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RESEARCH ARTICLE

Screening of Biofilm-Associated Genes in Antibiotic-Resistant Bacteria Isolated from Water Plumbing Systems

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Abstract

Biofilms in water plumbing systems promote microbial persistence, antimicrobial resistance, and water quality deterioration. This study aimed to identify biofilm-forming bacteria in household water plumbing systems and detect selected biofilm-associated genes. A total of 80 water and pipe-scraping samples were collected from household distribution and drainage systems in Colombo, Sri Lanka. Bacterial isolates were screened for biofilm production using the Congo Red Agar, Tube Method, and Tissue Culture Plate assays. The biochemically identified strong biofilm-producing isolates were subjected to antibiotic susceptibility testing. Twelve strong biofilm-producing, antibiotic-resistant isolates were selected for molecular analysis. Genomic DNA was extracted and subjected to polymerase chain reaction (PCR) amplification of the 16S rRNA gene and selected biofilm-associated genes. Representative 16S rRNA gene and biofilm-associated gene amplicons were sequenced to confirm bacterial species and gene identities. A total of 189 bacterial isolates were recovered, including 65 isolates from distribution lines and 124 isolates from drain lines. Among these, 125 (66.14%) isolates identified as biofilm producers. Of them, biochemical identification confirmed the presence of *Escherichia coli* (n = 71), *Pseudomonas aeruginosa* (n = 22), *Staphylococcus aureus* (n = 13), *Klebsiella pneumoniae* (n = 12), and *Staphylococcus epidermidis* (n = 7). Antibiotic susceptibility testing revealed that all 16 tested isolates were multidrug-resistant. PCR screening detected the *icaA* and *icaD* genes in two *Staphylococcus aureus* isolates, the *cupA* gene in one *Pseudomonas aeruginosa* isolate, the *fimB* gene in one *Escherichia coli* isolate, and the *luxS* gene in two isolates each of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus.* The findings demonstrate that household plumbing systems can serve as reservoirs of biofilm-forming multidrug-resistant bacteria carrying biofilm-associated genes. These microorganisms may contribute to persistent contamination and potential public health risks, highlighting the need for continuous monitoring and effective biofilm control strategies.

Keywords: antimicrobial resistance, biofilm, biofilm associated genes, polymerase chain reaction, water plumbing systems

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The authors declare no conflict

1 | Introduction

Bacterial biofilms are structured communities of bacteria enclosed within a self-produced extracellular polymeric substance (EPS) matrix, which enables attachment to surfaces and enhances survival under adverse environmental conditions. In domestic water plumbing systems, biofilms readily develop on internal pipe surfaces where moisture, nutrients and suitable attachment sites are continuously available. Once established, biofilms protect microorganisms from disinfectants, environmental stress, and antimicrobial agents, thereby facilitating their long-term persistence within water systems (Oliveira et al., 2024; Wang et al., 2014).

Biofilm formation in water plumbing systems is of considerable public health concern as these microbial communities can serve as reservoirs for opportunistic pathogens and antimicrobial-resistant bacteria. Several bacterial species frequently associated with plumbing environments, including *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Aeromonas* spp., and *Acinetobacter* spp., possess strong biofilm-forming abilities and exhibit enhanced resistance to environmental stress and antimicrobial treatments (Gomes et al., 2014; Oliveira et al., 2024; Microorganisms embedded within biofilms may periodically detach and contaminate flowing water, increasing the risk of human exposure through domestic water use (Douterelo et al., 2018; Proctor et al., 2018). Infections caused by biofilm-producing bacteria associated with waterborne diseases are often difficult to treat with antibiotics, resulting in prolonged hospital stays, increased morbidity, and higher mortality rates. Therefore, understanding the molecular mechanisms underlying biofilm formation is crucial.

Biofilm-associated genes play a critical role in the initiation, development, and maintenance of bacterial biofilms. Biofilm formation is regulated by several genetic determinants involved in bacterial adhesion, intercellular communication, and extracellular polymeric substance production. Among these, the *icaA* and *icaD* genes are responsible for the synthesis of polysaccharide intercellular adhesin (PIA), a key component promoting intercellular adhesion and biofilm accumulation in *Staphylococcus aureus*. In *Pseudomonas aeruginosa*, the *cupA* gene encodes components of the chaperone–usher fimbrial system that facilitates initial surface attachment and early biofilm development. In *Escherichia coli*, the *fimB* gene regulates the phase variation and expression of type I fimbriae, thereby enhancing bacterial adhesion to surfaces. Furthermore, the *luxS* gene is involved in the synthesis of autoinducer-2 (AI-2), an important quorum-sensing signaling molecule that regulates biofilm maturation, bacterial communication, and persistence in diverse bacterial species (Armoon et al., 2024; Cui & Kim, 2024; Peng et al., 2022).

Although several international studies have investigated biofilm formation in domestic water systems, limited information is available regarding the occurrence of biofilm-forming bacteria and biofilm-associated genes in household plumbing systems in Sri Lanka. Most local studies have focused on microbiological water quality assessments rather than the molecular characterization of biofilm-associated bacteria, their genetic determinants, and antibiotic resistance profiles. Understanding the occurrence of these microorganisms, their biofilm-associated genes and antibiotic-resistant profile is important for evaluating potential public health risks and improving water quality management strategies aimed at preventing waterborne diseases. Therefore, this study aimed to identify biofilm-forming bacteria isolated from household water plumbing systems in Colombo, Sri Lanka, using 16S rRNA gene sequencing. In addition, selected biofilm-associated genes (*icaA*, *icaD*, *cupA*, *fimB*, and *luxS*) were detected using PCR, and the antibiotic resistance patterns of the isolates were evaluated. This study provides one of the first comprehensive insights into the molecular characteristics and antimicrobial resistance of biofilm-forming bacteria in Sri Lankan household plumbing systems, thereby addressing a significant knowledge gap and generating baseline data to support future surveillance, risk assessment, and the development of effective biofilm control strategies in the country.

2 | Methods

All the examination was carried out by following the American standard methods for the examination of water and wastewater (Baird et al., 2017).

(i) Sample Collection

A total 80 samples including biofilm scrapings, water and wastewater samples was collected randomly from various water plumbing systems in and around Colombo area (Individual residence, apartments, university, etc). Bacteria isolated from drinking water distribution pipelines and drainage lines were evaluated separately (Table 1). Samples were collected from various components of water plumbing systems, including kitchen/canteen outlets, washroom outlets, water distribution lines, and vent pipes. Bacterial isolation was performed to obtain a sufficient number of biofilm-forming isolates for subsequent analyses.

Table 1 Distribution of Water and Pipeline Scraping Samples Collected from Domestic Plumbing Systems for the Isolation of Biofilm-Forming Bacteria

Sample Category	Number of Samples
Drinking water	20
Wastewater	20
Scrapings of Distribution line	20
Scrapings of Drain line	20
Total	80

Collection of Biofilm Scrapings from Distribution lines and Drainage Lines

Biofilm samples were collected by opening off the pipes, maintaining sterile conditions. Biofilms were removed from the inside of the pipes by swabbing and scrapping off the biofilms using sterile spatula and swab in 360-degree manner to ensure representation of whole biofilm samples growing on the whole pipe rather than only a subsection. The samples were immediately transferred aseptically into sterile falcon tubes. The samples were be kept on ice during collection, transported to the lab, maintaining cold chain and processed within 24 hours after collection.

Collection of Drinking Water and Wastewater Samples from the Pipelines

The inner and outer mouths of the drinking water tap and drain pipelines were cleaned with 70% alcohol prior to sample collection, and drinking water/wastewater was allowed to run for 2–3 minutes. Samples were then collected in sterile 50 ml Falcon tubes.

(ii) Culturing of Bacteria from Biofilm Scrapings and Water Samples

Culturing of bacteria was performed using standard microbiological techniques with general-purpose media, including Nutrient Agar (NA) (HiMedia, India) and Tryptic Soy Broth (TSB) (HiMedia, India), for the isolation and maintenance of bacterial isolates. Selective and differential media, such as Reasoner's 2A (R2A) agar (HiMedia, India) for drinking water samples, were also employed to enhance the recovery of specific bacterial populations. Following primary cultivation, samples were subjected to serial dilution and pour plate techniques to obtain isolated colonies and determine bacterial distribution. Distinct colonies were purified through repeated streaking to obtain single-colony isolates. Preliminary identification was carried out based on colony morphology and biochemical characteristics according to Bergey's Manual of Determinative Bacteriology and standard microbiological procedures (Baird et al., 2017; Bergey & Holt,

1994). Pure bacterial cultures were subsequently preserved for future studies by preparing glycerol stocks and storing them at −20°C.

