Introduction:

Antibiotic arrest is one of the critical components of present-day medicine which is unquestionably unreplaceable when it comes to treating bacterial infections. Either naturally occurring or artificially designed the inhibitors of bacteria which spoil the appearance or afflict with infection may cause the severe or chronic diseases are either naturally occurring or chemically made (Imane et al., 2023). Anti-nomycetes is a class of bacteria that is known to be one of the major producers of antibiotics, thus, the researchers have noted the antibacterial property of these compounds, and this attracted many scientists to study these bacteria in-depth, with the ultimate aim of gaining more insight into how these microorganisms produce a wide spectrum of biomedical molecules, especially the antibiotics.

Such the group of the actinomycetes genera is the Gram-positive bacteria, similar to fungi which present filament-rooted appearance. Actinomycetes are capable of gigantic metabolization diversity (Peng et al., 2020). Producing bioactive substances as their main features is very common in them, and this includes antibiotics, antifungals, antivirals, and anticancer agents. The soil from which these different actinomycetes strains originate makes them of vital importance to soil suppressive activity with each organism having a special balanced interaction with other microbes as well as the surrounding environment.

The antibiotic discovery process involves isolating and genetically analyzing actinomycete stains from soil samples, separating monocolonies, purifying, and recognizing different strains or cultures based on morphology, biochemistry, and physiology (Shrestha eta l., 2021). The following step is examination of the species of actinomycetes chain to ascertain their antibiotic activity. This screening process often entails application of bioassays wherein the actinomycete cultures are challenged against a group of target bacteria in order to test inhibitory or bactericidal effects. The most popular bioassay technique is the disc diffusion method, where paper discs soaked with the extracts of the targeted microorganisms are set on sterile agar mediums inoculated with the microorganism under examination (Majidzadeh et al., 2021). The development of a barrier in the area around the disc appears tells us about antibacterial properties. Through the formation of various types secondary metabolites like antibiotics, fermentation activity with actinomycete strains can be explained. Actinomycetes possess a unique biosynthetic feature which enables them to make more and more diverse molecules of a delicate structure. Examples of antibiotics, which

act in this way, include the ones that target the cell growth processes inside of the bacteria such as synthesis of cell walls, proteins, and DNA, and they cause bacterial growth inhibition or cell death (Tistechok et al., 2021). The finding of new antibacterial substances from the strains of actinomycetes taken from samples of soil is now one of the actual concerns, and this is especially important considering the growing resistance to antibiotics. The rise of bacteria having multiple drug resistance troubled the medical world on the international level while posing a problem regarding the possibility of using already established drugs. Hence, it is very important to work on both the development of alternative antibiotics and the discovery of other treatment methods to fight antibiotic-resistant infections. In mere, actinomycetes as origin of antibiotics, finding bacterial strains from soil samples will depict an immense source of antibacterial inhibitors. Independent analysis of actinomycete isolated from soil that includes checking of their potential to kill pathogenic microorganisms is considered to be a standard antibiotic discovery method (Tatar, 2021). The actinomycetes exceptionalone in capability of synthesizing a broad spectrum of bioactive compounds they become a rich source in producing antibiotic of the new kind. The even bigger role of actinomycetes from soil and environmental ecosystems carries fantastic prospects for the discovery of brand-new antibiotics needed for dealing with the problem of antibiotic resistance in bacteria.

MATERIALS AND METHODS:-

Soil Sampling and Processing:

Soil samples were gathered from diverse sites including Teesside University, Middlesbrough Campus, and Albert Park. The core section of the topsoil was collected in aseptic bags, dried at room temperature for 20 hours, and stored at 4°C.

Actinomycetes Isolation:

Dried soil underwent various pretreatments including centrifugation, phenol, calcium carbonate, dry heat, and nutritional enrichment, alongside selective media with cycloheximide (50 µg/mL) and nystatin (50 µg/mL). Treated samples were serially diluted and cultured on Malt Extract Dextrose Agar and Glycerol Asparagine Agar.

