



ANTIBIOTIC SENSITIVITY PATTERN OF MICROORGANISMS ISOLATED FROM REMNANT FOODS AND WASTEWATER FROM RESTAURANTS

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ABSTRACT

Antibiotic sensitivity of microorganisms isolated from remnant foods and wastewater in restaurants was assessed. Samples obtained from different restaurants were microbiologically examined and sensitivity to antibiotics by isolates was carried out by conventional methods. The microbial load in remnant foods and wastewater samples ranged from 7.3×10^8 cfu/ml to 8.4×10^8 cfu/ml and 1.0×10^5 sfu/ml to 1.3×10^5 sfu/ml for bacteria and fungi respectively. The following microorganisms; *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Rhizopus stolonifer*, *Aspergillus niger*, *Triscelophorus monosporus*, *Mucor mucedo* were isolated. Highest percentage occurrence was observed for *Aspergillus niger* (24.1%) while the lowest was observed for *Pseudomonas aeruginosa* (1.1%). The isolates exhibited various levels of resistance to augmentin (83.3%), nitrofurantoin (80%), gentamycin (83.3%), amoxicillin (75.0%), cotrimoxazole (80%) and ceftriazone (62.5%). The percentage resistance to tetracycline by *Escherichia coli* and *Salmonella typhi* isolated from remnant foods was 80 and 87.5 respectively. *Staphylococcus aureus* from remnant foods and wastewater have resistance percent of 58.3 and 50 to chloramphenicol. The multiple resistances displayed by isolates against commonly available antibiotic portends a great danger of laden the environment with potential pathogens by the indiscriminate disposal of remnant foods and wastewater.

Key words: antibiotics resistance, restaurants, remnant foods, wastewater, environment

INTRODUCTION

The implications of untreated wastes on environment and public health currently required a regular monitoring and appropriate legislation. This is because wastes or effluents with constituent beyond permissible limit are directly discharged into public water source, open space, or as an underground injection without treating such wastes. This act will definitely impose negative impact on communities (Ogunfowokan *et al.*, 2005). Restaurants in developing and underdeveloped countries dispose their generated wastes without any treatment. These wastes accumulate different kinds of microorganism that are sourced directly or indirectly contact of contaminated kitchen equipment, human transmission of faecally contaminated hands from infected handlers, transfer of pathogenic organisms from unhealthy slaughter animals and most importantly the hygienic disposal of restaurant wastes. Invariably, the unknown

microbial quality and quantity of wastes being discharged from restaurants may be considered as a source of resistance pathogen. Van *et al.* (2007) indicated alarming multidrug resistance frequencies for isolated organisms from food-borne bacterial contaminants.

Environmental stresses on microorganisms during food preparation such as cooking, washing with detergent, addition of preservative and food component may lead to change in nature and induce multiple drug resistance (Rowan, 1999). McMahon *et al.* (2007) reported the decrease in susceptibility of organisms to a range of currently used antibiotics as a result of environmental stress. Thus, environmental bacteria had received more focus as another source of reservoir of antibiotics resistance gene and potential source of novel resistance gene in clinical pathogen (Dantas *et al.*, 2008). The wide spread emergence of antibiotics resistance among pathogenic microorganisms had become serious challenge in clinical therapy (Li

et al., 2008). The mechanism by which these microorganisms exhibited resistance includes modification or alteration of target site and alteration of metabolic pathway (Katzung, 2004). Frost *et al.* (2005) highlighted the means of acquiring resistance to be transferred of gene between bacteria strains, which could be facilitated by mobile genetic elements such as plasmid, transposons, interferon, bacteriophages and insertion elements. However, the co-existence of resistance microorganisms in restaurant wastes that are directly discharged into environment would definitely lead to rapid spread of antibiotics resistance gene among other organisms in ecosystem. Thus, the apparent increase of the occurrence of antibiotics resistance among microorganisms from various areas such as clinical, foods, water and its possible implications require adequate surveillance to detect and proffer solution to the emergence of antimicrobial resistance mechanisms. The present study is therefore undertaken to assess the antibiotic sensitivity pattern of isolated organisms from untreated remnant foods and wastewater that are directly discharged into the environment in Akure metropolis.

