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Culture-dependent characterization of microbes in biofilms from selected microhabitats in Rivers State, Nigeria

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Biofilms from different microhabitats such as air conditioner outlets, drainage system pipes, laboratory and kitchen sinks were sampled twice from the same sample sites at interval of four weeks. The sample sites were University of Port Harcourt, Abuja campus, University of Port Harcourt Teaching Hospital and Alakahia town, Rivers State. The significance of the study was to determine the various groups of bacteria and fungi involved in biofilm formation within the microhabitats sampled. Such knowledge will help us to understand the health implications of the individuals at risk within the environment sampled. A total of 48 bacterial and 34 fungal species covering 10 and four genera, respectively were isolated and characterized as *Bacillus, Enterobacter, Escherichia, Klebsiella, Salmonella, Staphylococcus, Streptococcus, Proteus, Pseudomonas*, *Vibrio; Aspergillus, Mucor*, *Penicillium* and *Saccharomyces*. Drainage system pipes had the highest frequency of bacterial and fungal microbial composition. On the other hand, kitchen sinks had the lowest frequency of bacterial isolates while laboratory sinks had the lowest fungal microbial communities isolated from the biofilms. The variation in microbial load was attributed to varying favourable conditions which aided in biofilm development at various stages in the respective microhabitats. Industrial and medical equipment need to be monitored for biofilm attachment and such discourage their formation where it is not beneficial with appropriate biocide applications.

Key words: Biofilms, health, microhabitats.

INTRODUCTION

Microorganisms have developed many defence mechanisms to ensure survival under hostile environmental conditions, the formation of biofilms is one of such defence mechanisms. Biofilms are aggregate of sessile adherent microorganisms embedded within self produced extracellular polymeric substance (EPS) consisting mainly of extracellular DNA, proteins and polysaccharides (Banerjee et al., 2012). They are usually formed on moist living or non- living solid surface. Microorganisms form biofilm to ensure survival, upon forma-

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tion there are several beneficial interactions which they derive such as change in extracellular polymeric substance composition, improved nutrient utilization, metabolic cooperation, community-based regulation of gene expression, increase resistance to antimicrobials and human immune responses, and spatial distribution within the mixed biofilm community (Hall-Stoodley et al., 2004; Hoiby et al., 2010; Banerjee et al., 2012; Elias and Banin, 2012). Among factors which affect the rate of bacterial attachment and biofilm formation are electrochemical properties of the surface, liquid flow, nutrient availability, pH, temperature, texture (Donlan, 2002; Mattila, 2002; Banerjee et al., 2012).

Biofilms present serious problems to human as they can be formed in every environment inhabited by bacteria and fungi. They only require presence of hydrated environ-ment and minimum amount of nutrients for their formation. The benefits of biofilm formation to bacteria and fungi include increased anti-biotic resistances, synergism between species, and domination of immediate environment. Their impacts upon formation include damage of industrial equipment, contamination of food which can cause food-borne infections and food poisoning, nosocomial infections. It is necessary that we monitor our medical devices, water distribution system pipes, and other drainage accessories for biofilm formation. Although biofilm formations in different environments have many implications, they can be beneficial in bioremediation of hazardous materials and waste sites, biofilteration of industrial waste water, formation of natural biological barriers to protect soil and groundwater from contamination. Microorganisms within biofilms benefit from various interactions which increase their survival in hostile environment, competition and growth inhibition may arise resulting in some species out competing others thereby rendering the biofilm architecture vulnerable to antimicrobials and other unfriendly environmental conditions (Elias and Banin, 2012).

The research was carried out to analyze the diversity of bacteria and fungi in biofilms from selected microhabitats using culture-dependent techniques and compare if the various groups of isolated bacteria and fungi have any health implications to individuals at risk in the various microhabitats and sites used for the study.

