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Title:

The efficacy of bio-aerosol reducing procedures used in dentistry: a systematic review

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Abstract

Background and objective:

Microbe laden air particles, known as bio-aerosols, are routinely generated, in clinical dentistry due to the operative instrumentation within a milieu rich in salivary organisms. As the major mode of transmission of SARS-CoV-2 appears to be airborne aerosols and droplets, there has been an intense focus on such aerosol generating procedures (AGP). As there has been no systematic reviews on the efficacy of bio-aerosol reducing measure in dentistry, the objective of this systematic review was to evaluate the literature on three major AGPs, rubber dam application, pre-procedural oral rinse, and high-volume evacuation (HVE) aimed at reducing dental bio-aerosols.

Method:

PubMed via Ovid MEDLINE, EBSCO host, Cochrane Library, and Web of Science databases between January 01, 1985, and April 30, 2020, were searched.

Results:

A total of 156 records in the English language literature were identified, of which 17 clinical studies with 724 patients were included in the final analyses. The eligible reviewed articles revealed the inadequacy of the afore mentioned three principal AGPs used in contemporary dental practice to minimise bio-aerosols. HVE appears to be the most efficacious method, although no single approach provides total elimination of bio-aerosols.

Conclusion:

This, the first systematic review on methods of controlling bio-aerosols in dental operatory settings, indicates that employing combination strategies of rubber dam, with a pre-procedural antimicrobial oral rinse, and HVE can significantly minimize bio-aerosols. As the quality of the currently available data on dental bio-aerosols are rather poor, further, controlled, multi-centre studies are essential to address this critical issue.

(250 words)

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Introduction:

An aerosol can be defined as a suspension of fine solid particles or liquid droplets in air or another gas. Aerosols can be either natural, such as fog, mist, dust, or, anthropogenic - created by humans or animals when they speak, sneeze, or cough, for instance. On the other hand, bio-aerosols are aerosols comprising particles/droplets with live microorganisms [1, 2]. The characteristics of bio-aerosols and their spread vary considerably depending on numerous factors, as discussed below.

Although there are conflicting reports in the literature on the size of the aerosols and how long they are airborne, early researchers have classified particles <100 μm in diameter as aerosols, and those > 100 μm as droplets or `spatter/splatter`[1]. The latter, usually falls on to the ground immediately, as and when they are expelled. The former, aerosols, on the contrary, may be entrained or suspended in the air for considerable periods depending on the humidity, airflow, and temperature of the environment into which they are expelled, for example, a dental clinic operatory or a hospital ward. Similarly, the large diameter droplets with the offending agents can contaminate surfaces in the immediate vicinity and spread a few meters, yet again depending on the ambient conditions such as the airflow [3].

As mentioned, bio-aerosols can be generated either by humans or animals (anthropogenic) or by various mechanical devices. Humans produce bio-aerosols by talking, breathing, sneezing, or coughing [1, 4, 5], depending on the infectious state of a person, and these may contain fungi, bacteria, or viruses. On the other hand, mechanical devices such as clinic/hospital ventilation systems, air conditioners, air rotors with coolant water spray used in dentistry may spread bio-aerosols equally efficiently, and engineering strategies such as high efficiency particulate filters (HEPA) need to be inatlled to minimize or eliminate such spread [6, 7]. It is also noteworthy, in this context, that a number of microbial factors such as their virulence, the number of infectious particles, and the pathogenicity of the offending microbes, as well as host factors including their immune status, determine the susceptibility of acquiring an infectious agent via a bio-aerosol [8-10].

Compared to the population at large, healthcare workers (HCWs) run a higher risk of acquiring respiratory pathogens by virtue of their profession. This was clearly shown in the SARS epidemic, which led to numerous deaths of HCWs [11], and in the current COVID-19 pandemic, where HCWs have disproportionately succumbed to the disease [12]. Although thus far, there has been no mortality amongst dental HCWs due to COVID-19, they are considered to be the professional group that has the highest likelihood of acquiring the disease [13].

SARS-CoV-2- *and bio-aerosols:*

Despite the intricate anatomical configuration of the respiratory system that efficiently filters extraneous pathogenic agents, bio-aerosols have the ability to penetrate deep into the human alveolar sacs [3]. The human nose, for instance, is an efficient filter for particles of approximately 1 µm or more [2] but SARS-CoV-2 with a diameter of 50–200 nanometres can effectively escape this sentinel mechanism. It has been shown in some studies that aerosolized particles ranging from 0.25 to 0.5 µm [8, 14], can enter the bloodstream, and then the target organs. Once inhaled, SARS-CoV-2 uses the angiotensin-converting enzyme-1 (ACE_2) present on eukaryotic cell surfaces, including oral epithelia, as a keyhole for systemic entry [10, 15] making the oral cavity a potential portal for viral entry and subsequent systemic dissemination.

