

## Oxalobacter formigenes May Reduce the Risk of Calcium Oxalate Kidney Stones

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### ABSTRACT

Most kidney stones are composed primarily of calcium oxalate. *Oxalobacter formigenes* is a Gram-negative, anaerobic bacterium that metabolizes oxalate in the intestinal tract and is present in a large proportion of the normal adult population. It was hypothesized that the absence of *O. formigenes* could lead to increased colonic absorption of oxalate, and the subsequent increase in urinary oxalate could favor the development of stones. To test this hypothesis, a case-control study involving 247 adult patients with recurrent calcium oxalate stones and 259 age-, gender-, and region-matched control subjects was performed. The prevalence of *O. formigenes*, determined by stool culture, was 17% among case patients and 38% among control subjects; on the basis of multivariate analysis controlling demographic factors, dietary oxalate, and antibiotic use, the odds ratio for colonization was 0.3 (95% confidence interval 0.2 to 0.5). The inverse association was consistently present within strata of age, gender, race/ethnicity, region, and antibiotic use. Among the subset of participants who completed a 24-h urine collection, the risk for kidney stones was directly proportional to urinary oxalate, but when urinary factors were included in the multivariable model, the odds ratio for *O. formigenes* remained 0.3 (95% confidence interval 0.1 to 0.7). Surprisingly, median urinary oxalate excretion did not differ with the presence or absence of *O. formigenes* colonization. In conclusion, these results suggest that colonization with *O. formigenes* is associated with a 70% reduction in the risk for being a recurrent calcium oxalate stone former.

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Kidney stones represent an important health problem in many countries. In the United States, the lifetime risk for developing a stone is approximately 5 to 15%,<sup>1</sup> and the 5-yr risk for a recurrence is approximately 30 to 50%. Annual incidence rates are approximately three cases per 1000 for men and one to two per 1000 in women.<sup>2–5</sup> In addition to the effects on individuals, the impact of renal stone disease on the medical care system is substantial; stones account for approximately 0.1% of hospital admissions and have an economic impact of \$2 billion dollars per year.<sup>6,7</sup> It has been estimated that medical prevention of stones may produce savings of up to \$2500 per patient per year.<sup>8</sup>

Up to 80% of kidney stones are predominantly composed of calcium oxalate (CaOx).<sup>9</sup> Urinary oxalate is a major risk factor for CaOx stone formation.<sup>10</sup> Oxalate is derived from both endogenous and exogenous sources, with absorbed dietary ox-

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alate rapidly excreted by the kidney.<sup>11</sup> One approach to preventing recurrent calculi is to decrease consumption of foods high in oxalate, but the effectiveness of this treatment is uncertain. The concept of reducing oxalate absorption by a microbiological approach has received increasing attention in recent years.<sup>12,13</sup>

*Oxalobacter formigenes* is a Gram-negative, anaerobic bacterium that metabolizes oxalate in the intestinal tract.<sup>12,14–16</sup> Little is known about when and how individuals become colonized or the persistence of the bacterium over time, but it seems to be present in a large proportion of the normal adult population, with reported prevalence ranging from 46 to 77%.<sup>17–26</sup> The bacterium is known to be susceptible to some antibiotics,<sup>27</sup> although the sensitivity pattern is incompletely characterized. The absence of *O. formigenes* could permit more absorption of dietary oxalate in the colon and decreased secretion from endogenous sources,<sup>28</sup> resulting in higher oxalate excretion in the urine and thus predisposition to CaOx calculus formation. Data from a number of relatively small studies show that patients with renal calculi and some conditions related to hyperoxaluria have a lower prevalence of *O. formigenes* in the stool than control subjects.<sup>17–26</sup> There is also some evidence that patients with nephrolithiasis who have *O. formigenes* in their stool experience lower urinary oxalate excretion than those who do not.<sup>25,26,29</sup> To provide a more definitive evaluation of the hypothesis that colonization with *O. formigenes* is associated with a reduced risk for CaOx kidney stones, we conducted a case-control study of 247 patients presenting with a recurrent episode of CaOx nephrolithiasis and 259 individuals without stone disease.

