

Microbial contamination on toothbrush storage



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The study of microbial contamination on storing a toothbrush in a bathroom with a toilet

Abstract

Aims: To examine the microbial contamination of storing a toothbrush in the bathroom with a toilet among the Colony Forming Unit (CFU) of microbial groups.

Methods and Results: The results are analyzed by the counting of CFU of agar plates

Conclusion: The toothbrush storing in a bathroom with a toilet or without toilet is being contaminated and it is a bad place for storage

Introduction

Escherichia coli (E. coli) is recognized as a coliform bacterium which is gram negative, anaerobically developed and shaped like a rod. It is generally found in the intestine of warm-blooded animals such as humans. In addition, E. coli is able to discharge into the environment with fecal substance under airborne condition especially by the flushing of toilet and the bacteria can grow numerously in fresh fecal substances aerobically for short periods of time. The bacterial aerosols by the flushing can move as far as six to eight feet away from the toilet. A humid, warm surface permit more bacteria to grow and the bristles of the toothbrush would increase the surface area for microbial adhesion, hence, the fecal-oral transmission can be used as a major route via which pathogenic strain of bacteria to bring into oral diseases. Apparently, the occurrence of fecal coliforms in water is not straightforwardly

harmful and does not essentially express the presence of feces (Doyle, M. P., and M. C. Erickson. 2006).

Five types of plates were used with the microbial groups in this experiment.

Chocolate blood agar, CBA, is an unselective and a medium with enriched development used for fastidious bacterial isolation. ^{[1] [2] [3]} It is an alternative of the blood agar plate which contains red blood cells lysed by moderate heating to 80 °C.

Reasoner's 2A agar, R2A, is used for the isolation of heterotrophic bacteria from treated drinkable water (Sandle, T, 2004). These bacteria are likely to grow slowly and would rapidly be restrained by speedy-growing species on a rich medium.

Diagnostics Pseudomonas Isolation Agar, PYO, is used for the isolation and differentiation of *Pseudomonas aeruginosa* selectively by raising the pyocyanin production. Pyocyanin production is indicated as a bluish-green, water-soluble pigment that gives a greenish color into the media (Bodey, G. D., et al., 1989). Malt Extract Agar, ME, is used for the cultivation and isolation of yeasts and molds by suitable nutrient supply ⁽⁷⁾. The pH is modified to nearly 5.5 for upgrading the fungi growth and to lightly hinder bacterial growth which usually determined as environmental contaminants.

⁽⁶⁾ Lauryl Sulfate Broth, LTA, is used for the isolation of coliforms in water and foods. The coliform groups involves aerobic and facultative anaerobic, gram-negative and the bacilli without spores which is able to progress the fermentation of lactose and generate acid and gas at 35°C. Additionally the

LTA44°C makes faecal coliforms to be enumerated and the Sodium Lauryl Sulfate demonstrates excellent inhibition of organisms except the coliforms.

The technique of spreading plates is used for the isolation and enumeration of microorganisms in a mixed culture by even allocation. This method can measure the bacteria easier by using a sterilized spreader and applying a small quantity of bacteria suspension on the surface of plate. The plate is required to be dry before incubation so the bacteria can be absorbed into the agar rapidly. The plate counting approximate the amount of cells depend on the capability to produce colonies under particular states of nutrient medium, temperature and time. The counting of CFU believes that every colonies is aggregate and observed by a single viable cell. [11] Moreover, CFU/mL of the original suspension is figured out mathematically and then factored in the quantity plated and the dilution factor. This study aims to examine the microbiological contamination of toothbrush to prove whether a bathroom with a toilet is a good place to store a toothbrush or not.

Material and methods

Sample collections and suspensions preparation of samples

Group 1, Stored normally at the sink in a bathroom which contained a toilet situated 0.5 - 2 m away from the sink. Group 2, not stored near a toilet. Brush either stored in bedroom or in bathroom devoid of toilet.

