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Antibiotic Resistance and Biofilm Formation of Bacterial Profile Isolated from Postoperative Drainage Fluid of Patients Admitted in Hilla Hospitals in Iraq

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Abstract

Surgical drains are catheters placed near the surgical wound to remove blood, pus, or other fluid and prevent it from accumulating in the body. These surgical drains are considered the major determinant for development of biofilm forming bacteria. Biofilm is an assemblage of the microbial cells that is irreversibly associated with a surface and usually enclosed in a matrix of polysaccharide material. The aim of this study is to investigate and identify biofilm formation bacteria with antibiotic resistance profile isolated from drainage tube after surgical operative. One hundred piece of drainage tube were collected from November 2022 to March 2023, catheterized inpatients of varying ages and sexes were enrolled in the study. Microbiology diagnosis and antibiotic sensitivity test were carried by routinely laboratory tests and VITEK system. The biofilm formation, detection by Congo red agar. The results that included 100 drainage tube post operation patients of different clinical surgical operation revealed that out of 47 Bacterial growth, 32 (68.0%) biofilm forming bacteria, gram negative bacteria were the most 29 (*Escherichia Coli 11, Pseudomonas Aeruginosa 8, Serratia Marcescens 6, Entrobacter cloacae complex 3, Citrobacter freundil 1).* While the gram positive were 18 (*Enterococcus faecalis 6, Staphyiococcus hominis ssp 5, Staphyiococcus haemolyticus 3, Staphyiococcus epidermidis 3, kocuria rosea 1).* The bacterial isolates that generated biofilm on Congo red media were identified by the black coloration caused by the dye's capacity to stain the polysaccharide matrix that was formed throughout the biofilm formation process. A biofilm gram positive and gram negative bacteria (61.1%), (72.4%) respectively, showed that some types of bacteria were very good at making biofilms, while others were not very good at it, and the differences were very big ($p<0.05$). The current study concludes that in spite of the sterility of drainage tubes in postoperative patients, but they may be considered a source of multi-drug resistance bacterial infection in patients.

Introduction

In order to prevent the buildup of fluids such as blood, pus, or other substances, surgical drains are catheters that are inserted close to the surgical incision. An assembly of microbial cells often encased in a polysaccharide matrix and permanently attached to a surface is called a biofilm [1]. Bacteria develop biofilms when attached to surfaces, become sessile, and secrete a slimy glue-like material for anchoring [2]. A negative pressure gradient is maintained by these active drains. When an active drain's vacuum is lost, the drain may stop collecting fluid since it relies on exchanging negative pressure. Catheter is a thin tube which is made from medical grade materials to serve a broad range of functions. Catheters are medical devices that can be inserted in the body to treat diseases or perform a surgical procedure [3]. As biofilm bacteria ultimately develops on of these devices depending on the device, gram-positive or gram-negative bacteria, yeasts, or other microorganisms can form a biofilm. Isolated microorganisms from these devices often include gram-positive bacteria like Escherichia coli and Pseudomonas aeruginosa, as well as gram-negative bacteria like Staphylococcus aureus and Staphylococcus epidermidis. These creatures could have an internal or external source of origin. Endotracheal tubes, central venous catheters, and urine catheters are the most prevalent indwelling medical devices from which biofilm-forming microorganisms are isolated (30%). These microorganisms are more likely to form biofilms—Escherichia coli, Klebsiella pneumonia, Streptococcus spp., and Staphylococcus epidermidis the longer the catheter stays in place.

Because they make microbes less susceptible to antimicrobials, biofilms are very important in medicine. Additionally, plasmid exchange can be facilitated by the close proximity of cells within a biofilm which can promote the propagation of antibiotic resistance [4]. The aim of this study is to find out more about the biofilm that forms on indwelling catheters, as well as qualitative and quantitative detection methods in vitro and antibiotic susceptibility testing so that doctors can make an informed choice about how to treat these kinds of infections. Due to the lack of studies on the subject of examining the catheters inside the patient’s body and the lack of health procedures in this regard, we carried out this research.

Materials and methods

Collections of samples

From November 2022 to March 2023, a total of 100 catheterized inpatients of varying ages and sexes were enrolled in the study. These patients were being admitted at three different hospitals in the city of Hilla: AL- Hilla Teaching Hospital, Al-Imam Sadiq Hospital and Marjan Medical City. Patient

information (including age, catheter duration, concomitant clinical conditions, and antibiotic use) was gathered using a standardized questionnaire.

