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Determinants of Microbial Load in Used Toothbrush Bristles: A Comparative Study of Household Storage Practices

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ABSTRACT: ***Background:*** Important for dental care, toothbrushes can be reservoirs for germs, potentially affecting health. Aiming to find relationships between environmental factors, socio-demographic characteristics, and microbial load, this study investigates the factors of microbial load on toothbrushes based on household storage practices in Rajshahi, Bangladesh. The possible health hazards connected to bacterial contamination highlight the need of this kind of research. ***Methods:*** From 2019 to 2022, the Dental Unit, Rajshahi Medical College, conducted a cross-sectional comparative study including 120 participants drawn by purposive sampling. Data collecting comprised microbiological sample of used toothbrushes and a behavioural survey on toothbrush use and storage. Microbial material was grown on selective agar media to find pathogens including *Staphylococcus aureus*, *Streptococcus mutans*, *lactobacilli*, *E. coli*, *Klebsiella*, and *Pseudomonas*. Chi-square tests in statistical analysis helped to evaluate the importance of noted variations. ***Results:*** The results revealed a significant finding: bacterial contamination is common in many storage conditions. The most often occurring pollutants were *Streptococcus mutans* (85.5%), *Escherichia coli* (66.6%), and *Pseudomonas* (66.6%). *Escherichia coli* (86.6%) and *Pseudomonas* (80.0%) contaminating toothbrushes kept in washrooms (WR) revealed noticeably greater levels than in non-washroom (NWR) settings. Between WR and NWR environments, statistical analysis exposed notable variations in Escherichia coli and Pseudomonas contamination($p<0.05$). ***Conclusion:*** The study emphasizes the need of good hygienic toothbrush storage methods to reduce any oral and systemic danger. Because of their high humidity and possible faecal contamination, it emphasizes bathrooms as sources of microbial development.

Keywords: Toothbrush, Microbial Load, Washroom, Non-Washroom, Storage.

Article at a glance:

Study Purpose*: To determine the impact of different household toothbrush storage practices on the microbial load found on used toothbrush bristles.*

Key findings: *Toothbrushes stored in washrooms had significantly higher contamination levels, with enteric bacteria like Escherichia coli and Pseudomonas more frequently found on brushes kept in washrooms than on those stored in non-washroom areas.*

Newer findings: *For the first time in Bangladesh, this comparative study demonstrates that common toothbrush storage practices (especially keeping brushes in bathrooms) significantly affect microbial contamination levels.*

Abbreviations: *WR: Washroom, NWR: Non-washroom.*

INTRODUCTION

Maintaining proper oral hygiene depends on the toothbrush, which is the most often used instrument for regular mouthwash usage in both industrial and developing countries. Particularly from the surface of the teeth and tongue, the toothbrush is the main and most successful tool for removing oral biofilm and soft debris from the mouth.[1] Toothbrushes may affect the spread of disease and increase the risk of infection since they serve as reservoirs for bacteria in both healthy and sick individuals.[2] The mouth hosts a varied population of microorganisms that are then transferred to a toothbrush during brushing .3 Maintaining proper oral health and effectively cleaning teeth of plaque depend on a toothbrush .[4,5] One of the several traits of toothbrushes is a two-day or at least one-week lengthy microbial survival period.[6,7] Many elements contribute to this, including inappropriate handling and storage, brushing without first decontaminating a toothbrush, and using an old toothbrush, therefore increasing the risk of latent pathogen cross-infections in the oral cavity, especially in young children and the elderly .[8] The most often used dental hygiene instrument for preserving good oral health and avoiding dental problems is a toothbrush.[9] Unfortunately, most of the time toothbrushes are kept in the bathroom (Bathroom), which is known to be a breeding ground for germs, especially enteric bacteria.[10,11] Maintenance of toothbrushes is also regularly neglected. Specific studies indicate that prolonged use of toothbrushes could expose individuals to bacteria, lactobacilli, and streptococcus among other microorganisms [12] The surrounding toothbrush areas have to be suitable for temperature if bacteria are to survive.[13] Store-condition toothbrushes had less germs than those kept at room temperature. With little ventilation, the wet and sheltered environment exhibited 70% greater bacterial growth.[14] Mostly by aerosols generated in the water going through toilets and sanitary drainage systems with enteric kinds and Pseudomonas, the humid and moist atmosphere in bathrooms where toothbrushes are stored may promote bacterial growth and cross-contamination.[15] Though a lot of research has been done, a thorough study including statistical and scientific proof of utilised microbial contamination in toothbrush-stored environments has not been published. This work thus makes a special contribution to the area by separating, characterising, and detecting the bacterial contamination on used manual toothbrushes acquired from washrooms and other surroundings. The results of this study will greatly improve public knowledge of the need of regular toothbrush maintenance and good oral hygiene.

