ORIGINAL ARTICLE

Exploring the Link Between Cephalosporin Antibiotic Use and Urolithiasis: A Case–Control Study

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Abstract

Purpose: The study aimed to explore the association between cephalosporin antibiotics and urolithiasis, specifically focusing on *Oxalobacter formigenes* (*O. formigenes*) a colonization process. **Materials and Methods:** An observation was made that 50 were among patients, with kidney stones and 110 without stones had participated in the study. *O. formigenes*, a bacterium known to metabolize oxalates in the gastrointestinal tract, was detected in 36 of the 50 kidney stone patients and in 97 of the 110 control participants. **Results:** It was found that calcium and oxalate levels in urine. There was a significant increase in kidney stone patients than in controls, suggesting that these elevated levels may contribute to stone formation. Given the sensitivity of *O. formigenes* to certain antibiotics, including cephalosporins, frequent antibiotic use may reduce *O. formigenes.* This reduction could lead to increased oxalate absorption, as *O. formigenes* is not present to metabolize oxalates effectively. **Conclusion:** Thereby heightening Formation of kidney stones is at risk. According to these findings, the absence or reduction of species colonization may contribute to calcium oxalate stone pathogenesis, especially in patients taking antibiotics such as cephalosporins. Consequently, this study highlights the need for cautious antibiotic use to preserve beneficial gut microbiota, which assists protecting against diagnosis and treatment of kidney stones. Further research could confirm these associations and guide antibiotic stewardship practices to prevent disruptions in gut microbiome balance that might contribute to urolithiasis.

Key words: Calcium oxalates, cephalosporins, oxalobacter formigenes, urolithiasis

INTRODUCTION

In sheep, you would find bacteria that are anaerobic, Gram-negative, and enteric; *Oxalobacter formigenes* (*O. formigenes*).[1] It has since been in the digestive tract various animals, including mice, rats, guinea pigs, and other experimental animals, as well as in humans.[2-4] *O. formigenes* is an oxalate-dependent bacterium, meaning it relies entirely on oxalates for survival.[1] Both humans and animals depend on this bacterium for maintaining oxalate balance.[5]

Oxalates are significant due to their role in calcium urolithiasis, or kidney stone formation, which has spurred interest in studying *O. formigenes*. Research indicates that *O. formigenes* may be prevalent in human adults, with an occurrence rate between 46% and 77%.[6,7] While it is known that *O. formigenes* is sensitive to certain antibiotics,[8] further research is required to fully understand its antibiotic sensitivities, as only limited studies have been conducted.

When *O. formigenes* is absent, oxalate absorption in the colon increases,[9] This increases a higher concentration of oxalate in urine can lead to calcium oxalate poisoning stones forming. There are a caution should be taken when dealing with kidney stones containing calcium oxalate in composition.

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The incidence of urinary stones continues to rise, and current therapeutic interventions remain limited. Urolithiasis affects approximately 12% of the global population.[10] It occurs across all demographics, although men aged 20–49 experience the highest prevalence.[11] Without proper treatment, kidney stones can lead to severe complications, with relapse rates estimated at 10–23% annually, 50% within 5–10 years, and 75% over 20 years for individual patients.[11] Frequent monitoring and advancements in medical treatments are essential taking this medication reduces kidney stone recurrences. Certain lifestyle factors, such as physical inactivity, dietary habits,[12-14] and potentially even global warming, may contribute to the increasing incidence of kidney stones. In India, 12% of the population is affected by kidney stone disease. Among younger individuals, *O. formigenes* is commonly present, but its presence declines by 60-70% with age.[15] Although the reasons for this decrease are not fully understood, exposure to antibiotics is suggested as a possible cause. As a result, there may be a greater likelihood of forming stones. Sidhu *et al*.[16] observed a complete absence of *O. formigenes* in the fecal A sample of tetracycline-treated rats. The strains and the HC1 strains are sensitive to various antibiotics.[8]

We conducted this study to determine if cephalosporins affect colonization of *O. formigenes* in the human GI tract, as well as analyze interactions between *O. formigenes* colonization and kidney stones.