MATERIALS AND METHODS

Location and collection of samples

This study was carried out in Akure metropolis, Southwest, Nigeria between September, 2010 and June, 2011. Eighty samples consisting of remnant foods (n=40 samples) and wastewater (n=40 samples) were collected from various restaurants in Akure metropolis. The samples were transferred to laboratory immediately for analyses.

Isolation and identification of microorganisms

Serial dilution of remnant foods and wastewater samples were carried out by standard microbiological techniques until the required dilution was obtained. The inoculums were aseptically and evenly spread on the surface of the Plate Count Agar (Lab M) for bacteria and Potato Dextrose Agar (Biomark) for fungi in triplicate. The plates were incubated aerobically at 37°C for 24 hours and 28±2°C for 48 hours for bacteria and fungi respectively. The total colonies were counted in colony forming unit per millilitre (cfu/ml) for bacteria and spore forming unit per millilitre (sfu/ml) for fungi. The morphological and biochemical tests were carried out using the methods of Cappuccino and Sherman (1999); Olutiola *et al.* (2000). Bacteria isolates were

identified to species level according to Cowan and Steel (1993) and microscopic identification of fungi was done according to the method of Chander (2002).

Antibiotics sensitivity test

Antibiotics sensitivity test of the bacterial isolates were determined by disc diffusion method as described by Cheesbrough (2000). Standard inoculum of 18 hours broth was spread on Muller Hinton agar using sterile swab in triplicate. The plates were dried before placing the antibiotic disc at equidistance. The plates were incubated for 24 hours at 37°C and diameter of zone of inhibition were measured and recorded. The commercial antibiotics discs (Fondoz Laboratories Ltd, Nigeria) used were; Amoxicillin (AMX) 25µg, Ofloxacin (OFL) 5µg, Ceftriazone (CEF) 30µg, Gentamycin (GEN) 10µg, Pefloxacin (PFX) 5µg, Cotrimoxazole (COT) 25µg, Ciprofloxacin (CPX) 10µg, Augmentin (AUG) 30µg, Nitrofurantoin (NIT) 20µg, Tetracycline (TET) 30µg, Erythromycin (ERY) 5 µg, Chloramphenicol (CHL) 30 µg and Streptomycin (STR) 10 µg.

Antifungal sensitivity was determined by Poison Food Technique (Parajuli *et al.*, 2005). One millilitre of each antifungal; Griseofulvin (G) 50mg, Fluconazole (F) 20mg, Ketoconazole (K) 20mg, Itraconazole (20mg) and Clotrimazole (C) 10mg were aseptically poured into petri dish followed by the addition of equal amount of Potato Dextrose Agar (PDA). The petri dish was kept swirling while adding the PDA to get even mixture of the content. Seven day old culture of the test fungi was used to prepare inoculum disc using a sterile cork borer of 8mm diameter. A single disc was aseptically placed upside down in the centre of each labelled plates in triplicate and incubated. The control set was devoid of antifungal agents. The diameter of fungi colonies were measured on the 7th day after inoculation and percentage of mycelia growth inhibition were calculated using the formula below.

The Percentage growth inhibitions in different concentrations = $\frac{g_c - g_t}{g_c} \times 100$

where, g_c = Growth of mycelia colony after incubation period in control set subtracting the diameter of inoculums disc.

g_t = Growth of mycelia colony after incubation period in treatment set subtracting the diameter of inoculums disc.

Statistical Analysis

All experiments were carried out in triplicate. Data obtained were analyzed by one way analysis of variance and means were compared by Duncan Multiple Range Test (SPSS 15.0 version). Differences were considered significant at P = 0.05.

RESULTS

The bacterial and fungal counts for remnant foods were found to be 8.4×10^8 cfu/ml and 1.3×10^5 sfu/ml while 7.3×10^8 cfu/ml and 1.0×10^5 cfu/ml for bacterial and fungal count from wastewater samples (Fig 1). Table 1 shows the distribution of microbial isolates. The most frequently isolated microorganisms are *Aspergillus niger* (24.1%), *Staphylococcus aureus* (23.9%), *Aspergillus fumigatus* (21.5%) *Shigella dysenteriae* (16.3%), *Escherichia coli* (12.0%)

