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Synergistic Effects of *Candida* strains and *Staphylococcus aureus* in Promoting Biofilm Formation and Therapeutic Resistance

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Abstract

Background: Polymicrobial biofilms are often the source of chronic infections. Crucially, biofilms resistance to antimicrobial therapy is one of the main reasons that treating these illnesses is so challenging. **Objective:** The purpose of the present study was to evaluate the resistance of biofilms to antibiotics and antifungal drugs formed by clinical *Candida* strains, once in monomicrobial biofilms and another in compatible with *Staphylococcus aureus* strains via co-culture biofilm formation. **Methodology:** This study was designed as a laboratory-based cross-sectional investigation. The clinical samples were collected from patients at Al-Hussein Teaching Hospital in Al-Muthanna Governorate, southern Iraq, from October 2024 to January 2025. All microbiological assays, including isolation, identification, and biofilm assays for both *Staphylococcus aureus* and *Candida* species, were performed in the microbiology laboratory of the biology department, College of Science, University of Thi-Qar, Iraq. *Staphylococcus aureus* (29 isolates) and *Candida* species (26 isolates) were obtained from several body sites, including burns, urine, blood, vagina, oral swabs, diabetic foot, pus, and sputum. Biofilm formation was evaluated using flat-bottom microtiter plate assay with crystal violet. The anti-biofilm assay was achieved by using the microdilution method in 96-well flat-bottomed microplate, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. **Results:** According to the anti-biofilm assay, 29 isolates of *Staphylococcus aureus* developed biofilms even with presence of antibiotic. However, strains of *Staphylococcus aureus* were more resistant to ampicillin and tetracycline (13.8% biofilm formation) than vancomycin (10.3%) at high concentrations. Thirteen *Candida tropicalis*, seven *Candida krusei* and six *Candida albicans* strains were able to form biofilms even in the presence of antifungal drugs. At 256 µg/ml, itraconazole was more effective than fluconazole (19.2%) and voriconazole (50.0%) at inhibiting the *Candida* species (0.0% biofilm). Compared to monomicrobial biofilms, polymicrobial biofilms of *Staphylococcus aureus* and *Candida* species exhibited 100% biofilm formation with greater resistance to antimicrobial treatments. **Conclusion:** In conclusion, biofilms composed of compatible strains of both *Staphylococcus aureus* and *Candida* species enhanced antibiotic resistance, compared to monomicrobial biofilms. [*Bangladesh Journal of Infectious Diseases, December 2025;12(2):205-214]*

Keywords: *Candida* species; *Staphylococcus aureus*; biofilms; antibiotic resistance; antifungal resistance

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Introduction

Fungi and bacteria commonly coexist to form complex colonies called polymicrobial biofilms[1].it is described as a three-dimensional exopolysaccharide matrix which contains a diverse collection of microorganisms from different species[2]. Most common are polymeric biofilms, which are composed of from proteins, polysaccharides, ions, and nucleic acids. These are referred to as the extracellular polymeric substances matrixome[3]. There are five crucial phases in the development of polymicrobial biofilms. Primary reversible binding to the substrate, stable adhesion, microcolony development, maturation, and dispersion are the steps involved in the production of polymicrobial biofilms.

The processes of biofilm development and maturation are dynamic and influenced by ecological cues, interactions between microorganisms, and time[4]. In contrast to biofilms of a single species, the literature analysis highlights antagonism and cooperative interactions as microbial interactions that affect biofilm mass, tolerance, and function[5]. Numerous illnesses, such as respiratory conditions, wounds, diabetic foot ulcers, oral infections, and urinary tract infections have been shown to exhibit cross-species and cross-kingdom biofilm development[6].

Mixed fungal-bacterial biofilms pose an even more challenging immune system challenge and are associated with inferior clinical outcomes[7]. Compared to their mono-infections, both pathogens become more virulent in these biofilms, and these infections are associated with higher rates of morbidity and mortality[8]. Managing biofilm infections with traditional antimicrobials has become more difficult. Antibiotic and antifungal medication is part of standard treatment, antimicrobial-coated implants to decrease microbial adherence, immunomodulators, quorum sensing inhibitors, bacteriophage therapy, and combination antimicrobial therapies as opposed to monotherapy[9].

