

# Comparison of Bacterial Contamination and Antibacterial Efficacy in Bristles of Charcoal Toothbrushes versus Noncharcoal Toothbrushes: A Microbiological Study

## Abstract

**Background:** Charcoal toothbrushes have been marketed widely claiming lesser bacterial contamination owing to the presence of activated charcoal. **Aim and Objective:** The aim of this study was to evaluate the bacterial contamination and antimicrobial efficacy of charcoal bristles compared to noncharcoal bristles in used toothbrushes. **Materials and Methods:** A total of 50 patients met inclusion criteria which were given standard brushing instructions on the use of a charcoal toothbrush and were asked to return the used brushes after 1 week of usage. After a washout period of 1-week, the participants were then provided with noncharcoal toothbrush and given similar brushing instructions to both groups and were instructed to return the brush after another week of usage. Bristles of the used toothbrushes were sectioned and placed in a 5 ml of saline, and 0.1 ml was inoculated on blood agar plates, which were then placed in a gas pack jar for anaerobic culture. Colony forming units (CFU) were measured after 48 h of incubation. To evaluate the antibacterial efficacy of charcoal bristles, the zone of inhibition was evaluated for charcoal versus noncharcoal after 24 h of incubation. Data collected were analyzed using a paired sample *t*-test. **Results:** The mean CFU count for noncharcoal bristles was almost double that of charcoal bristles. About 10 mm of the zone of inhibition was found around charcoal bristles as compared to 3 mm for noncharcoal bristles. **Conclusion:** This study shows the statistically significant difference in bacterial counts between bristle types and lower CFUs in the charcoal bristles compared with noncharcoal bristles, after 1 week of use. The zone of inhibition that was found around charcoal tooth bristles supported the antimicrobial properties of the charcoal toothbrush.

**Keywords:** Anaerobic, antibacterial, charcoal, noncharcoal, toothbrushes

## Introduction

About 95% of tooth loss in humans are caused by dental caries and periodontal disease.<sup>[1]</sup> Several microorganisms have been implicated in the pathogenesis and perpetuation of these important oral diseases.<sup>[2-4]</sup> Intraoral transmission (translocation) of microorganisms can occur, and therefore, infect sites that were previously treated by scaling and root planning.<sup>[1]</sup> Moreover, several studies have found that cariogenic and periodontopathic bacteria can be transmitted by means of dental instruments, dental floss, and toothbrush.<sup>[5-7]</sup> The concept that toothbrushes are contaminated, after use, was proposed as early as 1920 by Cobb, who implicated that the contaminated toothbrush as a cause of repeated infections of the mouth.<sup>[8]</sup> Microorganisms can gain

entry into a toothbrush from the oral cavity or from the external environment, such as contaminated fingers, aerosols from toilet flushing, and bacteria present in moist and humid conditions found in the bathroom. The toothbrush can harbor a variety of microorganisms including bacteria, fungi, and viruses, facilitating translocation and transmission of these organisms.<sup>[1]</sup> Previously, it was demonstrated that a actinomycetemcomitans and herpes simplex virus Type I, survived at least for 3 days on toothbrushes, and *Enterobacter cloacae* could survive for 16 days.<sup>[9]</sup> A more recent study demonstrated that periodontopathic organisms and super-infecting *Enterobacteriaceae* and *Pseudomonadaceae* species were cultured from toothbrushes in patients with destructive periodontitis and by this way might facilitate bacterial transmission and

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translocation.<sup>[10]</sup> In recent years, the issue of toothbrush disinfection has gained importance. Toothbrush disinfection should be recommended as a routine practice to the patients. The use of chlorhexidine (CHX),<sup>[11]</sup> Listerine, and several dentifrices<sup>[12-14]</sup> have shown varying degrees of efficacy, none are widely used as a home-based application. A possible reason for the noncompliance with these methods is that they are time-consuming and may result in unwanted product residues. Recently, few studies indicated that the use of microwave<sup>[15]</sup> and ultraviolet (UV) light<sup>[16,17]</sup> are the most effective household method to sanitize the toothbrushes after contamination. Furthermore, due to the ease of use, these techniques may increase compliance in toothbrush bacterial decontamination. However, the extent of bacterial decontamination using the microwave and UV light has not been determined in a clinical setting.

