

Screening of Bacteria from Soil Involved In the Control of Nosocomial Infections in Hospitals

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Abstract: Hospital-acquired infections (HAIs), sometimes referred to as nosocomial infections, are a significant issue for healthcare systems worldwide since they often lead to increased rates of mortality, longer hospital stays, and greater medical costs. Finding and screening bacterial strains from soil with possible antagonistic action against prevalent nosocomial infections was the goal of this investigation. Samples were taken from the air, surfaces, and equipment of the hospital, and they were analysed microbiologically.

Soil isolated bacteria were evaluated for their inhibitory effects against selected nosocomial pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* using agar well diffusion and cross-streak methods. Several isolates demonstrated significant antagonistic activity, suggesting their potential role in the biocontrol of hospital-acquired infections. These findings open avenues for the development of alternative biological strategies to complement traditional disinfection and infection control measures in healthcare settings as well as further study focused on purification of potent antimicrobial compound involved in control of Nosocomial Infections.

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Introduction:

Since the discovery of the first antibiotics over 80 years ago, the treatment of infections has been transformed, turning formerly fatal illnesses into treatable conditions and helping to stop the spread of those specific diseases.[1]

One of the most significant global public health concerns is bacterial resistance. Antibiotics are currently ineffective against superbugs, which emphasizes the necessity of finding novel compounds with antibacterial properties to fight them. [2]

Infections caused by resistant bacteria cause more severe symptoms, require more time in the hospital, and raise patient morbidity and death rates in both developed and developing nations as a result of bacterial resistance's ongoing advancement in spite of coordinated attempts to counteract it. Related

expenditures rise significantly as a result of this increase in the burden of healthcare. [3]

Antibiotics' inefficiency against resistant bacteria emphasizes the need to develop novel antimicrobial compounds in order to combat the worldwide resistance problem. Antimicrobial compounds produced by *Bacillus* species are among the most intriguing possibilities currently under investigation.[4,5]

One of the most important methods for increasing the synthesis of a wide range of secondary metabolites that are vital to the pharmaceutical sector is microbial fermentation. Optimizing culture parameters, such as nutrient supply, agitation, pH, temperature, inoculum concentration, and incubation period, results in the highest output yields.[6] Many previously undiscovered natural products from a variety of fungi and actinomycetes were successfully isolated by researchers by properly modifying these simple culture conditions.[7]

Healthcare-associated infections, also known as nosocomial infections, are thought to be the most common adverse event that poses a risk to patient safety and has major impacts on society and the economy. Hospitals frequently have cockroach infestations, particularly in developing nations. Recently *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* are common nosocomial pathogens that have been isolated from cockroaches. [8]

This study's primary goal was to use the streak plate method and the agar well diffusion method to assess the antibacterial activity of *Bacillus* species against prevalent pathogens implicated in nosocomial infections in hospitals.

Material and methods:

Sample collection and isolation of hospital-acquired *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*:

The hospital surface environment is crucial to infection risk because it can serve as a reservoir for germs. Swabbing method used for a sample collection for isolation of hospital born common pathogens [9] in this swab are taken from different areas of hospital and pathogens are isolated by using selective medias of respective pathogen.

The 10x10 area designated at the point of sampling location was swabbed multiple times in a vertical as well as horizontal direction using a cotton swab saturated in a sterile sampling solution. The cotton swab was then screened for *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* using mannitol salt agar (MSA), Cetrimide agar, and MacConkey agar, respectively, to isolate the organisms[10-12]. The culture dish was incubated at 37 °C for 24- 48 h then identify bacterial colonies by using After proper incubation on selective media well isolated colonies are confirmed by colony colour morphology, Gram staining and antibiotic sensitivity test. [13,14]

Screening of antagonistic Bacteria from soil against hospital born *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* :

Following the collection of the rhizosphere soil sample from Baghdad Teaching Hospital, soil bacteria were isolated using the conventional serial dilution plate technique [15].