One of the most alarming consequences of polymicrobial interaction is antimicrobial resistance. The biofilms numerous microbial populations work together to make it resistant to biocides[10]. The hypothesis of this study was that the interaction between *Candida* strains and *Staphylococcus aureus* contributes to constructing highly resistant biofilms to antimicrobial agents based on *Candida* strain type, compared to monomicrobial biofilms. Therefore, this study targeted to evaluate the resistance of biofilms to antibiotics and antifungal drugs formed by clinical *Candida* strains, once in monomicrobial biofilms and another in compatible with *Staphylococcus aureus* strains via co-culture biofilm formation.

Methodology

Study Settings and Population: This study was designed as a laboratory-based cross-sectional investigation. The clinical samples were collected from patients at Al-Hussein Teaching Hospital in Al-Muthanna Governorate, southern Iraq, from October 2024 to January 2025 for a period of 4 months. All microbiological assays, including isolation, identification, and biofilm assays for both *Staphylococcus aureus* and *Candida* species, were performed in the microbiology laboratory of the biology department, College of Science, University of Thi-Qar, Iraq.

Collection of Samples: Under sterile settings, 180 clinical samples were taken from patients who were thought to have bacterial or fungal illnesses connected to biofilm development. These samples included blood, pus, burns, Sputum, urine, diabetic foot, vaginal, and oral swabs. These samples were collected from Al-Hussein Teaching Hospital in Al-Muthanna Governorate, southern Iraq during the period from October 2024 to January 2025.

Samples Processing, Transportation and Identification of Organism: Samples were transported directly to the microbiology laboratory after collection, and then cultivated aerobically. *Staphylococcus aureus* was isolated on blood agar and incubated for 24h at 37°C, then sub-cultured on mannitol salt agar as a differential medium[11]. Vitek 2 technique and traditional biochemical assays such as the Novobiocin, hemolysis, and catalase tests were utilized to confirm the diagnosis of *Staphylococcus aureus*[12]. Candida was isolated on Sabouraud Dextrose Agar (SDA) and incubated for 24-48h at 37°C, then subcultured on CHROM-agar of Candida to phenotypic differentiation among their species[13]. The Vitek 2 technique was also used for more precise identification of Candida species. In addition, the germ tube test and thermotolerant temperature (45°C) were performed to specifically confirm *Candida albicans*[14].

Biofilm Formation Assay: The flat-bottom microliter plate assay using crystal violet was used to create the biofilm. Microbial suspension of each bacterial and fungal isolates was prepared from

Brain Heart Infusion Broth (BHIB) (24h), with adding 1% NaCl and 1% glucose. A McFarland standard of 0.5 was used to adjust the microbial turbidity. Each treatment was performed in triplicate. microliter plate was incubated for 24h at 37°C then, micro-titer plates were emptied, washed with Phosphate Buffer Solution (PBS) two times, and incubated for one hour at 65°C. Subsequently, 200µl of 1% crystal violet was added to each well, and incubated for five minutes. then by using PBS, the micro-plates were washed two times. After 30 minutes at room temperature, each well received 200 µl of glacial acetic acid. An ELISA reader was used to measure optical density (OD) at 630 nm. Sterile BHIB was used as the negative control. BHIB was inoculated with each one of bacterial and fungal isolates as positive control[15].

Anti-Biofilm Formation Assay: Minimal inhibitory concentration (MIC) assay of monomicrobial biofilm formation for either Candida species or S. aureus was performed by microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) guideline[16], using a set of serial dilutions of antibiotic (vancomycin- tetracycline- ampicillin) /antifungal (fluconazole- itraconazole- voriconazole) from 8 to 256 µg/ml. All concentrations were prepared by Roswell Park Memorial Institute Medium 1640 (RPMI-1640) in 96-well flat-bottomed microplate[17]. All steps of biofilm formation mentioned above were also applied. The polymicrobial biofilm formation was evaluated in two ways. First, a serial dilution of antifungal drugs was prepared with Candida and incubated for 4h to allow the organism to develop, then antibiotics were added with S. aureus isolates and again incubated overnight at 37°C. Second, the same method of polymicrobial biofilm formation is used but in reverse: A serial dilution of antibiotics was prepared with S. aureus isolates and incubated for 4h to allow the organisms to develop, then antifungal drugs were added with Candida and again incubated overnight at 37°C. After that, all steps of biofilm formation mentioned above were also applied.

