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Antimicrobial resistance profile of *Staphylococcus spp.* and *Listeria spp.* isolated from refrigerators of retailers and students in the Tolon District, Ghana

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ABSTRACT

Refrigeration is one of the common and safest food methods aided by the refrigerator which works by distorting the activities/growth of microorganisms. Nevertheless, the refrigerator could be a transmission source of non-pathogenic and pathogenic bacterial strains involved in food spoilage and foodborne infections. The study seeks to assess the knowledge of students and retailers on microbial contamination of refrigerators. Also, the study would determine the occurrence and antimicrobial resistance profile of *Staphylococcus* spp. and *Listeria* spp. isolates. The swab-rinse method was employed for sampling refrigerators used by students (n=86) and retailers (n=38) with a total of 248 samples (Interior surfaces n=124, Exterior surfaces n=124). The swab samples were directly streaked on Mannitol Salt Agar and Oxford *Listeria* Agar Base for the isolation of *Staphylococcus* spp. and *Listeria* spp., respectively. Antimicrobial-resistant profile was determined using the Kirby-Bauer disc diffusion method against vancomycin (30 µg), ampicillin (10 µg), and penicillin-G (10 µg). Of the 110 isolates identified, *Staphylococcus* spp. were (95) with a prevalence of (86.36%) and *Listeria* spp. were (15) with a prevalence of (13.64%). Antibiotic resistance was most common to penicillin-G (67.27%) followed by ampicillin (60%), and vancomycin (22.73%). This study confirms the presence of cold-tolerant bacteria in refrigerators. Thus, making refrigerators used by students and retailers a potential source of bacterial transmission and/or contamination.

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1. Introduction

A refrigerator is one of the major indispensable household appliances utilized by the population in Ghana for its comfort (1, 2).

It is a low-temperature device fundamentally utilized for safeguarding food items from spoilage (3). It works by slowing down the metabolic activities of the microorganisms present (4). Despite its invaluable importance, Sultana (5) reported that perishable foods can, however, deteriorate even at low (refrigeration) temperatures due to the actions of bacteria.

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According to Otu-Bassey et al., (6), microorganisms are most abundant in an environment with optimal nutrition, humidity, and temperature for their development and replication. This suggests that microbes (bacteria) can dwell on fomites including refrigerators. Bacteria from unwashed hands, unwashed raw foods (fruits and vegetables), and leaking packaged food items can immediately infect other stored foods in refrigerators, which subsequently can cause infections in humans when such contaminated foods are consumed (7).

The interior of a refrigerator is generally expected to be an unsuitable environment for microbial growth meanwhile studies have reported microbial contamination of household refrigerators (8, 9). Bacillus and Acinetobacter were the most common genera found in the interior of refrigerators (10). *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli,* and *Yersinia enterocolitica* were isolated from interior surfaces of some residential refrigerators in a study by (11). Unquestionably, it has been reported by earlier studies including (12) and (13) that, refrigerators are predominately home to Staphylococcus, Pseudomonas, Bacillus, and Enterobacteriaceae.

Foodborne pathogens have developed resistance to antibiotics (14). Munita and Arias (15) reported antibiotic resistance as one of the three most severe public health problems in the twenty-first century which was emphasized by the World Health Organization in 2014.

The fast growth of antibiotic-resistant bacteria in the food chain has become a major source of concern (16), putting antibiotic efficacy in jeopardy. Earlier investigations by (5, 16, 17) reported the existence of antibiotic-resistant bacteria in refrigerators. Antibiotic-resistant bacteria may increase the risk of users acquiring infections that is nearly untreatable and leaving clinicians with no viable options for treating infected patients (15).

With the increased use of refrigerators by students and retailers, it is imperative to assess the knowledge of students and retailers on microbial contamination and to determine the presence and antibiotic-resistance profile of *Staphylococcus* spp. and *Listeria* spp. in students and retailers' refrigerators. The outcome of the work would provide a microbial database that would help predict potential contamination sources of foodborne pathogens and the potential antibiotics for treatment.