PATIENTS AND METHODS

Post approval of the Subham Ethics Committee, Chennai (ECR/323/Indt/TN/2020) subjects have been enrolled. In each independent sample t-test, a 1:1 ratio for independent samples were used, In this experiment, the alpha error was 0.05, the power was 80%, the mean difference was 8.5%, and the standard deviation was 0.05.

A total of 110 individuals (aged 24–54 years; 27 women and 83 men) leaving out kidney stone disease were recruited as controls. Among the controls, 78 had not used antibiotics in the previous 3 months, while 32 had taken collection of stool with antibiotics.

An additional 50 those affected (aged 24–54 years; 9 women and 41 men) kidney-infected stone disease were recruited as cases. Kidney stones were confirmed through plain X-rays, An X-ray crystallographic analysis verified that all stones were calcium oxalates using ultrasonography or intravenous urography. Thirty-two patients in the study did not take antibiotics in the preceding 3 months, while 18 had used antibiotics during stool sample collection. All 18 patients (16 males and 2 females) had taken cephalosporins (500 mg twice daily) for 5–7 days, with stool samples collected on the 8th day.

Eligible patients were selected based on specified criteria, and informed consent was obtained. An application for data collection documented demographic information. A stool sample was collected to test for *O. formigenes* and to determine Levels of oxalate and calcium in the urine.

Determination of *O. Formigenes*, Oxalate Levels and Urinary Calcium

Patient consent, stool samples (20–30 mg) were collected Becton Dickinson Cary Blair medium was used to collect and transport feces. In preparation for analysis, samples were stored at −80°C for *O. formigenes* presence, with all experiments performed in triplicate. Stool samples were cultured in a medium containing liquid oxalates for 10 days.[5] Oxalate content was then treated with CaCl2. In the absence of *O. formigenes*, oxalates remain unmetabolized and precipitate on calcium Treatment A measure of this is OD 600. By demonstrating stool oxalate, the method cannot direct identify the microorganism breakdown in a selective medium for *O. formigenes*.[17]

A 24-h urine collection was obtained from both study groups. Analyzing urinary calcium and citrate levels by means of an automatic was used for determining oxalate levels in the urine. Quantification of urinary oxalate excretion was performed. In the experiment, a commercial enzyme kit was used (Sigma Diagnostics Inc., MO, USA). Baseline urinary calcium and oxalate levels were compared to formerly reported normal values (calcium 100-250 mg/24 h, oxalate <24 mg/24 h, citrate>320 mg/24h).[18]

Statistics

The data were analyzed using an unpaired *t*-test evaluate the significance of *O. formigenes* presence between patients and healthy controls, given that O. formigenes distribution differed between these groups ($P = 0.01$). There was a two-tailed test. To determine statistical significance, a $P = 0.05$ was used set as the threshold. An analysis of the data was A SPSS version 15.0 programs was used for the study.

RESULTS AND DISCUSSION

Oxalobacter formigenes (O. formigenes) is a type of bacteria naturally found GIT (gastrointestinal tract) in humans. Its primary function is to metabolize oxalates, a dietary component associated with kidney stone formation. Through oxalate metabolism, *O. formigenes* reduces. It is possible for the GI tract to absorb a certain amount of oxalate, thus potentially lowering the risk of among the most common kidney stones are calcium oxalate stones. Previously, discussed, *O. formigenes* is highly sensitive to specific categories of antibiotics. Repeated or prolonged exposure to these antibiotics can result in a decline or even complete eradication of *O. formigenes* from the gut microbiome. This susceptibility to antibiotics raises concerns about the impact

of certain treatments on *O. formigenes* populations and, consequently, on oxalate homeostasis.

