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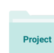
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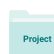
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ORIGINAL ARTICLE

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Prevalence of *E. coli*, *Salmonella*, and *Listeria* spp. as potential pathogens: A comparative study for biofilm of sink drain environment

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Abstract

Since knowledge and understanding of waterborne pathogens and their diseases are well illuminated, a few research publications on the prevalence of pathogenic microorganisms in various household sink drain pipes are often not extensively examined. Therefore, this study aims to (a) assess and monitor the densities of the bacterial community in the different natural biofilm that grow on plastic pipelines, (b) to detect *Escherichia coli*, *Salmonella*, and *Listeria* spp. from natural biofilm samples that are collected from the kitchen (n = 30), bathroom (n = 10), laboratories (n = 13), and hospital (n = 8) sink drainage pipes. Three bacterial species selected were assessed using a culture-dependent approach followed by verification of isolates using both BIOLOG GEN III and polymerase chain reaction. The estimated number of each bacterium was 122 isolates, while 60, 20, 26, and 16 isolates were obtained from the natural biofilm samples, kitchen, bathroom, laboratories, and hospital, respectively. As for the tests, in all types of biofilm samples, the overall bacterial counts at low temperature (22°C) were higher than those at high temperature (37°C). Meanwhile, *E. coli* had the most significant number of bacterial microorganisms compared to the other two pathogens. Additionally, the most massive cell densities of *E. coli*, *Salmonella*, and *Listeria* species were discovered in the biofilm collected from the kitchen, then the hospital. Statistically, the results reveal that there is a positive correlation ($p \geq .0001$) with significance between the sources of biofilm. This work certainly makes the potential of household sink drain pipes for reservoir contagious pathogens more explicitly noticeable. Such knowledge would also be beneficial for prospective consideration of the threat to human public health and the environment.

1 | INTRODUCTION

Although the enlarged reports regarding the occurrence of bacterial biofilms in domestic sink drain pipes, the microbial community composition of this environment is less documented until now (Hemdan, El-Liethy, ElMahdy, & EL-Taweel, 2019). Many studies have identified the potential health risks of biofilm microbial contamination (Awoke, Kassa, & Teshager, 2019; Mcbain et al., 2003). Biofilm usually develop on hydrated surfaces, such as drinking water distribution systems, storage tanks, showerheads, and sink drainage pipes (Elias & Banin, 2012; Moore et al., 2002). The biofilm microbial cells have behaviors that differ from the free cells. Whereas the majority of microbiomes have adhered to the inner surface of water pipes as sessile cells, and about 5% are free in the water column (Hemdan, Sedik, Kamel, & El-Taweel, 2015; Liu et al., 2014). Bacteria can form biofilms and colonize for an extended period in kitchens and bathroom sink, where people may be exposed to a wide range of bacterial pathogens and become more reliable for infection (Sinclair & Gerba, 2011). The

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contact ways of these bacterial pathogens carried out either by the direct way like handling, preparing and eating food or by the indirect way like, contact with surfaces that carry a considerable number of bacterial pathogens that come from different sources, including humans, food and aerosolized water (Medrano-Felix et al., 2011). Domestic sink drain biofilms consider a favorable environment for potentially pathogenic bacteria (Winder & Bonheyo, 2015). In addition to these, it is well-known that sink drains in the hospital considered a significant source of pathogens (Hemdan et al., 2019; Mcgeer et al., 1990). Sink drainage pipes of hospitals are made of different solid surfaces that are suitable substrates for biofilm formation (Niquette, Servais, & Savoir, 2000). Moreover, Sink drainage pipes in laboratories contain different types of bacterial pathogens involved in biofilm formation (Hemdan et al., 2019). Heterotrophic bacteria are commonly enumerated to verify the number of cultivable bacteria in the biofilm by scraping this biofilm from the inner surface of pipes (Gagnon et al. 2005). *Escherichia coli* and *Salmonella* species are gram-negative, rod shape, motile, and belong to the family Enterobacteriaceae (Singleton, 1999). *E. coli* is a normal inhabitant in the intestine of warm blooded animals. *E. coli* is usually used as an indicator for fecal pollution in water and foodstuff (Feng, Weagant, Grant, & Brukhardt, 2002). The presence of *E. coli* in water and food is indicated for the probability presence of enteric pathogens such as *Salmonella* spp. and hepatitis A virus (Odonkor & Ampofo, 2013) and also *E. coli* includes some pathogenic serotypes containing virulence genes (El-Shatoury, El-Leithy, Abou-Zeid, El-Taweel, & El-Senousy, 2015). *E. coli* has been determined in biofilm samples of drinking water and domestic drainage pipes (Juhna et al., 2007; Mcbain et al., 2003). *Salmonella* species are widely distributed in farmhouse wastes, human wastes, and polluted fecal matter. *Salmonella* is frequently detected in large numbers in raw wastewater (10^3–10^4 CFU/L) (Davidson, White, & Surette, 2008; El-Lathy, El-Taweel, El-Sonosy, Samhan, & Moussa, 2009). *Salmonella* is able to form biofilm on plastics, glass, and stainless steel (Hemdan, El-Liethy, Eissam, Kamel, & El-Taweel, 2016; Momba & Kaleni, 2002). Some studies on the biofilm formation process have confirmed that *E. coli*, and *Salmonella* in addition to many other species of the Enterobacteriaceae family, generate cellulose as a vital constituent of the bacterial extracellular matrix (EPS) and its formation is essential for the prolong the survival of these bacteria in the surroundings for an extended period (Lasa, Del Pozo, Penadés, & Leiva, 2005). *Listeria* species are gram-positive, facultative anaerobic, nonspore-forming, and commonly distributed in different environments and can be found in freshwater, soil, animal fecal matter, and sewage (Pagadala et al., 2012). At present the genus *Listeria* contains 20 species. Two out of 20 *Listeria* species namely *L. monocytogenes* and *L. ivanovii* are considered pathogens (Leclercq et al., 2019; Orsi & Wiedmann, 2016). *L. monocytogenes* is the third pathogen among microbes causing foodborne deaths in the United States (Scallan et al., 2011). Besides, *Listeria* species can be trapped on various materials, like those of plastic surfaces, polyethylene, leather, stainless steel, and glass, and it can be easily damaged on the substratum once attached (Pan, Breidt, & Kathariou, 2006). Several previous studies have examined bacterial biofilms, including *E. coli*, *Salmonella*, and *Listeria* species in potable water delivery system pipelines and their simulated prototypes (Pizarro, Vargas, Pastén, & Calle, 2014; Hemdan et al., 2015, 2016). Since the microbial population diversity of these potential bacterial pathogens in the natural biofilm of different sink drainage pipes are not extensively investigated primarily in lab and hospital drainage pipes. The present research aims to investigate the bacterial densities of biofilms that are full-grown on dissimilar drainage pipes and also to study the occurrence of *E. coli*, *Salmonella*, and *Listeria* species amid natural biofilms of kitchen, bathroom, laboratories, and hospital sink drainage pipes. In addition to this, their isolates were confirmed by both BIOLOG and conventional polymerase chain reaction (PCR).

