International Journal of Research Studies in Microbiology and Biotechnology (IJRSMB)
Volume 10, Issue 1, 2025, PP 21-27
ISSN 2454-9428 (Online)
https://doi.org/10.20431/2454-9428.1001004
www.arcjournals.org

Detection of Pathogenic Bacteria on the Hands of Workers at a Fish Processing Plant

Sydney Malama[1], Adriace Chauwa[2], Beatrice Kankhuni[3], Aron Rezene Mehbratu[1]

[1]*University of Zambia, School of Natural and Applied Sciences, Biosciences and Biotrechnology Department Lusaka Zambia*

[2]*Enteric Disease and Vaccine Research Unit, Centre for Infectious Disease Research in Zambia, Lusaka, Zambia*

[3]*Levy Mwanawasa Medical University, Department of Public Health Nutrition, Lusaka, Zambia.*

Corresponding Author:** ***Sydney Malama, *University of Zambia, School of Natural and Applied Sciences, Biosciences and Biotrechnology Department Lusaka Zambia.*

Abstract: *Annually the World Health Organisation (WHO) reports about two million fatal cases of diarrheal bacterial infections attributed to poor handling of food and general poor hygiene. The faecal-oral transmission is a major cause of enteric infections if good personal hygiene is not practiced, and is often associated with food handlers in big food processing companies. This study aimed to evaluate detect pathogenic bacteria in a fish factory. Further the study assessed the effectiveness of alcohol-based hand washing against enteric bacteria on the hands of food processors. This study showed that there exists a high carriage of pathogenic bacteria by food workers and emphasises that hygienic practices especially hand washing is essential in reducing bacterial contamination and ultimately reduce infections.*

Keywords: *Fish processing, Disinfectant, Pathogenic bacteria, Hands.*

1. INTRODUCTION

Hand hygiene is a practice that keeps hands free of pathogens, it is a procedure that can be taken prior or post touching surfaces or anything that may be a cause of contamination to prevent cross-contamination (Toney-Butler, Gasner and Carver, 2017). Every year the World Health Organisation (WHO) reports about two million fatal cases of diarrheal bacterial infection attributed to poor handling of food and general hygiene (WHO, 2018). Faecal-oral transmission is a major cause of enteric infections if good personal hygiene is not practiced, and is often associated with food handlers in large food processing companies (Ifeadike *et al.*, 2012). Food handlers are people who work for a food business and handle food regardless of whether they prepare or serve it. Hands are a main source of bacteria transmission in both human-to-human and human-to-food contact hence the need for proper hand hygiene. Hands serve as a vehicle for pathogenic bacteria such as *Salmonella spp, Escherichia coli, Vibrio, Shigella spp and Campylobacter jejuni* (Alemu, 2014). Enteric bacteria are a large family of gram-negative bacteria found in the gastrointestinal tract of mammals (Chlebicz and Śliżewska, 2018). Diseases caused by enteric bacteria are often characterised by gastroenteritis, nausea, vomiting, stomach cramps and bloody diarrhoea (Ehuwa, Jaiswal and Jaiswal, 2021).

To maintain a good hygienic way of life, it is important to know the ways of hygiene that will yield a high level of efficacy. One of the many effective ways of managing pathogens on the hands of food handlers is by practicing proper hand hygiene such as; (i) social hand wash, which is the cleaning of hands with plain, non-medicated bar or liquid soap and water for removal of dirt, soil and various organic substances; (ii) hygienic or antiseptic hand wash, which is the cleaning of hands using bar or liquid soap preparations containing an antiseptic agent; and (iii) hygienic hand disinfection, which normally consists of the application of an alcohol-based hand rub onto dry hands without the use of water, which all work in different ways but more effective when used together. Alcohol-based hand sanitizers have become a popular alternative to hand washing with soap and have been shown to improve the compliance to hand hygiene significantly, thus the World Health Organisation (WHO) and Centre for Disease Control (CDC) have provided guidelines on how they should be used to stop the spread of bacteria. It is therefore important to study and understand the effectiveness of hand sanitizer