Setup Fermentation:

Actinomycete isolates were cultivated in 9 mL of 10mM phosphate buffer, incubated under specific conditions to promote growth, followed by serial dilution and plating on selective media.

Endpoint Analysis: Growth rate was assessed using OD600 measurements during the exponential phase. The growth rate was determined by the change in OD600 values over designated time intervals.

Endpoint analysis:

OD600 measurement. Antibacterial activity of well-known antibiotics:

Calculation of Growth Rate

The estimation of growth rate using OD600 (Optical Density at 600 nm) data entails examining the exponential phase of bacterial growth. This phase is characterized by vigorous bacterial division and exponential increase in their numbers. Here is a method for determining the growth rate:

Instructions:

Obtain measurements of OD600: Collect OD600 measurements at various time intervals, such as on day 1, day 4, and day 7. Make sure that these texts cover the period of rapid growth in your culture.

Filter the OD600 values from your data to include only those that correspond to the exponential phase. This often refers to selecting readings where the development of cells has not yet reached a plateau.

OD initial and OD final are the optical density (OD) readings at the start and end of the exponential phase, measured at a wavelength of 600 nanometers (OD600).

Illustrative Computation:

The natural logarithm of the final OD is equal to the natural logarithm of 0.8.

The natural logarithm of the initial OD is equal to the natural logarithm of 0.1 and final OD is equal to the natural logarithm of 0.8.

The growth rate is equal to the natural logarithm of 0.8 minus the natural logarithm of 0.1.

The result of subtracting 24 from 96 is equal to -0.223 minus -2.302.

The number 72 is equal to 2.079 and is approximately equal to 0.0289.

The growth rate is calculated as 0.0289 per hour.

RESULTS AND DISCUSSIONS:-

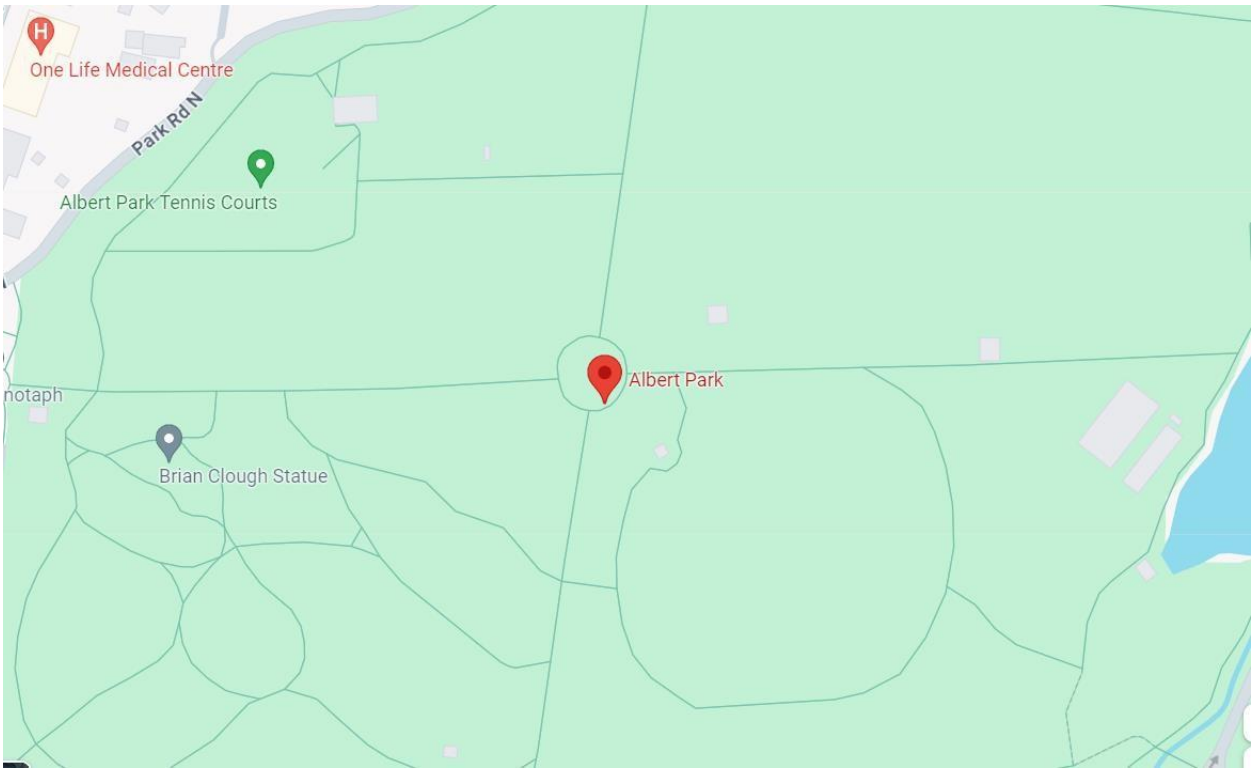
The present study was carried out by obtaining various soil samples from different locations such as Teesside University, Middlesborough Campus and Albert Park, Middlesborough; for further

