ANTIMICROBIAL SUCEPTIBILITY PATTERN OF MICRORGANISMS ISOLATED FROM ~~DIFFERENT~~ TAP~~S~~ WATER SURFACES IN FUTA (OBAKEKERE CAMPUS)

ABSTRACT

Resistance of microorganisms to only available antimicrobial agents have resulted in pernicious effect to human health over the last decades. The increase in antimicrobial resistance of pathogenic microorganisms particularly in tap~~s~~ water surfaces is a major concern to the society. This research focused on the investigation of antimicrobial resistance pattern of pathogenic organisms (bacteria and fungi) isolated from various tap~~s~~ water surfaces in the Federal University of Technology Akure (FUTA) Obakere campus, in Akure, Ondo State, Nigeria. Samples were obtained from different locations (laboratories, hostels and toilets) in ~~(~~FUTA~~)~~. The samples were analyzed microbiologically on selective, differential and general purpose media. The isolated organisms were identified by the routine ~~convectional standard~~ microbiological methods. Among the pathogenic microorganisms isolated were *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Escherichia coli, Bacillus cereus, Citrobacter freundii, Acinectobacter bourmanii, Staphylo~~s~~coccus aureus, Aspergillus fumigatus, Aspergillus niger, Candida albicans* and *Fusarium oxysporium* ~~with different loads~~. The percentage distribution varies with *P.~~seudomonas~~ aeruginosa* (26.~~09~~%) having the highest percentage ~~occurrence~~ and *Klebsiella* spp. (4.~~45~~%) recorded at the lowest percentage of occurrence ~~of percentage distribution~~. All fungi isolated have ~~equal~~ simillar percentage distribution. The isolates displayed various levels of resistance to piperacillin (100%), ampicillin (67~~6.6~~%). ceftazidime (67~~6.6~~%), tetracycline (83.~~3~~%), cefepime (67~~6.6~~%) and chloramphenicol (67~~6.6~~%). The ~~percentage of~~ resistance to amoxicillin-clavulanate by *E. coli* and *C.~~itrobacter~~ freundii* isolated from tap~~s~~ water surfaces were 60% and 50%, respectively. *K.~~lebisella~~ pneumoniae* from tap water surface has 100% resistance ~~percent of 100%~~ to cefepime. The high resistance of microorganisms to antimicrobial agents indicated great danger to people living within FUTA community that constantly have contact with this tap water surfaces.

Keywords: Antibiotic~~s~~ resistance, ~~tap~~ water ~~surfaces~~, environment, society

INTRODUCTION

Water is a universal solvent and it remains the ~~———~~only component responsible and play greater parts in human and domestic lives. It dominates the highest component of biosphere and the earth's surface (Petraccia *et al.*, 2005). Being the basic needs of man and domestic lives, it is require for drinking, cooking, bathing and others which are essential for metabolism and reproduction of lives. The means of obtaining this water varies depending on the source of it. Most are obtained from rivers and well, while water obtained from taps are considered to be more reliable and safe for human consumption. In Federal University of Technology, Akure (FUTA), tap water remains major source of water which has provided relieve for staff and students most especially those who live in the hostels and quarters. The popularity of tap water during

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Comment [J2]: Unnecessary. Do you mean pandrug-resistance?

Comment [J3]: Do you mean Acinetobacter baumannii. If yes, delete this bacterium since it could not be identified by the convectional standard microbiological methods

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the 20th century and becoming the thing of challenges among poor people especially in developing countries where squalor is very rampant (Petraccia *et al.*, 2005). Tap water is most times assumed to be pure and drinkable most especially in developing countries.

Ideally, water obtained from taps are supposed to be free of microorganisms especially the pathogenic microbes but, due to the sources from which this water is released which harbour and serve as the reservoir of pathogenic microbes render the water unsafe for human consumption (Samie and Makonto, 2011). In addition, due to the ubiquitous of microorganisms, some of them colonized tap~~s~~ water surfaces and contaminate the safe water when such waters are released and running from the tap~~s~~ surfaces. Bacteria and fungi are the most serious concern pathogens in waterborne disease, and these organisms cause gastrointestinal outbreaks around the world (World Health Organization, 2005). Many human diseases are transmitted as a result of ingestion of contaminated water containing infectious stage of the pathogens. Over 2.2 billion case of diarrhea per year around the world has been enumerated as a result of contaminated water; this condition is the second leading cause of death in children (Samie and Makonto, 2011). The challenges of health are common in rural area especially in developing countries where there is lack of adequate potable water (World Health Organization, 2005). Enteric bacteria~~l~~ are common in human intestine and feaces of animals together with spore forming fungi that ~~which are~~ commonly colonize~~d~~ taps water surfaces. Bacterial pathogens potentially transmitted through water ingestion include~~d~~ *Escherichia coli*, *Klebsiella* spp., *Salmonella* spp., ~~*enteric sub* spp*+* and~~ other coliforms, and ~~the~~ protozoa, while the fungi ~~were~~ are *Aspergillus fumigatus, A. niger* and *flavus* (Omojowo and Omojaosola, 2013).

