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Assessment of Bacterial and Fungal Contaminants from Some Restaurants Wastewater in Karji, Chikun Local Government Area, Kaduna State, Nigeria

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KEYWORDS
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ABSTRACT
The relevance of restaurants in a society can never be overemphasized. However, improper management of waste water being generated by these restaurants poses a great deal of health risks by the interacting pathogenic microorganisms from the waste water with man. This study investigated the presence and diversity of bacteria and fungi in wastewater from selected restaurants at Karji Chikun Local Government Area, Kaduna State. Wastewater samples were collected from 3 restaurants in duplicate and analyzed for bacterial and fungal contaminants using standard microbiological methods. The results showed that all wastewater samples contained high levels of bacterial and fungal contaminants, including pathogenic species. Eight bacterial species (*E. coli, Klebsiella pneumoniae, Bacillus sp, Salmonella sp, Enterobacter sp, Shigella sp, Staphylococcus sp, and Pseudomonas sp)* accounted for 8 (55.12%) while Six fungal isolate (*Mucor sp, Aspergillus fumigatus, Aspergillus niger, Candida sp, Rhizopus stolonifer and Penicillium sp*) accounted for 6 (44.88%). The most common bacterial isolates were *Escherichia coli, Salmonella sp.*, *Pseudomonas sp., Klebsiella pneumoniae, Bacillus sp.*, *Enterobacter sp., Shigella sp.,* and *Staphylococcus sp*, while the dominant fungal species were *Aspergillus fumigatus, Penicillium spp*., *Candida spp., Rhizopus stolonifer, Aspergillus niger,* and *Mucor sp*. The study highlights the need for effective wastewater management practices in restaurants to prevent environmental pollution and protect public health. The findings of this study can inform policy decisions and wastewater management strategies in Karji and similar settings. The research recommends regular monitoring of restaurant wastewater, implementation of proper wastewater treatment systems, and education and training programs for restaurant staff on wastewater management and best hygiene practices.

CITATION
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INTRODUCTION

Access to clean and safe water is a fundamental necessity for many countries worldwide, including Africa, yet Water pollution and scarcity are pressing issues confronting humanity globally. Factors such as industrialization, population growth, and inadequate wastewater treatment, among others, are responsible for this crisis. The practice of discharging untreated wastewater into the environment continues to persist in developing countries despite advancements in wastewater treatment. (Onu *et al*., 2023). Wastewater is commonly contaminated with a variety of biological agents, including bacteria, fungi, viruses, protozoa, and helminths, with pathogenic bacteria representing the most serious epidemiological threat (Sorber & Sagik, 1980; Cyprowski *et al*., 2005; Gerardi & Zimmerman, 2005). When improperly managed, wastewater becomes a significant source of environmental pollution which could be a hazard. The environmental consequence of wastewater degradation may result in physical changes to receiving waters, increased level of dissolved oxygen, bioaccumulation in aquatic life, release of toxic substances and increased ground water quality (Mahmood and Maqbool, 2006). Untreated wastewater is associated with numerous diseases caused by bacteria, viruses and protozoa. Many microbial pathogens in wastewater can cause chronic diseases with long-term effects such as degenerative heart disease and stomach ulcer (Paillard *et al*., 2005). These debilitating ailments can be fatal and have been known to impair human productivity. Wastewater also consists of vast quantities of bacteria, most of which are harmless to man. Although, pathogenic forms that causes diseases such as typhoid, dysentery and other intestinal disorder may be present in the wastewater (Absar, 2005).

Poor wastewater management in several African countries has led to severe health risks for humans, animals, and aquatic ecosystems. This poses a particular threat to vulnerable groups like children, women, and the disabled residing in rural and remote areas with limited access to healthcare. Population inflation has led to the unprecedented increase in urbanization, thus causing negative impacts on environmental sustainability. The restaurant industry, in particular, generates significant amounts of wastewater containing organic matter, nutrients, and microorganisms. Improper management of this wastewater can lead to environmental pollution, harm aquatic life, and pose health risks to humans (Sulieman *et al*., 2010).

