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Evaluation of the Biosafety of Domestic and Restaurant Wastewater and the Antibiotic Sensitivity of Microorganisms associated with them

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ABSTRACT: *Biosafety and antibiotic sensitivity ofmicroorganisms obtainedfrom domestic and restaurant wastewater was assessed. Wastewater samples were collected from different residential home and restaurant facility within Port Harcourt Metropolis. The tyndallization test, total and faecal coliform counts and sensitivity to antibiotics were done using conventional methods. The total heterotrophic count for restaurant and domestic wastewater samples ranged from $1.06x10^6$cfu/ml to $1.19x10^6$cfu/ml and $1.0x10^4$cfu/ml to $1.2x10^4$cfu/ml for bacteria and fungi respectively. The coliform analysis revealed that fecal and total coliform had an MPN-index of $2.4x10^3$cfu/100ml. The microflora obtained from the restaurant wastewater are Corynebacterium*sp., *Hafnia*sp., *Klebsiella*sp., *Staphylococcus* sp., *Bacillus* sp., *and Pseudomonas* sp., *while the domestic samples contained Micrococcus* sp., *Aerococcus*sp., *Acinetobacter*sp., *Providencia*sp. *and Tatumella*sp. *Dominant fungal isolates were Penicillium*sp., *Mucor*sp., *Rhizopus*sp. *and Aspergillus*sp. *Highest percentage occurrence was observed for Corynebacterium*sp. *(30.77%) while the lowest was observed from Morganella*sp. *and Aerococcus (1.92%). Biosafety evaluation saw a reduction from 120cfu/ml to 0cfu/ml on the third day. The isolates showed various level of resistance to Pefloxacin (50%), Ciprofloxacin (50%), Gentamycin (50%), Amplicox (50%), Septrin (50%), Streptomycin (40%), Penicillin (40%), Ceporex (30%), Ofloxacin (20%) and Nalidixic Acid (20%). The level of resistance to Pefloxacin by Acinetobacter* sp. *and Bacillus* sp. *isolated from domestic and restaurant wastewater was 20.5 and 20 respectively. Bacillus* sp. *from the domestic wastewater sample has 17.5% to gentamycin and Nalidixic acid. While 0.0% of Corynebacterium* sp. *and Klebsiella* sp. *isolated from restaurant wastewater samples were resistance to gentamycin. The multiple drug resistances displayed by these isolates against commonly available antibiotic indicates high health implication to the environment.*
Key words: *Antibiotics resistance, biosafety, coliform, environment, wastewater.*

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I. INTRODUCTION

The consequence of untreated wastes on ecological and public health requires normal monitoring and proper legislation. The amount of waste or effluents with huge microbial load are directly released into open space, open water source, or as underground infusion without treating is a great threat to the health of the inhabitants in communities where such practices are carried out (Ogunfowokan*et al*., 2005). Domestics and restaurant wastes in undeveloped nations dispose their produced wastes without any treatment. This waste accumulates causing various types of harm and proliferation of microorganism directly or indirectly from different sources. Mostly, they emanate from contaminated equipment used in food processing, contaminated hands from food handlers and other sources (Ofor*et al*., 2009). Consequently, the unexplored microbial quality and amount of wastewater that is released from domestics and restaurants might be regard as a source of contamination. Ecological stresses on microorganisms rising from food processing,washing with cleanser, preservativeaddition,cooking of food and food pigment may promptransformation in ecosystem and rise different drug resistance (McMahon *et al*., 2007). Rowan, (1999) detailed a reduction in the susceptibility of microorganisms to a range of presently antibiotics used according to ecological stress. Van *et al*. (2007) showeda disturbing multidrug resistance frequency for isolated microorganism from food borne contaminants. Thus, ecological microbes had gotten more focus as another mean of acquiring antibiotic resistance mechanisms by utilizing the enzymes in initiation and efflux transport as a means for producing potential source of novel resistance geneand antibiotic resistance genein clinical disease causinginfections (Dantas*et al*., 2008). The multiple riseorspread of antibioticresistance among disease causing microbes had turned into an intense test in clinical treatment (Liasi*et al*., 2009). The

system by which these microbes demonstrated resistance modification of target site or incorporated adjustment and changes in metabolic pathway (Frost*et al.*, 2005).Katzung, (2004) highlighted different methods for getting resistance to the exchange of gene between microorganism strains, which could be supported by mobile genetic component, for example, bacteriophages,transposons,insertion component, interferonandplasmid. However, the combination of resistance microbes in domestic and restaurant wastewater that are released straight into ecological system prompt fast spread of antibiotic resistance gene in the ecosystem. In this way, the increase of antibiotic resistance spam from different areas and its conceivable ramifications require sufficient observation to provide solution. The present investigation in this manner evaluates the biosafety and antibiotic pattern of microorganisms obtained from untreated domestic and restaurant wastewater that are specifically release into environment.

