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Research Article

The Effect of Sewak on Oral Microbiota Composition

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Abstract

Considerable data by many experimental studies have shown that the *Salvadora persica* plant and its chewing sticks (sewak) display beneficial effects for oral hygiene. The aim of this study was to evaluate the potential effects of sewak on salivary microbiome profiles using 16s metagenomics sequencing approach. Surveys and mouthwash samples with and without the use of sewak were obtained from six healthy volunteer participants. Samples were processed and DNA was isolated using QlAamp kit. The variable V3 and V4 regions of the 16S rRNA gene were sequenced. Each sample was sequenced to a depth of 380,000 reads in the paired-end 2x300 read format. Taxonomic identification and statistical analysis revealed that after using the sewak treatment, the proportions of 27 species level taxa decreased and those of 16 taxa increased ($P < 0.05$). *Streptococcus fryi* and *Streptococcus vestibularis* were two streptococcus species that were significantly lowered after sewak treatment in comparison to the control samples ($P < 0.05$). Other bacterial species were not changed among tested samples between the two conditions. There were also differences in the proportion of different taxa between samples without sewak treatment. It is possible that sewak use may reduce oral bacteria in a global, proportional manner, such that the microbiome approach could not detect such changes since the method is designed to measure composition changes, not absolute changes.

Keywords: Sewak; Chewing Stick; Miswak; Oral Microbiome; Bacteria; *Salvadora persica*

Introduction

The history of oral microbiota study began in 1683 when Anthony van Leeuwenhoek decided to examine the film growing in his mouth using his handmade microscope. It has been a long journey from these simple experiments to the comprehensive study of the human oral microbiota. Oral microbiota is considered the most complex and most significant microbial community in the individual's body and includes an unknown number of archaea, fungi, viruses, and bacteria. To date, there are only about 750 different bacterial species identified, including those of genera *Prevotella, Streptococcus, Leptotrichia, Actinomyces, Eikenella, Peptostreptococcus, Veillonella, Fusobacterium, Porphromonas, Haemophilis, Treponema, Nisseria, Eubacteria, Lactobacterium, Capnocytophaga, Staphylococcus*, and *Propionibacterium* [1-3]. Stability of the oral microbiome composition is essential to prevent dysbiosis-a microbial shift toward a disease [4]. Significant increase in the abundance range of some oral bacterial species could be considered as a sign for some serious diseases. Several studies have linked specific oral bacterial abundances with increased risk cell cancer [5]. For example, *Streptococcus anginosus* is linked the carcinogenesis of head and neck squamous cell carcinoma [6], while the oral microbiota in head and neck squamous cell carcinoma patients is distinguishable from non-cancer patients [7]. To control disease-causing bacteria, dental hygiene, includes using a toothbrush, toothpaste and mouthwash, must be practiced every single day [2,8]. Interestingly, the earliest common method of dental hygiene is the use of a wood stick called sewak that was used 7000 years ago and is still used by some people [9]. Sewak is an Arabic word that refers to a tooth-cleaning stick, made from the branches or twigs of *Salvadora persica* used as a natural toothbrush for obtaining good oral hygie-

ne and as an oral remedy for religious and social purposes. *S. persica* plant is grown initially in Rajasthan (India), Nepal, and Malaysia, Pakistan, Iran, Iraq, Saudi Arabia, Sudan, Ethiopia, Egypt and Mauritania [9]. Other names of sewak are "arak" or "miswak" in Arabic, "koyoji" in Japanese, "qesam" in Hebrew, "qisa" in Aramaic, and "mastic" in Latin. It was first used by Babylonians, followed by the Greek and Roman empires, and then by ancient Egyptians and Muslims. Moreover, the mechanical cleansing, anti-plaque, anti-caries, anti-bacterial, and anti-decay actions have been linked to regular use of sewak in some previous studies. The inhibitory action of sewak extract against certain bacterial species that were growing on Petri dishes has been extensively studied [10-12]. These studies were often focused on a few microbial species. No study reported so far has examined the effect of sewak use on the comprehensive composition of oral microbiota. Thus, this study aims to comprehensively analyze oral microbiota composition, using 16S rRNA gene sequencing, in oral fluids samples collected from young healthy individuals, before and after the use of sewak.

