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Antimicrobial Susceptibility Pattern of Microorganisms Isolated from Tap Surfaces in Nigeria Campus

T. S. Aderanti[1*], D. J. Arotupin[1], A. T. Adeyemo[2] and T. V. Adegoke[1]

[1]Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Nigeria.
[2]Department of Medical Microbiology and Parasitology, Obafemi Awolowo University Teaching Hospital complex, Ile-Ife, Osun State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors TSA and DJA designed the study, author TSA carried out the research. Author DJA supervised the research, authors TSA and TVA performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author ATA managed the analysis of the research. Author TSA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Resistance of microorganisms to 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 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 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 microbiological methods. Among the pathogenic microorganisms isolated were *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, K. oxytoca Escherichia coli, Bacillus cereus, Citrobacter freundii, Acinectobacter baumannii, Staphylococcus aureus, Aspergillus fumigatus,*

**Corresponding author: E-mail: drtemitopsa@gmail.com;*

Aspergillus niger, Candida albicans and *Fusarium oxysporium*. The percentage distribution varies with *P. aeruginosa* (26.09%) having the highest percentage and *Klebsiella* sp. (4.45%) recorded at the lowest percentage of occurrence. All fungi isolated have simillar percentage distribution. The isolates displayed various levels of resistance to piperacillin (100%), ampicillin (66.67%). ceftazidime (66.67%), tetracycline (83.33%), cefepime (66.67%) and chloramphenicol (66.67%). The resistance to amoxicillin-clavulanate by *E. coli* and *C. freundii* isolated from taps surfaces were 60% and 50%, respectively. *K. pneumoniae* from tap water surface has 100% resistance to cefepime. The high resistance of microorganisms to antimicrobial agents indicated a great threat to people living within FUTA community that constantly obtaining water from these taps surfaces.

Keywords: Antibiotic resistance; water; environment; society.

1. 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 [1]. 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 staff quarters for drinking and other house chores activities. The popularity of tap water during the 20th century is becoming the thing of challenges among poor people especially in developing countries where squalor is very rampant [1]. 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 [2]. In addition, due to the ubiquitous nature of microorganisms, some of them colonized tap water surfaces and contaminate the safe water when such waters are released and running from the tap surfaces. Bacteria and fungi are the most serious concern pathogens in waterborne disease, and these organisms cause gastrointestinal outbreaks around the world [3]. 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 [2]. The challenges of health are common in rural area especially in developing countries where there is lack of adequate potable water [3]. Enteric bacteria are common in human intestine and feaces of animals together with spore forming fungi that commonly colonize taps water surfaces. Bacterial pathogens potentially transmitted through water ingestion include *Escherichia coli*, *Klebsiella* spp., *Salmonella* spp., + other coliforms, and protozoa, while the fungi are *Aspergillus fumigatus, A. niger* and *flavus* [4].

Antibiotic resistance of microorganisms are the major concern and challenges since it existence which has resulted in death and state of dyeing of 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 [4]. 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* [5].

Therefore, this study focused on the antimicrobial susceptibility pattern of microorganisms isolated from different tap water surfaces in the Federal University of Technology Akure (FUTA), Nigeria, with the aim to put in place good sanitation measures in regard to drinking water supply.

2. MATERIALS AND METHODS

2.1 Location and Collection of Samples

This research work was carried out from June 2018 to August 2018 in Akure metropolis, Ondo state, Nigeria. Fifteen different tap surfaces (laboratory, hostels and toilets) were swabbed with sterile swab stick and transferred aseptically to the laboratory for further analysis.

2.2 Isolation of Microorganisms

Microbial identification was carried out using standard conventional microbiological methods [6]. 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 removed 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°C for 15 minutes. The plates were incubated at 25°C/72h for fungi and at 37°C/24h for bacteria.

2.3 Biochemical Characterization

Biochemical characterization and presumptive identification of isolates were carried out as described by Olutiola et al. [7].

2.4 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 CLSI [8]. 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 sulphanilamide and cloxacilin 5µg.

Antifungal susceptibility was determined according to Bauer et al. [9]. Antifungal agents used were griseofulvin (50mg), ketoconazole (20mg), fluconazole (20mg) and itraconazole (20mg).

2.5 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 $p \leq 0.05$.

3. RESULTS

Tables 1 and 2 show total bacterial and fungal viable counts in different taps surfaces in FUTA with highest loads of viable bacteria in microbiological laboratory. Fig. 1 shows the load of isolates from taps surfaces. The most frequently isolated microorganisms were *Pseudomonas aeruginosa* (26.09%), *Escherichia coli* (21.74%), *Klebsiella pneumoniae* (13.04%), *Candida albicans* (25.0%), *Aspergillus niger* (25.0%). The results of antibiotic and antifungal sensitivity of isolates to commonly available agents are shown in Tables 3 and 4, respectively. The percentage resistance of bacteria isolated from different taps surfaces (Fig. 2) where mostly the bacteria isolates which exhibited high resistance (33-100%) topiperacillin, ceftriazone, cefepime, amoxicillin, ampicillin, ceftazidime. *Pseudomonas aruginosa* exhibited more resistance (100%) to piperacillin and *Staphylococcus aureus* resistance to ciprofloxacin, ceftazidime. Fig. 3 shows percentage of resistance of mycelia obtained from different taps surfaces in which *Fusarium oxysporium* and *Aspergillus fumigatus,* were resistance to itraconazole, sivoketonazole, griseoufulvin and fluconazole??

