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Research Article

Bacterial Load on the Palms of Market Women at Different Times of the Day: A Case Study of Madina Market

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Abstract

Background: Bacteria thrive on and within the human body, with previous works revealing vast diversity in several human-associated bacterial communities. One of the largest human-associated microbial habitats is the skin where bacterial density may be as high as 10^7 cells per square centimetre. This study sought to enumerate the bacterial load on the palms of market women in Madina at different times of the day.

Methods: A cross-sectional study design was carried out on market women with palm swabs collected aseptically from 30 participants who consented to the study. The samples were transported on ice immediately after collection to perform total heterotrophic counts and hygienic quality testing.

Results: The total quality mean counts for samples taken in the morning (8.09×10^6) was significantly higher than afternoon samples (2.9×10^6) ($P = 0.025$) However, the hygienic quality of samples obtained in the morning (mean count of 5.9×10^3) was lower than samples obtained in the afternoon (mean count of 6.9×10^3). The difference observed was statistically insignificant ($p = 0.065$).

Conclusion: In conclusion, the bacterial load on the palms of the market women was high, particularly on the palms of the educated women and among those who presumed they practiced good hand hygiene.

Keywords: *Bacterial Load; Palm Swabs; Total Quality Mean Counts; Hygienic Quality; Food Borne Diseases*

Introduction

Hands may be the most effortless means by which enteric pathogens are transmitted. Skin hygiene, particularly of the hands, has been accepted as a primary mechanism to control the spread of infectious agents [1]. Hand hygiene is an encompassing term referring

to any action of hand cleansing, thus, it is the act of cleaning one's hands with or without the use of water or another liquid, or with the use of soap, for the purpose of removing soil, dirt, and or microorganisms [2]. Informal market and street traders in Accra are faced with a number of occupational health and safety risks, which have significant physical and psychological impact on the traders. The disregard for hygienic measures on the part of market women enables pathogens to get access to food and in some cases to survive and multiply in sufficient numbers to cause illness to the consumers [3]. Hand hygiene is the most effective measure for interrupting the transmission of microorganisms which cause infection both in the community and in the market setting. Hand washing with antibacterial soap and water is more effective for the removal of bacteria of potential faecal origin from hands than hand washing with water alone [4].

Food-borne diseases represent a persistent global health burden, and market women play a major role in their transmission [4]. An association between measured faecal indicator bacteria on hand and diarrhoeal illness have been proven, although a causal relationship has not been established [5]. Microbial contamination of the hands has become a global health problem despite all the public health campaigns that promotes washing of hands. Studies have found that hand washing is poorly practiced outside the healthcare profession [6], but little has been done to enumerate the microbial load on the palm of individuals outside the healthcare profession especially the market women.

Even though the sources of food contamination are diverse, personal hygiene and environmental sanitation are among the key factors in preventing the transmission of food borne diseases [7]. This study sought to determine the bacterial load found on the palms of market women in Madina at different times of the day.

Methodology

Study site and sample size

The study was conducted on samples taken from Madina Market in Accra. This market is often congested with market women and consumers. The women sold at any convenient space, some along the gutters and ditches which were sometimes choked with rubbish and breeding houseflies. Various commuters, shoppers and pedestrians could be observed and they regularly patronized the huge market for all kinds of goods and services. A total of 30 individuals engaged in food vending (7 persons), cloth vending (9 persons), cosmetic vending (7 persons) and utensil vending (7 persons) volunteered to participate in this study.

Sample collection

The 30 samples were collected aseptically by swabbing the palms of consenting market women that were selected randomly at different times of the day. The swabs were sent to the laboratory within an hour after collection on ice.

Laboratory examination

Culture media

All media used in this study were prepared according to the manufacturer's instructions. The bacterial colonies grown on the agar media were presumptively identified. All colony forming units less than five were ignored and identical colonies were carefully sub-cultured on either the Blood agar (Techno PharmChem, India) or MacConkey agar plate (Accumix™ Belgium) depending on their Gram reactions.

