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Research article

Antimicrobial Effect of Soil Microorganisms' Products against Different Clinical Bacterial Isolates

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ABSTRACT

Finding an effective antibacterial against a wide spectrum of antibiotic-resistant bacteria is a major challenge for scientists. The current study aims to investigate microorganisms that produce antibacterial agents in soil. In the present study, 65 soil samples were collected from different areas of Baghdad. Microorganisms were grown in nutrient broth to collect the extracellular secretions of these microorganisms. The effect of substances secreted by microorganisms in each sample into the growth media against four species of pathogenic bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Enterobacter* spp) was estimated using well diffusion method. The study showed that 9.1% of soil samples produced antibacterial substances against four pathogenic bacteria, but with different levels and effectiveness. The highest effect of these secretions was against *S. aureus* and *E. coli*, as the study showed that 8 samples produced anti-*S. aureus* substances. Five samples containing microorganisms that produce anti-*E. coli*. While the study showed that the microorganisms present in one sample produced anti-*S. pneumoniae* and Enterobacter spp. *Aspergillus* and *Penicillium* represent the highest percentage of microorganisms in the soil samples, followed by *Saccharomyces*. It can be concluded from the current study that there is a large percentage of microorganisms that can produce antibacterial substances, and this opens the door to the possibility of obtaining new antibiotics that can help treat diseases caused by antibiotic-resistant bacteria.

Keywords: *Aspergillus,* Antibacterial agents, Pathogenic bacteria.

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1. INTRODUCTION

Microbial interactions within ecosystems have garnered significant attention due to their profound implications in various fields, including medicine, agriculture, and biotechnology [1]. The interplay between fungus and yeast shows antagonistic behavior against bacterial pathogens such as *Escherichia coli*, *Staphylococcus aureus, Streptococcus pneumoniae*, and *Enterobacter* spp. presents a complex and intriguing area for investigation. The intricate dynamics of microbial communities have long been an area of interest, especially considering their role in shaping diverse environments and their potential applications in various industries [1]. Aspergillus is a ubiquitous genus of filamentous fungi that encompasses various species with diverse ecological roles and biotechnological potential. Its versatile metabolic capabilities and ability to produce an array of

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bioactive compounds have positioned it as a significant organism for scientific inquiry and industrial applications [2]. *Saccharomyces*, notably *Saccharomyces cerevisiae*, stands as a prominent yeast species extensively studied for its fermentative abilities in various biotechnological processes, including brewing, baking, and bioethanol production. Its genetic tractability and well-characterized metabolism have propelled its use as a model organism for molecular studies [3].

The antagonistic relationship between Aspergillus *spp*. and E. coli, a gram-negative bacterium and a common inhabitant of the human gut, has piqued interest in recent years. Investigations into the mechanisms underlying this interaction reveal potential avenues for understanding microbial competition and the role of secondary metabolites, a previous study showed the that genus *Aspergillus*, was able to inhibit *E. coli* [4]. The interactions between *Aspergillus* and *Staphylococcus*, a genus encompassing pathogenic strains like *Staphylococcus aureus*, hold implications for human health. Previous studies showed that the antimicrobial effect of Aspergillus products inhibits S. *aureus* growth [5]. Souza et al. (2016) found that Pereskia aculeata leaf extracts showed inhibitory activity against *Staphylococcus aureus*, with Aspergillus versicolor being more effective in inhibiting growth [6].

Saccharomyces, often associated with fermentation processes, exhibits intriguing interactions with Escherichia coli and *Staphylococcus*. The mechanisms involved in these interactions and the potential for utilizing *Saccharomyces*-derived compounds in antibacterial strategies warrant comprehensive investigation. Seddik et al. (2014) found that S. cerevisiae P9L1 has the potential as a probiotic, inhibiting the growth of Escherichia coli and *Staphylococcus aureus*. Other investigators found that *S. boul*ardii in the presence of inulin forms aggregates with *E. coli* and *Enterococcus faecalis*, decreasing the number of microorganisms in feces [7]. Another study of Srirama et al. (2019) reported the inhibition of *S. pneumoniae* by the Bactericidal that produced by S. boulardii [8].