Materials and Methods

Project approval, participant enrollment and survey

The research was approved by the Institutional Review Board at Tennessee State University (IRB# FWA00007692). Ten healthy, randomly selected participants were recruited for this study. Written consent was obtained from each of the volunteer participants. Of this group, one was excluded, and three were lost to follow up. The six healthy individuals who continued in this study were from both genders aged between 24 and 34 with no symptoms of oral diseases, as claimed themselves. A survey questionnaire containing questions of health history, food habits, oral health and general information that needed 10 minutes to complete was given to volunteers before samples collection. Written permission forms were signed from all subjects after providing information about this study and details about sample collection and use of sewak.

Sewak brand and instructions of use

Sewak sticks, Alfalah brand, labeled with the instruction of use were shipped from Saudi Arabia to the USA. Participants were requested to chew sewak tip repeatedly until the fibers appeared like a toothbrush (the fibers were trimmed by participants every 24 hours). Then, they were asked to apply the cleaning action directed away from the gingival margin of the teeth.

Collection criteria and processing of samples

Each participant was asked to provide 3 mouthwash samples, each about 10 ml, using Scope mouthwash (original brand) in a two-week period. The first two mouthwash samples were collected one week apart in the morning of Friday, as a control before any eating or drinking. After the second sample collection, participants were asked to use sewak for one week besides their regular brush routine, at least five times a day or frequently whenever possible. Then, each participant was asked to collect the third mouthwash sample early in the morning on Friday. All eighteen samples were centrifuged, vortexed; then, the buccal cells were suspended and stored in the 80°C freezer until use.

DNA extraction and amplification

The QlAamp Mini kit (Qiagen) was used for genomic DNA isolation from the eighteen samples, following the manufacturer's protocol. Briefly, the bead beating lysis was done in ATL buffer. Three cleaning solution steps were applied, and ATL buffer was added to the cleansing number 3 solution and samples were incubated at 55 °C for 1 hour with proteinase-K (Sigma-Aldrich, 100 ug/ul). Samples were washed twice with AW1 and AW2. Finally, pellets were resuspended in DNase-RNase-free water (Sigma-Aldrich) and the purity and concentration of each extracted DNA were assessed using a Nano Drop spectrophotometer (NanoDrop Technologies, Willmington, DE). The amplification of extracted DNA was done using the NEXTflex 16S V1-V3 amplicon sequencing kit (196-4202-04).

16S rRNA gene sequencing

Phylogenetic classifications analysis of the oral microbiota in each mouthwash sample were done using the 16S metagenomics sequencing library approach (on the Illumina platform, by Omega Bioservices). The protocol includes the amplification of the V3 and V4 regions using a primer pair that creates a single amplicon of approximately ~460 bp. A full complement of Nextera XT indices was used for library preparation. For sequencing on MiSeq, paired-end 300-bp reads and MiSeq v3 reagents were used. For taxonomic classification, kingdom, phylum, class, order, family, genus and species, the Metagenomics Workflow was performed using the Greengenes database (http://greengenes.lbl.gov/) showing genus and species level classification in a graphical format. The Illumina protocol with a benchtop sequencing system, primary analysis, and secondary analysis using BaseSpace (cloud-based software) has been applied to provide a comprehensive workflow for 16S rRNA amplicon sequencing.

Statistical analysis

Recorded data were statistically analyzed using a paired t-test, ANOVA and Chi-square test.

Results and Discussion

All previous studies of sewak used traditional methods to test its inhibition activity against some selected bacterial species. In contrast, this study used 16s rRNA gene sequencing to obtain a comprehensive profile and comparisons of the diversity and community structure changes of oral microbiota in saliva samples (before and after the use of sewak) of six healthy, non-smoker participants (aged ≥24 years, in Nashville, TN, USA). Each participant provided two samples along with his or her oral hygiene routine and one sample after one week of sewak treatment.

Around 384,344 reads per sample were obtained. Among the 6,620,906 reads in total, 6,620,630 reads were bacteria, while 235 and 41 reads were viruses and Archaea respectively (Figure 1). Bacterial reads were classified based on the Greengenes database (http://greengenes.lbl.gov/) at different taxonomic levels (Kingdom, phylum, class, order, family, genus, and species).