II. MATERIALS AND METHODS

Collection of samples

Three different domestics and restaurants wastewater samples were collected from various residential home and restaurant in Choba Port Harcourt, Rivers State, Nigeria. The samples were kept in an ice pack and immediately transported to laboratory for analyses.

Biosafety evaluation of the wastewater samples

The microbial quality of the domestic and restaurant wastewater were determined by conventional methods. The serial dilution of domestic and restaurant wastewater samples was carried out to reduce the microbial load. Nutrient Agar was used for bacteria while Potato Dextrose Agar for the growth of fungi, the organisms were aseptically prepared and spread on the surface of the agar plate. The bacteria plates wereaerobically incubated at 37^0C for 24 h and the fungi 28 ± 2^0C for 96 h. The countedplates were express in colony forming unit per milliliter (cfu/ml) for bacteria and the mycelia plate were spore forming unit per milliliter (sfu/ml) for fungi.Biosafety of domestic and restaurant wastewater was determined, to ascertain the possible deteriogens and pathogen of health importance. The biosafety was determined using tyndalization technique, the wastewater were heated for 100^0C for 30mins and was plated out. This process was repeated for three successive days and incubated at ambient temperature during the intervening time. The plate count assay was used to study the viability of the cells after sterilization, while the incubation was done to encourage sporulation of the heat-resistant bacterial strains. Biochemical test was carried out using the methods of Olutiola*et al*. (2000) and Analytical profile index (API) was used to identify the pathogens that survive the second stage sterilization.

Total and Fecal Coliform Count using most probable number

The most probable number (MPN) test is used to ascertain the presence of fecal and totalcoliforms. Thereafter, incubation was done at 37^0C for 48h (total coliform) and 44.5^0C for 48h (fecal coliform). The microbial count obtained will be compared with the most probable number (MPN) of coliforms per 100 ml of the water sample (Uzoigwe and Agwa, 2012).

Antibiotics sensitivity test

Antibiotic sensitivity test was performed from the isolated bacterial using disc diffusion techniques shown by Cheesbrough (2000). Prepared subculture plate of 18h culture was standardized and swabbed were used to spread on Muller Hinton agar in duplicate. The petri dishwas allowed todry and the antibiotic circle disc was placed on the top of the agar. The petri dish was incubated for 24h at 37^0C and observed for zone of inhibition. The inhibition zone was taken and recorded for level of inhibition. The antimicrobial disc used were: Pefloxacin (PEF) 10µg, Gentamycin (CN) 10µg, Ampiclox (AU) 10µg, Ciprofloxacin (CPX) 10µg, Septrin (SXT) 30µg, Streptomycin (S) 30µg, Penicillin (PN) 30µg, Ceporex (CEP) 10µg, Ofloxacin (OFX) 10µg, and Nalidixic Acid (NA) 10µg. Antifungal disc method was carried out by using Food Poison Technique (Parajuli*et al*., 2005). One milliliter of every antifungal; Clotrimazole(C) 10mg,Ketoconazole (K) 20mg, Fluconazole (F) 20mg, Itraconazole (20mg) and Griseofulvin (G) 50mg. They were aseptically added to petri dish and subsequently mixed with the same volume of Potato Dextrose Agar (PDA) to get even blend of the substance. Using sterile plug borer of 8mm measurement, a disc was aseptically put on top cover in the focal point of each marked plates in duplicate and incubated. The plate without antifungal substance was set as control. The zone of inhibition of mycelia growth were observed and taken on the fifth day after incubation and mycelia inhibited was determined.

Statistical Analysis

The one-way ANOVA were used to analyze points of significant. The statistical package for social sciences (SPSS 20.0 version) provided the platform for Duncan multiple range test, sheffe and tukey-HSD tools were used to compare the parameters and locate points of significance at a confidence level of P= 0.05 (95%).