The intricate interplay between Aspergillus *fungus*, *Saccharomyces*, *E. coli*, and *Staphylococcus* embodies a complex network of interactions with far-reaching implications. Understanding the mechanisms underlying these interactions offers prospects for novel therapeutic interventions, biotechnological advancements, and insights into microbial ecology. This paper aims to delve into the antagonism effect of Aspergillus and *Saccharomyces* on the in vitro growth of *E. coli*, and *S. aureus*. *S. pneumoniae*, and *Enterobacter* spp

2. MATERIALS AND METHODS

2.1. Clinical bacterial isolates

In the current study, the clinical isolates of *S. aureus*, *E. coli,* S. *pneumoniae*, and *Enterobacter* spp. were procured from the Medical Microbiology Laboratory at the Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq. The pure isolates were maintained for a short time by being inoculated onto a nutrient agar slant and after incubation for 18 h at 37 ^{o}C, the slants were stored at 4 ^{o}C for several weeks. The pure isolates were stored for a long time by being inoculated into the sterile nutrient broth (20 % glycerol) and after being incubated for 18 at 37 ^{o}C, the tubes were stored at – 20 ^{o}C for several months.

2.2. Soil samples

In this method, clean and sterile plastic containers were used. The sampling sites were selected representing diverse environments (different land uses, vegetation types, moisture levels, etc.) to get the highest probability of isolating the Aspergillus spp. and *Saccharomyces* spp. The 100 soil samples were collected from different sites in Baghdad city and surrounding areas. The spots were chosen randomly within the site to collect the samples. The samples of soil were collected from the topsoil layer (typically the top 5-15 cm) to capture the most active microbial community. The sterilized tool was used to scoop the soil sample into the container. The surface debris and organic matter have to be avoided. Multiple samples were collected across the area of interest, and mixed to create a composite sample for a representative analysis [9]. The containers were labeled with unique identifiers, including location, date, depth, and any other relevant information. The samples were Stored in a cool, dark place or refrigerated until processing to maintain microbial viability [10]. The samples should remain sealed and at appropriate temperatures to prevent changes in microbial composition during transit until reach the lab.

2.3. Anti-bacterial effect of soil culture

One gram of soil samples was added to 10 ml of sterile nutrient broth (NB). The inoculated NB tubes were incubated at 30 ^{o}C for 4 days. The supernatants were collected and the anti-bacterial effect of supernatants was checked against four clinical isolates (*S. aureus*, *E. coli,* S. *pneumoniae*, and *Enterobacter* spp) using well diffusion methods [11].

2.4. Isolation of Aspergillus and *Saccharomyces* spp.

A hundred microliters of the supernatants that gave antibacterial effects against the four clinical isolates of bacterial isolates were cultured in Sabouraud dextrose agar (SDA) [12]. Incubate the plates at a suitable temperature (25-30°C). Monitor regularly for fungal and yeast colonies [13]. The colonies resembling Aspergillus morphology and *Saccharomyces* spp (characterized by color, texture, and spore formation) were selected and sub-cultured the individual colonies onto fresh agar plates for obtaining pure cultures.

2.5. Statistical analysis

The results are presented as mean and standard deviation (SD). All data were statistically analyzed by a one-way analysis of variance ANOVA test to determine whether there were differences within the groups. P values smaller than 0.05 were considered to be significant. The analyses were performed using Origin Pro 7.5 software (Origin Lab Corporation, North Hampton, MA).

3. RESULTS

In the current study, 65 soil samples were collected from different areas of Baghdad. The antibacterial effect of the microorganisms present in these samples was investigated. It was demonstrated the anti-bacterial effect by the well diffusion method. The effect suspension showed the clear zone around the well that was filled with a suspension of soil microorganisms. It was found that 14 samples (9.1 %) produced anti-bacterial materials against one or more of the pathogenic bacterial isolates used in this study. Table 1 shows that the highest effect of the extracts was effected against *S. aureus* and *E. coli*. While the lowest effect was seen against S. pneumoniae and Enterobacter. Fig. 1 shows the morphological features of the microorganisms that produced the anti-bacterial materials.

Table 1. The microorganisms that were isolated from the different soil samples showed the anti-microbial effect against four clinical isolates, *S. aureus*, *E. coli,* S. *pneumoniae*, and *Enterobacter* spp. R, Resistance response, S, sensitive response.