A new variant of toothbrushes, charcoal toothbrushes, has been introduced into the market; these toothbrushes are popular in South-East Asian countries such as Malaysia, Singapore, and Indonesia.<sup>[18]</sup> Bristles of charcoal toothbrushes are black in color and are prepared by blending binchotan charcoal into nylon bristles.<sup>[18]</sup> Manufacturers of these toothbrushes claim that they have antimicrobial properties in them, resulting in less bacterial contamination. Activated carbon has proven to remove bacteria such as *Pseudomonas aeruginosa* and *Escherichia coli* from fresh and potable water systems (Percival and Walker, 1999; Quinlivan *et al.*, 2005). Despite electrostatic repulsion between negatively charged microorganisms and carbon surfaces, microorganisms attach to activated carbon particles through strong Lifshitz van der Waals forces (Jucker *et al.*, 1996). Potable water systems are considered low in ionic strength, so electrostatic interactions can offer the possibility of enhancing the efficacy of activated carbon to remove microorganisms from water by positive charge modification of the carbon surfaces.

Since it has been suggested that charcoal may have bacterial resistant properties, toothbrushes have been created with charcoal infused into the bristles. To best of our knowledge, this is the first study in which anaerobic bacterial contamination along with antibacterial efficacy of charcoal bristles was evaluated.

### Materials and Methods

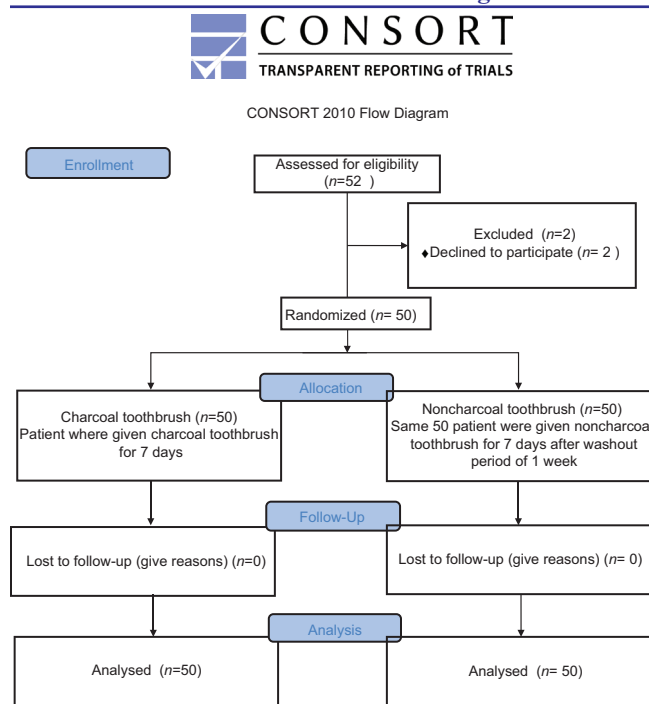
The study was conducted in MGV’s KBH Dental College and Hospital, Nashik. Those ages were 18–35 years and with the tooth brushing frequency of two times daily were eligible for inclusion in the study. Patients with open carious lesions, poor plaque scores (plaque index scores of >2),<sup>[19]</sup> severe gingivitis (gingival index score >2),<sup>[19]</sup> throat infections, irregular brushing frequency, as well as those unwilling to use a charcoal toothbrush, those using mouthwash and/or antibacterial toothpastes, smokers, or those medically compromised were excluded from the

study. A total of 50 patients were randomly chosen [Table 1] for the study from the Department of Periodontology and Implantology. These 50 participants were manual brush users and were informed about the study and signed the consent form before participation.