III. RESULTS

The total heterotrophic counts for bacterial and fungal counts for domestic wastewater were$1.19x10^6$cfu/ml and $1.2x10^4$cfu/ml while $1.06x10^6$cfu/ml and $1.0x10^4$cfu/ml for bacterial and fungal count from restaurant wastewater samples. The total heterotrophic count for bacterial and fungal isolates (Fig.1). The coliform analysis which revealed that fecal and total coliform had an MPN-index of $2.4x10^3$cfu/100ml for domestic and restaurant wastewater. Biosafety evaluation shows reduction from 120cfu/ml to 0cfu/ml on the third day (Fig 2 and Fig 3). The most frequently isolated microorganisms are

*Corynebacterium*sp. (30.77%),*Klebsiella*sp. (11%), *Pseudomonas* sp. (11%), *Staphylococcus* sp. (11%), and *Bacillus* sp. (8%).The antibiotic sensitivity results of the isolates subjected to commercially antibiotic(Tables 1 and 2). The bacteria obtained from domestic and restaurant wastewater were more resistance (11.5% - 100%) to pefloxacin, gentamycin, ciprofloxacin and penicillin. *Acinetobacter*sp.and*Bacillus*sp. isolated from domestic's wastewater have high level of 20.5% and 20% to pefloxacin. *Bacillus* sp. from the domestic wastewater sample possesses 17.5% to gentamycin and nalidixic acid while 0.0% of *Corynebacterium*sp. and *Klebsiella*sp. obtained from restaurant samples were resistance to gentamycin. Parts of the isolated microbes were shown to be inhibited to pefloxacin and ofloxacin except *Tatumella*sp.,*Pseudomonas*sp. and *Klebsiella*sp. are resistance to these penicillin, ceporex, nalidixic acid and streptomycin. The level of inhibited mycelia of the fungi isolates from domestic and restaurant wastewater to antifungal agents (Table 3). The high level of mycelia susceptible was shown in the following fungi isolates; *Rhizopous* sp., *Penicillium*sp., and *Mucor*sp., to griseofulvin, fluconazole and ketoconazole. The Analytical Profile Index of isolates from domestic and restaurant wastewater (Fig 4 and 5).