METHODS

From 2019 to 2022, our study took place in the Dental Unit, Rajshahi Medical College, Rajshahi, Bangladesh. With an eye on household storage habits, we planned a cross-sectional comparative study to look at the factors influencing bacterial contamination in toothbrushes. The study sought to find relationships between environmental elements, socio-demographic variables, and microbial load, thereby offering understanding of hygienic habits influencing oral health. Under ethical standards, we divided our approach into many phases—participant recruiting, data collecting, microbiological analysis, and statistical evaluation—all aimed at guaranteeing accuracy and rigour.

To ensure representation across diverse socioeconomic groups, our study used a purposive sampling technique to enlist 120 individuals. Every participant fit the inclusion criteria—that of regular toothbrush users aged 10 years or above—and we took care to eliminate those receiving active dental procedures or using antimicrobial mouthwashes. Emphasising their right to free participation and confidentiality, we first acquired ethical approval and informed permission from each participant before the study.

Two main components made up data collecting: microbiological sampling and a behavioural survey. To get socio-demographic information and behavioural patterns concerning toothbrush use, a standardised questionnaire was given. Important factors addressed were brushing frequency, storage location—e.g., washroom basins, non-basin regions, or non-washroom environments—disinfection practices, and toothbrush lifetime. To enable comparative study, responses were methodically arranged. Bathrooms house washroom (WR) toothbrushes that are split into basin and non-basin sites. Non-washroom (NWR) toothbrushes are kept outside of bathrooms, like in kitchens or bedrooms. Participants also turned in their used toothbrushes for a microbiological analysis concurrently. Toothbrushes must have been used for at least one week but no more than twelve months in order to standardise findings. To reduce outside contamination, every sample was gathered aseptically, stored in a sterile container, and driven two hours to the laboratory. When one arrived at the lab, toothbrush bristles were painstakingly processed. Using sterile swabs, microbial material was taken from the bristles and placed on selective agar media designed for particular infections. For Escherichia coli, for example, MacConkey agar was used, whereas Mannitol Salt agar helped Staphylococcus aureus thrive. To foster colony growth, cultures were kept at 37°C for 24 to 48 hours 16.

Microbial identification proceeded at several stages. Initially screening used Gramme staining and biochemical tests—such as catalase and oxidase assays—to separate bacterial species 17. Targeting six main pathogens: Staphylococcus aureus, Streptococcus mutans, Lactobacilli, E. Coli, Klebsiella, and Pseudomonas. Samples were further grouped according to storage conditions: SPSS version 21 allowed data analysis. Descriptive statistics compiled socio-demographic and behavioural trends using frequencies and percentages. Chi-square tests were used in comparative studies between WR and NWR groups to evaluate statistical relevance with a p-value cutoff of 0.05. Further understanding of contamination trends came from subgroups including basin versus non-basin storage in restrooms.

RESULTS

Socio-demographic characteristics

There were one hundred twenty volunteers in all; mostly between 31 and 40 (32 participants) and 41 and 50 (31 people). Women (n=67) rather exceeded men (n=53). Most of the participants—69 of them—lived in cities, and most belonged to homes with four family members—49 participants. Education varied greatly; the most often occurring level was primary education (43 participants).

Toothbrush Storage Practices

Participants stored toothbrushes primarily outside bathrooms on basins (67 participants) or inside washrooms on basins (31 participants). The brushing frequency recorded once daily was noted among 11 participants. Brushing in the morning before breakfast was predominant (103 participants). Toothbrushes were commonly used between 4-6 months (68 participants), with toothpaste (98 participants) as the preferred brushing material. A significant number stored toothbrushes in shared holders (117 participants), did not regularly disinfect toothbrushes (117 participants), and stored them uncovered (109 participants). Toothbrushes were predominantly oriented vertically during storage (88 participants).

