Bacteria Abundance Positively Correlates with Hand Dominance Independent of Time and Dependent on Physical Activity

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Abstract: Human hands serve as vectors for the transmission of infectious diseases due to their continuous exposure to diverse biomes and their capacity to adapt by selection of spontaneous mutants. Hand hygiene, particularly routine washing, reduces transient microbial flora on both dominant and nondominant hands, limiting the spread of gram-positive and gram-negative pathogens. This study investigated bacterial distribution across dominant and nondominant hands concerning activity level and time of day. Samples were collected from 30 subjects at three intervals—morning, noon, and evening—and processed using a standardized six-step methodology: 1) nutrient agar preparation, 2) petri dish segmentation for bacterial isolation, 3) swab sampling from both hands, 4) 72-hour incubation at 25 °C, 5) colony quantification via ImageJ analysis, and 6) sterilization and Gram staining. Results indicated significantly higher bacterial counts on the dominant hand, with approximately 50% more colonies than the nondominant hand, independent of time of day. However, bacterial distribution across individual fingers was relatively uniform. Growth rate correlated strongly with subject activity rather than circadian variation. Additionally, dormant periods yielded reduced bacterial counts, reflecting the stable presence of resident skin flora. Most isolated bacterial colonies were gram-positive, attributed to limited exposure to enteric or contaminated environments necessary for gram-negative proliferation. These findings highlight the role of dominant-hand activity in microbial transmission and emphasize the necessity of rigorous hand hygiene practices. The implications of this study extend to clinical and food-handling settings, where preventing disease transmission to immune-compromised individuals and ensuring sanitization in food preparation are critical.

Keywords: Bacteria; Bacterial Flora; Left Hand; Right Hand; Gram-negative; Gram-positive; Hand Hygiene; Infectious Diseases.

1. Introduction

Our hands carry pathogenic microorganisms that can facilitate the transmission of infectious diseases, posing a risk to human health [1]. Based on empirical evidence, the prevalence of the flu season supports the claim that human hands serve as vectors for transmitting global diseases[16]. Some studies demonstrate that flu transmission heavily relies on frequent hand contact with contaminated surfaces containing respiratory droplets from ill patients, where disease manifestation occurs in the first 24-48 hours, which accounts for over half of all the flu transmission events[9]. Hand contact activity is an effective vector for diseases due to the sheer abundance of bacteria dwelling on the hands. Their omnipresence stems from their versatile nature, which accounts for their presence in diverse environmental conditions[3]. Bacteria can spread through a variety of media such as water, soil, food, and even animals or insects[5]. Given their adaptability, it is plausible that hands carry at least a million viable microorganisms because both hands are exposed to different media that are non-sterile surfaces, such as public places[10]. Therefore, hand hygiene is warranted and hand washing reduces transient flora in the superficial skin layers, which ultimately protects the health of immunecompromised individuals, who typically have an immune system defect that makes them more prone to primary and secondary immunodeficiencies[4]. The minimization of the amount of transient organisms on our hands also serves as a crucial factor in many aspects, like maintaining the sanitization of food preparation[15]. When routine handwashing is not promoted, it develops various pathogenic infections that may cause diseases. In low and middle-income countries, particularly in Africa, suboptimal handwashing practices has resulted in neglected tropical diseases, such as intestinal worms, schistosomiasis, and trachoma[9].

Our hands carry gram-positive and gram-negative microbes that can be identified by the gram-staining, a staining method that classifies bacteria based on their cell wall characteristics[7]. Gram-positive bacteria lack LPS, which is responsible for activating an inflammation response, while gram-negative bacteria produce endotoxins that can cause tissue destruction, shock, or even death[10]. Gramnegative bacteria pose a greater risk to public health due to having a stronger resistance to hand washing[8]. Gramnegative bacteria possess an outer membrane consisting of rich lipopolysaccharides, which acts as a permeability barrier from the penetration of biocides and antimicrobial agents[11]. Therefore, hand cleanliness is important as it reduces the distribution of transient microorganisms on the hands. Humans have a dominant hand that we tend to use more frequently, which may contribute to whether one hand has more concentrated bacteria than the other hand. This study aims to investigate if hand dominance influences the total abundance and distribution of bacteria. This study is crucial as it provides a novel understanding of the differences between dominant and nondominant hand bacteria and whether their distinct traits and abundance contribute to diseases being transmitted equally. We posit that bacteria distribution on the fingers and hands is different based on hand dominance, independent of daytime and activity.