(iii) Screening of Biofilm Production among the Bacterial Isolates

The detection of biofilm production was performed using three well-established phenotypic methods: Tube Method (TM), Congo Red Agar (CRA) Method, and Tissue Culture Plate (TCP) Method, as described by Hassan et al., (2011). The TM, CRA, and TCP methods were selected as they represent complementary qualitative and quantitative approaches for the detection of biofilm formation. While TM and CRA serve as rapid screening methods, TCP provides quantitative assessment and is widely regarded as the reference method for phenotypic biofilm evaluation. The results were compared with clinically identified bacterial isolates obtained from the standard laboratory of the Medical Research Institute (MRI), Sri Lanka. These isolates were previously identified by MRI and were subsequently confirmed as biofilm-producing bacteria using the Tube Method, Congo Red Agar method, and Tissue Culture Plate assay before being used as reference biofilm-forming isolates in this study.

a) Tube Assay for Qualitative Biofilm Assessment

Bacterial isolates were inoculated into test tubes containing TSB. After incubation at 37°C for 24–48 hours, the tubes were emptied, rinsed with phosphate-buffered saline (PBS) (HiMedia, India), and stained with 0.1% crystal violet solution. The tubes were then rinsed with distilled water and dried in an inverted position. Biofilm formation was assessed by visual examination of the tube walls, and the intensity of staining was qualitatively graded as strong, moderate, weak, or negative (Hassan et al. 2011).

b) Congo Red Agar Method for Biofilm Visualization

In the CRA method, the bacteria were cultured on a specially prepared agar medium containing Congo red dye, sucrose and Brain Heart Infusion Agar (BHI) (HiMedia, India) which enables the detection of biofilm-producing bacteria through the formation of black, crystalline colonies. The CRA plates were inoculated with bacterial isolates and incubated at 37 °C for 24–48 hours. Colony morphology was evaluated according to standard CRA criteria for biofilm detection.

c) Tissue Culture Plate Assay for Quantitative Biofilm Analysis

Bacterial isolates were grown in TSB and inoculated into individual wells of 96-well flat-bottom polystyrene plates. Plates were incubated at 37°C for 24 hours. Non-adherent cells were removed by rinsing with PBS, and adherent biofilms were fixed with sodium acetate and stained with crystal violet. The optical density (OD) of the stained biofilm was measured using a micro-ELISA reader at a wavelength of 630 nm. The intensity of the staining directly correlated with the amount of biofilm formed. Biofilm production was categorized as absent, weak, moderate, or strong based on the OD values (Table 2) (Hassan et al., 2011; Stepanovic et al., 2007).

*Optical Density cut-off value (ODc) = Average OD of negative control + 3x Standard Deviation (SD) of negative control

Table 2: Interpretation of results of Tissue Culture Plate Method

Average OD Value	Biofilm Production
≤ ODc / ODc < ~ ≤ 2x ODc	Non/weak biofilm producer
2x ODc < ~ ≤ 4x ODc	Moderate biofilm producer
> 4x ODc	Strong biofilm producer

(iv) Biochemical Identification of Biofilm Producers

Bacterial isolates were identified based on the morphological studies (i.e. colony morphology, Gram staining, etc.) and a series of biochemical tests according to Cowan and Steel's Manual for the Identification of Medical Bacteria (Barrow & Feltham, 2003). The biochemical profiles obtained were compared with the published reference characteristics of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus epidermidis* (Forbes et al., 2007; Winn et al., 2006). Enterobacterales isolates were cultured on MacConkey Agar (MAC) (HiMedia, India) to assess lactose fermentation and subsequently tested for oxidase, catalase, motility, IMViC reactions (Indole, Methyl Red, Voges–Proskauer, Citrate) and urease activity. Typical CLSI-consistent biochemical profiles were used to differentiate *K. pneumoniae* (IMViC −−++, urease-positive, non-motile) and *E. coli* (IMViC ++−−, motile, urease-negative). Non-fermenting isolates suspected as *P. aeruginosa* were evaluated on MAC examined for pigment production, oxidase and catalase reactions, citrate utilization and urease activity. Presumptive *S. aureus* isolates were confirmed using catalase and coagulase reactions, mannitol fermentation on Mannitol Salt Agar (MSA) (HiMedia, India) and Gram stain characteristics. Coagulase-negative staphylococci, including *Staphylococcus epidermidis* were identified based on catalase positivity, coagulase negativity, and colony morphology, with additional supportive biochemical characteristics where necessary.

The identified isolates were subsequently grouped according to their presumptive species identification. From each bacterial group, the isolate demonstrating the strongest biofilm-forming ability was selected for further molecular confirmation by 16S rRNA gene sequencing.

(v) Evaluation of Antibiotic Susceptibility in Biofilm-Forming Isolates

Antibiotic susceptibility testing was performed to determine the antimicrobial susceptibility profiles of selected strong biofilm-producing bacterial species that were biochemically identified and confirmed by all three biofilm detection methods, using the Kirby–Bauer disk diffusion method according to CLSI guidelines (CLSI, 2022). The Kirby Bauer disc diffusion technique which entails inoculating a McFarland concentration onto the surface of a sterile Mueller Hinton agar (MHI) (HiMedia, India) plate was used to determine the resistance profile of bacterial isolates. The process entails standardizing the bacterial isolates to a turbidity standard equal to 0.5 McFarland. Before inserting the commercially available antibiotic-impregnated filter-paper discs using sterile forceps, the surface of freshly prepared Mueller-Hinton Agar was thoroughly swabbed with the bacterial isolate. The following antibiotics were included in the study: The following antibiotics were selected based on their relevance in treating biofilm-associated infections: Amoxicillin/Clavulanic acid (20/10 µg), Tetracycline (30 µg), Ceftriaxone (30 µg), Ciprofloxacin (10 µg), Gentamicin (10 µg), Cotrimoxazole (25 µg), Cefuroxime (30 µg), Nitrofurantoin (300 µg), Nalidixic acid (30 µg), Meropenem (10 µg), Imipenem (10 µg), Chloramphenicol (30 µg), Ceftazidime (10 µg), Amikacin (30 µg), Erythromycin (10 µg), Cefoxitin (30 µg), Clindamycin ((2 µg). Following a 24-hour incubation period at 37°C, the zone of inhibition was measured to the closest millimeter and classified as Susceptible (S), Intermediate (I) and Resistant (R).

(vi) Molecular Identification of Bacterial Isolates Using 16S rRNA Gene Sequencing

A total of 12 strong biofilm-producing isolates were selected for molecular analysis. Genomic DNA was extracted from all 12 isolates and subjected to PCR amplification of the 16S rRNA gene and relevant biofilm-associated genes. However, due to resource limitations, only ten representative PCR products were selected for sequencing at Macrogen, Republic of Korea. Of these, five representative 16S rRNA gene amplicons, each corresponding to a predominant bacterial species, were sequenced for bacterial species confirmation, while five representative biofilm-associated gene amplicons (*icaA*, *icaD*, *cupA*, *fimB*, and *luxS*) were sequenced to confirm the identity of the target biofilm-associated genes.

(a) *Genomic DNA Extraction*

Genomic DNA was extracted from bacterial cell pellets harvested from overnight pure cultures of the selected bacterial isolates using a commercial extraction kit (Presto™ Mini gDNA Bacteria Kit, (Geneaid Biotech Ltd., Taiwan) following the manufacturer's protocol. Briefly, cells were pelleted by centrifugation and resuspended in GT buffer, followed by enzymatic digestion with Proteinase K. Cell lysis was achieved using GB buffer with incubation at elevated temperature. The lysate was then mixed with absolute ethanol and transferred to a spin column for DNA binding. The bound DNA was washed sequentially using W1 and wash buffers to remove impurities. Finally, purified DNA was eluted using pre-heated elution buffer and stored at −20 °C for further analysis.
The extracted DNA was used directly for PCR amplification, and successful amplification was confirmed by agarose gel electrophoresis prior to sequencing.

