

Research Article

Comparison of Bacterial Contamination in Bristles of Charcoal Toothbrushes versus Non-Charcoal Toothbrushes: A Randomized Clinical Trial

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ABSTRACT

Background: Toothbrush may serve as a reservoir for harbouring diverse colonies of microorganisms. For protection against toothbrush mediated oral contamination, toothbrushes with charcoal infused bristles are also available in the market. They are regarded as antibacterial in their role but limited evidence is available on their performance over an extended period of use. This study compares bacterial contamination in the bristles of charcoal versus non-charcoal toothbrushes after two weeks of routine use.

Methods: A randomized clinical trial was carried out for comparing bacterial colonization of charcoal and non-charcoal toothbrushes in n=230 dental students and house officers after two weeks of routine brushing. Both groups received identical toothpaste and standardized brushing instructions and used their assigned brush for two weeks. Bristles from their respective toothbrushes were then aseptically excised, suspended in nutrient broth, and cultured on blood agar for 24-48 hours under standard laboratory conditions. Colony-forming units (CFUs) were counted, and the two groups were compared using an independent-samples t-test, with $p \leq 0.05$ taken as significant.

Results: Charcoal bristles carried a mean of 5.1 CFUs compared with 15.0 CFUs for non-charcoal bristles, a highly significant difference ($p=0.001$).

Conclusions: After two weeks of daily use, charcoal-infused bristles carried substantially low bacterial load as compared to conventional non charcoal toothbrushes. Therefore charcoal infused toothbrushes resist bacterial colonization supporting their potential role in reducing toothbrush-related contamination.

Keywords: Tooth Brushing, Plaque Removal, Oral Hygiene, Oral Health, Charcoal-Infused Bristles, Cross-Contamination, Colony-Forming Unit.

INTRODUCTION

Toothbrushes are a vital tool in maintaining oral hygiene, and the type of toothbrush used may influence the effectiveness of oral hygiene practices. In recent years, charcoal toothbrushes have gained popularity due to their purported antimicrobial properties, with some claiming that they offer superior

cleaning and antibacterial efficacy compared to traditional non-charcoal toothbrushes(1). The efficacy of mechanical plaque control is associated with the technique and frequency of the dentifrices application (2). Microorganisms may accumulate, adhere, and survive in toothbrushes. Moreover, these microbes have a transmission capacity, which

can in turn cause diseases in an individual (3). Therefore, decontamination of pathogenic microorganisms that are transmitted into toothbrushes while brushing from a storage area, oral cavity, or other toothbrushes is inevitable(4).

Mechanically brushing the teeth, preferably with fluoride toothpaste, is the most popular plaque control approach. The buildup of microbiological plaque from gingiva and tooth surfaces may be avoided by using a toothbrush(5).

Unfortunately, tooth brushes may harbour many microorganisms from the mouth and the environment(6). Use of toothbrushes for an extended period can also alleviate different microorganisms such as Streptococcus, staphylococcus, and lactobacilli. These microorganisms may be a secondary cause for dental caries, gingivitis, stomatitis, and infective endocarditis in an individual affecting both oral and systemic health (7,8). Multiple bactericidal agents have been introduced to reduce the bacterial contamination of toothbrushes. These include chlorhexidine, triclosan, cetylpyridinium chloride, Listerine, ultraviolet (UV), spraying of disinfecting solutions on bristles and use of microwave ovens, etc.(9).

Activated charcoal infusions have also gained popularity in medical applications such as an antidote in use of acute poisoning, overdose of drugs and skin infection management, wound healing and malodor reduction and pruriti(10). Charcoal has been added to the bristles of toothbrushes that are usually black in colour. Manufacturers of such charcoal infused tooth brushes claim that blending charcoal with nylon bristles reduces halitosis, plaque buildup, and decrease bacterial load that develops during tooth brush storage and use (11,12). They are regarded as antibacterial in their role but limited evidence is available on their performance over an extended period of use. Therefore the current study was undertaken to compare bacterial contamination in the bristles of charcoal versus non-charcoal toothbrushes after two weeks of routine use.

METHODS

A randomized clinical trial was conducted in the department of periodontology, HITEC Dental College, Taxila over a period of three months from November 2023 to January 2024. This study was approved by the ethical committee of Gandhara University Peshawar

Pakistan vide letter No. GU/Ethical Committee/2023/216.