2. Materials and Methods

2.1. Study area

The study was carried out on the Nyankpala campus of the University for Development Studies where convenient sampling was employed to obtain swab samples from refrigerators used by students (86) and retailers (38). Nyankpala campus is situated within the Nyankpala town, which is in the Tolon District, about 10 miles southwest of Tamale, the capital of the Northern region of Ghana. Laboratory procedures and experiments were performed at the Spanish Laboratory Complex, Nyankpala Campus.

2.2. Study design

The present study was a two-phase study. The first phase was a cross-sectional survey where two sets of semi-structured questionnaires were administered to assess the knowledge of students and retailers on microbial contamination whereas the second phase involved primary sample collection and laboratory analysis.

2.3. Sample collection

The swab-rinse method was employed to obtain 248 swab samples (both interior and Exterior) from 124 refrigerators accessed with the consent of the owners. The swabs taken were transported to the Spanish Laboratory Complex for analysis.

2.4. Isolation of bacteria

Swab samples were directly streaked on different selective media plates (Mannitol Salt Agar-Oxoid, UK) and Oxford *Listeria* Agar Base-Alpha Biosciences, USA) for the isolation of *Staphylococcus* spp. and *Listeria* spp. respectively. Plates were incubated at 37 °C for 24 h. Presumptive bacteria were confirmed following gram staining and biochemical tests (Catalase, citrate, and Triple sugar iron test) as described by (16, 18, 19).

2.5. Antimicrobial-resistant profile

Antimicrobial-resistant profile was carried out using Kirby-Bauer's disc diffusion method (19) on Mueller-Hinton Agar (MHA; Oxoid, UK). Briefly, fresh overnight pure cultures on nutrient agar (Oxoid, UK) plates were suspended to attain a concentration that commensurate with a 0.5 McFarland standard. Sterile cotton swabs were used to streak the suspension evenly on the MHA plates.

Resistance was tested against vancomycin (30 µg), ampicillin (10 µg), and penicillin-G (10 µg). The inoculated MHA plates were incubated at 37°C for 24 h.

2.6. Data analysis

The questionnaire data was analyzed using One-Way ANOVA finding means, standard deviation, and checking for significant differences whiles laboratory data were subjected to descriptive statistics and result presented in mean and percentages using [Statistical Package for the Social Sciences, version 20 and Microsoft Office Excel (2016).

3. Results

3.1. Qualitative analysis of questionnaires

To evaluate the correlation between refrigerator usage by students and retailers and microbial contamination, a total of 124 respondents on the Nyankpala campus were included in the survey which comprised both males (39.50%) and females (60.50%). The survey revealed that the ages of the sampled refrigerators ranged from three (3) weeks to 24 years (Table 1).

It was found that 80.50% of the respondents do not wash their hands before handling the refrigerator. Most of the respondents use detergent (61.30%), soap (17.70%), detergent and soap (2.40%), disinfectant (9.70%), and only water (8.90%) to wash their refrigerators. Cleaning was revealed to be done once a week by most respondents. On average, a student shares his/her refrigerator with 10 other students while 65.80% of the retailers' refrigerators were accessible to everyone (customers).

The survey revealed that refrigerators on the Nyankpala campus were not used for any other purpose but to store and preserve food items such as vegetables, fruits, meat, fish, pastries, dairy products, minerals, and cooked foods. Irrespective of the need to wash purchased vegetables and fruits from the market before introducing them into the refrigerator, 32.10% of the respondents do not wash them. Per the survey, the electricity supply on the Nyankpala campus was revealed to be stable but 51.70% of the respondents complained of food spoilage even under refrigeration conditions.

The survey data revealed that 73% of retailers are aware of refrigerators as a potential source of microbial contamination compared to students' knowledge of 56% (Fig. 1). Further analysis of the data revealed a significant difference in knowledge among the respondents depending on their educational level. There was no significant difference observed among respondents in the following educational levels; SHS, tertiary, and postgraduate (Table 2).