(b) *Agarose Gel-Based Confirmation of Genomic DNA*

PCR Amplification of the 16S rRNA Gene

PCR amplification of the 16S rRNA gene was performed on genomic DNA extracted from bacterial isolates using universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). Each reaction was carried out in a total volume of 25 µL, containing 2.5 µL $MgCl_2$ (2.5 mM), 0.5 µL dNTPs (0.2 mM), 0.5 µL of each primer (0.2 µM), 0.2 µL Taq DNA polymerase (1 U/µL), 2 µL template DNA, and nuclease-free water to volume.
Thermal cycling conditions consisted of an initial denaturation at 94 °C for 5 minutes, followed by 30 cycles of denaturation at 94 °C for 1 minute, annealing at 54 °C for 1 minute, and extension at 72 °C for 2 minutes. A final extension step was carried out at 72 °C for 10 minutes, after which the reaction was held at 4 °C. The amplified products were analyzed by electrophoresis on a 1% agarose gel stained with ethidium bromide (0.5 µg/mL).

(vii) Screening of Biofilm Associated Genes

Biofilm-associated genes (*icaA, icaD, cupA, luxS, and fimB*) were amplified from genomic DNA of bacterial isolates using gene-specific primers. PCR amplification was carried out in a total reaction volume of 25 µL, comprising $MgCl_2$, Taq DNA polymerase, dNTPs, specific primers, and template DNA. The thermal cycling protocol included an initial denaturation at 95 °C for 2 minutes, followed by 30 cycles of denaturation at 95 °C for 1 minute, annealing at gene-specific temperatures ranging from 48–57 °C for 1 minute, and extension at 72 °C for 2 minutes. A final extension step was performed at 72 °C for 10 minutes. The amplified products were resolved on 1% agarose gel stained with ethidium bromide and visualized under UV illumination (Table 3).

Table 3: Target biofilm-associated genes, primer sequences, annealing temperatures, and expected amplicon sizes used for PCR amplification. F = Forward primer; R = Reverse primer.

Target gene	Primer sequence (5′–3′)	Annealing temperature (°C)	Expected band size (bp)
16S rRNA	F: AGAGTTTGATCCTGGCTCAG R: TACGGYTACCTTGTTACGACTT	48	1500
icaA	F: AAGTCATACACTTGCTGGCG R: CTGTCTGGGCTTCACCATGT	48	630
icaD	F: ACCCAACGCTAAAATCATCG R: CCTGTAACCGCACCAAGTTT	48	211
cupA	F: AATTCGATGATCGCCTGTT	54	448

	R: GCGATAGAGGTTGGTGTCGT		
fimB	F: CGAATCACTCCTTAAAGCA	50	379
	R: GGCGTAACATGTGCGGA		
luxS	F: GCTTCCTGCAACGAGTGCAT	57	112
	R: ATGCCTGGAAAGCGGCAATG		

(viii) DNA Sequencing and BLAST-Based Identification

Representative PCR amplicons corresponding to the target biofilm-associated genes (*icaA, icaD*, *cupA, fimB*, and *luxS*) were selected for sequencing confirmation. The PCR products were submitted to Macrogen Inc. (Seoul, Republic of Korea) for purification and Sanger sequencing. PCR product purification was performed using ExoSAP-IT™ Express PCR Product Cleanup (Thermo Fisher Scientific). Cycle sequencing was carried out using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), and sequence analysis was performed using an ABI PRISM 3730XL Genetic Analyzer (96-capillary system).

The obtained chromatograms were quality-checked and trimmed to remove low-quality regions. Forward and reverse sequences were aligned using BioEdit software, and the resulting sequences were compared with reference sequences available in the NCBI GenBank database using the Basic Local Alignment Search Tool (BLAST) to confirm gene identity.

3 | Results

Isolation of Bacteria from Water Plumbing Systems

Table 4: Total Number of bacteria isolated from Whole Water Plumbing Systems

Water Plumbing System	Number of Samples	Number of Bacterial Isolates
Distribution Line	40	65
Drain Line	40	124
Total	80	189

A total of 189 bacterial isolates found in the entire water plumbing system, including both distribution and drain line samples. Among these, 65 isolates were obtained from distribution lines, whereas a higher number of 124 isolates were recovered from drain lines (Table 4).

Screening of Biofilm Production

In this study, out of 189 bacterial isolates examined, 125 demonstrated biofilm formation as determined by standard methods, including the Congo Red Agar, Tissue Culture Plate, and Tube assays. Among the 65 bacterial isolates recovered from the distribution lines, 38 (58.5%) were biofilm producers and 27 (41.5%) were non-biofilm producers, indicating a higher prevalence of biofilm-forming bacteria. Among the 124 bacterial isolates recovered from the drain lines, 87 (70.2%) were biofilm producers and 37 (29.8%) were non-biofilm producers, demonstrating a higher prevalence of biofilm-forming bacteria in drain line than distribution line samples.

Biochemical Identification of Biofilm-Producing Bacteria Isolated from Water Plumbing Systems

Biochemical identification of biofilm-producing isolates revealed that the majority were *Escherichia coli* (56.8%, 71/125), followed by *Pseudomonas aeruginosa* (17.6%, 22/125), *Staphylococcus aureus* (10.4%, 13/125), *Klebsiella pneumoniae* (9.6%, 12/125) and *Staphylococcus epidermidis* (5.6%, 7/125) (Table 5).

Table 5: Biochemically identified Biofilm Bacteria from Whole Water Plumbing Systems

Biochemically Identified Biofilm Bacteria	Distribution Line	Drain Line	Total
Klebsiella Pneumoniae	05	07	12
Escherichia coli	22	49	71
Pseudomonas aeruginosa	05	17	22
Staphylococcus aureus	03	10	13
Staphylococcus epidermidis	03	04	07

Assessment of Antibiotic Susceptibility in Biofilm-Producing Bacteria

Table 6: Antimicrobial resistance profiles of biofilm producing Klebsiella Pneumoniae

Antibiotic	Resistant (%)	Intermediate (%)	Susceptible (%)
AMC	100	-	-
CXM	100	-	-
CRO	25	-	75
CAZ	62.5	25	12.5
MEM	100	-	-
IMP	62.5	25	12.5
NAL	25	75	-
CIP	62.5	-	37.5
GEN	75	-	25
TE	100	-	-
SXT	12.5	-	87.5
NIT	87.5	-	12.5
C	87.5	12.5	-

Table 7: Antimicrobial resistance profiles of biofilm producing Escherichia coli

Antibiotic	Resistant (%)	Intermediate (%)	Susceptible (%)
AMC	100	-	-
CXM	100	-	-
CRO	25	-	75
CAZ	12.5	87.5	-
MEM	100	-	-
IMP	25	-	75
NAL	25	-	75
CIP	25	-	75
GEN	100	-	-
TE	100	-	-
SXT	50	-	50
NIT	100	-	-
C	100	-	-

AMC- Amoxicillin/Clavulanic acid; CXM- Cefuroxime; CRO- Ceftriaxone; CAZ- Ceftazidime; MEM- Meropenem; IMP- Imipenem; NAL- Nalidixic acid; CIP-Ciprofloxacin; GEN- Gentamicin; TE- Tetracycline; SXT- Cotrimoxazole; NIT- Nitrofurantoin; C- Chloramphenicol

Table 8: Antimicrobial resistance profiles of biofilm producing Pseudomonas aeruginosa

Antibiotic	Resistant (%)	Intermediate (%)	Susceptible (%)
CAZ	100	-	-
MEM	87.5	12.5	-
IMP	12.5	87.5	-
CIP	100	-	-
GEN	25	-	75
AK	87.5	12.5	

CAZ- Ceftazidime; MEM- Meropenem; IMP- Imipenem; CIP- Ciprofloxacin; GEN- Gentamicin; AK- Amikacin

Table 9: Antimicrobial resistance profiles of biofilm producing Staphylococcus aureus

Antibiotic	Resistant (%)	Intermediate (%)	Susceptible (%)
E	87.5	12.5	-
GEN	100	-	-
TE	100	-	-
FOX	87.5	12.5	-
DA	100	-	-
NIT	87.5	12.5	-
CIP	25		75
SXT	25		75
C	-	-	100

E- Erythromycin; GEN- Gentamicin; TE- Tetracycline; FOX- Cefoxitin; DA- Clindamycin; NIT- Nitrofurantoin; CIP- Ciprofloxacin; SXT-Cotrimoxazole; C- Chloramphenicol