The results of antibiotic sensitivity of isolates to commonly used antibiotic are shown in Tables 2 and 3. Bacteria isolated from remnant foods and wastewater exhibited more resistance (33.3% - 100%) to augmentin, ceftriazone, nitrofurantoin, gentamycin, amoxicillin and cotrimoxazole. *E.*

coli and *S. typhi* isolated from remnant foods have highest percentage of 87.5 and 80 to tetracycline. *S. aureus* from remnant foods sample have 58.3% to chloramphenicol and Streptomycin while 50% of *S. aureus* and *Micrococcus luteus* isolated from wastewater samples were resistance to chloramphenicol. Most of the isolates were found to be susceptible to pefloxacin and ofloxacin except *Pseudomonas aeruginosa*, *E. coli*, *S. aureus*, *S. typhi* and *S. dysenteriae*.

Table 4 shows the proportional resistance of the total bacteria isolates from remnant foods and wastewater to commercial antibiotics. This reveals the number of organisms that are resistance to the indicated numbers of tested antibiotic(s). Table 5 shows the percentage of mycelia inhibition of fungi isolates by commercial antifungal agents. The highest mycelia inhibition was observed in the following fungi isolates; *Rhizopus stolonifer*, *Penicillium italicium*, *Fusarium oxysporum*, *A.niger*, *A.fumigatus* and *Mucor mucedo* to fluconazole, ketoconazole, itraconazole and griseofulvin.

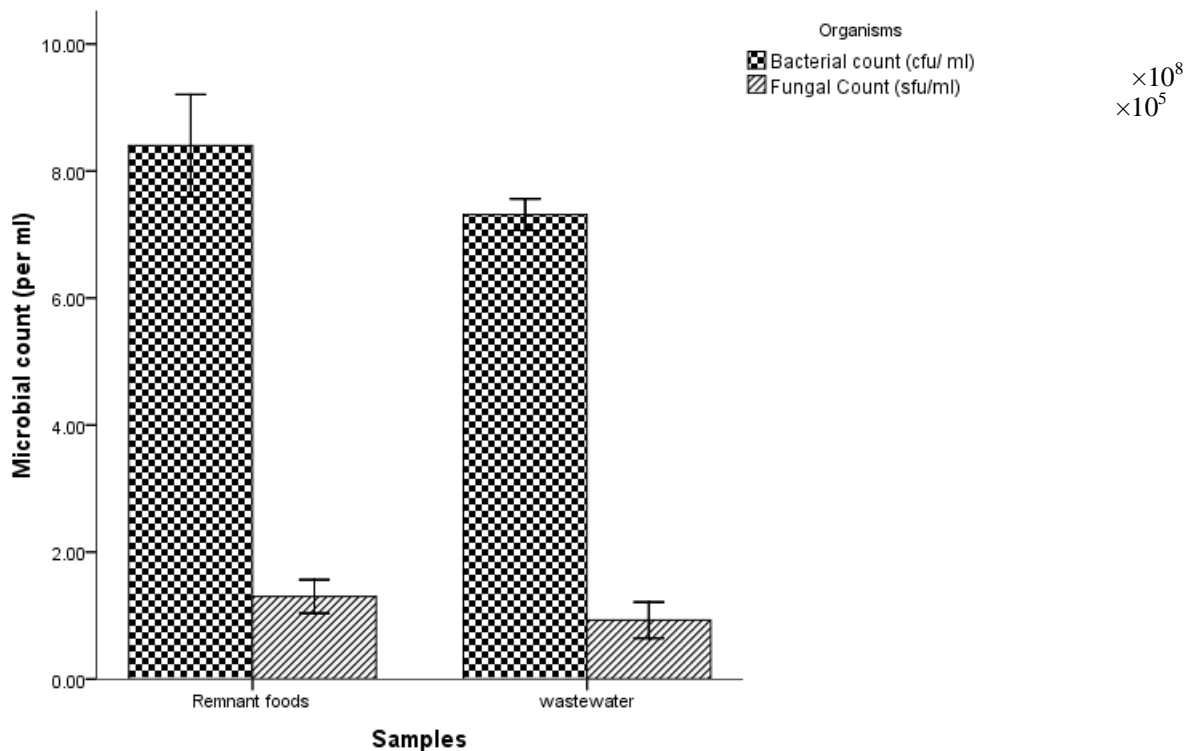


Table 1: Distribution of bacterial and fungal isolates (%) in remnant foods and wastewater from restaurants
Figure 1: Microbial load of sampled remnant foods and wastewater from restaurants in Akure metropolis

