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RESEARCH ARTICLE

ENUMERATION AND CHARACTERIZATION OF MICROORGANISMS FROM LABORATORY EFFLUENTS IN MADONNA UNIVERSITY LABORATORIES, ELELE CAMPUS, RIVERS STATE, NGERIA

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ABSTRACT

The enumeration and characterization of microorganisms from laboratory effluents in Madonna University laboratories were carried out. The total aerobic plate count, coliform count, *Escherichia coli* count and fungal count were investigated using pour plate technique in nutrient agar, MacConkey agar, eosine methylene blue agar and Sabouraud dextrose agar respectively. The statistical analysis used ANOVA. The mean total aerobic plate count for the treated and untreated effluents ranged from $0Log_{10}$cfu/mL to $4.47 \pm 0.01Log_{10}$cfu/mL and $6.97 \pm 0.10Log_{10}$cfu/mL to $7.21 \pm 0.20Log_{10}$cfu/mL respectively. The fungal count ranged from 0 Log_{10}cfu/mL to $3.82 \pm 0.17Log_{10}$cfu/mL and $5.98 \pm 0.10Log_{10}$cfu/mL to $6.57 \pm 0.14Log_{10}$cfu/mL respectively. The mean coliform and *Escherichia coli* counts for the untreated effluents ranged from $6.82 \pm 0.14Log_{10}$cfu/mL to $7.09 \pm 0.10Log_{10}$cfu/mL and $0Log_{10}$cfu/mL to $2.2 \pm 1.24Log_{10}$cfu/mL respectively. There was no coliform and *Escherichia coli* counts for the treated effluents. Bacterial genera isolated were *Escherichia coli*, *Klebsiella* species, *Staphylococcus aureus*, *Streptococcus* species, *Micrococcus* species and *Pseudomonas* species. The fungal genera isolated were *Penicillum* species, *Aspergillus* species, *Rhizopus* species and *Yeast* species. High microbial counts observed in the untreated effluents when compared with the treated effluents showed the need for proper treatment of laboratory effluents before disposal.

Key words: Enumeration, microorganisms, characterization, laboratory, effluents.

INTRODUCTION

A laboratory is a room or building used for scientific research, experiments and testing (Hornby, 2000). In carrying out research, experiments and testing, materials used are washed, sterilized and disinfected. Washing with plain soaps generally does not destroy organisms. It simply aids in the mechanical removal of transient microbes, including most pathogens, as well as dirt, organic material and cells of the outermost layer of skin. However, washing and scrubbing with detergents and disinfectants achieves routine control of undesirable microorganisms and viruses (Nester *et al*., 2001). The water generated after thorough washing of laboratory materials and scrubbing of the floor in the laboratory is called laboratory effluent. Effluents are wastewater draining out of homes, septic tanks, industries/factories. Effluents may also be referred to as sewage. Effluents usually contain wide varieties of chemicals, debris and various microorganisms and are usually carried away through special underground pipes called sewers. The growth in the economic activities in under developed and developed nations has led to the deposition of industrial and domestic effluents in water sources (Uzor, 2001).

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Laboratory effluents are of concern because of the pollutants they contain. These include disease casing pathogens which can make the water unfit for drinking and contaminate fish; chemicals, which may either be acutely or chronically toxic to aquatic organisms and poses a health risk hazard to human damaging organic wastes which use up the water's dissolved oxygen and threaten the survival of fish and other aquatic plants giving rise to eutrophication, odour and some case, contamination of shellfish; grit, debris and suspended solids which can discolour the water making it unfit for recreational , domestic and industrial uses and eventually smother and contaminate plant and animal life at the bottom of the receiving water body (APHA, 2005). Treatment of effluent before disposal into water and soil environment tends to reduce the quantities of these pollutants to acceptable limit of discharge into the environment (Talaro and Tararo, 2004). The aim of the work is to animate and identity the microorganism present in the laboratory effluents in Madonna University Laboratories.

MATERIALS AND METHODS

Effluents samples from different laboratories in Madonna University Elele Campus were collected in sterile bottles. The laboratories were microbiology clinical laboratory, heamatology clinical laboratory, chemical pathology clinical

laboratory, biochemistry department laboratory and microbiology department laboratory. A total of thirty effluent samples which comprised fifteen samples each from effluents treated with disinfectants and untreated effluent were collected. They were transported to the laboratory in an ice packed cooler and immediately analyzed on reaching the laboratory.

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; Sabouraud dextrose agar used for the isolation of fungi and MacConkey broth for the isolation of coliforms.

Enumeration of Total Heterotrophic Bacteria and Fungi

Samples of the sawdust wastes were serially diluted in ten folds. Total viable heterotrophic aerobic counts were determined using pour plate technique. Then the molten nutrient agar, MacConkey and Sabouraud dextrose 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 room temperature for 48h and preserved by sub culturing into nutrient agar slants which were used for biochemical tests.