5. 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 [10]. 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 [10].

The continuous release of water through unwashed and contaminated taps surface into the environment (Federal University of

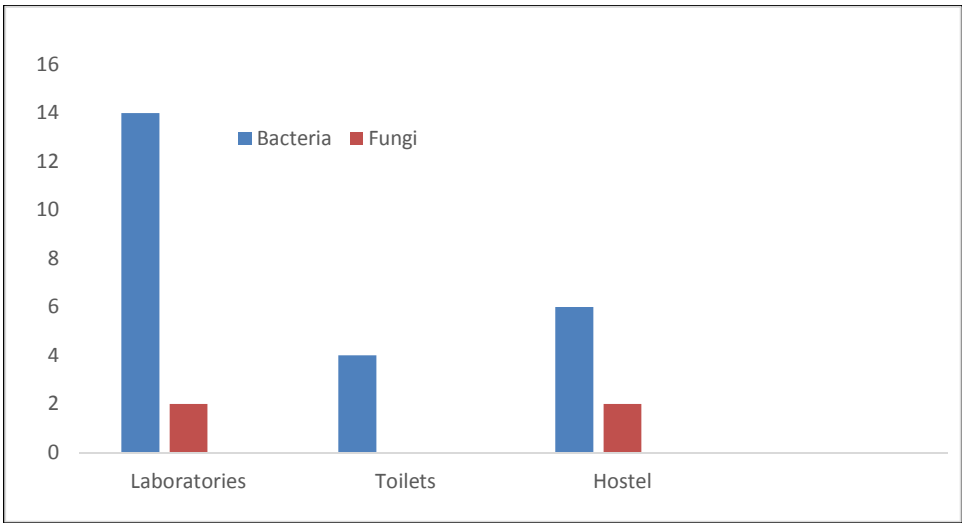

Fig. 1. Number of microorganisms from different taps surfaces in FUTA (Obakere campus)

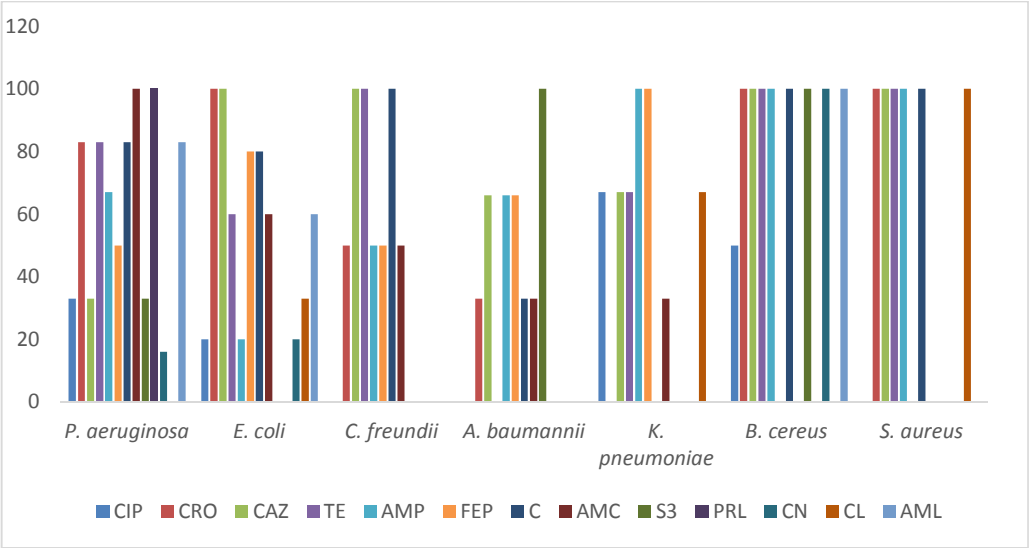

Fig. 2. Percentage resistance of bacteria isolated from different taps surfaces in FUTA (Obakere campus)

Table 1. Total number of bacteria (CFU/ml) on taps surfaces in FUTA (Obakere campus)

Locations	Sample No.					
	1	2	3	4	5	6
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
Microbiology and Physics Toilets	4.0×10^3	No Growth	3.0×10^3	NG	7.0×10^3	No Growth
Hostel (Akindeko)	No Growth	7.0×10^3	5.0×10^3	NG	No Growth	4.0×10^3

Table 2. Total number of fungi on different taps surfaces in FUTA (Obakere 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 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
Staphylococcus aureus	1	4.35
Klebsiella oxytoca	1	4.35
Total	23	100
Fungi		
Candida albicans	1	25
Aspergillus niger	1	25
Aspergillus fumigatus	1	25
Fusarium oxysporium	1	25
Total	4	100

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 [11].