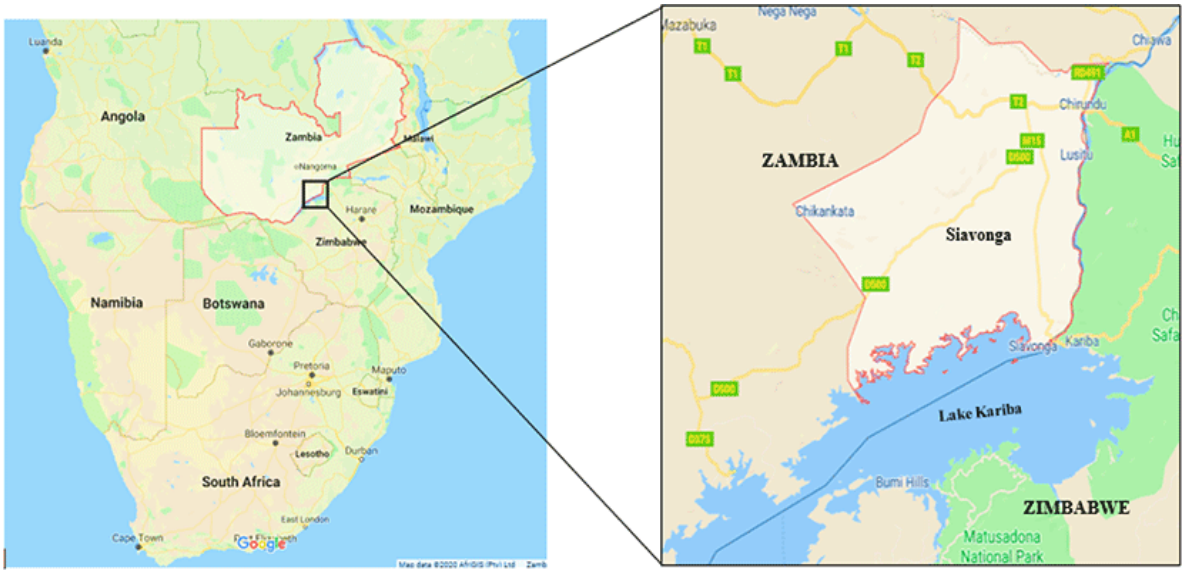
in elimination of pathogens. Hand sanitizers containing alcohol can include ethanol, isopropyl alcohol and a combination of all and in most cases, sanitizers with about 60% to 95% alcohol are more effective. While hand sanitizers are demonstrably effective, their efficacy relies on proper application. However, prolonged use may disrupt the skin's natural pH balance, potentially altering the normal microbial flora.

Food handlers, while playing a crucial role in food production and distribution, they can inadvertently become significant vectors of bacterial transmission within communities and around the world if proper hand hygiene is not followed. For instance, Shamaila *et al*., (2018) reported a prevalence of 2% of virulent and multi-drug resistant *Salmonella* isolated from processed broilers in commercial poultry abattoirs in Zambia. This finding raises concerns about the hygiene levels in other similar fresh food processing plants, as well as the hygiene of food handlers. This study aimed to identify pathogenic bacteria and evaluate the effectiveness of alcohol-based hand washing against enteric bacteria on the hands of food processors.

2. Materials And Methods

2.1. Study Site

This study was done at a fish farm and processing plant located in Southern Province of Zambia (coordinates: 16.4646°S, 28.6392°E). The facility is involved in the entire supply chain including inputs, breeding, growing, and distribution to retail.