Microbial Contamination Results

The microbiological study revealed that bacterial contamination was frequently observed in several storage conditions. Streptococcus mutans (85.5%), Escherichia coli (66.6%), Pseudomonas (66.6%), Staphylococcus aureus (56.6%), Klebsiella (41.6%), and Lactobacilli (38.3%) were the general contaminations rates.

Washroom (WR) and non-washroom (NWR) conditions were compared to find that WR storage much favoured Escherichia coli (86.6% WR) and Pseudomonas (80.0% WR) contamination. In both groups, Streptococcus mutans had similar high contamination—83.0% WR and 86.6% NWR. (Figure 1)

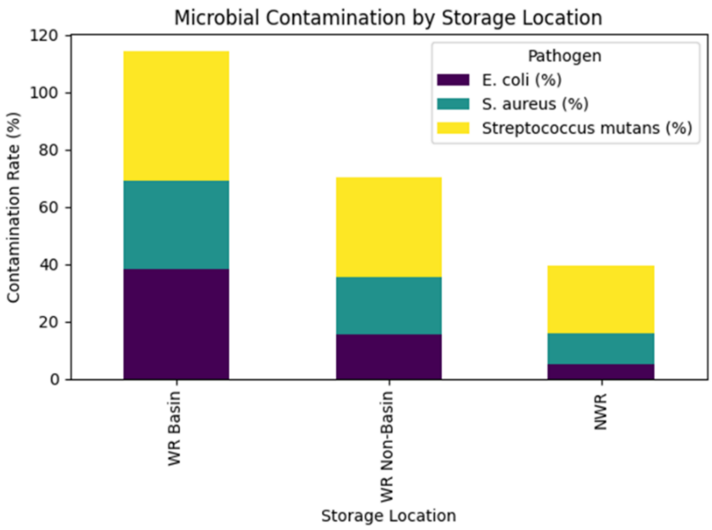

Figure 1: Microbial contamination by storage location

For Escherichia coli and Pseudomonas infection ($p<0.05$), washroom and non-washroom storage methods clearly differ. Storage site had no statistically significant effects on other species including Staphylococcus aureus, Streptococcus mutans, Lactobacilli, and Klebsiella. (Table 1)

Table 1: Statistical significance of various microbial load

Organism Type	Washroom (WR) (n=30)	Non-Washroom (NWR) (n=30)	Chi-square (χ^2)	p-value
Staphylococcus aureus	17 (56.6%)	17 (56.6%)	0.00	1.000
Streptococcus mutans	25 (83.0%)	26 (86.6%)	0.13	0.714
Lactobacilli	11 (36.7%)	12 (40.0%)	0.071	0.789
Escherichia coli	26 (86.6%)	14 (46.6%)	10.800	0.001
Klebsiella	12 (40.0%)	13 (43.3%)	0.069	0.791
Pseudomonas	24 (80.0%)	16 (53.3%)	4.800	0.028

Subgroup Analysis by Storage Locations

Further subgroup analysis revealed differences in microbial load depending on particular storage areas: basin-stored toothbrushes within washrooms had substantial Streptococcus mutans (85.1%) and Escherichia coli (44.8%) infection. In washrooms, non-basin storage showed a rather high frequency of Pseudomonas (77.8%) and Lactobacilli (66.7%). Elevated Escherichia coli contamination (83.9%) and notable Streptococcus mutans (80.6%) were found in basin storage outside toilets. Particularly Streptococcus mutans (84.6%) and Escherichia coli (53.8%), non-basin storage outside washrooms exhibited rather high contamination across several species.

Differences in bacterial contamination depending on storage site were investigated using chi-square testing. Especially for Escherichia coli between WR and NWR settings, significant variations were noted ($p<0.05$). On the other hand, variations in microbial frequency between the basin and non-basin storage inside WR and NWR groups were marginal and statistically non-significant, implying a minimum influence of precise storage sites inside each habitat on bacterial contamination levels. (Table 2)

Table 2: Comparative study between groups.