This research revealed the microbial load and the types of microorganisms associated with taps surfaces in FUTA, which are directly discharged into the FUTA community and evaluation of resistance pattern of these microorganisms against antimicrobial agents. The percentage of bacterial and fungal counts from taps surface are in agreement with the finding of [12], who reported such index of $6x10^3$ to $4x10^4$ in well water sample in Ijebu metropolis. Moreover [13], who worked pipe-borne chlorinated water and untreated water in Ilorin and [2] 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 Enterobacterales family could result in contamination of taps 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 laboratory. The high percentage of microorganism obtained in the laboratory was probably due to genetic mutation. Total number of seven bacteria *Staphylococcus aureus*, *E. coli, P. aeruginosa, C. freundii, K. pneumoniae* and *B. cereus* and four fungal isolates (*Candida albicans Aspergillus niger, Aspergillus fumigatus* and *Fusarium oxysporium*) were isolated from tap water surface. The presence of these organisms conformed to the work of [13], 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 [13]. This organism causes food poison when present in contaminated water used to prepared food. The presence of this organism in the tap surface was a great reflection of poor hygiene and sanitary condition of taps surfaces in study area.

The multidrug-resistance of *Pseudomonas aeruginosa* to piperacillin, ceftriaxone, amoxicillin, augumentin, tetracycline, ceftazidime and chloramphenicol was recorded. This is in conjunction with the finding of [13]. High infection caused by *Pseudomonas aeruginosa* are difficult to treat due to it multidrug resistance [11].

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

Antibiotics	*C. freundii*	*E. coli*	*P. aeruginosa*	*B. cereus*	*K. pneumoniae*	*S. aureus*	*K. oxytoca*
CIP	32.33±0.58^{i}	20.33±0.58^{e}	6.00±1.00^{b}	32.67±0.58^{e}	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}	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}	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}	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}	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}	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}	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}	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}	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}	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}	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}	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}
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}	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- Chloromycetin, AML- Amoxicillin, S3- Compound sulphanilamide

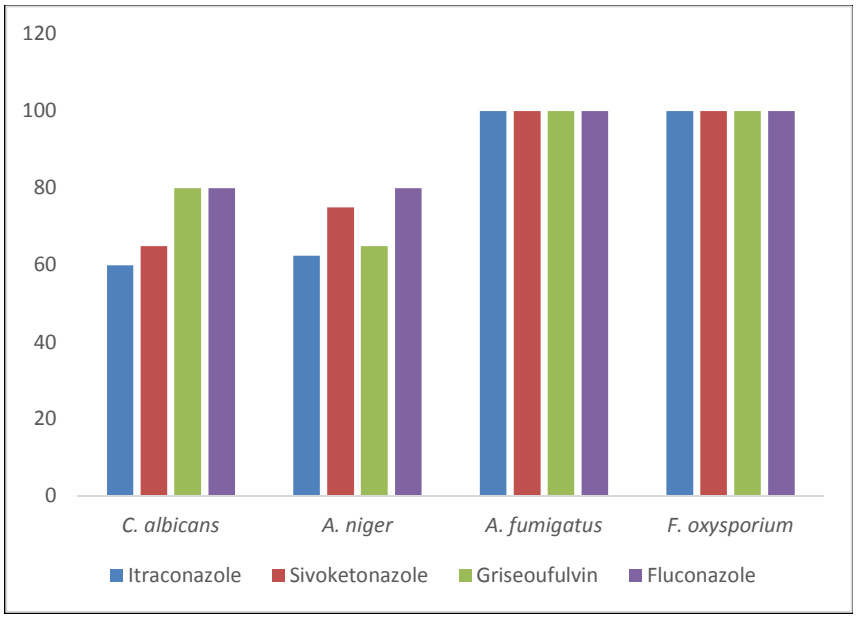

Fig. 3. Percentage resistance of isolated fungi from different taps surfaces in FUTA (Obakere campus)

Table 4. Antifungal sensitivity pattern of fungi isolated from various taps 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

The presence of these organisms in tap surfaces are of public concerns as these organisms are likely to cause an increased incidence of waterborne diseases and thereby against the rules of life sustainability [11]. The high prevalence of *Klebsiella pneumoniae* in taps surfaces could be due to high persistence adherence to iron pipe and the ability to caused resistance to commonly available antibiotics could occur as result of it high virulence factor.

Bacillus cereus is known of medical importance. The presence of this organism in taps 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 [14,13].

The isolated fungi from taps surfaces are similar to the finding of [11], who worked on susceptibility pattern of microorganisms isolated from remnant foods and waste water from restaurants in Akure metropolis and [11] 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 [11] and susceptibility of the *Candida albicans* to these agents justified the finding of [11]. The inhibition activity of itraconazole was due to cytochrome P-450 dependent enzymes that inhibit the fungi ergosterol synthesis [15].

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

6. CONCLUSION

This research has revealed the high load of microorganism colonized the taps surfaces and

their capability to cause infections, if water coming out from these taps surfaces are been consumed daily either for drinking or in other forms of house chores activities.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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