In recent years, there has been an upsurge in the number of restaurants due to the changing lifestyles of the people round the globe. This growth has resulted in highly polluted restaurant wastewater (RWW), generated during the cooking, washing, and cleaning operations. RWW typically contain fat, oil, and grease (FOG) resulting from residues of meat, deep-fried food, baked items and butter, and contributing on blockages of sewer due to clogging and eventually sewage backup. This has increased the required frequency of cleaning and sanitary sewer overflows (SSOs) (Imran *et al*., 2022). additionally, wastewater generated by restaurants contains grease, food residues, detergents, and pathogenic microorganisms that pose serious risks to human health and the environment (Smith, 2020). Poor disposal practices of wastewater further threaten aquatic ecosystems and wildlife due to the present of pollutants and contaminants. Everyday 80% of the world's waste water enters our environment completely untreated, jeopardizing, nature and public health, with far reaching consequences for climate resilience, aquatic biodiversity and food and water security and access (Davis *et al*., 2017; Lee and Kim, 2018). Bacteria and fungi in wastewater from restaurants can indicate poor hygiene practices, improper waste disposal, or potential source of contamination (Hall 2020). In kaduna State and Nigeria at large, previous studies have primarily centered on abattoir wastewater, municipal wastewater, and industrial effluents and Environmental wastewater, with findings highlighting high microbial loads and significant public health concerns. However, restaurant wastewater remains underexplored despite the rapid growth of the food service sector and the increasing release of untreated restaurant effluents into the environment. The absence of studies on restaurant wastewater in this locality represents a critical gap, making it difficult for policymakers, environmental agencies, and public health authorities to assess the actual risks and design appropriate management strategies. In rapidly growing urban areas such as Karji in Chikun Local Government Area, Kaduna State, untreated wastewater is often discharged directly into the environment, increasing the risk of waterborne diseases such as cholera, typhoid, and dysentery. This study aimed at isolating, identifying, and evaluating the prevalence of bacterial and fungal contaminants in wastewater from selected restaurants in Karji.

MATERIALS AND METHODS

The study was carried out in Karji of Chikun Local Government Area of Kaduna State. Karji is a residential area with a localized markets and makeshift restaurants. It is situated in the northwestern part of Kaduna states, with the latitude 10.4333^{o}N and longitude 7.0667^{o}E. The experiment was carried out at the Department of Biological Sciences zoology laboratory, in Kaduna State University located at Tafawa Balewa way, kaduna north, Kaduna State with the latitude 10.51761^{o}N and longitude 7.45009^{o}E.

