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Inhibition of *Rothia* Species by Over-the-Counter Products and Bacterial Antagonists

Cannon L Mark[1,2*], Kabat B[1], Yogev R[1,2], Jantra L[1], Awan A[1], Le C[1], White K[1] and Vorachek A[1]

Affiliation:
[1]Ann and Robert Lurie Children's Hospital of Chicago
[2]Northwestern University Feinberg School of Medicine

[*]**Corresponding author:** Mark L Cannon, Professor, Feinberg School of Medicine, Northwestern University, Chicago, USA, Tel: 847-899-6720, E-mail: drmarkcannon@outlook.com

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Abstract

The interaction between the human host micro biome and over the counter products has recently been investigated, with surprising results. Some over the counter items may negatively affect the health of the host, supporting the concept of the "hygiene hypothesis", that is, that disease may be actually caused by the lack of beneficial commensal bacteria. Recent reports on the gluten metabolizing genus, *Rothia*, and a possible association with Celiac Disease beg the question, what happened to the *Rothia*? In this study inhibitory factors, such as, Over The Counter oral hygiene products and antagonistic bacteria were investigated and, *in vitro*, significantly inhibited the gluten metabolizing bacteria, possibly affecting human digestion and contributing to gluten sensitivity.

Keywords: Gluten oral bacteria, *Rothia mucilaginosa* and *Streptococcus salivariu*.

Introduction

The human body is host to trillions of microorganisms, including bacteria, molds, yeasts, viruses and archaea. In addition, the contribution of the microbiome to human health has become thoroughly established with roles such as educating the immune response, resisting pathogens, and digestion. As a result, the human microbiome project was designed to ascertain the microbial composition of the entire human body. Meanwhile, the oral microbiome has been extensively determined and reported in the literature. The current reported microbiome of the oral cavity region contains 619 taxa, derived from 13 phyla [1-4].

An additional 36,043 gene clones have been sequenced, identifying an additional 434 unique oral taxa that (after further validation) may be added to the database. Amongst the oral strains sequenced to date, two important gluten metabolizing species, *Rothia mucilaginosa* and *Rothia aeria* have been identified [4,5]. *R. mucilaginosa* and *R. aeria* are of the *Rothia* genus under the phyla *Actinobacteria*. *R. aeria* was named after its isolation from air in the Russian space laboratory Mir and is an oral inhabitant [6,7].

R. mucilaginosa is primarily found in the oral cavity but has been reported in the upper respiratory tract and also the duodenum [8-11]. Interestingly, mucosal damage in celiac disease is mostly found in this area of the gastro-intestinal system [12]. Oral micro-organisms that *in vitro* degrade dietary proteins may mean that they play an *in vivo* role in food metabolism. During mastication, ingested food is mixed with stimulated whole saliva and oral micro-organisms. This process accelerates food digestion while the bolus is still churning in the oral cavity [13]. For example, nitrate reducing bacteria have been described as being indispensable in the production of nitric oxide which regulates blood pressure and cardiovascular health and this further emphasize the importance of the oral microbiome in systemic health [14-16]. A favorable and potential source for gluten-degrading enzymes would be the micro-organisms inhabiting the human gastro-intestinal tract. It is well reported that bacteria residing in and on the human body supply the host with numerous functions that are not encoded by the human genome [17]. For instance, bacteria that colonize the large intestine ferment starches that are resistant to mammalian digestive enzymes [18].

In addition, it has been reported that human breast milk contains a number of oligosaccharides that are only digested by gut bacteria, not the breast-feeding child [19,20]. Therefore, recent publications that report gluten-degrading bacteria as natural residents of the oral cavity are not surprising after all [21,22]. This discovery is also very significant, since the oral cavity represents the gateway to the gastro-intestinal system in which gluten is mixed with the oral microorganisms in human saliva. The finding of gluten-degrading oral microbes then begs the questions, what are they susceptible to and what common source may reduce the gluten metabolizers or decrease their effectiveness of gluten processing, leading to gluten "sensitivity"?

Objective

The purpose of this study was to determine if there is any inhibition of beneficial oral biofilm species such as *Rothia aeria*, *R. mucilaginosa*

and *R. dentocariosa*, *Streptococcus mutans* (pathogen-negative control) and also *Lactobacillus reuteri* strains (isolated from periobalance Probiotic) by Over The Counter (OTC) oral antimicrobials utilizing *in vitro* laboratory technique. The secondary objective was to determine the antagonism, if any, of the *Rothia* genus by *Streptococcus* species (*mutans* and *salivarius*) and known pathogens. *Rothia aeria* and *R. mucilaginosa* are reported to be important in the processing of gluten. Inhibition of these beneficial bacteria by OTC products, either directly or indirectly, would increase gluten sensitivity in patients. Beneficial bacteria may be indirectly inhibited by certain antagonistic bacteria that are relatively less sensitive to OTC products.