MATERIALS AND METHODS

Sampling

Mature biofilm samples from air conditioner outlets, drainage system pipes, laboratory and kitchen sinks from Alakahia town, University of Port Harcourt Abuja Campus and University of Port Harcourt Teaching Hospital were aseptically collected with sterile swabs and transported to Microbiology Laboratory University of Port Harcourt. In the laboratory, 2 ml phosphate buffer saline was added to each swab containing the biofilm samples and were stored in a refrigerator at 4°C for 24 h before analyses were carried out. In order to ensure the isolation of indigenous microorganisms involved in the biofilm attachment and formation, sampling was done twice from the same site at interval of four weeks. Prior to each sample collection, each microhabitat was intensively cleaned (Invistky et al., 2007) to ensure that the isolated microorganisms were in biofilms and not contaminants.

Enumeration and isolation of microorganisms

Different media were used including MacConkey agar, mannitol salt agar, nutrient agar, plate count agar, phosphate buffer saline, Sabouraud dextrose agar, Salmonella-shigella agar (SSA). 1 ml of each sample was transferred into test tubes containing 9 ml of 0.1% peptone water as diluents (Banerjee et al., 2012) and was serially diluted up to 10^{-6}. 0.1 ml of each dilution was aseptically transferred onto the media mentioned herein and was spread uniformly with sterile glass rod. The inoculated agar plates for bacteria isolation were incubated at 37°C for 24-48 h and the colony forming units (CFU) were recorded from the plate count agar (PCA). Sabouraud dextrose agar plates supplemented with a known concentration of antibiotics (Chloramphenicol 0.5 g/L) which was used for fungi isolation were incubated at 28°C for 10 days. Purification of the isolates was done on the respective agar plates and the isolates were maintained on nutrient agar slants in a freezer at 4°C for further analyses. MacConkey agar plates served as a differential medium for the isolation of the bacterial isolates while mannitol salt agar plates were used for the isolation of *Staphylococcus* spp. *Salmonellae* were isolated on SSA.

Microscopic and biochemical characterization of bacterial and fungal isolates

Bacterial and fungal isolates were tentatively characterized based on morphological features such as cell shape, size, and Gram reaction. Biochemical tests were also carried out on the bacterial isolates according to Cheesbrough (2006). After the various tests, the bacterial isolates were identified according to Bergey's Manual of Determinative Bacteriology (Holt et al., 1994). On the other hand, fungi atlas was used to identify the fungi to genus level using the microscope (Malloch, 1940).

RESULTS

Only samples sites which had the same microorganisms isolated in the first and second sampling were considered as microhabitats having biofilms. Other sample sites which had different microorganisms isolated during the different sampling were not considered as having biofilm, the isolated microorganisms from such sites were considered to be allochthonous (temporal or transient) or contaminating microorganisms. A total of 48 bacteria covering 10 genera and 34 fungi covering four genera were isolated on differential, routine and selective media and were characterized as: *Bacillus, Enterobacter, Escherichia, Klebsiella, Salmonella, Staphylococcus, Streptococcus, Proteus, Pseudomonas, Vibrio; Aspergillus*, *Mucor*, *Penicillium,* and *Saccharomyces* (Tables 1-8). Members of the isolated bacteria belong to various groups of Gram negative and Gram positive

