

Electronic Nicotine Delivery Systems and the Human Oral Microbiome – A Systematic Review of the Literature

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Introduction

Electronic nicotine delivery systems (ENDS), also called ”vapes” or “e-cigarettes”, have become very popular in the last decade but there is very little data regarding the effects of these devices on oral health. The oral microbiome is the collection of the genomes of all the organisms in the mouth including bacteria, viruses, and fungi. The composition of the oral microbiome has been associated with a variety of health conditions, including cancer. It is well established that cigarette use is associated with dysbiosis of the oral microbiome, but there is limited data about the impact of ENDS. Therefore, the goal of this systematic literature review was to add more clarity regarding how the use of electronic nicotine delivery systems affects the oral microbiome.

Methods

A literature search was conducted via PubMed using the following search terms: ("e-cigarette" OR "electronic nicotine" OR vape OR vaping OR juul OR "electronic cigarette") AND (microbiota OR microbiome OR flora OR bacteria OR yeast OR biofilm OR microflora). For inclusion, manuscripts needed to be original peer-reviewed English language research studies addressing the oral microbiome or individual components in which ENDS users were compared to a non-ENDS-user control group. References of all eligible studies and applicable review articles were additionally screened to ensure capture of all applicable studies.

Results

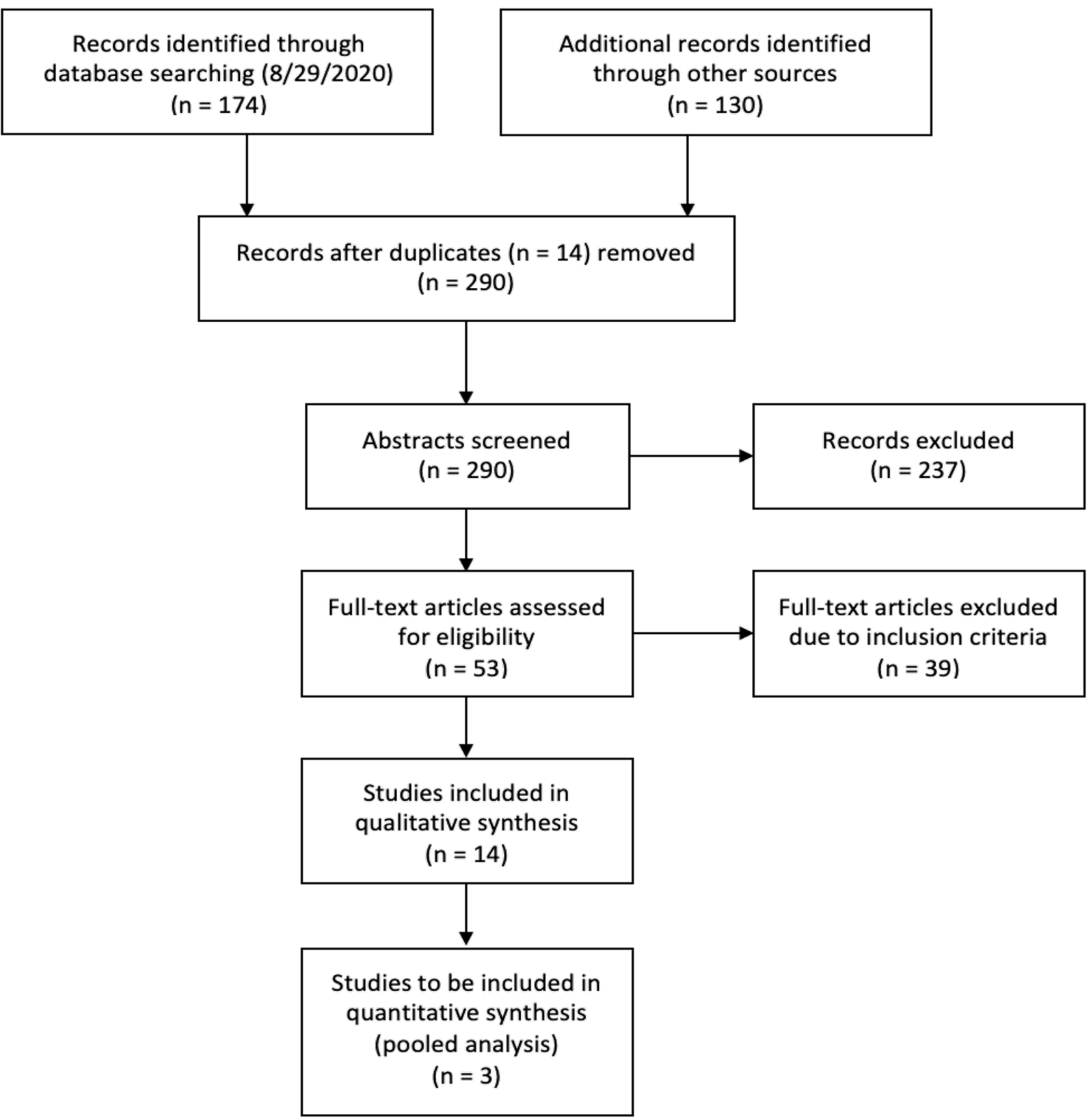


Figure 1: Flow chart for article identification and selection

Table 2: Activity of Individual Microbes of Interest

Microbe/Microbiome/Bio-diversity/Biofilm	Gram (+) or Gram (-)	Control (NS)	Cigarette (CS) Compared to Control	ENDS (ES) Compared to Control	Evidence
Aggregatibacter	Gram (-)	-	-	Increased	Ganesan, et al.
C. albicans	Gram (+)	-	Significantly increased	Significantly increased	Mokeem, et al.
C. albicans	Gram (+)	-	N/A (not tested)	Increased	Alanazi, et al.
Corynebacterium durum	Gram (+)	-	-	Increased	Pushalkar, et al
Fusobacterium nucleatum subsp. Vincentii	Gram (-)	-	Increased	Increased	Pushalkar, et al
Kingella	Gram (-)	-	-	Increased	Ganesan, et al.
Neisseria	Gram (-)	Increased (compared to CS)	No change	Increased (compared to CS)	Pushalkar, et al
P. gingivalis	Gram (-)	-	-	Increased	Pushalkar, et al

