Journal of Advanced Zoology
ISSN: 0253-7214
Volume 45 Issue 1 Year 2024 Page 1185-1190

Microbiological Examination of Hand Towels Used in Public Restaurants

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Abstract

A total of five hand towels were collected from 5 different restaurants and taken to the laboratory in a sterile container. Nutrient agar, Macconkey agar, and Sabouraud dextrose agar media were prepared for the isolation of heterotrophic bacteria, coliforms, and fungi respectively. The total heterotrophic bacteria count for the various samples was (8.7×10^5 cfu/ml) with the highest total bacterial count, while (1.0×10^5 cfu/ml) had the lowest bacterial count. The mean values for the total coliform count showed (7.4×10^5 cfu/ml) as the highest coliform count, whereas (2.4×10^5 cfu/ml) was the lowest coliform count. Also, the mean total fungal count from the restaurants showed that the highest fungal count occurred (8.6×10^5cfu/ml), and (1.2×10^5 cfu/ml) lowest fungal count. The bacteria species identified and their prevalence include *Staphylococcus sp* 10 (4.4%), *E. coli* 22 (9.6%), *Sarratia sp*, 23 (10%), *Proteus sp* 14 (6.1%), *Pseudomonas sp* 21 (9.2%), *Shigella sp* 3 (1.3%), *Bacillus sp* 85 (37.1%), *Micrococcus sp* 14 (6.1%), and *Salmonella sp* 37 (16.2%). Fungi species identified were *Penicillium sp, Fusarium sp, Mucor sp, Aspergillus tarmani, Aspergillus flavus, and Penicillium sp.* Hand towels used in public restaurants contain different species of bacteria and fungi, which can be a source of infection to other users or customers. It is recommended that restaurant owners and their staff should regularly wash their hand towels, and ensure that sanitizers and disinfectants are made available to customers to prevent the spread of potentially harmful microorganisms.

Keywords: Public Restaurants, Hand Towels, Microbial Contamination, Infection.

1. INTRODUCTION

Hand towels are small cotton materials often used to dry hands after washing them either in public or private residences. In restaurants, hand towels can be placed strategically or folded and kept around sinks so that customers can access them easily. Eating away from home is a very common practice among some categories of individuals, and visits to fast food restaurants are growing more rapidly. This phenomenon involves people from practically all walks of life, ranging from children, teenagers, the elderly, and immune-compromised individuals (Amelda & Maviluo, 2015). Within the restaurant surroundings, the risk of microbial transmission to the customers who visit the facility is high. Used hand towels in fast food joints or restaurants can serve as

one important source of microorganisms as they can retain these microbes (Naja' atu *et al*, 2021). These microorganisms can be transmitted through direct contact with the hands or inanimate objects that are present in the environment. Washing hands with disinfectants has proven to be an effective way of decontamination, but the same contaminated hand is being cleaned on restaurant towels which have been unwashed and used by many customers. Most microorganisms found on restaurant towels can cause diseases when in contact with our foods, leading to food poisoning (Sleigh & Tilbury, 2017).