All participants were given standard instructions on tooth brushing and toothbrush storage. Standard brushing instructions included brushing twice daily (once each in the morning and night) for 2 min. Students were instructed to place the brush at a 45° angle to the gums and gently move the brush back and forth in short strokes. Participants were instructed to brush the outer surfaces, the inner surfaces, and the chewing surfaces of all teeth. They were also instructed to clean the inside surface of the front teeth, tilting the brush vertically, and making several up-and-down strokes. They were also advised not to use any type of mouthwash, to wash the toothbrush bristles under running water without using their fingers to clean the bristles, not to cover the toothbrush bristles with a cap, and to place the toothbrush upright after use with the bristles on top at least 2 feet away from the toilet.

Each participant was then given a charcoal toothbrush and asked to return the toothbrush after 1 week of use. After a washout period of 1 week, noncharcoal toothbrushes were given to the participants, and again, they were asked to use the brushes for 1 week and to return the noncharcoal toothbrushes after the week. Both the charcoal and noncharcoal brushes were similar in design with a compact head, soft bristles, and a bristle tip that was <0.01 mm ([Figure 1a and b]; Colgate® Slim Soft

**Table 1: Consort 2010 flow diagram**



Charcoal Toothbrush). The participants received individual sterile pouches into which to place each used toothbrush for return. On return of the toothbrushes, one-third of the bristles were cut and collected on separate sterile Petri dishes [Figure 2]. Using sterile forceps, toothbrush bristles were placed in separate test tubes containing a 5 mL of saline swirled. A sterile pipette was used to extract 0.1 mL of saline, which was poured onto a blood agar plate. A sterile cotton bud was used to smear the solution on the agar plate [Figure 3]. These agar plates were then placed in gas pack jar for anaerobic culture [Figure 4a]. The agar plates with gas pack jar were then placed in the incubator for 48 h [Figure 4b], after which the colony forming units (CFU) present on each agar plate of microbial growth were noted [Figure 5a and b]. To evaluate antibacterial efficacy of charcoal bristles, the zone of inhibition was evaluated for charcoal versus noncharcoal after 24 h of incubation [Figure 6A and B].

### Results

A total of 100 toothbrushes – 50 charcoal and 50 noncharcoal – were collected from participants. Out of 100 agar plates (50 charcoal and 50 noncharcoal), all plates (50 charcoal and 50 noncharcoal) were seen to have microbial colonies and included in the analysis. There were no growths seen in blood agar plates after 24 h of incubation; however, the colonies were then seen after 48 h of incubation. Higher counts of CFUs were seen on the agar plates from used noncharcoal brushes compared with those from used charcoal brushes. Table 2 presents the results of the paired sample *t*-test comparing the number

of CFUs between the two types of bristles. The mean CFUs for noncharcoal bristles were almost double (151.36) those of the charcoal bristles. *P* value was set at 0.03; the value which was obtained was <0.001 which is significant; hence, there was significant difference between the two products (The results for testing the antibacterial efficacy of charcoal toothbrush found the 10 mm of the zone of inhibition for charcoal tooth bristles compared to noncharcoal toothbrush, which was found to be 3 mm.

### Discussion

In this study, the charcoal and noncharcoal toothbrush were compared for bacterial contamination after 7 days of use. Study participants were instructed to keep the toothbrushes at least 2 feet away from the toilet, researchers from the University of Alabama found that brushes stored in the bathroom are very likely to have fecal matter lingering in the bristles.<sup>[20]</sup> Toilet flushing was shown to produce an aerosol spray of bacterium-tainted water, which can contaminate the bristles.<sup>[20]</sup> Results revealed substantially lower CFU counts in agar plates for used charcoal bristles compared with used noncharcoal bristles, and the difference