Table 1. Bacterial isolates from air conditioner outlets

Isolate code	Gram reaction	Shape	Oxidase	Indole	Methylred	Voges-Proskauer	Motility	Urease	Citrate	Catalase	Slant/butt	H_2S/Gas	Glucose	Lactose	Mannitol	Maltose	Sucrose	Tentative Identity
AOA_1	+	C	-	-	-	+	-	-	+	+	K/K	-/-	+	+	+	+	+	*Staphylococcus* sp.
AOA_2	-	R	+	-	-	-	+	-	+	+	K/K	-/-	-	-	+	-	-	*Pseudomonas* sp.
AOA_3	-	R	-	+	+	-	+	-	-	+	A/A	-/+	+	+	-	+	-	*Escherichia* sp.
SOA_1	+	C	-	-	-	+	-	-	+	+	K/K	-/-	+	+	+	+	+	*Staphylococcus* sp.
SOA_2	-	R	-	+	+	-	+	-	-	+	A/A	-/+	+	+	-	+	-	*Escherichia* sp.
TOA_1	+	C	-	-	-	+	-	-	+	+	K/K	-/-	+	+	+	+	+	*Staphylococcus* sp.
THA_1	+	C	-	-	-	+	-	-	+	+	K/K	-/-	+	+	+	+	+	*Staphylococcus* sp.
ThA_1	+	C	-	-	-	+	-	-	+	+	K/K	-/-	+	+	+	+	+	*Staphylococcus* sp.
ThA_2	-	R	+	-	-	-	+	-	+	+	K/K	-/-	-	-	+	-	-	*Pseudomonas* sp.

Positive (+), negative (-), cocci (C), rod (R), alkaline (K), acid (A). Isolate code: the first and second letters represent the sample location while the third (last) letters represent the sample type (air conditioner outlets). The Arabic numerals represent the isolate number.

Table 2. Bacterial isolates from drainage system pipes.

Isolate code	Gram reaction	Shape	Oxiase	Indole	Methylred	Voges-Proskauer	Motility	Urease	Citrate	Catalase	Slant/butt	H_2S/Gas	Glucose	Lactose	Mannitol	Maltose	Sucrose	Tentative identity
HD_1	-	R	-	-	+	-	+	-	+	-	K/A	+/+	+	+	-	+	+	*Salmonella* sp.
HD_2	-	R	+	+	+	-	+	-	+	+	A/A	-/-	+	-	-	+	+	*Vibrio* sp.
HD_3	+	C	-	-	-	+	-	-	+	+	K/K	-/-	+	+	+	+	+	*Staphylococcus* sp.
HD_4	-	R	-	+	+	-	+	-	-	+	A/A	-/+	+	+	-	+	-	*Escherichia* sp.
HD_5	-	R	+	-	-	-	+	-	+	+	K/K	-/-	-	-	+	-	-	*Pseudomonas* sp.
hD_1	-	R	+	-	-	-	+	-	+	+	K/K	-/-	-	-	+	-	-	*Pseudomonas* sp.
hD_2	+	C	-	-	-	+	-	-	+	+	K/K	-/-	+	+	+	+	+	*Staphylococcus* sp.
hD_3	-	R	-	+	+	-	+	-	-	+	A/A	-/+	+	+	-	+	-	*Escherichia* sp.
MD_1	+	C	-	-	-	+	-	-	+	+	K/K	-/-	+	+	+	+	+	*Staphylococcus* sp.
MD_2	-	R	-	+	+	-	+	-	-	+	A/A	-/+	+	+	-	+	-	*Escherichia* sp.
MD_3	-	R	-	-	-	+	-	-	+	+	A/A	-/+	-	+	+	-	+	*Klebsiella* sp.
MD_4	+	R	+	-	-	+	+	-	+	+	K/A	-/-	-	-	-	-	-	*Bacillus* sp.
ND_1	-	R	-	-	+	-	+	-	+	-	K/A	+/+	+	+	-	+	+	*Salmonella* sp.
ND_2	-	R	-	+	+	-	+	-	-	+	A/A	-/+	+	+	-	+	-	*Escherichia* sp.
ND_3	-	R	-	-	-	+	+	-	+	+	K/A	-/+	-	+	-	-	+	*Enterobacter* sp.
ND_4	-	R	-	-	-	+	-	-	+	+	A/A	-/+	-	+	+	-	+	*Klebsiella* sp.
ND_5	+	R	+	-	-	+	+	-	+	+	K/A	-/-	-	-	-	-	-	*Bacillus* sp.
ND_6	+	C	-	-	-	+	-	-	+	+	K/K	-/-	+	+	+	+	+	*Staphylococcus* sp.
SD_1	+	C	-	-	-	+	-	-	+	+	K/K	-/-	+	+	+	+	+	*Staphylococcus* sp.
TD_1	-	R	-	+	+	-	+	-	-	+	A/A	-/+	+	+	-	+	-	*Escherichia* sp.
TD_2	-	R	-	-	-	+	-	-	+	+	A/A	-/+	-	+	+	-	+	*Klebsiella* sp.
tD_1	-	R	-	+	+	-	+	-	-	+	A/A	-/+	+	+	-	+	-	*Escherichia* sp.
tD_2	-	R	-	-	-	+	-	-	+	+	A/A	-/+	-	+	+	-	+	*Klebsiella* sp.

