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Article

Bacteriological Assessment of Waste Water in a Tertiary Hospital in Port Harcourt, Nigeria

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Abstract: Hospital wastewater often contains pathogenic microorganisms and chemical contaminants that may pose risks to public health and the environment if discharged without adequate treatment. The bacteriological assessment of wastewater from the University of Port Harcourt Teaching Hospital in Port Harcourt was carried out. A total of 96 wastewater samples were collected from six hospital units: kitchen, surgery, theatre, paediatrics, laboratory, and laundry over a four-month period. Standard microbiological methods including serial dilution, culture on nutrient and MacConkey agar, morphological and biochemical identification were used for bacterial isolation. The results showed that *Escherichia coli* had the highest occurrence with 21 isolates (21.9%), followed by *Staphylococcus spp.* (18.8%) and *Micrococcus spp*. (14.6%). *Pseudomonas spp*. and *Klebsiella spp*. accounted for 13.5% and 12.5% respectively, while *Proteus spp*. (10.4%) and *Bacillus spp*. (8.3%) had lower prevalence. The distribution of isolates varied across hospital units, with the laboratory recording the highest prevalence of *E. coli* (43.8%), while *Staphylococcus spp*. was most prevalent in the kitchen and surgery units (25.0% each). *Pseudomonas spp*. showed highest occurrence in the surgery unit (25.0%), whereas *Klebsiella spp*. was more common in the theatre and laundry units (25.0% each), and *Proteus spp*. was predominantly found in the theatre and laundry sections. The findings highlight significant microbial contamination of hospital wastewater and emphasize the need for effective wastewater treatment and monitoring to prevent environmental pollution and the spread of antibiotic-resistant pathogens.

Keywords: Bacteriological Assessment, Waste Water, Tertiary Hospital, Port Harcourt.

Introduction

Waste water generation is a phenomenon that is common to the whole world and treatment of waste water is imperatively demanded by authorities before it is discharged into the environment. Waste water facility´s obligation is to provide quality ways of managing their wastewater effluents before its release into the environment but most of these facilities still release final effluents that contain certain amounts of pathogenic bacteria [1]. The release of wastewater with contaminants such as pathogens into the environment threatens the environmental biota as well as water bodies needed in society [2]. Increased pollution of the environment via the discharge of effluents from diverse sources including hospitals had resulted in many waterborne infectious disease epidemics in both developing and some developed countries. Wastewater could be composed of physical (odour, colour), chemical (toxic organic substances, alkaline), and biological materials (pathogens, animals) which is a function of the source of the wastewater [3].

Water supply to healthcare facilities is frequently overlooked yet essential for safe patient care and can be a manageable source of infections. Numerous healthcare-associated outbreaks have been linked to contaminated water used for patient care, particularly maternal sinks, faucets, or shower heads associated with hand washing and cleaning of medical devices [4]. Altin et al. [5] documented that among the biggest water consumers in cities, hospitals are on the list and that the quality of water supplied to hospitals is very critical since they play important roles in the health of the patients as well as the hospital's daily operation. Because of the multiple exposure or use of water [6], water should be regarded as a significant source of infection. These scenarios include intricate hospital water systems and water-containing equipment and tools utilised in medical facilities [7].

Most monitored wastes are industrial and domestic wastes and yet wastes that create severe challenges to public health are hospital wastes. For example, several medical personnel have reportedly contacted several infections through their interaction with the hospital environmental wastes [8].

