Published in final edited form as:

Urology. 2012 June ; 79(6): 1286–1289. doi:10.1016/j.urology.2011.11.017.

Sensitivity of Human Strains of *Oxalobacter formigenes* to Commonly Prescribed Antibiotics

Jessica N. Lange, M.D.^a, Kyle D. Wood, M.D.^a, Hayes Wong^a, Richard Otto^a, Patrick W. Mufarrij, M.D.^a, John Knight, Ph.D.^a, Haluk Akpınar, M.D.^a, Ross P. Holmes, Ph.D.^a, and Dean G. Assimos, M.D.^a

^aDepartment of Urology, Wake Forest University School of Medicine, Winston-Salem, NC, 27157

Abstract

Objective—To determine the sensitivity of 4 strains of *Oxalobacter formigenes* (OxF) found in humans, HC1, Va3, CC13, and OxF, to varying concentrations of commonly-prescribed antibiotics. OxF gut colonization has been associated with a decreased risk of forming recurrent calcium oxalate kidney stones.

Methods—For each strain and each antibiotic concentration, 100 μ L of an overnight culture and 100 μ L of the appropriate antibiotic were added to a 7 mL vial of oxalate culture media containing 20 mM oxalate. On the fourth day, vials were visually examined for growth, and a calcium oxalate precipitation test was performed to determine whether OxF grew in the presence of the antibiotic.

Results—All 4 OxF strains were resistant to amoxicillin, amoxicillin/clavulanate, ceftriaxone, cephalexin, and vancomycin while they were all sensitive to azithromycin, ciprofloxacin, clarithromycin, clindamycin, doxycycline, gentamicin, levofloxacin, metronidazole, and tetracycline. One strain, CC13, was resistant to nitrofurantoin while the others were sensitive. Differences in minimum inhibitory concentration between strains were demonstrated.

Conclusions—Four human strains of OxF are sensitive to a number of antibiotics commonly utilized in clinical practice; however, minimum inhibitory concentrations differ between strains.

Keywords

Oxalobacter formigenes; Calcium; Oxalate; Kidney stone; Antibiotics

© 2011 Elsevier Inc. All rights reserved.

Corresponding Author: Dean G. Assimos, Professor of Surgical Sciences—dassimos@wakehealth.edu, 336-716-5692.

Financial Conflicts of Interest

Jessica Lange – none
 Kyle Wood – none
 Hayes Wong – none
 Richard Otto – none
 Patrick Mufarrij – none
 John Knight – none
 Haluk Akpınar – none
 Ross Holmes – none
 Dean Assimos – none

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Oxalobacter formigenes (OxF) is a Gram-negative, anaerobic bacterium that often inhabits the colon of vertebrates including humans. It is unique in that oxalate is its main carbon and energy source, which it metabolizes into formate and CO₂¹. Dietary oxalate makes a significant contribution to the amount of oxalate excreted in urine². Since even small changes in urinary oxalate excretion can have a significant impact on calcium oxalate stone disease³, there has been speculation that colonization with OxF may help protect against stone disease⁴. A case-control study has confirmed that OxF colonization is lower in stone formers compared to non-stone formers, and OxF colonization is associated with a 70% reduction in the risk of recurrent calcium oxalate kidney stone formation³. Although increased utilization of antibiotics may contribute to the decreased OxF colonization observed in stone formers⁵, limited information exists on the sensitivity of this organism to commonly-used antibiotics. Our study determined the sensitivity of 4 different strains of OxF known to be present in humans to antibiotics that are commonly used in clinical practice.