A soil sample weighing one gram was suspended in ten milliliters of sterile physiological saline. After serially diluting the samples, 100 µl of each of the four dilutions (10⁻¹, 10⁻², 10⁻³, and 10⁻⁴) were used to screen each sample for strong soil bacteria that were resistant to nosocomial infections. In this 0.1 ml of serial dilution spread on sterile Muller Hinton agar. Then these plates were incubated at room temperature for 24 hrs to 48 hrs. After incubation period plates were observed for colonies showing zone of inhibition around it. The isolated organisms further purified by subculturing and studies for its morphological characteristics.

These isolates were further checked for antimicrobial activity against above isolated nosocomial pathogens like *staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* by streak method

on MH agar plates isolate showing more zone of inhibition selected and used for further process like gram staining, production of antimicrobial secondary metabolites etc.

Production and extraction of Antimicrobial secondary metabolite by *Bacillus* species:

The sterile nutrient broth was used as a medium for antimicrobial secondary metabolite production. A 500 µl inoculant was added to 250 ml Sterile nutrient broth and incubated at 35 °C for 24 to 36 hours. After incubation period 5ml aliquot was collected aseptically and centrifuged at 8000 rpm for 10 min at 4°C. Then the supernatant subjected to antibacterial activity by using agar diffusion assay.[16]

After centrifugation broth is collected and subjected to extraction of antimicrobial secondary metabolite by using solvent extraction. The extraction of bioactive compounds from crude cell free culture broth was done using solvent ethyl acetate [SRL] for this the solvent were mixed in the proportion of 1:1 (v/v) in separating funnel. After mixing whole mixture is Aqueous layer and organic layers were separated and tested for the presence of antimicrobial secondary metabolites using well diffusion method using above nosocomial isolates (*Staphylococcus aureus*, *Escherichia coli*) as a test organism. The partially purified form of bioactive compounds was subjected to further purification by silica gel column chromatography.[17,18]

Partial purification of Antimicrobial secondary metabolite Silica gel column chromatography:

The precipitate collected after ethyl acetate extraction was subjected to silica gel column chromatography. In this, column was packed by activated silica gel [mesh size 60-120, Hi-Media] of 20 cm with 2.0 cm diameter. Then the sample (approximately 5 ml) was loaded on the column. After that column was washed with 20 ml chloroform and further column was eluted by chloroform.[19] The 10 fractions of 5 ml were collected at the flow rate of 1ml/min. The eluted all fractions were checked for antibacterial activity.

Results :

Isolation of hospital born *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* :

hospital born nosocomial *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* were isolated and purified on respective media from hospital sample i.e. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* left to right according to colonies colour on selective media as well as antibiotic susceptibility test confirms the pathogens.

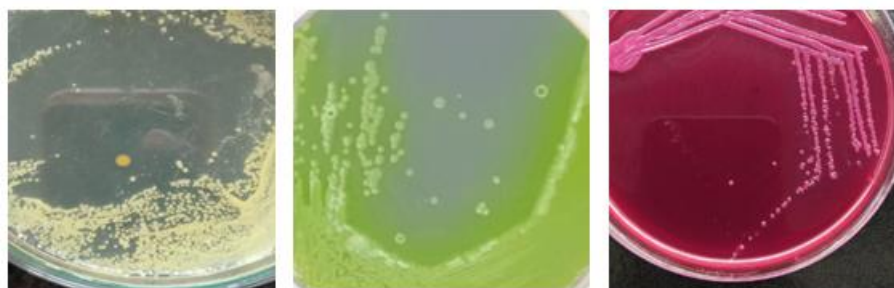


Figure 1. Isolation of hospital born nosocomial pathogens on respective selective media.