| Microorganisms | REF | WEW | Total |
|----------------------------------|------------------|------------------|------------------|
| Isolated Bacteria | | | |
| <i>Staphylococcus aureus</i> | 12(25.5%) | 10(22.2%) | 22(23.9%) |
| <i>Salmonella typhi</i> | 8(17.0%) | 2(4.4%) | 10(10.9%) |
| <i>Escherichia coli</i> | 5(10.6%) | 6(13.3%) | 11(12.0%) |
| <i>Shigella dysenteriae</i> | 5(10.6%) | 10(22.2%) | 15(16.3%) |
| <i>Enterobacter aerogenes</i> | – | 8(18.0%) | 8(8.7%) |
| <i>Klebsiella pneumoniae</i> | 5(10.6%) | 3(6.7%) | 8(8.7%) |
| <i>Serratia marcescens</i> | 3(6.4%) | 2(4.4%) | 5(5.4%) |
| <i>Micrococcus luteus</i> | 3(6.4%) | 2(4.4%) | 5(5.4%) |
| <i>Bacillus subtilis</i> | 4(8.5%) | – | 4(4.3%) |
| <i>Proteus vulgaris</i> | – | 2(4.4%) | 2(2.2%) |
| <i>Bacillus cereus</i> | 1(2.2%) | – | 1(1.1%) |
| <i>Pseudomonas aeruginosa</i> | 1(2.2%) | – | 1(1.1%) |
| Total | 47(100.0) | 45(100.0) | 92(100.0) |
| Isolated Fungi | | | |
| <i>Aspergillus niger</i> | 9(19.2%) | 10(31.3%) | 19(24.1%) |
| <i>Aspergillus fumigatus</i> | 10(21.3%) | 7(21.9%) | 17(21.5%) |
| <i>Triscelophorus monosporus</i> | – | 5(15.6%) | 5(6.3%) |
| <i>Penicillium chrysogenum</i> | 5(10.6%) | 4(12.5%) | 9(11.4%) |
| <i>Penicillium italicium</i> | 4(8.5%) | 4(12.5%) | 8(10.1%) |
| <i>Fusarium oxysporum</i> | 5(10.6%) | 2(6.2%) | 7(8.9%) |
| <i>Mucor mucedo</i> | 6(12.8%) | – | 6(7.6%) |
| <i>Aspergillus flavus</i> | 5(10.6%) | – | 5(6.3%) |
| <i>Rhizopus stolonifer</i> | 3(6.4%) | – | 3(3.8%) |
| Total | 47(100.0) | 32(100.0) | 79(100.0) |

Key:

– = isolate is absent

REF = number of isolates (%) from remnant foods

WEW = number of isolates (%) from wastewater

Table 2: Percentage resistance of bacteria isolated from remnant foods to antibiotics

| Tested isolates | N | AUG | CEF | NIT | STR | GEN | CHL | COT | OFL | AMX | ERY | CPX | TET | PFX |
|-------------------------------|------|------|------|------|------|------|------|------|------|------|-----|------|------|------|
| <i>Staphylococcus aureus</i> | (12) | NT | 41.7 | NT | 58.3 | 16.7 | 58.3 | 33.3 | 8.3 | 66.7 | 25 | 41.6 | NT | 16.7 |
| <i>Salmonella typhi</i> | (8) | 62.5 | 62.5 | 75 | NT | 62.5 | NT | 37.5 | 12.5 | 75 | NT | 37.5 | 87.5 | 0.0 |
| <i>Escherichia coli</i> | (5) | 80 | 40 | 80 | NT | 60 | NT | 80 | 0.0 | 60 | NT | 40 | 80 | 40 |
| <i>Shigella dysenteriae</i> | (5) | 60 | 40 | 60 | NT | 80 | NT | 40 | 0.0 | 40 | NT | 20 | 0.0 | 0.0 |
| <i>Klebsiella pneumoniae</i> | (5) | 60 | 60 | 40 | NT | 60 | NT | 20 | 0.0 | 0.0 | NT | 0.0 | 0.0 | 0.0 |
| <i>Serratia marcescens</i> | (3) | 33.3 | 33.3 | 66.6 | NT | 0.0 | NT | 33.3 | 0.0 | 0.0 | 25 | 0.0 | 0.0 | 0.0 |
| <i>Micrococcus luteus</i> | (3) | NT | 0.0 | NT | 33.3 | 0.0 | 33.3 | 0.0 | 0.0 | 33.3 | 0.0 | 0.0 | NT | 0.0 |
| <i>Bacillus subtilis</i> | (4) | NT | 50 | NT | 25 | 75 | 25 | 0.0 | 0.0 | 25 | 25 | 0.0 | NT | 0.0 |
| <i>Bacillus cereus</i> | (1) | NT | 100 | NT | 0.0 | 0.0 | 0.0 | 100 | 0.0 | 100 | 0.0 | 0.0 | NT | 0.0 |
| <i>Pseudomonas aeruginosa</i> | (1) | 100 | 100 | 100 | NT | 100 | NT | 0.0 | 100 | 100 | NT | 0.0 | 0.0 | 0.0 |