According to Robinson et al. [9], the bacteriological quality of the water samples, coagulase, haemolysis, biofilm, starch, and antibiogram were determined using standard microbiological procedures. The mean range of the total heterotrophic bacterial, staphylococcal, faecal coliform, and total coliform counts of the water samples were 8.5±0.7×105 to 3.8±2.1×107, 1.2±0.2 to 2.8±0.3×105, 0.0±0.0 to 8.0±4.2×103 and 0.0±0.0 to 1.1±0.1×105 CFU/mL, respectively. The prevalence of the isolated bacteria is *Staphylococcus sp* (17.5%), *Bacillus sp* (12.5%), *Enterobacter sp*. (12.5%), *Klebsiella sp*. (10%), *Citrobacter* sp. (5%), *Escherichia coli* (2.5%) and *Siccibacter sp*. (2.5%). *Staphylococcus, Bacillus, Enterobacter, Klebsiella, Citrobacter, Escherichia coli, and Siccibacter sp*. were positive for haemolysis and α-amylase production, 80% of *Staphylococcus sp* were coagulase positive while 46, 40, 57 and 25% of *Staphylococcus, Bacillus, Enterobacter,* and *Klebsiella sp* produced biofilm. The antibiogram showed multi-drug resistance (0.2-1.0). Levofloxacin was 60% effective against *Staphylococcus* sp, while susceptibility of *Klebsiella* and *Citrobacter sp* to ofloxacin, gentamycin, nalidixic acid, and levofloxacin 66.7%. The water samples from these hospitals might not be good for drinking.

Wastewater generated from tertiary hospitals contains a wide variety of pathogenic microorganisms, including bacteria originating from infected patients, laboratory activities, surgical procedures, and other clinical operations. When such wastewater is discharged into the environment without adequate treatment, it can serve as a reservoir for pathogenic and antibiotic-resistant bacteria, posing serious risks to public health and environmental safety. Therefore, it becomes necessary to conduct a bacteriological and physicochemical parameters assessment of wastewater from University of Port Harcourt teaching hospital.

The study was aimed at assessing the bacteriological contamination of waste water from a tertiary hospital in Port Harcourt. The objectives were to:

a) Determine the overall prevalence of bacterial isolated from waste water at the University of Port Harcourt teaching hospital.

b) Assess the overall prevalence of bacterial isolated from waste water in relation to different units in the study area.

Materials and Methods

Study Area

The study was carried out in University of Port Harcourt Teaching Hospital (UPTH) Rivers

State. Port Harcourt is located along the Bonny River (of the Niger River) 41 miles (66km) upstream from the Gulf of Guinea (Encyclopediae & Britannica, 2010). Its lies on latitude 40491 27" N and 7^0 2' 1" E on an area of 109km^2 (42sqm), with a population of over 541, 115 as of the 2015 and it grew to 2million with an urban density of 14,800/km^2 (38,000/sqm).

Study Design

A facility based cross-sectional study was conducted in University of Port Harcourt Teaching Hospital (UPTH), Choba, Port Harcourt.

Sample Collection

A total of ninety six waste water samples were collected from six different locations in the Hospital, the laboratory section, Hospital canteen (Kitchen), Hospital laundry, surgical ward (male and female), theatre and Pediatrics ward with a sterile capped container. About one liter of Wastewater samples were collected once a week, for over a period of four months from each main manhole discharging point of the various departments in the hospital.

However, a total number of sixteen waste water samples were collected from the month of July to October from the six different locations in the Hospital, the laboratory section, Hospital canteen (Kitchen), Hospital laundry, surgical ward (male and female), theatre and Pediatrics ward.

All samples were collected on weekdays in the morning between 9:00 am and 11:00 a.m. at ambient temperatures ranging from 24°C to 31°C and was transported to the laboratory within 10 to 20 minutes for microbiological analyses [10].

Control Sample

One control sample (drinking source) was processed together with each batch of sample to demonstrate that the analytical system and plastic containers were free of contamination [11].

Purity Plate

Each colony in the macConkey plate was picked with the aid of sterile wire loop and inoculated into another differential media MacConkey agar) for identification. With the aid of a sterile wire loop, the sample were streaked to enable distinct colonies visible under a controlled laboratory condition. The plates were incubated for 24hours at the optimal temperature (37^0 C).