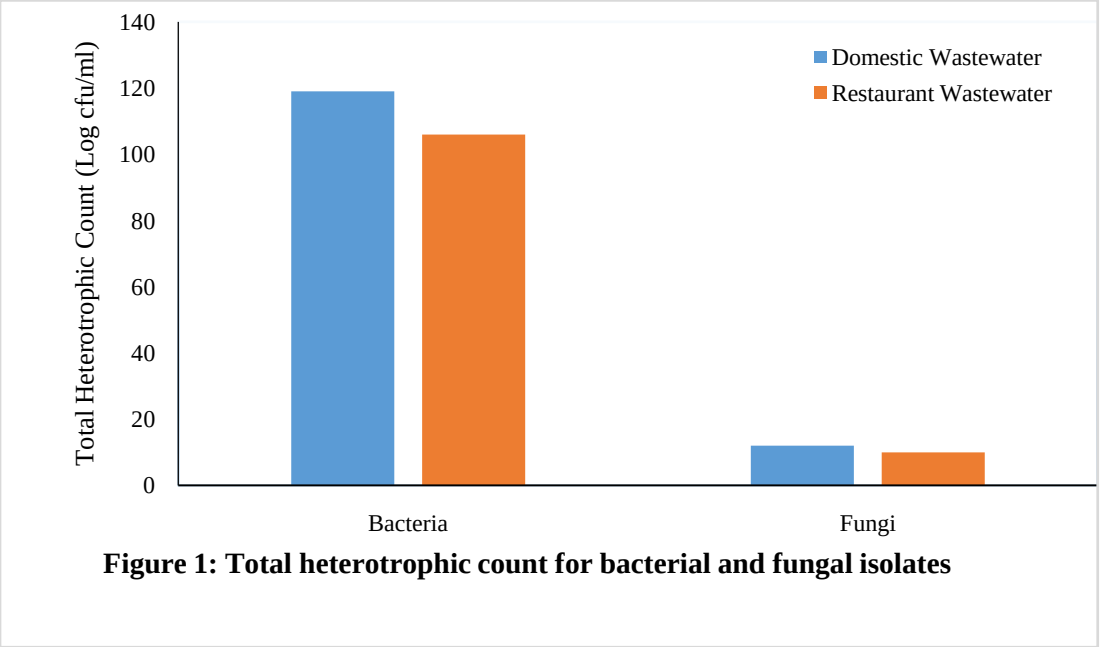

Figure 1: Total heterotrophic count for bacterial and fungal isolates

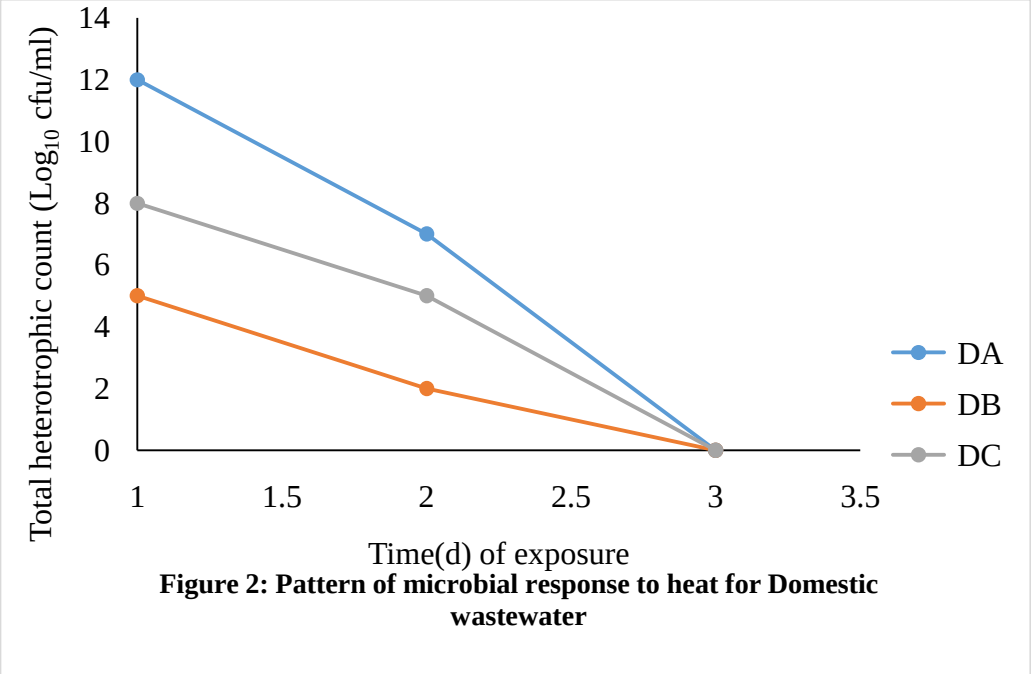

Figure 2: Pattern of microbial response to heat for Domestic wastewater

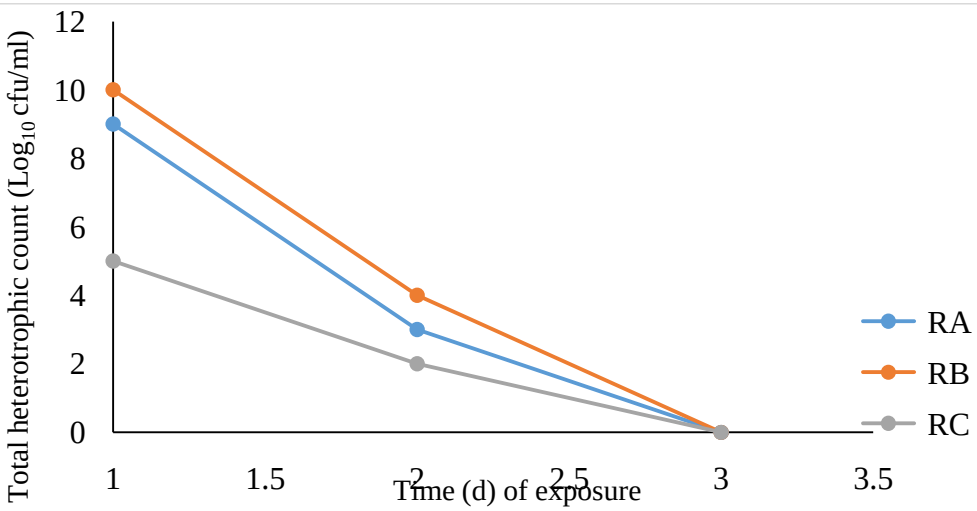

Figure 3: Pattern of microbial response to heat for Restaurant wastewater

Table 1: Percentage zone of inhibition of bacterial isolates from domestic wastewater to antibiotics