Values are mean of Replicates

Key: NT= not tested (antibiotics are absent in selected disc for Gram +ve or Gram -ve)

0.0 = Isolates are susceptible

n = Number of tested isolates

Antibiotic codes are defined under materials and methods

Table 3: Percentage resistance of bacteria isolated from wastewater to antibiotics

| Tested isolates | n | AUG | CEF | NIT | STR | GEN | CHL | COT | OFL | AMX | ERY | CPX | TET | PFX |
|-------------------------------|------|------|------|------|-----|------|-----|------|------|------|-----|------|------|------|
| <i>Staphylococcus aureus</i> | (10) | NT | 40 | NT | 30 | 30 | 50 | 30 | 20 | 60 | 30 | 20 | NT | 0.0 |
| <i>Shigella dysenteriae</i> | (10) | 70 | 30 | 60 | NT | 20 | NT | 40 | 0.0 | 50 | NT | 10 | 10 | 20 |
| <i>Enterobacter aerogenes</i> | (8) | 25 | 25 | 75 | NT | 37.5 | NT | 12.5 | 0.0 | 37.5 | | 37.5 | 25 | 0.0 |
| <i>Escherichia coli</i> | (6) | 83.3 | 50 | 66.6 | NT | 83.3 | NT | 33.3 | 16.6 | 50 | NT | 0.0 | 0.0 | 33.3 |
| <i>Klebsiella pneumoniae</i> | (3) | 66.6 | 33.3 | 0.0 | NT | 66.6 | NT | 33.3 | 0.0 | 66.7 | NT | 0.0 | 33.3 | 33.3 |
| <i>Salmonella typhi</i> | (2) | 50 | 50 | 50 | NT | 50 | NT | 50 | 0.0 | 0.0 | NT | 0.0 | 50 | 0.0 |
| <i>Proteus vulgaris</i> | (2) | 100 | 50 | 0.0 | NT | 0.0 | NT | 100 | 0.0 | 0.0 | NT | 0.0 | 50 | 50 |
| <i>Serratia marcescens</i> | (2) | 50 | 100 | 100 | NT | 0.0 | NT | 0.0 | 0.0 | 0.0 | NT | 0.0 | 0.0 | 0.0 |
| <i>Micrococcus luteus</i> | (2) | NT | 0.0 | NT | 0.0 | 50 | 50 | 50 | 0.0 | 0.0 | 50 | 100 | NT | 0.0 |

Values are mean of Replicates

Key: NT= not tested (antibiotics are absent in selected disc for Gram +ve or Gram -ve)

0.0 = Isolates are susceptible

n = Number of tested isolates

Antibiotic codes are defined under materials and methods

Table 4: Proportional resistance of bacteria isolates from remnant foods and wastewater to antibiotics