Materials and Methods

Four different strains of *Oxalobacter formigenes*, OxF, CC13, Va3, and HC1, were obtained from Dr. Milton Allison, Iowa State University, Ames, Iowa, and grown in a medium as previously described⁶. These specific strains were chosen because they have previously been isolated from human feces. HC1 and CC13 are Group 1 strains and OxF and Va3, Group 2. Antibiotics were purchased from Sigma Aldrich (St. Louis, Missouri). Antibiotic sensitivity testing was conducted as follows. A frozen preparation of each strain was thawed, and 100 µL of that preparation was injected into a 7 mL vial containing 6 mL of a 20 mM oxalate culture media. These vials were incubated at 37°C for 24 hours. One hundred µL of an appropriate antibiotic concentration was then injected into a fresh 7 mL oxalate culture vial, and 100 µL of the overnight culture was added. Antibiotic concentrations were chosen based on reported plausible colonic concentrations as derived from drug information sheets when available. Otherwise, antibiotic concentrations were modeled after the previous work of Duncan and associates⁴. This new vial was placed in a 37°C incubator. On day four, a calcium oxalate precipitation test⁷ was performed to determine whether OxF grew in the presence of the antibiotic. A calcium oxalate precipitation test involves mixing 50 µL of 0.1% calcium chloride solution to 50 µL of oxalate culture media in a 200 µL glass microinsert tube (National Scientific, Rockwood, TN). Precipitation of calcium oxalate is indicative of a lack of OxF growth in the presence of the antibiotic. We intended to include trimethoprim/sulfamethoxazole in our study, but it could not be tested due to the poor solubility of both drugs. Intravenous trimethoprim/sulfamethoxazole could not be obtained due to an ongoing national shortage. It is currently only available for emergency clinical use and thus could not be obtained for this study.

Results

The antibiotic sensitivity and resistance patterns of all 4 OxF strains are listed in Table 1. Two- to four-fold differences between strains in the minimum inhibitory concentration of all antibiotics except clarithromycin were observed (Table 2). The antibiotic concentrations used are listed in Table 3.

Comments

The reported rates of colonization with OxF in certain populations around the globe vary widely (Table 4). In normal adult populations, they range from 38% in the U.S.⁷ to 77% in

Korea⁸. The reported variation in children is slightly lower, ranging from 26% in Poland⁹ to 59% in the Ukraine¹⁰. It is not known whether the incidence of calcium oxalate stones inversely correlates with OxF status in non-U.S. populations. Colonization was determined with PCR testing in the majority of these studies, although the culture method appears to be more sensitive⁷. The culture method uses 50 mg of stool, whereas the PCR method uses DNA isolated from 1 mg of stool. Some individuals may have mean levels of 5 OxF/mg of stool¹¹ and could fall below the detection limit for the PCR technique. The reasons for these geographic differences are not known due to the paucity of knowledge about factors that affect colonization and the loss of colonization. Such factors could include diet, exposure to OxF, composition of the gut microbiome, host factors influencing colonization, and exposure to antibiotics. Studies in rodents suggest that colonization results from horizontal transfer, not vertical transfer, indicating that exposure to OxF is likely to be a central factor in colonization¹². The tendency to make the environment of infants as sterile as possible could be one factor reducing colonization in Western countries. Colonization rates are much lower in diseases where the bowel is affected, such as inflammatory bowel disease and cystic fibrosis¹³. Such patients are known to have increased oxalate excretion and supersaturation of calcium oxalate, and are also at risk for developing kidney stones^{14, 15}. Antibiotic therapy may contribute to this lower rate, as many may have required stone-removing procedures where antibiotic therapy is typically administered.