No of sample	The microorganism produced anti-bacterial agents	Pathogenic bacteria isolates sensitive to anti-microbial materials			
		S. aureus	*E. coli*	S. *pneumoniae*	*Enterobacter* spp
1	*Saccharomyces*	S	R	R	R
4	*Penicillium*	S	R	R	R
5	Bacteria	S	S	R	R
7	*Alternaria*	S	R	R	R
9	*Penicillium*	R	S	R	R
11	*Bacteria*	S	R	R	R
12	Aspergillus	S	R	R	R
13	*Saccharomyces*	S	S	R	R
16	*Penicillium*	R	S	R	R
19	Aspergillus	S	R	R	R
20	Aspergillus	R	S	R	R
26	Aspergillus	S	S	R	R
27	Actinomycetes	S	S	R	R
40	Bacteria	S	S	S	S

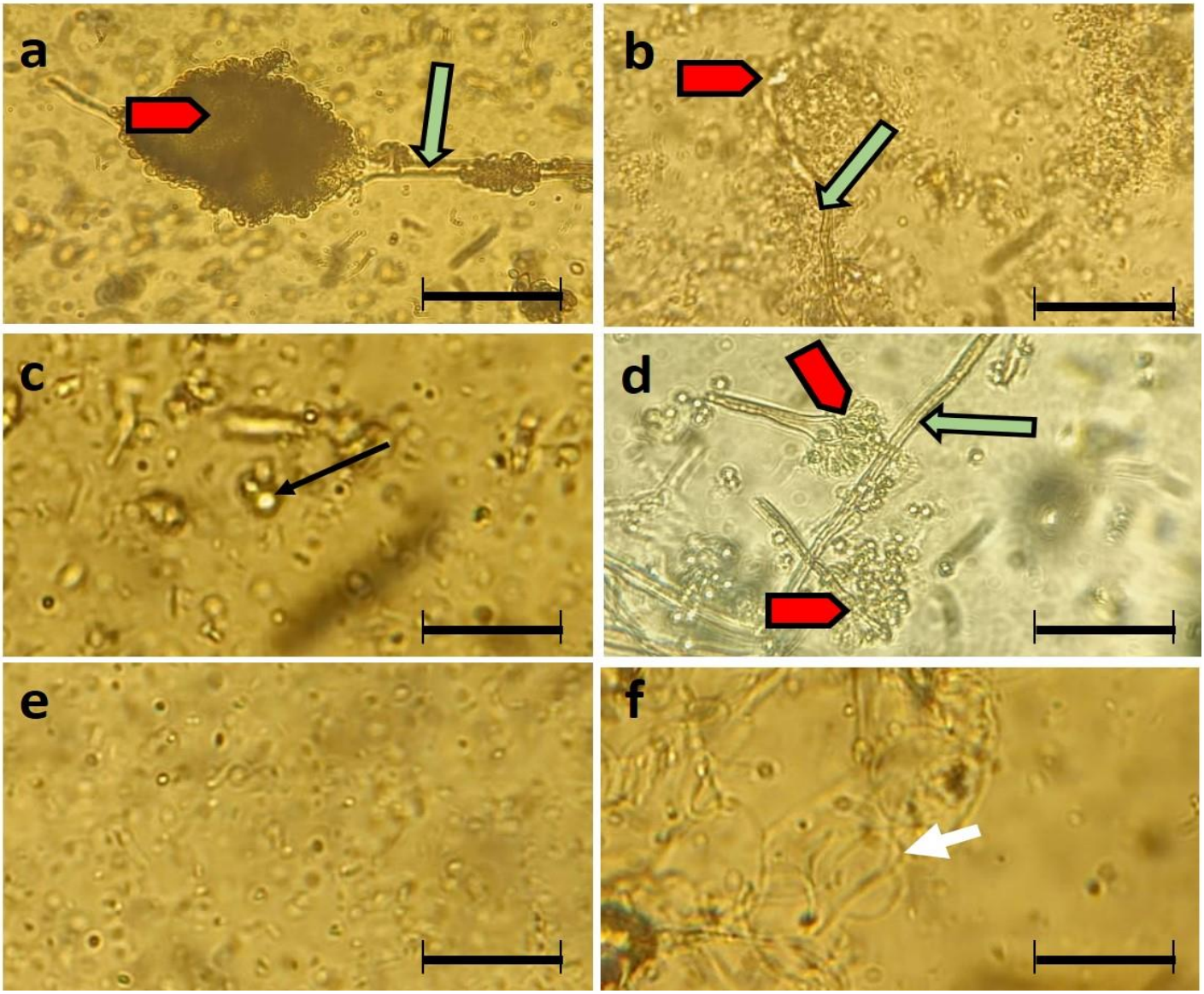

Fig 1. Images of microorganisms that were isolated from soil and showed antimicrobial effects against four clinical isolates (*S. aureus*, *E. coli,* S. *pneumoniae*, and *Enterobacter* spp), a, Aspergillus, b, *Alternaria*, c *Saccharomyces* spp., d, *Penicillium*, e, true bacteria, f, Actinomyces. Red arrow, head of fungus; green arrow, fungal hyphae; back arrow, yeast; white arrow; actinomyces hyphae.

4. DISCUSSION

Soil microorganisms have antimicrobial effects that are important for their survival and reproduction. These microorganisms, including bacteria, fungi, and actinomycetes, produce antibiotics as a natural defense mechanism against other microbes in their environment [14]. The production of antimicrobials is a potent strategy for adaptation in soil microorganisms [15]. However, the indiscriminate use of antibiotics and disinfectants has led to the emergence of multidrug-resistant pathogenic microbes, highlighting the need for effective antibiotics [16]. Soil microbes remain a significant source for antibiotic research [17]. Paint effluents from industries can negatively impact soil microorganisms, causing a decrease in their population. The environment soil fungi from the Arctic and Antarctic show antimicrobial activity against human pathogenic bacteria, and their secondary metabolite production is influenced by environmental conditions [18].

In the current study, the effect of secretions of some microorganisms isolated from soil against several clinical bacterial isolates was studied. It showed that 9% of the soil isolates contained microorganisms capable of producing clinical antibacterial substances used in this study, and it was found that fungi represented the highest percentage of microorganisms producing antibacterial substances. This study requires further investigation, which is related to determining the ability of these microorganisms that were isolated from the soil to produce antibiotics, in addition to determining the type of these antibiotics, whether they are known or new. The current study opens new horizons for investigating new antibiotics that may contribute to reducing the problem of the aggravation of the spread of bacteria resistant to a wide spectrum of antibiotics.