2. Results

This survey investigated the bacteria present on the fingers of multiple research subjects at different times of the day. In this section, we compared the hand and finger bacterial colonies of the test subjects. As the nondominant hand samples show, fewer bacterial colonies were isolated in the morning, with only 102 bacterial colonies in total (Figure 1). This increased to 316 bacterial colonies at noon, and subsequently 421 bacterial colonies in the evening (Figure 1). The number of colonies in the nondominant hand in the morning was approximately 110% less concentrated than that collected at noon, and the evening plates show the maximum bacterial concentration, approximately 33% and 212% more concentrated than noon and morning plates, respectively (Figure 2). The significant statistical difference in the number of bacterial colonies between dominant and nondominant groups is supported by an error bar plot, where the statistical difference is supported by a one-way ANOVA test of 30 participants (Figure 3). The nondominant hand's bacterial colonies grew in a consistent positive trend as the day progressed, while the dominant hand's bacterial colonies grew inconsistently (Figure 3). By taking the mean of the number of bacterial colonies in both hands over morning, noon, and evening periods, the study confirmed that the dominant hand has an average of 140 more bacterial colonies than those on the nondominant hand (Figure 4). Based on the results seen in Figure 5, the bacteria distribution was the most concentrated in fingers 3 and 2, based on the right and left hands, respectively. In Table 1, a one-way ANOVA revealed a significant statistical difference in the mean number of bacterial colonies between dominant and nondominant groups in the morning, noon, and evening periods, F (5, 174) = 6005.220, P < 0.01, with a high effect size. In Table 2, the test subject with the greatest variance in the types of activities performed was selected to see how his activity, performed between the intervals morning, noon, and evening, impacted the growth of bacterial colonies in both hands. In Table 3, the number of gram-positive and gram-negative bacteria is shown based on a comparison of gram-staining from six different bacteria variants isolated from both hands. The results indicate more gram-positive bacteria than gram-negative across both hands. These results indicate more bacterial colonies on the left hand on fingers 1 and 2 and fewer bacterial colonies on the left hand on fingers 3,4, and 5.

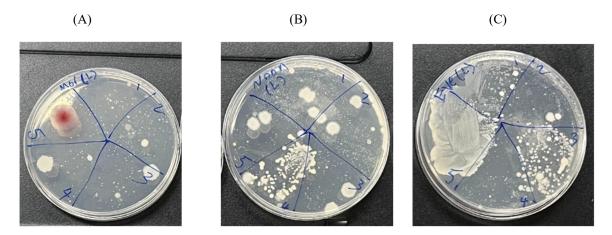
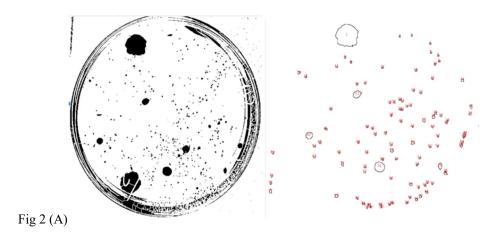


Figure 1. The abundance of bacteria increases on the nondominant hand as the day progresses. (A) Isolated bacteria from the morning nondominant hand sample. (B) Isolated bacteria from noon nondominant hand sample. (C) Isolated bacteria from the evening nondominant hand sample



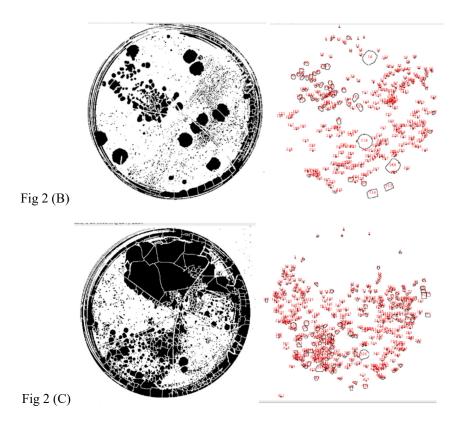


Figure 2. The analysis of bacteria colonies on the nondominant hand of all ten fingers as the day progresses using ImageJ software. (A) Isolated bacteria from the morning nondominant hand sample. (B) Isolated bacteria from the noon nondominant sample. (C) Isolated bacteria from the evening nondominant sample.