Table 1: Manuscripts Included in Systematic Review

Study	Year Published	Sample Size (n)	Population	Sample Type (saliva, etc.)	Outcome Interested In	Sequencing Method	ENDS Type (if specified)
Pushalkar, et al.	2020	119	ENDS users vs nonsmoker and cigarette smoker controls	Scrapings of oral mucosa/saliva	Dysbiotic oral microbial ecology	16S	Not specified
Stewart, et al.	2018	30	ENDS users and tobacco smokers vs non-smoker controls	Cross sectional fecal, buccal swabs, & saliva	Does ENDS use or smoking alter the oral and gut microbiota	V4 16S rRNA	Not specified
Bagale, et al.	2020	6	in vitro assays and in vivo mouse model	in vitro virulence assays and in vivo mouse model of acute pneumonia	Exposure to nicotine-containing and nicotine-free EV on the TIGR4 transcriptome	Other	Strawberry flavored e-liquid formulations (APII by Bomb Sauce, Atlanta GA) with 70% vegetable glycerin and 30% propylene glycol, either with 3mg/ml nicotine or nicotine free, purchased from a local vape shop.
Kumar, et al.	2019	30/125	Patients with periodontitis	Saliva	The impact of ENDS use on oral community membership	16S and Shotgun	Not specified
Ganesan, et al	2020	123 (5 groups)	Systemically and periodontally healthy individuals	Subgingival plaque samples from 15 sites on six maxillary and mandibular anterior teeth using sterile endodontic paper points. Also collected gingival crevicular fluid (GCF)	Effects of ENDS use on the subgingival microbiome	Shotgun	Not specified
Alanazi, et al.	2019	5	C. albicans culture	1 control and 4 50mL sterile culture tubes with 10*6 cells of C. albicans and 4 experimental conditions	The impact of e-cigarettes on C. albicans growth and expression of different virulence genes, such as a secreted aspartic proteases (SAPs), and the effect of ENDS exposed C. albicans on sites in the oral cavity	Other	eGo ONE CT electronic cigarette devices
Cichonska, et al.	2019	120	ENDS users	Saliva (mixed unstimulated whole saliva)	Estimate changes in selected physicochemical properties of saliva	Other	N/A
Cuadra, et al.	2019	4	Streptococci culture	S. gordonii DL1, S. intermedius 0809, S. mitis UF2 and S. oralis SK139 grown in brain heart infusion (BHI) broth with 5micrograms/mL hemin or BHI agar at 37 degrees C and 5% CO2	Effect of flavorless ECIG aerosol on the variability of oral commensal streptococci	Other	E-liquid composed of 50% propylene glycol and 50% vegetable glycerin with or without (+/-) 20 mg/mL of (S)-(-)-nicotine
Mokeem, et al.	2018	129	ENDS and waterpipe-smokers vs. ENDS users and never-smoker controls	Oral rinse with 9mL sterile 10% phosphate buffered saline for 60s and expectorated	Compare oral Candida carriage	Other	Not specified
Nelson, et al.	2019	10	Growth medium containing streptococci	S. gordonii DL1, S. mitis UF2, S. oralis SK139 grown in brain heart infusion (BHI) broth with 5micrograms/mL bovine hemin or BHI agar at 37 degrees C and 5% CO2	Does e-liquid (from ENDS) have less less impact on oral commensal streptococci than cigarette smoke	Other	E-liquid composed of 50% PG and 50% VG (aka glycerol) with or without (+/-) 20 mg/mL of 99% (S)-(-)-nicotine. No flavoring added. 20/80 propylene glycol/glycerin (by volume fraction). 10 mg/ml nicotine, and flavors. Aerosols generated by a Universal Electronic-Cigarette Testing Device (49.2 W, 0.2 Ohms)
