ANTIBIOTIC SUSCEPTIBILITY OF MICROORGANISMS ISOLATED FROM WASTEWATER AT AL-HIKMAH UNIVERSITY FEMALE HOSTELS, ILORIN, NIGEERIA

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ABSTRACT

Physicochemical properties and antibiotic susceptibility of microorganisms isolated from wastewater samples collected from Al-Hikmah University female hostels were assessed. Samples obtained from different female hostels were examined physiochemically and microbiologically using standard procedures. Phytochemical result revealed the low temperature value of 28°C from Oladimeji and Commaissie hostels, while Deremi and Owaish had a value of 29.5°C. Dissolved oxygen of 0.1 ml/L was recorded from all the samples collected. Electrical conductivity ranged from 658 to 997µs, while chocking odour was recorded in all the four samples collected. In terms of pH, Oladimeji hostel had a pH of 10.5, Deremi had a pH of 10.6, while Owaish and Commaissie had a pH of 10.4. The wastewater samples from the four hostels were highly contaminated. Oladimeji hostel showed a mean bacterial count of 25.4×10^5cfu/ml, Deremi hostel, Owaish hostel and Coomassie hostels, showed a mean bacterial count of 5.7×10^4cfu/ml,16.8×10^4cfu/ml and 3.5×10^4cfu/ml respectively. Percentage occurrence of bacteria isolates were *Salmonella* sp. (12.5%), *Klebsiella pneumoniae* (12.5%), *Escherichia coli* (25%), *Staphylococcus aureus* (12.5%), *Actinomyces* (12.5%), *Shigella* sp. (6.25%), *Pseudomonas aeruginosa* (12.5%) and *Proteus vulgaris* (6.25%). Antimicrobial sensitivity tests revealed that all the organisms were susceptible to gentamycin except *Actinomyces*. High microbial load in wastewater samples could have negative effects on human health. This study, further confirms the need to treat wastewater rather than directly discharging it to the environment.

Keywords: physiochemical, microbiological, sensitivity, domestic, wastewater, load
Accepted date: 6 June 2018

INTRODUCTION

Unhygienic practices, the preponderance of diarrhoea and other water related diseases among infants and children has continued to be a major health problems worldwide (Tortora *et al*., 2002). Land pollution caused by nitrogenous compounds leading to water body contamination can be attributed to wastewater from agricultural, domestic and industrial discharge (MPCA, 2008). Pollution is caused when a change in the physical, chemical and biological conditions in the environment harmfully affects quality of human life, including other animals' life and plant (Okoye *et al*., 2002).

Wastewater, which is the water spent by a community contains impurities added as a result of human's daily activities on water, since the major and primary means of waste disposal globally are through water. When a minute wastewater is added to water bodies, it alters the physical, chemical and biological nature of the receiving water bodies, and as a result the biological degradation becomes evident in terms of number and establishment of microbial pathogens in the water (Gray and Wang, 1999).In developing countries, numerous water related diseases result in an epidemic compared with developed nations, due to unhygienic practices, unawareness and ineffective legislator monitoring waste disposal (Okoh *et al*., 2007 and WHO, 2002).

Microbial pathogens through ingestion of faecal matter present in wastewater gain access to their new host and establish themselves within the host. Bacteria are the dominant microbial pathogens found in wastewater. Bacterial pathogens of enteric origin have predominantly been found in

wastewater. However, those of non-enteric origin, such as *Legionella* sp., *Mycobacterium* sp. and *Leptospira* sp. have been isolated also in wastewater (Fliermans, 1996). According to Grant *et al*. (1996), numerous gastrointestinal infections are caused by bacterial pathogens isolated from wastewater. They include *Vibrio cholera,* which causes cholera, Salmonellosis caused by *Salmonella* sp. and dysentery caused by some species of *Salmonella* and *Shigella*. Some strains of *Escherichia coli* have been linked with dysentery-like infections. *Salmonella typhi* which causes typhoid has been associated with foods supplied with wastewater (Bryan, 1977). Some opportunistic pathogens such as *Streptococcus, Aeromonas* and *flavobacterium* sp. have been isolated from wastewater (Ashbolt *et al*., 1995).