Table 1. One-way ANOVA showing the statistical difference between the dominant and nondominant hand number of bacterial colonies of 30 test subjects in the morning, noon, and evening periods.

ANOVA: Single Factor						
SUMMARY						
Groups	Count	Sum	Average	Variance		
Morning Dominant	30	14183	472.7666667	79.63333333		
Noon Dominant	30	10171	339.0333333	87.4816092		
Evening Dominant	30	13363	445.4333333	97.4954023		
Morning Nondominant	30	3143	104.7666667	84.04712644		
Noon Nondominant	30	9270	309	103.5862069		
Evening Nondominant	30	12537	417.9	91.54137931		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2721291.183	5	544258.2367	6005.220951	0	2.266061706
Within Groups	15769.76667	174	90.63084291			
Total	2737060.95	179				

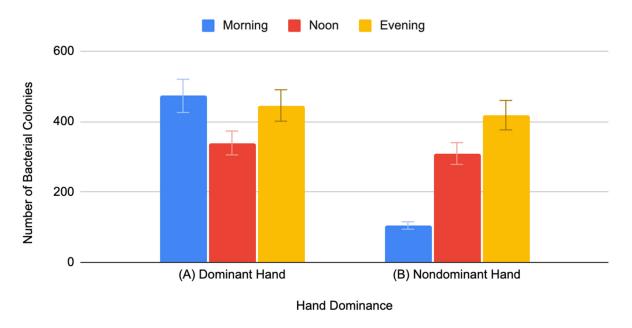


Figure 3. The number of bacterial colonies isolated from both hands after 72 hours of 25 °C incubation reflects the dominant hand having more bacteria than the nondominant hand as the day progresses. (A) The Isolated bacterial colonies from the dominant hand in the morning, noon, and evening. (B) The Isolated bacterial colonies from the nondominant hand in the morning, noon, and evening

Table 2. Activities completed between intervals of bacterial isolation of one test subject

Time of Day	Number of Hand Washings	Number of times in contact with food	Number of times in contact with other media
Morning	0	0	2
Morning-Noon	3	1	0
Noon-Evening	0	0	5

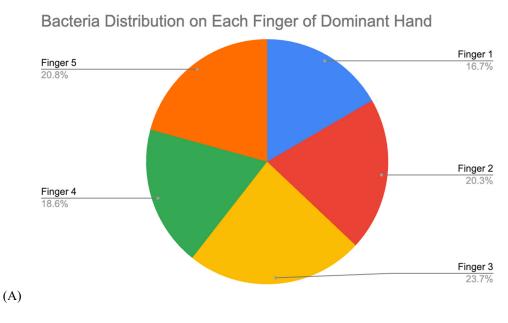


Figure 4. The average number of bacteria colonies isolated from the dominant and nondominant hands after 72 hours of 25 °Cincubation. (A) Average number of bacterial colonies isolated from the dominant hand. (B) Average number of bacterial colonies isolated from the nondominant hand.

Bacteria Distribution on Each Finger of Nondominant Hand

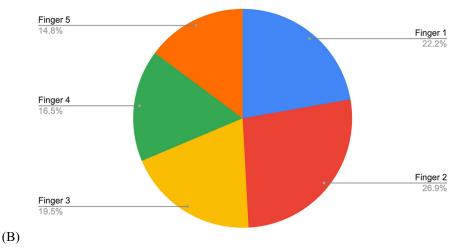


Figure 5. The comparison of bacteria distribution on each finger after evening of both hands. (A) The percentage of bacterial colonies isolated from each finger on the dominant hand. (B) The percentage of bacterial colonies isolated from each finger on the nondominant hand

Table 3. A comparison of Gram staining from the six different variants of bacteria isolated from both hands

Different types of bacteria				
Different Gram-positive bacteria	Different Gram-Negative Bacteria			
41778	20889			

3. Discussion

Bacteria are colonies of microbes that are omnipresent in the environment, including the human body [10]. This study demonstrated that the dominant hand carried more bacteria than the nondominant hand, regardless of the time of day. One possible justification is due to hand dominance. The isolation of more bacteria from the test subjects' dominant hand is expected, given that their dominant hand comes into contact with more surfaces and objects. This engenders an increase in the diversity of bacterial species, making the dominant hand more susceptible to transmitting diseases than the nondominant hand. There was no significant difference between the number of isolated colonies in each finger since each finger constantly carried bacteria. A plausible explanation is that the test subject may have performed each activity with roughly all five fingers of each hand, eliminating the possibility of having substantially different bacterial distributions on each finger.