Antibiotic resistance of microorganisms are the major concern and challenges since it existence ~~which~~ has ~~leads to~~resulted in death of ~~and state of dyeing of an~~ infected individuals. The wrong use and misuse of antibiotics among hospital and community acquired infections have led to this phenomenon. The increase of antibiotics resistance among bacteria have actually render the effectiveness of these various groups of antibiotics inactive and causing bacteria to proliferate in their number (Omojowo and Omojaosola, 2013). ~~—~~Of particular concern are the multidrug-resistant pathogens, for example ~~:~~ *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, methicillin-resistant *Staphylococcus aureus*, penicillin-resistant *Streptococcus pneumoniae*, vancomycin-resistant *Enterococcus*, and extensively drug-resistant *Mycobacterium tuberculosis* (Alekshun and Levy, 2007). Therefore, this study focused in the antimicrobial susceptibility pattern of microorganisms isolated from differen~~ce~~t tap~~s~~ water surfaces in the Federal University of Technology Akure (FUTA), Nigeria, with the purpose ~~of knowing the nature, types of the microbes so as~~ to put in place good sanitation measures in ~~as~~ regard to water supply.

MATERIALS AND METHODS

Location and collection of samples

This research work was carried out from June 2018 to August 2018 in Akure metropolis, Ondo state, Nigeria. About

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fifteen different tap~~s~~ water surfaces (laboratory, h~~H~~ostels and t~~T~~oilets) were swabbed with sterile swab stick and transferred aseptically to the laboratory for further analysis.

Isolation ~~and identification~~ of microorganisms

Examination of microorganisms was carried out using standard convectional microbiological methods (William, 2017). Each sample was aseptically transferred into Bijou bottles containing 9 ml of sterile water and serial dilution was performed on each sample. The dilution was continued until the fourth (10^{-4}) dilution was attained for each of the sample and 1 ml of third (10^{-3}) of each of the sample was aseptically collected and poured plated on already prepared solid media: ~~(~~Nutrient, MacConkey, Eosin methylene blue, Sarbraoud dextrose (SDA) and Potato dextrose agar (PDA) according to the manufacturer's instruction and sterilized by autoclaving at 121^{0}C for 15 minutes. ~~All~~ T~~t~~he plates were incubated at 25^{0}C/72h for fungi~~)~~ ~~for 72 hours~~ and at 37^{0}C/24h for bacteria ~~for 24 hours~~.

Biochemical characterization

Biochemical characterization and presumptive identification of isolates were carried out as described by (Olutiola *et al.* 2000).

Antimicrobial susceptibility

Antibiotics susceptibility of all the isolates was carried out as described by disk diffusion method and interpreted as susceptible, intermediates and resistance according to the Clinical Laboratory Standard Institute (CLSI), 2017. The commercial antibiotics used were ciprofloxacin (CIP) 10µg, ceftriazone (CRO) 30µg, ceftazidime (CAZ) 30µg, tetracycline (TE) 30µg, ampicillin (AMP) 10µg, cefepime (FEP) 30µg, chloramphenicol (C) 30µg, amoxicillin-clavulanate (AMC) 30µg, trimethoprim/sulphamethoxazole (SXT) 25µg, piperacillin (PRL), gentamicin (CN) 20µg, compound sulphurnamide and cloxacilin 5µg.

Antifungal susceptibility was determined according to (Bauer *et al.* 2010). Antifungal agents used were griseofulvin (50mg), ketoconazole (20mg), fluconazole (20mg) and itraconazole (20mg).

Statistical analysis of data

All experiments were carried out in triplicate, and data obtained were subjected to one way analysis of variance, while the means were compared by Duncan's New Multiple Range Test at 95 % confidence interval using Statistical Package for Social Sciences version 23.0. Differences were considered significant at p≤0.05.