2 | MATERIALS AND METHODS

2.1 | Collection and preparation of the natural biofilm samples

The kitchen (n = 30) and bathroom (n = 10) sink drainage pipes have been obtained from household areas in the Helwan city, 30 km south of Cairo, Egypt. Whereas the pipelines for laboratory sink drainage were obtained from the National Research Centre in the Dokki region, Giza Governrate, Egypt, the laboratories (n = 13), and hospital (n = 8). The collected biofilm ages varied from 1 to 5 years (The period that extend from installing sink drain pipelines to removing them), and all samples were deposited in the icebox and conveyed to the laboratory for microbiological analysis instantly, according to American Public Health Association (APHA) (2017). All procedures in the collection and processing of biofilm samples were undertaken throughout optimal conditions. The existing biofilm samples were extracted using disposable cotton buds to remove 10 cm^2 from the inner layer of the plastic pipes. The swab was dipped into a test tube containing 10 ml of saline solution and homogenized for 5 min with a vortex troublemaker (Zhou, Zhang, & Li, 2009).

2.2 | Determination of total bacterial counts in biofilm samples

In biofilm samples, total heterotrophic bacterial numbers were executed in accordance with APHA (2017) to evaluate the existing bacterial flora at both 37°C and 22°C. The temperature at 37°C was chosen for referring to the presence of enteric bacterial pathogens. Moreover, temperature at 22°C was chosen for referring to the presence of natural bacterial flora in the environment. The suspensions of existing heterogeneous cells were serially diluted in a sterile saline solution to acquire the most appropriate dilution. Specific bacterial numbers on plate count agar (BD DifcoTM) were determined using the pour plate approach. The quantities for total bacterial cells were evaluated and displayed in CFU/cm^2 using an automated colonization monitor (Stuart, FL).

2.3 | Determination and confirmation of potential pathogens in biofilm samples

2.3.1 | Determination of *E. coli*, *Salmonella*, and *Listeria* species

In the natural biofilm samples, the target possible bacterial pathogens were evaluated using a culture-dependent approach on specific agar media. A 100 μl of the sufficient biofilm suspension was transported to the HiChrome ECC agar media to evaluate the cell viabilities of *E. coli*. The inoculated plate was incubated at 37°C for 24 hr. The colonies of *E. coli* appeared in color blue/purple. HiCrome improved salmonella agar was used to enumerate the *Salmonella* species. After optimum incubation, the plates at 37°C for 24–48 hr, the standard morphological characterizing of *Salmonella* species colonies seemed in light pink and pink to red color. Besides, the HiCrome Listeria agar base, modified supplemented HiCrome Listeria selective supplement was being used to count the biofilm cells of *L. monocytogenes*, *L. ivanovii* and *L. innocua*. The inoculated plates have also been kept in the incubator for 24–48 hr at 37°C. The typical colonies of *Listeria* turned up in bluish-green color. All the specific agar media used for chemometrics were supplied from HiMedia, India. Biofilm accumulation was expressed in CFU/10 cm^2 in all of the experiments. For further validation using BIOLOG and PCR, two bacterial isolates from each sample were held at −20°C in tryptic soya broth (TSB) (Oxoid, UK) with 10% glycerol after bacterial growth at 37°C for 18–24 hr.