The initial claim that bacteria distribution is independent of activity is unwarranted based on empirical evidence from Figure 3a, which displays a positive correlation between activity and bacteria growth from all 30 test subjects. However, both hands have different rates of bacterial expansion. Bacteria distribution on the nondominant hands of all test subjects had a general linear increase in trend from morning to noon, while the dominant hands of all test subjects' bacterial colonies increased substantially in the morning and evening, but decreased at noon. A plausible explanation of this phenomenon is due to hand activity. Perhaps, in the morning, test subjects used their dominant hands more frequently, which accounts for why initial amounts of isolated bacteria were greater in the dominant hand than the nondominant hand. Nearing noon, hand sanitization may have

occurred, potentially decreasing the amount of bacteria substantially on both hands. Between noon to evening, test subjects may have exercised using both hands, which could account for the similar increasing trend for dominant and nondominant hands. This justifies the different rates of bacterial expansion in both hands based on the degree and inclusivity of the activity performed by each test subject, such as the case of one particular test subject in Table 2 who used their hands for hand sanitization, food preparation, and contact with other media.

Despite the test subjects' dominant hand having a pronounced decrease in the number of bacterial colonies between morning and noon due to activities that promoted "cleaner" hands, there were still isolated bacterial colonies on the test subjects' hands. One plausible explanation of this phenomenon is the residence of bacterial flora in the body, such as the skin. Bacterial flora are not typically pathogens in a healthy person but can become harmful in immunecompromised individuals[2]. Immune-compromised individuals have a higher likelihood of contracting diseases because of a genetic immune system defect that hinders the efficiency of their defensive barriers[17]. These immune system defects can lead to either primary immunodeficiency diseases, such as X-linked agammaglobulinemia, which restricts the production of **B-lymphocytes** panhypogammaglobulinemia, or secondary immunodeficiencies, such as impaired phagocytosis from increased corticosteroid usage[6]. Therefore, excessive residence of bacterial flora is detrimental to the health of immune-compromised individuals because its abundance increases the probability of a healthy person, who may not be ill or contract diseases, transmitting a particular disease to an immune-compromised individual. Even in dormant periods, when a healthy individual is not physically active, a simple

hand-to-hand contact can increase the risk of disease contraction because the human body contains a natural concentration of transient bacteria[13]. By promoting hand hygiene, communities can significantly reduce the likelihood of disease transmission to immune-compromised individuals. As a broader societal implication, it is also imperative to consider safety procedures in food preparation industries. As rapidly dividing organisms, bacteria can grow in food materials and contaminate the environment, given their high adaptability to various media. Considering the safety procedures, such as having a habit of hand sanitization techniques, is crucial to protect food production from bacterial contamination. According to Nizame et al., young people, especially children, had poor hand-washing habits. Lack of habit and a convenient place for hand washing while eating food were observed as common barriers[6]. Washing hands regularly, meaning up to four times a day, and not sporadically, results in 24% fewer sick days because of respiratory illness and 51% fewer sick days because of gastrointestinal diseases[13]. This highlights the necessity of hand hygiene for public and medical health.

Gram staining was conducted on bacteria isolated from the subject's hands to study the type of bacteria. More than 67% were gram-positive bacteria and were mostly bacilli. 23% were gram-negative bacteria[14]. As gram-positive bacteria are more abundant in nature than gram-negative bacteria, the present study result suggests that during the activity, the body parts absorbed the naturally existing bacteria from the environment[6]. Regarding the outcome of this study, the isolated gram-negative bacteria is fewer than the grampositive bacteria. Since gram-negative bacteria are more prevalent in animal digestive systems, the isolation of gramnegative bacteria is related to contaminated places by enteric materials[14]. This accounts for why there are more grampositive bacteria than gram-negative bacteria because the subject's activity did not extend to places contaminated with enteric materials[12].