There is evidence of antagonistic relationships between soil microbial organisms and clinical isolates of bacteria. Studies have isolated microorganisms from natural sources such as soil, water bodies, and plants, and found that they possess antimicrobial properties against drug-resistant pathogens [19]. Actinomycetes isolated from soil have also shown antagonistic activity against multi-drug resistant bacterial strains [20]. Additionally, bacteria and fungi isolated from the rhizosphere of healthy onion plants have demonstrated the ability to inhibit the growth of different pathogenic microorganisms [21]. These findings suggest that soil microbial organisms have the potential to antagonize clinical isolates of bacteria, highlighting the importance of studying natural sources for the development of new pharmaceutical substances and biological control agents.

5. CONCLUSION

The current study shows that a high percentage of microorganisms can be isolated from soil, which produces antibacterial substances. The current study showed that 14 soil samples were identified out of 65 soil samples collected from different areas of Baghdad. The microorganisms that were isolated from the soil were almost fungus followed by yeast and bacteria. It showed that the microorganisms present in these samples can produce anti-bacterial substances against four clinical isolates used in this study (*S. aureus*, *E. coli,* S. *pneumoniae*, and *Enterobacter* spp). The study showed that *S. aureus* and *E. coli* showed a high response, while the lowest response was against *S. pneumoniae* and *Enterobacter*.

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Conflict of interest

The authors declare that they have no conflict of interests.

Ethical Approval

This review was approved by the University of Baghdad, Baghdad, Iraq (No 223, 2023).

Author contributions

Sajjad A Khudhair: Investigation; Methodology; and Resources.

Hussain A Ismael: Methodology, and Conceptualization.

Noor al-huda Rashak: Methodology; and Data curation.

Aya M Mallooki: Methodology; and Visualization.

Jenan A. Ghafil: Conceptualization; Formal analysis; Project administration; Resources; and Writing - review & editing.

Ayaid K. Zgair: Conceptualization; Formal analysis; Project administration; Resources; Supervision; Validation; Roles/Writing - original draft; and Writing - review & editing.

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