In the antibiotic susceptibility test, *Klebsiella pneumoniae* exhibited a high level of multidrug resistance, showing complete resistance to amoxicillin/clavulanic acid, tetracycline, and cefuroxime. Most isolates were also resistant to imipenem, ceftazidime, ciprofloxacin, nitrofurantoin, and chloramphenicol. However, most isolates remained susceptible to ceftriaxone, and cotrimoxazole, indicating its potential effectiveness against these strains (Table 6). *Escherichia coli* demonstrated extensive multidrug resistance, with all isolates resistant to several antibiotic classes, including amoxicillin/clavulanic acid, cefuroxime, meropenem, gentamicin, tetracycline, nitrofurantoin, and chloramphenicol. These findings indicate a widespread and concerning resistance profile among the isolates (Table 7). *Pseudomonas aeruginosa* showed a pronounced multidrug-resistant phenotype, with complete resistance to ceftazidime and ciprofloxacin. Resistance to meropenem was also common, while intermediate resistance to imipenem was observed among several isolates. In addition, resistance to amikacin was frequently detected, although most isolates remained susceptible to gentamicin (Table 8). *Staphylococcus aureus* exhibited extensive resistance to multiple antibiotics, with all isolates showing resistance to gentamicin, tetracycline, and clindamycin. High levels of resistance were also observed for erythromycin, cefoxitin, and nitrofurantoin. In contrast, most isolates remained susceptible to ciprofloxacin, cotrimoxazole, and chloramphenicol (Table 9). Overall, all four bacterial species tested for antibiotic susceptibility demonstrated multidrug resistance, with resistance detected against several commonly used antibiotics. However, susceptibility to certain antibiotics, particularly cotrimoxazole, ceftriaxone, ciprofloxacin, and chloramphenicol, was retained in some species, suggesting potential therapeutic options. The findings highlight the presence of clinically significant antibiotic-resistant bacteria within water plumbing systems and their potential public health implications.

Genomic DNA Extraction from Selected Strong Biofilm-Forming Bacterial Isolates and PCR Amplification of the 16S rRNA Gene

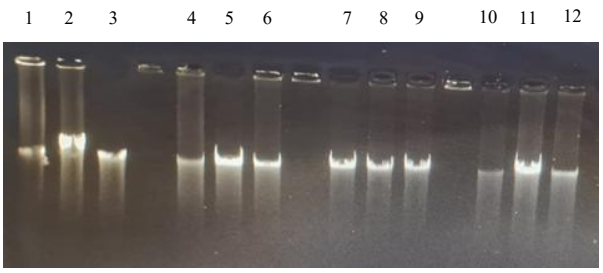

Figure 1: Agarose gel (0.8 %) electrophoresis of genomic DNA extracted from biofilm-forming bacterial isolates obtained from water plumbing systems

Lane 1-3: *Klebsiella pneumoniae*.; Lane 4-6: *Pseudomonas aeruginosa*; Lane 7-9: *Escherichia coli*; Lane 10-11: *Staphylococcus aureus*; Lane 12: *Staphylococcus epidermidis*

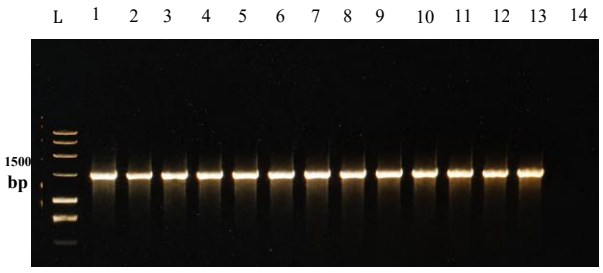

Figure 2: Agarose gel electrophoresis (1%) showing PCR amplification of the 16S rRNA gene from biofilm-forming bacterial isolates obtained from water plumbing systems.

Lane L- 1 Kb Ladder, Lanes 1–3: *Klebsiella pneumoniae*; Lanes 4–6: *Pseudomonas aeruginosa;* Lanes 7–9: *Escherichia coli;* Lanes 10–11: *Staphylococcus aureus*; Lane 12: *Staphylococcus epidermidis* Lane 13: Positive reference; Lane 14: Negative control.

Genomic DNA extracted from selected strong biofilm-forming bacterial isolates showed intact high-molecular-weight DNA bands during agarose gel electrophoresis (Figure 1). The extracted DNA samples demonstrated sufficient quality for downstream molecular applications. PCR amplification using universal 16S rRNA primers (27F and 1492R) generated amplicons of approximately 1500 bp in all tested bacterial isolates (Figure 2). The positive control produced the expected band, while no amplification was observed in the negative control.

Molecular Identification of Biofilm-Forming Bacterial Isolates

Table 10: Molecular Identification of Bacterial Isolates Based on 16S rRNA Gene Sequencing

Sample No.	Forward Sequence Similarity (%)	Reverse Sequence Similarity (%)	Bacterial Identification
1	98.52	98.54	*Klebsiella pneumoniae*
2	98.21	99.05	*Escherichia coli*
3	96.82	97.95	*Pseudomonas aeruginosa*
4	97.14	99.45	*Staphylococcus aureus*
5	97.03	98.60	*Staphylococcus epidermidis*

Molecular identification based on 16S rRNA gene sequencing showed sequence similarity values ranging from 96.82% to 99.45% with reference sequences in the NCBI GenBank database (Table 10). The sequenced isolates were identified as *Klebsiella pneumoniae* (98.52–98.54%), *Escherichia coli* (98.21–99.05%), *Pseudomonas aeruginosa* (96.82–97.95%), *Staphylococcus aureus* (97.14–99.45%), and *Staphylococcus epidermidis* (97.03–98.60%).

PCR-Based Detection of Biofilm-Associated Genes

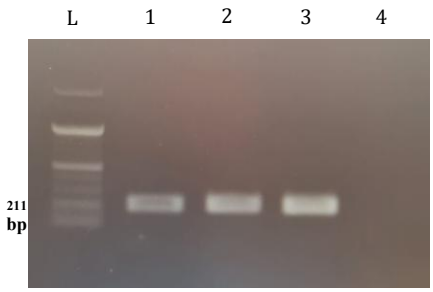

Figure 3: Detection of Ica D Gene.
Lane L: 100 bp DNA ladder; Lanes 1–2: *Staphylococcus aureus* samples; Lane 3: positive control; Lane 4: negative control.

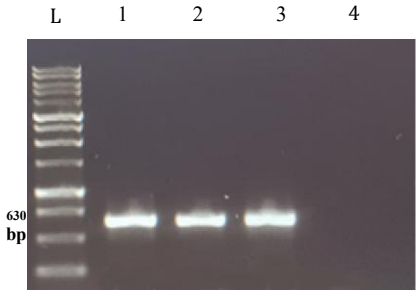

Figure 4: Detection of Ica A Gene.
Lane L: 1 kb DNA ladder; Lanes 1–2: *Staphylococcus aureus samples*; Lane 3: positive control; Lane 4: negative control.

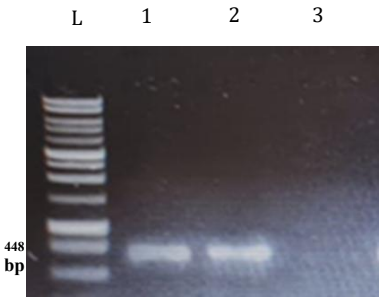

Figure 5: Detection of Cup A Gene.
Lane L: 1 kb DNA ladder; Lane 1: *Pseudomonas aeruginosa* sample Lane 2: positive control; Lane 3: negative control.

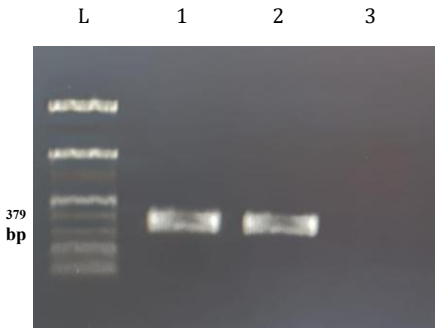

Figure 6: Detection of Fim B Gene.
Lane L: 100 bp DNA ladder; Lane 1: *Escherichia coli* sample; Lane 2: positive control; Lane 3: negative control.

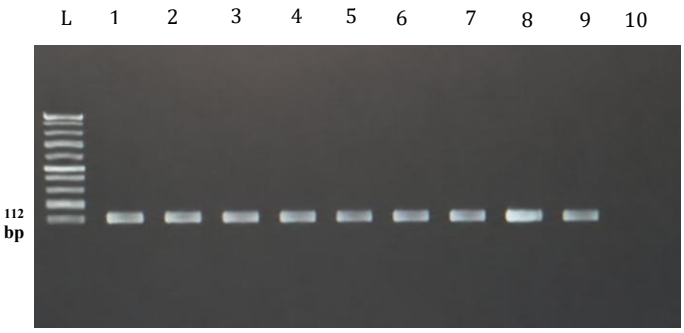

Figure 7: Detection of Lux S Gene.
Lane L: 100 bp DNA ladder; Lanes 1–2: *Escherichia coli* samples; Lane 3: *Escherichia coli* positive control; Lanes 4–5: *Pseudomonas aeruginosa* samples; Lane 6: *Pseudomonas aeruginosa* positive control; Lanes 7–8: *Staphylococcus aureus* samples; Lane 9: *Staphylococcus aureus* positive control; Lane 10: negative control.