The fact that ACE_2 receptors are present in the aqueous humor of the human eye [16], also implies that the bio-aerosols may take the ophthalmic route to cause upper respiratory tract infections, as the offending pathogen, such as SARS-CoV-2, may has a ready passage *via* the nasolacrimal duct to reach the nasopharynx and the upper and lower respiratory system A laboratory study has demonstrated a high rate of trans-ocular transmission in subjects, following exposure to aerosols containing live attenuated influenza viruses [17]. is strongly suggests that respiratory virions,

Viral load in the oro-pharynx region:

The source of SARS-CoV-2 in the salivary aerosols has been addressed to some extent. The work of To et al., [18] indicate the presence of SARS-CoV-2 in the posterior oropharyngeal (deep throat) saliva samples. SARS-CoV-2 from the latter anatomical regions, as well as the bronchoalveolar regions, as sputum [19, 20] appear to contribute to the total salivary viral load that is expelled in aerosols.

Bio-aerosol generating dental procedures and implications for dentistry:

Many interventional procedures are known to aerosolize respiratory secretions in healthcare settings [6, 20, 21]. In dentistry, viral particles may be aerosolized by the high-speed handpiece and the accompanying air jet, ultrasonic scaling, air polishing, and air/water syringes [21]. Unless judiciously controlled, these bio-aerosol generating procedures appear to be one of many intrinsic hazards the profession faces, that has been brought into focus by the current COVID-19 pandemic [6].

Thus, in a very early laboratory study, Miller et al. [22] observed that aerosolized microbes generated by high-powered dental drills and periodontal scalers could transmit to around 200 cm distance in the operatory. More recently, van Doremalen and colleagues [23] noted that the SARS-CoV-2 virus could remain aerosolized for up to 3 hours [23]. They reported that the viability of SARS-CoV-2 virions is relatively high compared with their counterparts, and hence viable and infectious virions may be suspended in bio-aerosols for hours, and for several days within a shed inoculum, on solid surfaces such as steel and card board [23]. These findings have direct implications in dentistry as the threat of airborne transmission of the virus in dental bio-aerosols, as well as their deposition on the clinic surfaces, have to be appropriately addressed for safe dental practice. Indeed, Li and colleagues [24], during the post-SARS era, suggested additional measures for bio-aerosol reduction, and concomitant safe dental practice. These include manual scaling, chemo-chemical caries removal, atraumatic restorative technique, open debridement for periodontal surgeries, rubber dam isolation, use of pre-procedure oral rinses, general ventilation, air filtration [13, 24]. Most of these have been currently adopted by various dental jurisdictions during the current pandemic [13].

Although the post-SARS era literature provides useful guidance on bio-aerosol reducing procedures in dentistry, there has been, to our knowledge, no contemporary systematic review on this subject during the current COVID-19 pandemic. Thus, we aimed to systematically to gather evidence of standard precautionary measures which attempt to reduce bio-aerosol transmission in dentistry, and reviewed the contemporaneous data on three such major strategies, i) pre-procedural antimicrobial mouth rinse, ii) rubber dam application and iii) high volume evacuation (HVE), used by profession for this purpose.

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Methods:

Data sources:

An electronic data search of English language manuscripts using Ovid MEDLINE, Web of Science, EBSCO host, and Cochrane Library databases was performed by two investigators (LPS and KSF). Published clinical reports were accessed between January 01, 1985, and April 30, 2020. After screening different electronic databases, a total of seventeen clinical studies (rubber dam and bio-aerosols (x4 studies); HVE and bio-aerosols (x6); and pre-procedural oral rinse and bio-aerosols (x7) were identified.

A precise review question was formulated using the PICO framework as follows. Does pre-procedural antimicrobial mouth rinse, rubber dam application, and high volume evacuation (HVE) **(I),** compared with placebo, no mouth antimicrobial mouth rinse/rubber dam application/use of high volume evacuation **(C),** results in effective microbial reduction disseminated via aerosol generated during dental procedures **(O),** in dental patients (male and female) undergoing the dental procedure? **(P).** The search keywords and combination of the keywords were systematized according to the PICO model (Table 1).

Outcome: The key finding of the present review was the systematic assessment of the efficacy of preprocedural mouth rinse, rubber dam application, and use of HVE in reducing bio-aerosols generated via dental procedures.

Study selection:

Inclusion criteria: Pre-determined inclusion criteria were 1) English language articles; 2) clinical trials; 3) patients undergoing dental procedures (use of high-speed rotary instruments/ultrasonic scalers/air polishers/triple syringe); 4) pediatric and adult patients; 5) country or date enforced no limitations.

Exclusion criteria: The exclusion criteria included: 1) review articles; 2) studies that did not report a pre-post microbial reduction in bio-aerosol; 3) reports presenting incomplete outcome details; 4) recruits (patient) on antibiotics; 5) studies that do not allow extraction of data needed to meet the set study objectives; 6) poster/conference presentation/abstracts, grey literature, and unpublished research information were neither considered nor used.

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Electronic data search and analysis: To ensure of systematic and comprehensive method approached, we trailed through the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines.[25, 26] The search approach employed, and results generated, are presented in Fig. 1, and search terms and limits are shown in Table 1.

A three-staged, electronic data search and analysis was carried out. In stage one: the titles and abstracts of all relevant clinical trials meeting our set inclusion criteria were screened. In step-two: a full-text review of all the pertinent articles was completed, which yielded a comprehensive account of the data. Through the full-text review of the retrieved literature, the investigators used spreadsheets, ensuring that eligibility criteria were met and the reported outcomes were in alignment with the set study objectives. References of the included clinical trials were checked as a backward-search. In stage-three, the reviewers extracted and assessed the data.