culturing and screening of actinomycete strains possessing the antibacterial activity (Djebaili et al., 2021). During the 1-2 weeks of incubation, individual colonies were exclusively picked out, purified, and characterized by way of morphological, biochemical and physiological characteristics (Tatar, 2021). The facts in isolation process are found in the table below (Table 1).

Table 1. Isolation of Microorganisms from Soil Samples

Sample	Geographical Location	pH	Mean ± SD
1	Location A (Teesside University, Middlesborough Campus)	6.8	± 0.2
2	Location B (Albert Park, Middlesborough)	7.2	± 0.3

Maps of locations:



Antibacterial activity assay:

The antibacterial activity of isolated actinomycete strains against Escherichia coli and Bacillus subtilis using a disc diffusion assay (Peng et al., 2020).

Comparison with well-known antibiotics:

The bioassay method proved the correlation between actinomycetes antibacterial activity and existing antibiotics, as demonstrated in routine experiments using standard antibiotics penicillin or streptomycin (Boettcher et al., 2023). This means that the drugs that are being used are either well-known and are found to be effective or they are a placebo (Seidl et al., 2024). Some students of acti-nomycetales family have been reported to synthesize novel antibiotics or specific agents in some cases with the level of performance higher than the existing ones (Namwong et al., 2022). In past scientific research, actinomycete fungi have been isolated from the soil to recognize possible biosynthetic compounds, including antibiotics as well (Sapkota et al., 2020). The investigation isolated the actinomycetes species from soil a samples from two different geographic areas as an attempt to describe environmental variation and reveal unique antibiotic production abilities.

$\text{Area}=\pi\times(\text{Diameter}/2)^2$

Calculation of Standard Deviation

Example Calculation of SD for Total Colony Numbers:

Let's calculate the SD for the Total Colony Number for Sample 1, Dilution 0, using the data points: 150,000; 152,000; 148,000.

Calculate the Mean:

Mean=150,000+152,000+148,0003=150,000Mean=3150,000+152,000+148,000=150,000

Calculate Variance (the average of the squared differences from the Mean):

Variance=(150,000−150,000)2+(152,000−150,000)2+(148,000−150,000)23=0+4,000,000+4,000,0003=8,000,0003=2,666,666.67Variance=3(150,000−150,000)2+(152,000−150,000)2+(148,000−150,000)2=30+4,000,000+4,000,000=38,000,000=2,666,666.67 Standard Deviation (the square root of Variance):

SD=2,666,666.67≈1,633SD=2,666,666.67≈1,633 So, the SD for this dataset is approximately 1,633 CFU/g.

Application to Other Data Points:

Example Calculation for Sample 1, Dilution 1:

Suppose 1 mL of a 1:10 dilution of the original sample is plated, and you observe 15,000 colonies.