PCR screening revealed the presence of several biofilm-associated genes among the tested isolates. The *icaA* and *icaD* genes were detected in two *Staphylococcus aureus* isolates (Figure 3 & 4). The cupA gene was detected in one *Pseudomonas aeruginosa* isolate (Figure 5), while the *fimB* gene was detected in one *Escherichia coli* isolate (Figure 6). The *luxS* gene was identified in two isolates each of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Figure 7). The expected amplicon sizes were observed for all target genes, namely 630 bp for *icaA*, 211 bp for *icaD*, 448 bp for *cupA*, 379 bp for *fimB,* and 112 bp for *luxS*.

Sequence Similarity Analysis of Biofilm-Associated Genes

Table 11: Percentage sequence similarity of biofilm-associated gene sequences obtained from selected isolates compared with reference gene sequences in the NCBI GenBank database based on BLAST analysis

Bacterial Species	Target Gene	Forward Sequence Similarity with the reference gene (%)	Reverse Sequence Similarity with the reference gene (%)
Staphylococcus aureus	*icaA*	99.72	100
Staphylococcus aureus	*icaD*	100.00	99.01
Pseudomonas aeruginosa	*cupA*	95.99	95.43
Escherichia coli	*fimB*	96.77	97.10
Escherichia coli	*luxS*	95.00	95.31

Sequence analysis of the amplified biofilm-associated genes was performed using the Basic Local Alignment Search Tool (BLAST) against reference sequences available in the NCBI GenBank database. The forward and reverse sequencing reads of each gene were analyzed separately, and the percentage similarity was calculated based on sequence alignment with the closest matching reference sequence. Similarity values ranged from 95.00% to 100.00%. The *icaA* and *icaD* genes of *Staphylococcus aureus* exhibited the highest sequence similarity to reference sequences, whereas *cupA*, *fimB*, and *luxS* showed slightly lower similarity values, ranging from 95.00% to 97.10%, indicating minor sequence variations while maintaining a high degree of homology with the corresponding reference genes (Table 11).

4 | Discussion

This study demonstrated a high prevalence of biofilm-forming bacteria in household water plumbing systems, particularly in drain lines. *Escherichia coli* was the predominant biofilm-forming species, followed by *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae,* and *Staphylococcus epidermidis*. The detection of multidrug resistance and biofilm-associated genes further highlighted the persistence and potential public health significance of these microorganisms within plumbing environments.

Biofilm formation is considered one of the most important survival strategies adopted by microorganisms in water distribution and plumbing systems. Within biofilms, microorganisms are embedded in EPS matrix that provides protection against environmental stress conditions, disinfectants, antimicrobial agents, and hydraulic shear forces. The persistence of biofilm-associated microorganisms in plumbing environments represents a significant public health concern as biofilms can act as reservoirs for opportunistic pathogens and facilitate continuous microbial contamination of domestic water supplies. Furthermore, microorganisms residing within biofilms frequently exhibit enhanced antimicrobial resistance and increased tolerance to conventional disinfection procedures compared to their planktonic counterparts (Douterelo et al., 2018; Flemming et al., 2023; Oliveira et al., 2024).

The detection of biofilm-forming bacteria and biofilm-associated genes is therefore important for understanding the molecular mechanisms involved in bacterial adhesion, colonization, persistence, and survival within plumbing systems. Genes such as *icaA*, *icaD*, *cupA*, *fimB*, and *luxS* play critical roles in exopolysaccharide production, quorum sensing, fimbrial adhesion, and biofilm maturation, thereby contributing to the establishment and long-term stability of microbial biofilms (Armoon et al., 2024; Cui & Kim, 2024: Flemming et al., 2023). Molecular identification combined with PCR-based detection of these genes provides valuable information regarding the pathogenic potential and biofilm-forming capability of

bacterial isolates recovered from water plumbing systems. Therefore, the present study contributes to the understanding of biofilm-associated bacterial communities and their genetic determinants in household plumbing environments.

Distribution of Bacterial Isolates Recovered from Water Plumbing Systems

A total of 189 bacterial isolates were recovered from the water plumbing system, with 65 isolates obtained from distribution lines and 124 isolates from drain lines (Table 4). The higher number of isolates recovered from drain lines suggests that these environments provide more favorable conditions for microbial growth and persistence than distribution lines. Drain lines are continuously exposed to wastewater containing organic matter and other nutrients that support bacterial colonization and biofilm development. Biofilms formed in wastewater environments can serve as reservoirs for diverse microbial communities and facilitate the persistence and spread of antimicrobial-resistant bacteria to the surrounding environment (Nahum et al., 2025). The presence of bacterial isolates in both distribution and drain lines further indicates that water plumbing systems provide suitable ecological niches for microbial survival and biofilm establishment (Bello et al., 2024; Meradji et al., 2025). The recovery of nearly twice as many bacterial isolates from drain lines as from distribution lines highlights the greater microbial burden associated with wastewater environments. These findings emphasize the importance of monitoring water plumbing systems, particularly drain lines, because of their high microbial load.

Biofilm Formation Potential of Bacteria Isolated from Water Plumbing Systems

The use of three complementary biofilm screening methods, Congo Red Agar, Tissue Culture Plate, and Tube Method, enhanced the reliability of biofilm detection in this study. The TCP method, widely regarded as the gold standard for biofilm assessment, enables quantitative evaluation of biofilm biomass, while the CRA and TM methods provide rapid qualitative confirmation of biofilm-producing phenotypes (Stepanović et al., 2007). The combined application of these methods strengthened the validity of the biofilm detection results.

Screening for biofilm production revealed that 125 (66.14%) of the 189 bacterial isolates recovered from the water plumbing system were biofilm producers, whereas 64 (33.86%) were non-biofilm producers. Among the 65 isolates recovered from distribution lines, 38 (58.5%) were identified as biofilm producers and 27 (41.5%) as non-biofilm producers. In comparison, 87 (70.2%) of the 124 isolates recovered from drain lines were biofilm producers, while only 37 (29.8%) were non-biofilm producers. The greater prevalence of biofilm-forming bacteria in drain lines indicates that wastewater-associated environments are more conducive to biofilm development than distribution lines.

The higher occurrence of biofilm producers in drain lines may be attributed to the continuous presence of moisture, organic matter, nutrients, and suitable attachment surfaces. Compared with distribution lines, drain lines are exposed to higher microbial loads, greater nutrient availability, and lower levels of residual disinfectants, all of which favor biofilm establishment and persistence. These conditions promote microbial adhesion and growth, facilitating the development and maturation of biofilms on pipe surfaces (Nahum et al., 2025). In contrast, distribution lines carry treated water and generally contain lower nutrient concentrations, which may limit microbial growth. Nevertheless, the recovery of a considerable number of biofilm-producing isolates from distribution lines demonstrates that bacterial colonization can still occur within drinking water distribution networks. Once established, biofilms on pipe surfaces can protect microorganisms from environmental stresses and support their long-term persistence within plumbing systems (Bello et al., 2024).

The predominance of biofilm-producing bacteria observed in the present study is of particular concern as biofilms are recognized reservoirs of opportunistic pathogens and antimicrobial-resistant bacteria. Hayward et al. (2025) identified plumbing biofilms as important hotspots for antimicrobial-resistant pathogens, with

drain biofilms representing the most common reservoir, highlighting their role in microbial persistence and dissemination. Furthermore, cells embedded within biofilms are protected by an extracellular polymeric matrix that limits the penetration of antimicrobial agents and disinfectants, thereby enhancing bacterial survival and tolerance to control measures (Nahum et al*.,* 2025).

The presence of biofilm-producing bacteria in both drain lines and treated drinking water distribution lines indicates that microbial colonization can occur throughout water plumbing systems despite water treatment processes. These findings highlight the importance of continuous monitoring and effective control strategies to minimize biofilm development within water plumbing systems and reduce the public health risks associated with the persistence and dissemination of pathogenic and antimicrobial-resistant bacteria.