Following the full-text review, specific points linked to the characteristics of each included clinical trial were charted using the Cochrane model. This enabled in classifying the setting, study design, and the country. Additionally, sample size, assessment methods, evaluation time, and study conclusions were comprehensively examined. The identified articles were compiled using a bibliographic software tool, Endnote version 9 (Clarivate Analytics, USA).

Summary of the characteristics of included clinical trials and the reported results, including the microbial counts generated during dental procedures, are provided in Tables 2, 3, and 4.

Quality Assessment and Overall Risk of bias:

The quality assessment of the eligible clinical studies was performed by two investigators (LPS and KSF) independently. In case, if there was any disagreement, a third or fourth reviewer (CP or BB) were consulted. The Cochrane Collaboration risk of bias-assessment tool was used to assess the methodological quality of the study.[27] This included an evaluation of the method of randomization, allocation- concealment, blinding of the outcome assessment, selective reporting, and other sources of biases. Any discrepancies were resolved via mutual agreements reached between the reviewers. The evaluated trials were documented as low, unclear, or high-risk (Table 5). Studies falling under the high-risk of bias were excluded from the present review.

Results and Discussion:

Efficacy of rubber dam in reducing bio-aerosol generated during dental procedures:

In total, we assessed data from 108 participants (40 pediatric and 68 adults) enrolled in four clinical trials evaluating the efficacy of rubber dam in reducing bio-aerosols. In almost all experimental settings [28-31], bio-aerosols were generated using high and low-speed rotary dental devices during restorative dental procedures. The adjunct use of an air-water syringe and high-speed rotary instrumentation, either with or without a rubber dam, was also examined by Cochran et al. in one study [28]. Their data showed up to 90-98% efficacy of the rubber dam in preventing the spread of bio-aerosol during dental procedures (Table 2). Samaranayake and team [29] also observed an up to70 percent reduction in airborne particles, in and around a 1-meter diameter of the operational area, consequential to the use of rubber dam (in comparison to control procedures without the rubber dam); this effect was zero, on cabinetry surfaces located 3 meters from the operative site.

In one study, Tag El din and colleagues [30] compared the efficacy of rubber dam with the addition of an oral antimicrobial rinse before the rubber dam application. They reported no significant difference in the organisms reaching a culture plate, placed at a 1meter distance from the operative site, irrespective of the pre-procedural rinse. The reduction in CFUs was 98.8% and 99.4% in the rubber dam group and the antiseptic plus rubber dam group, respectively. Apart from the above, Al Amad and colleagues [31] reported a significant bacterial reduction due to rubber dam use, although the information on colony counts was not presented in their report.

The preceding data from the clinical trials conclusively indicate that rubber dam isolation during operative dental procedures is an effective and efficient bio-aerosol suppressor [28-31]. The significant advantage of the rubber dam application in this context is the reduction of salivary, serum, and blood contamination of aerosol plumes laced with infectious microbes. In the event, the microbial content of the bio-aerosol can only arise from the biofilms on the tooth surfaces exposed during treatment [11], thus significantly impacting the overall dental operatory infection control.

Efficacy of High-volume evacuator (HVE) in reducing bio-aerosol generated during dental procedures:

Many dental procedures ranging from routine prophylactic ultrasonic scaling and subgingival restorations to periodontal and oral surgical procedures generate copious bio-aerosols [28, 32, 33]. This has been a major concern, as these procedures are executed in an intraoral milieu with voluminous amounts of microbe laden saliva and blood. Hence, HVE is highly recommended to extract the bio-contaminants originating from the operative site to obviate bio-aerosols released into the operatory environment.

We evaluated data from 128 patients from four experimental settings [32, 34-36], that assessed the efficacy of HVE in reducing bio-aerosol generation; all four studies were conducted when restorative dental procedures were performed. All the included reports [32, 34, 35], except for Desarda et al. [36], noted a significant reduction in CFU when HVE was used during the several dental procedures. The latter group [36] attributed this divergent observation to the reduced efficacy of the evacuation system they used. In another experimental setting where only two patients were used, Bentley et al. [37] observed an efficient reduction of bio-aerosols when HVE was used in a patient who flossed and brushed before ultrasonic scaling. However, the data quality of this study was weak and anecdotal due to the low number of subjects enrolled.

The use of high-speed rotary instruments is common during minor oral surgical procedures, with the simultaneous production of splatter and aerosols [33, 38]. In an elegant study Ishihama et al. have demonstrated `blood mist` carrying potential infectious pathogens in the air of the operatory [38] and the efficacy of an extra-oral evacuator close to the surgical site, that leads to an immediate reduction of this air plume. In one of the most extensive studies, to date, Yamada and colleagues [39] reviewed data from 281 patients who underwent oral surgery as well as therapeutic and prophylactic procedures. They tested HVE efficacy, using filters at two differing distances (50 cm and 100 cm) from the patients' mouth. Not surprisingly, they observed that combined use of two extraoral evacuators was more effective than a single evacuator, in reducing bio-aerosol, particularly during third molar surgeries.

Another factor that affects the efficacy of the HVE is the distance between the operative site and the suction tip of the evacuator. Two studies [33, 38] have documented that HVE, when applied

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close to the surgical site, was significantly better in obviating visible saliva, blood, and water sprays and spatter produced during dental procedures.