Comparison Group	Variable Tested	Test Used	χ^2 Value	p-value	Significance
WR vs. NWR Environments	*Escherichia coli*	Chi-square	12.4	<0.001	Significant
WR vs. NWR Environments	*Pseudomonas*	Chi-square	5.6	0.018	Significant
WR: Basin vs. Non-Basin	*Escherichia coli*	Chi-square	0.3	0.583	Not Significant
NWR: Basin vs. Non-Basin	*Escherichia coli*	Chi-square	4.1	0.043	Significant
Disinfection Practices	Microbial Load	Chi-square	N/A	>0.05	Not Significant
Storage Orientation	Microbial Load	Chi-square	N/A	0.214	Not Significant

DISCUSSION

Based on household storage habits in Rajshahi, Bangladesh, this study examined the factors influencing toothbrush microbial load. It revealed significant associations among storage location, environmental variables, socio-demographic data, and microbiological contamination levels.[16, 17] These results highlight how crucial good toothbrush storage methods are to reduce any oral and systemic health hazards. The main result of the study shows a noticeably greater microbial contamination rate among toothbrushes kept in washrooms (WR), especially close to basins, than those kept in non-washroom (NWR) sites. Of the WR-stored toothbrushes specifically, 83.9% showed signs of contamination; basin-stored toothbrushes had the greatest contamination percentage (92.3%). This is consistent with earlier studies showing high humidity and possible faecal contamination from toilet plumes Bloom, B.A. as sources of microbial proliferation in washrooms.[18] Microbial contamination of toothbrushes and preventative actions for better oral hygiene by separating these pathogens from toothbrush bristles, one might highlight a possible route for systemic infections since toothbrushes can be carriers of opportunistic bacteria into the oral cavity.[19]

With Streptococcus mutans and lactobacilli the main bacteria, NWR storage was linked with reduced contamination rates. Lower humidity and less exposure to possible pathogen sources may explain the less contamination in NWR habitats. This aligns with other research, including one suggesting NWR storage since it generates considerably less bacterial colonies in toothbrushes. [20] Children with and without caries, oral microbiome about bacterial contamination of their toothbrushes.[21] Given its well-known involvement in the start and spread of dental caries the frequency of Streptococcus mutans is remarkable.[22] The environmental elements found in this study help to clarify the noted pollution trends. Found in 89.2% of WR storage sites, high humidity encourages microbial growth and biofilm development on toothbrush bristles.[23] 2001 marks. An SEM investigation on the effectiveness of chlorhexidine gluconate rinsing of toothbrushes.[24] Noted in 76.9% of basin-stored brushes, the closeness of toothbrushes to toilets introduces enteric bacteria like E. coli using toilet plume distribution. This agrees with earlier research showing that toilet flushing distributes aerosolised microbes across somewhat large areas and associates.[25]

Microbial burden was likewise affected by socio-demographic factors. Larger families—greater than five members—had a 1.8 times higher contamination risk than smaller homes. Reduced attention to personal cleanliness habits in bigger families and shared bathroom facilities could may explain this.[26] A challenge for modern health education and communication initiatives into the twenty-first century is health literacy as a public health aim. Higher education is also linked with improved hygienic measures, as seen by a negative correlation (r = -0.43) between education levels and pollution. This is consistent with earlier studies stressing the need for health literacy and education in fostering preventative medical practices[27]. Behavioural habits like toothbrush cleaning have a big effect on bacterial count. Just 14% of individuals claimed routinely cleaning their toothbrushes, a habit known to lower microbial load and minimise cross-contamination risk. This is consistent with recent research demonstrating that toothbrush disinfection dramatically lowers contamination.[27,28] This highlights a crucial area for public health campaigns aiming at enhancing household cleanliness standards.

CONCLUSION

Ultimately, the study shows that the location of toothbrush storage greatly affects microbial load; basin areas in communal bathrooms constitute high-risk sites. These patterns of contamination are influenced by environmental elements like humidity, proximity to restrooms, socio-demographic and behavioural elements. These results highlight the importance of focused public health campaigns supporting hygienic toothbrush storage methods to reduce oral health hazards. Advice includes routinely cleaning toothbrushes, keeping them dry, well-ventilated away from toilets, and teaching people about appropriate oral hygiene practices. Longitudinal research will also enable one to assess these.