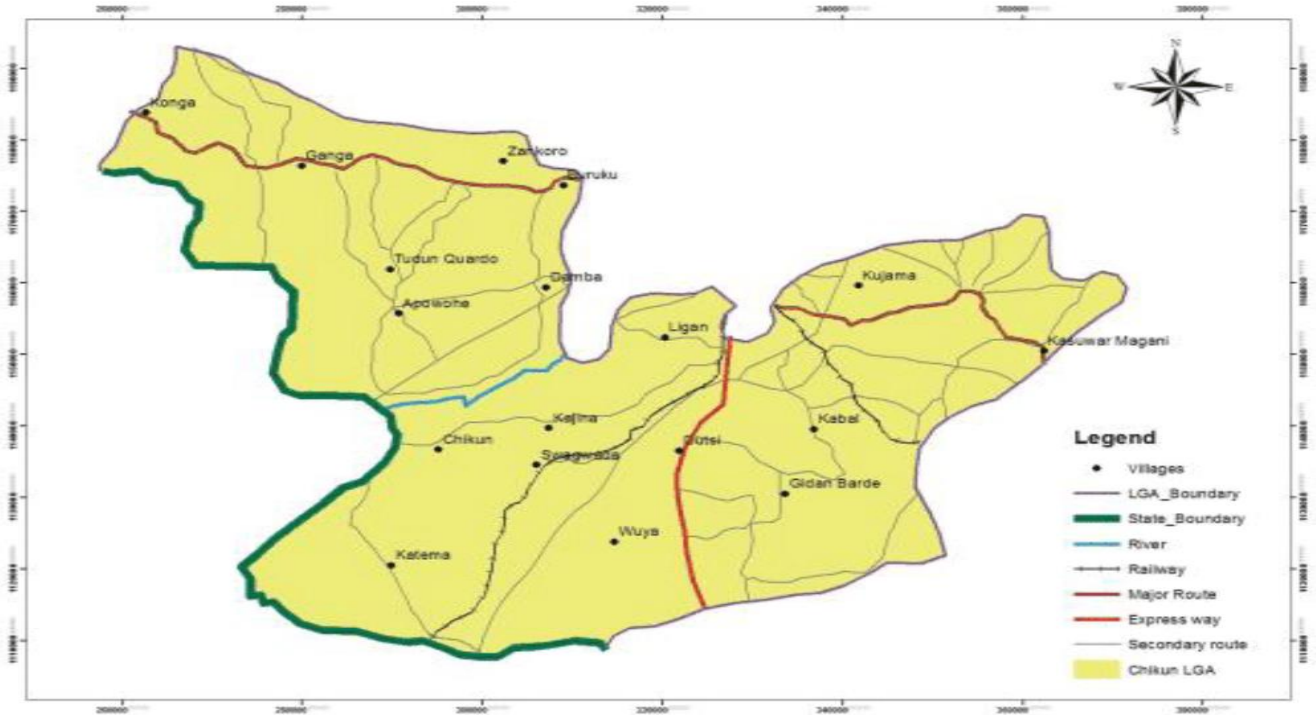

Figure 1: Map of the study Area

Sample collection

Wastewater samples was collected from three (3) different makeshift restaurants in Karji designated A, B, and C. The samples were collected in duplicate from each restaurant which makes it a total of six (6) samples to ensure representativeness and accuracy. In each of the restaurant A, B, and C, sample was collected from the Kitchen sink, and the floor drain. The samples were collected aseptically in sterile containers with hand gloves on and transported to the laboratory in ice bags container. In the laboratory samples were kept to acclimatized and were analysis within 24 hours. Wastewater samples collected from the three (3) selected restaurants in triplicate form were analyzed at the Laboratory at the department of microbiology, Kaduna State University.

Sample Analysis

Serial dilution and Preparation of Culture media

An aliquot (1ml) of the wastewater was transferred into 9ml of distilled water, thoroughly shaken and serially diluted up to 10^{-5}5 dilution. The stock and dilutions 10^{-1} and 10^{-3} were inoculated onto Nutrient agar, Eosin methylene blue agar, Mannitol salt agar, MacConkey agar, *Salmonella Shigella* agar plates for bacteria and Potato Dextrose Agar plates for fungi.

Media used in this study was prepared and sterilized according to manufacturer's instruction. The concentration of a medium is always given by the manufacturer in grams per liter. They were sterilized in an autoclave at 121℃ for 15minutes, then it was allowed to cool to 45℃ before use. The media are Nutrient Agar (NA), Eosin Methylene Blue Agar (EMB), MacConkey Agar (MCA), Mannitol salt agar (MSA) and *Salmonella shigella* Agar (SSA) for bacteria and Potato Dextrose Agar (PDA) plates for fungi.

Inoculation and Incubation

Inoculation is the process of introducing microorganisms into a growth medium, such as the petri dish to initiate growth and multiplication. Bacteria and fungi were inoculated using a sterile wire loop to make streaks onto the growth medium (in a petri dish).

The plates were incubated for 24 hours at 37℃ and 37℃ for 72 hours (3 days) for bacteria and fungi respectively.

Bacterial Isolation and Identification

The microbial load and cultural characteristics were observed according to standard microbiological techniques (culturing techniques, staining techniques and isolation techiques). Pure cultures were isolated, Gram stained, followed by biochemical tests to identify the isolates. The biochemical tests include; coagulase, catalase, indole production, oxidase, Voges Proskauer (VP test), methyl red. These tests were used to identify the isolates to strain level according to Cowan and Steel (1993).

Gram Staining

Smears of isolates were prepared and heat fixed on clean grease free slides. The smears were stained for one minute with crystal with violet. This was washed out with sterile distilled water. The slides were covered with Gram's iodine

solution for one minute. The slides were washed with distilled water and the smears were decolorized with 95% alcohol for 30 seconds and rinsed off with distilled water. The smears were then counter stained with safranin solution for 1 minute. Finally, the slides were washed off with sterile distilled water, air dried and observed under oil immersion objective.

Catalase test
Using a dropper, 1 drop of 3% Hydrogen peroxide was placed onto the test organisms on a microscope slide, then covered using a cover slip and view under 40x magnification. The production of gas bubble indicates presence of the catalase enzyme.

Oxidase Test
A piece of filter paper was wet with a few drops of the dilute 1% solution of oxidase reagent (tetramethyl-phenylenediamine-dihydrochloride) which was prepared by standard procedure. A bit of growth from the nutrient slant was obtained using sterilized platinum wire loop and smeared on the wet piece of paper. Development of an intense purple color by the cells within 30 seconds indicates a positive oxidase test.

