprim, sulfamethoxazole, sulfa NOS, and antiobiotic NOS.

drugs, erythromycin and azithromycin, and one nonsensitive drug, amoxicillin. The prevalence was 18% among 40 azithromycin users, 26% in 19 erythromycin users, and 29% in 24 subjects who took other sensitive antibiotics. The ORs were similar, ranging from 0.2 to 0.3, all with upper confidence limits below 1.0. The prevalence among 21 amoxicillin users was 38% (OR, 0.8), compared with 33% (OR, 0.6) among 58 subjects who took other nonsensitive antibiotics.

An examination of selected nutrient factors is displayed in Table 5. We obtained information on numerous nutrients, but none were significantly associated with *O. formigenes* colonization, and many were highly correlated with each other. Here we present results only for oxalate, a source of food for *O. formigenes*, calcium and magnesium, which bind with oxalate, and vitamin C, which is metabolized to oxalate. The prevalence of *O. formigenes* was lowest for the quartile of lowest oxalate consumption and increased somewhat with increasing intake (32%–45%). The ORs for the three quartiles of higher consumption relative to the lowest reflected this linear pattern, but none of the individual estimates was significantly elevated, nor was there a statistically significant trend ($p = 0.14$). *O. formigenes* prevalence estimates did not differ according to level of consumption for the remaining nutrients.

Among other factors, we also examined *O. formigenes* prevalence according to body mass index, history of urinary tract infection, family history of renal stones, and diuretic use. There were no significant differences in colonization, with ORs ranging from 1.0 to 1.2 (data not shown).

Discussion

Considerable evidence indicates that *O. formigenes* is the primary organism that degrades oxalate in the colon.[29,30] Although a few other species of intestinal bacteria, including strains of *Lactobacillus* and *Bifidobacterium*, are also capable of consuming oxalate and have recently been shown to carry the same *oxc* and *frc* genes as *O. formigenes*.[31–37] these other bacteria are generalists that consume other substrates as well as oxalate.

The present results suggest that the use of certain antibiotics is the main factor affecting colonization with *O. formigenes* among U.S. adults. Compared with nonusers, we observed a markedly lower prevalence of colonization among individuals who, in the last 5 years, had taken antibiotics to which the bacterium has been reported to be sensitive, including macrolides, tetracyclines, chloramphenicol, rifampin, and metronidazole. The reduction in colonization among users of these drugs persisted after multivariate analysis, which adjusted for several factors, including the use of nonsensitive antibiotics. The prevalence was also reduced, but to a lesser extent, among those who took these drugs >5 years ago. These findings provide *in vivo* confirmation of unpublished *in vitro* sensitivity testing (H. Sidhu, pers. comm.); there is only minimal published information about the antibiotic sensitivity of the bacterium.[22,23] Among individual drugs, it was possible to estimate the prevalence of colonization only for users of erythromycin and azithromycin; both were clearly associated.

Results for use in the last 5 years of antibiotics that were previously not thought to affect colonization were equivocal: the prevalence estimates were somewhat lower than among nonusers, particularly for recent use, but the ORs were not significant. When mutually exclusive categories of the two types of antibiotics were examined, the above findings were largely confirmed. It is of interest that the use of sensitive antibiotics >5 years ago had a more marked effect on prevalence than more recent use of nonsensitive antibiotics. While it remains possible that *O. formigenes* might be sensitive to at least some of the antibiotics that have not been previously identified as affecting the bacterium, the only nonsensitive antibiotic with a sufficient number of users to examine individually was amoxicillin; the prevalence was actually higher than that among users of other drugs in that category.

The results were consistent with some recolonization or recovery to detectable levels of colonization after use of antibiotics. For both sensitive and nonsensitive drugs, the prevalence of *O. formigenes* was lowest when use was comparatively recent. However, with the relatively small numbers of users, the estimates were statistically compatible with those for use in the more distant past. In the mutually exclusive analysis, the median interval since last use was generally higher for those who were positive for *O. formigenes*.

Table 4. Prevalence of *Oxalobacter formigenes* Among 240 Control Subjects According to Mutually Exclusive Categories of Antibiotic Use

	O. formigenes			
Antibiotic use	*Positive No. (%)*	*Negative No. (%)*	*Crude OR*	*MVOR (95% CI)*
None[a]	36 (59)	25 (41)	1.0[b]	1.0[b]
Sensitive+nonsensitive ≤5 year	4 (15)	23 (85)	0.1	0.1 (0.03–0.3)
Median interval since last use (month)	34	17		
Sensitive only ≤5 year	15 (27)	41 (73)	0.3	0.2 (0.1–0.5)
Median interval since last use (month)	19	15		
Nonsensitive only ≤5 year	15 (48)	16 (52)	0.7	0.6 (0.2–1.5)
Median interval since last use (month)	26	21		
Sensitive >5 year only	16 (36)	28 (64)	0.5	0.3 (0.1–0.8)
Median interval since last use (month)	123	155		
Sensitive >5 year + nonsensitive ≤5 year	8 (38)	13 (62)	0.5	0.3 (0.1–1.0)
Median interval since last use (month)	179	119		

[a]No use of sensitive antibiotics at any time, and no use of nonsensitive antibiotics in the previous 5 years.
[b]Reference category.