2.2. Sample Collection

Twelve individuals were conveniently selected from the processing plant to be evaluated for bacteria contamination. The palms of each of the 12 participants were swabbed before taking any form of hand hygiene, and were properly labelled. The group was then requested to split into three (3) groups of four (4). The first group were requested to wash their hands with plain water and air dry them then swabbed (labelled as "W"). The second group were requested to wash their hands using a named anti-microbial hand washing liquid soap and then dry their hands before swabbing (labelled as "WS"). The third group were requested to wash their hands with anti-microbial soap followed by use of an alcohol-based hand sanitizer and then swabbed (labelled as "SHS"). The swabs were then transferred aseptically to the lab for analysis. Upon arrival at the lab, the swabs were transferred into peptone water to preserve the microbes for analysis the following day.

2.3. Inoculation and Analysis

Nine mL (9mls) of peptone water was transferred into 24 sterile test tubes and all the swabs collected were transferred into their respective test tubes. The first 12 swabs labelled BL1 –BL12 were cut into 12 test tubes labelled BL1 PW- BL12 PW. Swabs labelled W1 –W4 were cut into test tubes labelled W1 PW- W4 PW. Another set of swabs labelled WS1 – WS4 were cut into the test tubes labelled WS1 PW – WS4 PW. The last set of swabs labelled SHS1 – SHS4 were cut into test tubes labelled SHS1 PW- SHS4 PW, and all the test tubes were then vortexed for a minute each and incubated for 24 hours at 37°C.For faecal and total coliform testing, serial dilutions were prepared from the first incubated test

tubes. One (1) ml of sample from each tube was transferred using a new sterile dropper to four sets of new sterile test tubes. These sets were labelled BL1 10^{-1} – BL12 $^{10—1}$, W1 10 $^{-1}$ – W4 10^{-1}, WS1 10^{-1} – WS4 10^{-1}, SHS1 10^{-1} – SHS4 10^{-1}, respectively. Each new tube contained 9 ml of sterile normal saline water. After transferring the sample, the tubes were vortexed for 1 minute each. This represents the first dilution (10^-1).

The first dilution (1:10) was further diluted to create a second dilution. One (1) ml of sample from each tube in the first dilution set was transferred using a new sterile dropper to another set of 24 sterile test tubes containing 9 ml of normal saline water. These new tubes were labelled BL1 10^{-2} – BL12 10 $^{-2}$, W1 10^{-2} – W4 10^{-2}, WS1 10^{-2} - WS4 10^{-2}, SHS1 10^{-2} – SHS4 10^{-2}, maintaining the sample identification. Each tube was then vortexed for 1 minute. This process resulted in a second dilution with a dilution factor of 1:100. ^{2} From the second dilution all samples were transferred onto PCA and MacConkey plates labelled BL1 10^{-2} –BL12 10^{-2}, W1 10^{-2} – W4 10^{-2}, WS1 10^{-2} – WS4 10^{-2}, SHS1 10^{-2} – SHS4 10^{-2}.This was done by transferring 3 drops of sample onto the media plate using droppers and then spreading the sample across the plate ensuring full coverage of the media using a spreader. The inoculated media was then incubated for 24 hours at 37°C. For control purposes, one plate of the first dilution from each group was incubated. After 24 hours, plates were checked for growth and results recorded accordingly.

For vibrio testing 1ml of each sample was collected from the primary incubated test tubes containing swabs and transferred into 24 new sterile test tubes containing 9 ml alkaline peptone water (APW) which is an enrichment for *vibrio*. The tubes were labelled BL1 APW – BL12 APW, W1 APW - W2 APW, WS1 APW - WS4 APW, SHS1 APW – SHS4 APW. The tubes were vortexed for 1 minute and incubated for 24 hours at 37 °C. After 24 hours, all samples were vortexed to ensure a homogeneous mixture of growth in the broth, then each sample was transferred onto semi-solid Thio Citrate Bile Salt (TCBS) agar plates labelled BL1 – BL12, W1 – W4, WS1 – WS4, SHS1 – SHS4, by inserting a sterile new swab into the test tube and swabbing onto the plate.