ISOLATES	PEF 10µg	CN 10µg	AU 10µg	CPX 10µg	SXT 30 µg	S 30µg	PN 30µg	CEP 10µg	OFX 10µg	NA 10µg
*Tatumella*sp.	12.5	11	13	20	14	13.5	0.0	0.0	13	0.0
*Acinetobacter*sp.	*20.5*	*13.5*	*14*	*20*	*17.5*	*15.5*	*16.5*	*15*	*20*	*15.5*
Pseudomonas sp.	*17*	*12*	*6.5*	*15.5*	*13.5*	*0.0*	*11*	*0.0*	*16*	*11*
*Morganella*sp.	*14*	*13.5*	*15*	*13.5*	*0.0*	*11*	*9*	*12*	*14.5*	*0.0*
Bacillus sp.	20	17.5	16	8.5	8.5	7.5	9.5	8	10	17.5
*Hafnia*sp.	10	13	13	12	19	14	12	11	20	13
*Klebsiella*sp.	20	19.5	0.0	15.5	15	0.0	0.0	0.0	18	0.0

Key: PEF=Pefloxacin, CN=Gentamycin, AU=Ampiclox, CPX= Ciprofloxacin, SXT=Septrim, S=Streptomycin, PN=Penicillin, CEP=Ceporex, OFX=Ofloxacin,NA=Nalidixic Acid

Table 2: Percentage zone of inhibition of bacterial isolates from restaurant wastewater to antibiotics

Samples	PEF 10µg	CN 10µg	AU 10µg	CPX 10µg	SXT 30µg	S 30µg	PN 30µg	CEP 10µg	OFX 10µg	NA 10µg
Staphylococcus	13	15.5	17	18.5	11.5	18.5	18.5	18	14.5	12.5
Corynebacterium	7.5	11	15.5	12	17	0.0	9	11.5	11.5	11.5
Pseudomonas	9.5	12.5	14.5	14.5	14	17	14	12	9.5	12
Providencia	0.0	13	15	11	14	12	10	14	12	13
Bacillus	10.5	17.5	16	8.5	8.5	7.5	9.5	8	10	16.5
Hafnia	10	13	13	12	19	14	12	11	20	13
Klebsiella	20	19.5	0.0	15.5	15	0.0	0.0	0.0	18	0.0

Key: PEF= Pefloxacin, CN= Gentamycin, AU=Ampiclox, CPX= Ciprofloxacin, SXT= Septrim, S=Streptomycin, PN=Penicillin, CEP=Ceporex, OFX=Ofloxacin, NA=Nalidixic Acid

Table 3: Percentagezone of inhibition of fungal isolates from domestic and restaurant wastewater by commercial antifungal agents

Tested Isolates	G (50mg)	F (20mg)	K (20mg)	I (20mg)	C(10mg)
Domestic wastewater					
*Mucor*sp	63.4	43.7	57.3	44.9	43.2
*Rhizopous*sp	50.0	70.2	50.0	54.2	0
*Aspergillus*sp	0	68.8	26.9	0	0
*Penicillium*sp	38.6	57.2	47.8	0	30.7
Restaurant wastewater					
*Mucor*sp	60.1	45.3	56.4	41.8	40.1
*Aspergillus*sp	0	0	19.5	11.5	0
*Penicillium*sp	54.7	62.9	68.1	37.8	0
*Rhizopous*sp	50.0	68.5	47.3	53.2	0

KEY: K=Ketoconazole, F= Fluconazole, G=Griseofulvin,I=Itraconazole, C= Clotrimazole