Table 5. Prevalence of *Oxalobacter formigenes* Among 240 Controls According to Dietary Factors

	O. formigenes *positive*			
Nutrient mg/day	*No.*	*(%)*	*Crude OR*	*MVOR (95% CI)*
Oxalate[a]				
<115	19/60	(32)	1.0[b]	1.0[b]
115–169	23/60	(38)	1.3	1.3 (0.5–3.0)
170–239	25/60	(42)	1.5	1.6 (0.6–4.1)
≥240	27/60	(45)	1.8	2.1 (0.8–5.7)
Calcium				
<550	23/58	(40)	1.0[b]	1.0[b]
550–819	25/63	(40)	1.0	0.7 (0.3–1.7)
820–1199	24/58	(41)	1.1	0.7 (0.2–1.7)
≥1200	22/61	(36)	0.9	0.6 (0.2–1.7)
Vitamin C				
<75	23/61	(38)	1.0[b]	1.0[b]
75–139	23/61	(38)	1.0	0.7 (0.3–1.8)
140–244	24/59	(41)	1.1	1.0 (0.4–2.6)
≥245	24/59	(41)	1.1	0.8 (0.3–2.2)
Magnesium				
<235	22/60	(37)	1.0[b]	1.0[b]
235–319	24/58	(41)	1.3	1.1 (0.4–3.2)
320–419	29/62	(47)	1.6	1.1 (0.3–3.8)
≥420	19/58	(33)	0.9	0.3 (0.1–1.4)

[a]Test for trend, $p = 0.14$.
[b]Reference category.

With regard to other factors, there were no clear patterns in the likelihood of being colonized with *O. formigenes* according to age, sex, race, and education; there was also no evidence of geographic variability. The only significant finding among the demographic variables was a higher prevalence among subjects in a middle age category (50–59 years), and with the numerous subgroups evaluated, such a finding might be expected to occur by chance. Body mass index, history of urinary tract infection, family history of renal stones, and use of diuretics were not associated with colonization.

It is somewhat surprising that we did not observe a stronger relation of colonization with oxalate consumption, since this nutrient is one of two sources of energy for *O. formigenes* (endogenous oxalate being the other). There was a modest increase in prevalence with increasing consumption, but this was not a significant trend. The equivocal results could be a reflection of imprecision in the measurement of dietary oxalate muting a real effect. Among other dietary factors, the ORs for quartiles of calcium, vitamin C, and magnesium consumption, relative to the lowest levels, produced no clear differences.

A limitation to the evaluation of antibiotics was the lack of information on use of nonsensitive drugs >5 years in the past. It is also possible that antibiotic use was incompletely reported, with the resulting misclassification of users as nonusers blurring differences. This could particularly affect nonsensitive drugs, which were not asked about by name. However, we deem it unlikely that reporting of antibiotic was affected by *O. formigenes* status, since this was not known by study subjects.

Other potential limitations that should be considered are information and selection bias. We judge that information bias is unlikely for several reasons. Upon enrollment, study subjects were unaware of the hypothesis and did not know whether they were colonized with *O. formigenes*. Laboratory testing of stool specimens was performed blind to case–control status and to all other factors. Other information was obtained directly from the study subjects, by interview and self-administered dietary questionnaire. The interview was designed to maximize recall and was conducted by an experienced nurse-interviewer; the self-completed dietary questionnaire has been validated.[26,38,39] Selection bias is a theoretical possibility, given the participation rate of 76% among control subjects; however, the decision to participate could not have been related to *O. formigenes* status.

A caveat to the current analysis is that the original study was not designed to explore patterns and determinants of *O. formigenes* colonization, but rather to evaluate the relation of the bacterium to the risk of recurrent calcium oxalate kidney stones. The data collected from controls reported on here provide a valuable opportunity to shed some light on factors affecting the bacterium itself, about which little is known, but there are limitations to using the study population for this purpose. These include the incomplete information on antibiotic use that has already been discussed, geographic restriction to two regions of the United States, and confining the study to adults. Specifically with regard to the latter restriction, it was reported from a study of *O. formigenes* colonization among Ukrainian children that the bacterium was not detectable in neonates but was present in nearly all 6–9 year olds; the prevalence then declined in adolescence.[25] This suggests that *O. formigenes* may be acquired in infancy, a key aspect of its natural history that we were not able to evaluate.

In conclusion, the present analysis has demonstrated that colonization with *O. formigenes* is markedly affected by use of antibiotics previously suspected to have an effect on the bacterium. Questions remain concerning recolonization after eradication and the effects of individual drugs. Although no other factor was identified as having a material influence on the prevalence of the bacterium, there is much to learn about how an individual acquires the organism and which factors affect persistence of colonization. As *O. formigenes* has no known adverse effects and appears to have a greater capacity to metabolize oxalate than other bacteria, there is potential for its use as a probiotic to reduce the risk of commonly occurring calcium oxalate renal stones.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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Abbreviations Used

CI = confidence interval
OR = odds ratio
PCR = polymerase chain reaction