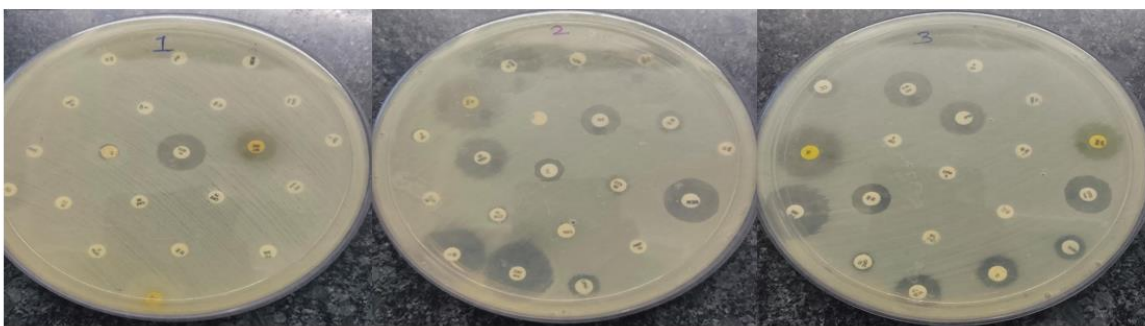


Fig 2. Antibiotic sensitivity test Here 1. *S. aureus* 2. *P. aeruginosa* 3. *E. coli*

Screening of antagonistic Bacteria from soil against hospital born *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* :

The soil samples from Baghdad Teaching Hospital were used for the screening of antimicrobial compound producers. We obtained different colonies showing zone of inhibition on different Muller Hinton agar plates. The colony showing larger zone of inhibition as compared to others selected for further study.

Isolated organisms have shown different potency of antimicrobial activity against test organism as shown in fig.3. From these organisms one organism shown highest zone of inhibition, so it was selected for further studies. The potent organism studied for its colony characteristics as shown in table 1 and it was found to be Gram positive rods in nature which confirmed that isolated microorganism is *Bacillus* spp.

colony showing zone of inhibition

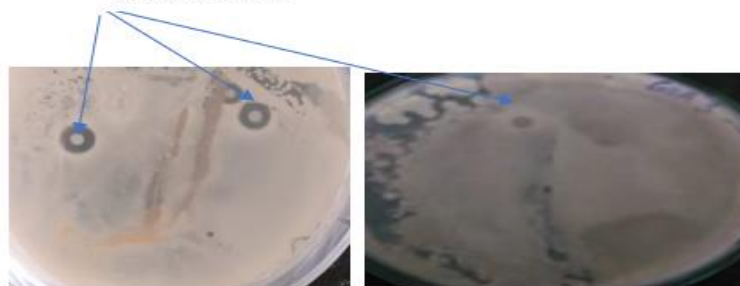


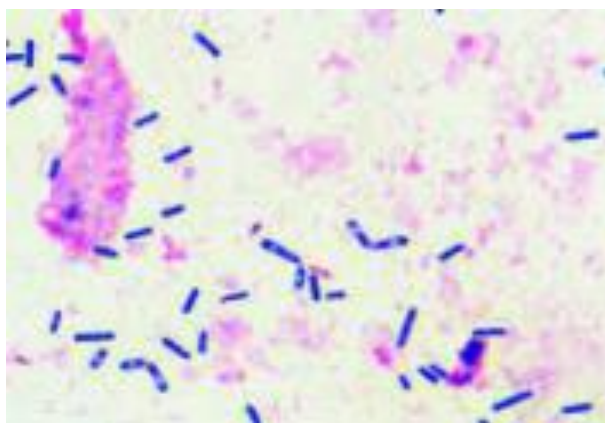
Fig 3. Screening of antimicrobial compound producers on Muller and Hinton agar medium.



Fig 4. isolated potent organism shows zone of inhibition by streak plate method *staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* left to right

Table 1. Colony characteristics of potent isolate

Size	Shape	Colour	Margin	Surface	Elevation	Opacity	Consistency
1.5 mm	Circular	Off white	Erose	Rough	Convex	Opaque	Non-Sticky
Gram nature			Motility				
Gram positive			Non motile				

**Fig 5. Gram staining of potent isolate**

Production and extraction of Antimicrobial secondary metabolite by *Bacillus* species:

The ethyl acetate extracted sample was loaded on silica gel column chromatography [Fig 6] and eluted by Chloroform. All fractions tested for antimicrobial activity in that fraction no 4 show good antimicrobial activity against pathogens. [Fig 7 and Table No.2]