Statistical Analysis: p- and f-values were computed using the ANOVA single factor as implemented in SPSS program (version 25). These values quantitatively demonstrated the variations across different groups, including microbiological organisms, concentrations, and the efficacy of antibiotics or antifungals.

Ethical Clearance: All procedures of the present study were carried out in accordance with the principles for human investigations (Helsinki et al., 2013) and also with the ethical guidelines of the Institutional Research Ethics. Formal ethics approval was granted by the local ethics committee. Participants in the study were informed about the procedure and purpose of the study and the confidentiality of information provided.

Results

Out of 180 clinical samples, 26 species of Candida and 29 isolates of S. aureus were isolated and identified. The appearance and color of the colonies on CHROM-agar were used to identify the yeasts; the 6 Candida albicans, 7 Candida krusei, and 13 Candida tropicalis isolates were identified. The impact of antifungals on the production of Candida biofilms was evaluated at serial concentrations ranging from 8 to 256 µg/ml. Except for C. albicans, voriconazole and fluconazole were less effective against Candida species. However, itraconazole had greater efficacy, attaining complete inhibition (0%) at high concentration (256 µg/ml). Overall, the results revealed that the ability of Candida species to form biofilm was inversely correlated with drug concentrations. A breakpoint where biofilm production dropped was observed at 256 µg/ml. Conversely, at 8 µg/ml, all Candida isolates produced 100% biofilms (Table 1).

Table 1: Effect of Antifungal Drugs on Biofilm Formation by Candida Species Isolates at Different Concentrations

Concentration µg/ml	Antifungal Agents	*Candida tropicalis*	*Candida krusei*	*Candida albicans*
8	VOR	100.0	100	100
	ITR	92.31	85.71	83.33
	FLU	84.62	71.43	83.33
16	VOR	92.31	100	83.33
	ITR	61.54	85.71	66.67
	FLU	69.23	71.43	66.67
32	VOR	92.31	85.71	83.33
	ITR	46.15	71.43	50.0
	FLU	53.85	57.14	50

Concentration µg/ml	Antifungal Agents	*Candida tropicalis*	*Candida krusei*	*Candida albicans*
64	VOR	92.31	85.71	33.33
	ITR	30.77	42.86	33.33
	FLU	46.15	42.86	33.33
128	VOR	84.62	57.14	33.33
	ITR	7.69	0	16.67
	FLU	38.46	28.57	33.33
256	VOR	61.54	42.86	16.67
	ITR	0	0	0
	FLU	15.38	14.29	33.33

VOR: Voriconazole; ITR: Itraconazole; FLU; Fluconazole

Depend on their ability to produce biofilm without the use of an antifungal drugs (Control), C. tropicalis isolates were categorized into two groups: moderate and strong. The distribution of C. tropicalis isolates resistance to antifungal drugs was based on the profiles of resistant yeasts. Two strains of C. tropicalis that were categorized as having moderate biofilm development were sensitive to voriconazole at all other concentrations but resistant to it at 8 and 64 µg/ml. Eleven strains of *Candida tropicalis* exhibited the highest voriconazole resistance, with concentrations of 128 and 256 µg/ml. In control, five of these strains were categorized as having high biofilm development. The resistance of *Candida tropicalis* isolates has decreased against itraconazole where there were no resistant strains at 256 µg/ml. One strain was resistant at 128 µg/ml, and all other strains showed breakpoints at 64, 32, and 8 µg/ml. Depending on the strain and concentration, different C. tropicalis strains had varying levels of resistance to fluconazole. All concentrations caused resistance in two strains. However, two other strains showed sensitivity to any concentration. Based on their capacity to resist fluconazole, nine strains were distributed to different concentrations. The three antifungal drugs effects on *Candida tropicalis* ability to produce biofilms differ significantly (Table 2).