**Table 2: The results of the paired sample *t*-test comparing the number of colonies forming units between the two types of bristles**

|                            | Used charcoal brushes (n=50) | Used noncharcoal brushes (n=50) |
|----------------------------|------------------------------|---------------------------------|
| CFU mean (SD)              | 37.960                       | 151.36                          |
| Standard error of the mean | 1.948                        | 2.648                           |
| Mean difference (SD)       | 115.40                       | 115.40                          |
| Two-tailed probability     | <i>P</i> <0.001              | <i>P</i> <0.001                 |

CFU: Colony forming units; SD: Standard deviation

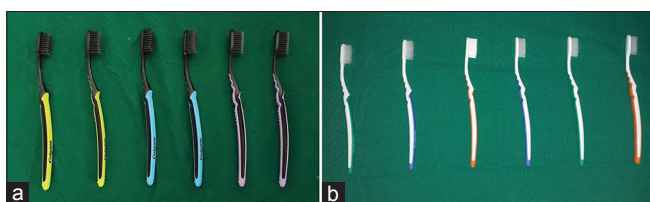


Figure 1: (a) Charcoal toothbrush. (b) noncharcoal toothbrush

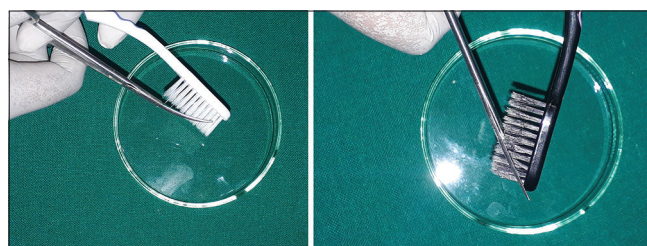


Figure 2: Bristles were cut and collected on separate sterile Petri dishes

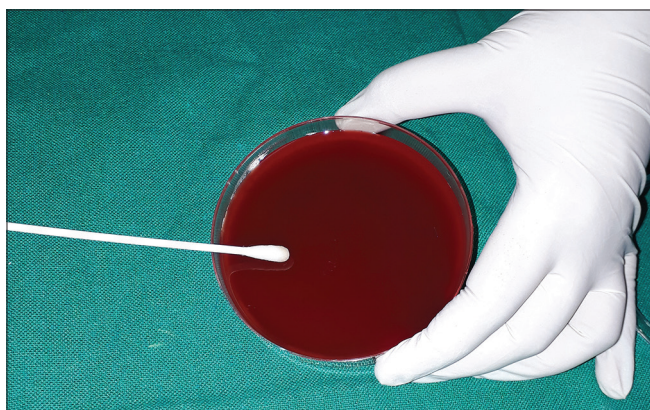
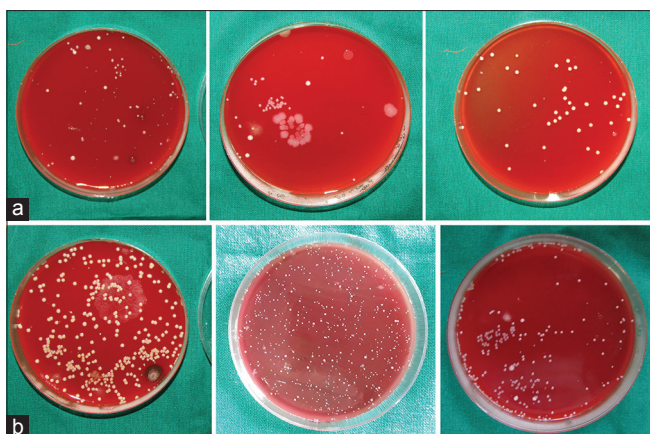


Figure 3: A sterile cotton swab was used to smear the solution on the agar plate



Figure 4: (a) Agar plates were then placed in gas pack jar for anaerobic culture. (b) Gas pack jar were then placed in the incubator for 48 h