## RESULTS

### Study Population

A total of 379 eligible cases were identified at hospitals in Boston, MA, and Durham, NC; 247 (65%) completed the study procedures (by design, not all patients provided urine collections). Among 339 eligible control subjects initially matched to case patients, 259 (76%) completed the study (including 12 control subjects matched to potential case patients who were subsequently excluded). Among the control subjects, 40 were nominated by 39 patients who had kidney stones and were included as case patients, 121 were nominated by ineligible patients, and 98 were volunteers. Because of the matched design, the distributions of participating case patients and con-

trol subjects were identical with regard to age (median 48 yr), gender (62% male), and region (75% from Boston area); 85% of the case patients and 83% of the control subjects were non-Hispanic whites. Participating case patients were similar in age to eligible case patients who did not complete the study; they were more often female (38 versus 33%), non-Hispanic white (85 versus 80%), and from North Carolina (25 versus 14%). Participating control subjects were older (48 versus 42%) and more often male (61 versus 41%), non-Hispanic white (83 versus 77%), and from North Carolina (25 versus 21%) than control subjects who were replaced because they did not complete the study.

### Results of Analyses

The overall prevalence of *O. formigenes* was 38% among the control subjects (33% among control subjects nominated by included case patients, 39% among control subjects nominated by ineligible patients, and 40% among volunteers) and 17% among the case patients, giving a crude odds ratio (OR) of 0.3 (Table 1); the multivariate estimate was also 0.3 (95% confidence interval 0.2 to 0.5). When the analysis was restricted to the 209 case patients who did not contribute any nominated control subjects to the final series and the 219 control subjects who were not nominated by included case patients, the crude OR was 0.3. The inverse association was consistently present within strata of age, gender, race/ethnicity, and region (Table 2). Among participants aged 18 to 39 yr, the estimate was 0.7 (95% CI 0.3 to 1.6); in all remaining strata, the OR ranged from 0.1 to 0.4 and were statistically significant.

The relation of *O. formigenes* to kidney stones according to antibiotic use is shown in Table 3. Antibiotic use was related to colonization: Among control subjects, the prevalence of colonization was lowest (29%) in those who had used antibiotics to which *O. formigenes* is sensitive at any time in the past (regardless of whether they also took other antibiotics), intermediate (46%) in those who had taken other antibiotics in the past 5 yr (with no use of *O. formigenes*-sensitive antibiotics), and highest in nonusers (59%); a similar relationship was observed among case patients. The inverse association with *O. formigenes* was present in both groups of antibiotic users, with multivariate OR of 0.4 and 0.1, respectively. The OR was 0.4 among nonusers, but the upper confidence limit was 2.2. Among participants who had not taken antibiotics in the previous 3 mo (but may have taken them in the past), the OR was 0.4 (95% CI 0.3 to 0.8).

**Table 1.** *O. formigenes* in stool among patients with recurrent CaOx kidney stones and control subjects

<i>O. formigenes</i> Status	Case Patients (n = 247)		Control Subjects (n = 259)		Crude OR	Multivariate OR (95% CI) <sup>a</sup>
	n	%	n	%		
Positive	42	17	99	38	0.3	0.3 (0.2 to 0.5)
Negative	205	83	160	62	1.0 <sup>b</sup>	1.0 <sup>b</sup>

<sup>a</sup>OR based on unconditional logistic regression with the following factors in the model: *O. formigenes*, age, gender, region, education, race, dietary oxalate, antibiotic use, and family history of stones. OR calculated using conditional logistic regression: 0.3 (0.1 to 0.5).

<sup>b</sup>Reference category.