Occurrence and Identification of Biofilm-Producing Bacteria in Water Plumbing Systems

The biochemical identification of biofilm-producing isolates revealed the presence of *Escherichia coli* (n = 71), *Pseudomonas aeruginosa* (n = 22), *Staphylococcus aureus* (n = 13), *Klebsiella pneumoniae* (n = 12), and *Staphylococcus epidermidis* (n = 7) within the water plumbing system.

Among the identified isolates, *E. coli accounted for 56.8% of biofilm-producing isolates, followed by P. aeruginosa (17.6%), S. aureus (10.4%), K. pneumoniae (9.6%), and S. epidermidis (5.6%).* The successful extraction of high-quality genomic DNA and amplification of the 16S rRNA gene confirmed the suitability of the bacterial isolates for molecular identification. The obtained amplicons of approximately 1500 bp are consistent with previous studies that used universal 16S rRNA primers for bacterial identification (Johnson et al*.,* 2019). The sequencing results validated the biochemical identification and confirmed the occurrence of these bacterial species in household plumbing systems.

Similar bacterial species, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*, have been reported in drinking water distribution systems and premise plumbing environments, where they contribute to biofilm formation and may pose potential public health risks (Bello et al., 2024; Hayward et al*.,* 2025). The predominance of *E. coli* suggests widespread bacterial contamination and highlights its ability to adapt to plumbing environments through biofilm formation. The occurrence of *E. coli* in plumbing systems is considered an important indicator of fecal contamination and inadequate sanitary conditions, suggesting possible contamination from wastewater sources or cross-connections within plumbing infrastructure (WHO, 2022). Comparable findings have been reported in drinking water distribution systems, where *Escherichia coli* has been identified as one of the predominant bacterial contaminants capable of forming biofilms and persisting within water distribution networks (Bello et al., 2024; Hayward et al., 2025). Its predominance in the present study may also reflect its strong ability to adhere to pipe surfaces and establish biofilms under favorable environmental conditions.

The occurrence of *P. aeruginosa* is particularly significant because of its well-documented ability to survive in low-nutrient aquatic environments and form highly resilient biofilms within plumbing systems (Oliveira et al., 2024; Hayward et al., 2025). Similarly, the detection of *K. pneumoniae*, *S. aureus*, and *S. epidermidis* indicates that plumbing systems can serve as reservoirs for opportunistic pathogens capable of persistent colonization and biofilm formation. *Staphylococcus aureus*, in particular, possesses several virulence and adhesion factors that facilitate attachment to abiotic surfaces and long-term persistence within biofilms (Armoon et al., 2024).

The presence of these opportunistic pathogens in household plumbing systems is of considerable public health concern, particularly for immunocompromised individuals, hospitalized patients, elderly populations, and young children. Exposure to water contaminated with biofilm-associated bacteria may increase the risk of opportunistic infections and recurrent microbial contamination (Oliveira et al., 2024). Furthermore, biofilm-associated microorganisms often exhibit enhanced resistance to disinfectants and

antimicrobial agents due to the protective effect of the extracellular polymeric substance matrix and the altered physiological state of cells within mature biofilms (Flemming et al., 2023;).

Overall, the combined biochemical and molecular identification results demonstrate that water plumbing systems provide favorable environments for the persistence of diverse biofilm-forming bacteria, including several opportunistic pathogens. The predominance of *E. coli* and the detection of *P. aeruginosa*, *K. pneumoniae*, *S. aureus*, and *S. epidermidis* highlight the microbiological risks associated with plumbing-associated biofilms and emphasize the importance of continuous monitoring and effective biofilm control strategies to improve the safety and quality of domestic water supplies.

Antibiotic Resistance Profiles of Biofilm-Producing Bacteria

The antibiotic susceptibility testing revealed that all four bacterial species exhibited multidrug resistance against several commonly used antibiotics. *Klebsiella pneumoniae* showed complete resistance to amoxicillin/clavulanic acid, tetracycline, and cefuroxime, while *Escherichia coli* demonstrated extensive resistance to multiple antibiotic classes, including β-lactams, aminoglycosides, tetracyclines, and nitrofurantoin. *Pseudomonas aeruginosa* exhibited complete resistance to ceftazidime and ciprofloxacin, with additional resistance to carbapenems and amikacin. Similarly, *Staphylococcus aureus* showed high levels of resistance to gentamicin, tetracycline, clindamycin, erythromycin, cefoxitin, and nitrofurantoin. These findings indicate the widespread occurrence of multidrug-resistant bacteria within the water plumbing system. The high level of antibiotic resistance observed may be associated with the biofilm-forming ability of these bacteria. Biofilms limit antibiotic penetration and promote the survival of resistant bacterial populations, contributing to the multidrug-resistant phenotypes observed in this study (Nahum et al., 2025).

Despite the widespread resistance observed, certain antibiotics remained effective against some isolates. Cotrimoxazole showed good activity against *K. pneumoniae* and *S. aureus*, while ceftriaxone retained effectiveness against most *K. pneumoniae* isolates. In addition, susceptibility to ciprofloxacin and chloramphenicol was observed among most *S. aureus* isolates. These findings suggest that although resistance is prevalent, some therapeutic options may still be available for the management of infections caused by these organisms.

Overall, the results demonstrate the presence of clinically significant multidrug-resistant biofilm-forming bacteria in water plumbing systems. The occurrence of resistant *E. coli, P. aeruginosa, K. pneumoniae,* and *S. aureus* highlights the potential public health risks associated with plumbing-associated biofilms, as these systems may facilitate the persistence and dissemination of antimicrobial-resistant bacteria and resistance genes within the environment (Hayward et al., 2025; Meradji et al., 2025). Continuous monitoring and effective biofilm control measures are therefore essential to reduce microbial contamination and limit the spread of antimicrobial resistance.

Detection of Biofilm Associated Genes

The detection of *icaA*, *icaD*, *cupA*, *fimB*, and *luxS* genes confirms the presence of key genetic determinants associated with biofilm formation among plumbing-associated bacterial isolates. Similar observations have been reported in drinking water systems, where these genes facilitate bacterial adhesion, biofilm development, and persistence under environmental stress conditions. (Flemming et al., 2023, Oliveira et al., 2024).

The simultaneous detection of *icaA* and *icaD* in *Staphylococcus aureus* indicates the presence of the intercellular adhesion (*ica*) operon involved in polysaccharide intercellular adhesin (PIA) synthesis, an important factor in biofilm accumulation. The *icaA* gene encodes N-acetylglucosaminyltransferase involved in polysaccharide synthesis, while *icaD* enhances the enzymatic activity required for efficient PIA

production. Previous studies have demonstrated that co-expression of *icaA* and *icaD* is strongly associated with increased biofilm-forming capacity and enhanced antimicrobial resistance among staphylococcal isolates (Armoon et al., 2024). The detection of these genes in the present study therefore suggests that *Staphylococcus aureus* isolates recovered from plumbing systems possess considerable biofilm-forming potential, which may contribute to their persistence under adverse environmental conditions.

The detection of the *cupA* gene in *Pseudomonas aeruginosa* isolates further highlights the ability of this organism to establish stable biofilms within plumbing environments. The *cupA* gene is associated with the chaperone–usher pathway, which mediates fimbrial assembly and facilitates bacterial attachment to abiotic surfaces during the early stages of biofilm formation (Böhning et al., 2023). *Pseudomonas aeruginosa* is widely recognized for its exceptional ability to colonize water systems and survive under nutrient-limited conditions through efficient biofilm formation (Li et al., 2023; Oliveira et al., 2024). Similar studies have reported the presence of *cupA*-associated adhesion systems in environmental *P. aeruginosa* isolates recovered from drinking water systems and hospital plumbing networks, emphasizing their role in bacterial persistence and resistance to disinfectants (Gholipour et al., 2024; Oliveira et al., 2024). Likewise, the *fimB* gene identified in *Escherichia coli* isolates is associated with the regulation of type I fimbriae expression, which contributes to bacterial adhesion and initiation of biofilm development on abiotic surfaces. Type I fimbriae play a crucial role in the early attachment phase of biofilm formation by enabling bacterial adherence to pipe surfaces and facilitating colonization within plumbing systems. The detection of the *fimB* gene in the present study therefore indicates the potential of *E. coli* isolates to establish persistent biofilms within household water systems. Similar findings have been reported in environmental and clinical *E. coli* isolates, where fimbrial adhesion factors were associated with enhanced colonization, surface attachment, and increased persistence within biofilm communities (Ageorges et al., 2020; Kallas et al., 2020).