To test for salmonella spp, 1ml of each sample from the previously incubated test tubes containing swabs was transferred into 24 new sterile test tubes containing 9 ml Rappaport, an enrichment for salmonella and labelled BL1 – BL12, W1 – W4, WS1 –WS4, SHS1 – SHS4, then vortexed for a minute each and incubated for 24 hours at 37°C. After 24 hours all samples were vortexed to ensure thorough mixing of growth in the broth then each sample was transferred to semi-solid XLD agar plates labelled BL1 – BL12, W1 – W4, WS1 – WS4, SHS1 – SHS4 by inserting a sterile new swab into the test tube and swabbing onto the plate. After 24 hours, results on plates of TCBS and XLD were checked for growth and results were recorded. After 24 hours the sample IDs that had growth on them were sub-cultured onto new plates of TCBS and XLD. This was done by picking a colony from the plates and swabbing them onto new plates of TCBS and XLD using sterile swabs and incubated for 24 hours at 37°C. The sub-cultured colonies were then subjected to gram staining and biochemical testing for all those that came out gram-negative.

Biochemical tests were carried out using an API kit 20 E (non-fastidious G- rod identification system for enterobacteria). Sterile swabs were used to transfer inoculum from the sub-cultured plates into test tubes containing normal saline. Colonies were transferred into test tubes labeled BL2, BL3, BL12, and W2, each containing normal saline. Using sterile swabs, a colony was picked from the plates and cut into the test tubes with sterile scissors. The tubes were then vortexed to ensure all the colonies from the swab were dispersed in the saline.

Next, using sterile pipettes, sufficient amounts of each sample were collected and transferred into the test kit. The compartments indicated by underlined words were filled just to the base of the capsule, and emission oil was added on top to create an anaerobic environment. The compartments indicated by boxed words were filled to the top with the sample. All capsules were then incubated for 24 hours at 37°C. After 24 hours, the following reagents were added to the respective capsules: VP 1 and VP 2 to the VP capsule, tryptophan deaminase (TDA) reagent to the TDA capsule, Kovac's reagent to the IND capsule, and NIT 1 and NIT 2 to the GLU capsule. Results were then noted and recorded.

3. RESULTS

The total bacterial count of every palm of the food handlers showed that all had bacterial count above the acceptable limit before washing hands. Eight (8) of the total food handlers under our study had

faecal coliform count above the acceptable limit before washing hands. The isolated bacteria were *Salmonella spp* and *Vibrio spp* from which *Salmonella spp* were found in all of our study participants. The results are shown in figure 1,2 and 3.

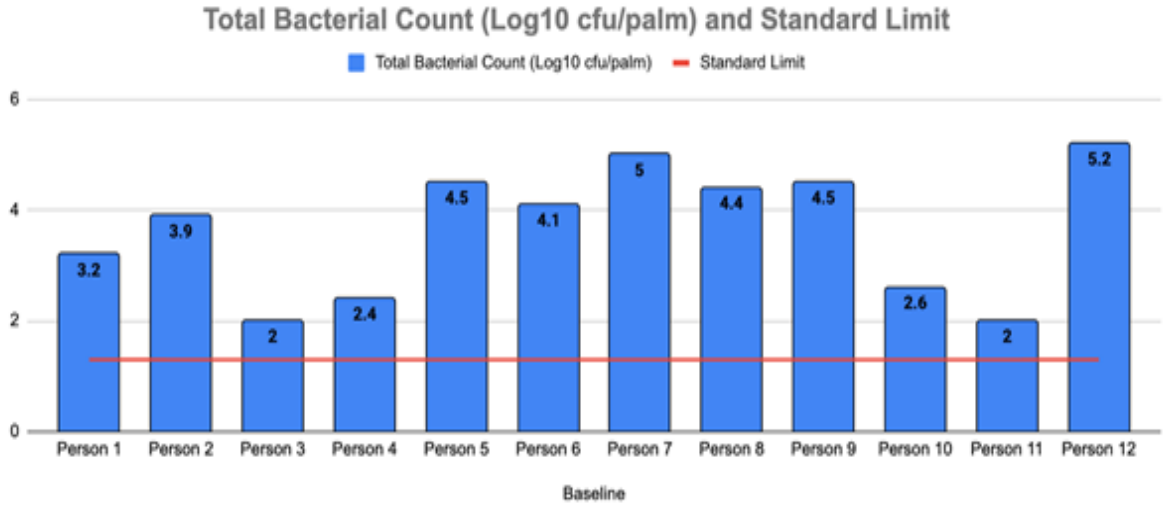


Figure 1. *Total bacterial count of every palm of the food handlers before hand washing.*

