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Detection of Enteric Bacteria in Municipal Wastewater in Agbor Metropolis, Delta State

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ABSTRACT

Municipal wastewater is a significant reservoir for enteric bacteria and poses substantial risks to the health of the public, specifically in underdeveloped countries. where wastewater treatment infrastructure is inefficient. This study investigated the detection of enteric bacteria in municipal wastewater samples collected from five selected sources in Agbor, Delta State, Nigeria. The sources were, Restaurant, Tomato Effluent from Ebu Owa Market, Laundry, Shower, and Kitchen Sink sources. A total of five (5) wastewater samples were aseptically collected and analyzed using standard microbiological techniques. Total heterotrophic bacterial counts ranged from $47.33 \pm 1.70 \times 10^6$ CFU/mL in Shower wastewater samples to $64.00 \pm 3.74 \times 10^6$ CFU/mL in Restaurant wastewater samples, indicating heavy microbial contamination across all sample types. Coliform counts ranged from $25.33 \pm 1.25 \times 10^3$ CFU/mL in Kitchen Sink to $57.67 \pm 3.40 \times 10^3$ CFU/mL in Restaurant samples. The bacteria that were isolated from the samples were phenotypically identified as; *Staphylococcus* sp., *Enterococcus* sp., *Bacillus* sp., *Citrobacter* sp., *Klebsiella pneumoniae, Salmonella* sp., *Klebsiella* sp., and *Escherichia coli*. The isolate varied across sampling sources with restaurant having the highest bacteria load and diversity. The presence of known enteric pathogens such as *Salmonella* sp., *Escherichia coli,* and *Klebsiella pneumoniae* in untreated municipal wastewater studied indicates significant public health risks associated with inadequate wastewater management in Agbor. The findings highlight the urgent need for wastewater treatment, regular microbiological monitoring, and targeted public health interventions to mitigate the environmental dissemination of enteric bacteria and prevent waterborne disease outbreaks in Agbor metropolis.

Keywords: Metropolis, Wastewater, Enteric Bacteria, *Salmonella sp., Escherichia coli, Klebsiella pneumoniae*, Public Health.

Introduction

Municipal wastewaters are liquids waste produced by a community of people, from their houses, from academic institutions, from hospitals, urban runoff and diverse establishment (Corpuz *et al*., 2024). These wastes consist of organic compounds, certain chemical contaminant, and microbes (Khanam *et al*., 2022). In developing regions, such as Nigeria, Municipal waste water has become overly substantial to public health concern, due to an increase in population in Nigeria, a country with inadequate hygiene and sanitation system (WHO, 2017). These waste water system, serves as a collection of faeces, liquid waste as well as s dissoluble solid waste from various locations, thus it serves as a reservoir for pathogenic microorganisms.

Municipal waste water contains diverse pathogens including enteric pathogens which are of great concern to public health Cabra, (2010). Pathogenic bacteria species like the *Vibrio cholerae*, *Shigella* spp., *Samonella* spp., and viral agent like *Norovirus*, as well as pathogenic protozoa like *Cryptosporidium* species (Bivins 2024). In many underdeveloped and developing countries, water waste is released into surrounding water bodies such as rivers, and streams, which in certain communities serves as source of water for domestic activities, this therefore increases the prevalence of disease outbreak to the public.

Children of under five years of age and the elderly are mostly affected leading to morbidity and mortality (WHO 2021; Meier *et al*., 2020).

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Recent advancement in molecular biology, has increased and improve the detection of pathogenic microbes in waste water, using molecular methods like (PCR), Next generation sequencing, quantitative polymerase chain reaction technique and more which aids identification of pathogens (McCall *et al*., 2024). In Nigeria, the use of molecular methods has revealed ground water contamination (Olalemi *et al*., 2022). The epidemiology (WBE) of waste water has elevated as an innovative approach for monitoring, analyzing sewage for pathogen genetical material (Zahedi *et al*., 2021; Acosta *et al*., 2024). This approach has been applied successfully for viruses and is now being used to track multiple pathogens simultaneously (Nnaji *et al*., 2022). In Nigeria, waste water management systems are not efficient as the challenges of poor infrastructure, and inadequate treatment facilities frequently increases, septic tanks and open drainage are directly discharge into nearby water bodies (Ajonina *et al*., 2022).