**Fig 6. Silica gel column chromatography**

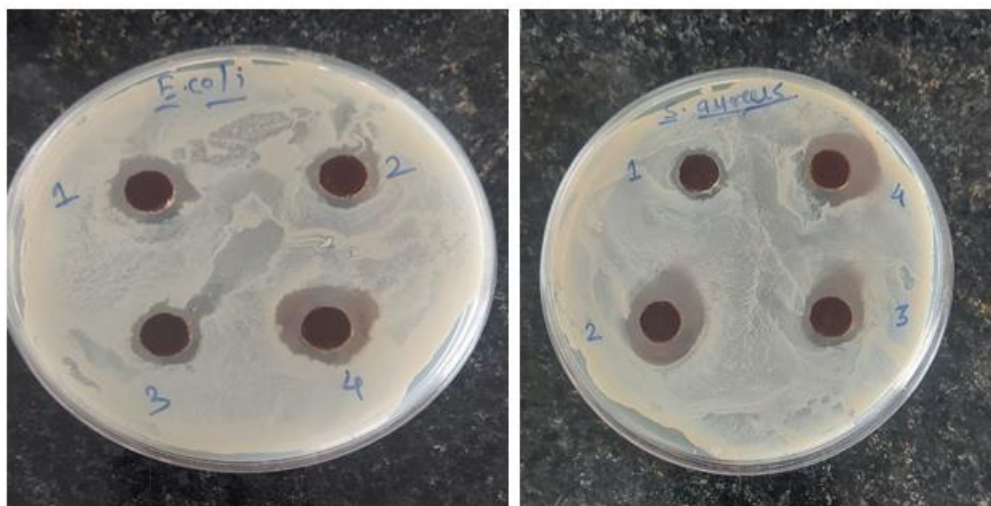


Fig 7. Antimicrobial activity of silica gel column fractions against pathogens

Table 2. Zone of inhibition of silica gel column chromatography fractions against pathogens

Test pathogens	Zone of inhibition in mm of respective fractions			
	1	2	3	4
<i>S. aureus</i>	11	15	20	23
<i>E. coli</i>	13	17	21	24

Discussion:

Drug-resistant bacterial infections have emerged as a major global issue, rendering treatment ineffective worldwide. It is currently crucial to develop efficient control mechanisms for the management and treatment of drug resistance. In many instance soil bacteria are play crucial role in screening of antimicrobial compound producing bacteria or microorganisms. Current study focuses on isolation of antimicrobial secondary metabolite producer and partial purification of secondary metabolites by solvent extraction method and silica gel column chromatography. In addition, the previous study showed that some endophytic fungi are isolated the Medicinal Plant *Hyssopus officinalis* and its secondary metabolite show antimicrobial activity against some nosocomial and human pathogens [20] some researchers isolate *Bacillus* species from soil samples from different regions in India which produces antibiotic like compounds [21] and they possess antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*. Present study also confirmed that soil act as a good reservoir for different kind bacteria which produces secondary metabolites that are mainly active against the different pathogens.

Conclusion:

The current research emphasized Screening of Bacteria from soil involved in the control of nosocomial infections in hospitals. The continuous use of antibiotics has resulted in a situation where the pathogens have acquired resistance against the conventional drug candidates. So, now there is an urgent need for the development of novel, safe and effective secondary metabolites which show antimicrobial activity against nosocomial pathogens as well as drug resistant super bugs . The goal of the current investigation was to identify and separate soil microorganisms that showed production of extracellular antimicrobial secondary metabolites which are potent antibiotic source and confirmed by agar well diffusion method and partial purification achieved by silica gel column chromatography. The isolates screened from the soil sample had strong activity and created the highest zone of inhibition against the test nosocomial pathogens, *E. coli* and *S. aureus*. Following purification and appropriate standardization, our findings

support the commercial application of the microbial isolates from this specific strain for the synthesis of antimicrobial secondary metabolites.

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