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Isolation and Identification of Microorganisms from Domestic Effluent in Michael Okpara University of Agriculture Umudike, Abia State, Nigeria

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Abstract
The isolation and identification of the microorganisms from domestic effluent in Michael Okpara University of Agriculture Umudike were carried out. The mean total aerobic heterotrophic plate count ranged from 6.10 ± 0.01Log_{10}cfu/mL to 6.29 ± 0.09Log_{10}cfu/mL, *Salmonella* count ranged from 2.63 ± 0.14Log_{10}cfu/mL to 2.93 ± 0.16Log_{10}cfu/mL, *Escherichia coli* count ranged from 3.63 ± 0.14Log_{10}cfu/mL to 3.93 ± 0.20Log_{10}cfu/mL, *Vibrio cholerae* ranged from 1.54 ± 0.10Log_{10}cfu/mL to 1.87 ± 0.20Log_{10}cfu/mL and fungal count ranged from 3.48 ± 0.33Log_{10}cfu/mL to 3.88 ± 0.07Log_{10}cfu/mL while the coliform count ranged from 35 ± 2.0MPN/100mL to 160 ± 5.0MPN/100mL. The microorganisms isolated and their percentage occurrence were *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* species, *Pseudomonas* aeruginosa, *Enterobacter* species, *Klebsiella* species, *Vibrio cholerae Aspergillus* species, *Rhizopus* species, *Penicillium* species, and *Trichoderma* species. The results showed that the effluent samples were heavily contaminated with known pathogenic microorganisms and should therefore be treated before discharge.
Keywords: contaminated, pathogenic, heterotrophic, effluents, domestic, identification, microorganisms, Umudike

1.0 Introduction

Effluent is a liquid waste flowing out of a factory, farm, commercial establishment or a household into a water body such as a river, lake or lagoon or a sewer system or reservoiur. Waste discharged into air is called emission (Kristen, 2009). Effluents may also be referred to as sewage. Effluent discharges from domestic, municipal, industrial and agricultural set-ups contain various pollutants such as debris, microorganisms and heavy metals and are transported in untreated forms through drains, water ways and soils into inland water-bodies (Anderson *et al*., 2000; Uzor, 2001; Eze and Korie, 2012). The receiving water-bodies loads of pollutants are increased during the rainy season due to water run-off and atmospheric precipitation.

Domestic wastes are a consequence of housekeeping activities such as food preparation, sweeping and vacuum cleaning. They also contain fuel residue, empty containers and packaging wastes from repairs and redecorating. Microorganisms in domestic effluents use the waste constituents as nutrients, thus detoxifying the materials as their digestive processes breakdown complex organic molecules into simpler less toxic molecules (Al-A'ama *et al*., 1995). A waste is hazardous if it is infectious, meaning containing viable microorganisms or their toxins, which are known or suspected to cause disease in animal or human (Abbaszadegan *et al.,* 1997a).

Therefore, whenever untreated domestic effluent is dumped into water bodies, it builds up certain pathogenic organisms and when the water is consumed, it causes major diseases such as dysentery, diarrhoea and other gastroenteritis caused by coliform and other related species of bacteria. The pollution of land and water environments with untreated domestic effluent can affect the inhabitant of the area (Abbaszadegan *et al.,* 1997b).

The aim of the study is to isolate and identify microorganisms from the domestic effluent samples in Michael Okpara University of Agriculture Umudike

2.0 Materials and Methods

2.1 Sample Collection

The domestic effluent samples were collected from four different sites in Michael Okpara University of Agriculture, Umudike. The sites were PG hostel, female hostel, school restaurant and male hostel. The samples were collected using clean sterile one litre plastic containers. They were transported in ice packed cooler to the Microbiology Laboratory of the institution and analysis was immediately carried out.