Standard plate counts

This procedure provided a standardized means of determining the quantity of bacteria on the palms of market women. This was an empirical measurement because organisms occurred singly, in pairs, clusters, or packets, and no single growth medium or set of physical and chemical conditions could satisfy the physiological requirements of all the organisms in the samples. Swabs were placed in 1 ml of phosphate buffered saline (PBS) and afterwards filled up to 10 ml and a 1:10 dilution was made from this, cultured and examined by

means of the pour plate method [8]. Thus, each plate was carefully labeled and 1 ml of sample from each dilution that was analyzed was transferred using a sterile pipette into the plates and 25 ml of cooled molten agar (Plate Count Agar-Thermo Fisher Scientific) was poured over it, giving a total of twenty six milliliters. The sample was mixed thoroughly with the medium and then allowed to set on a flat-top bench. Solidified plates were incubated at 37°C for 18 - 24 hours. All platings were performed in duplicates. After overnight incubation, counts were made using an automatic colony counting device that allowed viewing of individual colonies. All discrete colonies were counted where possible and expressed in colony forming units per milliliter (CFU/mL) for liquid samples.

Enterobacteriaceae count (EC)

0.1 ml of each sample analyzed was transferred using a sterile pipette onto 25 ml of solidified MacConkey agar (Accumix™ Belgium) after which an L-rod spreader was used to spread the inoculum evenly over the surface of the agar to ensure confluent growth. The plates were incubated at 37°C for 18 - 24 hours. All plating was performed in duplicates. After incubation, counts were made using an automatic colony counting device that allowed viewing of individual colonies. All discrete colonies were counted and expressed in colony forming units per milliliter (CFU/mL) for liquid samples.

Isolation of organisms

Bacterial isolation, purification, and identification were the first steps to bacteriological studies.

2 ml portions of the diluents were centrifuged at 1000 rpm for 30 minutes in a centrifuge and the supernatants were decanted. A loop full of the sediment was inoculated into Selenite F broth (enrichment medium for the growth of *Salmonella spp.* and *Shigella spp.*) and incubated at 37°C for 18 - 24 hours after which it was sub-cultured on *Salmonella/Shigella* Agar (Techno PharmChem, India). Two separate loopful of the sediment were inoculated into Deoxycholate Citrate Agar (Accumix™ Belgium) and MacConkey agar (Accumix™ Belgium) for the detection of *Shigella spp, Escherichia coli*, *Klebsiella spp,* and other Enterobacteriaceae. An enriched general purpose medium (Blood Agar, Techno PharmChem, India) was used to culture the sediment to enable other organisms (e.g. Gram positive organisms) to grow and Thiosulfate-Citrate-Bile-Sucrose Salt agar (Sigma-Aldrich, Inc.) was used for the isolation of *Vibrio spp.* after initial inoculation into Alkaline Peptone Water for enrichment**.** All incubations were done at 37°C under aerobic conditions for 18 - 24 hours. In cases of mixed growth, purity plating was carried out and suspected colonies were further identified using standard biochemical methods.

Purity plating

To obtain a pure culture, a pool was made on MacConkey agar (Accumix™ Belgium) and blood agar (Techno PharmChem, India) using a loopful of the inoculum obtained from an isolated colony on the mixed growth culture. This was then streaked on the plate using the four-dimensional method (whiles flaming the loop in-between strokes to obtain isolated colonies) to obtain parallel overlapping strokes. It was incubated at 37°C under aerobic conditions for 18 - 24 hours. The entire process was repeated for each distinct representative bacterial colony that was observed in the mixed growth culture plate.