A total number of n=230 BDS undergraduate students and house officers were recruited using a non-probability consecutive sampling technique, with sample size calculated at 80% power and a 5% margin of error. To be eligible, participants needed a Basic Periodontal Examination (BPE) score of 1 or 2 and a habit of brushing twice daily. People were excluded if they had a throat infection, were unwilling to use a charcoal toothbrush, used an antibacterial mouthwash, or smoked cigarettes or used naswar. Every participant signed an informed consent before enrolment. Participants were allocated by simple lottery to Group A (charcoal toothbrush) or Group B (non-charcoal toothbrush); both brush types and the toothpaste supplied came from the same manufacturer to avoid bias arising for formulation differences. Everyone received identical brushing instructions. Participants were asked not to use any mouthwash, to rinse bristles under running water without scrubbing them with a finger, and to keep using the same brush for two weeks before returning it in an individually supplied sterile pouch.

At the two-week mark, bristles were cut from the brush head with a single-use sterile blade, placed into nutrient broth using sterile forceps, and 0.1 ml of the broth was transferred onto blood agar plates. Plates were incubated for 24–48 hours under standard laboratory conditions appropriate for anaerobic/aerobic culture, after which colony-forming units were counted under a microscope.

Data were analysed in SPSS version 26.0. Descriptive statistics summarised participant demographics, and an independent-samples t-test (supplemented by chi-square testing for categorical comparisons) was used to compare mean CFU counts between the charcoal and non-charcoal groups. A p-value of $p \leq 0.05$ was considered significant.

RESULTS

Among all the included participants of the study n=230, the age range observed was from 18 years to 24 years (Fig 1), with the mean age= 21.5 years. The majority of the participants (85%) were in the second decade of their life. The largest population included were 23 years old (19.13%) The youngest participants were 18 years old and least in number (4.7%).

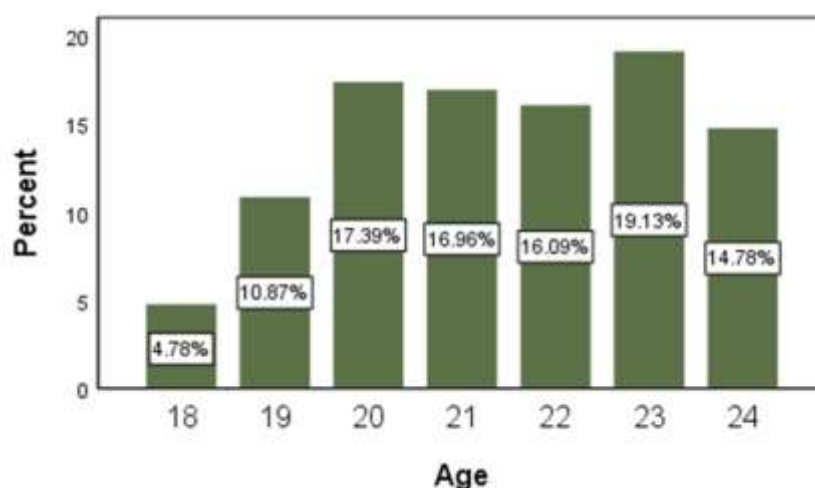


Figure 1: Age wise Distribution of all participants

Microbiological analysis of the bristles from the toothbrushes revealed differences in the contamination level between charcoal and non-charcoal toothbrushes. Bristles from the charcoal group carried a mean of 5.1 CFUs (SD 1.7), while bristles from the non-charcoal group carried a mean of 15.0 CFUs (SD 4.1) about thrice as much as the former group. The

overall sample mean across both groups combined was 10.0 CFUs (SD 5.8). This difference was highly statistically significant ($p=0.001$), as shown in table 1. This findings support rejection of the null hypothesis that bacterial contamination would be equal between the two bristle types.