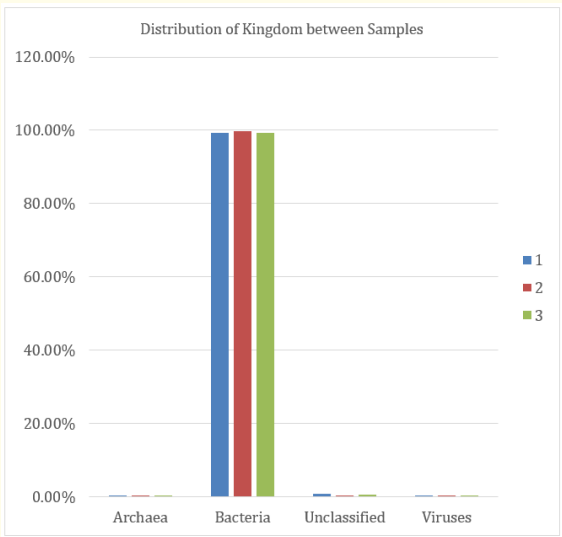

Figure 1: Reads distribution in saliva samples.

We were able to detect 28 phyla, 58 classes, 110 orders, 236 families, 644 genera, and 1,643 species in total. The mean proportions of bacterial phyla and families among samples from all participants was shown in Figures 2 and 3. Phyla *Firmicutes* had most abundant reads, followed by *Bacteroidetes, Protobacteria*, and *Actinobacteria* (see Figure 2). About 50% of reads belonged to *Firmicutes* in which *Streptococcaceae* was found most abundant at family level in all samples (see Figure 3), which agrees with previously published literature that shows that the relatively higher frequencies of this family correspond with the healthier oral cavity [13].

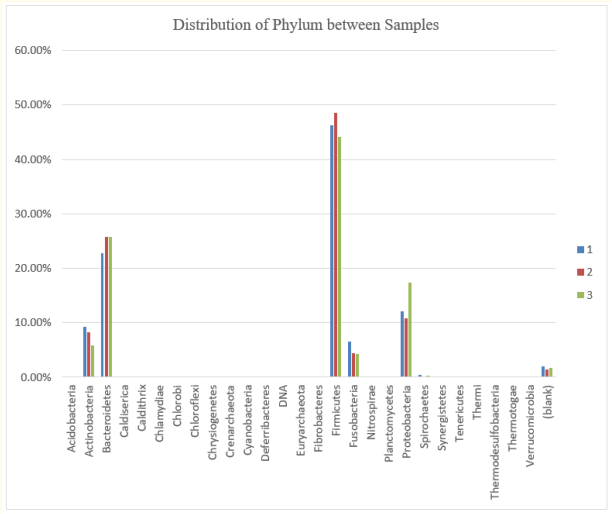

Figure 2: The distribution of the major phyla found in the 18 mouthwash samples, 1=first control samples, 2=second control samples control, and 3= after treatment samples.

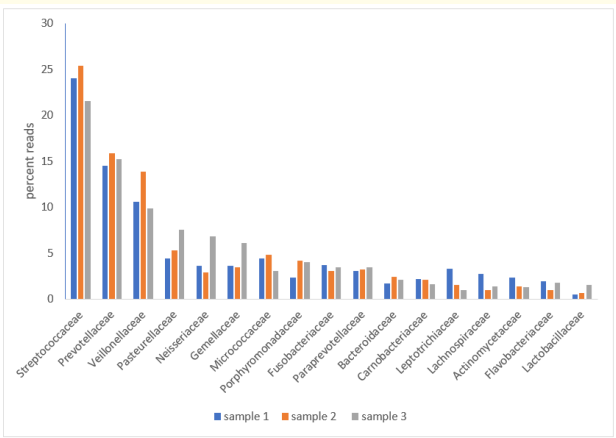

Figure 3: The distribution of major families (abundance > 1%) found the 18 mouthwash samples, 1=first control samples, 2=second control samples, and 3= after treatment samples.

The proportions of oral bacteria species were compared between samples collected before and after sewak treatment. Differences were found at several taxonomic levels when paired-end t test was applied to compare each "taxa" of bacteria between the control and

the treatment (P < 0.05). At the species level, the proportions of 27 "taxa" were reduced with the sewak treatment; whereas those of 16 taxa were increased (P < 0.05, see Table 1, 2). However, the proportions of the majority of bacterial species level taxa were unchanged (Data not shown).