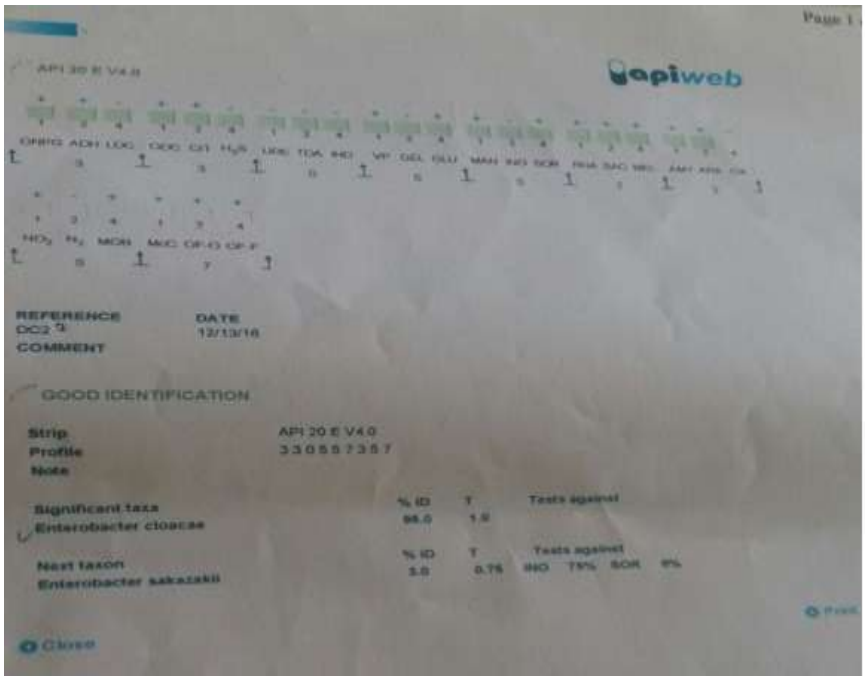

Figure 4: Analytical profile index of isolates from domestic wastewater sample

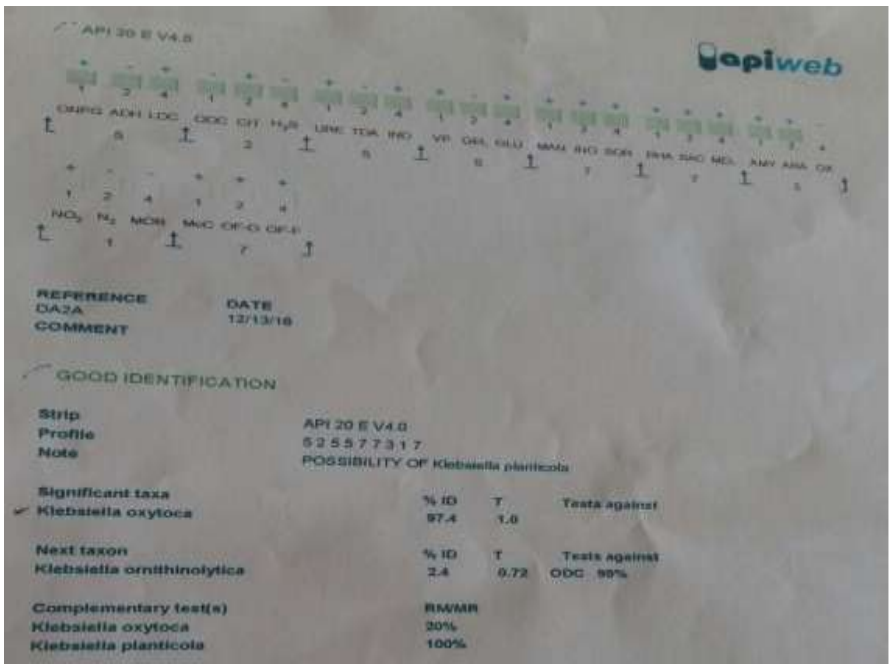

Figure 5: Analytical Profile Index of isolates from restaurant wastewater sample

DISCUSSION

The constant release or arrival of untreated waste into the ecosystem will not just cause serious impact in contamination, odour issue but generally increase the microbial load which will lead to disease and other health implications in the ecosystem. This demonstration will along these lines prompt an irregular impact on economic growthand human health (Egun, 2010;Adebisi and Fayemiwo, 2011). The resultof this investigation shows thekind of microorganism and microbial load that are involved in domestic and restaurant wastewater, when discharged or release directlyinto the environment. The result demonstrated that improper disposal of untreated waste from domestic and restaurant to the ecosystem will increase the microbial load of the ecosystem and cause serious health danger on human health and water bodies (Adebisi and Fayemiwo, 2011). The fungal and bacterial count from domestic and restaurant wastewater are in line with the discoveries of Bukar*et al.*