Despite posing health risk to the public at large, waste management system and practices are limited, routine monitoring for enteric pathogenic organisms in municipal water is finite and most recent assessment focus on bacterial indicators rather than diverse pathogens in municipal waters (Iwu *et al*., 2024). Having the knowledge and understanding the prevalence, types as well as potential risk of diverse pathogens in municipal waste water is essential for improving waste water treatment, thereby reducing environmental contamination, as well as safeguarding public health. Thus, investigation to detect these pathogens in municipal water is important. Due to the continuous discharge of fecal by humans, as well as animals, in to sewage system, municipal water waste, stands as a reservoir for enteric pathogens (UNICEF and WHO 2019; Corpuz *et al*., 2024). In developing country like Nigeria, wastewater treatment infrastructure are inadequate, poorly maintained or absent resulting in releasing of waste that is not yet treated into the surroundings. This therefore increases the danger of contamination in the environmental (Olalemi *et al*., 2022). Although fecal indicators of organisms are monitored in waste water, they do not accurately show the existence of certain other pathogens like viruses, protozoans and more (Bonetta *et al*., 2024). In Nigeria, similar limitations have been observed in surface water surveillance, where molecular methods could bridge detection gaps (Nnaji *et al*., 2022). Anti-microbial resistance pathogens in wastewater further aids public health risk thereby presenting an additional challenge. Without systematic detection and characterization of these pathogens, communities remain vulnerable to waterborne disease outbreaks and the transmission of resistant infections. Recent Nigerian studies have documented multidrug-resistant enteric bacteria in household wastewater, indicating community-level AMR reservoirs (Adenipekun *et al*., 2024).

Despite the recognized public health implications, available indigenous data are insufficient on the occurrence and detection of enteric pathogens in municipal wastewater in many urban centers. This lack of data hinders the formulation of policies that are evidence based; it hinders an effective management of waste water, and disease prevention strategies (Kim *et al*., 2024). Thus, there arise needs to investigate pathogens that are enteric to municipal water waste (Iwu *et al*., 2024; Olalemi, 2022).

Materials and Methods

Study Area

Experimentation of the study assesses the detection of enteric bacteria in municipal wastewater across five waste water sites in Agbor. Agbor is located in Ika , within Delta State, Nigeria. It is located around the rain forest zone of southern Nigeria, with latitude 6.25°N and longitude 6.19°E . Agbor experiences rainy and dry climatic seasons which runs from April to October for rainy seasons, and November to March for dry season. The residents of Agbor are mainly, civil servants, traders, artisans, student as well as farmers. The market was selected for tomato effluent and other samples were from residential areas that poses public health risk.

Sampling Sites and Sample Collection

This study employed a cross-sectional descriptive design to detection of enteric bacteria in municipal wastewater in Agbor metropolis, Delta State. The study selected randomly, five waste water sampling sites were collected from residential areas across Agbor, the locations were chosen to represent areas with high human activities and potential sources of microbial contamination.

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Wastewater samples were collected aseptically using a new, clean unopened 500 mL plastic bottles. Prior to collection, each bottle was rinsed three times with the wastewater at the sampling point to minimize contamination and ensure sample representatives. The bottles were then filled, properly capped, and handled carefully to avoid external contamination. Five (5) samples in total were collected for the study. All samples were clearly labeled for identifying accuracy. The collected samples were immediately transported in ice-packed containers to the Laboratory, within two (2) hours of collection for microbiological analysis, following standard procedures as recommended by APHA, (2017).