Positive (+), negative (-), cocci (C), rod (R), alkaline (K), acid (A). Isolate code: the first letters represent the sample site while the second letters represent the sample type (drainage system pipes). The Arabic numerals represent the isolate numbers.

Table 3. Bacterial isolates from laboratory sinks.

Isolate code	Gram reaction	Shape	Oxidase	Indole	MethylRed	Voges-Proskauer	Motility	Urease	Citrate	Catalase	Slant/butt	H_2S/Gas	Glucose	Lactose	Mannitol	Maltose	Sucrose	Tentative identity
US_1	-	R	+	-	-	-	+	-	+	+	K/K	-/-	-	-	+	-	-	*Pseudomonas* sp.
US_2	-	R	-	+	+	+	+	+	+	+	A/A	+/+	+	+	+	+	+	*Proteus* sp.
US_3	-	R	-	+	+	-	+	-	-	+	A/A	-/+	+	+	-	+	-	*Escherichia* sp.
US_4	+	C	-	-	-	+	-	-	+	+	K/K	-/-	+	+	+	+	+	*Staphylococcus* sp.
BS_1	+	C	-	-	-	-	-	-	-	-	K/K	-/-	-	-	-	+	+	*Streptococcus* sp.
BS_2	+	C	-	-	-	+	-	-	+	+	K/K	-/-	+	+	+	+	+	*Staphylococcus* sp.
SS_1	+	C	-	-	-	+	-	-	+	+	K/K	-/-	+	+	+	+	+	*Staphylococcus* sp.
SS_2	+	C	-	-	-	-	-	-	-	-	K/K	-/-	-	-	-	+	+	*Streptococcus* sp.
SS_3	-	R	+	-	-	-	+	-	+	+	K/K	-/-	-	-	+	-	-	*Pseudomonas* sp.
SS_4	-	R	-	+	+	-	+	-	-	+	A/A	-/+	+	+	-	+	-	*Escherichia* sp.

Positive (+), negative (-), cocci (C), rod (R), alkaline (K), acid (A). Isolate code: the first letters represent the sample site while the second letters represent the sample type (laboratory sink). The Arabic numerals represent the isolate numbers.

Table 4. Bacterial isolates from kitchen sinks.

Isolate code	Gram reaction	Shape	Oxidase	Indole	MethylRed	Voges-Proskauer	Motility	Urease	Citrate	Catalase	Slant/butt	H_2S/Gas	Glucose	Lactose	Mannitol	Maltose	Sucrose	Tentative identity
RS_1	-	R	-	+	+	-	+	-	-	+	A/A	-/+	+	+	-	+	-	*Escherichia* sp.
RS_2	+	C	-	-	-	+	-	-	+	+	K/K	-/-	+	+	+	+	+	*Staphylococcus* sp.
RS_3	-	R	-	-	-	+	-	-	+	+	A/A	-/+	-	+	+	-	+	*Klebsiella* sp.
ES_1	+	C	-	-	-	+	-	-	+	+	K/K	-/-	+	+	+	+	+	*Staphylococcus* sp.
ES_2	-	R	-	+	+	-	+	-	-	+	A/A	-/+	+	+	-	+	-	*Escherichia* sp.
ES_3	-	R	-	-	-	+	-	-	+	+	A/A	-/+	-	+	+	-	+	*Klebsiella* sp.