(2010) who revealed the presence of numerous microorganisms in preparedready-to-eat foods investigated in Kano state. Uzeh*et al*. (2009) reported thatserving mixed vegetable blended greens in retail outlet in Lagos was synonymouswith large microbes. The type of organisms isolated were the same with the studies of Prasai*et al*. (2007); Akoachere*et al*. (2008); Ofor*et al*. (2009) and Makun*et al*. (2009).The population of microorganism and kind of microbes in domestic and restaurant wastewater gives an impression of thecross contamination of microorganisms with food ingredients, preparation and handling operations, The presence of these microorganism in domestic and restaurant wastewater are worries as these are likely going to trigger an expansion in the occurrence of waterborne disease prompting serious general health hazard.In this way,they are mitigated against the guideline of sustainable and millennium development goal.

Many reviews have focused on antibiotic resistance microorganism discovered from various regionsaround the world (Lateef*et al*., 2005; Van *et al*., 2007).The antibiotic sensitivity investigations exhibited high level of resistance to the organism with commercially sold antibiotic. The levels of multidrug resistance of a portion of the isolate are in accordance with the discoveries ofChung *et al*. (2003) andLateef*et al*. (2005). The high resistance predominance is as a result of the occurrence of multidrug resistance in the microorganisms obtainedfrom domestic and restaurant wastewater which normally release and discharge wastes into the environment inan unsanitary practice. This demonstration had been proposed as a medium that makes the ecosystem worse, as it supports increase of waterborne pattern of *Corynebacterium*sp. and *Klebsiella*sp. to various antibiotics such as gentamycin. This result is similar with the discoveries of Van *et al.* (2007) whose pathogens and toxic substance deliver from Cyanobacteria (Akpor and Muchie, 2011). The resistance demonstrated that the antibiotic resistance of*Corynebacterium* sp. and *Salmonella* sp. fromfood sold in various vended business areas in Vietnam. The discoveries of Li *et al.*(2010) have shownthat gentamycin antibiotic resistance gene in *Corynebacterium* sp. and *Salmonella* sp. from natural wastewater samples. Besides, the disturbing multidrug resistance frequencies for *Corynebacterium* sp., *Klebsiella* sp. and *Salmonella* sp. had been credited to the use of antibioticin animals that produce food and food additives (McMahon *et al.*, 2007). *Corynebacterium* sp.*Tatumella* sp., *Klebsiella* sp. and *Pseudomonas*sp were resisted to the class of aminoglycosides antibiotic. This can be due to the nearness of aminoglycoside-modifying enzymes, alteration of the ribosomal target site and decrease permeability by mutations influencing membrane transport (Willey *et al*., 2008).This is in accordance with the discoveries of Franklin and Snow (2005) and Olaniran*et al*. (2009) who expressed the purposes behind resistance as an enzymatic catalyzed inactivation of anti-microbial because of transformation in the ribosomes and change in cell penetrability. The resistance level of *Corynebacterium* sp. and *Salmonella* sp. to cotrimazole is in line with the discoveries of Franklin and Snow (2005) who prescribed the resistance to such antibiotic could be examined by a change in the chromosomal gene that intervene with dihydropteroate amalgamation. The fungi isolated from domestics and restaurant wastewater showed better vulnerability to antifungal agent butdiffers from the discoveries ofArora*et al*. (2006) andPeraea and Patterson (2002)who detailed the resistance profile of clinical growths of the mycelia. The most antifungal inhibited are fluconazole and ketoconazole, which could be a direct result of its properties, which is pharmacokinetic (Arora*et al*., 2006). Moreover, it is a serious risk to the general public as domestic and restaurant wastewater harboring diverse antibiotic resistance microbes as it remain in the ecosystem without treating the wastewater. The developing resistance of test microorganism to antibiotic and other fundamental kind of antifungal agent build up a means of change in genetic material, which offers resistance to antimicrobial agent between microbial vegetation's in the environment.

IV. Conclusion

The microbial quality of domestics and restaurant wastewater samples and their antibiotic sensitivity requires very fast solution. Legislations and regulations must be put in place on appropriate disposal of wastewater and its consequences to the water bodies. This will lead to a specific goal of defending the health of individuals within the urban and rural area from disease causingresistance organisms.

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