Cultivation and Identification of Bacterial Isolates

Standard microbiological methods including culture on Nutrient and MacConkey agar plates using the streak plate method according to the method described by Miles and Misra [12]., morphological and biochemical identification: motility test, catalase, coagulase, methy red, indole, Voges- Proskaeut, oxidase, citrate, starch hydrolysis, sugar fermentation tests and the Kirby–Bauer disk diffusion method were used for bacterial isolation and antibiotic susceptibility testing.

Gram Staining Techniques Gram Staining

This test was carried out to group bacteria into Gram positive and Gram negative and also show the cellular morphologies and forms [13]. A smear was made from a 24-hours culture on properly labelled grease free glass slide. This was achieved by dropping one to two drops of water on the slide and emulsifying with a loopful of bacteria on the grease free glass slide. The smear was air dried and heat fixed by passing the slide under a Bunsen burner flame three times. Each smeared slide was flooded with the primary stain (Crystal violet) for 60 seconds, rinsed in slow running tap water. Smears were the flooded with Lugol's iodine for 60 seconds and rinsed in slow running tap water. The smears were then decolorized with 95% ethanol for 30 seconds and rinsed with tap water and then flooded with a counter stain (Safranin) for 30 seconds and again rinsed with slow running tap water. The slide was allowed to air dry on a slide rack. The stained smear was examined microscopically using oil immersion lens (X100) for better magnification. Purple or violet colour showed Gram positive while pink or red colour showed gram negative.

Motility Test:

A sterile needle was used to pick a loop of a 24hrs old culture and was stabbed onto nutrient

agar in glass vials. The vials were incubated at 37°C for 24-48 h. Non-motile bacteria had growth confined to the stab line with definite margins without spreading to surroundings area while motile bacteria gave diffused growth extending from the surface [14].

Catalase Test:

A small quantity of 24 h old culture was transferred into a drop of 3% Hydrogen peroxide solution on a clean slide with the aid of sterile inoculating loop. Gas seen as white froth indicates the presence of catalase enzyme [15].

Coagulase Test:

Coagulase is an enzyme capable of coagulating certain blood plasma, notably human and rabbit plasma. This test differentiates pathogenic from non-pathogenic Staphylococcus spp.; the test was carried out using 18-24 h old culture. A loopful of isolated bacterium was emulsified with normal saline solution on a microscope slide. A drop of undiluted plasma was added to the suspension and stirred for five seconds. A coagulase-positive result was indicated by clumping of colonies together.

Methyl red Test:

Five millimeters of glucose phosphate broth (1 g glucose, 0.5% KH_2PO_4, 0.5% peptone and 100 mL distilled water) were dispensed in clean test tubes and sterilized. The tubes were then inoculated with the test organisms and incubated at 37°C for 48 h. At the end of incubation, few drops of methyl red solution were added to each test and colour change was observed. A red colour indicates a positive reaction.

Voges-Proskaeur Test:

Five millimeter of glucose phosphate broth (1 g glucose, 0.5% KH_2PO_4, 0.5% peptone and 100 mL distilled water) were dispensed in clean test tubes and sterilized. The tubes were then inoculated with the test organisms and incubated at 37°C for 48 h. After incubation, 6% α-naphhtol and 6% Sodium hydroxide were added to about 1 mL of the broth culture. A strong red colouration formed within 30 min indicates positive reaction.

Indole Test:

Tryptone broth (5 mL) was placed into different test tubes after which a loopful of the bacterial isolates was inoculated into the test tubes, leaving one of the test tubes uninoculated to serve as control. The test tubes were then incubated at 37°C for 48 h. After incubation, 0.5 mL of Kovac's reagent was added and shaken gently; it was allowed to stand for 20 min to permit the reagent to rise. A red or red-violet colour at the top surface of the tube indicates a positive result while yellow colouration indicates a negative result.