The samples of plaque, toothbrush, sink and tap water were collected by respective sterile method. The plaque sample was put into a 2.5ml Ringer's solution and sonicated for 10 minutes. Then the weight of plaque sample is determined by weighing the bijou bottle and the toothpick is removed by

aseptic techniques. The toothbrush sample is put into the 10ml Ringer's solution into a Universal bottle and the toothbrush head is cut and removed aseptically. The swab originated from 2 x 2 cm square of the sink sample is put into 2.5 Ringer's solution of the bijou bottle and is vortexed for approximately 3 minutes. Then the swab is taken out by sterile techniques and the suspension is ensured to squeeze out of the swab. The sample of tap water is put into the 15ml of sterile tube.

10-fold dilutions and plates spreading

Each of the samples were processed with orders of plaque, toothbrush, sink and tap water. Different dilutions of samples are prepared by the 10-fold dilutions except the tap water samples. 0.5ml of undiluted original suspension (10^0) is removed and inoculated into 4.5 ml of another Ringer's solution aseptically and mixed well to be a 10^{-1} dilution. All the samples are prepared down to 10^{-5} dilution.

Four plates of CBA, R2A, ME and two plates of PYO, LTA37 and LTA44 of each samples were collected and labelled. For the plaque sample, 2 PYO, 2 LTA37 and 2 LTA44 plates with 10^0 , 2ME plates with 10^{-1} , 2ME plates with 10^{-2} , 2 CBA and 2 R2A plates with 10^{-4} , 2 CBA and 2 R2A plates with 10^{-5} were inoculated. For the toothbrush sample, 2 PYO, 2 ME, 2 LTA37 and 2 LTA44 with 10^0 , 2 ME plates with 10^{-1} , 2 R2A with 10^{-2} , 2 CBA and 2 R2A plates with 10^{-3} , 2 CBA plates with 10^{-4} were inoculated. For the sink sample, 2 PYO, 2 LTA37 and 2 LTA44 plates with 10^0 , 2 ME plates with 10^{-1} , 2 ME and 2 R2A plates with 10^{-2} , 2 CBA and 2 R2A plates with 10^{-3} , 2 CBA plates

with 10^{-4} were inoculated. For the tap water sample, all 18 plates were inoculated with 10^0 undiluted suspension. 0.2 ml of relative dilutions were spread across the surface of the agar plates with aseptic methods. All the plates were allowed to dry before inoculation. 2 LTA44 plates were put in the incubator with 44, f, 2 PYO plates, 2 LTA37 and 4 CBA plates were put in the incubator with 37, f, 4 R2A and 4 ME plates were put in the incubator with 25, f (United States Pharmacopeia, 2015).

Data collection

The inoculated plates were collected and colonies were counted to determine the Colony Forming Units (CFU) by multiplying the average number of colonies by 5 to convert from 0.2 ml to 1 ml and is multiplied by the dilution factor. The best number of colonies (30-60 colonies) were counted for the plates which have two dilutions plated onto them. The CFU was calculated as the $\text{CFU ml}^{-1} 10^0$ suspension and needed subsequent conversions except for the tap water sample. For the plaque, $\text{CFU ml}^{-1} 10^0$ suspension was multiplied by 2.5 and divided by the weight of plaque to determine the CFU g^{-1} in 2.5 ml bijou bottle. For the toothbrush, $\text{CFU ml}^{-1} 10^0$ suspension was multiplied by 10 to determine the CFU head^{-1} in the 10 ml Universal bottle. For the sink surface, $\text{CFU ml}^{-1} 10^0$ suspension was multiplied by 2.5 and divided by 4 to determine the CFU cm^{-2} in the 2.5 ml bijou bottle from a 4 cm^2 square.

Results

Mean CFUs per unit with SD : a measure that is used to quantify the amount of variation or dispersion of a set of data values. ^[11]A low standard deviation indicates that the data points tend to be close to the mean (also called the expected value) of the set, while a high standard deviation indicates that the data points are spread out over a wider range of values.