Table 2: Numbers of Resistance Profiles of *Candida t*ropicalis Biofilms to Different Antifungal Drugs Concentrations

MIC Breakpoints (µg/ml)								NO. strains
Candida tropicalis	**Control**	**Voriconazole**		**Itraconazole**		**Fluconazole**		
		S	**R**	**S**	**R**	**S**	**R**	
1	Moderate	256	128	128	64	64	32	11
2	Moderate	256	128	16	8	256	0	12
3	Moderate	0	256	256	128	0	256	7
4	Moderate	0	256	128	64	128	64	5
5	Moderate	256	128	16	8	256	128	13
6	Moderate	16	8	16	8	16	8	9
7	Moderate	128	64	32	16	32	16	10
8	Moderate	0	256	32	16	256	0	2
9	Strong	0	256	64	32	32	16	3
10	Strong	0	256	256	0	0	256	8
11	Strong	0	256	16	8	16	8	1
12	Strong	0	256	64	32	256	128	4
13	Strong	0	256	128	64	256	128	6
P-value		≤ 0.05		≤ 0.05		≤ 0.05		13 strains out of 13 isolates
F-value		7.73		17.55		7.59		

Control: biofilm categories formed by *Candida krusei* isolates without antifungal drugs; S: sensitive, indicating to isolates that did not form biofilms with the concentrations of antifungal drugs; R: resistance, indicating to isolates that formed biofilms with the concentration of antifungal drugs.

Table 3: Resistance profiles of *Candida krusei* biofilms to different antifungal drugs concentrations

MIC Breakpoints (µg/ml)								NO. strains
Candida krusei	**Control**	**Voriconazole**		**Itraconazole**		**Fluconazole**		
		S	**R**	**S**	**R**	**S**	**R**	

MIC Breakpoints (µg/ml)								NO. strains
Candida krusei	**Control**	**Voriconazole**		**Itraconazole**		**Fluconazole**		
		S	**R**	**S**	**R**	**S**	**R**	
1	Moderate	0	256	256	0	256	0	1
2	Moderate	128	64	128	64	32	16	5
3	Moderate	0	256	128	64	256	128	2
4	Strong	0	256	64	32	64	32	3
5	Strong	128	64	128	64	64	32	6
6	Strong	256	128	32	16	128	64	7
7	Strong	0	256	64	32	256	0	4
P-value		0.01		≤ 0.05		0.03		7 strains out of 7 isolates
F-value		3.43		12.23		2.82		

Control: biofilm categories formed by *Candida krusei* isolates without antifungal drugs; S: sensitive, indicating to isolates that did not form biofilms with the concentrations of antifungal drugs; R: resistance, indicating to isolates that formed biofilms with the concentrations of antifungal drugs.

The isolates of *Candida krusei* were categorized into two groups: moderate and strong. One *Candida krusei* strain that was classified as moderate biofilm formation was resistant to voriconazole at 64 µg/ml. On the other hand, two *Candida krusei* strains were resistant to all other concentrations. The highest resistance to voriconazole was recorded in five *Candida krusei* strains at 128 and 256 µg/ml. In control, four of these strains were categorized as having strong biofilm development. Isolates of *Candida krusei* showed reduced resistance to itraconazole. Nonetheless, breakpoints were showed in all strains of *Candida krusei* at 64, 32, and 16 µg/ml. Two strains of *Candida krusei* showed resistance to fluconazole at all concentrations. However, according to strain type and concentration, five strains were distributed according to their capacity to resist fluconazole. Compared to itraconazole, voriconazole and fluconazole produced very different results (Table 3).

The isolates of *Candida albicans* were divided into two weak strains and four moderate strains. At 8 µg/ml, strains of C. albicans showed resistance to voriconazole. However, two *Candida albicans* strains were recorded highest resistance to voriconazole at 128 and 256 µg/ml. While decreased the ability of strains to form biofilms at 128 µg/ml of itraconazole. At 256 µg/ml of fluconazole, two strains of *Candida albicans* showed resistance. However, one strain exhibited resistance to any concentration. The breakpoints for the other strains were 8, 16, and 32 µg/ml. The results for voriconazole, fluconazole, and itraconazole were significant (Table 4).

Table 4: Resistance Profiles of *Candida albicans* Biofilms to Different Antifungal Drugs Concentrations

MIC Breakpoints (µg/ml)								NO. strains
Candida albicans	**Control**	**Voriconazole**		**Itraconazole**		**Fluconazole**		
		S	**R**	**S**	**R**	**S**	**R**	
1	Moderate	16	8	64	32	256	0	1
2	Moderate	256	128	256	0	32	16	2
3	Moderate	64	32	256	128	16	8	3
4	Moderate	0	256	128	64	0	256	4
5	weak	64	32	32	16	64	32	5
6	weak	64	32	16	8	0	256	6
P-value		≤ 0.05		0.008		0.14		6 strains out of 6 isolates
F-value		14.44		3.85		1.83		

Control: biofilm categories formed by C. albicans isolates without antifungal drugs; S: sensitive, indicating to isolates that did not form biofilms with the concentrations of antifungal drugs; R: resistance, indicating to isolates that formed biofilms with the concentrations of antifungal drugs.