One previous study by Duncan and associates reported the sensitivity of the HC1 and Va3 strains of OxF to a small number of antibiotics⁴. They found that OxF was resistant to ampicillin, amoxicillin, and streptomycin and sensitive to doxycycline and clarithromycin. We similarly found resistance to amoxicillin and sensitivity to doxycycline and clarithromycin. They noted a differential strain response to chloramphenicol, nalidixic acid, erythromycin, and amoxicillin/clavulanic acid, some of which was antibiotic concentration dependent, suggesting that antibiotic resistance of commonly-occurring strains may be variable. Our results demonstrated pan-resistance to amoxicillin/clavulanate whereas Duncan and associates reported Va3 sensitivity to 50 µg/mL concentration, which is within the range of concentrations we assessed. We have no explanation for this discordance. Kharlamb and associates have recently prospectively examined the effects of antibiotic treatment to eradicate *H. pylori* infection on OxF gut colonization¹⁶. Antibiotic regimens administered included 10-14 day courses of amoxicillin 1 g twice daily/clarithromycin 500 mg twice daily, metronidazole 250 mg 4 times daily/tetracycline 500 mg 4 times daily/bismuth 525 mg 4 times daily, or metronidazole 250 mg 4 times daily/clarithromycin 500 mg twice daily. Of the 16 colonized patients treated with the amoxicillin/clarithromycin regimen, 37.5% (6/16) remained colonized at 1 month and 43.8% (7/16) were colonized at 6 months. Based on our sensitivity results, we would have expected that none of the subjects would remain colonized. Of the 2 patients treated with the metronidazole/tetracycline/bismuth regimen, 1 remained colonized at 1 month follow-up and neither remained colonized at 6 months. This coincides with our findings that OxF is sensitive to both metronidazole and tetracycline. The one patient treated with metronidazole/clarithromycin was not colonized at 1 or 6 months, which would also be consistent with our results. Reasons for the difference in findings between Kharlamb et al. and our study include our experiments being performed in broth cultures, which may not closely mimic *in vivo* phenomena, as well as the possibility that some antibiotics (perhaps clarithromycin in this example) may not reach concentrations in the large intestine as high as those we tested in our vials. In addition, the subjects in their study may have been colonized with an OxF strain or strains different from the 4 we studied.

There are several potential factors which could influence whether an antibiotic can eliminate OxF from the fecal microbiome. These could include the amount administered, route of administration, amount reaching the colon, the population of OxF present in the colon, and

host factors. The amount of antibiotic reaching the colon is impacted by its proximal absorption and potential metabolism in the gut. These factors may vary between antibiotics as well as individuals. In addition, the resistance and sensitivity characteristics may vary for different strains of OxF. Furthermore, the distribution and number of OxF strains across different populations is unknown.

The influence of OxF colonization on kidney stone formation is presently poorly defined. OxF colonization may limit net gastrointestinal oxalate absorption by degrading oxalate in the colon. OxF has also been demonstrated to induce colonic oxalate secretion in animal models, which may impact oxalate delivery to the kidney^{17, 18}. A recent study in a mouse model of primary hyperoxaluria (PH) type 1 by Hatch et al. demonstrated that both plasma and urinary oxalate were 50% lower in colonized mice compared to non-colonized mice¹⁸, suggesting OxF may be a therapeutic strategy to reduce the oxalate burden in patients with PH. Both processes could be significant, as small increases in oxalate excretion have been demonstrated to increase stone risk³. While some reports have indicated that colonized individuals have lower oxalate excretion than non-colonized individuals, other analyses have differed^{7, 19, 20}. In addition, subjects in these studies consumed self-selected diets, and the lack of control of dietary oxalate and calcium may have clouded the interpretation of results. It is also not possible to decipher whether the elimination of OxF colonization is a cause or consequence of stone formation. The relationship of OxF with other oxalate-degrading organisms in the fecal microbiome may also be important but has not yet been elucidated.

Conclusions

In conclusion, we have demonstrated that 4 strains of OxF are sensitive to a number of antibiotics commonly utilized in clinical practice. The impact of antibiotic therapy on OxF colonization and its consequences need to be determined.

Acknowledgments

Support

This study was supported by the National Institutes of Health Grant R01 DK62284.

References

1. Ruan ZS, Anantharam V, Crawford IT, et al. Identification, purification and reconstitution of OxlT, the oxalate: formate antiport protein of *Oxalobacter formigenes*. J Biol Chem. 1992; 267:10537–10543. [PubMed: 1587834]
2. Holmes RP, Goodman HO, Assimos DG. Contribution of dietary oxalate to urinary oxalate excretion. Kidney Int. 2001; 59:270–276. [PubMed: 11135080]
3. Curhan GC, Taylor EN. 24-h uric acid excretion and the risk of kidney stones. Kidney Int. 2008; 73:489–96. [PubMed: 18059457]
4. Duncan SH, Richardson AJ, Kaul P, et al. Oxalobacter formigenes and its potential role in human health. Appl Environ Microbiol. 2002; 68:3841–7. [PubMed: 12147479]
5. Mittal RD, Kumar R, Bid HK, et al. Effect of antibiotics on Oxalobacter formigenes colonization of human gastrointestinal tract. J Endourol. 2005; 19:102–6. [PubMed: 15735393]
6. Allison MJ, Dawson KA, Mayberry WR, et al. Oxalobacter formigenes gen. nov., sp. nov.: oxalate-degrading anaerobes that inhabit the gastrointestinal tract. Arch Microbiol. 1985; 141:1–7. [PubMed: 3994481]
7. Kaufman DW, Kelly JP, Curhan GC, et al. Oxalobacter formigenes may reduce the risk of calcium oxalate kidney stones. J Am Soc Nephrol. 2008; 19:1197–203. [PubMed: 18322162]