As this is only a pilot study with preliminary data showing that the dominant hand contained more bacteria than the nondominant hand, further investigation is required. This study could consider growing isolated bacteria colonies in a more inclusive medium. The nutrient agar medium is not inclusive of all bacterial variants, given that it only contains vitamins, carbohydrates, nitrogen, and salts, ingredients that non-fastidious bacteria cultivate in, but not fastidious ones like Abiotrophia, which requires pyridoxal or cysteine[18]. This results in selectively growing bacteria colonies that thrive in the nutrients that the nutrient agar medium provides. Another factor of further investigation is using 16s rRNA to extract bacterial DNA for sequencing instead of relying on 25 °C for growth. Room temperature may inhibit bacteria cultivation because not all bacteria variants grow well in 20-25 Celsius. Some, like human microbes, thrive in the temperature interval 28-37 Celsius[19]. Even for the ones that cultivate at room temperature, they may compete for growth.

Considering the results and implications of this study, it is warranted to conclude that hand dominance positively correlates with the quantity of bacterial colonies based on the degree of activity performed, but the time of day has no direct relationship with the quantity of bacterial colonies. As participants used their dominant hand more frequently, more bacterial colonies grew on their dominant hand. Depending on whether they exercised, ate, or slept, bacterial colonies for each participant varied significantly. This resulted in an

inconsistent trend of growth in the morning, noon, and evening periods. Regardless of the trend, bacterial colonies were still present during dormant or sanitization periods, highlighting the presence of bacterial flora. This continuous presence ultimately provides insights into the importance of maintaining good hand hygiene habits. Because bacteria cannot be eliminated, it is imperative to regulate them so that the health of immune-compromised individuals and the sanitization in food preparation are effectively safeguarded.

4. Methods

The general methodology includes 1) preparing the nutrient agar medium in a petri dish 2) Dividing the petri dish into five areas in preparation for bacterial isolation 3) collecting bacteria samples from the dominant and nondominant hands of 30 research subjects in the morning, noon, and evening, 4) incubating the bacteria culture plates at 25 °C for 72 hours, 5) performing ImageJ software analysis to count the number of bacteria colonies on both hands and analyze the bacteria distribution on each finger, and 6) perform sterilization and gram staining procedures.

Two media were prepared, one for each hand of 30 test subjects, by mixing Nutrient Agar Seaweed Solution Laboratories and Nutrient Broth HIMEDIA M002-100G with 100 ml of distilled water. The mediums were then microwaved approximately 30 times, 10 seconds each time, with brief pauses between heating. The nutrient agar mediums were poured into the petri dishes and then left to solidify. Each petri dish was labeled into five sections, with each section denoting each finger: the thumb (1), index (2), middle (3), ring (4), and pinky (5). Sterility is maintained in the petri dishes by sealing each plate with tape to prevent accidental contamination with the hands. The tape was removed from each petri dish only during periods of bacterial transmission onto the nutrient agar media. Bacteria were first isolated from the test subjects' hands into petri dish samples between 10:30-40 A.M. It is important to note that participants did not wear gloves overnight to prevent environmental contamination before the morning sampling. Then, nearing noon, bacteria were isolated from the test subjects' hands between 11:45-55 A.M. Finally, between noon and evening, bacteria were isolated from the test subjects' hands between 6:11-22 P.M. During the process of transferring bacteria from each finger to the nutrient agar mediums, the test subjects were told swab each finger onto the corresponding labeled sections (1), (2), (3), (4), (5) and wait for approximately four seconds before releasing their finger from the petri dishes. After culturing hand bacteria, the petri dishes were incubated at 25 °C for 72 hours and examined every 12 hours for visible formation of bacterial colonies. After examination, a picture of all the bacterial colonies on the petri dishes was taken and imported to ImageJ to count the number of bacterial colonies on the dominant and nondominant hands. The picture was then converted to an 8-bit type image, and a threshold between 182-255% was used with a dark background. Subsequently, the 8-bit type image was made binary and underwent watershed to make the bacterial colonies quantifiable. Upon finishing with ImageJ analysis, the bacterial colonies were isolated from 60 petri dishes and sterilized using standard sterilization procedures with an inoculum. Then, six different types of bacterial colonies were identified in the petri dishes by performing gram staining procedures using Innovating Science Gram Staining materials, which involved a compound microscope, microscopic slides, and standard

gram staining materials.

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