Starch Hydrolysis:

This is used to assay the ability of microorganisms that can produce enzymes that degrade substrate with carbon compounds. Nutrient agar was prepared with 1% soluble starch and was sterilized. The medium was poured into sterile plates and were inoculated by streaking the organisms once across the plates after solidifying. The plates were incubated at 37°C for 24 h after which they were flooded with Gram's iodine. Unhydrolysed starch forms a blue colour with the iodine. Hydrolysed starch appears as a clear zone due to alpha amylase activity while reddish brown zones around the colony indicates partial hydrolysis of starch to dextrans.

Citrate Test:

This test detects the ability of an organism to use citrate as a sole source of carbon and energy. About 2.4 g of citrate agar was dissolve in 100 mL of distilled water. About ten milliliter (10 mL) of citrate medium was dispensed into each tube and covered, then sterilized and allowed to cool in a slanted position. The tubes were inoculated by streaking the organisms once across the surface. A change from green to blue indicates utilization of the citrate.

Oxidase Test:

A piece of filter paper was soaked with few drops of oxidase reagent. Sterile inoculating loop was used to pick a colony of the test organism and smeared on the filter paper. If the organism is oxidase producing, the phenylenediamine in the reagent will be oxidized to a deep purple colour.

Sugar Fermentation:

Sugar fermentation test was carried out to determine the ability of organisms to ferment sugars with production of acid and gas. Sugar indicator broth was prepared using peptone water medium containing 1% fermentable sugar and 0.01% phenol red. About ten milliliters of sugar broth was dispensed into each of the test tubes, Durham tube which would trap the gas if produced was inverted carefully. The test tubes were autoclaved and inoculated with a loopful of 24 h old culture of the test organisms after then incubated for 2-7 days at 36±1°C and observed daily for acid and gas production. Yellow colouration indicates acid production while gas production was indicated by displacement of the medium in the durham tube.

Statistical Analysis

This was performed using Graphpad for Analysis of variance (ANOVA)

RESULT

Table 1 shows the overall prevalence of bacterial isolates recovered from the 96 wastewater samples. *Escherichia coli* had the highest occurrence with 21 isolates (21.9%), followed by *Staphylococcus spp.* with 18 isolates (18.8%). *Micrococcus spp.* accounted for 14 isolates (14.6%), while *Pseudomonas spp.* and *Klebsiella spp.* were detected in 13 (13.5%) and 12 (12.5%) samples respectively. *Proteus spp.* occurred in 10 samples (10.4%), whereas *Bacillus spp.* had the lowest prevalence with 8 isolates (8.3%). The results indicate that *E. coli* was the most predominant bacterium in the hospital waste water samples.

Table 1: **Overall prevalence of bacterial isolate**

Bacterial Isolate	Number of Positive Samples	Prevalence (%)
Escherichia coli	21	21.9
Staphylococcus spp.	18	18.8
Micrococcus spp.	14	14.6
Pseudomonas spp.	13	13.5
Klebsiella spp.	12	12.5
Proteus spp.	10	10.4
Bacillus spp.	8	8.3
Total	**96**	**100**

Table 2 presents the distribution and percentage prevalence of bacterial isolates across different hospital units. The laboratory recorded the highest prevalence of *E. coli* with 7 isolates (43.8%), while the kitchen and laundry units also showed notable occurrences. *Staphylococcus spp.* was most prevalent in the kitchen and surgery units (25.0% each). *Pseudomonas spp.* showed its highest occurrence in the surgery unit (25.0%), whereas *Klebsiella spp.* was more prevalent in the theatre and laundry units (25.0% each). *Proteus spp.* occurred mainly in the theatre and laundry sections.