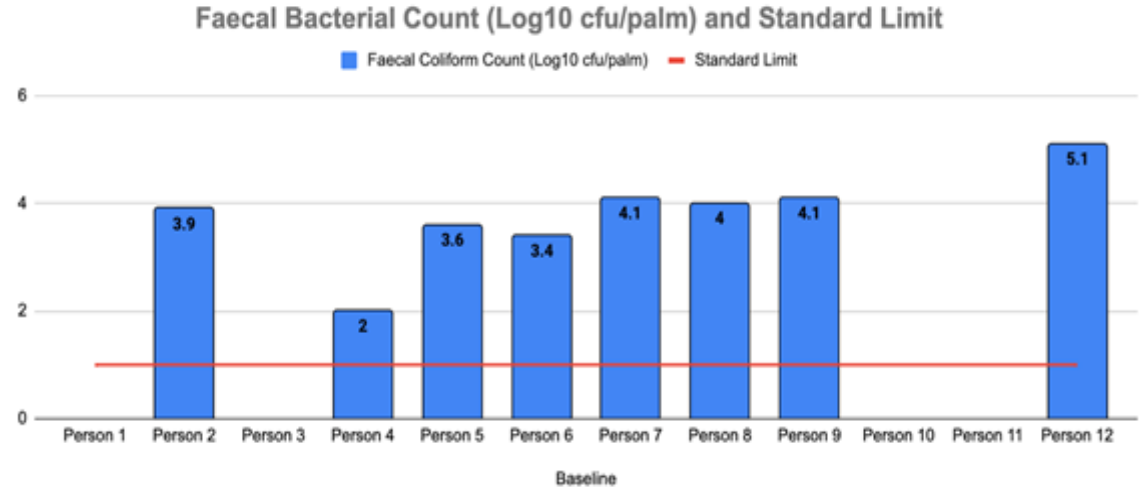


Figure 2. *Faecal coliform count of every palm of the food handlers before hand washing.*

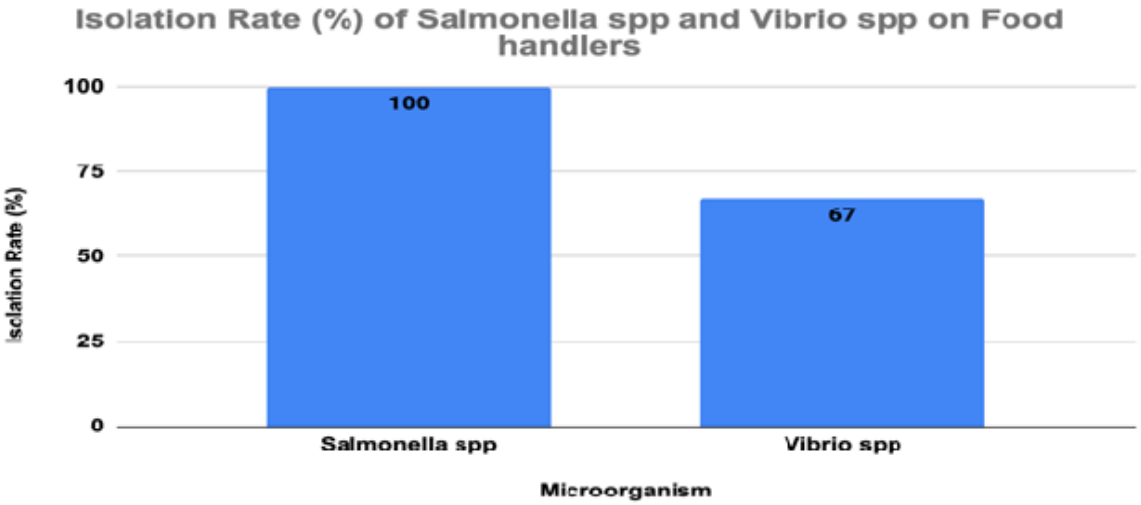


Figure 3. Isolation of *Salmonella spp.* and *Vibrio spp.* before hand washing.