Preparation and Sterilization of Media

General-purpose and selective/differential media for isolating enteric bacteria from wastewater samples was utilized (Maidamma *et al* (2023). The media used included Nutrient Agar (NA) for total heterotrophic bacterial count, MacConkey Agar (MAC) and Eosin Methylene Blue Agar (EMB) for the isolation of coliforms and *E. coli*, Triple Sugar Iron Agar (TSI) for differentiation of enteric bacteria based on sugar fermentation, and Simmons Citrate Agar for citrate utilization testing (Maidamma *et al* (2023).

Cultivation of Bacterial and Enumeration

The process followed the serial dilution of mixing 9ml of sterile distilled water, with a ml of waste water (sample), to make a dilution, this was the initial dilution, as dilution was done afterward, step by step, by a factor of 10, until the 6^{th} time as required. An aliquot of 1.0mL was then introduced into a Petri dish that was already sterilized and afterward, about 15mL of freshly prepared sterile molten agar was aseptically poured into the plates. The plates were gently rotated in all directions to ensure even mixing and allowed to cool and harden (Maidamma *et* al., 2023), and then it was incubated at 37^0c for 24hours. The colonies which developed after the incubation period were counted using a colony counter and the results per dilution were recorded (Nfor *et al*., 2022). The number of colony forming units per millilitre (CFU/mL) of each wastewater sample was calculated using the formula of Maidamma *et al* (2023):

$$\text{CFU} = \frac{number\ of\ colonies}{Volume\ plated\ (\text{ml}) \times\ \ dilution\ factor}$$

Subculturing of Pure Isolates

Distinct colonies were subcultured onto freshly prepared Nutrient Agar plates to obtain pure cultures. Isolates were further subjected to morphological, microscopic, and biochemical characterization for proper identification (Nfor *et al*. (2022).

Identification of Bacteria isolates

Pure isolates were obtained by subculturing individual bacterial colonies and repeatedly transferred onto Nutrient agar (Nfor *et al.*, 2022). Each pure isolate then undergo two forms of assessment such as size, shapes. The pure isolates were subjected to Gram stain procedure (Nfor *et al.*, 2022; Maidamma *et al*., 2023), physiological and biochemical characterization tests such as; and the KOH string test (Roberts and Sandle, 2008), Catalase Test, and Citrate Utilization Test (Khatoon *et al.,* 2022), Urease Test, Hydrogen Sulphide (H2S) Test, Indole Test and Coagulase test (Cheesbrough, 2005).

Sugar Fermentation Test

All isolate was tested for sugar fermentation, by checking for acid or acid with gas, and peptone water served as the medium (1% sugar) and bromocresol was used as the indicator. And the mixture was poured into tubes and was autoclaved for 15mins at 121°C and cooled after wards, then incubated for 24 h at 37°C (Ejikeugwu, 2022), and colour changed from purple to yellow, which shows acid production and gas production was also noted but separately and lactose, fructose, maltose, starch and sorbitol was the sugar tested.

Results

The results of the microbial counts are as presented in Table 1. The total heterotrophic bacterial counts of municipal wastewater samples are presented in Table 1. The Restaurant sample recorded the highest mean bacterial count of $64.00 \pm 3.74 \times 10^6$ CFU/mL, while the Shower sample had the lowest count of $47.33 \pm 1.70 \times 10^6$ CFU/mL. Tomato Effluent and Laundry samples also showed high bacterial loads of $57.67 \pm 2.05 \times 10^6$ CFU/mL and $57.67 \pm 2.49 \times 10^6$ CFU/mL, respectively.

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The high bacteria counts suggest heavy microbial contamination of the wastewater samples, especially in the food-related environments. The Restaurant sample recorded the highest mean count of 57.67 ± 3.40 × 10^3 CFU/mL, followed by Laundry with 55.67 ± 2.87 × 10^3 CFU/mL, while the Kitchen Sink sample had the lowest count of 25.33 ± 1.25 × 10^3 CFU/mL. The Restaurant sample again recorded the highest mean count of faecal coliform 37.67 ± 2.05 × 10^2 CFU/mL, whereas the Shower sample showed the lowest count of 5.00 ± 0.00 × 10^2 CFU/mL. Laundry and Kitchen Sink samples recorded moderate counts of 30.67 ± 1.70 × 10^2 CFU/mL and 20.00 ± 2.16 × 10^2 CFU/mL, respectively. These findings indicate the presence of Gram-negative enteric bacteria in the wastewater samples, particularly in Restaurant and Laundry effluents.