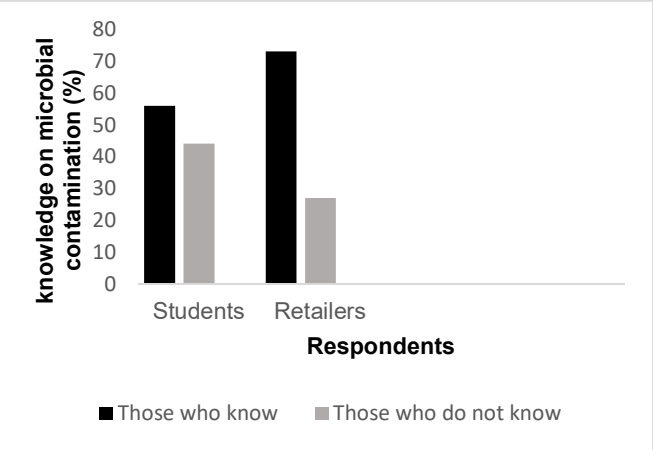

Figure 1. Students and Retailers Knowledge on Microbial contamination; Retailers were more aware of refrigerators as source of microbial contamination compared to students.

Table 1. Ages and frequencies (F) of the refrigerators sampled for the study

Ages of refrigerators	Students (F)	Retailers (F)
0 – 11 months	29	8
1 – 10 years	56	23
11 – 20 years	0	7
20 – 30 years	1	0

Table 2. Influence of educational level on knowing refrigerators as source of germs transmission

RLE	Frequency	(RSGT) Mean±SD
None	7	1.86±0.38a
JHS	3	1.33±0.58b
SHS	5	1.00±0.00c
Tertiary	6	1.17±0.41d.c
Postgraduate	9	1.00±0.00e.c

RLE = respondent's level of education; RSGT = refrigerator as source of germs transmission; SD = standard deviation; means (± standard deviation) in column followed by different letters are significantly while those with same letters are not significantly different.

3.2. Microbiological analysis of samples

One hundred and ten (110) bacteria isolates were obtained from students and retailers' refrigerators. These isolates were classified as *Staphylococcus* spp. (95) and *Listeria* spp. (15) (Table 3). Among the bacteria isolated from student refrigerators, *Staphylococcus* spp. was the predominant on the different parts (32.60% and 30.20% for interior and exterior parts respectively) while *Listeria* spp. showed the same frequency for the different parts (4.70%)

(Table 4). On the other hand, the exterior part showed more contamination with *Staphylococcus* spp. (65.80%) and *Listeria* spp. (10.50%) for retailers' refrigerators (Table 5). Again, the study revealed that students' refrigerators had the most bacteria-contamination with 56.40 % while retailers had a 43.60% prevalence rate (Fig. 2).

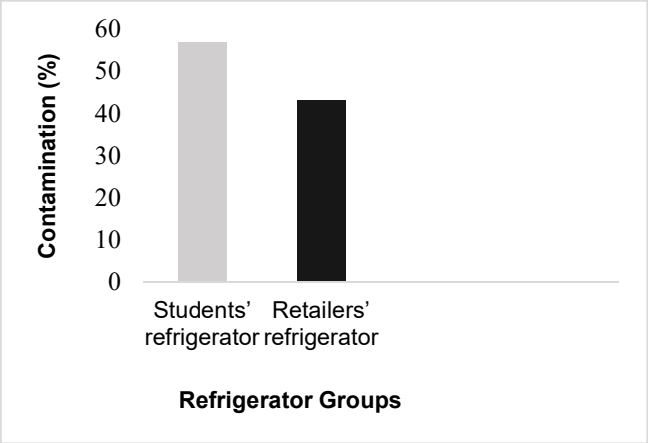

Figure 2. Contamination of Refrigerators; student refrigerators were more contaminated than retailers.

3.3. Antimicrobial-resistant profile of *Staphylococcus* spp. and *Listeria* spp. isolates

Of the 95 *Staphylococcus* spp. isolates, 65 (68.42%) were resistant to penicillin, isolates showed 60% resistance to Ampicillin and showed the least resistance (5.26%) to vancomycin. With regards *Listeria* spp., isolates showed 60% resistance against penicillin and ampicillin, and 40% resistance to Vancomycin. Also, 39.09% of the 110 isolates were resistant to at least two of the tested antibiotics.