Research has shown that 80% of infections are spread through direct contact with hands or other objects (Al-Ghamdi *et al*, 2011). The common occurrence of enteric bacteria in hand towels, sponges, and cloths suggests that they can play a role in the cross-contamination of foods, fomites, and hands by food-borne pathogens (Charles *et al*., 2014). According to Charles *et al*., (2014) bacterial occurrence in kitchen hand towels, a total of 82 hand towels were collected from households in five major cities in the United States and Canada, and the number of heterotrophic bacteria, coliform bacteria, and *Escherichia coli in* each towel were determined. Coliform bacteria were detected in 89% and *E. coli* in 25.6% of towels. The presence of *E. coli* was related to the frequency of washing. Some researchers have also assessed the microbial quantity of hand towels used in restaurants in Nigeria. Ibrahim *et al*., (2011), in their studies, identified a total of 296 bacteria isolates belonging to 9 different genera of both gram-positive and gram-negative, isolated from three different hand towels used for drying hands in different restaurants. They identified *Staphylococcus aureus* 66 (22.3%), *Staphylococcus epidermis* 57 19.3%), *Klebsiella sp* 31 (10.5%) *Pseudomonas sp* 23 (7.8%), *Enterobacter sp* 28 (9.5%), *proteus sp* 18 (6.1%), *Serratia sp* 21 (7.1%), *E. coli* 22 (7.5%), coliforms 30 (10.1%). Hand washing is a fundamental cautionary measure to protect against the spread of disease and is one of the primary practices to reduce the transfer of bacteria from person to person, or from person to food and contact surfaces (Chinakwe *et al*, 2012). It is established that unwashed hands can transmit pathogens to food products after visits to the toilet. Investigation of food-borne illness showed that poor personal hygiene, primarily effective hand washing is an important contributor to food-borne illness (Lambrechts *et al*, 2014). Used towels left unwashed provide a perfect place for pathogenic and non-pathogenic microbes to grow (Curtis & Cairncross, 2013). Most contaminated hands play a major role in transmitting microbes. Microbes that are found on the palm encompass both inhabitants and transient pathogenic and non-pathogenic flora (Dorathy & Noble, 2017). Transient flora takes over the apparent cover of the skin, and are simply detached by washing, which may be transmitted through direct contact with human hands and the surroundings, this temporary or transient flora includes microbes linked with nosocomial infections such as *Staphylococcus aureus, Enterococci, Pseudomonas spp, Klebsiella spp and Acinetobacter spp* (Dorathy & Noble, 2017). Poor personal hygiene by food handlers frequently contributes to the outbreak of foodborne illness, most contaminated hand towels are handled by restaurant customers and may sometimes be contaminated during cooking and serving of meals. Although regular washing of hand towels and the availability of disinfectant may seem trivial to some restaurant owners, failing to do it can also have tragic consequences (Shojaei *et al*, 2006). Hand towels in restaurants have been found to harbor microorganisms, hence it is appropriate that necessary actions be taken by restaurant owners to prevent customers from being contaminated. This study aims to examine the microbiological quality of hand towels used in selected public restaurants in Gondia, Maharashtra, India.

2. METHODOLOGY

Study Area

The Gondia, Maharashtra community is an industrial area, hence it accommodates lots of Food Vendors and Restaurants to meet the food needs of individuals and other workers who cannot eat at home.

Sample Collection, Culture Media and Inoculation

Different hand towels were collected from five different restaurants and placed in different stabilized air-tight containers with hand gloves. The different containers were taken to the Research Laboratory for microbial analysis. Each of the towels was dipped into their respective bowls containing distilled water for 2 hours. 1 ml of the original sample solution was serially diluted in 5 different test tubes. This procedure was repeated for each of the samples. The test tubes labeled 10^3 were then used for the inoculation of their different plates respectively. Nutrient agar, MacConkey agar, and Sabouraud agar media were used for culture. After the solidification of the nutrient media, 1 ml of appropriate dilution 10^{-3} of the samples were pipette into petri dish containing molten nutrients agar and Mac-Conkey agar respectively. The plates were incubated at 37^0 C for 24 hours. After 24 hours, the total heterotrophic and coliform count was done using the most probable number technique. After 24 hours of incubation, the bacteria populations were counted, and the morphological characteristics of the isolates were examined. Pure cultures of the bacteria species were obtained by picking a

specific colony and being transferred into a newly prepared medium. After 24 hours the pure culture was subject to gram staining reaction and biochemical tests such as oxidase, catalase, regular citrate, and sugar utilization (Okwelle, 2019). The fungal colonies were identified macroscopically and microscopically after staining with cotton blue in lactophenol, using the detailed drawings of the diagnostic features and identification manual used (Snowdon, 1991).

Antibiotics Susceptibility Test

The method employed for antibiotic susceptibility was the Disc Diffusion Method. After preparing the respective nutrient media; nutrient agar, Macconkay agar, and Sabaroud Dextrose agar, a pure culture was introduced. The disc containing the impregnated antibiotics was placed carefully in the petri dish. For gram-negative bacteria the following antibiotics were used: Oflatoxin, Nalidixic acid, Pefloxin, Gentamycin, Augmentin, Ciprofoxaxin, Septrin, Streptomycin, and Ceprox. While for the gram-positive bacteria, the following antibiotics were used: Ampiclox, Amoxil, Norfloxacin, Chloramphenicol, Gentamicin, Streptomycin, Rifampicin, Erythromycin, Levofloxacin and Ciprofloxacin. After 24 hrs the zone of inhibition was carefully measured.