The viruses are by far the deadliest microbial pathogens found in wastewater. The upper respiratory infections, aseptic meningitis, poliomyelitis, conjunctivitis, and hepatitis are few among the diseases caused by enteroviruses (International Nomenclature of Diseases, 1983). Although, virtually all members of the population are susceptible to enteric virus infections, however, the immune-compromised individuals and small children have greater risk and higher infection rate from these viruses, especially from rotaviruses, being the most infectious of the enteric viruses (Gerba *et al*., 1996).

Before helminthes develops fully and becomes infectious to human, they need an intercessor host. The two most commonly isolated helminthes in wastewater are roundworm (*Ascaris lumbricoides*) and hookworm (*Ancylostoma duodenale*). According to Ellis *et al*. (1993), about 25% of the global population are infected with roundworm. The use of wastewater for irrigation has been the easiest way by which intestinal nematodes affect human with an endemic level of infection when the human faecal matter is used as fertilizer to grow vegetables(Udonsi*et al*., 1996and WHO,1995).*Entamoebahistolytica*, *Giardia intestinalis* and *Cryptosporidium parvum* are protozoans that are discovered more in wastewater contaminated with faecal matter than in any other environmental sources. The enteric pathogens, *Giardia intestinalis* and *Cryptosporidium parvum* have been discovered in many countries and are omnipresent in estuarine and fresh water (Ferguson *et al*., 1996). It is undoubtedly clear that wastewater creates a major environmental and public health hazard. Mosquitoes, house flies, rodents, and other vectors of communicable diseases use wastewater as their breeding sites. This research work aimed at analysing the wastewater generated from Al-Hikmah University female hostels, determining the physicochemical composition of the wastewater, isolating and identifying the microorganisms in the wastewater, and determining the susceptibility of the isolated microorganisms to standard drugs. By initiating this study, a better understanding of the typical susceptibility profile of microorganisms in wastewater can be obtained that will help improve the environment.

MATERIALS AND METHODS

Sample Collection

Wastewater samples were collected from the female hostels in Al-Hikmah University, Ilorin as described by (Onajobi *et al*., 2013). The wastewater samples were from Oladimeji, Owaish, Deremi and Coomaissie hostels. The samples were collected into four screw cap sterile containers which were labelled 1-4 appropriately, and were immediately taken to the laboratory for analysis.

Determination of physicochemical properties of the samples

The physicochemical properties of the samples were determined by measuring parameters such as temperature, pH, odour, colour, conductivity, total dissolved solid and dissolved oxygen(Onajobi*et al.,* 2015b).

Bacteria Isolation and Biochemical Characterization

Media used include Nutrient agar, MacConkey agar, *Salmonella Shigella* agar, Eosin Methylene Blue agar and MacConkey broth. All media were prepared in accordance to manufacturer's specifications. Three dilution factors were chosen and samples were aseptically inoculated onto agar media in duplicate using standard pour plate (Harrigan and McCane, 1976). It was then inverted and incubated at 37°C for 24 hours. Distinct

colonies were sub-cultured, stored and subjected to biochemical characterization. The bacterial isolates were characterized using morphological and biochemical characteristics described by (Onajobi *et al*., 2015a). Isolates were identified by comparing their characteristics with those known taxa prescribed by Jolt *et al*. (1994), Cheesbroough (2006), Oyeleke and Manga (2008), Adebayo-Tayo *et al*. (2012).

Antibiotic Sensitivity of Bacterial Isolates

The standard disc diffusion method described by Beathy *et al.*(2004) was used. The bacterial cell suspension was prepared at 1 x 10^8cfu/ml following McFarland of 0.5 turbidity standard. The susceptibility test was carried out in line with Onajobi *et al.* (2015b) using streptomycin gentamycin, augumentin, sparfloxacin, tofloxacin, chloramphenicol, amoxillin, cotimoxazole, ciprofloxacin, pefloxacin, erythromycin and ampicloxdiscs.