The *luxS* gene was detected in multiple bacterial species, including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, indicating the potential role of quorum sensing in regulating microbial communication and coordinated biofilm formation within plumbing systems. The *luxS* gene is responsible for the production of autoinducer-2 (AI-2), a universal signaling molecule involved in interspecies bacterial communication and the regulation of virulence-associated traits (Cui & Kim, 2024). Quorum-sensing mechanisms have been recognized as important contributors to bacterial persistence, virulence, stress adaptation, and antimicrobial resistance in mature biofilms (Cui & Kim, 2024; Juszczuk-Kubiak, 2024). Previous studies have demonstrated that AI-2-mediated signaling pathways enhance biofilm maturation and stability by coordinating gene expression among bacterial populations under environmental stress conditions (Juszczuk-Kubiak, 2024). Overall, the findings of the present study demonstrate that household water plumbing systems can serve as important reservoirs of biofilm-forming bacteria, including opportunistic pathogens with the potential to persist under both treated drinking water and wastewater conditions. The high prevalence of biofilm-producing isolates, the occurrence of biofilm-associated genes involved in adhesion and quorum sensing, and the detection of antimicrobial resistance among selected isolates collectively indicate the potential public health risks associated with plumbing-associated biofilms. These characteristics may facilitate bacterial persistence, dissemination, and reduced susceptibility to conventional disinfection and antimicrobial treatments. Therefore, continuous microbiological and molecular surveillance is essential for the early detection of contamination risks and for improving the management of water quality within domestic plumbing systems. Furthermore, the implementation of effective biofilm control strategies, including antimicrobial nanocoating, improved pipe materials, optimized disinfection practices, and nanoparticle-based technologies, may provide promising approaches for minimizing biofilm formation and limiting the spread of antimicrobial-resistant bacteria. Effective management of plumbing-associated biofilms is therefore crucial for safeguarding public health and ensuring the long-term microbiological safety and sustainability of domestic water supplies.

Public Health Significance and Implications

The detection of clinically important opportunistic pathogens such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis,* and *Klebsiella pneumoniae* in the present study is particularly concerning as these microorganisms are commonly associated with gastrointestinal, respiratory, urinary tract, wound, and bloodstream infections (Bello et al., 2024; Hayward et al., 2025). Their persistence within household plumbing systems increases the risk of human exposure through routine domestic activities such as drinking, handwashing, bathing, food preparation, and cleaning. Previous studies have demonstrated that biofilm-associated microorganisms can detach from mature biofilms and enter bulk water, contributing to recurrent microbial contamination and the dissemination of opportunistic pathogens within drinking water systems (Douterelo et al., 2018; Oliveira et al., 2024).

The public health significance of these findings is further amplified by the detection of important biofilm-associated genes, including *icaA*, *icaD*, *cupA*, *fimB*, and *luxS*. These genes are involved in bacterial adhesion, quorum sensing, extracellular polysaccharide production, and biofilm maturation, all of which contribute to enhanced bacterial persistence and pathogenicity (Cui & Kim, 2024; Flemming et al., 2023). The ability of bacteria to form mature biofilms significantly reduces the effectiveness of conventional water treatment and sanitation procedures, as the EPS matrix restricts disinfectant penetration and protects embedded bacterial cells (Oliveira et al., 2024).

The antibiotic susceptibility testing conducted in the present study further demonstrated the occurrence of antimicrobial resistance among selected biofilm-producing isolates. The detection of resistance to commonly used antibiotics among opportunistic pathogens highlights the potential for plumbing systems to act as environmental reservoirs of antimicrobial-resistant bacteria. The coexistence of biofilm-forming ability and antimicrobial resistance is particularly concerning as biofilms facilitate the survival, persistence, and dissemination of resistant microorganisms while reducing the efficacy of antibiotic treatment. This may increase the risk of waterborne infections that are difficult to treat and contribute to adverse health outcomes, including increased morbidity and mortality.

The presence of opportunistic pathogens in plumbing systems poses an even greater risk to vulnerable populations, including immunocompromised individuals, elderly persons, hospitalized patients, pregnant women, and young children, whose immune defenses may be insufficient to prevent infection following exposure to contaminated water (WHO, 2022). *Pseudomonas aeruginosa*, for example, is recognized as an important opportunistic pathogen frequently associated with hospital-acquired infections and chronic respiratory infections, particularly among immunocompromised patients (Mena & Gerba, 2009). Similarly, *Staphylococcus aureus* and *Klebsiella pneumoniae* are well-known causes of healthcare-associated infections and have been detected in water and plumbing-associated biofilms, where they may persist and contribute to environmental dissemination (Bello et al., 2024; Hayward et al., 2025). The occurrence of these pathogens in domestic water systems therefore highlights the potential role of household plumbing infrastructures as reservoirs for infection and antimicrobial resistance dissemination.

In addition to direct health impacts, biofilm formation within pipelines may negatively affect overall water quality and infrastructure performance. Biofilms can contribute to pipe corrosion, unpleasant taste and odor, discoloration of water, reduced hydraulic efficiency, and accumulation of organic and inorganic contaminants within water distribution systems. Persistent biofilm contamination may also increase operational costs associated with cleaning, maintenance, pipe replacement, and disinfection procedures. Conventional chlorination and flushing practices are often insufficient for complete biofilm removal as residual bacterial populations may rapidly recolonize pipe surfaces following treatment (Oliveira *et al.,* 2024). Similar observations have been reported in previous investigations where mature biofilms remained detectable even after repeated disinfection procedures (Douterelo et al., 2018).

Overall, the findings of the present study demonstrate that household plumbing systems can function as reservoirs of biofilm-forming and antimicrobial-resistant bacteria, posing significant challenges to water quality management and public health. Continuous microbiological and molecular surveillance, together with the implementation of effective biofilm control strategies, is therefore essential to minimize contamination risks, limit the dissemination of antimicrobial resistance, and ensure the long-term safety of domestic water supplies.

Limitations and Future Recommendations

The present study was limited to selected household plumbing systems within Colombo, Sri Lanka, and focused on a limited number of bacterial species and biofilm-associated genes, including *icaA*, *icaD, cupA, fimB, and luxS*. Although these genes are important in biofilm formation and persistence, several other clinically significant biofilm-forming bacteria such as *Acinetobacter baumannii and Enterococcus faecalis* as well as additional biofilm-associated genes including *psl, pel, algD, bap*, and *csgA*, were not investigated. Furthermore, the present study evaluated only the presence of biofilm-associated genes and did not assess their expression levels under environmental stress conditions. Future studies should therefore incorporate larger sampling areas, additional bacterial species, quantitative gene expression analysis, metagenomic sequencing, and antimicrobial resistance profiling to provide a more comprehensive understanding of plumbing-associated biofilm communities and their pathogenic potential.

5 | Conclusions

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The present study confirmed the presence of biofilm-forming bacteria in household water plumbing systems, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Staphylococcus epidermidis*. Molecular identification and PCR analysis revealed the occurrence of important biofilm-associated genes (*icaA, icaD, cupA, fimB,* and *luxS*), which are involved in bacterial adhesion, biofilm development, quorum sensing, and persistence. The detection of these genes supports the observed biofilm-forming phenotypes and demonstrates the genetic basis for biofilm establishment within plumbing environments. The multidrug-resistant nature of the identified bacterial isolates further highlights the potential public health risks associated with plumbing-associated biofilms. The coexistence of biofilm-forming ability, antimicrobial resistance, and biofilm-associated genes suggests that household plumbing systems can serve as reservoirs for persistent and potentially pathogenic microorganisms. Therefore, regular monitoring of water plumbing systems and the implementation of effective biofilm control strategies are essential to improve water quality and minimize health risks. Further studies involving a wider geographical coverage and additional biofilm- and resistance-associated genes are recommended to enhance understanding of microbial persistence in domestic water systems.

Author Contributions

S.T.: Conceptualized the study, contributed to the methodology, performed the investigation and experiments, conducted the formal analysis, curated and validated the data, prepared the original draft of the manuscript, contributed to the review and editing of the manuscript, and contributed to project administration.
K.V. & I.A.: Contributed to the conceptualization and methodology, supervised the study, contributed to data validation and interpretation, critically reviewed and edited the manuscript, and contributed to project administration.
All authors: Have read and approved the final version of the manuscript.

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Ethics Statement

Ethical approval was not required for this study, as it involved environmental water samples and did not include human or animal subjects.