Processing Sample (Catheter Piece)

One hundred of catheter pieces were collected from patients after surgical operative. Catheter samples were cultured in enrichment media (brain heart infusion broth and incubated at 37 ℃ for 24 h, then cultured in routinely media for bacterial growth (nutrient and MacConkey agar) and incubated at 37 ℃ for 24 h. Bacterial culture growth was diagnosed by the automated system VITEK 2.

Finding Biofilm Formation

The Tissue Culture Plate (TCP) method and the Congo red agar method (TCP) were used to find biofilms [5] and [6].

The statistical analysis

Showed that all the experiments were done three times to make sure they could be repeated. One-way ANOVA with a p-value of 0.005 was used for the statistical study in SPSS Statistics 24.0.

Results and Discussion

Among 100 drainage tube samples, 47 bacteria isolates were isolated after culturing on routing culture media. The highly incidence of bacterial isolates among catheter tube sample indicate the potent bacterial pathogenicity and virulence. Usually, the drained fluid after postoperative must be sterile which the current study revealed the tube sample contain pathogenic bacterial. Total studies pointed the presence of bacterial isolate in drain fluid after surgical post operation [7]. The presence of medical advice (catheter) inside the patient's body stimulates the bacterial infection by courage, the attachment of pathogenic bacteria, and persist on medical advice which leads to cause huge problem effect on public health. Klebsiella, Enterobacter, and Serratia are found in the intestines of many healthy people and rarely cause infections. Infections with these bacteria are often contracted in hospitals and long-term care homes. They usually infect people with weak resistance to infection or people who carry medical devices (such as catheters, drains, or breathing tubes) [8].

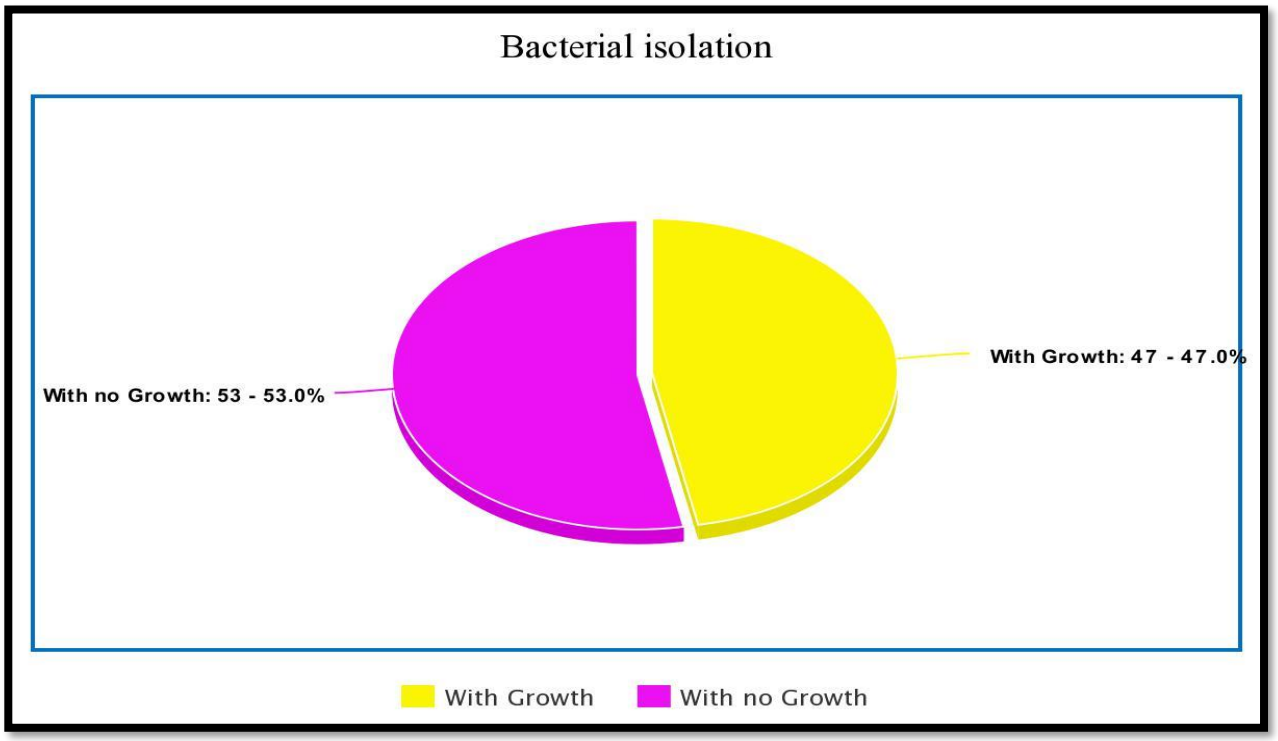

Figure 1: Pie chart of bacterial growth from drainage tube.