2.3.2 | Confirmation and metabolic fingerprint of bacterial isolates using BIOLOG

In particular, for confirmatory testing, 366 of the stored bacterial isolates were entered using BIOLOG and PCR and 122 isolates from each of the species *E. coli*, *Salmonella*, and *Listeria*. Confirmations were rendered of presumed bacterial isolates and their biochemical metadata using BIOLOG GEN III (Biolog Inc.) according to El-Liethy, Hemdan, and El-Taweel (2018). On a microplate, each bacterial isolate was checked for 71 styles of carbon and 23 chemical issues. Every storage of bacterial isolates in TSB (Oxoid, UK), *E. coli*, *salmonella*, and *Listeria* species are suspended. The inoculated tubes have already been incubated for 24 hr at 37°C. After that the isolates were streaked onto the plate of tryptic soya agar (TSA) (Oxoid, UK) then the plates were incubated for 18–24 hr at 37°C. Using a sterile removable inoculator swab, a colony was assembled and inoculated into 10 ml of inoculated fluid A (IF-A). The organism-containing IF-A was allocated to 96 microplate wells (100 μl per well). The microplates were incubated at 37°C for 18–24 hr. The reading for each microplate has been taken mechanically by the computerized MicroStation system (Biolog Inc.) with the fingerprint data, which were previously fed into the software.

2.3.3 | Confirmation of bacterial isolates using PCR

Bacterial preparation and DNA extraction

The preserved bacterial isolates were suspended on the TSB medium and then the tubes were kept in the incubator at 37°C for 18–24 hr. According to Kapperud, Vardund, Skjerve, Hornes, and Michaelsen (1993), bacterial DNA extraction was implemented with some adjustments. Approximately, 100 ml of the bacterial isolate was centrifuged at 12,500 rpm for 5 min at 7°C. The obtained pellets by centrifugation were suspended in 50 μl of 1X PCR buffer containing 0.2 mg of proteinase K/ml. For the lysis of the bacterial cell wall, the suspension was incubated at 37°C for 1 hr. The suspension was then heated for 10 min and then centrifuged for 5 min at 4°C at 12,500 rpm. The aqueous phase is used to achieve PCR. The consistency of the obtained DNA was analyzed using Nanodrop (NanoDropTM2000/2000C) spectrophotometers) to test their absorbance at 260 and 280 nm. According to Lucena-Aguilar et al. (2016), the suitable array of the removed DNA for PCR is amid 1.6–1.8 ng/μl.

2.3.4 | The PCR amplification

The PCR amplification was carried out in separate PCR for the selected microorganism isolates. All the primers and PCR conditions used in this study are given in Table 1. The Primers used were properly manufactured by Macrogen Co. (Soul, Republic of Korea). In this study, the primers URL-301 and URR-432 were used for amplification of *E. coli* isolates and these primers targeting regulatory region of *uidA* structural gene in *E. coli* (Bej et al., 1991). Nevertheless, the primers SAL-1F and SAL-2R were selected from conserved sequences within a 2·3 kb randomly cloned DNA fragment from the *Salmonella* Typhimurium chromosome (Aabo et al., 1993). On the other hand, the primers S1F and S1R were selected for the amplification of the 23S rRNA gene in *Listeria* species (Paillard et al., 2003). The PCR reaction mixture of the bacterial isolate was carried out in 20 μl total volume as follows: 4 μl of 5x FIREPol Master Mix Ready to Load with 12.5 mM $MgCl_2$, 0.5 μl (10 pmol) from each primer, and 2.5 μl from DNA extracted isolate. *E. coli* ATCC 25922, *S. enterica* Typhimurium ATCC 14028, *L. monocytogenes* ATCC 25152 were used as positive controls. Agarose gel electrophoresis assessed the amplified materials. Gels were stained with ethidium bromide (0.005%, wt/vol), and use of 100 bp ladder and UVP BioDoc-it Imaging System was used to image the gel under UV transilluminator.

2.4 | Statistical analysis

The statistical study was conducted using GraphPad Prism 5.0. A two-way difference test (ANOVA) and a student *t* test were implemented to determine the values between viable cells using incubation

TABLE 1 Primer sets and PCR conditions used for bacterial isolates confirmation

Bacterial isolates	Primer name	Primer sequence (5′ to 3′)	T*m*°C	PCR conditions (cycle)	Product size (bp)	References
E. coli	URL-301	TGTTACGTCCTGTAGAAAGCCC	62.1	94°C for 1 min, 55°C for 1 min and 72°C. (30x)	153	Bej, Dicesare, Haff, and Atlas (1991)
	URR-432	AAAACTGCCTGGCACAGCAATT	60.3			
Salmonella spp.	SAL-1F	GTA GAAATTCCCAGCGGGTAC TG	64.7	95°C for 30 s, 60°C for 1 min and 72°C for 1.5 min. (40×)	438	Aabo, Rasmussen, Rossen, Sorensen, and Olsen (1993)
	SAL-2R	GTATCCATCTAGCCAACC ATT GC	62.9			
Listeria Spp.	S1F	AGT CGG ATA GTA TCC TTA C	53	94°C for 1 min, 60/55°C for 1 min and 72°C for 1 min (35×)	460	Paillard et al. (2003)
	S1R	GGCTCTAACTACTTGTAG GC	58.4			

Abbreviation: PCR, polymerase chain reaction.

temperatures of 22°C and 37°C. As well, the relationship between three studied bacterial microorganisms is already verified.