The clinical and demographic characteristics of the participants, including age, sex, and recent antibiotic use, were carefully documented and summarized in Table 1. Initially, 175 individuals were recruited for the study. However, due to personal reasons, 15 participants withdrew early, ensuring no impact on the validity or statistical power of the results. Following this attrition, an analysis of 160 subjects was performed, including 110 control participants without kidney stones and 50 case participants diagnosed with calcium oxalate kidney stones.

In the control group of 110 individuals, 97 (88%) tested positive for *O. formigenes* colonization, indicating a high prevalence of the bacterium among healthy individuals. Conversely, in the case group of 50 individuals with confirmed calcium oxalate kidney stones, only 36 (72%) tested positive for *O. formigenes* [Table 2]. This significant difference in *O. formigenes* prevalence between controls and cases suggests a potential. An association has been found between the presence of *O. formigenes* and reduced kidney stone risk. According to this research, *O. formigenes* reduces the absorption and subsequent excretion of oxalates in the intestine, thereby protecting against calcium oxalate stones.

Further analysis revealed additional distinctions in urinary parameters between cases and controls. Urinary calcium levels, among the case group and control group, a well-known kidney stone risk factor was significantly different [Table 3]. In addition, urinary citrate concentrations were statistically lower in cases than in controls [Table 4]. Citrate is recognized for its role in inhibiting calcium stone formation by binding to calcium ions, reducing their availability to form calcium oxalate crystals. Lower urinary citrate concentrations, therefore, increase the propensity. For calcium oxalate stones to form, these factors must be present seen in the case group. Moreover, urinary oxalate levels, which directly correlate. The incidence of calcium oxalate stones increases significantly was observed among cases than controls [Table 5].

The amount of urine oxalate excreted determines how likely it is for calcium oxalate stones to form. Even average levels of urinary oxalate can be sufficient to promote calcium oxalate crystal formation, particularly when urinary citrate levels are low, as found in the cases in this study. Previous research supports that *O. formigenes* colonization influences urinary oxalate excretion, as *O. formigenes* primarily uses oxalate as an energy source. Through this unique dependence, *O. formigenes* reduces the amount of oxalate in the gastrointestinal tract, ultimately decreasing oxalate absorption and urinary excretion. However, reduced colonization of *O. formigenes* has been observed among kidney stone formers, with significantly diminished levels. Risks of hyperoxaluria and hypercalcemia are increased hypercalciuria conditions that heighten the likelihood of deficiency of calcium oxalate lead to stone formation.[19,20]

The current study's findings align with and support these previous observations, illustrating a clear interconnections

Table 1: Comparison of clinical findings and other characteristics in patients and controls subjects

Characteristics	Patients	Controls	*P* value
Age (year)	45.2±9.8	44.8±10.4	0.064
BMI (kg/m^2)	26.6±2.2	26.2±1.8	0.45
Urine calcium (mg/24 h)	258.3±97	214.3±101.6	0.072
Urine citrate (mg/24 h)	257.6±118.6	526.4±254.9	<0.001
Urine oxalate (mg/24 h)	25.9±14.2	27.2±12.8	0.002

BMI: body mass index. [1]*P* values were separately calculated for comparison of each parameter. [2]A total testosterone concentration of < 285 ng/ml is considered low.

Table 2: Oxalobacter formigenes in stool sample among patients with kidney stones and control subjects

O. formigenes Status	Case (n=50)		Control (n=110)		Crude OR	MVOR (95% CI)
	n	%	n	%		
Positive	36	72	97	88	0.4800	0.4 (0.4–1.8)
Negative	14	28	13	12	0.4900	0.4 (0.5–2.2)

The overall prevalence of *O. formigenes* was 12% among the control subjects and 28% among the case patients, giving a crude odds ratio (OR) of 0.49 and 0.48.