Table 1: The number, percentage and types of bacteria according to the clinical cases.

Source	Organisms	No.	%
Cholecystectomy Surgery	*Enterococcus Faecalis*	2	33.3%
	Staphylococcus hominis ssp	2	40%
	Staphylococcus haemolyticus	1	33.3%
	Staphylococcus Epidermidis	1	33.3%
	Escherichia Coli	4	36.3%
	Pseudomonas Aeruginosa	3	37.5%
	Serratia Marcescens	2	33.3%
	Enterobacter cloacae complex	1	33.3%
Cesarean Surgery	*Enterococcus Faecalis*	2	33.3%
	Staphylococcus hominis ssp	1	20%
	Staphylococcus haemolyticus	1	33.3%

	Escherichia Coli	**3**	**27.2%**
	Pseudomonas Aeruginosa	**2**	**25%**
	Serratia Marcescens	**4**	**66.7%**
Hernia Surgery	***Staphylococcus Epidermidis***	**2**	**66.7%**
	Kocuria rosea	**1**	**100%**
	Escherichia Coli	**3**	**27.2%**
	Pseudomonas Aeruginosa	**2**	**25%**
	Enterobacter cloacae complex	**2**	**66.7%**
	Citrobacter freundil	**1**	**100%**
Thyroidectomy Surgery	***Enterococcus Faecalis***	**2**	**33.3%**
	Staphylococcus hominis ssp	**2**	**40%**
	Staphylococcus haemolyticus	**1**	**33.3%**
	Escherichia Coli	**1**	**9.3%**
	Pseudomonas Aeruginosa	**1**	**12.5%**

Table 2: Type of Bacterial isolates isolated from drainage tube

Bacterial isolation	**+Gram Positive**	**-Gram Negative**	**N (%)**
Bacterial Growth	18	29	47%
No Growth	53		53%

Isolated bacterial strains were able to develop biofilm on Congo red medium. Whereas the black color signified the capacity of Congo red dye to stain the polysaccharide matrix that was formed during the process of biofilm formation, a biofilm + Gram Positive - Gram Negative (61.1%), 72.4% of the samples indicated that certain bacterial species exhibited a high ratio of biofilm formation, while others exhibited a low ability, and the differences were statistically significant ($p< 0.05$).

Table 3: Bacterial isolates based on biofilm formation

Bacterial Biofilm	+Gram Positive	-Gram Negative	N(%)
Biofilm formation	11(61.1%)	21(72.4%)	68%
No Biofilm formation	7(38.9%)	8(27.6%)	32%
Total	47		100%
χ^2	0.653		
P	0.41 (*not* significant at $p < 0.05$)		