RESULTS

Table 1 and 2 show total bacterial and fungal viable counts in different tap~~s~~ water surfaces in FUTA with highest loads of viable bacteria in m~~M~~icrobiologicaly~~y~~ laboratory. Figure 1 shows the load of isolates from different ta~~p~~s water surfaces ~~in FUTA (Obakere campus)~~. The most frequently isolated microorganisms we~~a~~re *Pseudomonas aeruginosa* (26.09%), *Escherichia coli* (21.74%), *Klebsiella* p~~P~~*neumoniae* (13.04%), ~~*Acinetobacter baumannii* (13.04%),~~ *Candida albicans* (25.0%), *Aspergillus niger* (25.0%). The results of antibiotic and antifungal sensitivity

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of isolates to commonly available agents are shown in Table 3 and 4, respectively. The percentage resistance of bacteria isolated from different taps water surfaces ~~in FUTA (Obakere campus)~~(Figure 2) where mostly ~~of~~ the bacteria isolates which exhibited high~~ly~~ resistance (33.~~3~~-100%) to piperacillin, ceftriazone, cefepime, amoxicillin, ampicillin, ceftazidime. *Pseudomonas* exhibited more resistance (100%) to piperacillin and *Staphylococcus aureus* resistance to ciprofloxacin, ceftazidime. Figure 3 shows percentage of resistance of mycelia obtained from different taps water surfaces in which *Fusarium oxysporium* and *Aspergillus fumigatus*, were resistance to itraconazole, sivoketonazole, griseoufulvin and fluconazole??

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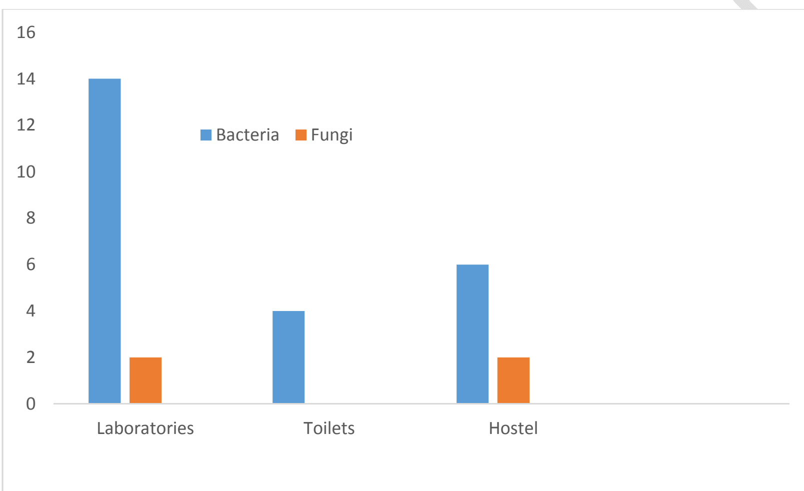

Figure 1: Number~~Load~~ of microorganisms~~bial isolates~~ from different taps water surfaces in FUTA (Obakere campus).

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Table 1: Total number of bacteria ~~loads~~ (CFU~~cfu~~/ml) on~~in~~ ~~different~~ taps water surfaces in FUTA (O~~o~~bakere campus).

	SAMPLE no.~~S~~					
locations	1	2	3	4	5	6
~~A~~ Microbiology and Physics Laboratories	4.0×10^3	6.0×10^3	4.0×10^3	5.0×10^3	7.0×10^3	4.0×10^3
~~B~~ Microbiology and Physics Toilets	4.0×10^3	NG	3.0×10^3	NG	7.0×10^3	NG

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C Hostel (Akindeko).	NG	7.0×10^3	5.0×10^3	NG	NG	4.0×10^3

Keys

A- Microbiology and Physics Laboratories, B- Microbiology and Physics Toilets, C- Hostel (Akindeko).

Table 2: Total number of fungi loads on in different taps water surfaces in FUTA (Oobakere campus).

Sample locations	**Load (sfu/ml)**
Laboratory	2.0×10^3
Hostel	2.0×10^3

Table 3: Bacterial and fungal isolates (%) in various taps water surfaces in FUTA

Bacteria	Number of tap water tested positive	Frequency distribution (%)
Pseudomonas aeruginosa	6	26.09
Escherichia coli	5	21.74
Citrobacter~~.~~ freundii	2	8.70
Bacillus cereus	2	8.70
Klebsiella pneumoniae	3	13.04
~~*Acinetobacter baumannii*~~	~~3~~	~~13.04~~
Staphylococcus aureus	1	4.35
Klebsiella oxytoca	1	4.35
Total	23	100~~.01~~
Fungi		
Candida albicans	1	25
Aspergillus niger	1	25
Aspergillus fumigatus	1	25
Fusarium oxysporium	1	25
Total	4	100

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Comment [J17]: Number of bacteria?