Average with SD (Plaque CFUs per gram)

	Group 1 (with toilet)	Group 2 (without toilet)
CBA	$4.16 \pm 12 \times 10^8$	$4.71 \pm 16 \times 10^8$
R2A	$1.2 \pm 3.41 \times 10^8$	$1.96 \pm 3.55 \times 10^8$
PYO	$8.34 \pm 23.5 \times 10^2$	$7.41 \pm 38.5 \times 10^7$
LTA37	$4.7 \pm 23.4 \times 10^2$	$8.49 \pm 4.33 \times 10^3$
LTA44	0	$2.55 \pm 13.2 \times 10^2$
ME(yeasts)	$3.35 \pm 8.78 \times 10$	$9.51 \pm 49.2 \times$

	4	10^5
ME (fil fungi)	$1.72 \pm 6.2 \times 10^4$	$2.95 \pm 5.71 \times 10^3$

Average with SD (toothbrush CFUs per head)

	Group 1 (with toilet)	Group 2 (without toilet)
CBA	$1.92 \pm 3.52 \times 10^6$	$1.51 \pm 5.76 \times 10^7$
R2A	$2.35 \pm 5.71 \times 10^6$	$1.12 \pm 4.60 \times 10^7$
PYO	$1.27 \pm 4.07 \times 10^4$	$2.78 \pm 14.4 \times 10^8$
LTA37	$1.84 \pm 5.94 \times 10^4$	$2.54 \pm 12.9 \times 10^6$
LTA44	$1.18 \pm 5.88 \times 10^4$	$4.85 \pm 24 \times 10^4$
ME(yeasts)	$2.10 \pm 9.78 \times 10^4$	$3 \pm 13.6 \times 10^5$
ME (fil fungi)	$2.59 \pm 11.8 \times 10^4$	$1.34 \pm 2.26 \times 10^4$

4 10^2

Average with SD (sink per square cm)

Group 1 (with toilet)	Group 2 (without toilet)
CBA	$8.91 \pm 22.7 \times 10^4$ $6.33 \pm 24.7 \times 10^5$
R2A	$2.56 \pm 11.5 \times 10^5$ $7.82 \pm 36.7 \times 10^5$
PYO	$1.22 \pm 2.98 \times 10^2$ $4.65 \pm 16 \times 10^3$
LTA37	$4.55 \pm 12.4 \times 10^2$ $5.73 \pm 16.9 \times 10^3$
LTA44	$4.24 \pm 14.5 \times 10^2$ $1.54 \pm 6.19 \times 10^2$
ME(yeasts)	$4.84 \pm 1.57 \times 10^2$ $3.13 \pm 7.55 \times 10^3$
ME (fil fungi)	$1.40 \pm 1.76 \times 10^2$ $4.71 \pm 18.5 \times 10^2$

Average with SD (tap water CFU per mL)

	Group 1 (with toilet)	Group 2 (without toilet)	
CBA	$1.53 \pm 2.14 \times 10^4$	$1.5 \pm 7.69 \times 10^4$	
R2A	$9.04 \pm 21.6 \times 10^4$	$3.49 \pm 14.4 \times 10^4$	
PYO	$8.46 \pm 30.7 \times 10^{-1}$	$1.85 \pm 9.62 \times 10^{-1}$	
LTA37	0	$3.7 \pm 19.2 \times 10^{-2}$	
LTA44	$1.15 \pm 5.88 \times 10^{-1}$	0	
ME(yeasts)	7.12 ± 19.5	9.7 ± 22.1	
ME (fil fungi)	5.85 ± 19.5	4.44 ± 7.85	

p-value. Two-tailed independent samples t-test (Group 1 vs Group 2) - 95% = P < 0.05 is significant

plaque	toothbrush	sink	Tap water	
CBA	0.891	0.	0.269	0.