PRESENTATION OF RESULTS

The results obtained from the study are presented in the following tables below:
Table: The total heterotrophic bacteria count (cfu/ml) from selected restaurants.

Table 1: The mean values of the total heterotrophic bacteria count (cfu/ml)

Dilution factor	Coffee House	Shyambaba Pohewala	Shalimar restaurant	Gayatri cafe	AP Restaurant
10^3	6.4×10^5	4.7×10^5	8.7×10^5	1.0×10^5	2.1×10^5

Table 1 shows the mean value for the total heterotrophic bacteria count. **Shalimar Restaurant** (8.7×10^5 cfu/ml) has the highest total bacteria count while AP Restaurant (1.0×10^5 cfu/ml) has the least bacteria count.

Table 2: Mean total coliform bacteria count (cfu/ml) from the restaurants

Dilution factor	Coffee House	Shyambaba Pohewala	Shalimar Restaurant	Gayatri Cafe	AP Restaurant
10^3	4.6×10^5	2.6×10^5	7.4×10^5	6.5×10^5	1.3×10^5

Table 2 shows the mean values for the total coliform count. **Shalimar Restaurant** (7.4×10^5 cfu/ml) has the highest coliform count whereas **Shyambaba Pohewala** (2.6×10^5 cfu/ml) has the lowest coliform count.

Table 3: Mean total fungal count from the restaurants

Dilution factor	Coffee House	Shyambaba Pohewala	Shalimar Restaurant	Gayatri Cafe	AP Restaurant
10^3	4.9×10^5	8.6×10^5	7.5×10^5	3.5×10^5	1.2×10^5

Table 3 shows the mean total fungal count from the selected restaurants. The highest fungal count was seen in Shyambaba Pohewala (8.6×10^5cfu/ml) while **AP Restaurant** (1.2×10^5 cfu/ml) had the lowest fungal coun

Table 4: Bacteria and fungi isolates identified from the selected restaurants.

Restaurants	Bacteria	Fungi
Coffee House	*Sarratia sp* *Bacillus sp* *Micrococcus sp*	*Penicillium sp Fusarium sp* *Aspergillus tarmaii* *Candida sp*
Shyambaba Pohewala	*Bacillus sp* *Micrococcus sp* *Proteus sp* *E. coli* *Salmonella sp* *Shigella sp*	*Penicillium sp* *Fusarium sp* *Aspergillus flavus* *Candida sp*

Shalimar Restaurant	*Bacillus sp* *Salmonella sp* *Pseudomonas sp* *Proteus sp*	*Penicillium sp* *Mucor sp*
Gayatri Cafe	*E. coli* *Pseudomonas sp* *Bacillus sp*	*Candida sp*
AP Restaurant	*E. coli* & *Staphylococcus*	*Aspergillus niger*

Table 5: The frequency of occurrence of each isolated bacteria species

Organism	**Percentage (%)**
Staphylococcus sp	10 (4.4%)
E. coli	22 (9.6%)
Sarratia sp	23 (10%)
Proteus sp	14 (6.1%)
Pseudomonas sp	21 (9.2%)
Shigella sp	3 (1.3%)
Bacillus sp	85 (37.1%)
Micrococcus sp	14 (6.1%)
Salmonella sp	37 (16.2%)

The table shows the frequency of occurrence of each isolated bacteria, however, *Bacillus sp* 85 (37.1%) had the highest frequency of occurrence followed by *Salmonella sp* 37 (16.2%). *Shigella sp* 3 (1.3%) was the least occurring organism. *Proteus sp* and *Micrococcus sp* had equal levels of occurrence 14 (6.1%).

Table 6: Zone of inhibition of gram-negative isolated organisms

Organisms	**OFX**	**NA**	**PEF**	**CN**	**AU**	**CPX**	**SXT**	**S**	**PN**	**CPE**
E. coli	22	0	12	25	14	28	14	0	0	0
Proteus sp	12	18	9	0	12	22	0	0	0	0
Salmonella sp	18	0	11	15	19	22	8	10	0	0
Pseudomonas sp	28	1	24	37	36	30	2	1	0	0
Shigella sp	30	0	25	31	32	28	13	14	13	12
Sarratia sp	10	18	9	13	9	22	0	0	0	0