Table 1: Bacterial Contamination of Toothbrush Bristles

Variable		N	Mean	Standard Deviation	Standard Error	P value
Bristles type	Charcoal	115	5.1	1.7	1.7	0.001
	Non-Charcoal	115	15.0	4.1	4.1	
	Total	230	10.0	5.8	5.8	

DISCUSSION

A randomized clinical trial was carried out to compare bacterial contamination in charcoal-infused bristles versus non-charcoal bristles. From the beginning, 1920s, manual toothbrush has been a widely accessible and reasonably priced oral hygiene tool for the general public(13). In a study conducted by Bunetel et al., potentially harmful bacteria and viruses, including *Staphylococcus aureus*, *E. coli*, *Pseudomonas*, and the herpes simplex virus, were found on the toothbrushes of both healthy and sick patients. It was discovered that toothbrushes used by individuals who already had oral diseases caused a rapid deterioration of their health and caused secondary infections with systemic involvement(14). An increased toothbrush's efficacy and antibacterial activity has been made possible by a number of creative design innovations. There have been attempts for elongation of the duration of use of a single tooth brush by experimenting with different

materials. Charcoal has recently been infused into toothbrush bristles in an effort to reduce the bacterial load that contaminates toothbrushes(15). Thoothbrush coated with charcoal has a comparative lower bacterial load as reported by Turner et al who found that after 30 days of use, no statistically significant variation in the number of bacteria surviving on charcoal-coated filaments were found in contrast to the control group (16). Al-Ahmad and colleagues investigated the in vitro antibacterial activity of toothbrush heads coated with silver. *Actinomyces viscosus*, *Lactobacillus casei*, *Streptococcus oralis*, *Streptococcus mutans*, and *Streptococcus sanguis* were the organisms under investigation. The study found that, for the examined species listed above, there was no discernible decrease in CFUs caused by silver-coated toothbrushes(17). This difference may be due the change in chemical nature of charcoal and silver.

According to the current study, there was about three times less bacterial contamination in charcoal toothbrushes as compared to non-charcoal toothbrushes. This finding is consistent with another study by AL Dhawi et al. that showed a significant difference in bacterial contamination between charcoal and non-charcoal toothbrushes(12). The notable similarity in results may be due to the intrinsic antimicrobial activity of charcoal. The findings of this investigation also align with the findings of a previous study by Lee et al. in which the researchers assessed the aerobic bacterial contamination of charcoal toothbrushes and discovered that the microbial counts in non-charcoal toothbrushes were twice as high as those in charcoal bristles. According to the findings, charcoal bristles had significantly lower CFU counts in the blood agar plates than non-charcoal bristles(18). Our findings are also in line with a study by Mitali Vilas Thamke that found that after a week of use, charcoal toothbrushes had significantly fewer CFUs than non-charcoal toothbrushes (19). This significant similarity in the findings can be attributed by the anti microbial nature of infused charcoal. One of the observed drawback of charcoal toothbrushes is its less efficacy in plaque removal as compared to conventional nylon toothbrush. According to a study conducted by Kini V et al.(20), charcoal infused bristles are more efficient in plaque removal and more resistant to wear as compared to conventional nylon bristles, strengthening the results of this study. A study by Annu et al. showed no statistically significant differences in plaque removal efficacy between charcoal and non-charcoal toothbrushes(21). This study further suggested that charcoal infusion into nylon bristles not only reduce the plaque and halitosis but also reduces bacterial contamination of used toothbrushes during storage as per manufacturers' claim.

Thus toothbrushes infused with charcoal have lower levels of bacterial contamination than toothbrushes without charcoal, improving oral health. Therefore, it's advised to regularly use disinfectants such chlorohexidine or other antimicrobial agents to clean non-charcoal toothbrushes(22).

Limitations

The trial was conducted at a single centre using a single manufacturer's brushes, which limits how confidently these findings generalize across brands and populations.

CONCLUSIONS

After two weeks of daily use, charcoal-infused bristles carried substantially low bacterial load as compared to conventional non charcoal toothbrushes. Therefore charcoal infused toothbrushes resist bacterial colonization supporting their potential role in reducing toothbrush-related contamination.

Declarations

Ethics Approval and Consent to Participate: This study was approved by the Ethical Review Committee of Gandhara University. Written informed consent was obtained from all participants prior to enrolment.

Consent for Publication: Not applicable.

Availability of Data and Materials: The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests: The authors declare no competing interests.

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Authors' Contributions: All authors contributed to the study design, data collection, analysis, and manuscript preparation, and approved the final version.

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