Table 5: Resistance Profiles of *Staphylococcus aureus* Biofilms to Different Antibiotic Concentrations

MIC Breakpoints (µg/ml)								NO. strain
Staphylococcus aureus	**Biofilm categories**	**Vancomycin**		**Tetracycline**		**Ampicillin**		
		S	**R**	**S**	**R**	**S**	**R**	
1	Moderate	64	32	256	128	16	8	2
2	Moderate	128	64	16	8	256	0	6
3	Moderate	64	32	128	64	256	0	3
4	Moderate	256	128	64	32	256	0	16
5	Moderate	128	64	32	16	32	16	7
6	Moderate	128	64	16	8	32	16	8
7	Moderate	128	64	256	0	16	8	9
8	Moderate	256	128	256	0	16	8	20
9	Moderate	256	128	256	128	32	16	22
10	Moderate	256	128	64	32	128	64	17
11	Moderate	256	128	64	32	256	0	16
12	Weak	256	128	32	16	32	16	14
13	Weak	256	128	0	256	0	256	11
14	Moderate	256	128	32	16	32	16	14
15	Moderate	256	128	32	16	64	32	15
16	Moderate	256	128	256	128	128	64	23
17	Strong	64	32	16	8	64	32	4
18	Weak	128	64	64	32	256	128	10
19	Weak	0	256	256	128	0	256	24
20	Moderate	256	128	256	128	32	16	22
21	Moderate	64	32	16	8	128	64	5
22	Moderate	256	128	0	256	256	128	12
23	Weak	32	16	16	8	256	0	1
24	Moderate	256	128	0	256	128	64	13
25	Moderate	256	128	256	0	32	16	21
26	Moderate	256	128	128	64	64	32	19
27	Moderate	0	256	0	256	0	256	25
28	Moderate	0	256	128	64	256	128	26
29	Weak	256	128	64	32	0	256	18
P-value		≤ 0.05		≤ 0.05		≤ 0.05		26 strains out of 29 isolates
F-value		44.46		20.17		20.35		

Based on their ability to form biofilm, S. aureus isolates were categorized into three groups: strong, moderate, and weak. Depend on profiles of resistant bacterial strains, S. aureus isolates shown varying degrees of antibiotic resistance. Despite being sensitive to all other concentrations, seven S. aureus isolates that were categorized as moderate biofilm-forming were resistant to vancomycin at 32 and 64 µg/ml. However, twelve strains of S. aureus were sensitive to vancomycin at 256 µg/ml and resistant to it at 128 µg/ml. Four isolates of S. aureus had the highest vancomycin resistance, measuring 256 µg/ml. In control, one of these strains was categorized as having weak biofilm development. Four strains of *Staphylococcus aureus* isolates demonstrated resistance to all serial concentrations of tetracycline. Conversely, eight strains exhibited breakpoints between 8 and 16 µg/ml. The strains of *Staphylococcus aureus* that exhibited the highest resistance to ampicillin, four strains were sensitive only at 256 µg/ml and four strains were resistant to all serial concentrations. In contrast, eighteen strains were dispersed among different concentrations based on their ampicillin resistance. For each of the three antibiotics, the results revealed notable variations (Table 5).

The capacity of the *Staphylococcus aureus* and Candida species isolates to form biofilms at various concentrations of antimicrobial agents (8–256 µg/ml) was evaluated. The findings demonstrated that compared to monomicrobial biofilms, polymicrobial biofilms (Candida spp.–*Staphylococcus aureus*) based on strain types of