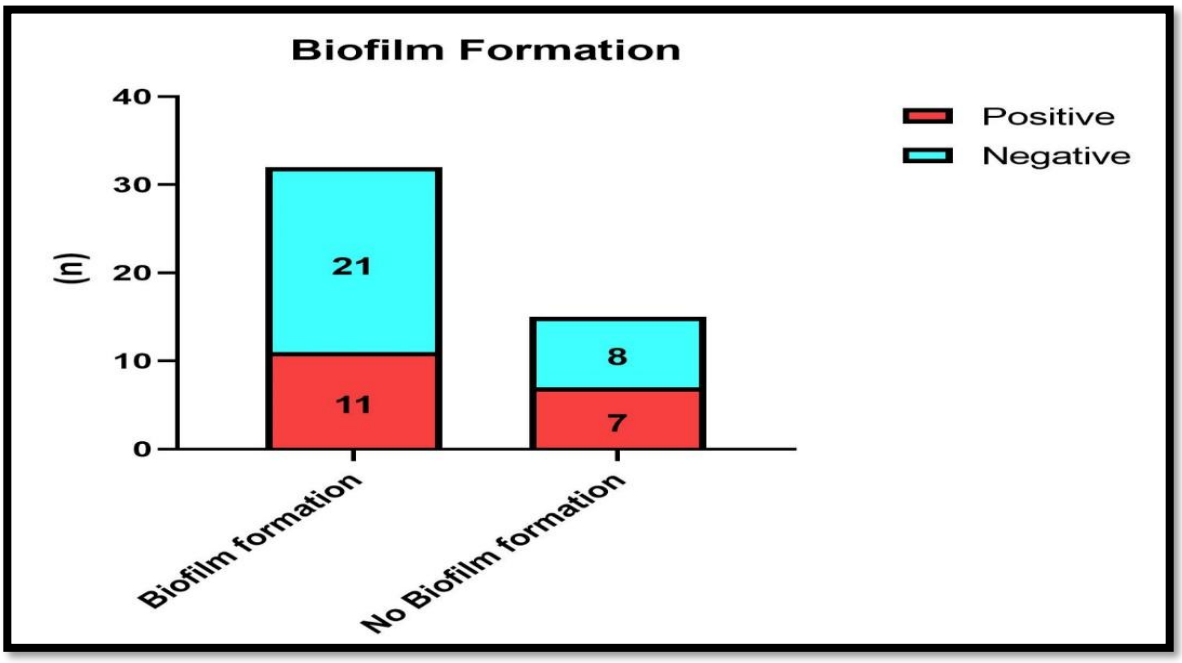

Figure 2:Biofilm formation according to gram positive and negative bacteria

Table 3:VITEK AST results of biofilm forming from Various bacteria

Antibiotics	MIC	Interp.
Ampicillin	**≥ 32**	**R**
Ampicillin/	**clavulanic ≥ 32**	**R**
Ampicillin/	**sulbactaum ≥ 32**	**R**
Cefazolin	**≥ 64**	**R**
Ceftazidime	**≥ 64**	**R**
Ceftriaxone	**≥ 64**	**R**
Impienem	**8**	**I**
Gentamicin	**≥ 16**	**R**
Tobramycin	**≥ 16**	**R**
Ciprofloxacin	**≥ 4**	**R**
Levofloxacin	**≥ 8**	**R**
Nitrofurantain	**≥ 512**	**R**
Trimetheprium/sulfamithaxzol	**≥ 320**	**R**

Figure 3:Bacteria growth on a plate and biofilm formation

The frequency of occurrence gram negative bacterial isolates reveals, Escherichia Coli (11%) Pseudomonas Aeruginosa (6%) Serratia Marcescens (8%), Entrobacter cloacae complex (3%) Citrobacter freundil (1%). Fluor quinolone antibiotic has a clear effect on all types of bacteria and is considered an important antibiotic (100%), (83.3%), (62.5%), (33.3%), (100%) p=0.04 considered significant with MDR. (MDRGN bacteria) are a type of Gram-negative bacteria with resistance to multiple antibiotics. They can cause bacterial infections that pose a serious and rapidly emerging threat for hospitalized patients and especially patients in intensive care units [9].

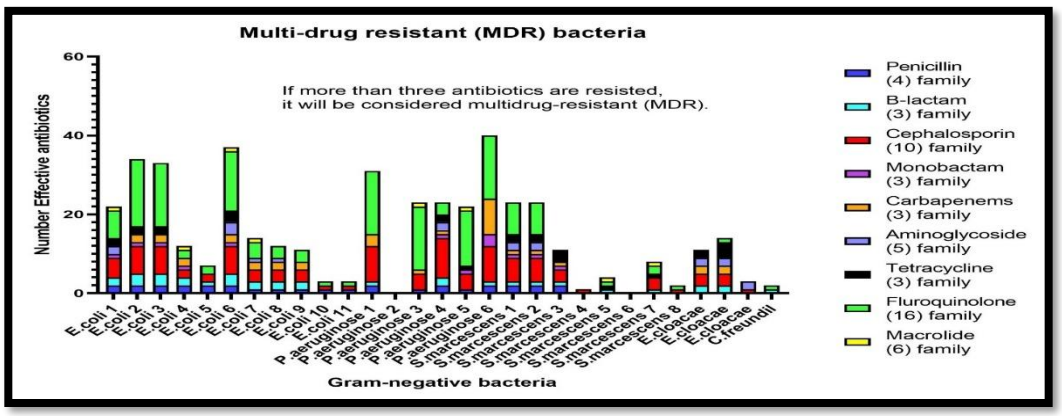

Figure 4: Multi-drug resistant (MDR) gram negative bacteria isolated from drainage tube