Positive (+), negative (-), cocci (C), rod (R), alkaline (K), acid (A). Isolate code: the first letters represent the sample site while the second letter represents the sample type (kitchen sink). The Arabic numerals represent the isolate numbers.

bacteria 60.42 and 39.58%, respectively. Based on the results, it was observed that samples sites of drainage system had the highest frequency of bacterial (47.92%) and fungal (38.24%) composition involved in the biofilm formation. Restaurants' kitchen sinks had the lowest frequency of bacterial occurrence, while laboratory sinks had the lowest frequency of fungal occurrence 12.50 and 11.76%, respectively (Figure 1). Out of the twenty-three bacteria isolated from the drainage system pipes, two genera (*Salmonella* and *Vibrio*) are well known pathogens which cause gastrointestinal discomfort in humans. Among the genera of bacteria isolated, the highest frequency of occurrence was recorded for *Staphylococcus* (31.25%) while *Enterobacter, Proteus* and *Vibrio* had the lowest frequency of occurrence (Figure 2). *Mucor* genera had the highest frequency of fungi occurrence, while the lowest fungal frequency of occurrence was recorded for *Penicillium* 38.24 and 14.71%, respectively (Figure 3). Sample site ND had more number of bacteria in biofilm compared to other sites (Table 2).

DISCUSSION

The result show that drainage system pipes biofilms

Table 5. Fungal isolates from air conditioner outlets.

Isolate code	Colonial characteristic	Morphological characteristic	Tentative identity
AOA_1	Whitish, pink fluffy surface	Branched conidiophores with some conidia in chains	*Mucor* sp.
AOA_2	Dark brown powdery surface	Septate hyphae with unbranched conidiophores vessicels which produced chain of conidia.	*Aspergillus* sp.
SOA_1	Flat filamentous green velvet colony	Septate hyaline hyphae with branched conidiophores, presence of conidia and phialides	*Penicillium* sp.
SOA_2	Whitish, pink fluffy surface	Branched conidiophores with some conidia in chains	*Mucor* sp.
TOA_1	Dark brown powdery surface	Septate hyphae with unbranched conidiophores vessicels which produced chain of conidia.	*Aspergillus* sp.
THA_1	Green velvet colony with flat surface	Septate hyphae with branched conidiophores	*Penicillium* sp.
THA_2	Whitish, pink fluffy surface	Septate hyaline hyphae with branched conidiophores with some conidia in chains	*Mucor* sp.
ThA1	Dark brown powdery surface	Septate hyphae with unbranched conidiophores vessicels which produced chain of conidia.	*Aspergillus* sp.
thA1	Creamy appearance, round raised colony shinny opaque colony	Conidia was produced by anelide, another type of conidia genus	*Saccharomyces* sp.

Table 6.

Isolate code	Colonial characteristic	Morphological characteristic	Tentative identity
HD_1	Whitish, pink fluffy surface	Branched conidiophores with some conidia in chains	*Mucor* sp.
hD_1	Creamy appearance, round raised colony shinny opaque colony	Conidia was produced by anelide, another type of conidia genus	*Saccharomyces* sp.
hD_2	Whitish, pink fluffy surface	Branched conidiophores with some conidia in chains	*Mucor* sp.
hD_3	Dark brown powdery surface	Septate hyphae with unbranched conidiophores vessicels which produced chain of conidia.	*Aspergillus* sp.
MD_1	Creamy appearance, round raised colony shinny opaque colony	Conidia was produced by anelide, another type of conidia genus	*Saccharomyces* sp.
ND_1	Dark brown powdery surface	Septate hyphae with unbranched conidiophores vessicels which produced chain of conidia.	*Aspergillus* sp.
ND_2	Dark brown powdery surface	Septate hyphae with unbranched conidiophores vessicels which produced chain of conidia.	*Aspergillus* sp.
ND_3	Whitish, pink fluffy surface	Septate hyaline hyphae with branched conidiophores with some conidia in chains	*Mucor* sp.
SD_1	Creamy appearance, round raised colony shinny opaque colony	Conidia was produced by anelide, another type of conidia genus	*Saccharomyces* sp.