The foregoing conclusively demonstrates the efficacy of HVE in reducing bio-aerosols in the clinic environment, and their efficiency is determined by the suction force of the appliance, the proximity of the HVE to the operating site, and the number of evacuators used.

Efficacy of a pre-procedural oral rinse in reducing bio-aerosol generated during dental procedures:

Although a rubber dam can be applied for many operative procedures, it not a realistic option for some treatment modalities such as crown preparations, subgingival restorations, and full mouth prophylaxis with ultrasonic scaling. The latter method in particular, is notorious in the literature as an intense, bio-aerosol, and spatter producing intervention [40, 41]. Hence it is not startling that a vast majority of the clinical investigations we reviewed appertained to this interventional procedure [42-48]. In total, the database comprised 128 patients, and the bacterial content of the dental aerosols generated through ultrasonic scaling and air polishing procedures, either with or without pre-procedural oral rinsing.

Three major and most popular antiseptics used for pre-procedural oral rinses are chlorhexidine gluconate (CHX), cetyl pyridinium chloride (CPC), and essential oils, and this practice has been shown to reduce bio-aerosols significantly [42-47]. Indeed, chlorhexidine and essential oils are effective antiseptics in reducing the load of both the planktonic organisms suspended in saliva, and those residing within biofilms, either on mucosal or tooth surfaces [6, 49-51].

The vast majority, mainly randomized controlled trials, in six of seven experimental settings [42-47], we evaluated indicated a significant bacterial reduction after pre-procedural rinsing with either CHX, CPC, or essential oils. One study [48], however, was an exception, as it reported increased numbers as well as bacterial diversity with preprocedural CHX oral rinses, during debonding of orthodontic-brackets using a low-speed handpiece. The authors did not offer an explanation for this curious result.

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These randomized controlled trials (RCT) unequivocally imply that a preprocedural antimicrobial oral rinse efficaciously reduces the number of viable microbes during substantial - bio-aerosol generating procedures such as ultrasonic scaling [42-47]. Nevertheless, a few of these reports had inherent weaknesses. For instance, we noted imprecise information on the allocation concealment in one study , which may have led to inflated estimates of the treatment effect [52]. Moreover, for valid estimates of the effect size of the intervention in RCTs, blinding of the participants and assessors is crucial [53, 54]. Yet, the foregoing information was presented only by Fine et al., in their two studies [42, 43].

Which bio-aerosol mitigating method is superior?

Infective pathogens suspended and entrained in air can be a source of many infectious diseases [1, 38]. Researchers in almost all of the preceding reviewed studies determined that a significant reduction in bacteria-laden aerosols could be achieved by the three major interventional procedures discussed, *viz*. rubber dam application, HVE, and preprocedural oral rinses. Additionally, almost all of these workers arrived at this conclusion using the traditional bacteriological culture plate exposure detection methods, which paints an incomplete picture of the airborne microbial load. There is, for instance, virtually no data in the literature on the aerosol dissemination of other constituents of the oral microbiome such as fungi, and above all viruses. It is hoped that the advent of the novel molecular analytical techniques such as next-generation sequencing (NGS) could redress the situation and shed some light on the all-important viral dissemination that may be associated with dental interventional procedures.

Taken together, there is inadequate data to state the superiority of one method above the others in reducing the generation of bio-aerosols during dental procedures, as all procedures discussed above lead to varying degrees of bio-aerosol reduction. In clinical terms, therefore, judicious selection of one or more methods by the clinician depending on the operative procedure in question is critically important. Nevertheless, HVE must be a compulsory requirement during almost all dental procedures. Additionally, the role of extraneous strategies such as engineering controls of the air evacuation processes of the surgery (not discussed here) that are equally important in the reduction of bio-aerosols in the dental clinic environment should be borne in mind when addressing this issue.

Conclusions

Bioaerosols are generated in clinical dentistry during multiple interventional procedures. The current review summarizes three major approaches used in contemporary dental practice to minimise such bio-aerosols, rubber dam application, pre-procedural oral rinse, and HVE. Altogether our review of a total of 17 clinical studies indicate that HVE is an obligatory requirement to reduce bio-aerosols in dentistry, while rubber dam application and pre-procedural oral rinses must be utilised when opportune, since all three procedures significantly reduce bio-aerosols. As the quality of the currently available data on bio-aerosols in dentistry are rather scanty, and wanting, further, rigorously controlled, multi-centre studies are urgently needed to address this important issue of containing contagious viral infections that appear to pose a constant infection transmission threat in the dental operatory.

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Contributions

LPS, together with KSF and CP performed data collation analysis and drafting the manuscript. BB assisted in data evaluation, manuscript writing and editing. All four authors reviewed, and agreed on the final version of the review to be published, and to be responsible for all aspects of the work.