**Table 2.** *O. formigenes* among patients with recurrent CaOx kidney stones and control subjects according to demographic factors

Factor	Case Patients		Control Subjects		Crude OR	Multivariate OR (95% CI) <sup>a</sup>
	<i>O. formigenes</i> /Total	%	<i>O. formigenes</i> /Total	%		
Age						
18 to 39	13/58	22	18/61	30	0.7	0.7 (0.3 to 1.6)
40 to 49	14/74	19	28/80	35	0.4	0.4 (0.2 to 0.9)
50 to 59	9/72	13	34/71	48	0.2	0.2 (0.07 to 0.4)
60 to 69	6/43	14	19/47	40	0.2	0.2 (0.08 to 0.7)
Gender						
male	27/154	18	59/158	37	0.4	0.4 (0.2 to 0.6)
female	15/93	16	40/101	40	0.3	0.3 (0.1 to 0.6)
Race/ethnicity						
non-Hispanic white	37/210	18	79/215	37	0.4	0.4 (0.2 to 0.6)
other	5/37	14	20/44	46	0.2	0.1 (0.04 to 0.5)
Region						
Massachusetts	35/186	19	74/193	38	0.4	0.4 (0.2 to 0.6)
North Carolina	7/61	12	25/66	38	0.2	0.2 (0.07 to 0.5)

<sup>a</sup>OR based on unconditional logistic regression with the model for each stratum including terms for the other factors listed above (e.g., for age 18 to 39 yr, gender, race, and region were controlled).

**Table 3.** *O. formigenes* among patients with recurrent CaOx kidney stones and control subjects according to antibiotic use

Antibiotic Use	Case Patients		Control Subjects		Crude OR	Multivariate OR (95% CI) <sup>a</sup>
	<i>O. formigenes</i> /Total	%	<i>O. formigenes</i> /Total	%		
Antibiotics that affect <i>O. formigenes</i> (ever)	24/168	14	47/162	29	0.4	0.4 (0.2 to 0.7)
Other antibiotics in previous 5 yr <sup>b</sup>	13/64	20	18/39	46	0.3	0.1 (0.02 to 0.5)
No antibiotics <sup>c</sup>	5/15	33	34/58	59	0.4	0.4 (0.08 to 2.2)

<sup>a</sup>OR based on unconditional logistic regression with the following factors in the model: *O. formigenes*, age, gender, region, education, race, dietary oxalate, and family history of stones.

<sup>b</sup>No antibiotics that affect *O. formigenes* at any time.

<sup>c</sup>No antibiotics that affect *O. formigenes* at any time and no other antibiotics in previous 5 yr. OR for participants who took no antibiotics in previous 3 mo: 0.4 (0.3 to 0.8), based on 27 of 125 case patients and 95 of 244 control subjects.

Case patients and control subjects were divided into approximate tertiles of oxalate consumption (<120, 120 to 199, ≥200 mg/d). Among control subjects, the prevalence of *O. formigenes* was 31% in the lowest tertile, 37% in the middle tertile, and 45% in the highest (data not shown). There was no consistent relationship among case patients. The crude OR for the development of kidney stones among those colonized by *O.*

*formigenes* status was 0.5 in the lowest tertile and 0.3 in each of the others.

Among the 139 case patients and 138 control subjects who completed 24-h urine collections, the OR for developing kidney stones increased with increasing urinary oxalate excretion ( $P_{\text{trend}} = 0.002$ ; Table 4). The inverse association with *O. formigenes* remained present in participants with urine collec-

**Table 4.** Urinary oxalate in patients with recurrent CaOx kidney stones and control subjects<sup>a</sup>

Urinary Oxalate (mg/24 h)	Case Patients (n = 139)		Control Subjects (n = 138)		Crude OR	Multivariate OR (95% CI) <sup>b</sup>
	n	%	n	%		
<25	27	19	44	32	1.0	1.0
25 to 34	47	34	50	36	1.5	1.6 (0.6 to 4.0)
35 to 44	32	23	30	22	1.7	3.3 (1.1 to 10)
≥45	33	24	14	10	3.8	7.2 (2.0 to 27)

<sup>a</sup>Based on the average values of repeat 24-h urine collections in a subset of participants.