Several streptococcus species (i. e., *S. vestibularis, S. fryi, S, milleri, S. anginosus*) were reduced significantly after sewak treatment (P<0.05, Treatment<Control). This finding agrees with the previous finding that sewak inhibits some streptococcus spp. *S. vestibularis* is the most abundant bacteria whose proportion was decreased 5 fold after one-week sewak use (P < 0.004). In recently years, *S. vestibularis* has been frequently reported as a pathogen associated with diseases [14-16]. *Butyrivitrio proteoclasticus* is the second most abundant species whose proportion was decreased markedly (P < 0.05). This bacterium is abundant in the rumen of ruminants, playing an important role in breakdown of plant polysaccharides [17]. The proportion of bacteria in genus *Leptotrichia* was also decreased about 6-fold. These bacteria were moderately abundant in the human oral cavity. Five species of *Leptotrichia* are known from human source [18]. They may be associated dental caries [19]. *Rothia dentocariossa* is also moderately abundant. After sewak treatment for one week, the proportion of *R. dentocariosa* was decreased by 2.5-fold. Rothia spp. are well-known to be involved in the formation of biofilm on teeth [20]. Another moderately abundant species whose proportion was decreased after one-week sewak use is *Atopobium parvulum*. This species is associated with halitosis (oral malodor), but not associated significantly with chronic periodontitis [21]. The rest of the bacteria species found significantly reduced after one-week sewak use were less abundant

Order	Family	Genus	Species	Control mean	Treatment mean	P
Lactobacillales	*Streptococcaceae*	*Streptococcus*	*vestibularis*	7033	1380	0.004
Clostridiales	*Lachnospiraceae*	*Butyrivibrio*	*proteoclasticus*	1272	454	0.02
Fusobacteriales	*Leptotrichiaceae*	*Leptotrichia*		950	160	0.01
Fusobacteriales	*Leptotrichiaceae*	*Leptotrichia*	*wadei*	950	160	0.017
Actinomycetales	*Micrococcaceae*	*Rothia*	*dentocariosa*	911	372	0.04
Coriobacteriales	*Coriobacteriaceae*	*Atopobium*	*parvulum*	746	353	0.007
Lactobacillales	*Streptococcaceae*	*Streptococcus*	*fryi*	452	128	0.001
Lactobacillales	*Streptococcaceae*	*Streptococcus*	*milleri*	401	225	0.02
Lactobacillales	*Streptococcaceae*	*Streptococcus*	*anginosus*	184	37	0.01
Fusobacteriales	*Fusobacteriaceae*	*Propionigenium*	*modestum*	163	56	0.02
Actinomycetales	*Actinomycetaceae*	*Actinomyces*	*turicensis*	120	33	0.04
Actinomycetales	*Actinomycetaceae*	*Actinomyces*		120	33	0.04
Bacillales	*Paenibacillaceae*	*Paenibacillus*	*darangshiensis*	82	57	0.04
Bifidobacteriales	*Bifidobacteriaceae*	*Bifidobacterium*	*bombi*	68	37	0.03
Actinomycetales	*Actinosynnemataceae*	*Actinokineospora*	*inagensis*	63	37	0.02
Fusobacteriales	*Leptotrichiaceae*	*Sebaldella*		63	37	0.02
Thermotogales	*Thermotogaceae*	*Marinitoga*		63	37	0.02
Coriobacteriales	*Coriobacteriaceae*	*Atopobium*		49	30	0.03
Coriobacteriales	*Coriobacteriaceae*	*Atopobium*	*fossor*	49	30	0.03
Clostridiales	*Lachnospiraceae*	*Blautia*	*wexlerae*	33	11	0.01
Actinomycetales	*Micromonosporaceae*	*Micromonospora*		33	11	0.01
Lactobacillales	*Streptococcaceae*	*Streptococcus*	*plurextorum*	24	19	0.03
Clostridiales	*Peptococcaceae*	*Peptococcus*		24	19	0.03
Clostridiales	*Veillonellaceae*	*Propionispora*	*hippei*	24	19	0.03
Lactobacillales	*Carnobacteriaceae*	*Trichococcus*		24	19	0.03
Clostridiales	*Clostridiaceae*	*Mogibacterium*		8	1	0.03
Actinomycetales	*Microbacteriaceae*			8	1	0.03

Table 1: Bacteria found decreased after the use of sewak.