Characterization and 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 Proskaur reaction and urease production. The tests were performed according to the methods of (Cheesbrough, 2005; Adeoye, 2007; Agwung-Fobellah and Kemajou, 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).

RESULTS

Table 1 shows the mean counts of microorganisms isolated from the treated laboratory effluents. The total aerobic plate court ranged from O Log_{10}cfu/mL to 4.47 $\pm$ 0.01 Log_{10}cfu/mL while the fungal count ranged from O Log_{10}cfu/mL to 3.50 $\pm$ 0.28 Log_{10}cfu/mL. The total aerobic plate count was recorded in only the Microbiology Department Laboratory while the fungal count was recorded in the Microbiology clinical and Departmental Laboratories. The ANOVA, $P < 0.05$ showed that there was significant difference in their mean counts.

Table 1: Mean Counts of Microorganisms isolated from Treated Laboratory Effluents

	Log_{10}cfu/mL			
Laboratory	TAPC	CC	EC	FC
MCL	0	0	0	3.50 $\pm$ 0.28
HCL	0	0	0	0
MDL	4.47 $\pm$ 0.01	0	0	3.82 $\pm$ 0.117
CPCL	0	0	0	0
BDL	0	0	0	0

Legend: MCL = Microbiology Clinical Laboratory, HCL = Haematology Clinical Laboratory, MDL = Microbiology Department Laboratory; CPCL = Chemical Pathology Clinical Laboratory; BDL = Biochemistry Department Laboratory, TAPC = total aerobic plate count; CC = Coliform count. EC = *Escherichia coli* count and FC = Fungal count.

Table 2 shows the mean counts of microorganisms isolated from the untreated Laboratory effluents. The total aerobic plate count ranged from 6.97 $\pm$ 0.10Log_{10}cfu/mL to 7.21 $\pm$ 0.20 Log_{10}cfu/mL while the coliform count ranged from 6.09 $\pm$ 0.16 Log_{10}cfu/mL to 6.94 $\pm$ 0.28 Log_{10}cfu/mL.The *Escherichia coli* count ranged from 0 Log_{10}cfu/mL to 2.20 $\pm$ 1.26 Log_{10}cfu/mL. Fungal count ranged from 6. 98 $\pm$ 0.10 Log_{10}cfu/mL to 6.10 $\pm$ 0.23 Log_{10}cfu/mL. The ANOVA, $P > 0.05$ showed that there was no significant difference in their mean counts.

Table 2: Mean counts of Microorganisms isolated from the untreated Laboratory Effluents

Laboratory	Log_{10}cfu/mL			
	TAPC	CC	EC	FC
MCL	7.07 $\pm$ 0.39	6.90 $\pm$ 0.14	0	6.11 $\pm$ 0.14
HCL	7.08 $\pm$ 0.12	6.94 $\pm$ 02.8	0	6.10 $\pm$ 0.23
MDL	7.21 $\pm$ 0.20	7.09 $\pm$ 0.16	2.2 $\pm$ 0.26	6.49 $\pm$ 0.24
CRCL	7.03 $\pm$ 0.20	6.94 $\pm$ 0.10	0	6.57 $\pm$ 0.14
BCL	6.97 $\pm$ 0.10	6.82 $\pm$ 0.14	0	5.98 $\pm$ 0.10

Legend: MCL = Microbiology Chemical Laboratory, HCL = Heamatology Chemical laboratory, MDL = Microbiology Department Laboratory; CPCL = Chemical Pathology Chemical Laboratory; BDL = Biochemistry Department Laboratory, TAPC = total aerobic plate court; CC = Coliform count. EC = *Escherichia coli* count and FC = Fungal count.

Table 3 shows the microorganisms isolated from the various laboratories and their percentage occurrence. The chi square analysis showed that *Staphylococcus aureus* (42%) was significantly the most frequently isolated bacteria while *Micrococcus* species and *Escherichia coli* (3.5%) were significantly the least isolated bacteria. Other bacteria isolated were *Klebsiella* species (24.6%) and *Streptococcus* species (26.3%). The fungal genera isolated included *Penicillium* species (5.2%), *Aspergillus* species (15.8%), *Rhizopus* species (13.2%) and *Yeast* (65.8%). The *Yeast* species had the highest occurrence of 65.8% while the *Penicillium* species had the least occurrence of 5.2%