Candida were more resistant to antimicrobial treatments. At the maximum concentration (256 µg/ml), monomicrobial biofilms produced by *Staphylococcus aureus* isolates were more resistant to ampicillin and tetracycline than vancomycin. However, *Staphylococcus aureus* isolates developed biofilms 100% with vancomycin, 89.66% with tetracycline, and 82.76% with ampicillin, their sensitivity decreased at 8 µg/ml. At high concentrations (256 µg/ml), itraconazole had a greater effect on monomicrobial biofilms produced by Candida species than voriconazole and fluconazole. However, at 8 µg/ml, all Candida isolates produced biofilms with voriconazole (96.15%), itraconazole (84.62%), and fluconazole (80.8%). Synergistic interactions between Candida species and *Staphylococcus aureus* in polymicrobial biofilms increased resistance to drug combinations based on strains of the microorganisms. Polymicrobial biofilms generated by Candida spp. - *Staphylococcus aureus* (Candida as a base layer) diminished to 73.3% to 86.9% with voriconazole-vancomycin, 86.9% with itraconazole-ampicillin, and 93.3% with fluconazole-tetracycline only at high concentrations of 128 to 256 µg/ml. Overall, every isolate in polymicrobial biofilm (*Staphylococcus aureus* – Candida spp.) showed development 100% biofilm formation and were not affected by any concentration of drug combinations. With the exception of fluconazole-tetracycline and voriconazole-vancomycin with *Candida* spp.- *Staphylococcus aureus*, every analysis demonstrated substantial differences (Table 6).

Table 6: Effect of Antibiotic–Antifungal Combinations on Polymicrobial Biofilm Formation by *Candida* species and *Staphylococcus aureus*

		MIC (µg/ml)							
Organism	**Antibiotics**	**256**	**128**	**64**	**32**	**16**	**8**	**F value**	**P value**
S. aureus (no.= 29 isolates)	VAN	10.34	65.52	79.31	96.55	96.55	100	44.46	≤ 0.05
	TET	13.79	31.03	34.48	58.62	75.86	89.66	20.17	≤ 0.05
	AMP	13.79	24.14	37.93	48.28	72.41	82.76	20.35	≤ 0.05
Candida species (no = 26 isolates)	VOR	50	65.38	76.92	88.46	96.15	96.15	15.47	≤ 0.05
	ITR	0	7.69	34.62	53.85	65.38	84.62	31.14	≤ 0.05
	FLU	19.23	34.62	42.31	53.85	69.23	80.77	11.86	≤ 0.05
Candida spp - *S. aureus*	VOR-VAN	73.33	86.67	100	100	100	100	2.13	0.07
S. aureus - *Candida* spp	VAN-VOR	100	100	100	100	100	100	2.84	0.02
Candida spp - *S. aureus*	FLU-TET	93.33	100	100	100	100	100	1.75	0.13
S. aureus - *Candida* spp	TET-FLU	100	100	100	100	100	100	3.44	0.007
Candida spp - *S. aureus*	ITR-AMP	86.67	100	100	100	100	100	4.49	0.001
S. aureus - *Candida* spp	AMP-ITR	100	100	100	100	100	100	3.62	0.005

Discussion

Antimicrobial drug resistance makes it difficult to treat many chronic infections[18]. The anti-biofilm assay findings demonstrated that three different species of *Candida* could form biofilms in the presence of antifungal drugs. Every strain of *Candida* produced biofilms that were resistant. This resistance pattern is mostly caused by these species ability to create a biofilm, which serves as a physical and biological barrier and increases fungal resistance to the drug[19]. According to a study, biofilm reformation is influenced by the reservoir of persistent cells[20].

In terms of effectiveness against Candida species, itraconazole outperformed voriconazole and fluconazole. Increased resistance may arise from the widespread and extended use of antifungal agents such voriconazole and fluconazole[21]. Yeast cells may become more resilient if a biofilm forms during antifungal treatment[22]. The results of the current investigation contradict those of a prior study that found itraconazole had no appreciable

impact on adherent cells or mature biofilm[23]. In contrast to *Candida krusei* and *Candida albicans* strains, *Candida tropicalis* strains shown a high level of resistance. *Candida tropicalis* resistance is a significant and worrisome problem, especially in light of the increasing use of azole in clinical practice[24]. Previous studies have demonstrated that fluconazole has trouble penetrating *Candida tropicalis* biofilms due to their slimy appearance, which implied an extensive matrix[25].

Different levels of antibiotic resistance were observed in *Staphylococcus aureus* isolates. One of the mechanisms that bacteria might become resistant to antibiotics is by forming biofilms, which function as a physical barrier and alter metabolism[26]. The study findings demonstrated that vancomycin, a member of the glycopeptide medication class, was superior to tetracycline and ampicillin in its ability to prevent *Staphylococcus aureus* biofilms. This result is consistent with another study that found that all strains of *Staphylococcus aureus* showed higher resistance to tetracycline than to vancomycin[27]. The presence of antibiotic resistance in biofilm-forming microbes may be due to different expression of biofilm resistance genes like antibiotic exclusion or efflux pumps than in planktonic cells[28].