		251		325
R2A	0. 437	0. 336	0. 488	0. 234
PYO	0. 341	0. 331	0. 155	0. 291
LTA37	0. 334	0. 324	0. 118	0. 331
LTA44	0. 341	0. 453	0. 375	0. 313
ME(yeasts)	0. 356	0. 300	0. 086	0. 654
ME (fil fungi)	0. 241	0. 283	0. 370	0. 731

From the table, the CFU in the plaque sample collected from storing the toothbrush with and without toilet are observed. For the CBA and R2A, the CFU in group 1(with toilet) is counted as $4.16 \pm 12 \times 10^8$ CFU g⁻¹ and $1.2 \pm 3.41 \times 10^8$ respectively, and the CFU in group2 (without toilet) is marginally greater than group1 which resulted as $4.71 \pm 16 \times 10^8$ and $1.96 \pm 3.55 \times 10^8$ respectively. For the PYO, LTA37, LTA44 and ME (yeasts), The CFU of group 2 are slightly more than the group 1 except the fewer CFU ($2.95 \pm 5.71 \times 10^3$) without toilet than in which with toilet ($1.72 \pm 6.2 \times 10^4$)

observed in ME (fil fungi). It is found that the fewest CFU is on LTA44 or even observed as 0 CFU per unit since 44,, fis over the optimal temperature and the bacteria can be inactive. Besides, for the other microbial groups, toothbrush, sink and the tap water samples, they showed the similar results as the plaque sample that the average CFUs per unit of the without toilet sets were slightly more than the sets with toilet on the CBA, R2A, PYP, LTA37, LTA44 and ME (yeasts) and the CFUs per unit of the without toilet sets were slightly less than the toilets sets. However, the samples in the tap water evaluated some differences from the other groups was that the small quantity of average CFUs per ml within each samples were indicated since the presence of fecal coliforms in water might not be directly harmful and did not necessarily indicate the presence of feces.

Overall, the CFU of the plaque sample collected without toilet is slightly greater than which with toilet but probably there is no obvious difference between two groups by the examination of p value.

P value is used to compare the significance of the tests. Significant difference between two groups can be determined if the p value is smaller than 0.05 and the null hypothesis is not be supported. From the table of two-tailed independent sample t-test, it indicated that all p values among the different agar plates of the 4 microbial groups are greater than 0.05 which showed that the difference between 2 groups for any sample type (with toilets and without toilets) or medium types are not significant. Although the tables showed large variability within a given sample, this is common for environmental samples and hence the null hypothesis is supported.

Discussion

The toothbrush, the plaque and the sink samples collected with or without the toilet sets, except the tap-water, were observed to contain large quantity of coliforms and it proved the presence of pathogenic bacteria, E. coli. The toothbrushes contamination acts as a crucial role in the expansion of various diseases such as respiratory infection and oral diseases (M. B. Dayoub, D. Rusilko, and A. Gross, 1977). Toothbrushes are often stored in the toilets and disclosed to contamination as it is a microbial atmosphere with the occurrence of pathogenic bacteria which is spread by aerosols and the flushing of toilets (Taji SS, Rogers AH, 1998). Moreover, the presence of the E. coli is correlated with the uncleaned bristles or the storage of brushes with high humidity (the sink) and warm environment which are near to the bristles and this can effectively enhance the spread and growth of bacteria than those stored in aerated conditions by 70%. (R. T. Glass, 1992).

However, the experimental results showed the colony forming units in the bathroom without toilets sets were greater than the sets with toilets which is apparently difference from the hypothesis. P-values of Two-tailed independent samples t-test can evaluate the situation. Although the large variability within a given sample was observed, this is common for environmental samples. Because of the large variability, there is no significant difference between the two groups for any sample type or medium type. The p values is greater than 0. 05 which showed 95% level of confidence that the two parameters are not the same and there is no enough difference within the samples to conclude a difference so the null hypothesis

is accepted. It is concluded that toothbrush would get contaminated regardless of storing near or far from the toilet.

There are some limitations existed in this experiment. Firstly, there is manageable amount of data for the analysis which can affect the precision of the results. In this experiment, only 26 samples and 27 samples for each groups were examined which were not excessive enough to evaluate the hypothesis. More sample sizes are suggested and hence sufficient statistical power to the final results can be determined. Another error would be the dilution error since the pipetting for 10- fold dilutions could lead to a considerable departure from the expected identity and inaccurate results. It is suggested that the precise pipetting from 10^{-1} down to 10^{-5} and proper vortex with vibration of suspension within each dilutions is required. Additionally, more time allowed for the first lab practical and appropriate aseptic techniques could be suggested to ensure the accuracy of results.

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