| ANTIBIOTICS | REF | WEW |
|-------------|---|--|
| AUG | B ₂ , B ₃ , B ₅ , B ₇ , B ₈ , C ₁ , C ₃ , C ₄ , C ₅ , D ₁ , D ₄ , D ₅ , E ₁ , E ₄ , E ₅ , F ₂ , J ₁ | D ₆ , D ₈ , D ₁₁ , D ₁₂ , D ₁₃ , D ₁₄ , D ₁₅ , K ₃ , K ₇ , C ₆ , C ₇ , C ₈ , C ₁₀ , C ₁₁ , E ₆ , E ₇ , B ₉ , M ₁ , M ₂ , F ₅ |
| CEF | A ₁ , A ₂ , A ₆ , A ₉ , A ₁₂ , B ₃ , B ₄ , B ₅ , B ₆ , B ₈ , C ₁ , C ₃ , D ₁ , D ₃ , E ₂ , E ₄ , E ₅ , F ₁ , H ₁ , H ₃ , I ₁ , J ₁ | A ₁₄ , A ₁₆ , A ₁₇ , A ₂₀ , D ₆ , D ₈ , D ₁₄ , K ₃ , K ₇ , C ₈ , C ₉ , C ₁₀ , E ₇ , B ₉ , M ₂ , F ₄ , F ₅ |
| NIT | B ₁ , B ₃ , B ₄ , B ₆ , B ₇ , B ₈ , C ₁ , C ₂ , C ₃ , C ₄ , D ₂ , D ₃ , D ₅ , E ₂ , E ₄ , F ₁ , F ₃ , J ₁ | D ₆ , D ₇ , D ₈ , D ₁₁ , D ₁₂ , D ₁₃ , K ₃ , K ₄ , K ₅ , K ₆ , K ₇ , K ₈ , C ₆ , C ₇ , C ₈ , C ₁₀ , B ₉ , F ₄ , F ₅ |
| STR | A ₂ , A ₆ , A ₈ , A ₉ , A ₁₀ , A ₁₁ , A ₁₂ , G ₁ , H ₁ | A ₁₄ , A ₁₅ , A ₂₁ |
| GEN | A ₂ , A ₆ , B ₃ , B ₄ , B ₆ , B ₇ , B ₈ , C ₁ , C ₂ , C ₃ , D ₁ , D ₃ , D ₄ , D ₅ , E ₂ , E ₄ , E ₅ , H ₁ , H ₃ , H ₄ , J ₁ | A ₁₄ , A ₁₈ , A ₂₀ , D ₆ , D ₁₅ , K ₃ , K ₄ , K ₇ , C ₆ , C ₇ , C ₈ , C ₉ , C ₁₁ , E ₆ , E ₇ , B ₉ , G ₄ |
| CHL | A ₂ , A ₃ , A ₄ , A ₇ , A ₉ , A ₁₀ , A ₁₂ , G ₁ , H ₃ | A ₁₃ , A ₁₄ , A ₁₆ , A ₁₇ , A ₂₀ , G ₄ |
| COT | A ₁ , A ₃ , A ₄ , A ₆ , B ₃ , B ₅ , B ₇ , C ₁ , C ₃ , C ₄ , C ₅ , D ₃ , D ₄ , E ₁ , F ₁ , I ₁ | A ₁₄ , A ₁₆ , A ₂₀ , D ₆ , D ₇ , D ₁₁ , D ₁₄ , K ₃ , C ₈ , C ₁₀ , E ₇ , B ₉ , M ₁ , M ₂ , G ₄ |
| OFL | A ₆ , B ₅ , J ₁ | A ₁₄ , A ₂₀ , C ₈ |
| AMX | A ₂ , A ₃ , A ₆ , A ₈ , A ₉ , A ₁₀ , A ₁₁ , A ₁₂ , B ₁ , B ₂ , B ₃ , B ₅ , B ₆ , B ₇ , C ₂ , C ₃ , C ₅ , D ₁ , D ₃ , G ₂ , H ₃ , I ₁ , J ₁ | A ₁₃ , A ₁₄ , A ₁₆ , A ₁₇ , A ₂₀ , A ₂₁ , D ₆ , D ₈ , D ₁₁ , D ₁₂ , D ₁₅ , K ₂ , K ₃ , K ₇ , C ₆ , C ₇ , C ₈ , E ₇ , E ₈ |
| ERY | A ₆ , A ₉ , A ₁₂ , H ₃ | A ₁₄ , A ₁₉ , A ₂₀ , G ₄ |
| CPX | A ₆ , A ₇ , A ₉ , A ₁₂ , B ₃ , B ₅ , B ₆ , C ₁ , C ₂ , C ₃ , D ₃ | A ₁₃ , A ₂₀ , D ₆ , K ₂ , K ₃ , K ₇ , G ₄ , G ₅ |
| TET | B ₁ , B ₂ , B ₃ , B ₅ , B ₆ , B ₇ , B ₈ , C ₁ , C ₂ , C ₃ , C ₄ | D ₆ , K ₂ , K ₇ , E ₈ , B ₁₀ , M ₂ sd |
| PFX | A ₂ , A ₇ , C ₁ , C ₃ | D ₆ , D ₁₁ , C ₆ , C ₁₀ , E ₇ , M ₁ |