Table 4:Antimicrobial Resistance of Gram-Negative Bacteria isolated from drainage tube

Antibiotics	***Escherichia Coli*** **N (11)**	***Pseudomonas Aeruginosa*** **N (8)**	***Serratia Marcescens*** **N (6)**	***Entrobacter cloacae*** **complex N (3)**	***Citrobacter freundil*** **N (1)**	**p.value**
Penicillin	11(100%)	5 (83.3%)	3(37.5%)	0(0)	0(0)	0.6
B-lactam	9(81.9%)	3 (50%)	5 (62.5%)	2 (66.6%)	1 (100%)	0.4
Cephalosporin	11 (100%)	5 (83.3%)	6 (75%)	3 (100%)	0(0)	0.3
Monobactam	5 (45.5%)	3 (50%)	3(37.5%)	0 (0)	0(0)	0.2
Carbapenems	7 (63.6%)	3 (50%)	3(37.5%)	2 (66.6%)	0(0)	0.5
Aminoglycoside	4 (36.3%)	1 (16.6%)	2 (25%)	3 (100%)	0(0)	0.3
Tetracycline	4 (36.3%)	2 (33.3%)	5 (62.5%)	2 (66.6%)	0(0)	0.2
Fluroquinolone	11 (100%)	5 (83.3%)	5 (62.5%)	1 (33.3%)	1 (100%)	0.04
Macrolide	4 (36.3%)	2 (33.3%)	2 (25%)	0(0)	0(0)	0.21

The frequency of occurrence gram-positive bacterial isolates were Enterococcus faecalis (6%) Staphylococcus hominis ssp (5%), Staphylococcus haemolyticus (3%), Staphylococcus epidermidis (3%), Kocuria rosea (1%). Penicillin antibiotic is considered to have a strong effect on bacteria, especially removing the presence of this bacteria in large quantities from the gallbladder p value = 0.04. The number of bacterial isolates and types of bacteria are shown per source table-6 that illustrates the percentages of sampling with several sources. Antibiotic prophylaxis is one of the

methods that can be utilized to prevent the occurrence of infection following surgical treatments. Cholecystectomies that involve certain risk factors for infection are the ones that cause this to happen. Some guidelines, on the other hand, recommend that the antibiotic prophylaxis be used prior to any and all cholecystectomies [10].