RESULTS

The physicochemical results of the wastewater samples analyzed are presented in Table 1. It showed that the temperature of samples from both Oladimeji and Coomaissie hostels had the same value of 28^{o}C, while Deremiand Owaish hostels were higher with 29.0^{o}C and 29.5^{o}C respectively. The hydrogen ion concentration, pH is an important quality parameter of wastewater. The pH of the wastewater samples was measured to be 10.4 to 10.6 (Table 1). The brownish colour of the waste water samples from Oladimeji and Commaisse indicate high turbidity. Conductivity values increase with increase in temperature. The total bacterial and coliform count of the wastewater samples of the four hostels is presented in table 2. The total bacterial and coliform counts of the samples ranged from 0.5×10^8 cfu/ml to 25.4×10^5cfu/ml and 0.2×10^8 cfu/ml to 9.7×10^5cfu/ml respectively. These figures indicate a very high microbial load. The MPN result of the wastewater samples is shown in table 3 with faecal coliform ranging from 350 to >2400.Frequency of occurrence revealed that *Escherichia coli* was the highest (25.0%), while *Klebsiella pneumonia*and *Proteus vulgaris* had lowest percentage occurrence of 6.5% each (Table 4).

The antibacterial sensitivity result of bacterial isolates is shown in table 5. *Actinomyces* sp. was resistant to gentamycin, pefloxacin, erythromycin, cotrimoxazole and ampiclox while *Pseudomonas aeruginosa* was resistant to chloramphenicol, cotimoxazole, ciprofloxacin and pefloxacin. *Staphylococcus aureus* was resistant to erythromycin, septrin and ampiclox. *Escherichia coli* was resistant to gentamycin and streptomycin while *Salmonella* sp. and *Proteus vulgaris* were resistant to gentamycin and streptomycin respectively. *Klebsiella pneumonia* was susceptible to all antibiotics used (gentamycin, augumentin, pefloxacin, of loxacin and cotimoxazole).

Table 1: Physiochemical Properties of wastewater samples

Parameters	Oladimeji	Deremi	Owaish	Commaisse
Temperature^{0}C	28	29	29.5	28
pH	10.5	10.6	10.4	28
Odour	Choking	Choking	Choking	Choking
Colour	Brown	Cream	Cream	Brown
DissolvedOxygen (mg/l)	0.1	0.1	0.1	0.1
Conductivity (µs)	847	997	996	658
TDS (ppm)	424	498	498	329

Abbreviations: TDS- Total Dissolved Solids.

Table 2: Microbial enumeration of wastewater samples (cfu/ml)

Sample	Heterotrophic count	*E. coli* Count	*Salmonella Shigella* count	total coliform	feacal coliform count
OL	25.4×10^5	$6.2.4\times10^4$	5.7×10^4	9.7×10^4	8.7×10^4
	6.4×10^6	0.5×10^6	2.4×10^6	0.2×10^6	1.9×10^6
	0.5×10^8	NG	NG	NG	NG
	5.7×10^4	3.3×10^4	3.7×10^4	5.7×10^4	5.7×10^4
DR	2.2×10^6	0.7×10^6	0.2×10^6	0.6×10^6	0.7×10^8
OW	0.8×10^6	0.2×10^6	0.5×10^6	2.4×10^6	0.7×10^6
	$0.7\times10_8$	NG	0.4×10^8	0.1×10^8	NG
COM	3.5×10^2	2.5×10^4	2.8×10^4	0.7×10^4	NG
	0.7×10^8	1.1×10^6	1.1×10^6	0.4×10^6	NG
	0.5×10^6	0.7×10^8	0.4×10^8	0.4×10^8	NG

COM- Commaissie, OL- Oladimeji, DR- Deremi, OW- Oweaish, NG-No growth

Table 3: Most Probable Number for wastewater samples

S/N	Sample Code	Double Strength 5 of 10ml	Single Strength 5 of 1.0ml	Single strength 5 of 0.1ml	MPN Index /100ml
1	OL WW	5/5	5/5	4/5	1600
2	DR WW	5/5	4/5	4/5	350
3	OW WW	5/5	5/5	5/5	=2,400
4	COM WW	5/5	5/5	5/5	=2,400

COM- Commaissie OL- Oladimeji, DR- Deremi OW- Oweaish WW- wastewater

Table 4: Frequency of occurrence of isolated organisms from wastewater samples

Isolates	Number of occurrences	Percentage of occurrence
Salmonella sp.	2	12.5
Klebsiella pneumonia	1	6.5
Escherichia coli	4	25.0
*Shigella*sp.	2	12.5
*Actinomyces*sp.	2	12.5
Staphylococcus aureus	2	12.5
Pseudomonas aeruginosa	2	12.5
Proteus vulgaris	1	6.5
Total	**16**	**100**