Methods

Susceptibility Experiment

Three colonies of *R. aeria*, *R. dentocariosa*, *R. mucilaginosa*, *S. mutans*, or *Lactobacillus* were obtained from isolation plates and grown in Mueller-Hinton media to a McFarland Standard of 0.5. Either Brucella agar plates, Rogosa agar, or Mueller-Hinton agar plates with 5% sheep blood were wholly spread to create a lawn with one cotton swab inoculation of chosen target bacteria. Five cotton discs were evenly distributed on the plate and 10 microliters of full strength OTC reagent was pipetted directly onto each corresponding disc. The plates were evaluated after 30 hours of growth at 36ºC. Calipers were used to measure zones of inhibition in millimeters.

Diffusion Experiment

Trypticase Soy Agar (TSA) was autoclaved and cooled to 56 degrees and aliquots of 25 mL were cooled and inoculated with 2 mL of 0.5 McFarland Standard suspensions of target organisms: *R. dentocariosa*, *R. mucilaginosa*, *Streptococcus salivarius*, *Escherichia coli* or *Pseudomonas aeruginosa* prior to pouring agar plates. Impregnated plates were then inoculated in punched zones using a disposable 10 microliter loop with 0.5 McFarland Standards of test inhibiting bacteria species: *Streptococcus salivarius*, *Staphylococcus aureus*, Vancomycin-resistant Enterococcus, *Pseudomonas aeruginosa*, Escherichia coli, and *R. dentocariosa* or *R. mucilaginosa*. The plates were evaluated after 24 hours of growth at 36ºC. Calipers were used to measure zones of inhibition.

Results

Bacterial growths of all tested bacteria were inhibited by Crest ProHealth™, ACT™, Listerine SmartRinse™, and Chlorhexidine. *R. aeria* and *R. mucilaginosa* were also inhibited by Embrace™ varnish (**Table 1**).

Reagent	*R. aeria* on blood agar	*R. dentocariosa*		*R. mucilaginosa*		perio probiotic (*Lactobacillus*)		*S. Mutans* on blood agar
		on blood agar	on Brucella	on blood agar	on Brucella	on blood agar	on Rogosa	
Spry Xylitol Mouthwash™	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Crest Prohealth™	9.9	12.12	11.11	14.16	14.10	15.13	16.13	12.12
ACT fluoride rinse™	10.10	11.12	14	12.14	16.14	17.15	16.15	13
Listerine Smartrinse™	9.9	10.11	9.9	14.14	9.8	14.12	13.12	11.11
Chlorhexidine (11.6% alcohol)	13.12	18.18	13.12	14.14	11.11	16.15	15.15	15.14
Listerine™ (27% Alcohol)	0.0	0.0	0.0	0.0	0.0	9.9	0.0	0.0
Phosphate Buffered Saline (PBS)	0	0.0	0	0.0	0	0	0	0
27% Alcohol	0.0	0.0	10	0.0	0	10	0	0
Embrace varnish™ (has xylitol)	8.9	0.0	0.0	12.12	0.0	0.0	0.0	0.0
Spry™ Xylitol toothpaste gel	0.0	0.0	0.0	10.12	0.0	0.0	0.0	0.0
50% Spry™ Xylitol toothpaste gel in PBS	-	0.0	-	0.0	-	-	-	-
Levoflaxacin (5 micrograms)	30	30	30	36	20	0	0	20

Table 1: Susceptibility Experiment: The effect of over the counter oral hygiene products on oral bacteria.

Spry™ Xylitol Toothpaste Gel inhibited *R. mucilaginosa*, *L. reuteri*, a probiotic that inhibits many oral and pathogens, was significantly inhibited by OTC oral products, except the xylitol based. Xylitol based oral products did not inhibit the commensal *S. salivarius* nor the pathogens, *S. aureus*, *E. coli* and *P. aeruginosa* (**Table 2**).

	S. aureus	*S. salivarius*	*E.coli*	*P. aeruginosa*	VRE
Spry™ Mouthwash	0	0	0	0	0
Embrace™ varnish	0	0	0	0	0
Spry™ Xylitol Gel diluted in PBS	0	0	0	0*	0
PBS control	0	0	0	0	0

Table 2: Susceptibility Experiment: The effect of OTC oral hygiene products on other bacteria of the human flora.

Xylitol based oral products do inhibit many oral pathogens and have been extensively used in dentistry for decades. Growth of P. aeruginosa was inhibited by *R. dentocariosa* and growth of S. aureus was inhibited by *R. mucilaginosa*. The zones of inhibition by the gluten metabolizers were demonstrably large. The inhibition of the beneficial gluten metabolizers and probiotic bacteria by OTC oral products may have been the result of fluoride concentration.

An alcohol based product, Listerine™, did not greatly inhibit the gluten metabolizers (**Figure 1**).

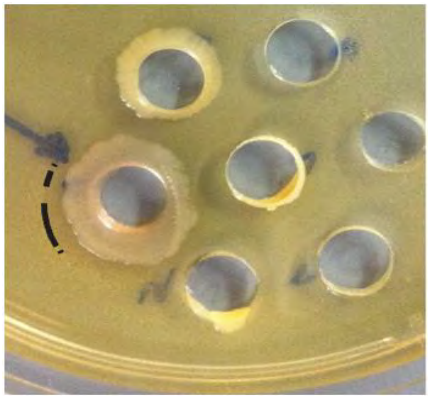
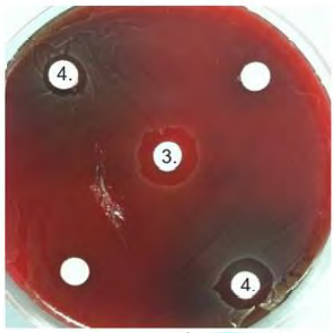


Figure 1: Example of Inhibition of pathogen *P. aeruginosa* by gluten metabolizer, *R. dentocariosa*.