3 | RESULTS AND DISCUSSION

3.1 | Determination of total bacterial counts in the biofilm samples

Biofilm formation may be present in any wet area inhabited by natural microbes, including pathogenic bacteria. They can be harmful to the health of individuals who are exposed to microhabitats, including bathroom, kitchen, hospital, and laboratories sink drainage pipes, and so on. (Chikere & Azubuike, 2014). Therefore, the total bacterial counts at both 22°C and 37°C are an essential parameter to determine the bacterial loads in the natural biofilm samples (Walker et al., 2000). In the present study, the total bacterial counts at 22°C were slightly higher than that at 37°C in all biofilm samples (Figure 1). This could be revealed that the lower temperature (22°C) showed the abundance of naturally occurring bacterial flora. In contrast, at high temperature (37°C), numerous bacterial pathogens, and members of Enterobacteriaceae that has direct effect on human health might be present (Gensberger, Gössl, Antonielli, Sessitsch, & Kostić, 2015). Moreover, the highest counts at 22°C may be due to the presence of autochthonous flora that already exists in water where the water temperature is usually between 20–22°C, this temperature considered the optimum for the microbial survival (Korhonen & Martikaine, 1991). In the present work, the highest bacterial counts were observed in biofilm samples collected from kitchen sink drainage pipes followed by the hospital biofilm samples (Figure 1). The kitchen sink is considered as a shelter for huge number of microbial pathogens, while the toilet showed little evidence of contamination with pathogenic microorganisms and microbes of fecal origin (Josephson, Rubino, & Pepper, 1997). Bacteria are capable of inhabiting the kitchen surfaces, and the direct exposure of these microorganisms between humans and the kitchen environment might have a direct outcome on human health (Flores,

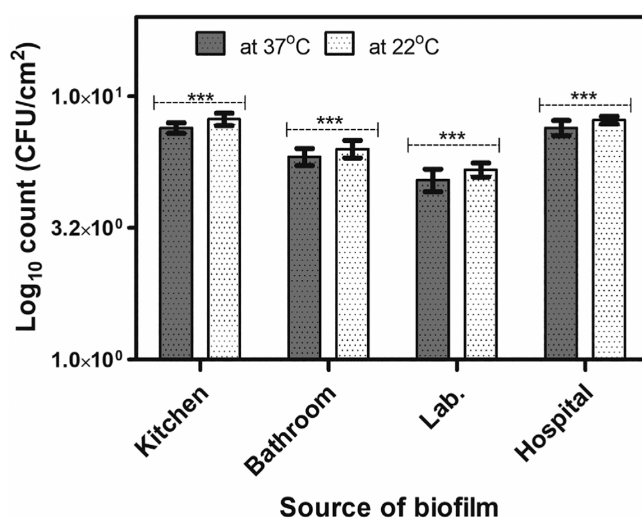

FIGURE 1 Log_{10} counts of the total bacteria at 37°C and 22°C in the natural biofilm cells harvested from different drainage pipes. Two-way analysis of variance states *** means that there is a high correlation ($p \le .001$) with significance between the counts of viable cells at both 22°C and 37°C

Bates, Caporaso, Lauber, & Leff, 2013). Also, the most observed results were that the kitchen sink which plays a role in carrying the large number of *E. coli* and *Enterobacter* spp., whereas, toilet area showed little evidence of contamination with organisms of fecal sources (Hemdan et al., 2019). In this study, the average counts of total bacteria in all collected natural biofilm samples were ranged between 10^4 and 10^8 colony forming unit (CFU/cm^2). In another study carried by Långmark, Storey, Ashbolt, and Stenström (2005) found that the concentrations of total cultivable bacteria in well-established biofilm varied from 10 to 10^6 CFU/cm^2. On the other hand, the total bacterial counts in polypropylene pipe materials biofilm ranged between 2.1×10^5 and 5.5×10^7 CFU/cm^2 (Rogers, Garrison, Grober, Hillis, & Franke, 1994). In the present study, it was found that high statistical correlation with significance ($p \le .001$) was observed between the bacterial counts at 37 and 22°C (Figure 1).

3.2 | Determination of *E. coli*, *Salmonella*, and *Listeria* species in the natural biofilm samples

About 60% of pathogenic microorganisms are involved in the biofilm that presents in drainage pipes, hence research into the development of biofilm communities, their cellular organization, and composition become necessary to evaluate (Cooper, 2011). Consequently, in any sanitation and hygienic programs, several attempts are needed to avoid and control pathogenic contamination for public health, and these can be supported by the assessment of fomite contamination as an indicator for improvement of household sanitation (Sinclair & Gerba, 2011). Hence, the present work was focused on providing data on detection, determination, and identification of *E. coli*, *Salmonella*, and *Listeria* spp. in the natural biofilm samples grown on plastic pipelines of different domestic sink drains. In this research, *E. coli*, *Salmonella* spp. and the most clinical relevant *L. monocytogenes* beside *L. ivanovii* and *L. innocua* isolates were detected in the all-natural biofilm samples. The *E. coli* counts were higher than *Salmonella*, and *Listeria* counts in all biofilm samples (Figure 2). Rusin, Orosz-Coughlin, and Gerba (1998) found that there were more *E. coli* and heterogeneous bacteria in places that were wet or regularly touched by human hands (kitchen faucet handles and kitchen sinks). The three counts of potential pathogens were the highest biofilm samples in the kitchen, followed by biofilm samples in the hospital. The three potential pathogens counts were the highest in the kitchen biofilm samples, followed by in hospital biofilm samples. This may be due to raw vegetables, meats, and chicken; besides, the dishes containing cooked food residue might harbor natural flora and some pathogenic microorganisms are directly washing in the kitchen sink (El-Liethy et al., 2018).