Comment [J18]: No of fungi?

Table 3a: Antibiotics sensitivity test pattern of bacteria isolated from various taps water surfaces in FUTA

Antibiotics	*C. ftreundii*	*E. coli*	*P. aeruginosa*	*B.~~acillus~~ cereus*	*A. ~~baumannii~~*	*K. pneumoniae*	*S.~~taphylococcus~~ aureus*	*K. oxytoca*
CIP	32.33±0.58^{i}	20.33±0.58^{e}	6.00±1.00^{b}	32.67±0.58^{e}	~~33.33±0.58^{i}~~	6.33±0.58^{b}	29.33±0.58^{d}	30.00±0.00^{i}
CRO	9.67±0.58^{c}	14.33±0.58^{c}	22.33±0.58^{f}	6.33±0.58^{b}	~~34.00±0.00^{j}~~	22.33±0.58^{f}	6.33±0.58^{b}	25.33±0.58^{g}
CAZ	6.00±0.00^{b}	6.33±0.58^{b}	6.33±0.58^{b}	6.00±0.00^{b}	~~14.00±0.00^{e}~~	6.00±0.00^{b}	6.00±0.00^{b}	20.67±0.58^{e}
TE	16.33±0.58^{e}	20.33±0.58^{e}	12.33±0.58^{d}	6.33±0.58^{b}	~~16.67±0.58^{f}~~	20.33±0.58^{e}	0.00±0.00^{a}	0.00±0.00^{a}
AMP	18.33±0.58^{g}	19.33±0.58^{d}	11.33±0.58^{c}	6.33±0.58^{b}	~~12.33±0.58^{d}~~	9.33±0.58^{c}	6.00±0.00^{b}	17.33±0.58^{d}
FEP	6.33±0.58^{b}	6.33±0.58^{b}	5.67±0.58^{b}	6.00±0.00^{b}	~~27.00±0.00^{g}~~	6.33±0.58^{b}	0.00±0.00^{a}	14.33±0.58^{c}
C	17.00±0.00^{f}	20.33±0.58^{e}	0.00±0.00^{a}	6.33±0.58^{b}	~~6.33±0.58^{b}~~	0.00±0.00^{a}	6.33±0.58^{b}	15.00±0.00^{c}
AMC	12.33±0.58^{d}	21.33±0.58^{f}	15.33±0.58^{e}	7.33±0.58^{c}	~~0.00±0.00^{a}~~	20.00±0.00^{e}	0.00±0.00^{a}	30.67±0.58^{i}
SXT	0.00±0.00^{a}	0.00±0.00^{a}	30.33±0.58^{g}	6.33±0.58^{b}	~~10.00±0.00^{e}~~	0.00±0.00^{a}	0.00±0.00^{a}	30.33±0.58^{i}
PRL	0.00±0.00^{a}	0.00±0.00^{a}	6.33±0.58^{b}	30.00±0.00^{d}	~~0.00±0.00^{a}~~	0.00±0.00^{a}	0.00±0.00^{a}	6.33±0.58^{b}
CN	28.00±0.00^{g}	26.33±0.58^{g}	0.00±0.00^{a}	0.00±0.00^{a}	~~28.33±0.58^{h}~~	10.33±0.58^{d}	10.33±0.58^{c}	22.67±0.58^{f}
CL	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	~~0.00±0.00^{a}~~	0.00±0.00^{a}	6.33±0.58^{b}	0.00±0.00^{a}
AML	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	~~0.00±0.00^{a}~~	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
S3	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	~~0.00±0.00^{a}~~	0.00±0.00^{a}	0.00±0.00^{a}	28.33±0.58^{h}

Data are presented as Mean± S.D. (n=3). Values with the same superscript letter(s) along the same column are not significantly different ($P<0.05$).