Key: REF = number of resistance isolates from remnant foods

WEW= number of resistance isolates from wastewater

Antibiotic codes are defined under materials and methods

A₁, A₂, A₃, A₄, A₆, A₇, A₈, A₉, A₁₀, A₁₁, A₁₂, A₁₃, A₁₄, A₁₅, A₁₆, A₁₇, A₁₈, A₂₀, A₂₁ : *S. aureus* isolates

B₁, B₂, B₃, B₄, B₅, B₆, B₇, B₈, B₉, B₁₀ : *Salmonella typhi* isolates

C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁ : *Escherichia coli* isolates

D₁, D₂, D₃, D₄, D₅, D₆, D₇, D₈, D₉, D₁₁, D₁₂, D₁₃, D₁₄, D₁₅ : *Shigella dysenteriae* isolates

E₁, E₂, E₄, E₅, E₆, E₇, E₈ : *Klebsiella pneumoniae* isolates

F₁, F₂, F₃, F₄, F₅ : *Serratia marcescens* isolates

G₁, G₂, G₄, G₅ : *Micrococcus luteus* isolates

H₁, H₃, H₄ : *Bacillus subtilis* isolates

I₁ : *Bacillus cereus* isolate

J₁ : *Pseudomonas aeruginosa* isolate

K₂, K₃, K₄, K₅, K₆, K₇, K₈ : *Enterobacter aerogenes* isolates

M₁, M₂: *Proteus vulgaris* isolates**Table 5: Percentage of mycelia inhibition of fungi isolates from remnant foods and wastewater by commercial antifungal agents**

| Tested Isolates | N | G (50mg) | F (20mg) | K (20mg) | I (20mg) | C(10mg) |
|----------------------------------|------|----------|----------|----------|----------|---------|
| Remnant foods | | | | | | |
| <i>Penicillium italicium</i> | (4) | 36.7 | 56.1 | 46.9 | – | 29.6 |
| <i>A. niger</i> | (9) | – | 67.7 | 25.8 | – | – |
| <i>Fusarium oxysporum</i> | (5) | 42.6 | 61.6 | 42.6 | 21.2 | 32.4 |
| <i>Mucor mucedo</i> | (6) | 62.3 | 42.6 | 56.2 | 43.8 | 42.1 |
| <i>Rhizopus stolonifer</i> | (3) | 49.0 | 69.1 | 49.0 | 52.1 | – |
| <i>Penicillium chrysogenum</i> | (5) | – | 50.1 | – | 10.8 | 21.8 |
| <i>A. fumigatus</i> | (10) | – | 59.7 | – | 20.0 | 23.0 |
| <i>A. flavus</i> | (5) | – | 13.6 | – | 18.4 | – |
| Wastewater | | | | | | |
| <i>Fusarium oxysporum</i> | (2) | 67.3 | 68.0 | 56.3 | – | 20.0 |
| <i>Penicillium italicium</i> | (4) | 53.6 | 63.7 | 69.0 | 38.9 | – |
| <i>Asperillus fumigatus</i> | (7) | – | 40.9 | – | 67.8 | – |
| <i>Triscelophorus monosporus</i> | (5) | 43.2 | 41.1 | 30.2 | – | – |
| <i>Penicillium chrysogenum</i> | (4) | 60.3 | 20.4 | 32.5 | – | 51.8 |
| <i>Aspergillus niger</i> | (10) | – | – | 18.4 | 10.4 | – |

Values are mean of replicates (n = 3)