Indole Test
Tryptophan broth of 5ml in each test tube was prepared by autoclaving at 15 psi 121℃. Using sterile technique, small amount of the experimental bacteria from 24hours old pure culture was inoculated into the test tubes by means of wire loop inoculation method with an inoculating loop and tubes were incubated for 48 hours at 37℃. In order to test for indole production, 5 drops of Kovac's reagent was added directly into the tubes a ring formation at the surface indicate positive indole production (Mac Williams, 2009).

Coagulase Test
Here, clean slide was divided into two sections, to one section the test organism was smeared on it using a sterile wire loop while a drop of distilled water was added to the other section serving as control. Then human plasma was added to both sections and the slide was rocked gently for some minutes. A clumping or agglutination of the plasma indicates the presence pf coagulase (Cheesbrough, 2006).

Methyl Red Test
A small quantity of the organism was inoculated into the sterile glucose phosphate peptone water medium and incubated at 37℃ for 48 hours. Then five drops of the methyl red reagent were added into the culture after incubation, mixed and read immediately (Madueke *et al*., 2014)

Voges Proskauer
Voges Proskauer test was done where the isolates were inoculated in the MR/VP broth and incubated for 24hrs at 37℃. Aliquots of 1ml was taken into a clean test tube and 0.6ml of 5% α-naphthol was added followed by 0.2ml of 40% potassium hydroxide (40% KOH). The tubes were shaken gently and allowed to remain undisturbed for 15minutes. Red colour development was reported as positive VP test while no colour development was reported negative VP test (Cheesbrough, 2006).

Fungal isolation and identification
Macroscopy was used to observe the isolate cultural and morphological features such as its size, shape, pigmentation, texture and colony growth pattern.
For microscopic identification a slide was prepared where a portion of the mycelium was picked from the isolates using a wire loop, mounted on a drop of Lactophenol Cotton Blue (LPCB) placed on a slide, it was teased and covered using a coverslip and viewed under the compound binocular microscope from low to high magnification.

Statistical analysis
The data that was generated from this study was organized and presented using simple descriptive statistics on Microsoft Excel 2019.

RESULTS AND DISCUSSION
Table 1 shows probable organisms that were isolated from respective colonial morphologies of bacterial isolates on selective media which were MacConkey Agar -MCA, Mannitol salt agar -MSA, Eosin methylene blue agar – EMB and *Salmonella Shigella* agar -SSA. The general-purpose medium which was the Nutrient Agar- NA favored the growth of most of the bacterial isolates prior to confirmatory biochemical tests.

Table 1: Colonial Appearance of Bacterial Isolate on Different Media from the Study Areas

S/N	Colony Morphology	Probable Organisms
1	Pinkish/mucoid/cloudy colonies on MCA, Green metallic sheen/slightly raised and entire on EMB	*E. coli*
2	Pinkish/mucoid colonies on MCA, very sticky/purple on EMB, greyish white dome and convex colonies on NA	*Klebsiella pneumoniae*
3	Whitish fuss on MCA with a circular and rough edges on NA after 24hrs	*Bacillus* sp
4	Red pinkish colonies with black centers on SSA	*Salmonella sp*
5	Pinkish/reddish on MCA, Purple dots of EMB	*Enterobacter* sp
6	Clear transparent growth on SSA, small smooth colorless colonies on NA.	*Shigella sp*

7	Produced raise and mucoid/yellowish colonies/zones on MSA; Pinkish smooth surface on MCA, round and raised, smooth, shiny and convex shape on NA.	*Staphylococcus so*
8	Greenish raised colonies on NA, colourless on MCA	*Pseudomonas* so

Table 2 Indicates the confirmed isolates by their respective reactions to the various bio-chemicals and their characteristic appearances following stain with gram reagents otherwise refers to as their microscopic morphologies and biochemical Properties. The following biochemical test; Indole, catalase, coagulase, methyl red, voges Proskauer and oxidase were used to confirm each probable bacterial isolate as a combined identification with the colony morphologies.

