REMOVAL OF *ESCHERICHIA COLI* ON HANDS WITH NATURAL OR ARTIFICIAL FINGERNAILS

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Abstract

Regions beneath fingernails are the physical locations on hands that harbor microorganisms. Long fingernails tend to harbor more microorganisms than short nails. Artificial fingernails, which are usually long and always polished, reportedly harbor higher microbial populations than natural nails. Hence, the efficiency of different hand washing methods to remove microorganisms from natural and artificial fingernails was evaluated. A strain of nonpathogenic *Escherichia coli* JM109 genetically transformed with a green fluorescent protein-labelled plasmid, pPGFPuv, was used as the indicator bacterium. The fingernails of eight volunteers with artificial nails and ten volunteers with natural nails were artificially contaminated with ground beef containing *E. coli* JM109. Volunteers then washed their hands by different methods, including tap water, liquid soap, antibacterial liquid soap, alcohol gel, liquid soap plus alcohol gel, and liquid soap plus a nailbrush. The inoculated *E. coli* were recovered by scrubbing fingernails with an electronic toothbrush soaking in Butterfield's phosphate buffer (BPB) before and during the scrub. Five inoculated fingernails on two different hands were scrubbed with the toothbrush before washing to obtain baseline data, whereas the other five nails were scrubbed after hand washing. Dilutions of BPB scrub suspensions were plated onto tryptic soy agar (TSA) containing 100 mg ampicillin/L, and colonies were counted under UV light. The greatest reduction (3 $\log_{10}$ CFU per volunteer) of *E. coli* populations was obtained by washing with liquid soap plus a nailbrush. Other washing methods achieved a 1-to 2- $\log_{10}$ CFU per volunteer reduction. Lower populations of *E. coli* were removed from artificial than natural fingernails; however, there was no statistical difference ($p>0.05$) in removal of *E. coli* from artificial or natural nails, except for washing with liquid soap plus a nailbrush. Based on the results of this study, physical elimination such as scrubbed by a nailbrush was a critical step to remove microorganisms from areas underneath fingernails.

Introduction

Hand washing has long been recognized as an important procedure in preventing the transmission of disease (Larson, 1989). Investigations of foodborne outbreaks have revealed that poor personal hygienic practices of food handlers have been a factor in transmitting causes for several outbreaks (Guzewich & Ross, 1999). Based on a survey conducted by the Centers for Disease Control and Prevention (CDC), poor personal hygiene of food handlers was responsible for about 36% of outbreaks from 1988 to 1992 (Bean et al., 1996). Even when hands are washed, microbes can still exist beneath

fingernails (Rayan et al., 1987). Higher populations of microorganisms (2 to 3 $\log_{10}$ CFU/fingernail) frequently occur beneath nails and are often more difficult to remove than on other locations of hands (McGinley et al., 1988). Fingernail length and texture also affect the efficiency of microbial removal from beneath nails. Long and polished nails generally harbor more microbes after hand washing than short and unpolished nails (Baumgardner et al., 1993). Hence, wearing artificial fingernails may be a factor influencing the efficacy of hand washing because artificial nails normally polished are usually longer than natural nails. Several studies have revealed that higher microbial populations are recovered from artificial nails than natural nails (Rubin, 1988; Pottinger et al., 1989; Hedderwick, et al., 2000). In fact, an Norwalk-like virus outbreak involving about 300 case was associated with cake icing contaminated with an infectious baker wearing artificial fingernails (Georgia Department of Human Resources, 2001). Hence, effective hand washing methods to remove microbes from artificial or natural fingernails are essential to preventing disease transmission.

Materials and Methods

Preparation of inoculum. Non-pathogenic *E. coli* strain JM109, which was genetically transformed with plasmid pGFPuv (Clontech Laboratories, Palo Alto, CA), was used as the indicator bacterium for this study. One ml of bacterial suspension was added into 25 g of fresh ground beef, and massaged by hands for one min to mix bacterial suspension into the beef.

Bacterial inoculation of fingernails of volunteers. Eight volunteers with artificial fingernails, all female, and ten volunteers with natural nails, an equal number of male and female, participated in the study. Fingernail lengths of volunteers were measured by placing a thin ruler underneath the nail. Before inoculation, volunteers washed their hands with liquid soap for 15 sec, then rinsed with warm water (40 to 45°C) for 10 sec. After drying their hands with paper towels, volunteers placed nails of their right hand into the contaminated ground beef, pushing their hands into the beef five times. Volunteers used the corresponding fingers on their other hand to rub the beef residue into the areas underneath nails for 30 sec. The same procedure was followed for inoculation of fingernails on the left hand. One 25-g ground beef sample was used for each volunteer for each test.