Identification of organisms

Bacterial isolates were identified using standard biochemical methods and microscopy. The first test that was performed in order to identify organisms after culture was gram stain. This was done to differentiate gram positive organisms from gram negative organisms. When the organism was positive to the stain, catalase test was performed to differentiate *Staphylococcus spp*. from *Streptococcus spp.* When catalase was positive, then coagulase test was performed to differentiate *Staphylococcus aureus* from other species of *Staphylococcus*. On the other hand, when the Gram staining procedure yielded Gram negative rods, oxidase test was performed to differentiate Enterobacteria (oxidase negative, e.g. *Klebsiella spp*) from non-Enterobacteria (oxidase positive e.g. *Pseudomonas spp*). Both oxidase positive and negative organisms were subjected to further biochemical test such as indole, motility, urea, citrate, and triple sugar iron (TSI) for the confirmation of species.

Inclusion and exclusion criteria

Samples from Madina market women who were directly involved in the sale of foodstuff, clothes, cosmetics, and cooking utensils were selected at random and at different times of the day. The participating women were not pre-informed about the study prior to the day of sampling. This was done to forbear supplementary hand hygiene practices. Market women who traded in products other than that which the study focused on were excluded, likewise street hawkers.

Results

Demographic characteristics

The general population consisted mostly of middle-aged market women (31 - 60 years). The subgroups are as follows: 16.7% of the market women aged between 15 to 30 years, and 83.3% of market women aged between 31 to 60 years. The latter accounted for two-thirds of the total number of participants (Table 1).

Age (Range)	Percentage (%)
15-30	16.7
31-60	83.3
Total	100

Table 1: *A table showing the age distribution of participants.*

The environmental sanitary levels were determined by observation of the surroundings and have been described in table 2.

Environmental Sanitation	Percentage (%)
Poor	53.3
Good	46.7
Total	100

Table 2: *A table showing the level of environmental sanitation of participants.*

Majority of the participants practiced hand washing in the right manner, whilst a few performed poorly in the rate of practice of hand washing.

Majority of the participants indicated that they rarely engaged in hand-washing activities whiles 10% of the participants washed twice daily (Figure 1). Moreso, two thirds of the study participants did not report using hand sanitizers during working hours (Figure 2).

Comparison of means

The mean counts for samples on the various categories studied were compared to each other as displayed in table 3-9. The morning samples were taken between 8am and 9am while the afternoon samples were taken between 2 pm and 3 pm.

A total of 8 microorganisms were isolated in this study. These are: *Staphylococcus spp., Bacillus spp.*, *Streptococcus spp., Klebsiella spp., Enterobacter spp., Citrobacter spp., Providencia spp.,* and *Proteus spp* (Table 10).