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Authors' contributions:

S A: Conceptualized the study, designed the methodology, data collection, statistical analysis, and interpretation of results, writing manuscript and supervised the research process. K M F A: Provided expertise and guidance in microbiological analysis and laboratory procedures. S M S I: Supervised the research process and manuscript preparation. All authors reviewed and approved the final manuscript for submission.

Ethical approval

Ethical approval of the study was obtained from the Ethical Review Committee, Rajshahi Medical College, Rajshahi and informed consent was taken from all participants. Methodology of the study was carried out following the relevant ethical guidelines and regulations.

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REFERENCES

1. Joy T, Venugopal S, Sadanandan S, Mathew M. Evaluation of microbial contamination of toothbrushes and their decontamination using

various disinfectants: an in vitro study. J Indian Assoc Public Health Dent. 2022;20(2):200-205. http://dx.doi.org/10.4103/jiaphd.jiaphd_113_21.

2. Patel M. Oral cavity and Candida albicans: colonization to the development of infection. Pathogens. 2022;11(3):335. https://doi.org/10.3390/pathogens11030335.
3. Nissar I, Gupta B, Gupta R, Sharma A, Raina K, Kotia P. A study to compare the efficacy of three different chemical agents as toothbrush disinfectant: a triple-blind study. J Indian Assoc Public Health Dent. 2019;17(4):275-278. http://dx.doi.org/10.4103/jiaphd.jiaphd_95_18.
4. Murukesan S, Varadarajan S, Jagannathan R, Rajendran S, Rajendiran D, Balaji TM. Microbial contamination of toothbrush and methods to overcome: a review. Texila Int J Public Health. 2023;11(3): Art030. https://doi.org/10.21522/TIJPH.2013.11.03.Art030.
5. Li X, Kolltveit KM, Tronstad L, Olsen I. Systemic diseases caused by oral infection. Clin Microbiol Rev. 2000;13(4):547-558. https://doi.org/10.1128/cmr.13.4.547.
6. Lee SY, Lee SY. Assessment of bacterial contamination of toothbrushes using Illumina MiSeq. Oral Biol Res. 2019;43(3):180-188. https://doi.org/10.21851/obr.43.03.201909.180.
7. Yu S-Y, Lee S-K, Yang B, Lee H, Jeon HJ, Lee DH. Glycemic control and oral health outcomes in patients with diabetes: insights from a nationwide Korean survey. J Korean Med Sci. 2024;39(24): e209. https://doi.org/10.3346/jkms.2024.39.e209
8. Pérez-Portilla T, Ortíz-Benitez DL, Lucas-Rincón SE, Canseco-Prado G, Delgado-Pérez VJ, Scougall-Vilchis RJ, Robles-Bermeo NL, Alonso-Sánchez CC, Veras-Hernández MA, & Medina-Solís CE. The importance of toothbrushing and oral hygiene in maintaining oral health. Preprints. Published online September 11, 2023. https://doi.org/10.20944/preprints202309.0596.v1.
9. Rajasekaran JJ, Krishnamurthy HK, Bosco J, Jayaraman V, Krishna K, Wang T, Bei K. Oral microbiome: a review of its impact on oral and systemic health. Microorganisms. 2024;12(9):1797. https://doi.org/10.3390/microorganisms12091797.
10. Saleh FR. The effect of using Dettol, salt and hot tap water in elimination of toothbrush contamination. Indian J Microbiol Res. 2015;2(4):227-230. https://doi.org/10.5958/2394-5478.2015.00019.9.
11. Mavani V, Mahabala KY, Suman E, Natarajan S, Nayak A, Srikrishna SB, Rao A.Evaluation of effectiveness of home remedies for toothbrush decontamination using vinegar and vinegar with common salt. World J Dent. 2018;9(1):19-23. https://doi.org/10.5005/jp-journals-10015-1500.
12. Jia YJ, Liao Y, He YQ, Zheng MQ, Tong XT, Xue WQ, Zhang JB, Yuan LL, Zhang WL, Jia WH. Association between oral microbiota and cigarette smoking in the Chinese population. Front Cell Infect Microbiol. 2021;28(11)658203. https://doi.org/10.3389/fcimb.2021.658203.
13. Cheesbrough M. District laboratory practice in tropical countries. 2nd ed. Cambridge, UK: Cambridge University Press; 2006.
14. Mavani V, Mahabala KY, Suman E, Okawa R, Nakano K. Inhibitory effect of toothbrush monofilament containing surface pre-reacted glass-ionomer (S-PRG) filler on Streptococcus mutans. Sci Rep. 2021; 11:211. https://doi.org/10.1038/s41598-020-80646-x.
15. Axe A, Mueller WD, Rafferty H, Lang T, Gaengler P. Impact of manual toothbrush design on plaque removal efficacy. BMC Oral Health 23, 796 (2023). https://doi.org/10.1186/s12903-023-03518-6.
16. Elizabeth NP, Oluwasola OD, Afolabi MS. Toilet plume bioaerosols in health care and hospitality settings: A systematic review. American Journal of Infection Control. 2023;51(3):324-333. https://doi.org/10.1016/j.ajic.2022.07.006.
17. Dogana SSA, Ugurb SG, Doganc O, Turedid OK, Sekerd E, Ulkerc G. Evaluation of bacterial contamination of miswak toothbrushes in different storage environments: an experimental study. Ann Med Res. 2024;31(3):190-198. https://doi.org/10.5455/annalsmedres.2023.10.292.
18. Jia YJ, Liao Y, He YQ, Zheng MQ, Tong XT, Xue WQ, Zhang JB, Yuan LL, Zhang WL, Jia WH. Association between oral microbiota and cigarette smoking in the Chinese population. Front Cell Infect Microbiol. 2021; 11:658203. https://doi.org/10.3389/fcimb.2021.658203.
19. Otto M. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015;28(3):603-661. https://doi.org/10.1128/cmr.00134-14.
20. Peker I, Akca G, Sarikir C, Alkurt TM, Celik I. Effectiveness of alternative methods for toothbrush disinfection: an in vitro study. Sci