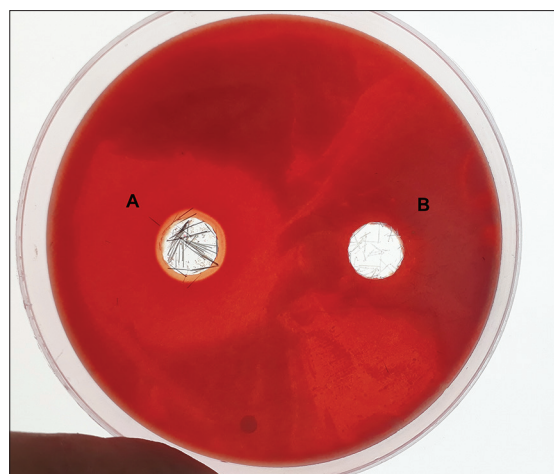


**Figure 5:** (a) Colony forming unit of charcoal bristles after 48 h of anaerobic incubation. (b) Colony forming unit of noncharcoal bristles after 48 h of anaerobic incubation

was statistically significant. This result can be supported by the fact that 10 mm of the zone of inhibition was found in the present study around charcoal bristles which support the theory of antibacterial efficacy of charcoal tooth bristles.

Charcoal in itself has the property of being absorbent, neutralizing toxins, poisons, and noxious gases. Additions of antiplaque and antimicrobial substances to toothbrush bristles in attempts to reduce contamination of used toothbrushes are not a new phenomenon. Turner *et al.* conducted a study to determine the effectiveness of CHX-coated toothbrush laments in reducing quantities of bacteria. The study concluded that there was no statistically significant difference in the quantity of bacteria surviving on CHX-coated laments compared with the control group after 30 days of use.<sup>[21]</sup> The manufacturer of the CHX-coated toothbrush, however, suggested that CHX-coated laments were only effective for a 30-day period, after which time the toothbrush should be replaced. Al-Ahmad *et al.*<sup>[22]</sup> studied the antimicrobial effect of silver-coated toothbrush heads *in-vitro*. The organisms investigated were *Streptococcus oralis*, *Streptococcus mutans*, *Streptococcus sanguis*, *Actinomyces viscosus*, *Lactobacillus casei*, and *Candida albicans*. The study concluded that there was no significant reduction in the CFUs by silver-coated toothbrushes for the above-mentioned tested organisms. On the contrary, the CFU counts for *S. sanguis* ( $P = 0.02$ ) and *C. albicans* ( $P = 0.01$ ) were significantly higher on silver-coated toothbrushes compared with the controls.<sup>[22]</sup>

In 2014, Tomar *et al.* evaluated the sanitization potential of UV-rays and 0.2% (CHX) solution for disinfection of used toothbrushes.<sup>[23]</sup> Toothbrushes were collected after 7 days of use and placed into three groups: Group I brushes were soaked in 0.2% CHX mouthwash. The results in this study are according to study conducted by Lee *et al.* in which they evaluated the aerobic bacterial contamination of charcoal toothbrush and they found



**Figure 6:** (A) Zone of inhibition of 10 mm found associated with charcoal bristles; (B) Zone of inhibition of 3 mm found associated with noncharcoal bristles

that the microbial counts in noncharcoal toothbrush was double than that of charcoal bristles.<sup>[24]</sup>

In this study, anaerobic microbial study was investigated since most of the causative periodontopathogenic are facultative anaerobes, and this study showed the significantly more counts in noncharcoal toothbrush. This is the first study in which the antibacterial efficacy along with the anaerobic microbial contamination for charcoal bristles was evaluated.

## Conclusion

This study showed the number of CFUs in charcoal toothbrushes was substantially less when compared with noncharcoal toothbrushes after 1 week of usage. The zone of inhibition that was found around charcoal tooth bristles supported the antimicrobial properties of charcoal toothbrush. Thus, the charcoal-infused tooth bristles can be considered a new product to prevent bacterial contamination.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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