The outcome of the antibiogram test further showed that 20.91% of the 110 isolates were found to be resistant to all three, with penicillin resistance accounting for the highest percentage with 74 (67.27%). Ampicillin followed as the next resistant antibiotic with 66 (60%) and vancomycin showed the least resistance with 25 (22.73%) (Fig. 3).

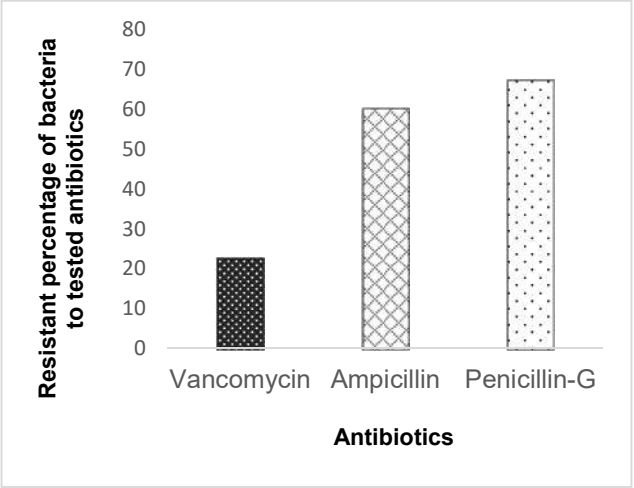

Figure 3. Resistance percentage of *Staphylococcus* and *Listeria* isolates to test antibiotics; highest resistance of bacteria isolates was recorded against Penicillin-G with the least resistance recorded against vancomycin

Table 3. Prevalence of bacterial species isolated from refrigerators

Sample source	Bacteria isolates	Number of the isolates	Prevalence (%)
Students' refrigerators	*Staphylococcus* spp.	54	49.10
Retailers' refrigerators		41	37.27
Students' refrigerators	*Listeria* spp.	8	7.27
Retailers' refrigerators		7	6.36
Total		110	100

Table 4. Contamination of different parts of refrigerators used by students

Sample source	Sampling part	*Staphylococcus* spp.	*Listeria* spp.	Percentage (%)
Students' refrigerators	Interior	28	4	37.21
	Exterior	26	4	34.88

Table 5. Contamination of different parts of refrigerators used by retailers

Sample source	Sampling part	*Staphylococcus* spp.	*Listeria* spp.	Percentage (%)
Retailers' refrigerators	Interior	16	3	50.00
	Exterior	25	4	76.32

4. Discussion

Microorganisms can be found in every region of the biosphere, and some can even grow at extremely low temperatures, including below freezing point (5). The presence of bacteria in refrigerators may potentially lead to direct or indirect contamination of stored foods (20). These bacteria can ruin food and cause foodborne illness if such contaminated foods are consumed. Most human activity is aided by the hands and may easily get contaminated. Meanwhile, the response to the questionnaires revealed that 80.48% of respondents do not wash their hands before opening the refrigerator.

This could be a potential reason for contamination of refrigerators with *Staphylococcus* spp. since the human skin serves as a natural flora. Data on usage of refrigerators suggest that 65.79% of retailers' refrigerators were accessible to all customers whereas one student shares his/her refrigerator with up to 10 other users. This practice could be a recipe for microbial transmission and/or contamination. Washing of vegetables before storing in the refrigerator might reduce microbial inoculation as this has been confirmed by Beumer et al., (21) that when vegetables are introduced into refrigerators without a prior wash, they can be contaminated with bacteria such as Listeria spp.. In the current study, 44% of students and 27% of retailers did not know that refrigerators could be a potential source of transmission of microbes. As a result of the perception that it is safe, they failed to adhere to hygienic practices such as regular hand washing and effective cleaning of the refrigerators. Contrary, adherence to hygienic practices could help reduce contamination with potential foodborne pathogens.