CIP- Ciprofloxacin, CRO- Ceftriaxone , CAZ-Ceftazidime , TE-Tetracycline, AMP-Ampicillin, FEP- Cefepime , C-Chloramphenicol, AMC- Amoxicillin-clavulanate , SXT- Trimethoprim-sulphamethoxazole, PRL- Piperacillin-, CN- Gentamicin, CL- , -AML- Amocilin, S3- Compound sulphurnamide

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Comment [J19]: Instead of these letters, indicate S, I or R pattern according to the CLSI

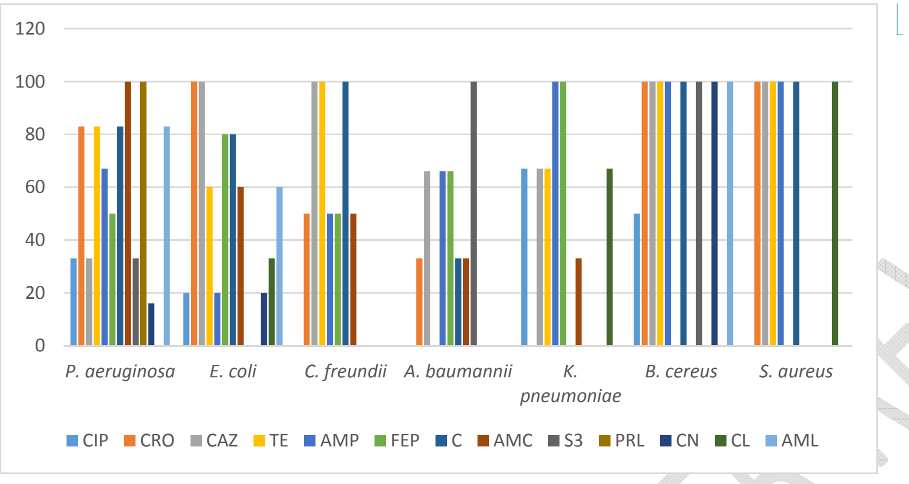

Figure 2: Percentage resistance of bacteria isolated from different taps water surfaces in FUTA (Obakere campus)

Table 4: Antifungal sensitivity pattern of fungi isolated from tap water in various taps water surfaces in FUTA

Antifungal	*C. albicans*	*A. niger*	*A. fumigatus*	*F. oxysporium*
Itraconazole	15.33±0.58[c]	13.33±0.58[d]	6.00±0.00[a]	6.33±0.58[a]
Sivoketonazole	11.33±0.58[b]	9.33±0.58[b]	6.67±0.58[a]	6.33±0.58[a]
Griseoufulvin	7.67±0.58[a]	11.00±0.00[c]	6.33±0.58[a]	6.00±0.00[a]
Fluconazole	7.33±0.58[a]	7.67±0.58[a]	6.33±0.58[a]	6.66±0.58[a]

Data are presented as Mean±S.D. (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05

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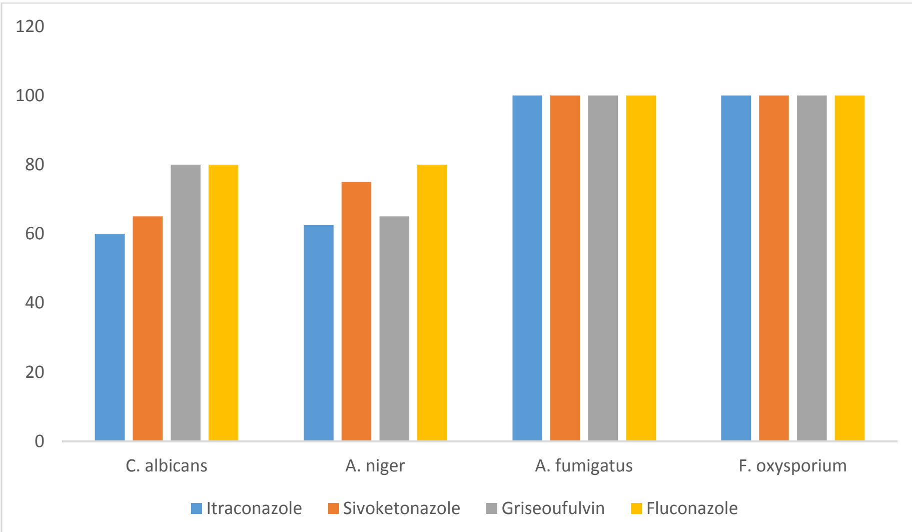

Figure 3: Percentage resistance of bacteria isolated from different taps water surfaces in FUTA (Obakere campus)

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DISCUSSION

The emergent of microorganism's resistance to commonly available antimicrobial agents have become the thing of concern and major challenges to the public for the past decades (Omoya and Ajayi, 2016). The high percentage of microorganisms being resistance to available antimicrobial agents are not farfetched, and this is because some of them are intrinsically resistance and others acquire resistance gene through their common exposure to the available agents. The acquired of multidrug resistance microorganisms of water origin occur through consumption of contaminated water that harbour pathogenic microorganisms (Omoya and Ajayi, 2016).