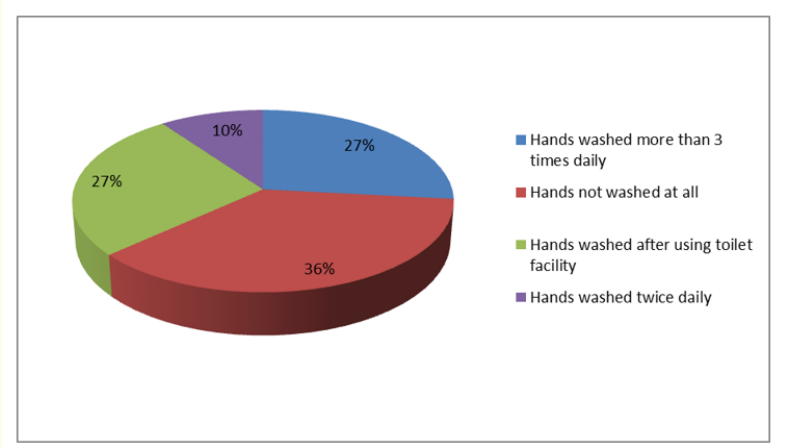

Figure 1: *A pie chart showing the frequency of hand washing by participants.*

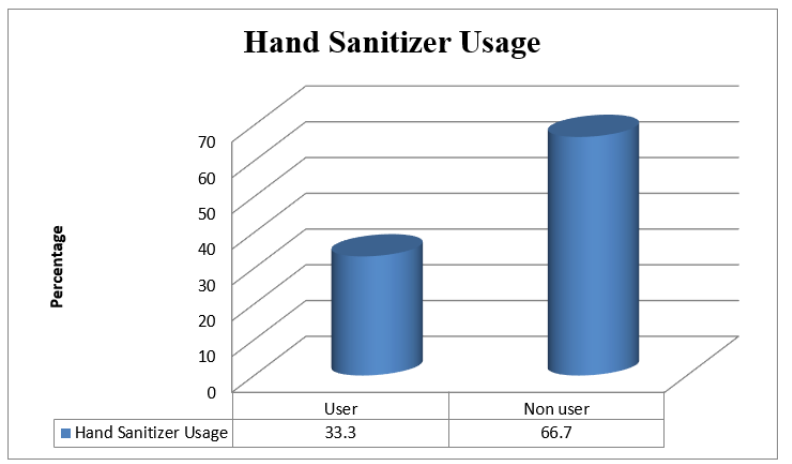

Figure 2: *Hand sanitizer usage among study participants.*

Sample Time	Morning	Afternoon	P-Value
Mean SPC	$8.1x10^6$	$2.9x10^6$	0.025
Mean EC	$5.9x10^3$	$6.9x10^3$	0.829
Mean CC	$8.7x10^2$	$1.3x10^3$	0.760

Table 3: *Mean counts of samples based on the sampling time among study participants.*

**SPC: Standard Plate Count; *EC: Enterobacteriaceae Count; *CC: Coliform Count.*

Educational Status	Educated	Uneducated	P-Value
Mean SPC	$3.0x10^7$	$8.7x10^6$	0.342
Mean EC	$23.9x10^3$	$3.8x10^2$	0.125
Mean CC	$3.6x10^3$	$1.2x10^2$	0.674

Table 4: *Mean counts of samples based on the educational status of the participants.*
**SPC: Standard Plate Count; *EC: Enterobacteriaceae Count; *CC: Coliform Count.*

Environmental Sanitation	Poor	Good	P-Value
Mean SPC	$11.0x10^6$	$6.5x10^6$	0.952
Mean EC	$13.7x10^3$	0.00	0.652
Mean CC	$2.3x10^3$	0.00	0.471

Table 5: *Mean counts of samples based on the environmental sanitation of the participants.*
**SPC: Standard Plate Count; *EC: Enterobacteriaceae Count; *CC: Coliform Count.*

Types of Goods Sold	Foodstuff	Clothing	Cosmetic	Utensils	P-Value
Mean SPC	$22.8x10^5$	$41.2x10^5$	$46.9x10^5$	$103.4x10^5$	0.074
Mean EC	$18.3x10^3$	$8.8x10^3$	0.00	$3.3x10^2$	0.027
Mean CC	$5.2x10^2$	$2.97x10^3$	0.00	$3.0x10^2$	0.277

Table 6: *Mean counts samples based on the category of vendor.*
**SPC: Standard Plate Count; *EC: Enterobacteriaceae Count; *CC: Coliform Count.*

Hand washing	Poor	Good	P-Value
Mean SPC	$55.0x10^5$	$11.8x10^6$	0.998
Mean EC	$6.9x10^3$	$15.0x10^3$	0.139
Mean CC	$5.0x10^2$	$3.4x10^3$	0.205

Table 7: *Mean counts of samples based on the practice of hand washing.*
**SPC: Standard Plate Count; *EC: Enterobacteriaceae Count; *CC: Coliform Count.*

Hand Sanitizer	User	Non-User	P-Value
Mean SPC	$58.1x10^5$	$54.1x10^5$	0.872
Mean EC	0.00	$95.9x10^2$	0.055
Mean CC	$5.0x10^1$	$15.9x10^2$	0.257

Table 8: *Mean counts of samples based on the usage of hand sanitizer.*
**SPC: Standard Plate Count; *EC: Enterobacteriaceae Count; *CC: Coliform Count.*