In contrast, the proportions of reads of *Haemophilus parainfluenzae, Neisseria mucosa, Snowella rosea, Selenomonas noxia, Gemella cunicula, Gemella sanguinis* and *Gemella haemolysans* increased after one-week sewak use (P<0.05, Table 2). *H. parainfluenzae* was one of the very abundant oral bacteria. The proportion of H. parainfluenzae was increased by 125% after one-week of sewak use. This is often non-pathogenic, but is increasingly recognized as opportunistic pathogen [22]. Neisseria mucosa was very abundant but increase by 160% after one-week sewak use. *N. mucosa* rarely causes disease [23]. *Gemella sanguinis* and *Gemella haemolysans* were abundant in mouthwash samples in which their proportions were significantly increase after sewak use. *G. haemolysans* is recognized to cause disease occasionally [24]. The rest of the increased species after one-week sewak use were in low abundance (Table 2).

Order	Family	Genus	Species	Control mean	Treatment mean	P
Pasteurellales	*Pasteurellaceae*	*Haemophilus*	*parainfluenzae*	6370	14387	0.04
Neisseriales	*Neisseriaceae*	*Neisseria*	*mucosa*	4725	12251	0.005
Gemellales	*Gemellaceae*	*Gemella*	*sanguinis*	2226	3839	0.03
Gemellales	*Gemellaceae*	*Gemella*	*haemolysans*	1816	5436	0.02
Flavobacteriales	*Flavobacteriaceae*	*Capnocytophaga*	*leadbetteri*	127	290	0.02
Clostridiales	*Peptococcaceae*	*Desulfotomaculum*	*indicum*	102	124	0.007
Clostridiales	*Veillonellaceae*	*Selenomonas*	*noxia*	58	111	0.033
Halanaerobiales	*Halanaerobiaceae*	*Halanaerobium*	*alcaliphilum*	23	55	0.011
Chrysiogenales	*Chrysiogenaceae*	*Desulfurispirillum*	*alkaliphilum*	23	55	0.03
Bacillales	*Planococcaceae*	*Lysinibacillus*	*parviboronicapiens*	18	26	0.03
Burkholderiales	*Oxalobacteraceae*	*Oxalobacter*	*vibrioformis*	13	22	0.04
Lactobacillales	*Lactobacillaceae*	*Lactobacillus*	*johnsonii*	6	121	0.03
Burkholderiales	*Burkholderiaceae*	*Burkholderia*	*ubonensis*	5	12	0.005
Chroococcales	*Gomphosphaeriaceae*	*Snowella*	*rosea*	5	19	0.02
Alteromonadales	*Alteromonadaceae*	*Marinobacter*	*arcticus*	4	8	0.01
Clostridiales	*Veillonellaceae*	*Selenomonas*	*infelix*	3	16	0.0003

Table 2: Bacteria increased after sewak use.

Statistical analysis in this study shows substantial inter-individual differences which confirms previous studies claiming that the structure of the human microbiota within one site varies even in the same individual and variation can be detected in the healthy people [25,26]. When comparing sample 1 and sample 2, we also found differences at the similar extent which suggest that the effect of sewak s relatively small. This could result from the short period of treatment. Moreover, the number of subjects in this study is relatively small, and inter-individual differences of microbiota associated with sewak antimicrobial activity might become apparent when larger numbers of subjects are included.

Conclusion

In this study, the use of 16s rRNA sequencing approach, taxonomic classification, and statistical analysis determined changes of oral microbiota composition in saliva samples after the use of sewak. Firmicutes was found to be most abundant taxon in all samples. Two streptococcus species, belonging to Firmicutes, significantly lower after sewak treatment were S. fryi and S. vestibularis (P < 0.05). Some bacterial species were increased after sewak, and the majority were unchanged. The microbiome approach could not detect overall abundancy changes since the method is designed to measure composition changes, not absolute changes. A greater number of samples are needed to minimize the possible effect of inter-individual variability. Taking all limitations into consideration, 16s r RNA metagenomics library sequencing technique will advance our understanding of sewak antimicrobial activity.

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Bibliography

1. Dewhirst FE., *et al*. "The human oral microbiome". *Journal of Bacteriology* 192 (2010): 5002-5017.

2. Yamashita Y and Takeshita T. "The oral microbiome and human health". *Journal of Oral Science* 59 (2017): 201-206.

3. Flynn KJ., *et al*. "Metabolic and Community Synergy of Oral Bacteria in Colorectal Cancer". *mSphere* 1 (2016).

4. Zaura E., *et al*. "Acquiring and maintaining a normal oral microbiome: current perspective". *Frontiers in Cellular and Infection Microbiology* 4 (2014): 85.

5. Karpinski TM. "Role of Oral Microbiota in Cancer Development". *Microorganisms* 7 (2019).

6. Shiga K., *et al*. "Presence of Streptococcus infection in extraoropharyngeal head and neck squamous cell carcinoma and its implication in carcinogenesis". *Oncology Report* 8 (2001): 245-248.