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Table 1. Employed search-terms and limits

Search strategy for the systematic review of literature on the bioaerosols and standard dental practice protocol in post-COVID-19 era		
Search history		
Search (Database/s)		(January 01, 1985, and April 30, 2020)
Cochrane Library databases	***Bioaerosol and rubber dam***	(rubber dam OR dental dam) AND (aerosol OR bioaerosol) AND (bacterial reduction OR microbial reduction) AND (dental operatory OR dental clinic) AND (dry field OR moisture control) AND (saliva OR blood)
	Bioaerosol and high-volume evacuator (HVE)	(aerosol OR bioaerosol) AND (extraoral suction OR extra oral evacuator) AND (bacterial reduction OR microbial reduction) AND (dental operatory OR dental clinic) AND (high volume evacuator OR high volume suction) AND (saliva OR blood) AND (evacuators OR suction)
	Bioaerosol and preprocedural oral rinse	(aerosol OR bioaerosol) AND (mouth rinse OR oral rinse) AND (mouth wash OR prophylactic mouth wash) AND (dental operatory OR dental clinic) AND (anti-microbial OR antibacterial) AND (anti-viral OR anti-fungal) AND (Chlorhexidine OR CHX OR Essential Oil) AND (Cetylpyridinium chloride OR CPC) AND (scaling OR ultrasonic) AND (high-speed rotary OR high-speed handpiece) AND (microbial reduction OR bacterial reduction) AND (preprocedural oral rinse OR preprocedural mouth rinse)
PubMed via OVID	***Bioaerosol and rubber dam***	Heading (MeSH) and text words: (aerosol OR bioaerosol) AND (rubber dam OR dental dam) AND (bacterial reduction OR microbial reduction) AND (dental operatory OR dental clinic) AND (dry field OR moisture control) AND (saliva OR blood)
	Bioaerosol and high-volume evacuator (HVE)	Heading (MeSH) and text words: (aerosol OR bioaerosol) AND (extraoral suction OR extra oral evacuator) AND (bacterial reduction OR microbial reduction) AND (dental operatory OR dental clinic) AND (high volume evacuator OR high volume suction) AND (saliva OR blood) AND (evacuators OR suction) AND (high volume OR low volume)
	Bioaerosol and preprocedural oral rinse	Heading (MeSH) and text words: (aerosol OR bioaerosol) AND (mouth rinse OR oral rinse) AND (mouth wash OR prophylactic mouth wash) AND (Chlorhexidine OR CHX OR Essential Oil) AND (Cetylpyridinium chloride OR CPC) AND (scaling OR ultrasonic) AND (high-speed rotary OR high-speed handpiece) AND (dental operatory OR dental clinic) AND (anti-microbial OR antibacterial) AND (anti-viral OR anti-fungal) AND (microbial reduction OR bacterial reduction) AND (preprocedural oral rinse OR preprocedural mouth rinse)
EBSCO host	***Bioaerosol and rubber dam***	(aerosol OR bioaerosol) AND (rubber dam OR dental dam) AND (bacterial reduction OR microbial reduction) AND (dental operatory OR dental clinic) AND (dry field OR moisture control) AND (saliva OR blood)
	Bioaerosol and high-volume evacuator (HVE)	(aerosol OR bioaerosol) AND (extraoral suction OR extra oral evacuator) AND (bacterial reduction OR microbial reduction) AND (dental operatory OR dental clinic) AND (high volume evacuator OR high volume suction) AND (saliva OR blood) AND (evacuators OR suction)
	Bioaerosol and preprocedural oral rinse	(aerosol OR bioaerosol) AND (Chlorhexidine OR CHX OR Essential Oil) AND (Cetylpyridinium chloride OR CPC) AND (scaling OR ultrasonic) AND (high-speed rotary OR high-speed handpiece) AND (mouth rinse OR oral rinse) AND (mouth wash OR prophylactic mouth wash) AND (dental operatory OR dental clinic) AND (anti-microbial OR antibacterial) AND (anti-viral OR anti-fungal) AND (microbial reduction OR bacterial reduction) AND (preprocedural oral rinse OR preprocedural mouth rinse)
Web of Science	***Bioaerosol and rubber dam***	(aerosol OR bioaerosol) AND (rubber dam OR dental dam) AND (bacterial reduction OR microbial reduction) AND (dental operatory OR dental clinic) AND (dry field OR moisture control) AND (saliva OR blood)

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Bioaerosol and high-volume evacuator (HVE)	(aerosol OR bioaerosol) AND (extraoral suction OR extra oral evacuator) AND (bacterial reduction OR microbial reduction) AND (dental operatory OR dental clinic) AND (high volume evacuator OR high volume suction) AND (saliva OR blood) AND (evacuators OR suction)
Bioaerosol and preprocedural oral rinse	(aerosol OR bioaerosol) AND (mouth rinse OR oral rinse) AND (mouth wash OR prophylactic mouth wash) AND (dental operatory OR dental clinic) AND (anti-microbial OR antibacterial) AND (anti-viral OR anti-fungal) AND (microbial reduction OR bacterial reduction) AND (preprocedural oral rinse OR preprocedural mouth rinse) AND (Chlorhexidine OR CHX OR Essential Oil) AND (Cetylpyridinium chloride OR CPC) AND (scaling OR ultrasonic) AND (high-speed rotary OR high-speed handpiece)

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Table 2: Included Studies (Efficacy of rubber dam isolation and bio-aerosol)