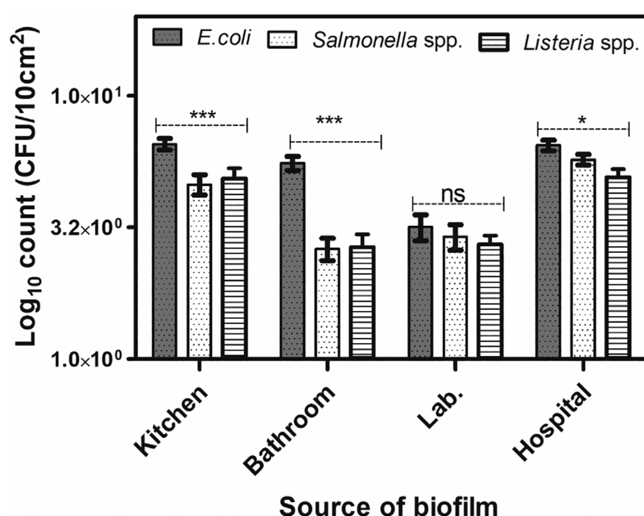

FIGURE 2 Log_{10} counts of *E. coli*, *Salmonella*, and *Listeria* species in the natural biofilm samples harvested from different drainage pipes Two-way analysis of variance between three tested bacterial species statuses show that ns: discloses nonsignificance; * indicates low correlation ($p \leq .05$), and *** discloses high correlation ($p \leq .0001$). At the same time, the statistical result for the source of variation reveals that there is a strong correlation between the source of biofilm with significance ($p \leq .0001$)

Furthermore, sink drains in hospitals receive huge amounts of microorganisms from hands and mouth washing and cleaning some medical matters with or without using detergents. Moore et al. (2002) confirmed that the hospitals sink drains are usually refuge for both pathogenic and nonpathogenic microorganisms and also considered as the core sources of contamination. In this work, the average counts of *E. coli* have fluctuated between 1.1×10^3 CFU/10 cm^2 (laboratory biofilm samples) and 3.6×10^6 CFU/10 cm^2 (kitchen biofilm samples) (Figure 2). Moreover, a few research confirmed that the presence of *E. coli* in natural biofilm (Juhna et al., 2007; Maes et al., 2019). Hemdan et al. (2015) found that the average of *E. coli* counts in sink drainage pipe biofilm samples was 8.8×10^4 CFU/10 cm^2. Meanwhile, household fomites have been observed to contribute to the dissemination of harmful bacteria (Stephens et al., 2019). Since several microorganisms can cause contaminations at minimal doses due to which they can survive from hours to some weeks on the humid surfaces of kitchen and bathroom (Reynolds, Watt, Boone, & Gerba, 2005; Sinclair, Choi, Riley, & Gerba, 2008). The gained results disclosed that the average *Salmonella* counts in all collected biofilm samples were ranged between 4.4×10^2 and 5.8×10^5 CFU/10 cm^2, and the maximum *Salmonella* counts were noted in the hospital and kitchen drainage pipes samples (Figure 2). These results are following Hemdan et al. (2015), who found that *Salmonella* species were the most dominant in kitchen drainage pipes biofilm samples. This may be due to *Salmonella* is environmentally persistent pathogens capable of forming the biofilm on different surfaces under different environmental conditions and may act as continuous sources of food contamination (Hemdan et al., 2019). In addition to that, *Salmonella* can form biofilm on plastics materials (Momba & Kaleni, 2002). Moreover, it can be considered possible found in water supplies due to its ability to colonize surfaces and replicate in the biofilm of distribution system pipes and other micro-inhabitants (Hemdan et al., 2019). In the present study, the averages log counts of *Listeria* species were ranged between 10^2 and 10^4 CFU/10 cm^2. The presence of *Listeria* spp. in kitchen and hospital drainage pipe biofilm samples was high compared with the other two samples, and these may be due to the washing of contaminated vegetables and fruits which irrigated by insufficiently treated wastewater that can provide a source of nonpathogenic and pathogenic microbes (Hemdan et al., 2019). Also, Berger et al. (2010) found that the kitchen as well-known that food items have been a reservoir for pathogenic bacteria. Similarly, several studies have identified sink drains in the hospital as possible sources of infections (Hemdan, 2015; Moore et al., 2002). In the present study, it can be cleared that the biofilm samples collected from laboratory, and hospital sink drainage pipes have numerous of pathogenic (e.g. *Salmonella* and *Listeria* spp.) and nonpathogenic (*E. coli*) microorganisms. Also, Hung and Henderson (2009) observed that biofilm-associated with medical and laboratory surfaces are often derived from the skin microflora. From statistical analysis it was concluded that there is no significant correlation between the three selected potential pathogens in lab biofilm samples. While, there is low correlation ($p \leq .05$) between the three selected microorganisms in hospital biofilm samples. Moreover, there is high significant correlation ($p \leq .0001$) between the three selected

microorganisms in both kitchen and bathroom biofilm samples. On the other hand, there is high significant correlation with significance ($p \leq .0001$) between the biofilm sources with each other (Figure 2).