Microbiological analysis revealed the existence of bacteria in refrigerators. It was observed that the refrigerators investigated had been contaminated with *Staphylococcus* spp. and *Listeria* spp.

Staphylococcus spp. was the most prevalent bacteria in both students' and retailers' refrigerators with a total rate of 86.37% which is similar to works reported in earlier studies; (12) and (22) confirming *Staphylococcus* spp. as the most occurring bacteria in domestic refrigerators. Other earlier studies (16, 18, 23, 24) confirmed *Staphylococcus* spp. as the most frequently isolated pathogen occurring in refrigerators but in lower percentages ranging from 5% - 30.60% as compared to the current study. However, Ye et al., (10) reported a different perspective on the most frequently isolated bacteria from refrigerators where *Bacillus* and *Acinetobacter* were reported as the most common occurring bacteria in domestic refrigerators. This discrepancy could be due to the environmental conditions existing in the study area.

The present study also isolated *Listeria* spp. with a prevalence rate of 13.63%. As a psychrotrophic organism, it can multiply in cold temperatures, making an important food preservation technique like refrigeration difficult to use (25). The results from this study were in agreement with a study by Beumer et al., (21) where *Listeria* spp. was isolated from both students' and retailers' refrigerators. Again, the study agrees with Kilonzo-Nthenge, Chen, and Godwin (17) findings where *Listeria* spp. were isolated in refrigerators. On the other hand, a lower incidence (3% or less) of *Listeria* spp. has been recorded in refrigerators (24, 26), which contradicts the current findings.

The interior surface of students' refrigerators showed more contamination as compared to the exterior surfaces whereas the exterior surfaces of retailers' refrigerators were more contaminated compared to the interior surfaces. The rate of contamination of the interior surface of students' refrigerators was 37.21% which is lower than the rate (52%) reported by Otu-Bassey (6) and this can be attributed to the fact that 31.65% of students do not wash fruits and vegetables before introducing them into the refrigerator. The exterior surfaces of students' refrigerators had a contamination rate of 34.88% and this may be attributed to the large number of students utilizing a single refrigerator. This study revealed a higher prevalence rate (76.32%) on the exterior surfaces of retailers' refrigerators, and this may be due to all customers having access to retailers' refrigerators. Also, the result affirms the fact that refrigerator door handles are the most contaminated part of the refrigerator as proposed by (27).

Antimicrobial-resistant profiles of bacteria isolated from students' and retailers' refrigerators were determined against three antibiotics. The highest percentage of resistance was observed against penicillin (67.27%). Hasan (16) reported high percentages of bacteria resistance to ampicillin (81.08%) and penicillin (75.60%).

However, another study by Sultana (5) found ampicillin to be the most resistant antibiotic with 73.17%. While in another study, erythromycin (39.90%) accounted for the highest percentage of resistance followed by ampicillin (33.80%) (17).

5. Conclusion

Despite students' and retailers' best attempts to keep their refrigerators free of bacteria, this study found that *Staphylococcus* spp and *Listeria* spp. persist on refrigerator exterior and interior surfaces, posing a threat of cross-contamination. Poor hygienic practices such as putting food (fruits, vegetables, meat, fish, etc.) into refrigerators without washing or no hand wash could all account for bacterial contamination recorded in this study. Mesophilic and psychrotrophic bacteria were prevalent in refrigerators sampled notwithstanding the initial belief that these bacteria may not survive refrigeration temperatures. The presence of *Staphylococcus* spp. and *Listeria* spp. in the refrigerators indicate poor personal hygiene among users. *Staphylococcus* spp. was the most prevalent. Both *Staphylococcus* spp. and *Listeria* spp. isolates showed the highest resistance to penicillin-G followed by ampicillin whilst vancomycin showed the least resistance. This may threaten the successful treatment of infections caused by these bacteria. Since refrigerators have proven to be home to some cold-tolerant bacteria, public sensitization on refrigeration best practices should be conducted. Furthermore, users should be mindful of good hygienic practices when handling food and refrigerator.

Conflict of Interest

The authors declare no conflict of interest.

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