Table 7: Shows the zone of inhibition (mm) of gram-positive isolated organisms

Organisms	**S**	**NB**	**CH**	**CPX**	**E**	**LEV**	**CN**	**APX**	**RD**	**AML**
Bacillus sp	2	22	15	12	15	8	18	11	16	10
Micrococcus sp	7	8	25	26	10	12	10	10	0	13
Staphylococcus sp	21	0	23	24	22	30	27	0	17	16

Legend:

OFX: Oflatoxin
NA: Nalidixique acid
PEF: Pefloxin
PN: Aplicin
CH: Chloramphenicol
LEV: Levofloxacin
NB: Norfloxacin
CPE: Ceprox

RD: Rifampicin CN: Gentamycin
CPX: Ciprofloxacin AML: Amoxil
AU: Augmentin SXT: Septrin
E: Erythromycin S: Streptomycin

Table 8: **Analysis of variance of bacterial count from the selected restaurants.**

SOV	DF	SS	MSS	F	0.05
BW	**4**	**966.19**	**241.55**	**4.72**	**3. 18**
ERROR	**13**	**665.42**	**51.19**		
TOTAL	**17**	**163.61**	**9. 62**	**----------**	

The ANOVA result in Table 8 tested the significant difference in the occurrence of bacteria from the hand towels collected from five different restaurants. The tabulated ANOVA value of 3.18 is less than the calculated value of 4.72 at the significant level of 0.05. There is a significant difference in the contamination of hand towels from the five different restaurants.

4. DISCUSSION

The results obtained from this study have confirmed reports that hand towels used in public restaurants can be contaminated with different species of microorganisms, which could cause infections. A total of 229 bacteria species belonging to nine different genera including; *Staphylococcus, Salmonella, Escherichia, Sarratia, Proteus, Pseudomonas, Shigella, Bacillus, and Micrococcus* were isolated. The nine genera of bacteria isolated in this study are following that reported by Ibrahim *et al*., (2011). This high level of bacteria isolated can be attributed to poor hand wash and poor personal hygiene. This number could be further reduced by frequent changes of hand wash water, use of detergent or hand wash solution during washing, and use of many hand towels to prevent microbial cross-contamination among food customers. This low level of microbial load observed in some of the restaurants can be traced to the regular washing of hand towels by the food vendor. *Bacillus sp* 85 (37.1%) was the highest isolated organism in this study. Though, Ibrahim *et al*., (2011) isolated *Staphylococcus epidermis* 57 (19.3%), while Chidi (2020) isolated *E. coli* (28.5%) as the highest organisms in their separate studies. The presence of *Shigella sp* in the current study disagrees with Ibrahim et al., (2011); Chidi (2020), and Charles *et al*., (2014) who did not recover *Shigella sp. Salmonella sp* is the second highest occurring organism 37(16.2%). The value of this study is under Ibeneme (2021) who isolated (16.7%) of *Salmonella sp* in her study. *E. coli* constitutes about 0.1% of gut microbial and fecal-oral transmission is the major route through which pathogenic strains cause disease. The 22(9.6%) *E. coli* isolated was low compared with (35.7%) recorded by Ibeneme, (2021) in his study. The *Pseudomonas sp* 21(9.2%) and *Staphylococcus sp* 10 (4.4%) isolated were also low concerning that of Ibrahim et al., (2011) and Chidi, (2020).

In this study, five different species of fungi including restaurants; *Penicillium sp, Fusarium sp, Aspergillus tarmani, Candida sp, Aspergillus flavus,* and *Mucor sp* were isolated. However, Shyambaba Pohewala had the highest fungal count of 8.6×10^5 cfu/ml as compare to 8.6×10^5 cfu/ml, 7.5×10^5 cfu/ml, 4.9×10^5 cfu/ml, 3.5×10^5 cfu/ml and 1.2×10^5 cfu/ml for Shalimar, Coffee house, AP and Gayatri Restaurant respectively. Antimicrobial susceptibility test was performed based on the Clinical Laboratory Standards Institute (CLSI) guidelines using the zone of inhibition measured in millimeters (mm). The organisms isolated were both gram-positive and gram-negative. The gram-negative bacteria were more susceptible to Ciprofloxacin with the zone of inhibition of *E. coli* and *Shigella sp* (28 mm), *Pseudomonas sp* (30 mm), *Proteus sp, Serratia sp* and *Salmonella sp* (22 mm) respectively. The isolates were most resistant to Ciprox and Apicilin with *Shigella sp* showing. The gram-negative bacteria were also susceptible to Augmentin, Oflatox, and Refloxin. *Shigella sp* showed a high range of susceptibility to all the antibiotics Ciprox and Apicilin; (12 mm). The gram-positive organisms were most susceptible to Gentamicin; *Bacillus sp* (18 mm), *Micrococcus sp* (10 mm), and *Staphilococcus sp* (30 mm). However, the organisms were most resistant to Ampiclox and Norfloxacin.