3.2.1 | Confirmation of bacterial isolates using Biolog GEN III

Conventional confirmatory tests of bacterial isolates depending on morphological identification followed by biochemical tests, then antisera identification, are time-consuming take from 3 to 7 days and also laborious (Chojniak et al., 2015). Recently, excessive attentions toward use of BIOLOG as a rapid tool to identify and characterize microbes including bacteria, yeasts, and fungi and to study their metabolic fingerprints (Al-Dhabaan & Bakhali, 2017). BIOLOG GEN III is generally utilized for microbial communities' analysis based on physiological profiles that are able to provide insight into microbial roles in changing ecosystems (Chojniak et al., 2015; El-Liethy et al., 2018). In this study, *E. coli*, *Salmonella*, and *Listeria* biofilm cells, which isolated from different biofilm samples, were verified and identified using BIOLOG. In which 77 out of 122 (63.1%) isolates were confirmed as *E. coli* that isolated from biofilm samples collected from sink drainage pipes, while, 37 out of 60 (61.6%) isolates from the biofilm samples that collected from kitchen sink drainage pipes, 16 out of 20 (80%) isolates from the bathroom biofilm samples, 15 out of 26 (57.6%) isolates from the lab. Biofilm samples, and 9 out of 16 (56.2%) isolates from biofilm samples of hospital sink drainage pipes (Table 2). The metabolic fingerprints of bacterial isolates are playing a significant key in biofilm formation and giving full information about their behavior and metabolic activities (Hemdan et al., 2019). Table 3 illustrates the metabolic fingerprints of *E. coli*, whereas *E. coli* was able to grow at 5 and 6 pH and also in 1 and 4% NaCl. Additionally, *E. coli* gives positive reaction with the following carbon sources and chemical substrates; Inosine, 1% sodium lactate, fusidic acid, glycerol, D-glucose 6- PO4, D-fructose 6-PO4, troleandomycin, rifamycin SV, L-lanine, L-spartic cid, L-serine, lincomycin, guanidine HCl, Niaproof 4, D-galacturonic, D-gluconic acid, vancomycin, tetrazolium violet and blue, L-lactic acid, L-malic acid, and sodium butyrate (Table 3). BIOLOG is a quick and standard technique for verification microbe by utilizing 71 carbon sources including sugars, carboxylic acids, amino acids and peptides and 23 of chemical tests including pH, NaCl, and other chemical tests. The BIOLOG test is depending on the oxidation of tetrazolium redox dye for positive reaction (Franco-Duarte et al., 2019).

In this study, 80 out of 122 (65.5%) isolates were confirmed as *Salmonella* spp., which isolated from biofilm samples of sink drainage pipes, where 43 out of 60 suspected *salmonella* isolates from biofilm samples collected from kitchen sink drainage pipes, 13 out of 20 isolates from the bathroom biofilm samples, 17 out of 26 isolates from lab. Biofilm samples and 7 out of 16 isolates from biofilm samples collected from hospital sink drainage pipes (Table 2). *Salmonella* isolates were confirmed utilizing the following carbon sources and chemical substrates; grown in 5 and 6 pH, 1 and 4% NaCl, inosine, 1% sodium lactate, fusidic acid, glycerol, D-glucose 6-PO4, D-fructose 6-PO4, D-aspartic acid, D-serine, troleandomycin, rifamycin SV, L-aspartic acid, L-serine, lincomycin, Guanidine HCL, Niaproof 4, D-glucuronic acid, mucic acid, D-scchric acid, vancomycin, tetrazolium violet and blue, P-hydroxy-phenylacetic acid, L-lactic acid, citric acid, L-malic acid, lithium chloride, γ-amino-butryric acid, propionic acid, and sodium butyrate (Table 3).

Our results showed that 76 out of 122 (62.2%) presumptive *Listeria* isolates were confirmed as *Listeria* which isolated from biofilm samples of sink drainage pipes, where 39 out of 60 (65%) isolates from kitchen biofilm samples, 14 out of 20 (70%) isolates from biofilm samples of bathroom, 12 out of 26 (46%) isolates from biofilm samples from lab sink drainage pipes and 11 out of 16 (68.7%) isolates from hospital sink drainage pipes (Table 3). *Listeria* isolates were confirmed utilizing the following carbon sources and chemical substrates; D-Maltose, D-Trehalose, D-Celobiose, Gentiobiose, grown at pH 5 and 6, D-Sallin, growing at 1, 4, and 8% of NaCl, A-D-glucose, D-mannose, D-fructose, 1% sodium lactate, serine, guanidine HCl, Tetrazolium violet, nalidixic acid, lithium chloride, potassium tellurte, aztreonam, and sodium butyrate (Table 3).

3.2.2 | Confirmation of bacterial isolates using PCR

In the last few decades, molecular techniques are extensively used in bacterial confirmation and documentation (Ina´Cio, Flores, &

TABLE 2 Number and percentage of bacterial biofilm isolates isolated from different sink drainage pipes confirmed by Biolog GEN III and PCR

	E. coli				*Salmonella* spp.				*Listeria* spp.			
	GEN III		PCR		GEN III		PCR		GEN III		PCR	
Biofilm samples (number)	+	%	+	%	+	%	+	%	+	%	+	%
Kitchen (60)	37	61.6	49	81.6	43	71.6	52	86.6	39	65	51	85
Bathroom (20)	16	80	18	90	13	65	20	100	14	70	18	90
Laboratories (26)	15	57.6	21	80.7	17	65.3	23	88.4	12	46.1	20	76.9
Hospital (16)	9	52.2	13	81.2	7	43.7	12	75	11	68.7	15	93.7
Total (122)	77	63.1	101	82.7	80	65.5	107	87.7	76	62.2	104	85.2

Abbreviation: PCR, polymerase chain reaction.