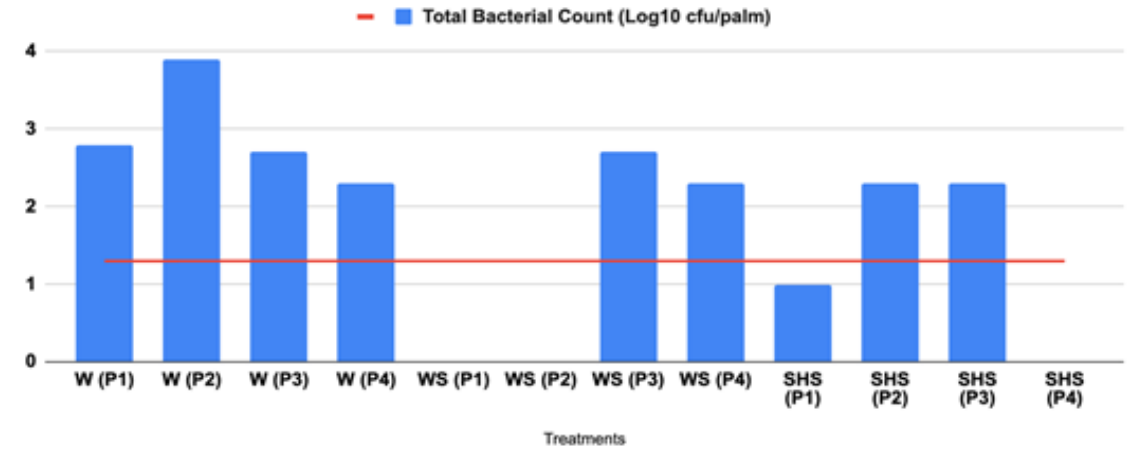


Figure 4. *Total bacterial count (Log 10 cfu/palm) after treatment which are in 3 groups of 4 each; water only [W (P1 to 4)], Water and soap [WS(P1 to P4)] and Soap and hand sanitizer [SHS(P1 to 4)].*