2.2 Chemical Reagents

The chemical reagents employed in the study were of analytical grade and were products of BDH Chemicals, Poole's England and Sigma Chemical Company St. Louis Missouri, USA. The microbiological media used were products of Oxoid and DIFCO Laboratories, England. They included nutrient agar used for the estimation of total heterotrophic aerobic bacteria, purification of isolates and for stock culture; Potato dextrose agar used for

the isolation of fungi; MacConkey broth for the isolation of coliforms; *Salmonella-Shigella* agar, eosin methylene blue agar and thiosulphate citrate bile salt sucrose agar for the isolation of *Salmonella*, *Escherichia coli* and *Vibrio cholerae* respectively

2.3 Enumeration of Total Heterotrophic Bacteria and Fungi

Samples of the domestic effluents were serially diluted in ten folds. Total viable heterotrophic aerobic counts were determined using pour plate technique. Then the molten nutrient agar, MacConkey, potato dextrose agar, *Salmonella-Shigella* agar, eosin methylene blue agar and thiosulphate citrate bile salt sucrose agar at 45^0C were poured into the Petri dishes containing 1mL of the appropriate dilution for the isolation of the total heterotrophic bacteria and fungi and coliforms respectively. They were swirled to mix and colony counts were taken after incubating the plates at 30^oC for 48h and preserved by sub culturing the bacterial isolates into nutrient agar slants which were used for biochemical tests.

2.4 Identification of Isolates

Bacteria isolates were characterized and identified after studying the Gram reaction as well as cell micro morphology. Other tests performed were spore formation, motility, oxidase and catalase production; citrate utilization, oxidative/fermentation (O/F) utilization of glucose; indole and coagulase production, starch hydrolysis, sugar fermentation, methyl red-Voges Proskauer reaction and urease production. The tests were performed according to the methods of (Cheesbrough, 2005; Adeoye, 2007; Ochei and Kolhatkar, 2007). Microbial identification was performed using the keys provided in the *Bergey's Manual of Determinative Bacteriology* (1994). Fungal isolates were examined microscopically using the needle mouth technique. Their identification was performed according to the scheme of Barnett and Hunter (1972) and Larone (1986).

3.0 Results

The Table 1 shows the mean counts of the microorganisms from the effluent samples. The total heterotrophic bacterial plate count ranged from $6.10 \pm 0.01 Log_{10}$cfu/mL to $6.29 \pm 0.09 Log_{10}$cfu/mL. The sample from female hostel recorded the highest mean count of $6.29 \pm 0.09 Log_{10}$cfu/mL and sample from postgraduate hostel had the least mean count of $6.10 \pm 0.01 Log_{10}$cfu/mL. The *Salmonella Shigella* count ranged from $2.63 \pm 0.14 Log_{10}$cfu/mL to $2.93 \pm 0.16 Log_{10}$cfu/mL. The sample from male hostel recorded the highest count of $2.93 \pm 0.16 Log_{10}$cfu/mL while school restaurant had the lowest count of $2.63 \pm 0.14 Log_{10}$cfu/mL. The *Vibrio* cholerae count ranged from $1.54 \pm 0.10 Log_{10}$cfu/mL to $1.87 \pm 0.20 Log_{10}$cfu/mL. The sample from male hostel had the highest count of $1.87 \pm 0.20 Log_{10}$cfu/mL and sample from school restaurant recorded the least count of $1.54 \pm 0.10 Log_{10}$cfu/mL. The *Escherichia coli* count ranged from $3.63 \pm 0.14 Log_{10}$cfu/mL to $3.93 \pm 0.20 Log_{10}$cfu/mL. The sample from female hostel had the highest count of $3.93 \pm 0.20 Log_{10}$cfu/mL and that of school restaurant recorded the least count of $3.63 \pm 0.14 Log_{10}$cfu/mL. The fungal count ranged from $3.48 \pm 0.33 Log_{10}$cfu/mL to $3.88 \pm 0.07 Log_{10}$cfu/mL. The highest count of $3.88 \pm 0.07 Log_{10}$cfu/mL was recorded by the female hostel while the school restaurant least count of $3.48 \pm 0.33 Log_{10}$cfu/mL. The coliform count ranged from 35 ± 2.0MPN/100mL to 160 ± 5.0MPN/100mL. The male hostel recorded the highest count of 160 ± 5.0MPN/100mL and postgraduate hostel had the least count of 35 ± 2.0MPN/100mL. The ANOVA, $P < 0.05$ showed that there was significant difference in the mean microbial counts in the bacterial groups among the locations.