Enumeration of bacterial populations. Nails on thumb, middle and little fingers on the left hand and the index and ring fingers on the right hand were scrubbed first by an electronic toothbrush according to the protocol described below to obtain baseline populations before washing. Each washing treatment was conducted at least twice with each volunteer. For the second study in which the same washing method was used, nails on the thumb and middle and little fingers of the right hand, and on the index and ring fingers of the left hand were scrubbed first to obtain baseline populations. The bristles of the electronic toothbrush were soaked in a petri-dish containing 10 ml of BPB before and during scrubbing for 2 sec at 10 sec intervals. Each fingernail was scrubbed for one min. After volunteers washed their hands, nails on the remaining fingers were scrubbed based on the same protocol but with another toothbrush. BPB was decimally (1:10) diluted and

plated onto duplicate trypic soy agar (TSA) containing 100 mg ampicillin/L. The plates were incubated at 37°C for 24 h and colonies were counted under UV light.

Two 25-g ground beef samples were selected randomly to enumerate *E. coli* JM109 populations in beef after inoculation. Populations of *E. coli* JM109 in the ground beef were maintained at 10^8 CFU/g throughout the study.

Hand washing methods. Five hand washing methods were evaluated, including washing hands with warm tap water, liquid soap, antibacterial liquid soap, alcohol gel, liquid soap plus alcohol gel, and liquid soap plus a nailbrush. Each volunteer used the same amount of liquid soap (2 ml). The flow rate of tap water (40 to 45°C) was 8 L per min. For washing hands with warm water only, volunteers rubbed their hands vigorously under warm tap water for 15 sec. Common brands of liquid soap or antibacterial liquid soap containing triclosan were used for the hand washing with soap. Volunteers first wet their hands and then 1 ml of liquid soap was placed on each hand. Volunteers rubbed their hands vigorously for 15 sec and then rinsed hands under warm tap water for 10 sec. Two paper towels were used by each volunteer to dry their hands. A common brand of alcohol gel containing 62% ethanol was used. The alcohol gel was used directly without washing their hands. After placing 1 ml of gel in the middle of each hand, volunteers rubbed their hands thoroughly for 15 sec then waited for 1 min for the alcohol to evaporate. For the combination of liquid soap and alcohol gel, volunteers washed with soap and dried their hands, then rubbed their hands with alcohol gel as previously described. For washing with liquid soap plus a nailbrush, volunteers washed their hands with soap for 15 sec, then used a nailbrush to scrub nails with soap on. Each fingernail was scrubbed with 10 firm stokes. Volunteers rinsed and dried their hands as previously described after nailbrush scrubbing.

Results

All female volunteers except one had longer natural fingernails than male volunteers. Length of artificial nails was generally longer than natural nails (Table 1). Length ranges for natural and artificial nails were from 0.1 to 5.7 mm and 2.75 to 10.2 mm, respectively. Most natural nails were shorter than 3 mm, except for two female volunteers. Similar populations (6 ± 1 $\log_{10}$ CFU/volunteer) of *E. coli* JM109 were recovered before washing despite different lengths of nails. The greatest reduction of *E. coli* JM109 was obtained with liquid soap plus a nailbrush and least reduction was obtained with alcohol gel rubbing (Fig. 1). During the study, laboratory personnel observed that more beef residue remained underneath fingernails when volunteers used alcohol gel than with other washing methods. Results indicate that physical removal of residue beneath fingernails is a critical factor affecting removal of bacteria.

Only the hand washing combinations of soap plus a nailbrush or alcohol gel provided greater than a 2-log CFU/volunteer reduction. However, there was less than 2-log CFU/volunteer reduction for long artificial nails washed with soap plus alcohol gel like other washing methods. A greater reduction of inoculated *E. coli* populations was obtained from short than long nails. However, there was no statistically significant

difference ($P \leq 0.05$) in results obtained from the two nail lengths. A significantly greater reduction was obtained from short natural than long artificial nails when nailbrushes were used. Greater than a 5-$\log_{10}$ CFU/volunteer reduction was obtained for two male volunteers with short natural nails when nailbrushes were used (Fig 2). Combined, there was a greater than 3-log CFU/volunteer average reduction for five male volunteers with short natural nails who washed their hands with liquid soap plus a nailbrush. In contrast, there was an ca. 2-log CFU/volunteer reduction for all female participants with long natural or artificial nails, with exception of one female volunteer (3-log reduction CFU/volunteer).