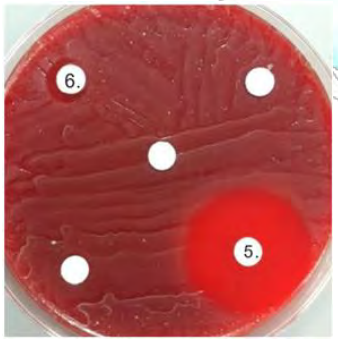
Discussion

In vitro results are not always applicable to the clinical situation. Indeed, the complexity of the human oral microbiome would make it difficult to predict a response to any oral intervention with certainty. The results of the present study are of a pilot nature, a negative finding would mean that there is little need for further investigation. However, *in vitro* studies are always necessary before progressing into more extensive, time consuming, and financially demanding clinical studies. The mere fact that OTC products, sometimes used ad libitum by patients, contribute to a reduction in beneficial bacteria should be a concern to all health practitioners. Of greater interest should be the extent of inhibition, as the zones of inhibition were quite significant in diameter. The average diameter of inhibition with an OTC product was 13 mm [Range: <6-18 mm] (**Figure 2**).

Note: *Rothia aeria* is inhibited by: 1. Chlorhexidine, 2. Listerine Smartrinse™.



Note: *Rothia mucilaginosa* inhibition by: 3. ACT fluoride rinse™, 4. Crest Prohealth™.



Note: *Rothia dentocariosa* inhibition by: 5. Levofloxacin, 6. 27% alcohol.

Figure 2: Examples of Inhibition Plates by OTC Products.

The mode of inhibition should be discovered, as it appears that the fluoride concentration of the OTC products may have been contributory. An alcohol based product was only inhibitory of the probiotic in this study, and not the gluten metabolizers. With dental disease at an increasing rate in developing countries due to the shift to a higher carbohydrate diet, with addition of processed foods containing added sugars, health professionals should be cautioning about the over use of OTC products. The dental caries rate is not decreasing, as would be expected with all the OTC utilization, and dental expenditures are increasing every year. Perhaps the OTC products help with limiting the pathogenic bacteria but only at the expense of also eliminating many beneficial bacteria. This is a no win situation for the population, spending vital resources on products that may indeed create more pathology, such as, gluten sensitivity, and fail to protect from dental caries.

The beneficial effect of fluoride for caries protection may be somewhat decreased by the possible inhibition of oral probiotic bacteria by over use of OTC products. Unsupervised use of a daily fluoride mouth rinse by a child could possibly create a gluten sensitivity issue, and due to lack of regulatory oversight, this severe side effect would never be discovered (**Table 3**).

	R. dentocariosa	*R. mucilaginosa*	*S. salivarius*	*E. coli*	*P. aeruginosa*
R.mucilaginosa	0.0	0.0	0.0	0.0	0.0
VRE	0.0	0.0	0.0	0.0	0.0
E. coli	0.0	0.0	0.0	0.0	0.0
P.aeruginosa	inhibits	0.0	0.0	0.0	0.0
S.Salivarius	0.0	0.0	0.0	0.0	0.0
R.dentocariosa	0.0	0.0	0.0	0.0	0.0
S. aureus	0.0	inhibits	0.0	0.0	0.0

Table 3: Diffusion experiment: Bacterial species inhibition of each other.

Another very important aspect of this study was the interaction between pathogenic and beneficial bacteria. The interaction, or rather, the inhibition of different bacterial species actually determines the health of the host and as such, is paramount in importance. The results were significant in that growth of *Rothia* species was inhibited by other bacteria. This suggests that if the oral flora equilibrium is changed by using OTC oral hygiene products, a domino effect can change the entire oral microbiome, which is the gateway to the digestive tract. The gastric microbiome is now recognized as a vital component of the host's health, both mental and physical. Increased oversight concerning the over uses of anti-microbial, food preservatives that are also anti-microbial, and OTC products that inhibit commensal bacteria, is essential. Required testing of OTC products and better population education into the importance of the holobiome should be a health priority. The connection between the increase in chronic diseases and the significant shift reported in the modern human microbiome should be further investigated.

Conclusion

Rothia and *Lactobacillus* species may be decreased in quantity by the overuse of oral antimicrobials. OTC products may alter the oral microbiome creating a situation less conducive for the survival of essential beneficial bacteria. The use of OTC products may decrease the enzymatic degradation of gluten containing foods by *Rothia* bacteria. This can possibly result in gluten sensitivity, thereby increasing the clinical prevalence of celiac disease. Further studies are required before any clinical implications may be concluded, but oral antimicrobials should be used only when necessary.

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