Compared to monomicrobial biofilms, polymicrobial biofilms (*Staphylococcus aureus*-Candida species) exhibited greater resistance to antimicrobial treatments. Dual-species biofilms cannot be eradicated by antimicrobials alone because the pathogenic fungus can proliferate and continue to form biofilms[29]. Synergistic interactions in polymicrobial biofilms may improve biofilm formation and antimicrobial resistance, according to a recent study[30]. *Staphylococcus aureus* isolates that were exposed to 256 µg/ml vancomycin generated a monomicrobial biofilm (10.34%). However, at the same antibiotic concentration, the biofilm formation was increased to 100% in mixed strains of *Staphylococcus aureus* and *Candida*.

When C. albicans and S. aureus are grown together in a biofilm, vancomycin effectiveness in eliminating the bacteria is diminished[31]. The biofilm serves as physical barrier that prevents the appropriate diffusion of the medications may be the reason why antibiotics are less effective against *Staphylococcus aureus* in a C. albicans biofilm than in a biofilm made up of a single type of microorganism. According to Kong et al., the extracellular matrix (ECM) of a C. albicans biofilm contains β-glucans that prevent penetrating of antibiotic by covering S. aureus surface[32].

Monomicrobial biofilm was generated 19.23% of Candida biofilm at a high concentration of 256 µg/ml of itraconazole. However, at the same antifungal concentration, the rate of biofilm formation in mixed isolates of Candida-S. aureus was 86.7%. A major challenge in the treatment of infections is the formation of biofilms, which reduces the efficacy of antibiotics against numerous species, including *Staphylococcus aureus* and *Candida*. In comparison to biofilms formed by a single microbe, *Candida-Staphylococcus aureus* biofilms showed higher resistance to antimicrobial drugs in addition to increasing biofilm growth and biomass[33].

Voriconazole-vancomycin was found to reduce polymicrobial biofilms at high concentrations (128 and 256 µg/ml). A study suggests that azoles may have an impact on polymicrobial biofilms. As anticipated, voriconazole can effectively reduce the biomass of polymicrobial biofilms[34]. However, at low concentrations, polymicrobial *Candida-Staphylococcus* biofilms were resistant to fluconazole, and it has been demonstrated that the bacteria can make *Candida* more resistant[35]. At all serial concentrations of medication combinations, 100% formation was showed in polymicrobial biofilms (*Staphylococcus aureus–Candida* spp.). *Staphylococcus aureus* strong adhesion to hyphae of *Candida albicans*, but not to the yeast form, has a significant impact on pathogenicity. Antibiotics like vancomycin have less of an effect on *Staphylococcus aureus* that is linked with hyphae[36]. The impact of antifungal and antibacterial medication combinations on polymicrobial biofilms varied[29]. Biofilms are challenging to remove, even after extended treatments, due to their resistance mechanisms. When treating persistent infections, biofilms continue to be a significant issue. There are currently numerous approaches being researched to successfully heal diseases linked to biofilms[37].

Conclusion

This study shows compared to monomicrobial biofilm, polymicrobial biofilm composed by S. aureus and Candida species shown stronger resistance to antimicrobial treatments. Even combined antibiotic–antifungal treatments were unable to eradicate mixed biofilms, despite the fact that vancomycin and itraconazole were the most efficient agents against their respective single-species biofilms. This suggests that beyond the impact of conventional or combination therapy, polymicrobial interactions greatly enhance biofilm resistance. Therefore, increasing infection control

and combating antibiotic resistance require the development of innovative anti-biofilm techniques and chemicals made especially to target mixed microbial communities.

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Authors' contributions
Conceptualization, methods and literature review, Preparation of draft manuscript, Finalization of manuscript: Ghadeer Ali Saad; Statistical analysis Mohammad Hashim AL-Yasiri; Both authors approved the final manuscript.

Data Availability
Any questions regarding the availability of the study's supporti ng data should be addressed to the corresponding author, who can provide it upon justifiable request.

Ethics Approval and Consent to Participate
The Institutional Review Board granted the study ethical approval. Since this was a prospective study, every study participant provided formal informed consent. Each method followed the appropriate rules and regulations.

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