Conclusion

Our results revealed that removal of *E. coli* from long artificial fingernails was more difficult than from short natural nails. Washing with liquid soap plus a nailbrush was the most effective method to remove bacteria from nails. Food handlers would be best advised to avoid wearing artificial nails and keep natural nails trimmed to less than 2 mm beyond the fingertips.

References

Baumgardner, C. A., C. S. Maragos, M. J. Walz, and E. Larson. 1993. Effects of nail polish on microbial growth of fingernails. Asso. Operation Room Nurses J. 58: 84-88.

Bean, N. H., J. S. Goulding, C. Lao, and F. J. Angulo. 1996. Surveillance for foodborne-disease outbreak-United States, 1988-1992. Morbid. Mortal. Weekly Rep. 45:1-55

Bidawid, S., J. M. Farber, and S. A. Sattar. 2000. Contamination of foods by food handlers: Experiments on hepatitis A virus transfer to food and its interruption. Appl. Environ. Microbiol. 66:2759-2763.

Centers for Disease Control and Prevention. 2000. Outbreaks of Norwalk-like viral gastroneteritis-Alaska and Wisconsin. Morbid. Mortal. Weekly Rep. 49:207-210.

Georgia Department of Human Resources. Division of Public Health. 2001. Georgia Epidemiol. Rep. 17 (6):1-3.

Guzewich, J. and M. P. Ross. 1999. Interventions to prevent or minimize risks associated with bare-hand contact with ready-to-eat foods. Online:fttp//www.cfsan.fda.gov.

Hedderwick, S. A., S. A. McNeil, M. J. Lyons, and C. A. Kauffman. 2000. Pathogenic organisms associated with artificial fingernails worn by healthcare workers. Infect. Control Hosp. Epidemiol. 21:505-509.

Larson, E. 1989. Handwashing: it's essential-even when you use gloves. Ameri. J. Nursing. 89: 934-937.

McGinley, K. J., E. L. Larson, and J. J. Leyden. 1988. Comparison and density of microflora in the subungual space of the hand. J. Clin. Microbiol. 26:950-953.

Pottinger, J., S. Burns, and C. Manske. 1989. Bacterial carriage by artificial versus natural nails. Amer. J. Infect. Control. 17:340-344.

Rayan, G. M., and D. J. Flournoy. 1987. Microbiologic flora of human fingernail. J. Hand Surg. 12A:605-607.

Rubin, D. M. 1988. Prosthetic fingernails in the operation room. Asso. Operation Room Nurses J. 47:944-948.

Table 1. Fingernail length (mm) of volunteers

Fingernail types	Natural		Artificial
Gender	Male	Female	Female
Length range	0.1-0.9	0.75-5.7	2.75-10.2
Average±SD	1.22±0.47	2.67±1.10	5.94±2.04

Fig. 1. Reduction of *E. coli* JM109 populations by different hand washing methods

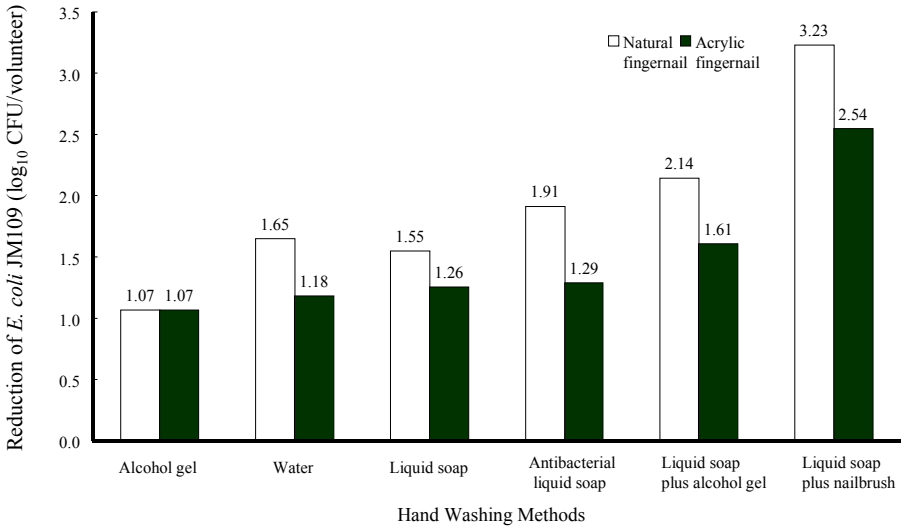

Fig. 2. Reduction of *E. coli* JM109 populations by liquid soap plus a nailbrush vs. nail length