Table 6: Contd.

TD_1	Creamy appearance, round raised colony shinny opaque colony	Conidia was produced by anelide, another type of conidia genus	*Saccharomyces* sp.
tD_1	Whitish, pink fluffy surface	Septate hyaline hyphae with branched conidiophores with some conidia in chains	*Mucor* sp.
tD_2	Flat filamentous green velvet colony	Septate hyaline hyphae with branched conidiophores, presence of conidia and phialides	*Penicillium* sp.
AD_1	Creamy appearance, round raised colony shinny opaque colony	Conidia was produced by anelide, another type of conidia genus	*Saccharomyces* sp.

Table 7. Fungal isolates from laboratory sinks.

Isolate code	Colonial characteristic	Morphological characteristic	Tentative identity
US_1	Creamy appearance, round raised colony shinny opaque colony	Conidia was produced by anelide, another type of conidia genus	*Saccharomyces* sp.
MS_1	Whitish, pink fluffy surface	Septate hyaline hyphae with branched conidiophores with some conidia in chains	*Mucor* sp.
SS_1	Flat filamentous green velvet colony	Septate hyaline hyphae with branched conidiophores, presence of conidia and phialides	*Penicillium* sp.
SS_2	Whitish, pink fluffy surface	Septate hyaline hyphae with branched conidiophores with some conidia in chains	*Mucor* sp.

Table 8. Fungal isolates from kitchen sinks.

Isolate code	Colonial characteristic	Morphological characteristic	Tentative identity
AS_1	Dark brown powdery surface	Septate hyphae with unbranched conidiophores vessicels which produced chain of conidia.	*Aspergillus* sp.
PS_1	Whitish, pink fluffy surface	Septate hyaline hyphae with branched conidiophores with some conidia in chains	*Mucor* sp.
pS_1	Creamy appearance, round raised colony shinny opaque colony	Conidia was produced by anelide, another type of conidia genus	*Saccharomyces* sp.
pS_2	Dark brown powdery surface	Septate hyphae with unbranched conidiophores vessicels which produced chain of conidia.	*Aspergillus* sp.
ES_1	Creamy fluffy surface	Branched conidiospores with some conidia in chains	*Mucor* sp.
SS_1	Creamy fluffy surface	Branched conidiospores with some conidia in chains	*Mucor* sp.
GS_1	Flat filamentous green velvet colony	Septate hyaline hyphae with branched conidiophores, presence of conidia and phialides	*Penicillium* sp.
MS_1	Whitish, pink fluffy surface	Septate hyaline hyphae with branched conidiophores with some conidia in chains	*Mucor* sp.

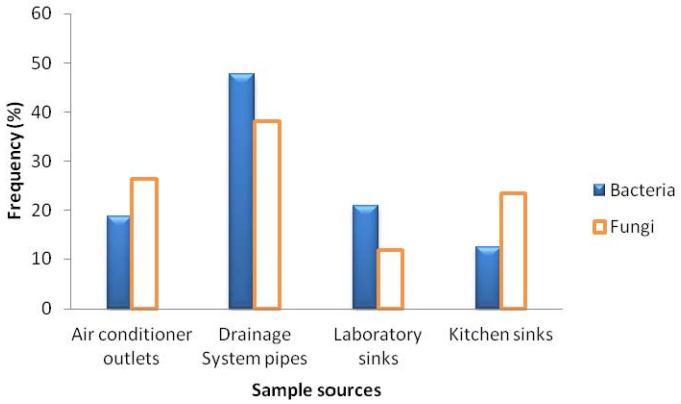

Figure 1. Frequency of occurrence of Microorganisms from the biofilm samples sites