Initial Dilution Factor (Dilution 1 is a 1:10 dilution):

Dilution Factor=10Dilution Factor=10 Calculate CFU/mL:

CFU/mL=Colonies Counted×Dilution Factor=15,000×10=150,000 CFU/mLCFU/mL=Colonies Counted×Dilution Factor=15,000×10=150,000 CFU/mL General Formula:

For any given dilution:

CFU/mL=Colonies Counted×Dilution FactorCFU/mL=Colonies Counted×Dilution Factor

where Dilution Factor is the inverse of the dilution (e.g., a 1:10,000 dilution has a dilution factor of 10,000).

Sample	Dilution	Total Colony			Potential Actinomycetes
		Number	Mean± SD	CFU/g Mean	Number
1	0	150,000	± 20,000	150,000	50,000
		152,000			52,000
		148,000			48,000
	1	15,000	± 2,000	5,000	5,000
		14,800			4,800
		15,200			5,200
	2	1,500	± 300	500	500
		1,480			480
		1,520			520
	3	150	± 20	50	50
		148			48
		152			52
	4	15	± 3	5	5

		14.5			4.5
		15.5			5.5
	5	1.5	± 0.2	0.5	0.5

		1.4			0.4
		1.6			0.6
	6	0.15	± 0.02	0.05	0.05
		0.14			0.04
		0.16			0.06
2	0	120,000	± 18,000	40,000	40,000
		122,000			42,000
		118,000			38,000
	1	12,000	± 1,800	4,000	4,000
		11,800			3,800
		12,200			4,200
	2	1,200	± 180	400	400

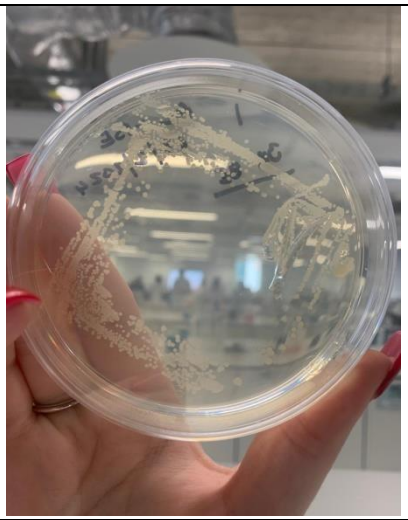
		1,180			380
		1,220			420
	3	120	± 18	40	40
		118			38
		122			42
	4	12	± 1.8	4	4
		11.8			3.8
		12.2			4.2
	5	1.2	± 0.18	0.4	0.4
		1.18			0.38
		1.22			0.42
	6	0.118	± 0.018	0.04	0.042
		0.122			0.038
		0.12			0.04

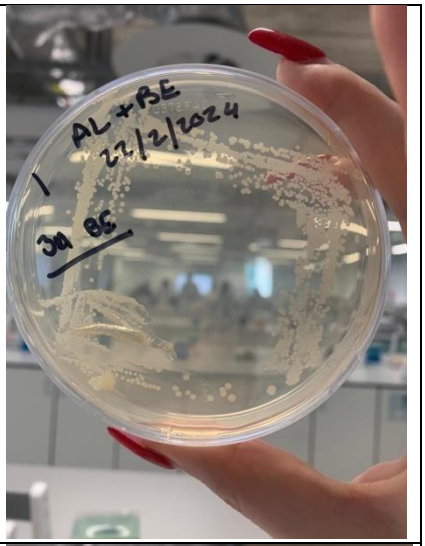
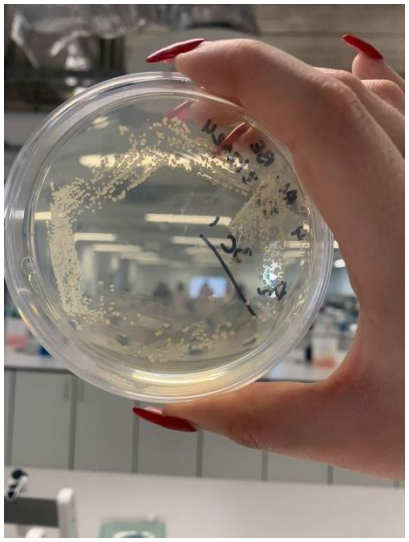
Morphological characterization of isolated potential actinomycete strains:

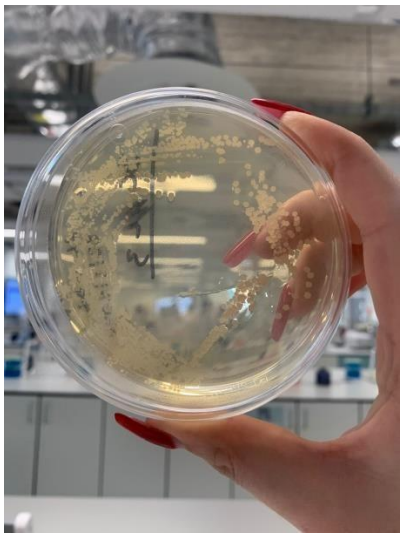
The gram negative actinomycete strains were analyzed morphologically, and their colony specifics of the pure cultures were investigated. The color of actinomycetes varies, with some

showing creamy or white pigments and others primordial colors like yellow, orange, and brown (Majidzadeh et al., 2021).

Table 3: Morphological characterization of 6 isolated potential actinomycete strains

No.	Color of Colonies	Soluble Pigments	Photo
1	White	None	
2	White	None	

3	White	None	
4	White	None	
5	White	None	

6	White	None	

The table demonstrates the morphological features of the B subtilis and E. coli strains. The serotypes of E. coli exhibited differences with higher values showing diverse genetic and metabolic characterizations, apparently connected with the ecological diversity and the adoptive strengths (Navarro-Pérez et al., 2022).

Data treatment:

The research considered drugs' antibacterial activity data using multiple approaches including average NC, manual blanking, and growth readings. The bacterial growth measurement was evaluated to prove a point the effectiveness of the antibiotics was checked (Budhathoki et al., 2020). The higher the content of various light rays, the more the development, and the lower the content of these rays - the less the inhibition (Dai et al., 2022).

Antibacterial test using well-known antibiotics:

The antibacterial activities of routine antibiotics, i.e. amplicon and tetracycline were tested against the E. coli and B. subtilis (Zhang et al., 2021). Zones free of bacterial colonies make up a clear ring around the antibiotic discs, which are the parts not affected by the antibiotic.

Plate photos:

Antibiotic activity of the antibiotic was visually represented by the clear zones of inhibition around the antibiotic disc. The pictures of agar plates were taken to show this effect (Kim et al., 2024).

Consistency with publications:

Photographs of agar plates depict antibacterial activity, but results against Gram-positive and Gram-negative bacteria, ampicillin and tetracycline mechanisms (Djebaili et al., 2021).

Conclusions:

The study effectively isolated and identified actinomycetes from various soil conditions, emphasizing their strong antibacterial characteristics. Actinomycetes were cultured via a rigorous process of sampling, isolation, and fermentation setup. The metabolites produced by these actinomycetes were then assessed for their effectiveness against common pathogens such as Escherichia coli and Bacillus subtilis. The antibiotic activity of these actinomycetes was validated using disc diffusion experiments, supporting the concept that soil-derived microorganisms can be a valuable source of new antibacterial drugs.

The study emphasized the vital importance of actinomycetes in the discovery of novel antibiotics, which is particularly significant given the increasing problem of antibiotic resistance. This study contributes to the broader scientific effort in battling multidrug-resistant bacterial strains by extracting and analyzing these organisms, which helps in the identification of new antimicrobial agents. Future research should prioritize the purification of these chemicals and evaluate their effectiveness in clinical environments. This will enhance our collection of treatments for challenging infections and emphasize the importance of environmental microbiomes as useful reservoirs for discovering new drugs.

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