References

Ageorges, V., Monteiro, R., Leroy, S., Burgess, C. M., Pizza, M., Trotter, A., Bottreau, E., & Desvaux, M. (2020). Molecular determinants of surface colonisation in diarrhoeagenic *Escherichia coli* (DEC): From bacterial adhesion to biofilm formation. *FEMS Microbiology Reviews, 44*(3), 314–350. https://doi.org/10.1093/femsre/fuaa008

Armoon, M., Babapour, E., Mirnejad, R., Babapour, M., & Taati Moghadam, M. (2024). Evaluation of *icaA* and *icaD* genes involved in biofilm formation in *Staphylococcus aureus* isolates from clinical sources using reverse transcriptase PCR. *Archives of Razi Institute, 79*(6), 1329–1335. https://doi.org/10.32592/ARI.2024.79.6.1329

Baird, R. B., Eaton, A. D., & Rice, E. W. (Eds.). (2017). *Standard methods for the examination of water and wastewater* (23rd ed.). American Public Health Association.

Barrow, G. I., & Feltham, R. K. A. (Eds.). (2003). *Cowan and Steel's manual for the identification of medical bacteria* (3rd ed.). Cambridge University Press.

Bergey, D. H., & Holt, J. G. (1994). *Bergey's manual of determinative bacteriology* (9th ed.). Williams & Wilkins.

Bello, O. O., Oni, M. O., Bello, T. K., Ilemobayo, A. M., Ajagunna, A. M., & Osho, A. (2024). Biofilm-forming antibiotic-resistant bacteria in water from distribution systems: Occurrence and public health implications. *International Journal of Microbiology, 2024*, Article 4147226. https://doi.org/10.1155/ijm/4147226

Böhning, J., Dobbelstein, A. W., Sulkowski, N., Eilers, K., von Kügelgen, A., Tarafder, A. K., Peak-Chew, S. Y., Skehel, M., Alva, V., Filloux, A., & Bharat, T. A. M. (2023). Architecture of the biofilm-associated archaic chaperone-usher pilus CupE from *Pseudomonas aeruginosa*. *PLoS Pathogens, 19*(4), e1011177. https://doi.org/10.1371/journal.ppat.1011177

Clinical and Laboratory Standards Institute. (2022). *Performance standards for antimicrobial susceptibility testing* (32nd ed.). CLSI.

Cui, S., & Kim, E. (2024). Quorum sensing and antibiotic resistance in polymicrobial infections. *Communicative & Integrative Biology, 17*(1), 2415598. https://doi.org/10.1080/19420889.2024.2415598

Douterelo, I., Fish, K. E., & Boxall, J. B. (2018). Succession of bacterial and fungal communities within biofilms of a chlorinated drinking water distribution system. *Water Research, 141*, 74–85. https://doi.org/10.1016/j.watres.2018.04.058

Flemming, H.-C., van Hullebusch, E. D., & Wuertz, S. (2023). The biofilm matrix: Multitasking in a shared space. *Nature Reviews Microbiology, 21*(9), 567–584. https://doi.org/10.1038/s41579-022-00791-0

Forbes, B. A., Sahm, D. F., & Weissfeld, A. S. (2007). *Bailey & Scott's diagnostic microbiology* (12th ed.). Mosby Elsevier. ISBN: 9780323030656.

Gholipour, S., Nikaeen, M., Mehdipour, M., Mohammadi, F., & Rabbani, D. (2024). Occurrence of chlorine-resistant *Pseudomonas aeruginosa* in hospital water systems: Threat of waterborne infections for patients. *Antimicrobial Resistance & Infection Control, 13*(1), Article 111. https://doi.org/10.1186/s13756-024-01468-4

Gomes, I. B., Simões, M., & Simões, L. C. (2014). An overview on the reactors to study drinking water biofilms. *Water Research, 62*, 63–87. https://doi.org/10.1016/j.watres.2014.05.039

Hassan, A., Usman, J., Kaleem, F., Omair, M., Khalid, A., & Iqbal, M. (2011). Evaluation of different detection methods of biofilm formation in the clinical isolates. *The Brazilian Journal of Infectious Diseases, 15*(4), 305–311. https://doi.org/10.1016/S1413-8670(11)70197-0

Hayward, C., Ross, K. E., Brown, M. H., Bentham, R., Hinds, J., & Whiley, H. (2025). Drinking water plumbing systems are a hot spot for antimicrobial-resistant pathogens. *Journal of Hospital Infection, 159*, 62–70. https://doi.org/10.1016/j.jhin.2025.02.018

Johnson, J. S., Spakowicz, D. J., Hong, B. Y., Petersen, L. M., Demkowicz, P., Chen, L., Leopold, S. R., Hanson, B. M., Agresta, H. O., Gerstein, M., Sodergren, E., & Weinstock, G. M. (2019). Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nature Communications, 10*(1), Article 5029. https://doi.org/10.1038/s41467-019-13036-1

Juszczuk-Kubiak, E. (2024). Molecular aspects of the functioning of pathogenic bacteria biofilm based on quorum sensing (QS) signal-response system and innovative non-antibiotic strategies for their elimination. *International Journal of Molecular Sciences, 25*(5), 2655. https://doi.org/10.3390/ijms25052655

Kallas, P., Haugen, H. J., Gadegaard, N., Stormonth-Darling, J., Hulander, M., Andersson, M., & Valen, H. (2020). *Adhesion of Escherichia coli to nanostructured surfaces and the role of type 1 fimbriae*. *Nanomaterials, 10*(11), 2247. https://doi.org/10.3390/nano10112247

Li, X., Gu, N., Huang, T. Y., Zhong, F., & Peng, G. (2023). *Pseudomonas aeruginosa*: A typical biofilm forming pathogen and an emerging but underestimated pathogen in food processing. *Frontiers in Microbiology, 13*, 1114199. https://doi.org/10.3389/fmicb.2022.1114199

Mena, K. D., & Gerba, C. P. (2009). Risk assessment of *Pseudomonas aeruginosa* in water. In D. M. Whitacre (Ed.), *Reviews of Environmental Contamination and Toxicology* (Vol. 201, pp. 71–115). Springer. https://doi.org/10.1007/978-1-4419-0032-6_3

Meradji, S., Basher, N. S., Sassi, A., Ibrahim, N. A., Idres, T., & Touati, A. (2025). The role of water as a reservoir for antibiotic-resistant bacteria. *Antibiotics, 14*(8), 763. https://doi.org/10.3390/antibiotics14080763

Nahum, Y., Muhvich, J., Morones-Ramirez, J. R., Casillas-Vega, N. G., & Zaman, M. H. (2025). Biofilms as potential reservoirs of antimicrobial resistance in vulnerable settings. *Frontiers in Public Health, 13*, 1568463. https://doi.org/10.3389/fpubh.2025.1568463

Oliveira, I. M., Gomes, I. B., Simões, L. C., & Simões, M. (2024). A review of research advances on disinfection strategies for biofilm control in drinking water distribution systems. *Water Research, 253*, 121273. https://doi.org/10.1016/j.watres.2024.121273

Peng, Q., Tang, X., Dong, W., Sun, N., & Yuan, W. (2023). A review of biofilm formation of *Staphylococcus aureus* and its regulation mechanism. *Antibiotics, 12*(1), 12. https://doi.org/10.3390/antibiotics12010012

Proctor, C. R., Reimann, M., Vriens, B., & Hammes, F. (2018). Biofilms in shower hoses. *Water Research, 131*, 274–286. https://doi.org/10.1016/j.watres.2017.12.027

Stepanović, S., Vuković, D., Hola, V., Di Bonaventura, G., Djukić, S., Cirković, I., & Růžička, F. (2007). Quantification of biofilm in microtiter plates: Overview of testing conditions and practical

recommendations for assessment of biofilm production by staphylococci. *APMIS, 115*(8), 891–899. https://doi.org/10.1111/j.1600-0463.2007.apm_630.x

Wang, H., Masters, S., Edwards, M. A., Falkinham, J. O., III, & Pruden, A. (2014). Effect of disinfectant, water age, and pipe materials on bacterial and eukaryotic community structure in drinking water biofilm. *Environmental Science & Technology, 48*(3), 1426–1435. https://doi.org/10.1021/es402636u

Winn, W. C., Jr., Allen, S. D., Janda, W. M., Koneman, E. W., Procop, G. W., Schreckenberger, P. C., & Woods, G. L. (2006). *Koneman's color atlas and textbook of diagnostic microbiology* (6th ed.). Lippincott Williams & Wilkins. ISBN: 9780781730143.

World Health Organization. (2022). *Guidelines for drinking-water quality* (4th ed., incorporating addenda). World Health Organization.