Key: – = no mycelia inhibition as in control set

G = griseofulvin, F= fluconazole, K=ketoconazole, I = itraconazole, C= Clotrimazole

n = Number of tested isolates

DISCUSSION

The continuous discharge of untreated wastes into the environment will not only contribute to deleterious effect of pollution, ordour problem but increase the microbial load that could be pathogenic in nature. This act will therefore lead to an unpleasant implication on human health and economic development (Adebisi and Fayemiwo, 2011; Egun, 2010). The results of this study revealed the microbial load and type of organisms that are associated with remnant foods and wastewater, which were directly discharged from restaurant to the open space. The higher bacterial and fungal counts from sampled remnant foods and wastewater are in agreement with the findings of Bukar *et al.* (2010) who reported such index of 10^5 to 10^8 in ready-to- eat foods in Kano metropolis. Moreover, Uzeh *et al.* (2009) also revealed that mixed vegetable salad in retail outlet in Lagos was laden with microorganisms. The result indicated that indiscriminate dumping of untreated wastes from restaurants to the environment without any caution in developing countries will increase the microbial load of the environment. Thus, this act will therefore contribute to hazards on water bodies and results to detrimental effects on human health (Adebisi and Fayemiwo, 2011).

The species of organisms isolated were similar to those obtained in other studies by Prasai *et al.* (2007); Akoachere *et al.* (2008) and Makun *et al.* (2009). The microbial load and type of organisms in restaurant wastes is a reflection of microbial cross contamination of ingredients, washing or rinsing water and handling during processing. Ofor *et al.* (2009) had earlier reported microbial contaminants in water used to rinse tomatoes. The occurrence of these organisms in remnant foods and wastewater are of public health concerns as these organisms are likely to cause an increased incidence of waterborne diseases and thereby against the principle of sustainable development.

In light of potential health risk posed by wastes containing microorganisms, many studies have focused on antibiotic resistance organisms recovered from various area of ecosystem (Lateef *et al.*, 2005; Van *et al.*, 2007). The result of antibiotics sensitivity showed varying degree of resistance by organisms to commercially available antibiotics. The percentage resistance and multidrug resistance of some of the isolates are in agreement with the findings of Chung *et al.* (2003) and Lateef *et al.* (2005). The high resistance prevalence and incidence of multidrug resistance in microorganisms isolated from

remnant foods and wastewater that are ready to discharge into the neighbourhood is an indication of unsanitary hygiene practises. This act had been suggested as a medium that create environmental condition, which favour proliferation of waterborne pathogens and toxin producing Cyanobacteria (Akpoy and Muchie, 2011).

The resistance showed by *E. coli* and *S. typhi* to tetracycline and gentamycin conformed to findings of Van *et al.* (2007) who revealed the antibiotics resistance of *Salmonella* sp and *E. coli* from food sold in market place in Vietnam. The earlier findings of Li *et al.* (2010) revealed tetracycline resistance gene in *Escherichia coli* and *Salmonella* sp from an oxytetracycline production wastewater. However, the alarming multidrug resistance frequencies for *E. coli*, *S. dysenteriae* and *S. typhi* had been attributed to the use of antibiotics in food producing animal and food preservatives (McMahon *et al.*, 2007). *S. typhi*, *E. coli*, *K. pneumoniae*, *S. dysenteriae* were resistance to class of aminoglycosides. This is in agreement with the findings of Olaniran *et al.* (2009) and Franklin and Snow (2005) who stated the causes of resistance as an enzymic catalysed inactivation of antibiotics as a result of mutation that affects the ribosomes and change in cellular permeability. The resistant percent of *E. coli* and *S. dysenteriae* to cotrimazole is in conformity with the findings of Franklin and Snow (2005) who suggested the resistance to such antibiotic could be as a result of mutation in the chromosomal gene that mediate dihydropteroate synthesis.

The isolated fungi from remnant foods and wastewater exhibited better susceptibility to antifungal agents compared to the findings of Peraea and Patterson (2002) and Arora *et al.* (2006) who reported the resistance profile of clinical fungi. The highest mycelia inhibition by fluconazole could be due to its pharmacokinetic properties (Arora *et al.*, 2006). However, it is a serious threat to public health as restaurant wastes harbouring the antibiotic resistant microorganisms and end up in the environment without treating their wastes. The emerging resistance of tested isolates to antibiotics and other principal type of antifungal agents establish a possible exchange of genetic material, which confers resistance to antimicrobial agents between microbial floras in the ecosystem. The microbiological quality of remnant foods and wastewater from restaurants and sensitivity of isolates to antibiotics require urgent attention by

making laws on proper disposal of wastes in order to safeguard the health of communities from resistance pathogenic organisms.

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