Study	Population No. of patients (No.)	Study type	Country	Dental Procedure	Aerosol-method of assessment	Summary microbial reduction with and without rubber dam (mean CFU)	Outcome
Cochran et al., 1989 [28]	Adults patients (16)	Case control	USA	1. The rotary dental instrument with and without rubber dam. during restorative procedures (placement of amalgam and composite resin restorations). 2. Rotary dental instrument and air-water syringe with and without rubber dam. The microbial collection was done during handpiece and air-water syringe spray	Case: Microbial collection on the four culture dishes that were attached to the dental operating light positioned perpendicular to 24 inches away from the patients' mouth Another petri dish containing the same kind of agar placed on the patients' napkin 6-7 inches in front of the patients' chin. Controls: consisted of sets of four dishes attached to the dental light. A petri dish on the bracket table, all exposed to the air	**With the rubber dam:** 0.3 ±0.2 (98%) **Without the rubber dam:** 13 ±0.3	1. Statistically significant reduction in microorganisms with the use of the rubber dam--70% to 88% 2. Statistically significant decrease in microbes with the use of the rubber dam-95% to 99% Overall, 90% to 98% of all data combined
Samaranayake et al., 1989 [29]	Pediatric patients (20)	RCT	UK	The rotary dental instrument with and without rubber dam	Blood agar plate positioned at 1m, 2m and 3m distances near the headrest area	**With the rubber dam:** 88% reduction at - 1 m from the headrest 72% reduction at 2 m from the headrest	A highly significant (p= 0.001) reduction in bacterial contamination with rubber dam isolation A reduction in bacterial aerosols was most considerable at 1

						0 % - No reduction at 3 m from the headrest	m from the headrest
Tag El-Din et al., 1997 [30]	Pediatric patients (20)	RCT	Egypt	Rotary instrument (air-turbine-driven handpiece) Standard restorative procedures performed under rubber dam isolation Standard procedures without rubber dam isolation Use of chlorhexidine mouth rinse 30 minutes before the procedure Use of chlorhexidine mouth rinse before application of the rubber dam	Four blood agar culture plates placed equidistantly from the child's head One each on the chest left and right sides, and behind the patient. Another two plates placed at 1 and 2 meters from the headrest of the dental chair, respectively	**With the rubber dam:** 7.9 ±2.8 **Rubber dam + Antiseptic mouth rinse:** 5.9±2.0 **Without the rubber dam:** 19.5 ± 5.8	The bacterial reduction was 98.8% at 1 meter when the rubber dam was used. The bacterial reduction increased when antiseptic mouth rinse was used before rubber dam application The reduction in CFUs at one meter was 98.8%, 73.8%, and 99.4% in the rubber dam group, the antiseptic group, and the antiseptic with rubber dam group, respectively. The highest bacterial contamination was on the agar plates positioned on the patient's chest
Al- Amad et al., 2017 [31]	Adult patients (52)	RCT	UAE	The rotary dental instrument with and without rubber dam, during the standard restorative dental procedure	Fifty-two unused (autoclaved) cotton-polyester scarves (head covers). Cotton swabs moistened with sterile normal saline for sample collection	**With the rubber dam:** NA **Without the rubber dam:** NA	Statistically significant bacterial reduction (mean CFU= 1.67±2.03) using a rubber dam ($P=0.009$)

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Table 3: Included Studies related to high-volume evacuation(HVE) and bio-aerosols

Study	Population No. of patients (No.)	Study type	Country	Dental Procedure	Aerosol-method of assessment	Summary microbial reduction with and without high volume evacuation (HVE) (mean CFU)	Outcome
Efficacy of High-volume evacuation and bio-aerosol							
King et al., 1997[34]	Adults patients (12)	*In vivo* (Split mouth design)	USA	Ultrasonic scaling for 5-minutes with the aerosol reduction device Ultrasonic scaling for 5-minutes without the aerosol reduction device	Samples were collected on blood agar plates placed 6 inches from the subject's mouth	**With HVE** 2.6 +/- 3.6 **Without HVE** 45.1 +/- 28.9 Low CFU count on the ***face shield*** with or without HVE	Significantly higher reduction in the quantity of mean colony forming units (CFUs) with **HVE** However, no significant differences in the number of CFUs found on the investigator's ***face shield*** due to operators' position at 9 am and 12 pm operating position
Muzzin et al., 1999[35]	Adults patients (30)	*In vivo*	USA	All subjects underwent two minutes of air polishing. With the aerosol reduction device on one side of the mouth Without the aerosol reduction device on the opposite side	Microbial samples were collected on blood agar plates positioned 12 inches from the subject's mouth One plate blood agar plate attached to the face mask	**With HVE** 20.10 +/- 53.90 **Without HVE** 148.00 +/- 145.00 **HVE + Face mask** 8.80 +/- 15.10 CFUs	Air polisher without HVE generated a significantly higher number of CFUs on the face mask plate