Age Group	15-30	31-60	P-Value
Mean SPC	$20.0x10^6$	$17.2x10^6$	0.321
Mean EC	$21.2x10^2$	$16.5x10^3$	0.248
Mean CC	$13.0x10^2$	$25.7x10^2$	0.482

Table 9: *Mean counts within the various age groups.*
**SPC: Standard Plate Count; *EC: Enterobacteriaceae Count; *CC: Coliform Count.*

Name of organism	Number of times isolated	Sample most frequently isolated from	Number of times isolated from sample
Staphylococcus spp.	42	Clothing	13
Streptococcus spp.	16	Utensils	7
Bacillus spp.	42	Utensils	13
Klebsiella spp.	9	Foodstuff	5
Enterobacter spp.	8	Foodstuff	6
Citrobacter spp.	6	Foodstuff	4
Proteus spp.	10	Foodstuff	7
Providencia spp.	1	Utensils	1

Table 10: *Bacteria isolated from the palms of the market women in the study.*

Discussion and Conclusion

Majority of the study participants were middle-aged people, mostly between the ages of 31-60 years. These individuals had advanced their education at least through the senior high school and were considered the educated in the study. However, the educated had a higher contamination level than the uneducated (See table 5). As opposed to popular belief, education had no bearing on the hygienic status of the market women. This could be due to the following reasons: samples collected from the educated were more than those collected from the uneducated, and as such this could have resulted in greater total quality counts amongst the educate. It could as well be assumed that the educated failed to practice personal hygiene as a result of inadequate resources or due to laziness.

The total number of participants who practiced good personal hygiene far exceeded those that did not. Washing hands more than 3 times daily, washing hands after using the toilet facility, as well as covering the head are all good hygienic practices which when done properly should curb the rate of spread of microbes to the barest minimum. However, the attitude of market women towards good hand hygiene remains questionable. Some studies have shown that the degree of wetness of hands appears to significantly influence bacterial transfer and dissemination to surfaces and items touched [1,9]. This probably occurs not only because of the physical aspects of moisture droplets transferring between surfaces but also because the bacteria may be maintained in a physiological state that makes them better able to thrive in the new environment due to the associated moisture. This study revealed that the market women who washed their hands had more contamination than those who did not wash their hands as the degree of wetness of hands appeared to greatly influence bacterial transfer. Majority of the women did not dry their hands effectively after washing, and this could possibly be a primary causal factor for this observation.

Samples taken in the morning were more contaminated than those taken in the afternoon. The assumption is that vendors have maximal contact with surfaces and materials before reaching their workplace from their respective homes.

The total plate count for women trading in utensils had the highest values as compared to those who traded in the other items studied. When vending sites are not cleaned properly, microbes can be carried by the wind onto surfaces (i.e. the utensils), and when the palms of the vendors come into contact with these surfaces there can be bacterial transfer as well from the surfaces to the palms contributing to the increased total quality counts observed. In terms of the hygienic quality, much was not observed, although, the difference between the counts were statistically significant between utensils and cosmetics. It has been reported that several species and strains of *Pseudomonas* and *Listeria monocytogenes* were found to attach to stainless steel surfaces within 20 to 30 minutes of contact. Some other microorganisms found were *Staphylococcus* species, *Escherichia coli*, *Bacillus* species and *Pseudomonas* species [10]. Cosmetics had the second highest total quality count; this could be due to handling of the products by the vendors both in showcasing the products at the vending site in the mornings to sell, and giving out the product to customers with their hands which may have been exposed to various degrees of contamination. When the vendors hand comes into contact with the pathogen from the washroom and touches the clothing with that same hand, there is the transfer of bacterial pathogens and this could also account for the high count observed in the clothing category, as compared to that of foodstuffs (Table 5). Foods are great sources of microbes since they are enriched with nutrients and the moisture that the bacteria needs to feed on for optimum growth. Hence foods are even the best source of microbes amongst all other categories of comparison. Food retailers and food service work involves high potential for wet hands and hands contaminated with proteinaceous material. Scientific research questions the efficacy of alcohol on moist hands and hands contaminated with proteinaceous material [11]. Hence, vendors who sold foodstuffs and visited the washrooms from time to time but failed to properly wash their hands, or used hand sanitizers without proper hand washing had potential of transferring microbes unto the products they retailed in. The hygienic quality of food vendors was the highest amongst the 4 categories compared. This could be due to the fact that, using alcohol gel instead of thorough hand washing in retail and food services did not adequately reduce important foodborne pathogens on the palm of food handlers as alcohols have very poor activity against bacterial spores [11].