<sup>b</sup>OR based on unconditional logistic regression with the following factors in the model: *O. formigenes*, age, gender, race, region, education, dietary oxalate, family history, body mass index, urine total volume, urinary calcium, urinary citrate, urinary uric acid, and antibiotic use. Test for trend based on a continuous term for urinary oxalate:  $P = 0.002$ . OR for *O. formigenes* in this model: 0.3 (0.1 to 0.7), based on 25 (18%) of 139 case patients and 52 (38%) of 138 control subjects.

tions, with an overall multivariate OR of 0.3 (95% CI 0.1 to 0.7) when urinary factors were included in the model. Within the four levels of increasing oxalate excretion, the crude OR were 0.3, 0.5, 0.3, and 0.4, respectively (data not shown).

The median oxalate excretion (Table 5) was 32 mg among *O. formigenes*-positive case patients and 35 mg among those who were *O. formigenes*-negative. The corresponding medians among control subjects were 28 and 27 mg, respectively.

## DISCUSSION

We observed a strong inverse association between colonization with *O. formigenes* and recurrent CaOx renal stones, with a 70% reduction in overall risk. The relationship was consistently evident within subgroups defined according to age, gender, race/ethnicity, and region. The prevalence of *O. formigenes* was related to the use of antibiotics to which the bacterium has been previously reported to be sensitive<sup>27</sup> and, to a lesser extent, other antibiotics; however, the association between the bacterium and kidney stones did not seem to be materially affected by antibiotic use. It also was not affected by oxalate consumption or urinary oxalate excretion, with OR ranging from 0.3 to 0.5 within categories of these parameters.

Our results, based on a large study population with rigorous selection of comparison subjects and control for potential confounding factors, extend the findings of previous, smaller studies.<sup>17–26,29,30</sup> These include comparisons of normal individuals with patients with kidney stones,<sup>17,18,20,22–26</sup> hyperoxaluria,<sup>21</sup> or diseases that cause hyperoxaluria, including cystic fibrosis<sup>19</sup> and inflammatory bowel disease.<sup>26</sup> OR were generally not reported, but in all of the previous studies of patients with kidney stones, the prevalence of *O. formigenes* was lower than in control subjects. The prevalence among control subjects varied widely, from 46 to 77%; our corresponding prevalence of 38% was somewhat below this range. Two potential explanations for the discrepancy are instability in some of the previous estimates as a result of small numbers and population differences. With one exception, a US study with 10 normal individuals,<sup>25</sup> all previous reports of the prevalence in normals were from other countries, where there might have been different patterns of antibiotic consumption, as well as genetic and dietary differences.

**Table 5.** Urinary oxalate in relation to *O. formigenes* in patients with recurrent CaOx kidney stones and control subjects<sup>a</sup>

	<i>O. formigenes</i> Positive		<i>O. formigenes</i> Negative	
	n	Median Oxalate (mg)	n	Median Oxalate (mg)
Case patients	25	32	114	35
Control subjects	52	28	86	27

<sup>a</sup>Based on the average values of repeat 24-h urine collections in a subset of 139 case patients and 138 control subjects.

Among control subjects, we observed an increase in the prevalence of *O. formigenes* with increasing oxalate consumption, expected because dietary oxalate is a major energy source for this bacterium, along with oxalate from endogenous production. This relation was not observed in the case patients, however, and there is no clear explanation for the latter finding. We also saw a strong trend in the risk for stones with increasing urinary oxalate excretion in individuals who provided 24-h urine collections, which has been reported in previous studies.<sup>23,24,26</sup> The relation of urinary oxalate to the presence of *O. formigenes*, however, was less clear cut in our data, which is unexpected given the strength of the primary association with kidney stones and the putative mechanism. The median oxalate values were marginally lower in case patients who were colonized compared with those who were not, and there was no such difference in control subjects. Previous studies found more pronounced differences in 24-h oxalate excretion according to *O. formigenes* status.<sup>19,24–26,29</sup> The reason for the inconsistency is not clear, although it has been suggested that postprandial urinary oxalate may be a more relevant measure of oxalate excretion.<sup>31</sup> *O. formigenes* may reduce postprandial spikes without a large impact on 24-h excretion. In addition, oxalate secretion into the intestine may be an important means of oxalate disposal, and this may be influenced by genetic factors.<sup>32</sup> Thus, the relation between *O. formigenes* status and urinary oxalate excretion requires further investigation; pending that clarification, the lack of a clear connection in this study should be considered a limitation to our findings. It is important to note, however, that the inverse association between colonization with the bacterium and kidney stones was unchanged when urinary oxalate was controlled in the analysis and was present at all levels of oxalate excretion.