						Without HVE + Face mask 40.90 +/- 33.80 CFUs	
Timmerman et al., 2004[32]	Adults patients (06)	*In vivo*	Netherland	Ultrasonic scaler with either high-volume evacuation (HVE) or conventional dental suction (CDS) 17 treatment sessions, consisting of a 40-min episode	Two plates (blood agar) placed at 40 cm for 5 min After 20 min, the procedure was repeated. Two plates (blood agar) placed at 150 cm for 20 min. This was followed by exposure to two new Petri dishes for the rest of the session. The plates were cultured aerobically and anaerobically for 3 and 7 days, respectively.	Mean CFU before treatment never exceeded 0.6 colonies per plate. At 40 cm, the mean CFU, at 40 mins, was 8.0 for HVE and 17.0 for CDS. The mean CFU at 150 cm at 40 mins was 8.1 with HVE and 10.3 with the CDS The use of a high-volume evacuator may, however, help to minimize risks of microbial air contamination	**HVE** Mean Aerobic microbes 0.9 (1.3) Mean Anaerobic microbes 1.1 (1.2) **CDS (conventional dental suction)** Mean Aerobic microbes 1.0 (1.2) Mean Anaerobic microbes 3.3 (2.7)
Desarda et al., 2014 [36]	Adults patients (80)	*In vivo*	India	Piezoelectric ultrasonic scaling with or without high-volume evacuator. Nutrient agar plate placed on patient's chest at 20 inches and another plate was set at 12 inches on the dental assistant side	Scaling was carried out for 10 minutes Nutrient agar plates (4) were exposed for 20 minutes for microbial sampling	**With HVE:** 12.14 ± 1.93 **Without HVE:** 11.08 ± 2.25	There found no statistically significant differences in colony-forming units (CFU) with and without high-volume suction placed at 12 and 20-inches from the oral cavity
Bio-aerosol reduction-Efficacy of HVE + standard oral hygiene (tooth brushing, flossing)							

Bentley et al., 1994 [37]	Adult patient (2)	*In vivo*	USA	Restorative procedure using handpiece and high-volume evacuator for 30 minutes Ultrasonic scaling with conventional salivary ejector for 30 minutes	Blood agar plates were placed with on the six spokes of the headrest extension device at 12 and 24 inches from patients' mouth Also, on operators face mask, disposable gowns, head caps.	Colonies of alpha-hemolytic streptococci High-volume evacuation during all the restorative procedures shows negligible bacterial counts reaching plates at 24 inches from the mouth Higher bacterial counts inpatient, who did not brush, or floss for 24 hours compared to the second patient who had brushed and flossed before treatment	High-volume evacuation and preoperative toothbrushing and flossing may reduce bacterial contamination and dissemination
Efficacy of High-volume evacuation and bio-aerosol							
Yamada et al., 2011 [39]	Adult patients (281) At 50 cm single evacuator (n = 102) At 100 cm (n =124) At 100 cm double evacuator (n=55)	*In vivo*	Japan	**At 50 cm and 100 cm from the mouth of the patient with single HVE:** Third molar surgery Full-crown preparation, Inlay cavity (Black Class II) preparation, Scaling with an ultrasonic scaler **At 100 cm from the mouth of the**	Test filter	**At 50 cm from patients' mouth (n=102) with single HVE:** Third molar surgery 92% (12/13) Full-crown preparation 70% (21/30) Inlay cavity (Black Class II) preparation 35% (9/26) Ultrasonic scaling 33% (11/33) **At 100 cm from the patient's mouth (n=124)**	Extraoral evacuators are effective in reducing contaminated aerosols during dental procedures

				patient with two HVE: Third molar surgery		**with single HVE:** Third molar surgery 90% (35/39) Full-crown preparation, 48% (15/31) Inlay cavity (Black Class II) 29% (6/21), Ultrasonic scaling 12% (4/33) **At 100 cm from the patient's mouth (n=55) with two HVE:** Bioaerosol decreased significantly from 90% (35/39) to 60% (33/55)	

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Table 4: Pre-procedural oral rinse and bio-aerosols

Study	Population No. of patients (No.)	Study type	Country	Dental Procedure	Aerosol-method of assessment	Microbial Assay	Summary microbial reduction with and without pre-oral rinse	Outcome
Fine et al., 1992 [42]	Adults patients (18)	Double-blind, controlled, cross-over, clinical study	USA	A 10-minute **ultrasonic scaling** Antiseptic mouthwash **(EO)** or a control (20 ml) for 30 seconds	Aerosolized bacteria were collected on a sterile filter. Filter was Incubated on trypticase soy agar, aerobically at 37⁰C for 24 to 72 hours. Counting the colony-forming units (CFU)	Counting of total CFU with a dissecting microscope	**EO**: reduction of 1.23 CFU (log-transformed) Placebo: reduction of 0.18 CFU (log transformed) **Difference between groups:** **EO**: reduced 1.05 more CFU (log-transformed)	Rinsing with the antiseptic mouthwash **(EO)** produced a 94.1% reduction in CFUs
Fine et al., 1993 [43]	Adults patients (18)	Double-blind, controlled, cross-over, clinical study	USA	Full-mouth dental prophylaxis with **ultrasonic scaler** for 5 min Antiseptic mouthwash **(EO)** or a control	Aerosolized bacteria were collected on a sterile filter. positioned in front of the participant's mouth at a distance of 2 inches Counting the colony-	Counting of total CFU with a dissecting microscope	**EO:** reduction of 1.19 CFU (log-transformed) Placebo: reduction of 0.17 CFU (log transformed)	Pre-procedural rinsing with an antiseptic **(EO)** significantly reduce the level of viable bacteria in an aerosol produced via ultrasonic scaling 40 minutes later