7. Guerrero-Preston., *et al*. "16S rRNA amplicon sequencing identifies microbiota associated with oral cancer, human papilloma virus infection and surgical treatment". *Oncotarget* 7 (2016): 51320-51334.

8. Beiruti N. "Views on oral health care strategies". *Eastern Mediterranean Health Journal* 11 (2005): 209-216.

9. Haque M., *et al*. "A review of the therapeutic effects of using miswak (Salvadora Persica) on oral health". *Saudi Medicine Journal* 36 (2015): 530-543.

10. Siddeeqh S., *et al*. "Estimation of Antimicrobial Properties of Aqueous and Alcoholic Extracts of Salvadora Persica (Miswak) on Oral Microbial Pathogens - An Invitro Study". *Journal of Clinical and Diagnostic Research* 10 (2016): FC13-FC16.

11. Al-Ayed MS., *et al*. "Antibacterial Activity of Salvadora persica L. (Miswak) Extracts against Multidrug Resistant Bacterial Clinical Isolates". *Evidence-Based Complementary and Alternative Medicine* 2016 (2016): 7083964.

12. Amir Alireza RG., *et al*. "Inhibitory activity of Salvadora persica extracts against oral bacterial strains associated with periodontitis: An in-vitro study". *Journal of Oral Biology and Craniofacial Research* 4 (2014): 19-23.

13. Palmer RJ., "Composition and development of oral bacterial communities". *Periodontology* 64 (2000): 20-39.

14. Tufan MA., *et al*. "Spondylodiscitis and endocarditis caused by S. vestibularis". *The Brazilian Journal of Infectious Diseases* 14 (2010): 377-379.

15. Koumaki D., *et al*. "Ecthyma gangrenosum caused by Klebsiella pneumoniae and Streptococcus vestibularis in a patient with acute myeloid leukemia: an emerging pathogen". *International Journal of Dermatology* 58 (2019): E83-E85.

16. Yilmaz F., *et al*. "Streptococcus vestibularis: A Rare Cause of Peritoneal Dialysis-Related Peritonitis". *Therapeutic Apheresis and Dialysis* 21 (2017): 418-419.

17. Kelly WJ., *et al*. "The glycobiome of the rumen bacterium Butyrivibrio proteoclasticus B316(T) highlights adaptation to a polysaccharide-rich environment". *PLoS One* 5 (2010): e11942.

18. Eribe ER., *et al*. "Genetic diversity of Leptotrichia and description of Leptotrichia goodfellowii sp. nov., Leptotrichia hofstadii sp. nov., Leptotrichia shahii sp. nov. and Leptotrichia wadei sp. Nov". *International Journal of Systematic and Evolutionary Microbiology* 54 (2004): 583-592.

19. Thompson J and Pikis A. "Metabolism of sugars by genetically diverse species of oral Leptotrichia". *Molecular Oral Microbiology* 27 (2012): 34-44.

20. Uppuluri P., *et al*. "Transcriptional Profiling of C. albicans in a Two Species Biofilm with Rothia dentocariosa". *Frontiers in Cellular and Infection Microbiology* 7 (2017): 311.

21. Copeland A., *et al*. "Complete genome sequence of Atopobium parvulum type strain (IPP 1246)". *Standards in Genomic Sciences* 1 (2009): 166-173.

22. Gonzalez-Diaz., *et al*. "Identification of polysaccharide capsules among extensively drug-resistant genitourinary Haemophilus parainfluenzae isolates". *Science Report* 9 (2019): 4481.

23. Osses DF., *et al*. "Neisseria Mucosa: A New Urinary Tract Pathogen?" *Current Urology* 10 (2017): 108-110.

24. Salceanu SO., *et al*. "Severe Gemella haemolysans endophthalmitis following ranibizumab intravitreal injection". *Indian Journal of Ophthalmology* 65 (2017): 1249-1251.

25. Thomas S., *et al*. "The Host Microbiome Regulates and Maintains Human Health: A Primer and Perspective for Non-Microbiologists". *Cancer Research* 77 (2017): 1783-1812.

26. Muszer M., *et al*. "Human Microbiome: When a Friend Becomes an Enemy". *Archivum Immunologiae et Therapiae Experimentalis* (Warsz) 63 (2015): 287-298.

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