A potential methodologic concern is measurement of *O. formigenes*, particularly given the relatively low prevalence among control subjects compared with previous studies. The cultures were grown in a medium selective for *O. formigenes* with detection based on a precipitation assay, whereas PCR was used in most of the previous studies. The identification of the bacterium by PCR in our study had a low sensitivity compared with the culture results; however, PCR conducted on the supernatant of a sample of positive cultures, in which the bacterial counts were amplified, was positive 96% of the time. We therefore conclude that the culture provided an acceptably accurate identification of *O. formigenes*. There was no bias in the testing, because it was blind to case/control status.

It was not possible in this study to determine the temporal sequence between colonization of case patients and the development of kidney stones, because the stool samples were collected after the episode—this issue applies to the previous studies as well. However, there is no reason to believe that having a stone would affect the bacterium beyond the potential impact of treatment with antibiotics, which is discussed next. We therefore consider it reasonable to conclude that the cross-sectional measurement of *O. formigenes* colonization generally reflects the status of case patients before their stone episodes.

Temporal sequence issues are not relevant for the determination of colonization among control subjects.

The widespread use of antibiotics in the study population is noteworthy because it could have affected *O. formigenes* colonization. Participants who had recently taken antibiotics to which the bacterium is sensitive<sup>27</sup> were excluded, but many patients with stones were treated with other antibiotics during the current episode, and *O. formigenes* status was determined after that. There is evidence in our data that previous use of antibiotics, even agents to which *O. formigenes* is not thought to be sensitive, affects colonization, with lower prevalences of the bacterium in both case patients and control subjects compared with those who had not taken antibiotics; however, the inverse association was observed in all categories of antibiotic use and among those who had taken no antibiotics in the recent past. Furthermore, antibiotic use was controlled in the multivariate analysis. A caveat is that intravenous antibiotics administered to some patients with stones during procedures were likely to have been underreported. Considering the overall lack of impact on the multivariate OR by identified antibiotic use, we judge the effect of any such misclassification to have been minimal.

Aside from antibiotics, little is known about factors that may affect *O. formigenes* or about other aspects of the bacterium's natural history in humans. A study in Ukrainian children<sup>33</sup> provides some evidence of early acquisition and colonization over time. *O. formigenes* was not detected in children who were younger than 1 yr; the prevalence rose to 100% (by PCR; approximately 80% by culture) between ages 6 and 8 and declined to approximately 75% at age 12. These results do not provide information on the long-term pattern of *O. formigenes* in individuals; there are no specific data on loss and reacquisition of the bacterium at any age.

As with the *O. formigenes* testing, analysis of the 24-h urine specimens was conducted blindly by an independent laboratory. A standard commercially available test was used. Other study information was obtained by interview and self-administered dietary questionnaire. Although there could have been differential reporting by case patients and control subjects, with potential underreporting of past antibiotic use by control subjects of most concern, the interview was designed to maximize recall and administered by an experienced interviewer; the dietary questionnaire has been validated.<sup>34–36</sup>

Selection bias is a theoretical consideration, particularly among case patients, for whom the participation rate was 65%. There were some differences in the demographic distributions of participating and nonparticipating case patients and control subjects; however, it is unlikely that the decision to participate in the study could have been related to *O. formigenes* status, which was assessed after study enrollment, and the consistency of the inverse association within strata of age, gender, race/ethnicity, and region argues against bias. To avoid potential correlation of colonization between the case patients and their nominated control subjects as a result of common lifestyle factors, it was a requirement that the control subjects be matched

to case patients who did not nominate them. Other control subjects were volunteers, and a connection between the decision to participate and *O. formigenes* status that was determined subsequently is implausible. Although the prevalence of colonization was somewhat lower in control subjects who were nominated by included case patients, this was a relatively small group; when these control subjects and the case patients who nominated them were excluded, the OR was unchanged.