Table 2: Microscopic Morphologies and Biochemical Properties of Bacterial Isolates from the Study Area

Gram Reaction	CM	IN	CA	CO	MR	VP	OX	Organisms
+ve	cocci	-	+	-	-	+	+	*Staphylococcus sp*
-ve	Rods	+	+	-	+	-	-	*E. coli*
-ve	Rods	-	+	-	+	-	-	*Klebsiella pneumoniae*
-ve	Rods	-	+	-	+	-	-	*Salmonella sp*
-ve	Rods	-	+	-	-	+	-	*Pseudomonas sp*
-ve	Rods	-	+	-	+	-	-	*Shigella sp*
+ve	Rods	-	+	-	-	+	-	*Bacillus sp*
-ve	Rods	-	+	-	-	+	-	*Enterobacter sp*

Keys: CM= Cellular morphology; IN = Indole; CA = Catalase; CO = Coagulase; MR = Methyl red; VP = Voges- Proskauer; OX = Oxidase

Table 3 Reveals the overall fungal isolates from the selected study area. Fungal isolates were sub cultured into fresh Potato Dextrose Agar (PDA) and subsequently onto slide culture. The colonial morphologies were examined followed by microscopy of the slide stained with Lactophenol Cotton Blue (LPCB) and comparing with the standard chart. The fungal isolates that were identified through the slide cultures by their sporulating or non-sporulating characters, cellular morphologies, nature of hyphae, types of spores or conidia, presence of columella, septate or aseptate hyphae were matched with standard chart identified as *Mucor sp, Aspergillus fumigatus, Aspergillus niger, Candida sp, Rhizopus stolonifer and Penicillium sp*.

Table 3: Colonial Morphologies and Microscopic Characteristics of Fungal Isolates from The Study Area

S/N	Colonial Morphology	Microscopic Characteristics From Slide Cultures Using LPCB	Isolates
1	Large whitish sporulating colonies turned blackish after 48hrs on PDA.	Unbranched sporangiophore that was clear without granules with smooth spores with columella.	*Mucor sp*
2	Dark green round and powdery colonies with white edges	Septate hyphae and conidiophores that are typically long, with a flask-shaped vesicle at the tip, from which conidia are formed in chains.	*Aspergillus fumigatus*
3	Black and round spreading colonies. Some have cracked bottoms	Darkly pigmented conidiophores that are swollen at the apex forming a vesicle from which radiate chains of round conidia	*Aspergillus niger*
4	Small creamy white colonies gradually turned yellowish and pasty white and distinct on PDA.	Showed distinct cells with buds stained bluish.	*Candida sp*
5	Showed cotton wool like spread colonies with black dots covering The entire plate.	Spherical sporangiospore produced inside a sporangium with columella on top of the sporangiophore and possessed root-like rhizoids.	*Rhizopus stolonifer*
6	Green powdery round and spreading growth after 48hrs on PDA	Branched conidiophores which bear chains of conidia that are typically smooth and round to oval in shape	*Penicillium sp*

Key: LPBC = Lactophenol Cotton Blue

Figure 2 presented the total number of both bacterial and fungal isolates from the study area according to their respective frequencies of occurrences. Eight bacterial species (*E. coli, klebsiella pneumoniae, Bacillus sp, Salmonella sp, Enterobacter sp, Shigella sp, Staphylococcus sp, and Pseudomonas sp)* accounted for 8(55.12) while Six fungal isolate (*Mucor sp, Aspergillus fumigatus, Aspergillus niger, Candida sp, Rhizopus stolonifer and Penicillium sp*) accounted for 6(44.88). Each isolate had 6 chances of occurrence according to the number of samples collected from the three selected restaurants in duplicate. *Staphylococcus sp, shigella sp, Enterobacter sp, Rhizopus sp and Aspergillus fumigatus* had the highest occurrence of 6 (100%) closely being followed by *klebsiella pneumoniae, Salmonella sp, Candida sp, and Penicillium sp* with the occurrence of 4 (66.6%) and the least occurred were the *E. coli, Bacillus sp, Pseudomonas sp and Mucor sp* of 2 (33.3%). Figure 4.1 shows the respective occurrence of each of the isolate in the three selected restaurants within the study period.