Table 3: Urinary Calcium in patients with kidney stones and control subjects

Urinary calcium (mg/24 h)	Case (n=50)		Control (n=110)		Crude OR	MVOR (95% CI)
	n	%	n	%		
100–250	14	28	98	89	0.4689	0.4 (0.3–1.8)
>300	36	72	12	11	0.4028	0.3 (0.3–2.1)

Urinary calcium levels were higher in patients and the difference was statistically significant (*P*<0.05)

Table 4: Urinary Citrate in patients with kidney stones and control subjects

Urinary Citrate (mg/24 h)	Case (n=50)		Control (n=110)		Crude OR	MVOR (95% CI)
	n	%	n	%		
>320	38	76	99	90	0.4676	0.4 (0.3–1.2)
<320	12	24	11	10	0.4559	0.4 (0.2–0.6)

Urinary citrate concentrations were lower in patients when compared with control subjects. The differences in the urinary citrates were found to be statistically significant (*P*<0.05).

Table 5: Urinary oxalates in patients with kidney stones and control subjects

Urinary oxalates (mg/24 h)	Case (n=50)		Control (n=110)		Crude OR	MVOR (95% CI)
	n	%	n	%		
<24	3	6	93	85	0.3926	0.3 (0.1–0.8)
25–34	11	22	8	7	0.3934	0.3 (0.1–1.2)
35–44	26	52	5	5	0.4722	0.4 (0.4–1.8)
>45	10	20	4	3	0.4898	0.4 (0.6–2.9)

Among the 50 case patients and 110 control subjects who completed 24-h urine collections, the OR for developing kidney stones increased with increasing urinary oxalate excretion. Within the four levels of increasing oxalate excretion, the crude OR was 0.3926, 0.3934, 0.4722, and 0.4898, respectively.

O. formigenes colonization and reduced calcium oxalate kidney stone risk. In our study population, *O. formigenes* colonization was notably higher in the control group than in individuals with kidney stones. This supports the hypothesis that *O. formigenes* colonization may play a protective role by reducing oxalate absorption and urinary excretion, thus lowering the likelihood of calcium oxalate crystal formation in the urinary tract.

Our findings contribute valuable insights into the potential protective role of *O. formigenes* in managing oxalate levels and suggest that promoting *O. formigenes* colonization might be beneficial for individuals at risk of calcium oxalate kidney stones. While antibiotic treatment is often unavoidable, the findings underscore the importance of judicious antibiotic use to minimize inadvertent reductions in beneficial gut bacteria such as *O. formigenes*.

Ultimately, our study adds to the growing body of evidence supporting the protective association between *O. formigenes* colonization and kidney stone formation. This association may prove significant in guiding future kidney stone prevention strategies and in recommending alternatives, such as probiotics, for preserving *O. formigenes* populations in individuals requiring frequent antibiotic therapy.

Our findings highlight the importance of maintaining *O. formigenes* within the gut microbiome and suggest that both clinicians and patients should consider antibiotic alternatives when possible, and use targeted antibiotic therapy with caution, to support oxalate homeostasis and potentially prevent kidney stones. Further research on a larger scale is needed to explore these relationships more deeply and confirm *O. formigenes*' role in calcium oxalate urolithiasis prevention.

Limitations

The primary limitation based on this study is insufficient number of samples. To strengthen the understanding of *O. formigenes*' association with kidney stones, larger-scale studies are necessary.

CONCLUSION

Regardless of appropriateness, antibiotic exposure is known to alter microbial resistance. As discussed, *O. formigenes* is particularly sensitive to certain antibiotics, yet antibiotic use is often unavoidable. To protect the gut microbiome, it is recommended that healthcare practitioners prescribe antibiotics only when absolutely necessary and choose the correct antibiotic type and duration. This approach can help preserve essential gut microbiota. In addition, probiotics may be an effective option for maintaining *O. formigenes* colonization. Further research is needed to explore the antibiotic sensitivity profile of *O. formigenes*, with the goal of safeguarding its presence in the gut and supporting oxalate homeostasis.

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