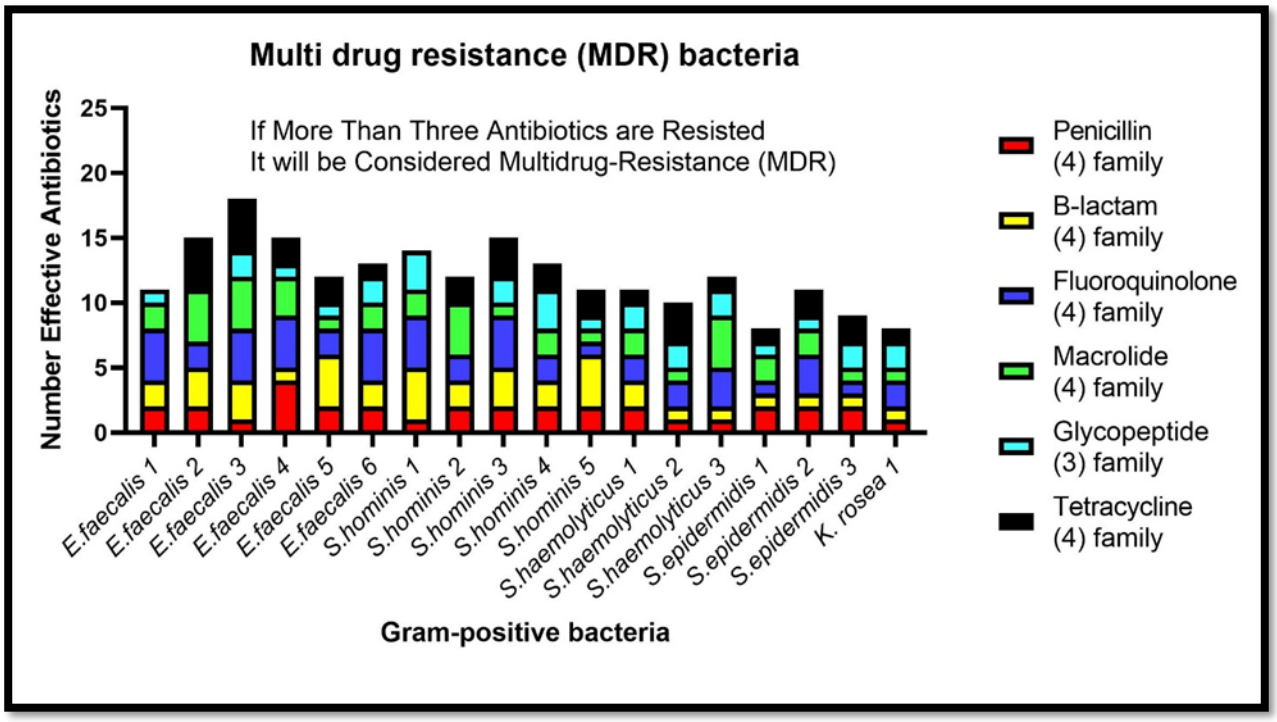

Figure 5: Multi-drug resistant (MDR) gram positive bacteria isolated from drainage tube

Table 5:Antimicrobial Resistance of Gram-Positive Bacteria isolated from drainage tube

Antibiotics	***Enterococcus faecalis* N (6)**	***Staphylococcus hominis ssp* N (5)**	***Staphylococcus haemolyticus* N (3)**	***Staphylococcus epidermidis* N (3)**	***Kocuria rosea* N (1)**	**p.value**
Penicillin	6 (100%)	5 (100%)	3(100%)	3 (100%)	1 (100%)	0.04
B-lactam	6 (100%)	5 (100%)	3(100%)	3 (100%)	1 (100%)	0.4

Fluroquinolone	6 (100%)	5 (100%)	3(100%)	3 (100%)	1 (100%)	0.4
Macrolide	6 (100%)	5 (100%)	3(100%)	3 (100%)	1 (100%)	0.4
Glycopeptide	5 (83.3%)	4 (80%)	3(100%)	3 (100%)	1 (100%)	0.2
Tetracycline	5 (83.3%)	4 (80%)	3(100%)	3 (100%)	1 (100%)	0.2

Table 6: Percentage Biofilm production and MDR pattern among bacterial isolates from drainage tube

Bacteria	**Multidrug-resistant bacteria**	**Biofilm formation**
Enterococcus faecalis (6)	6 (100%)	6 (100%)
Staphylococcus hominis ssp (5)	5(100%)	2 (40%)
Staphylococcus haemolyticus (3)	3(100%)	3(100%)
Staphylococcus Epidermidis (3)	3(100%)	0
Kocuria rosea (1)	1 (100%)	0
Escherichia Coli (11)	11(100%)	7 (63.6%)
Pseudomonas Aeruginosa (6)	5 (83%)	5 (83%)

Serratia Marcescens (8)	5(62.5%)	8 (100%)
Enterobacter cloacae complex (3)	2(66.6%)	1(33.4%)
Citrobacter freundil (1)	0	0

We found that Serratia Marcescens to be one of the most resistant to Multidrug and forms a biofilm. This agrees with the organized heterogeneous assemblages of microbial cells that are enclosed inside a matrix that were formed by the microorganisms themselves and are known as biofilms. Up to eighty percent of bacterial and archaeal cells are thought to be found in biofilms, according to the most recent estimations [11].

Duration of catheter time with biofilm

The current results showed that the length of catheter time at one day was more likely to result in biofilm formation, and this finding may be explained by the highly pathogenic bacteria that cause the biofilm and their capacity to form biofilms. This finding also aligns with other studies that suggested that biofilm formation may occur immediately after catheter insertion, which is related to microorganisms' ability to attach quickly to catheter surfaces [12]. Still, other research revealed that urinary catheters left in the urinary tract for extended periods of time were more likely to predispose to biofilm formation. A catheter left in the urinary tract for at least seven days had a 50% chance of being infected [13]. The length of catheterization and catheter maintenance determine the biofilm infection problem [14].

Conclusions

This study concluded that despite the use of sterile drainage catheters for post-operative patients, it is considered a source of infection with biofilm-forming and antibiotic resistance bacteria which has today become a problem that threatens global health. Therefore, it can be considered a hidden enemy, especially since testing the fluids from drainage catheters is not the focus of the attention of health staph in hospitals.

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