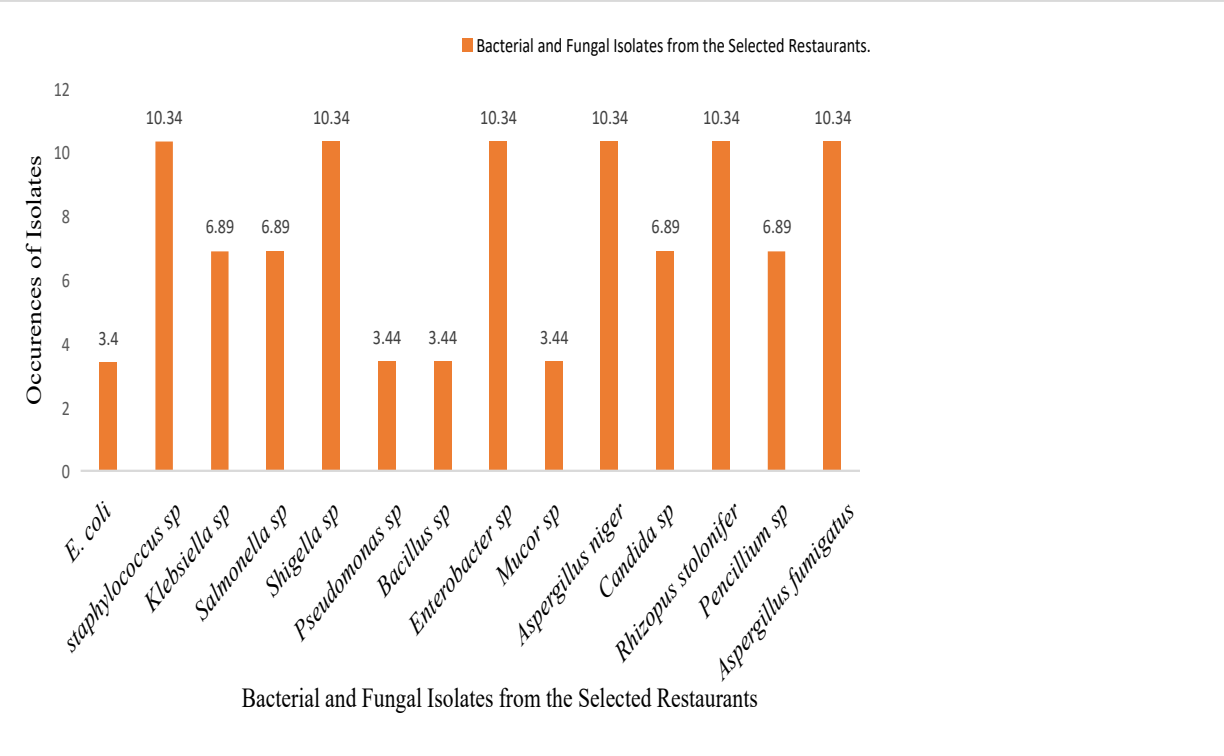

Figure 2: Percentage Occurrence of Isolates from the Selected Restaurants at Karji Chikun Local Government Area Kaduna State

Discussion

The high bacterial and fungal loads observed in restaurants wastewater could be due to the general degree of hygiene of restaurants and quality of water used by the restaurants. Generally, the higher microbial load could also be as a result of food particles, vegetables and oil which are the major source of organic matter in wastewater sample. Akpor and Muchie (2011) recently reported that water containing high organic matter require high microbial load to enhance degradation of solid wastes in such water. The isolated organisms (Tables 4.1 and 4.3) could be attributed to the microbiological quality of water used in preparation of food, sanitary quality of the equipment and health status of the employee. The high occurrence of *Staphylococcus aureus* may be due to shedding of resident *S. aureus* in human skin which may contaminate food and water during handling, processing, distribution and washing of hands after eating. Adams and Moss (2009) had earlier indicated that the principal habitat of *Staphylococci* is the skin, skin gland and mucous membrane of warm-blooded animal. The occurrence of *E. coli* from sampled wastewater in different locations could be an evidence of fecal contamination of water. The earlier finding of Lateef *et al*. (2006) and Makun *et al*. (2009) highlighted the public health importance of *E. coli* and other microorganisms. The presence of these organisms in wastewater disposed to the environment implies that there is need to provide proper waste disposal facilities to reduce the environmental hazards it may portend.