In addition to antibiotics, other relevant factors controlled in multivariate analyses included dietary and urinary oxalate, demographic factors, and family history of stones. The multivariate OR for *O. formigenes* was virtually identical to the crude estimate, suggesting minimal confounding by the factors in the models. Furthermore, the inverse association was remarkably consistent across strata of age, gender, race/ethnicity, and region. All of these points argue for the basic validity of the results.

Our findings are of potential clinical importance. *O. formigenes* is a naturally occurring bacterium that has no known adverse effects. CaOx renal stones are a recurring health problem that causes substantial morbidity and use of health care resources. The present results suggest that individuals who are colonized with *O. formigenes* have a 70% reduction in the risk for being a recurrent CaOx stone former. The possibility of using the bacterium as a probiotic is in the early stages of investigation. The results of a recent trial of patients with primary hyperoxaluria show some promise in this regard: Among 16 patients treated with *O. formigenes* as a frozen paste or enteric-coated capsules, 11 showed a reduction in urinary or plasma oxalate; there were no adverse effects, but on follow-up, none of the patients seemed to be permanently colonized.<sup>37</sup> In addition to more trials focusing on its potential as a treatment, further information is needed on the natural history of the bacterium in human populations, factors governing persistent colonization (particularly antibiotic sensitivity), and the bacterium's relation to first stone episodes and urinary oxalate.

## CONCISE METHODS

### Participants

Participants were enrolled from January 2004 to August 2006. Potential case patients were aged 18 to 69 yr, had a history of urolithiasis, and presented with a new stone episode to urology practices at three hospitals in Boston, MA—Massachusetts General Hospital, Boston Medical Center, and St. Elizabeth's Medical Center—and Duke University Medical Center in Durham, NC. Final inclusion required that the composition of the stone be confirmed by laboratory analysis conducted as part of normal clinical care to be  $\geq 50\%$  CaOx; when there was no analysis, a case patient was accepted when the previous episode was laboratory confirmed as CaOx. Potential case patients with any of the following were excluded: Spinal cord injury, inflammatory bowel disease, cystic fibrosis, other gastrointestinal condition that predisposes to malabsorption (including gastric bypass surgery), internalized double J ureteral stent or percutaneous nephrostomy tube in place for  $>4$  wk after the stone episode, chronic indwelling

Foley catheter, current chemotherapy, history of organ transplantation, and pregnancy within the preceding 2 mo. Patients were also excluded when they had received treatment with macrolides, tetracyclines, chloramphenicol, rifampin, or metronidazole in the 2-mo period before the stone episode (*O. formigenes* has been reported to be sensitive to these antibiotics<sup>27</sup>), as well as any use during or after the episode until the interview and stool collection, which usually occurred a few weeks later.

One control subject was matched to each case by gender, decade of age, and region (residence in the geographic areas surrounding Boston and Durham). Initially, control subjects were selected from among acquaintances of patients with stones, including housemates who were not biologically related. To minimize potential correlation between the *O. formigenes* status of case patients and control subjects as a result of shared living conditions, control subjects were not matched to the case patients who nominated them. Beginning in May 2005, volunteers were also sought as control subjects because of a shortage of nominated individuals; fliers, broadcast e-mails (at Massachusetts General Hospital), and newspaper ads (in Durham) were used to obtain volunteers. Potential control subjects were ineligible when they had a history of nephrolithiasis, use of the aforementioned antibiotics within the 3 mo before the interview (a period roughly comparable to the interval in the case patients), or other exclusion criteria that applied to the case patients. The project was approved by the institutional review boards of Boston University Medical Campus, Massachusetts General Hospital, and Duke University Medical Center.