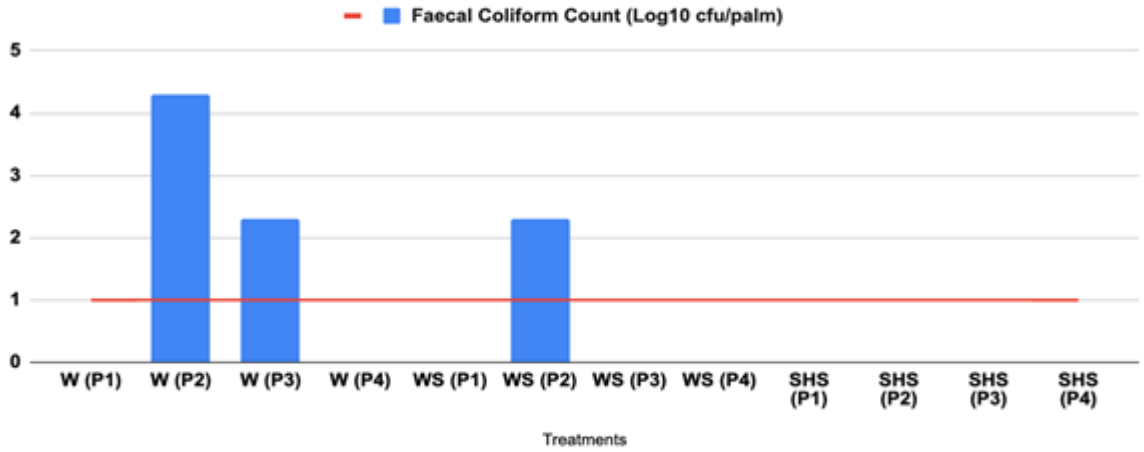


Figure 5. *Faecal coliform count (Log 10 cfu/palm) after treatment which are in 3 groups of 4 each; water only [W (P1 to 4)], Water and soap [WS(P1 to P4)] and Soap and hand sanitizer [SHS(P1 to 4)].*

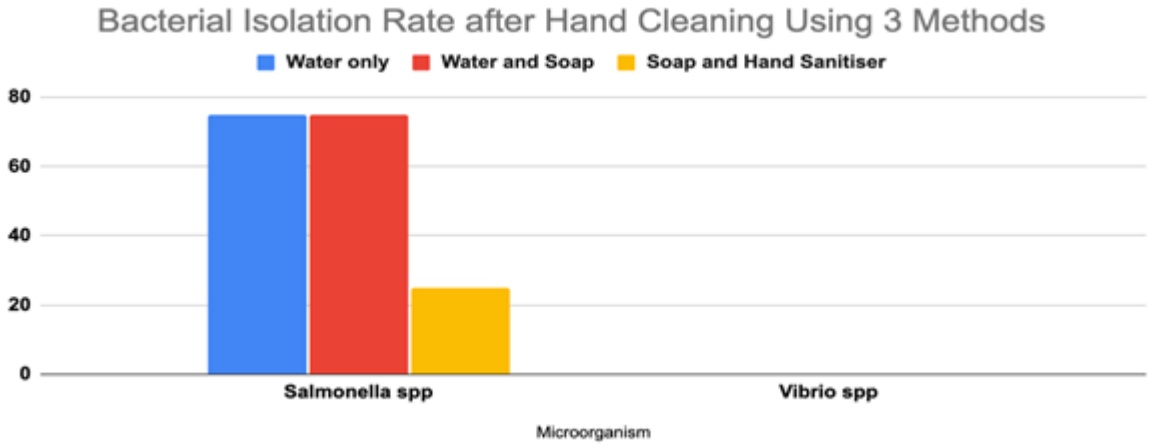


Figure 6. *Salmonella spp. and Vibrio spp. isolation rate after treatment in 3 groups; water only, Water and soap and Soap and hand sanitizer.*

There were four treatment groups: before washing hands, water only, using soap, and using soap and hand sanitizer. The average bacterial count was calculated for each group, revealing a decreasing trend in bacterial count from washing with water only to using soap and hand sanitizer.

Before hand washing, the total bacterial count was 3.7 CFU/palm, with a similar count for fecal coliforms. Washing with water only reduced the total bacterial count to 3.3 CFU/palm. Using soap

further reduced the count to 2.5 CFU/palm. The most significant decrease was observed in the group using both soap and hand sanitizer, where the total bacterial count dropped to 1.9 CFU/palm. Notably, no faecal coliforms were detected in the group using soap and hand sanitizer, indicating a significant reduction in bacterial contamination compared to before hand washing.

The results are shown in table 1, figure 4 and 5.

Table 1. *Average total bacterial count and faecal coliform count before and after hand washing*

	Average Counts (Log10 cfu/palm	
Treatment Groups	Total Bacterial	Faecal Coliform
Before Washing Hands	3.7	3.7
Water Only	3.3	2.9
Named commercial soap	2.5	2.3
Soap and Hand Sanitizer	1.9	0