Phenotypic characteristics of the bacteria isolated from the municipal wastewater samples revealed the presence of *Staphylococcus* sp., *Enterococcus* sp., *Bacillus* sp., *Citrobacter* sp., *Klebsiella pneumoniae*, *Salmonella* sp., *Klebsiella* sp., and *Escherichia coli*. Table 2 presents the distribution pattern of bacteria isolates in samples of wastewater sources. Restaurant wastewater harbored *Bacillus* sp. and *Salmonella* sp., while *Citrobacter* sp. was isolated from Tomato Effluent. Laundry sample yielded *Klebsiella pneumoniae* and *Escherichia coli*, indicating possible contamination from enteric sources. *Enterococcus* sp. was isolated only from the Shower sample, whereas Kitchen Sink sample contained *Staphylococcus* sp. and *Klebsiella* sp. The bacteria distribution indicates the presence of diverse bacteria contaminants associated with domestic and food-related activities.

Table 1: Bacteria Count (Mean ± SD) from Municipal Wastewater Samples

Source of Sample	**Bacteria Count (CFU/mL) from Municipal Wastewater Samples**		
	Total heterotrophic Bacteria (x 10^6)	**Total Coliform (x 10^3)**	**Fecal Coliform (x 10^2)**
Restaurant	64.00 ± 3.74	57.67 ± 3.40	37.67 ± 2.05
Tomato Effluent	57.67 ± 2.05	48.33 ± 2.05	10.00 ± 00
Laundry	57.67 ± 2.49	55.67 ± 2,87	30.67 ± 1.70
Shower	47.33 ± 1.70	38.00 ± 2.94	5.00 ± 0.00
Kitchen Sink	50.67 ± 1.70	25.33 ± 1.25	20.00 ± 2.16

Table 2: Distribution Pattern of Bacterial Isolates across Different Municipal Wastewater Sample Sources

Bacteria Isolate	**Source of Municipal Wastewater samples**				
	Restaurant	**Tomato Effluent**	**Laundry**	**Shower**	**Kitchen Sink**
Staphylococcus sp.	-	-	-	-	+
Enterococcus sp.	-	-	-	+	-
Bacillus sp.	+	-	-	-	-
Citrobacter sp.	-	+	-	-	-
Klebsiella pneumoniae	-	-	+	-	-
Salmonella sp	+	-	-	-	-
Klebsiella sp.	-	-	-	-	+
Escherichia coli	-		+	-	-

Keys: + = Present; − = Absent.