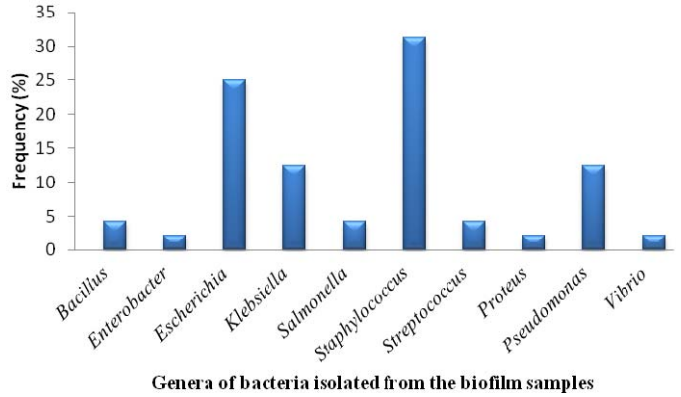

Figure 2. Frequency of bacterial genera isolated from the biofilm samples

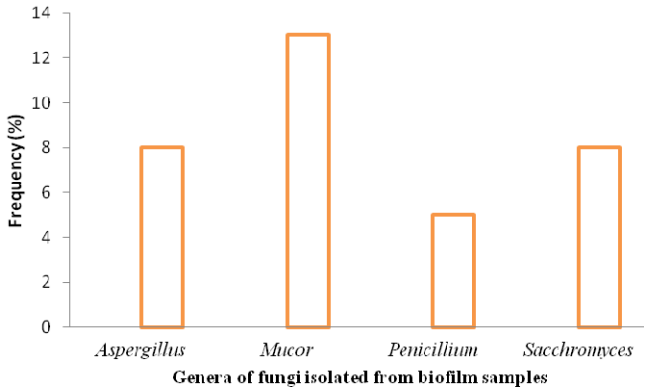

Figure 3. Frequency of fungal genera isolated from the biofilm samples

harboured large number of bacterial and fungal species; such observation was attributed to favourable conditions including increased nutrient availability, moisture and other environmental conditions which aided biofilm attachment and development at various stages when compared to other environments used in the study (McBain et al., 2003). Air conditioner outlets had more fungal than bacterial species in biofilm due to the cold nature of the microhabitat sampled. Some fungi are well known spore former; the presences of *Aspergillus* spp. in such microhabitats pose danger to the health of individuals at risk. Constant exposure to such air conditioner outlets may lead to some aspergillus-related lung disease such as allergic alveolitis, asthma, allergic bronchopulmonary aspergillosis, angioinvasive aspergillosis (Al-Alawi et al., 2005). Most of the bacteria from the hospital sinks were believed to have emanated from cultures and medical personnel in the laboratory. This supported the findings of Hung and Henderson (2009), who observed that biofilms associated with medical surfaces such as intravascular catheters, urinary catheters and prosthetic implants are often derived from the skin flora of patients or medical personnel during device insertion or implantation. They noted that the predominant organisms include: coagulase-negative *Staphylococci, S. aureus, Pseudomonas, Enterococcus, Stenotrophomonas* and *Candida* on intravascular catheters, *Escherichia coli*, Enterococcus*, Pseudomonas, Klebsiella, Enterobacter, Proteus mirabilis* and *Candida* on urinary catheters, and *Staphylococcus* spp. (predominantly *S. epidermidis*) and *Propionibacterium acnes* on prosthetic hip implants. These biofilm-associated bacteria have been implicated in some diseases include native valve endocarditis, otitis media, chronic bacterial prostaitis, cystic fibrosis, periodontitis and other opportunistic and nosocomial infections (Mahami and Adu- Gyamfi, 2011). The higher frequency of bacteria in biofilm (20.83%) from the hospital sites had also been reported by various researchers including Hassan et al. (2011), they applied several methods in detecting biofilm formation in clinical devices. Studies have shown that Gram negative and positive bacterial biofilms are known to be associated with many medical conditions including medical device, dental plaque, upper respiratory tract infections, peritonitis and urogenital infections and the bacteria commonly involved include *Enterobacter faecalis, E. coli, Klebsiella pneumonia, Proteus mirabilis, Psuedomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis* and *Streptococcus viridians* (Reid et al., 1999; Donlan et al., 2001; Hassan et al., 2011). *Staphylococci* are the most commonly cause of nosocomial infections, this was also observed in this study as they had the highest frequency of occurrence (31.25%).