## Data Collection

**Determination of *O. formigenes* Status.** Participants collected stool samples at home using transport swabs and sent them overnight to Ixion Biotechnology (Alachua, FL) to be tested for *O. formigenes*. All testing was blind to case/control status. The main approach was culture in selective liquid oxalate-containing medium for 10 d. The medium was tested for the presence of oxalate by the addition of calcium chloride ( $\text{CaCl}_2$ ). When *O. formigenes* is present, the oxalate is metabolized to calcium and formic acid. When oxalate remains in the medium, the addition of  $\text{CaCl}_2$  forms a white precipitate of  $\text{CaOx}$ , which can be quantified by the  $\text{OD}_{600}$ . 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*.<sup>14</sup> Specimens were also tested for *O. formigenes* by PCR,<sup>33</sup> but this proved to be unsatisfactory: Of 141 positive cultures, the PCR was positive for only 87, a sensitivity of 62%. Although these results could also be interpreted as a high false-positive rate for the culture, in a subset of participants, PCR was conducted on the positive culture supernatant, in which the bacterial count was amplified: The PCR was positive for 66 (96%) of the 69 supernatants tested. We therefore concluded that the culture provided an adequate identification of *O. formigenes* colonization, and those results are reported here.

**Urine Collection and Analysis.** A subset of participants provided two 24-h urine collections and sent aliquots overnight to Mission Pharmacal Laboratory (San Antonio, TX) for analysis (Urorisk), which provided excretion values for oxalate and other factors. The goal was to obtain urine samples from approximately half the case patients and

control subjects, and this part of the study was offered to consecutive participants until sufficient numbers completed the collections. Participants were instructed to follow their usual diet and fluid intake during the collections. The laboratory analysis was blind to case/control status. The urine profiles were blindly reviewed by one of the investigators who is a practicing nephrologist (G.C.C.). On the basis of the amount and variability of creatinine excretion, the collections were judged to be unsuitable for 11 case patients and 13 control subjects, and 139 case patients and 138 control subjects had valid information; average values from the two collections were used in the analysis.

**Interview.** A study nurse interviewed participants by telephone to obtain information on demographic factors; relevant medical history (e.g., family history of nephrolithiasis); history of use of antibiotics in the past 5 yr (lifetime histories for antibiotics to which *O. formigenes* is sensitive); and use of diuretics, allopurinol, potassium citrate, and potassium phosphate in the previous year.

**Dietary History.** A modified version of the Nurses Health Study food frequency questionnaire<sup>34</sup> was completed by all participants. This semiquantitative questionnaire has been validated.<sup>34–36</sup> The only change from the standard version was that the questions referred to food intake during the past month instead of the past year. Participants recorded the average frequency of use of more than 130 food items and 17 beverages, as well as vitamins and other supplements. The food frequency questionnaire is linked to a nutrient database from which the total intake of more than 100 nutrients, including oxalate, can be estimated. The oxalate content of foods was measured for the nutrient database by Dr. Ross Holmes at Wake Forest University, using capillary electrophoresis.

## Data Analysis

In the main analysis, case patients and control subjects were classified as *O. formigenes* positive or negative, and the OR was estimated, overall and among subgroups (e.g., according to gender). Confounding was controlled by conditional<sup>38</sup> and unconditional<sup>39</sup> logistic regression. When the analysis was confined to the 247 matched case patient–control subject sets, conditional and unconditional results were closely similar; therefore, unconditional logistic regression was used for the final analysis, which included the 12 unmatched control subjects, giving a total of 259. The following factors were included in the multivariate models: Age, gender, region, education, race/ethnicity, dietary oxalate consumption, use of antibiotics, and family history of kidney stones.

Urinary oxalate excretion was evaluated as a risk indicator for the development of stones and separately among case patients and control subjects according to *O. formigenes* status. These comparisons were confined to the participants who completed the urine collection. Unconditional logistic regression models included the previously mentioned factors, along with body mass index, urine total volume and urinary calcium, citrate, and uric acid.

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## DISCLOSURES

None.

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