Table 2 shows the microorganisms isolated from the effluent samples and their percentage occurrence. The bacteria isolated were *Escherichia coli*, 57 (20.21%), *Staphylococcus aureus* 34(12.06%), *Pseudomonas aeruginosa* 58(20.57%), *Enterobacter* species 29(10.28%), *Klebsiella* species 42(14.89%), *Vibrio cholerae* 25(8.87%) and *Salmonella* species, 37(13.12%). *Pseudomonas aeruginosa* had the highest percentage occurrence of 20.57% while *Vibrio cholerae* had the lowest percentage occurrence of 8.87%. The fungi isolated were *Aspergillus* species, 6(20.7%), *Rhizopus* species, 8(27.6%), *Trichoderma* species, 11(37.9%) and *Penicillium* species, 4(13.8%). *Trichoderma* species had the highest occurrence of 37.9% and *Penicillium* species recorded the least occurrence of 13.8%.

4.0 Discussion

Domestic effluents are good sources of microorganisms which may be due to the nature of human activities generating the effluents. The increased microbial load observed may be attributed to the presence of organic and inorganic component of the waste, which could act as source of nutrients to the microorganisms contributing in their multiplication (Prescott *et al.,* 2008).

The bacterial genera isolated were *Escherichia coli*, *Klebsiella* species, *Pseudomonas* species, *Staphylococcus aureus*, *Enterobacter* species, *Vibrio cholerae* and *Salmonella* species. The presence of *Staphylococcus aureus* could be as a result of contamination from the normal flora of the generators of the effluent and because it survives for prolonged periods in the environment, it is readily present in effluent. The

presence *Escherichia coli*, *Enterobacter* species, *Vibrio cholerae* and *Salmonella* species show faecal contamination of the domestic effluents. *Escherichia coli* is also a part of the normal intestinal flora and might enter the effluent through other sources as it is not specifically confined to the human intestine. It is also present in the faeces of many domestic animals and birds and can be a source of contamination of the effluents (Amanchukwu, 1991; Eze and Okpokwasili, 2008). These organisms are transmitted through water and food and can cause illness which may have symptoms such as severe abdominal pain, fever, profuse bloody diarrhoea and mild intestinal upset. The bacteria isolated are in line with the work of Ekundayo (1997).

The fungi isolated were *Aspergillus* species, *Penicillium* species, *Rhizopus* species and *Trichoderma* species. These fungi are spore formers and therefore can survive unfavourable conditions than the non spore forming bacteria. This makes them to be more persistent in the environment and also resulted in the increase in their counts

(Madsen, 2006; Eze *et al*., 2011). Domestic effluents should be properly treated before disposal in order to avoid the release of the spores of these organisms so as to avoid the spread of diseases such as aspergillosis, anthrax, gastroenteritis and food poisoning (Piet, 2009).

5.0 Conclusion

The study has shown that domestic effluent contains microorganisms of public health importance. It therefore becomes necessary the effluent should be treated properly before discharge into the environment to avoid contacting the diseases caused by these organisms as a result of contamination of water bodies and the soil environment.

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Table 1: The mean counts of microorganisms isolated from the effluents

	Log10cfu/mL					
Location	THBPC	SC	VC	EC	FC	CC (MPN/100mL)
Postgraduate Hostel	6.10 ± 0.01	2.70 ± 0.07	1.87 ± 0.20	3.64 ± 0.10	3.81 ± 0.12	35 ± 1.0
Female Hostel	6.29 ± 0.09	2.81 ± 0.05	1.64 ± 0.03	3.93 ± 0.20	3.88 ± 0.07	50 ± 2.0
School Restaurant	6.10 ± 0.01	2.63 ± 0.14	1.54 ± 0.10	3.63 ± 0.13	3.60 ± 0.21	90 ± 3.0
Male Hostel	6.13 ± 0.02	2.93 ± 0.16	1.63 ± 0.04	3.85 ± 0.09	3.48 ± 0.33	160 ± 5.0

Legend: THBC = Total heterotrophic bacterial count; SSC = *Salmonella-Shigella* count; VC = *Vibrio cholerae* count; EC = *Escherichia coli* count; FC = Fungal Count; CC= Coliform count

Table 2: Microorganisms isolated from the effluents and their percentage occurrence

Microorganisms	Number of isolates	% Occurrence
Bacteria		
Escherichia coli	57	20.21
Staphylococcus aureus	34	12.06
Pseudomonas aeruginosa	58	20.57
Enterobacter species	29	10.28
Klebsiella species	42	14.89
Vibrio cholerae	25	8.87
Salmonella species	37	13.12
Fungi		
Aspergillus species	6	20.7
Rhizopus species	8	27.6
Penicillium species	4	13.8
Trichoderma species	11	37.9

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