8. Kwak C, Kim HK, Kim EC, et al. Urinary oxalate levels and the enteric bacterium *Oxalobacter formigenes* in patients with calcium oxalate urolithiasis. *Eur Urol.* 2003; 44:475–481. [PubMed: 14499684]
9. Sikora P, Niedzwiedek J, Mazur E, et al. Intestinal colonization with *Oxalobacter formigenes* and its relation to urinary oxalate excretion in pediatric patients with idiopathic calcium urolithiasis. *Arch Med Res.* 2009; 40:369–73. [PubMed: 19766900]
10. Sidhu H, Yenatska L, Ogden SD, et al. Natural colonization of children in the Ukraine with the intestinal bacterium, *Oxalobacter formigenes*, using a PCR-based detection system. *Mol Diagnosis.* 1997; 2:89–97.
11. Prokopovich S, Knight J, Assimios DG, et al. Variability of *Oxalobacter formigenes* and oxalate in stool samples. *J Urol.* 2007; 178:2186–90. [PubMed: 17870112]
12. Cornelius JG, Peck AB. Colonization of the neonatal rat intestinal tract from environmental exposure to the anaerobic bacterium *Oxalobacter formigenes*. *J Med Microbiol.* 2004; 53:249–54. [PubMed: 14970252]
13. Sidhu H, Hoppe B, Hesse A, et al. Absence of *Oxalobacter formigenes* in cystic fibrosis patients: a risk factor for hyperoxaluria. *Lancet.* 1998; 352:1026–1029. [PubMed: 9759746]
14. Terribile M, Capuano M, Cangiano G, et al. Factors increasing the risk for stone formation in adult patients with cystic fibrosis. *Nephrol Dial Transplant.* 2006; 21:1870–5. [PubMed: 16595587]
15. Evan AP, Lingeman J, Coe F, et al. Renal histopathology of stone-forming patients with distal renal tubular acidosis. *Kidney Int.* 2007; 71:795–801. [PubMed: 17264873]
16. Kharlamb V, Schelker J, Francois F, et al. Oral antibiotic treatment of *Helicobacter pylori* leads to persistently reduced intestinal colonization rates with *Oxalobacter formigenes*. *J Endourol.* in press.
17. Hatch M, Cornelius J, Allison M, et al. *Oxalobacter* sp. reduces urinary oxalate excretion by promoting enteric oxalate secretion. *Kidney Int.* 2006; 69:691–8. [PubMed: 16518326]
18. Hatch M, Gjymishka A, Salido EC, et al. Enteric oxalate elimination is induced and oxalate is normalized in a mouse model of primary hyperoxaluria following intestinal colonization with *Oxalobacter*. *Am J Physiol Gastrointest Liver Physiol.* 2011; 300:G461–9. [PubMed: 21163900]
19. Troxel SA, Sidhu H, Kaul P, et al. Intestinal *Oxalobacter formigenes* colonization in calcium oxalate stone formers and its relation to urinary oxalate. *J Endourol.* 2003
20. Jiang J, Knight J, Easter LH, et al. Impact of Dietary Calcium and Oxalate, and *Oxalobacter Formigenes* Colonization on Urinary Oxalate Excretion. *J Urol.* 2011; 186:135–9. [PubMed: 21575973]