CONCLUSION

This study has revealed the presence substantial number of different species of microorganisms in hand towels used in public restaurants. The presence of these microorganisms in the hand towels is a clear indication that the hand towels were contaminated with pathogenic microorganisms. Hence, food vendors or restaurant owners are advised to wash their hands and regularly change the hand towels used by customers to avoid the transmission of disease-causing organisms.

REFERENCES

1. Al-Ghambi, A. K., Abdelmalek, S.M.A., Ashshi, A. M., Shukri, H & Jiman-Fatani, A. A. (2011). Bacterial contamination of computers and mice. *Journal of Biology, 3* (18): 2224-3208.
2. Amelda, C. L., & Mariluo, O. H. (2015). Level of Microbial Contamination of Table Napkins used in selected fast Food Chains. *Asian Journal of Health.* Lice de cagayan University. *5*(5-20): 112-134**.**
3. Charles, D. G., Akrum, H. T., Sherri, M, Laura, Y.S., Douglas, R. H., & David W. K (2014). Bacterial occurrence in kitchen hand towels. *International Association for Food Protection, 34*(5): 312-317.
4. Chidi, M. (2020). Evaluation of Bacterial Contamination of Shared Hand Towel in Restaurants in Umahia, Abia state. *Respiratory. Movav.edu.ng.*
5. Chinakwe, E. C., Nwogwugu, N.U., Nwachukwu I. N., Okoro Ndu, S. I., Onyemakara, N. N., & Ndubuisi, N. U. (2012). Microbial quality and public health implication of hand wash water samples of public adults in Owerri, South East Nigeria. *Internal Resource Journal of Microbiology, 3*(4): 144-14
6. Curtis, V., & Cairncross, S. (2013). Effect of Washing Hands with Soap on Diarrhoea Risk in the Community; a systematic review. *Lancets Infectious Disease,* (30): 2-8.
7. Dorathy, A. S., & Noble, W. C. (2017). Resident and Transient Bacteria of the Skin. *Journal of Cutaneous Pathology, 1*(6): 260-264.
8. Ibeneme, I. (2021). Microbial Evaluation of Contamination of Shared Hand Towel in Restaurant Located in Umahia. Respiratory. Mouau.edu.ng, 33.
9. Ibrahim, T. A., Adebowale, O., & Ola, I. (2011). Isolation and identification of bacteria from Hand Towels used in drying hands in Restaurants within Rufus Gwa Polytechnic, Owo, Ondo State, Nigeria. *Electronic Journal of Environmental, Agricultural and Food Chemistry, 10*(5): 2223-2239.
10. Lambrechts, A., Human, I. S., Doughari, J. H., & Lues, J. F. (2014). Bacterial Contamination of the Hands of Food Handlers as Indicator of hand washing efficacy in some Convenient Food Industries in South Africa. *Pakistan journal of medical science, 30*(4): 755-758.
11. Naja'atu, S. H., Uyi, G. O., Sani, B. F. A., Kabiru, A. S., & Sunday, O. O. (2021). Bacteriological Examination of Used Towels from Female and Male Hostel of Federal University of Lafia, Nigeria. *Journal of Advances in Microbiology, 21*(8): 28-34.
12. Okwelle, A. A. (2019). *Understanding Microbiology: Part One.* Zelon Integrated Services, Port Harcourt, Nigeria. 76-80.
13. Shojaei, H., Shooshtaripoor, J., & Amiri, M. (2006). Efficacy of Simple Hand-Washing in Reduction of Microbial Hand Contamination of Iranian Food Handlers. *Food Research International Journal,* 39:526-529.
14. Sleigh, D. J., & Timbury, M. C. (2017). Note on Medical Microbiology, 5th edition. Churchill-Livingstone, New York. 73.