TABLE 3 The metabolic fingerprints of *E. coli* (S1), *Salmonella* (S2), and *Listeria* (S3) isolates using BIOLOG GEN III

	Results				Results				Results				Results		
Properties	S1	S2	S3	Properties	S1	S2	S3	Properties	S1	S2	S3	Properties	S1	S2	S3
Negative ontrol	–	–	–	A-D-glucose	–/+	–/+	+	Gelatin	–	–	–	P-hydroxy-phenylacetic acid	–	+	–
Dextrin	–/+	–/+	–/+	D-mannose	–/+	–/+	+	Glycyl-L-proline	–/+	–/+	–	Methyl pyruvate	–/+	–/+	–
D-maltose	–/+	–/+	+	D-fructose	–/+	–/+	+	L-alanine	+	–/+	–	D-lactic acid methyl ester	–	–	–
D-trehalose	–/+	–/+	+	D-galactose	–/+	–/+	–	L-arginine	–/+	–	–	L-lactic acid	+	+	–
D-cellobiose	–	–	+	3 methyl glucose	–	–	–	L-aspartic acid	+	+	–	Citric acid	–	+	–
Gentiobiose	–	–	+	D-fucose	–	–	–	L-glutamic acid	–/+	–/+	–	A-keto-glutaric acid	–	–	–
Sucrose	–	–	–	L-fucose	–/+	–/+	–	L-histidine	–	–/+	–	D-malic acid	–/+	–	–
D-turanose	–	–	–	L-rhamnose	–/+	–/+	–/+	L-pyroglutamic acid	–	–	–	L-malic acid	+	+	–
Stachyose	–	–	–	Inosine	+	+	–/+	L-serine	+	+	–	Bromo-succinic acid	–/+	–/+	–
Positive control	+	+	+	1% sodium lactate	+	+	+	Lincomycin	+	+	–	Nalidixic acid	–	–	+
pH 6	+	+	+	Fusidic acid	+	+	–	Guanidine HCl	+	+	+	Lithium chloride	–/+	+	+
pH 5	+	+	+	Serine	–/+	–/+	+	Niaproof 4	+	+	–	Potassium tellurite	–/+	–/+	+
D-raffinose	–/+	–/+	–	D-sorbitol	+	–/+	–	Pecin	–	–	–	Tween 40	–	–/+	–
α-D-lactose	–/+	–	–/+	D-mannitol	–/+	–/+	–	D-galacturonic	+	–	–	γ-amino-butryric acid	–	+	–
D-melibiose	–/+	–/+	–	D-arabitol	–	–	–/+	L-galactonic acid lactone	–/+	–	–	α-hydroxy-butyric acid	–/+	–/+	–
β-methyl-D-glucoside	–/+	–/+	–/+	Myo-inositol	–	–/+	–	D-gluconic acid	+	+	–	β-hydroxy-D,L-butyric acid	–	–	–
D-salicin	–	–	+	Glycerol	+	+	–/+	D-glucuronic acid	+	+	–	A-keto-butyric acid	–/+	–/+	–/+
N-acetyl-D-glucosamine	–/+	–/+	–/+	D-glucose 6-PO_4	+	+	–	Glucuronamide	–/+	–/+	–	Acetoacetic acid	–	–	–/+
N-actyl-β-D-mannosamine	–/+	+	–/+	D-fructose 6-PO_4	+	+	–	Mucic acid	–/+	+	–	Propionic acid	–/+	+	–
N-actyl-D-galactosamine	–/+	–/+	–	D-aspartic acid	–	+	–	Quinic acid	–	–/+	–	Acetic acid	–/+	+	–
N-acetyl neuraminic acid	–/+	+	–	D-serine	–/+	+	–	D-Saccharic acid	–/+	+	–	Formic acid	–	–	–
1% NaCl	+	+	+	Troleandomycin	+	+	–	Vancomycin	+	+	–/+	Aztreonam	–/+	–/+	+
4% NaCl	+	–/+	+	Rifamycin SV	+	+	–	Tetrazolium violet	+	+	+	Sodium butyrate	+	+	+
8% NaCl	–/+	–/+	+	Minocycline	–/+	–/+	–	Tetrazolium blue	+	+	–/+	Sodium bromate	–/+	–/+	–/+

Note: +, positive; –, negative; –/+, intermediate.