Table 1

Sensitivity of OxF Strains to Various Antibiotics

Antibiotic	HC1	Va3	Cc13	OxK
Amoxicillin	R	R	R	R
Amoxicillin/Clavulanate	R	R	R	R
Azithromycin	S	S	S	S
Ceftriaxone	R	R	R	R
Cephalexin	R	R	R	R
Ciprofloxacin	S	S	S	S
Clarithromycin	S	S	S	S
Clindamycin	S	S	S	S
Doxycycline	S	S	S	S
Gentamicin	S	S	S	S
Levofloxacin	S	S	S	S
Metronidazole	S	S	S	S
Nitrofurantoin	S	S	R	S
Tetracycline	S	S	S	S
Vancomycin	R	R	R	R

R = resistant; S = sensitive

Table 2Minimum Inhibitory Concentration of Sensitive Strains ($\mu\text{g/mL}$)

Antibiotic	HC1	Va3	Cc13	OxK
Azithromycin	0.5	0.25	0.5	0.5
Ciprofloxacin	6	6	3	3
Clarithromycin	18.75	18.75	18.75	18.75
Clindamycin	32	16	8	16
Doxycycline	0.5	0.0625	0.25	0.0625
Gentamicin	125	500	250	67.5
Levofloxacin	2	1	4	0.5
Metronidazole	100	12.5	25	25
Nitrofurantoin	128	256	--	256
Tetracycline	4	4	2	2

Strains not listed were resistant at all concentrations.

Table 3

Antibiotic Concentrations Tested

Antibiotic	Lowest Concentration (µg/mL)	Highest Concentration (µg/mL)
Amoxicillin	15.6	500
Amoxicillin/Clavulanate	3.90/1.95	500/250
Azithromycin	0.03125	16
Ceftriaxone	0.5	128
Cephalexin	0.5	16
Ciprofloxacin	0.023	48
Clarithromycin	1.17	300
Clindamycin	0.0625	128
Doxycycline	0.0625	32
Gentamicin	7.8125	500
Levofloxacin	0.0625	16
Metronidazole	3.125	100
Nitrofurantoin	0.5	256
Tetracycline	0.0313	64
Vancomycin	1.17	300

Table 4**Reported OxF Colonization Rates**

Country	Population	N	Mean Age (Years)	Percent Colonized	Technique	Reference
Ukraine	Normal Children	100	-	59	PCR	10
Germany	Normal	21	-	71	PCR	18
Germany	Cystic Fibrosis	43	-	2.3	PCR	18
Germany India USA	CaOx Stone Formers	145	51.6	42.8	PCR	13
Germany	Normals	61	32	68.9	PCR	13
Germany	Normals	-	-	72	PCR	13
India	Normals	22	30.5	59	PCR	13
USA	Normals	26	-	62	PCR	13
USA	Crohn's Disease	16	-	9	PCR	13
Germany	Cystic Fibrosis	52	-	13.5	PCR	13
Japan	Normal Adults	88	-	72.7	PCR	19
India	CaOx Stone Formers	63	-	30	PCR	20
India	Normals	-	-	65	PCR	20
India	Adult Stone Formers	80	39	31.3	PCR	21
India	Normal Adults	70	45	62.2	PCR	21
Korea	Normal Adults	233	48	76.8	PCR	8
Korea	CaOx Stone Forming Adults	103	47	45.6	PCR	8
USA	Adult CaOx Stone Formers	35	-	26	PCR	16
USA	Normal Adults	10	-	60	PCR	16
India	Normal Adults	48	-	56	PCR	22
India	Adults with IBD	48	-	10.4	PCR	22
India	Adult CaOx Stone Formers	87	-	29	PCR	23
India	Normal Adults	80	38	41.3	PCR	5
India	Adult CaOx Stone Formers	100	39	27	PCR	5
USA	Normal Adults	259	-	38	PCR/Culture	7

Country	Population	N	Mean Age (Years)	Percent Colonized	Technique	Reference
USA	Adult Recurrent CaOx Stone Formers	247	-	17	PCR/Culture	7
Poland	Stone Forming Children	76	-	27.6	PCR	9
Poland	Normal Children	50	-	26	PCR	9

PCR = polymerase chain reaction; CaOx = calcium oxalate; IBD = inflammatory bowel disease