The continuous release of water through unwashed and contaminated tap water surface into the environment (Federal University of Technology Akure) (FUTA) will not only add to pernicious effect of pollution but increase the microbial load that could be pathogenic in nature. This will eventually lead to resistance in antimicrobial agents and the state of mortality in human health (Ogidi and Oyetayo, 2013).

This research revealed the microbial load and the types of microorganisms associated with taps water surfaces in FUTA, which are directly discharged into the FUTA community. The percentage of bacterial and fungal counts from taps water surface are in agreement with the finding of (Bello *et al.*, 2013), who reported such index of 10^3 to 10^4 in well water sample in Ijebu metropolis. Moreover (Orogu *et al.,* 2017), who worked pipe-borne chlorinated water and untreated water in Iloin and (Samie *et al.,* 2011) also revealed such load of microorganisms in borehole water used by school children in Mopani district, South Africa and these people proposed that the identity of pathogenic isolates indicated the high number of organisms including *Citrobacter freundii* and *Klebsiella pneumonieae* were present.

The prevalence of enterobacteriaceae Enterobacterales family could result in contamination of taps water surfaces and the prevalence of the organisms is probably due to degree of direct and indirect contact of people working in laboratory and exposing the pathogenic microorganism which already acquired resistance gene in the lLaboratory. The high percentage of microorganism obtained in the laboratory was probably due to genetic mutation. Total number of seven bacteria *Staphylococcus aureus*, *Acinectobacter bourmanii, E.scherichia coli, P.seudomonas aeruginosa, Citrobacter C. freundii, K.lebsiella pneumoniae* and *Bacillus cereus* and four fungal isolates (*Candida albicans Aspergillus niger*, *Aspergillus fumigatus* and *Fusarium oxysporium*) were isolated from taps water surface. The presence of these organisms conformed to the work of (Orogu *et al.,* 2017), who reported similar organisms in their work.

The presence of Gram positive *S. aureus* formed cluster, positive to catalase and coagulase and resistance to antibiotics was similar to the finding of Orogun *et al.,* (2017). This organism causes food poison when present in contaminated water used to prepared food. The presence of this organism in the tap water surface was a great reflection of poor hygiene and sanitary condition of taps water surfaces in study area.

The high multidrug-resistance of *Pseudomonas aeruginosa* to multidrug piperacillin, ceftriaxone, amoxicillin, augumentin, tetracycline, ceftazidime and chloramphenicol was recorded. This is in conjunction with the finding of Orogu *et al*, (2017). High infection caused by *Pseudomonas aeruginosa* are difficult to treat due to it multidrug resistance *(*Ogidi and Oyetayo, 2013).

The presence of these organisms in taps water surfaces are of public concerns as these organisms are likely to cause an increased incidence of waterborne diseases and thereby against the rules life sustainability (Ogidi and Oyetayo, 2013). The high prevalence of *Klebsiella pneumoniae* in taps water surfaces was due to high persistence adherence to iron pipe and the ability to caused resistance to commonly available antibiotics occur as result of it high virulence factor (Ayandiran *et al*., 2014).

Bacillus cereus is known of medical importance. The presence of this organism in taps water surface and the ability to caused resistance to available antibiotics could have occurred as result of the spore formation which enable it to adapt in extreme conditions Orogu *et al*, (2017).

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The isolated fungi from tap~~s~~ water surfaces are similar to the finding of Ogidi and Oyetayo, (2013), who worked on susceptibility pattern of microorganisms isolated from remnant foods and waste water from restaurants in Akure metropolis and Ogidi and Oyetayo, (2013) who worked on clinical fungi isolates. The resistance of *Aspergillus fumigatus, Fusarium oxysporium* and *Aspergillus niger* to all the antifungal agents was similar to the finding of Ogidi and Oyetayo, (2013) and susceptibility of the *Candida albicans* to these agents justified the finding of Ogidi and Oyetayo, (2013). The in inhibition activity of itraconazole was due to cytochrome P-450 dependent enzymes that in inhibit the fungi ergosterol synthesis (Kelley *et al.*, 1998).

However, it is of serious concern as the taps water surfaces in the Federal University of Technology Akure, (FUTA) habour pathogenic microorganisms that are multidrug-~~-~~resistant~~ce~~ hence these tap~~s~~ water surfaces could be vehicle of transfer of these organisms to the people in the study area.

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