Eight distinct bacterial species (*E. coli, klebsiella pneumoniae, Bacillus sp, Salmonella sp, Enterobacter sp, Shigella sp, Staphylococcus sp, and Pseudomonas sp*) and six fungal species (*Mucor sp, Aspergillus fumigatus, Aspergillus niger, Candida sp, Rhizopus stolonifer and*

Penicillium sp) were recovered for this investigation from waste water samples collected from three selected restaurants at Karji Chikun Local Government Area in Kaduna State. This result aligns with the findings of Sunday, (2023) in the "Assessment of water quality index and some enteric characteristics of Ossiomo" where he isolated. The physical characteristics and biochemical analyses carried out for their identification provided information about the organisms. The physical and biochemical characteristics examined to identify the isolates of bacteria are displayed while for fungal isolates is showed in *Staphylococcus sp., Klebsiella sp., Shigella sp.,* and *Enterobacter sp*. are dominance. These bacteria are commonly found in human gut, skin, and respiratory tract. Their presence in wastewater may be due to improper handwashing, poor hygiene practices, and inadequate wastewater treatment. *Staphylococcus aureus* was isolated from every sample of waste water. Among the significant bacteria that have been linked to human illnesses like folliculitis, carbuncles, scalded skin syndrome, and boils is *Staphylococcus aureus*. Extensive microbiological diversity, including species of *Staphylococcus aureus* on human hair, has been described and suggested in literature (Kanu and Achi (2017). *Staphylococcus aureus* is a common human flora and has the ability to spread infection (Ganguli. *et al*., (2017).

Salmonella sp. is often associated with foodborne illnesses. Its presence in wastewater may indicate contamination from food handling and preparation practices. Low occurrence of *E. coli, Bacillus sp., and Pseudomonas sp*. in the wastewater samples may be due to competition with other microorganisms or environmental factors. Since *Pseudomonas* is a highly adaptive bacterium that can be found in a wide range of conditions, including distilled water, the discovery that it was recovered from two out of the six samples was not concerning (Abubakar (2019).

For fungal contaminants *Rhizopus stolonifer* was dominant. It is a common fungus found in soil, decaying organic matter, and wastewater. Its dominance in the wastewater samples may be due to the presence of organic matter and nutrients. High occurrence of *A. fumigatus, Mucor sp., Candida sp.,* and *Penicillium sp*. These fungi are commonly found in soil, air, and water. Their presence in wastewater may be due to contamination from environmental sources or improper waste management practices. And the low occurrence of *A. niger* in the wastewater samples may be due to competition with other microorganisms or environmental factors.

A study by Sauer *et al*. (2017) investigated the microbial contamination of wastewater from restaurants in Germany. The study found that the most common bacterial isolates were *Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus*, which is consistent with the findings of the present study. Another study by Odonkor and Ampofo (2013) examined the microbial quality of wastewater from restaurants in Ghana. The study found that the most common fungal isolates were *Aspergillus niger, Aspergillus fumigatus, Rhizopus stolonifer, and Penicillium spp*., which is also consistent with the findings of the present study. These studies highlight the importance of proper wastewater management and hygiene practices in restaurants to prevent the spread of pathogenic microorganisms.

CONCLUSION

This research revealed the presence of various bacteria such as *E. coli, klebsiella pneumoniae, Bacillus sp, Salmonella sp, Enterobacter sp, Shigella sp, Staphylococcus sp, and Pseudomonas sp,* and fungal contaminants *Mucor sp, Aspergillus fumigatus, Aspergillus niger, Candida sp, Rhizopus stolonifer and Penicillium sp* in the selected restaurants wastewater at Karji, Chikun Local Government Area Kaduna State. The disposal of restaurant wastewater poses a potential risk to the environment in terms of contamination of the land and water, and decreased air quality due to the release of unpleasant odors and various gases from anaerobic decomposition as well as sporadic burning. Because it encourages the dispersion of bacterial infections into the air, either as free entities or attached to particles, it further poses a risk for air pollution and contamination. While these pathogens are not as serious when suspended in the air, they cause a variety of infectious diseases, respiratory symptoms, and lung function impairment when they settle on surfaces. These conditions can range from mild, acute conditions that barely affect daily life to severe, chronic respiratory diseases, cancer, etc. that require specialized care. International rules state that water with this degree of contamination is not fit for household use and should not be released untreated into the environment. However, Restaurant wastewater is dumped into untreated open drainages, and the leachates from these wastes' gradual breakdown can introduce enteric bacteria into the river, which can lead to gastrointestinal diseases. Overall, study highlights the need for proper wastewater management practices in the restaurants to prevent environmental pollution and protect public health.

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