Kim, et al.	2018	N/A	Streptococcus mutans UA157 (ATCC) culture	Streptococcus mutans UA157 (ATCC)	Changes in cariogenic potential when tooth surfaces are exposed to ENDS aerosols generated from well-characterized reference e-liquids with sweet flavors	Other	E-liquid composed of 50% PG and 50% VG (aka glycerol) with or without (+/-) 20 mg/mL of 99% (S)-(-)-nicotine. No flavoring added. 20/80 propylene glycol/glycerin (by volume fraction). 10 mg/ml nicotine, and flavors. Aerosols generated by a Universal Electronic-Cigarette Testing Device (49.2 W, 0.2 Ohms)
Gilpin, et al.	2019	N/A	ENDS, cigarette smoke, and non-exposed bacteria	Biofilm formation	Compared the effect of ENDS and cigarette smoke (CSE) on the virulence and inflammatory potential	Other	Vapourites VL-EGO 650
Aidakheel, et al.	2020	60	Cigarette smokers and ENDS users with periodontitis when compared to non-smokers without periodontitis	Subgingival oral-biofilm samples	Quantify pathogenic bacteria isolated from the subgingival oral biofilm samples	Other	Not specified
Rouabhiia, et al.	2020	N/A	Biofilms of Streptococcus mutans	Biofilms of Streptococcus mutans , permanent molar and premolar teeth which had been extracted.	Evaluate the effect of ENDS on the growth, the formation of biofilm, and the expression of certain virulence genes	Other	eGo ONE CT electronic cigarette devices

Conclusions

The use of ENDS is associated with changes in the oral microbiome that have deleterious effects on human health.

- Bacteria with known associations with malignancy, such as *P. gingivalis*, *F. nucleatum*, *Aggregatibacter*, and *Neisseria* species, were increased in concentration with the use of ENDS compared to non-users
- Candida* species were increased in concentration with the use of ENDS compared to non-users
- Candida* is known to convert ethanol into its carcinogenic byproduct, acetaldehyde
- Kingella* and *Corynebacteria* were also increased with the use of ENDS –interestingly, these bacteria are shown to have protective effects.

Future Directions

We are conducting a pooled-analysis with the available genomic sequence data to allow us to appraise overall trends in human oral microbiome composition that occur with the use of ENDS. We are still in the process of analyzing all the data, but trends we are seeing now include:

- A wide variety of alpha diversity in ENDS user samples
- Campylobacter* and *Micrococci* are enriched in ENDS user samples
- When we compared beta diversity, non-smokers (NS) are more similar to other non-smokers than to ENDS users (ES) or to cigarette smokers (CS) (p = <0.005), as seen in Figure 2.
- Other findings show that CS and ES are more similar to one another than to NS