Table 2: P**revalence of bacterial isolate in relation to hospital units**

Hospita	No. of	*E. coli*	*Staphyloc*	*Micrococ*	*Bacillus*	*Pseudo*	*Klebsiel*	*Proteus*

l Unit	Sampl es	(%)	occus spp. (%)	cus spp. (%)	spp. (%)	monas spp. (%)	la spp. n (%)	spp. n (%)
Kitchen	16	5 (31.3)	4 (25.0)	3 (18.8)	–	–	2 (12.5)	2 (12.5)
Surgery	16	–	4 (25.0)	2 (12.5)	2 (12.5)	4 (25.0)	2 (12.5)	2 (12.5)
Theatre	16	–	3 (18.8)	3 (18.8)	1 (6.3)	2 (12.5)	4 (25.0)	3 (18.8)
Paediatr ics	16	4 (25.0)	3 (18.8)	2 (12.5)	1 (6.3)	3 (18.8)	2 (12.5)	1 (6.3)
Laborat ory	16	7 (43.8)	2 (12.5)	2 (12.5)	1 (6.3)	2 (12.5)	2 (12.5)	–
Laundry	16	5 (31.3)	2 (12.5)	2 (12.5)	2 (12.5)	2 (12.5)	4 (25.0)	3 (18.8)
Total	**96**	**21**	**18**	**14**	**8**	**13**	**12**	**10**

Discussion

The findings of this study shows that wastewater from the University of Port Harcourt Teaching Hospital harbours a diverse population of bacteria, many of which are of clinical and public health importance. The predominance of *Escherichia coli* (21.9%) observed in this study is indicative of faecal contamination and reflects the continuous discharge of human waste and clinical effluents into hospital drainage systems. This observation aligns with previous reports which have identified *E. coli* as a common indicator organism in wastewater studies. The presence of other organisms such as *Staphylococcus spp.*, *Pseudomonas spp.*, and *Klebsiella spp.* further confirms the polymicrobial nature of hospital wastewater and its potential to serve as a reservoir of opportunistic pathogens.

The distribution of bacterial isolates across different hospital units highlights the influence of departmental activities on microbial load. The high prevalence of *E. coli* in the laboratory (43.8%) suggests contamination from clinical specimen handling and disposal of biological materials. Similarly, the significant occurrence of *Klebsiella spp.* and *Proteus spp.* in the laundry and theatre units may be attributed to the washing of contaminated linens and surgical materials. These findings are consistent with Awodele et al., who reported that hospital laundries are major sources of microbial contamination due to their role in handling infected materials. The detection of *Pseudomonas spp.* in surgical units also corroborates earlier studies that identified hospital water systems and moist environments as reservoirs for this organism).

The occurrence of *Staphylococcus spp.* across multiple units may be linked to its origin from human skin and hospital surfaces, suggesting possible contamination through patient-care activities and poor infection control practices. Suleyman et al. similarly reported that environmental surfaces within healthcare settings contribute significantly to the transmission of nosocomial pathogens. The presence of *Micrococcus spp.* and *Bacillus spp.* also reflects environmental contamination, as these organisms are commonly found in soil, air, and dust, and may enter wastewater through various routes.

Moreso, the findings of this study are in agreement with previous reports that hospital wastewater contains a high load of pathogenic and multidrug-resistant bacteria. The discharge of such untreated or inadequately treated wastewater into the environment poses serious risks, including contamination of water bodies, spread of infections, and dissemination of antimicrobial resistance.

Conclusion and Recommendation

This study assessed the bacteriological characteristics of wastewater generated from different departments of the University of Port Harcourt Teaching Hospital in Port Harcourt. The results showed the presence of several bacterial species including *Escherichia coli*, *Staphylococcus spp.*, *Pseudomonas spp.*, *Klebsiella spp.*, and *Proteus spp.*, indicating significant microbial contamination. The isolates also exhibited resistance to several commonly used antibiotics, suggesting the presence of multidrug-resistant organisms in hospital wastewater. This has shown that the general perspective that the disinfectants, detergents and some antibiotic reagents used in our wash-ups in the hospitals do not provide enough treatment to the waste water generated.

Since these organisms may be vital to safety and wellbeing of patients who are hospitalized and individuals susceptible to infection among the health workers, I therefore recommend that the hospital management should get waste water tanks for waste water storage/treatment in the different departments, where the disinfectant in right proportion will be added and allow a contact time for the

disinfectant to act.

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