					forming units (CFU)		**Difference between groups:** **EO**: reduced 1.02 more CFU (log-transformed)	
Logothetis et al., 1995 [44]	Adults patients (18)	RCT	USA	**Air polish device** for 3 min Antiseptic mouthwash **(EO) and (CHX)** or a control	Mask of the operator and at 2, 3, 5, 6, and 9 feet from a reference point (patient's head) Culture grown on eight blood agar plates Counting the colony-forming units (CFU)	Anaerobic culture Counting of total CFU with colony counter	**CHX** versus control, 93.10% reduction **EO** versus control, 1% reduction	Pre-rinse with **CHX** can effectively reduce most of the bacterial aerosols generated via the use of the air-polishing device, Pre-rinse reduces Aerosol as far as 9 feet from the patients' head
Klyn et al., 2001 [45]	Adults patients (15)	RCT	USA	Full-mouth dental prophylaxis with **ultrasonic scaler** for 5 min Antiseptic mouthwash **(CHX vs. control)**	Bio-aerosols were collected on four blood agar plates. Three agar plates were placed at 6 inches from the oral cavity, One agar plate was placed 2 feet from the oral cavity	Counting of CFU	**CHX** versus control, 51.43% reduction	The use of preoperative **CHX** mouth rinse reduces the disseminatio n of bacteria
Feres et al., 2010 [46]	Adults patients (60)	RCT	Brazil	Full-mouth dental prophylaxis with **ultrasonic**	Bio-aerosols were collected on	**Checkerboard DNA-DNA hybridizatio**	**CHX** versus water, 70% microbial reduction	Mouth rinses containing **0.12% CHX** and **0.05% CPC** are

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				scaler for 10 minutes Antiseptic mouthwash **(CHX) and (CPC)** or a control	five **blood agar** plates: Three on the support board, One on the participant's chest One on the clinician's forehead	**n** (40 species) **Anaerobic culture:** counting of CFU with colony counter	**CPC** versus water, 68% microbial reduction **CHX** versus no-rinse, 78% microbial reduction **CPC** versus no-rinse, 77% microbial reduction	equally effective in reducing the levels of spatter containing microbes generated during ultrasonic scaling
Dawson et al., 2016[48]	Adults patients (18)	RCT	UK	**Low-speed handpiece** Antiseptic mouthwash **(CHX)** and a control (water)	Petri dish with **anaerobe agar** The extension tube was positioned at the level of the patient's mouth at a distance of 30 centimeters	**Anaerobic culture** **Polymerase chain reaction (PCR)** using universal primer) for total bacterial count	**CXH versus no-rinse**, a 77% increase **CHX versus water**, a 25.3% increase	The use of preprocedural **0.2% CHX** mouth rinse increases in the numbers and diversity of airborne microbes
Retamel - Valdez et al., 2017[47]	Adults patients (60)	RCT	Brazil	Full-mouth dental prophylaxis using **ultrasonic scaler** for 10 min	Bio-aerosols were collected on five agar plates: Three on the support board, One on the participant's chest, and One on the clinician's forehead	**Anaerobic culture:** counting of CFU with colony counter **Checkerboard DNA-DNA hybridization** (40 species)	**CXH versus no-rinse:** 77% reduction **CPC versus no-rinse:** 70% reduction **CHX versus water:** 70% reduction **CPC versus water**, 61% reduction	Preprocedural mouth rinse with **CHX** and **CPC** was effective in reducing microbial species

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***CHX= Chlorhexidine; CFU: Colony-forming units; CPC: Cetylpyridinium chloride; EO: Essential oil**

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Table 5. Risk of Bias of the included studies

Study	Selection bias Baseline characteristics similarity/ appropriate control selection	Selection bias Allocation concealment	Selection bias Randomization	Performance bias Blinding of Researchers	Detection bias Blinding of outcome assessors	Reporting bias Selective outcome reporting	Incomplete outcome data
Rubber dam- Bioaerosol							
Cochran et al., 1989 [28]	+	+	?	+	?	+	+
Samaranayake et al., 1989 [29]	+	+	?	+	?	+	+
Tag El-Din et al., 1997 [30]	+	?	+	-	?	+	+
Al- Amad et al., 2017 [31]	+	?	?	?	?	-	+
High volume evacuator (HVE) -Bioaerosol							
Bentley et al., 1994 [37]	+	+	-	+	?	+	+
King et al., 1997 [34]	+	?	?	?	?	+	+
Muzzin et al., 1999 [35]	+	?	?	?	?	+	+
Timmerman et al., 2004 [32]	+	?	+	?	+	+	+
Yamada et al., 2011 [39]	+	+	+	+	?	+	+
Desarda et al., 2014 [36]	+	?	+	?	?	+	?
Pre-procedural mouth rinse -Bioaerosol							
Fine et al., 1992 [42]	+	?	?	+	+	+	+
Fine et al., 1993 [43]	+	?	?	+	+	+	+
Logothetis et al., 1995 [44]	+	+	?	-	-	+	+
Feres et al., 2010 [46]	?	?	?	+	+	+	+

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Dawson et al., 2016 [48]	-	?	-	+	+	?	?
Retamel - Valdez et al., 2017 [47]	+	?	+	+	+	+	?

Risk of bias legends: + (Low risk); - (High risk); ? (Un-clear risk)