The higher frequency of Gram negative bacteria (60.42%) involved in the biofilm formation in the various microhabitats has two possible explanations. Either the microhabitats sampled were inhabited mainly by Gram negative bacteria or that most Gram negative bacteria have developed various mechanisms which helped and

supported their growth within biofilms. Many Gram negative enteric bacteria which colonize urinary catheters carry plasmids encoding resistance to multiple antimicrobial agents (Donlan, 2002). *Salmonellae* posse cell-surface appendage (SEF-17 fimbrae) that facilitates adhesion to inanimate surface, and provide cell resistance to mechanical forces. Others such as *Escherichia* spp. use flagella, pili and membrane protein to initiate attachment. *Pseudomonas* spp. are known to be ubiquitous, they produce copious amount of extra-cellular polymeric substances which aid their attachment thereby facilitating their coexistence with other Gram negative bacteria forming multispecies biofilms which are more stable and resistant (Chmielewsky and Frank, 2003; Houdt and Michiels, 2005; Sarro et al., 2005). Invistky et al. (2007), reported the predominance of Gram negative bacteria particularly members of *Pseudomonas* genera in biofilm formation. They reported in their study that among the bacterial species isolated, most corresponded to *Proteobacteria* and only small fraction corresponded to Gram positive and others Gram negative. *Proteobacteria* were the most prevalent classes in all and *Pseudomonas, Burkholderia, Ralsotonia, Bacteroidetes* and *Sphingomonas* dominated. In a recent study carried out by Henne et al. (2012), they observed that each biofilm consist of a set of unique phylotype belonging to different classes such as *Alphaproteobacteria, Gammaproteobacteria* and *Deltaproteobacteria* which are present in most biofilms in comparable abundance thus building a community metabolism by system of complex interactions. Banerjee et al. (2012), also in their study of bacterial biofilms in water bodies of Cherrapunjee: the rainiest place on planet earth also observed the predominance of Gram negative bacteria in biofilms, 83.33% of their bacterial isolates were Gram negative among which *Proteus* and *Pseudomonas* predominated; they also observed that attachment and colonization by biofilm bacteria were more on stainless steel than glass surface.

Gram positive bacteria such as *Bacillus* spp. have also been reported for its impressive physiological diversity (Sarro et al., 2005). The specific role of each microbial community on the physiology of biofilms remains unclear (Invitsky et al., 2007). Researchers had shown that *Aspergillus* biofilms can also form on both abiotic and biotic surfaces, while conidia are the initial colonizing cells which adhere to surfaces, the mycelia (the hyphal form) develop as the biofilms mature (Mowat et al., 2009). Various factors such as transcription play important roles in both positive and negative regulation of biofilm formation through regulation of hyphal formation and cell surface proteins responsible for adherence (Finkel et al., 2011). The fewer bacterial spp. observed in the kitchen sinks should not be overlook because raw and minimally-processed food such as fruits and vegetable are high risk factors which aid in transfer of biofilm-associated infections to humans (Sapers, 2005).

Conclusion

Biofilms can be formed in any environment inhabited by microorganisms, their formation can either be beneficial or detrimental to the health of individuals who are exposed to the microhabitats where there are formed. Early detection of biofilms and their removal from equipment where their formation are not needed is of great importance. From the study, it was observed that biofilms as microbial communities can be likened to city of microbes.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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