4. DISCUSSION

This study was carried out to identify bacteria on the hands of factory workers and to assess the effectiveness of hand sanitizer on the hands of employees in the processing plant and three different steps were taken. This includes culturing bacteria after washing hands with plain water, washing hands with a named commercial anti-microbial liquid soap and using the soap and then sanitizer. The study showed the presence of medically important enteric bacteria such as Vibrio spp. (67%) and Salmonella spp. (100%) isolated from the palm hands of study participants who are employees at a fish processing plant in Zambia. In a similar study done in Guangxi Zhuang, China by (Yannong Zhou et al, 2007), 1012 Salmonella isolates with 68% antibiotic resistance were recorded from the hands of food workers. This shows that there exists a high carriage of salmonella by food workers and emphasises that hygienic practices by food workers especially hand washing is essential in reducing bacterial infection (Yannong, 2023). Several outbreaks associated with salmonella and Vibrio *spp*. have been reported from workers at food industries and public restaurants recently(Erin et.al (2009);(Donachie *et al.*, 2018). For instance, an epidemiological investigation by Erin and his colleagues showed that an outbreak of salmonella occurred from the contamination of an asymptomatic worker at a restaurant in the United States of America (USA)(Erin, 2009). Another epidemiologically documented outbreak of salmonellosis in Malta showed that the contamination from an asymptomatic food handler had lit the outbreak (Donachie *et al.*, 2018). Therefore, implementing routine microbiological check-ups is crucial to combat bacterial foodborne illnesses, especially given the isolation of medically important pathogens like Salmonella spp. and Vibrio spp.

The presence of Vibrio spp. on the hands of food workers is alarming and likely linked to the recent cholera outbreak that affected 62 districts in Zambia from 2023 to 2024. World Health Organization has reported that the Grade Three Cholera outbreak led to 14,900 reported cases and 560 deaths with a case fatality rate of 3.8% (data gathered until January of 2024) (WHO, 2023). The Ministry of Health of Zambia also reported that over 10% of the population, which is around 2 million people, reside in poor wash facilities (MOH, Zambia 2023). Another important perspective on the prevalence of *Vibrio spp*. on workers' hands is that, although this study calls for deeper microbiological typing of *Vibrio spp.*, workers in fish husbandries are particularly susceptible to contamination by Vibrio haemolyticus in seafood, such as in fish processing units. For instance, a study in China reported a total of 383 outbreaks of this bacteria from the year 2010 up to 2022 and addressed it as one of the leading foodborne bacterial agents in sea foods (Chen *et al.*, 2023).

This study has also discovered that hand sanitizers were more effective for vibrio spp. (eliminated 100%) compared to salmonella spp. (22%). This could be due to the load of bacterial count on individual hands but also due to the characteristic features of *Salmonella spp.* which can tolerate and stay in heavily contaminated areas for up to 4 days and on surfaces from one week to four weeks (CDC, 2023). This study has also demonstrated that using water alone or using water and soap alone is not effective in removing the bacteria without the use of hand sanitizers. In support of this, a study done by Magdalena and her colleagues showed a large decrease in colony-forming units of gram-negative bacteria after the use of alcoholic based hand sanitizers (Metzger *et al.*, 2024). Enteric bacteria such as *E.coli* and *Salmonella* are easily disinfected compared to gram-positive bacteria due to the latter containing a thick layer of peptidoglycan in their cell wall (Alajlan *et al.*, 2022).

5. Conclusion

This study isolated bacterial of public health and food safety concern. The study further showed that washing hands with plain water and anti-bacterial liquid soap did not completely eradicate the bacterial load on food handler's palms. However, using an alcohol-based hand sanitizer after hand washing with soap eliminated the faecal, total coliform and *vibrio* load, while *salmonella* was still present but very minimal. Therefore, its recommended that food processing companies implement effective sterilisation methods to avert public health risk and bacterial food contamination.

Conflict of Interest

All the authors read the full manuscript and have no conflict of interest to declare.

Funding

This research was not funded by any agency.

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Citation: *Sydney Malama et al. "Detection of Pathogenic Bacteria on the Hands of Workers at a Fish Processing Plant" International Journal of Research Studies in Microbiology and Biotechnology (IJRSMB), vol 10, no. 1, 2025, pp. 21-27. DOI: https://doi.org/10.20431/2454-9428.1001004.*