DISCUSSION

Laboratory effluents are good sources of microorganisms which may be due to the nature of activities that go on in the laboratory. Different microorganisms are constantly isolated from clinical specimen such blood vials, urine and stool

specimen from patients while others may be from the normal flora of laboratory users, laboratory equipment and benches, old cultures and isolates, which are washed up and the effluent emptied into the laboratory sink (Uzor, 2001; Antonine and Jean-Pierre, 2002). These resulted in high mean bacterial and fungal counts in the samples from the laboratories. The results obtained showed that the treated effluents had lower microbial counts than the untreated effluents. This can be attributed to the fact that disinfectants such as hypochlorite were used in the washing of laboratory equipment and other materials in the laboratories that generated the effluents. However, the result also showed that despite the use of disinfectants, some microorganisms were able to survive. This could be as a result of the number and location of the microorganisms, concentration and potency of the disinfectants and also the duration of exposure to disinfectants. Disinfectant can either be bactericidal or bacteria-static (Rice *et al*., 1999).

setting where they are a common cause of infection. They are a common cause of hospital-acquired pneumonia and infections of urinary tract and burn wounds (Brashaw, 2000; Nester *et al*., 2001, Eze and Okpokwasili, 2008; 2010).

The fungal genera isolated were *Aspergillus* species, *Penicillium* species, *Rhizopus* species and *Yeast* species. These fungi form spores which make them to survive unfavourable conditions than the non spore forming bacteria thereby making them to be more persistent in the environment and also resulting in the increase in their counts (Madsen, 2006; Eze *et al*., 2011). Most of these microorganisms isolated are pathogenic. It is therefore very necessary that laboratory effluents are properly disposed and treated to avoid the release of spores or organisms thus preventing the spread of diseases such as aspergillosis, anthrax and food poisoning and gastroenteritis (Piet, 2009). The laboratory users should therefore ensure that proper

Table 3: Microorganisms Isolated from the untreated Laboratory effluents and their percentage occurrence

Microorganism	MCL	HCL	MDL	CPCL	BDL	TNI	%occurrence
Bacteria							
Escherichia coli	0(0%)	0(0%)	2(100%)	0(0%)	0(0%)	2	3.4
Klebsiella species	5(35.7%)	0(0%)	4(28.6%)	3(21.4%)	2(14.3%)	14	24.1
Staphylococcus aureus	5(20.8%	4(16.7%)	5(20.8%)	5(20.8%)5	5(20.8%)	24	41.4
Micrococcus species	1(50%)	0(0%)	1(50%)	0(0%)	0(0%)	2	3.4
Pseudomonas species	3(18.8%)	2(12.5%)	4(25%)	3(18.8%)	4(25%)	16	27.6
Fungi							
Penicillium species	1(50%)	0(0%)	1(50%)	0(0%)	0(0%)	2	5.2
Aspergillus species	0(0%)	2(33.3%)	2(33.3%)	1(16.7%)	1(16.7%)	6	15.8
Rhizopus species	1(20%)	0(0%)	3(60%)	0(0%)	1(20%)	5	13.2
Yeast	5(20%)	5(20%)	5(20%)	5(20%)	5(20%)	25	65.8

Legend: Legend: MCL = Microbiology Chemical Laboratory, HCL = Heamatology Chemical laboratory, MDL = Microbiology Department Laboratory; CPCL = Chemical Pathology Chemical Laboratory; BDL = Biochemistry Department Laboratory and TNI = total number of isolates

The untreated laboratory effluents on the other hand, showed higher counts of microorganisms. This is as a result of the absence of any form of treatment to reduce their population. The genera of bacteria isolated were *Escherichia coli*, *Klebsiella* species, *Pseudomonas* species, *Staphylococcus aureus*, *Streptococcus* species and *Micrococcus* species. The presence of *Staphylococcus aureus* could be as a result of contamination from the normal flora of the laboratory users. It has been observed that many people including health care personnel are carriers of this organism. Because it survives for prolonged periods in the environment, it is readily transmissible on formites. It is a common cause of nosocomial pneumonia and surgical site infections. Hospital strains are often resistant to a variety of antimicrobial drugs. *Escherichia coli* though with the lowest occurrence signify faecal contamination of the laboratory effluents but to minimal level. It is also a part of the normal intestinal flora. The faecal materials might enter the effluent through other sources as *Escherichia coli* is not specifically confined to the human intestine. It is also present in the faeces of many domestic animals and birds and can be source of contamination of the effluents. *Pseudomonas* species can grow readily in many moist nutrient poor environments such as the water in the humidifier in a mechanical ventilator. They are not only resistant to many disinfectants and antimicrobial drugs but in some cases can actually grow in them. *Pseudomonas* species are of important in a hospital sanitary measures as well as safety procedures are observed while working in the laboratory. It has been shown that the microbial counts were higher in the untreated laboratory effluents when compared with the treated ones. This emphasizes the need for proper treatment of laboratory effluents before disposal in order to reduce the chances of contamination of the environment and laboratory personnel.

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