The usage of sanitizers had its short comings as it did not really rid the palms of bacterial spores and other pathogenic organisms. Despite their effectiveness towards microbes, non-water agents do not cleanse the hands of organic material, but simply disinfect them [12]. It is for this reason that hand sanitizers are not as effective as soap and water at preventing the spread of many pathogens, since the pathogens still remain on the hands after use [12]. Therefore, hand washing with soap and water is the recommended practice [13]. The following reasons could account for why those who used hand sanitizers had higher standard plate count values than those who did not:

1. Alcohol-based hand sanitizers are recommended as a component of hand hygiene [Boyce and Pittet, 2002]. For alcohol-based hand sanitizers, the Food and Drug Administration (FDA) recommends a concentration of 60% to 95% ethanol or isopropanol, the concentration range of greatest germicidal efficacy [11]. While non-healthcare groups also utilize alcohol-based hand sanitizers, there are no specific concentrations of alcohol recommended for commercial use. Hence it is possible the vendors did not use the sanitizers that contained the required concentrations and as such still had higher total quality counts as compared to those who did not use them.
2. Some products marketed to the public as antimicrobial hand sanitizers are not effective in reducing bacterial counts on hands as they claimed [14]. This could also account for why those who used hand sanitizers in this study had more counts in terms of total quality than those who did not use them.

This study sought to enumerate bacterial load on the palms of market women at various times of the day, while establishing how knowledgeable the market women were in terms of hand hygiene, with the intention of educating them about the relevance of good hand hygiene.

The total mean quality counts of vendors sampled in the morning were significantly higher than those sampled in the afternoon, indicating that there are lots of vending activities happening in the morning as compared to the afternoon when the days' work is almost done.

The total mean quality counts of the educated vendors was more than that of the uneducated. More so, it was realized that those who washed their hands at least 3 times as well as those who washed after visiting the washroom had higher total quality counts than those who did not wash at all due to inefficient drying.

The findings of this study revealed that 16 (53.3%) out of 30 participants had no idea of how enteric pathogens were transmitted. 20 participants (66.7%) did not use hand sanitizers at their workplaces, and had a higher total contamination than those who used the sanitizers, with the majority in this category being above 30 years of age.

Conclusively, the bacterial load on the palms of the market women was high, particularly on the palms of the educated women and among those who presumed they practiced good hand hygiene, as compared to the number of uneducated and those who admitted to practicing poor hand hygiene presumably because the number of educated participants sampled was higher [15].

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Data Availability Statement

All data regarding this research can be made available upon request.

Authors' Contribution

EUO and VNO developed the concept and directed the research as well as participated in the finalizing of the manuscript. CNA and EA carried out sample collection, data analysis and manuscript preparation. SAA, FCM coordinated and helped draft the manuscript as well as designed the sampling techniques. AM assisted in sample collection and participated in the finalizing of the manuscript. All authors read and approved the final manuscript

Ethics Approval and Consent to Participate

Ethic clearance for this study was obtained from Allied Health Ethical and Protocol Review committee (AHEPRC) with number AHEPRC048/11/20 of Radford University College prior to data collection and consent to participate was obtained from each participant without coercion, after which they signed as a form of authorization before recruiting into the study.

Consent for Publication

Not applicable.

Conflicts of Interest

There is no conflict of interest surrounding this study.

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