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Discussion

The research examines microbial contamination in municipal waste water in Agbor. Municipal wastewater collected from diverse sample site were analyzed, and the study observed heterotrophic bacterial count was higher in the restaurant wastewater with the value of $64.00 \pm 3.74 \times 10^6$ CFU/mL this reflects the high organic content in the effluent, as restaurant wastewater typically contains nutrient from food residues, cleaning agents and oils, and these nutrient promote the growth of bacteria, and this finding relates with that of Chukwu *et al*., (2024) and also Tenebe *et al*., (2024) that concluded that food processing activities contribute significantly to microbial loads in sewage systems (Chukwu *et al*., 2024; Tenebe *et al*., 2024). Similarly, high coliform count was also reported on restaurant with the value of $57.67 \pm 3.40 \times 10^3$ CFU/mL, followed by samples collected from laundry waste water with the value $55.67 \pm 3.40 \times 10^3$ CFU/mL. There was a considerable amount of fecal coliform count as well as environmental contamination. bacteria were detected, with samples from restaurant having the highest count ($37.67 \pm 2.05 \times 10^2$ CFU/mL). The pattern of municipal wastewater bacterial count agrees with a researched by Olalemi *et al*., (2016) and also the research by Sorensen *et al*., (2015) that detects bacterial contamination including gram negative bacteria as a result of fecal contamination and inadequate hygiene infrastructure. Experimentally, the research isolated eight bacterial isolates viz; *Klebsiella* spp., *Escherichia coli, Samonella* spp., *Klebsiella pneumoniae, Bacillus* spp., *Enterococcus* spp., *staphylococcus* spp. and *Citrobacter* (Cheesbrough, 2005). This reveals a broad diversity of microbial organisms, consisting of both opportunistic pathogens and enteric pathogens. *E. coli* and *Klebsiella pneumoniae* was isolated from laundry waste water, this is of great concern as it poses risk to the public health, and it suggest contamination from clothing soiled with human excreta due to poor hygiene practices. In effluents from restaurant *Samonella spp.* Was present this is traced to poor handling origins. Also, *Citrobacter* spp. was found in tomato effluents this could originates from contaminated produce or soil runoff. These source-specific distributions highlight how different human activities contribute distinct microbial profiles to the municipal wastewater stream in Agbor (Chukwu *et al*., 2024).

The bacterial load as well as diversity observed in this study relates with several researches across Nigeria like that of Tenebe *et al*., 2024 and also Nyandjou *et al*., 2018, where, high heterotrophic bacteria plate count and coliform bacteria level, exceeded regulatory limits, and this is commonly reported in most related researches in untreated domestic and municipal waste water (Nyandjou *et al*., 2018). According to the Okafor *et al.*, (2022), and in the research of karungamye 2023, predominant enteric bacteria like the *E. coli* and *Klebsiella* spp., are majorly reported to be seen in municipal waste water, as well as causative agent of several illnesses and diseases this is confirmed in most literary research works in Nigeria (Kagambèga *et al*., 2024; Okafor *et al*., 2022). In public health, the presence of pathogens including enteric microbes in waste water indicates persistent risk of disease transmission, if this wastewater is discharged into the surrounding environment; it would likely cause contamination and transmission of infection directly or indirectly. Pathogens such as the *Salmonella* spps and *E. coli* strains can facilitate disease outbreak like typhoid fever, and other infections. In addition, *Staphylococcus* spp. and *Enterococcus* spp. was isolated, and this raises a huge concern as opportunistic infection can have anti-microbial resistance genes . In areas like Delta state, findings like this shows the importance of improved sanitation systems. The reports of the bacterial count, from wastewater samples in restaurant and laundry contributed to grey water and food-related effluents and contamination. The general observation shows emphasis on major researches on municipal waste water especially in developing countries like Nigeria, which reported variable but frequently elevated levels of heterotrophic bacteria and coliforms. The differential distribution of isolates across sampling points food preparation sources yielding *Bacillus*, *Salmonella*, and *Citrobacter*, and hygiene-related sources favouring *Enterococcus*, *Klebsiella*, and *E. coli* supports the implementation of targeted pre-treatment strategies at high-load points before integration into the broader municipal system. Such source-specific profiling enhances the precision of wastewater management interventions in settings like Agbor. The presence of known enteric pathogens documented in municipal wastewater in Agbor, Delta State, highlights the inadequate treatment of sewage and the associated environmental and public health risks.

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Conclusion

The study revealed the presence of known enteric pathogens such as *Klebsiella pneumoniae*, *Salmonella* spp., *Klebsiella* spp., and *Escherichia coli*, *Staphylococcus* spp., *Enterococcus* sp., *Bacillus* spp., and *Citrobacter* sp in municipal wastewater studied in Agbor, Delta State. This call for concern as the effluents represents high-risk categories of pathogens and public health risks posed by inadequate municipal wastewater management in Nigerian Cities. There is therefore an urgent need by the relevant authorities for Strengthening wastewater collection, and effective management of municipal wastewater and the provision of wastewater treatment infrastructure to safe guard public health in Agbor Metropolis.

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