Table 5: Antibacterial sensitivity to standard drugs

Isolates	Antibiotics used	Zone of inhibition	Susceptibility
Escherichia coli	Streptomycin	No inhibition	Susceptible
	Gentamycin	30	Susceptible
	Augmentin	No inhibition	Resistant
	Sparfloxacin	24	Susceptible
	Travid	22	Susceptible
Pseudomonas aeruginosa	Chloramphenicol	No inhibition	Resistant
	Amoxicillin	17	Susceptible
	Septrin	No inhibition	Resistant
	Ciprofloxacin	No inhibition	Resistant
	Perfloxacin	No inhibition	Resistant
Staphylococcus aureus	Gentamycin	25	Susceptible
	Perfloxacin	24	Susceptible
	Erythromycin	No inhibition	Resistant
	Septrin	No inhibition	Resistant
	Ampiclox	No inhibition	Resistant
Klebsiella pneumonia	Perfloxacin	30	Susceptible
	Gentamycin	26	Susceptible
	Septrin	15	Susceptible
	Augmentin	26	Susceptible
	Travid	14	Susceptible
Salmonella spp.	Perfloxacin	24	Susceptible
	Gentamycin	No inhibition	Resistant
	Septrin	15	Susceptible
	Augmentin	23	Susceptible
	Travid	20	Resistant
*Actinomyces*sp.	Gentamycin	No inhibition	Resistant
	Perfloxacin	No inhibition	Resistant
	Erithromycin	No inhibition	Resistant
	Septrin	No inhibition	Resistant
	Ampiclox	No Inhibition	Resistant
Proteus vulgaris	Streptomycin	No inhibition	Resistant
	Gentamycin	22	Susceptible
	Sparfloxacin	21	Susceptible

DISCUSSION and CONCLUSION

The high temperature range recorded may be due to the chemical and biochemical reactions in the ground water (Onajobi *et al*., 2016). The choking odour was likely to be as a result of anaerobic reactions and this could be due to the high amount of dissolved solids in the wastewater (Onajobi *et al*., 2013). Going by international standard, any water contaminated to this level is neither good for domestic use, nor is it supposed to be discharged directly into the environment without treatment (Adeyemi *et al*., 2007). Many of the isolated bacteria from wastewater samples are known pathogenic microorganisms that are potential causative agents of various infectious diseases. These include enterobacteria. Some of the enterobacteria isolated were *Salmonella* sp., *Klebsiella pneumonia, Escherichia coli* and *Shigella* sp. The occurrence of these microorganisms may be as a result of the nature of the soil, and poor hygiene practices by the students. This is in accordance with Chapelle (1992) that Gram negative pathogenic bacteria are extensively found in underground water system where they constitute about 6% to 7% of the isolates recovered.

Actinomyces sp., *Staphylococcus aureus* and *Pseudomonas aeruginosa* were among the multiple resistant microorganisms isolated in this study. These findings conform to Onajobi *et al*.(2015a) that stressed the alarming rate of highly diverse antibiotics resistance by *Pseudomonas aeruginosa.* This is also comparable with the multi drug resistance reported by Aiyegoro *et al*.(2007) and Okonko *et al*. (2009).The sensitivity of *Klebsiella pneumonia* to gentamycin, perfloxacin, augumentin and ofloxacin in this study isin agreement with the previous reports of Reish *et al*. (1993) and Nkang *et al*. (2009).

The antibiotic resistant organisms from wastewater that contaminate water sources in the environment can cause infections among the human populace. This is the major health problems in the study areas. Today, the age long battle against antibiotic resistance in Africa is far from being won (Onajobi *et al*., 2015a)

The results obtained may be significant for Nigeria and other countries where large volumes of wastewater are generated from higher institutions with very few Universities efficiently monitoring their wastes. These wastes end up in the environment, most especially in water bodies. The result of this study shows that wastewater contains pathogenic microorganisms and therefore needs to be treated properly before disposal. Treatment of wastewater will help to reduce the pathogens and eliminate offensive odours so as to create a good and healthy environment. Therefore, proper disposal of waste water should be enforced.

Acknowledgments:

The authors gratefully acknowledge the support of Mrs. Jimoh Hamza, the Chief Technologist, Microbiology Laboratory, Al-Hikmah University, Ilorin, Nigeria.

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