Martins, 2008). PCR is beneficial, fast, and easy technique for the identification of microbial isolates especially bacterial threats (Franco-Duarte et al., 2019). Detailed PCR primers have been engaged to approve the presence or absence of target microbes and also used for the identification of these microbes (Spilker, Coenye, Vandamme, & Lipuma, 2004). Molecular typing assesses shown that bacterium is capable of surviving for months and even years deeply embedded in biofilm matrices, and work has shown that the bacterium can persist in human feces or fecally derived material outside the human host, on inorganic substrates, such as wood or metal and in both handled and unregulated water (Cooper, 2011). In the present study, 122 *E. coli* biofilm isolates (60 from the kitchen, 20 from the bathroom, 26 from lab and 16 from hospital sink drainage pipes) were confirmed and identified using PCR with specific primer. The result revealed in Table 2 demonstrated that the number of positive *E. coli* isolates was 49 out of 60 (81.6%) isolates from kitchen biofilm samples, 18 out of 20 (90%) isolates from bathroom biofilm samples, 21 out of 26 (80.7%) isolates from lab biofilm samples, and 13 out of 16 (81.2%) isolates from hospital biofilm samples. The results of this study reported that 122 *Salmonella* biofilm isolates (60 from the kitchen, 20 from the bathroom, 26 from lab and 16 from hospital sink drainage pipes) were confirmed and identified using PCR with specific primer. Results tabulated in Table 2, disclosed that the number of positive *Salmonella* isolates was as following; 52 out of 60 (86.6%) isolates from kitchen biofilm samples, 20 out of 20 (100%) isolates from bathroom biofilm sample, 23 out of 26 (88.4%) isolates from lab. Biofilm samples and 12 out of 16 (75%) isolates from hospital biofilm samples. The results of *Listeria* biofilm isolates are given in Table 3 and explained that the number of positive *Listeria* isolates was as follows; 51 out of 60 (85%) isolates from kitchen biofilm samples, 18 out of 20 (90%) isolates from bathroom biofilm, 20 out of 26 (76.9%) isolates from lab biofilm and 15 out of 16 (93.7%) isolates from hospital biofilm samples.

In ecological studies combination between genetic methods PCR as genotypic and phenotypic analysis by BIOLOG System, that allows differentiating a large number of isolates and thus contributes to the selection of the biotypes, which could be used as commercial starters (Cagno et al., 2010). From the obtained results in Table 2, it can be found that the accuracy percentage for confirmation of bacterial isolates using PCR were more reliable than BIOLOG. In a study conducted by Moraes, Perin, Júnior, and Nero (2013) mentioned that, the reliability of PCR technique target-specific genera and species is closed to be 100% while, the reliability of BIOLOG is ranged between 74 and 99.9% (Moraes et al., 2013). Where, the results demonstrated that the accuracy percentage for confirmation of *E. coli*, *Salmonella*, and *Listeria* isolates using BIOLOG were 61.6, 71.6, and 65%, respectively. Nevertheless by using PCR, the results were 81.6, 86.6, and 85%, respectively in bacterial isolates from kitchen biofilm. In case of bacterial isolates from bathroom biofilm, the results found that the accuracy percentage for confirmation of *E. coli*, *Salmonella* and *Listeria* biofilm isolates using BIOLOG were 80, 65 and 70%, respectively. But using PCR, the results were 90, 100, and 90%, respectively. Concerning bacterial isolates isolated from the laboratory biofilm, results showed that the accuracy percentages for confirmation of *E. coli*, *Salmonella*, and *Listeria* biofilm cells using BIOLOG were 57.6, 65.3, and 46.1%, respectively. While using PCR, the results were 80.7, 88.4, and 76.9%, respectively. Also bacterial isolates from hospital biofilm, results found that the accuracy percentage for confirmation of *E. coli*, *Salmonella*, and *Listeria* biofilm cells using BIOLOG were 52.2, 43.7, and 68.7%, respectively. While using PCR, the results were 81.2, 75, and 93.7%, respectively. The obtained results were compatible with Morgan, Boyette, Goforth, Sperry, and Greene (2009) who suggest that PCR provides more accurate identification of typical bacteria than the BIOLOG system. In addition to that, the genotypic characterization of bacteria using PCR is advantageous when compared with phenotypic methods by using BIOLOG. Moreover, the phenotypic require a prolonged cultivation period for suspected bacteria and pure bacterial cultures for various biochemical assays (Järvinen et al., 2009). The phenotypic investigation might be used as a trial examination; nevertheless, using PCR is more accurate and should be used as a confirmatory tool for isolates identification (Moraes et al., 2013). The number of the confirmed isolates using BIOLOG was lower than that of the PCR; therefore, PCR is considered more accurate for the confirmation of bacterial isolates than the phenotypic method using BIOLOG. The high accuracy of PCR is returning to that the PCR is targeting the nucleic acids of bacteria, but BIOLOG depends on metabolic activities of the bacterial isolates. Therefore, it can be concluded that, the PCR method has superior hand over BIOLOG identification (Hemdan et al., 2019; Janda & Abbott, 2007).

4 | CONCLUSIONS

The natural biofilm collected from kitchen, bathroom, laboratory, and hospital sink drainage pipes is considered the most crucial source for the dissemination of nonpathogenic and pathogenic microorganisms in the environmental surroundings and playing a vital role in the transamination of the infection to humankind. Four different sources of natural biofilm (kitchen, bathroom, laboratory, and hospital sink drainage pipes) contained *E. coli*, *Salmonella*, and *Listeria* species. Likewise, *E. coli* cell densities in all examined biofilm samples were higher than the other two pathogens (*Salmonella* and *Listeria* spp.). The most substantial numbers of bacterial pathogens were discovered in biofilm samples from the kitchen, followed by biofilm samples from the hospital. As a confirmatory test for the bacterial isolates, PCR was significantly more effective than the BIOLOG. Moreover, the biochemical evaluation of pathogen-specific isolation by BIOLOG is only a useful tool for providing information to pathogenic bacterial cells on the development of biofilms, age, and physiological characteristics. It can be concluded that drains banks are a haven for opportunistic and harmful microorganisms that pose a danger to humans, especially the elderly and low-immune people.

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CONFLICT OF INTERESTS

The authors stated that there is no conflict of interest.

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