World J. 2014; 2014:726190. doi:10.1155/2014/726190.

21. Eriksson S, van der Plas MJA, Mörgelin M, Sonesson A. Antibacterial and antibiofilm effects of sodium hypochlorite against Staphylococcus aureus isolates derived from patients with atopic dermatitis. Br J Dermatol. 20177(2):513-521. https://doi.org/10.1111/bjd.15410.
22. Brookes ZLS, Bescos R, Belfield LA, et al. Current uses of chlorhexidine for management of oral disease: a narrative review. J Dent. 2020; 103:103-110. https://doi.org/10.1016/j.jdent.2020.103497.
23. Harrison F, Blower A, de Wolf C, Connelly E. Sweet and sour synergy: exploring the antibacterial and antibiofilm activity of acetic acid and vinegar combined with medical-grade honeys. Microbiology (Reading). 2023;169(7):001351. https://doi.org/doi:10.1099/mic.0.001351.
24. Assari SA, Mahrous MM, Ahmad YA, Alotaibi F, Alshammari M, AlTurki F, AlShammari T.Efficacy of different sterilization techniques for toothbrush decontamination: an ex vivo study. Cureus. 2022;14(1): e21117. https://doi.org/10.7759/cureus.21117.
25. Frazão P, Narvai PC. Toothbrush contamination: a review of the literature. Braz Oral Res. 2010;24(1):1-6. https://doi.org/10.1590/S1806-83242010000100002.
26. Eichenauer J, von Bremen J, Ruf S. Microbial contamination of toothbrushes during treatment with multibracket appliances. Head Face Med. 2014; 10:43. https://doi.org/10.1186/1746-160X-10-43.
27. Kaur D, Neal P. Bacterial survival and biofilm formation on conventional and antibacterial toothbrushes. Biofilms. 2004; 1:123-130. https://doi.org/10.1017/S1479050504001334.
28. Chawla H, Anand P, Garg K, Bhagat N, Varmani SG, Bansal T, McBain AJ, Marwah RG. A comprehensive review of microbial contamination in the indoor environment: sources, sampling, health risks, and mitigation strategies. Front Public Health. 2023; 11:1285393. https://doi.org/10.3389/fpubh.2023.1285393.

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