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ANTIBIOTIC RESISTANCE PROFILE OF BACTERIA ISOLATED FROM VARIOUS SOIL SAMPLES

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ABSTRACT: Soil represents a major reservoir of diverse microorganisms, including bacteria capable of developing resistance to antibiotics. The present study aimed to isolate and characterize bacteria from soil samples collected from different locations in Durgapur, West Bengal, India, and to evaluate their antibiotic resistance patterns. Ten soil samples were collected and bacterial isolates were obtained using standard microbiological techniques. The isolates were characterized by colony morphology, Gram staining and biochemical tests, and their antibiotic susceptibility was determined by the Kirby–Bauer disc diffusion method following CLSI guidelines. The results revealed the presence of both Gram-positive and Gram-negative bacteria, including *Staphylococcus*, *Micrococcus*, *Bacillus*, *Proteus*, *Pseudomonas* and *Alcali genes* species. Several isolates showed resistance to one or more antibiotics, and multiple antibiotic resistance (MAR) indices ranged from 0.4 to 1.0. The findings suggest that soil environments may act as reservoirs of antibiotic-resistant bacteria. However, species identification in the present study was based on phenotypic characteristics and should be considered presumptive. Further molecular characterization is required to confirm the identity of the isolates.

INTRODUCTION: A broad range of microorganisms are present in soil. Since ancient times, soil bacteria play an important role on crop growth and yield by genetic transformation naturally. But the continuous use of chemical fertilizers reduces their number and proper environment for multiplication. Soil microorganisms are increasingly becoming an important source in the search for industrially important molecules¹.

In India, however, adequate information is available about the prevalence of microorganism strains in various parts of the country. In soil 80 to 99% of microorganisms remain unidentified whereas these biological communities are known to play a dominant role in maintaining a sustainable biosphere². Antibiotic resistance (AR) is a global problem especially in the developing countries, it also threatens public health in both developed and developing countries.

The bacterial antibiotic resistance has been reported as one of the most serious threats to the human as well as environmental health of this time, though antibiotics had tackled in the past many critical situations in clinical practices^{3,4}. The rapid global emergence of resistant bacteria threatens the

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efficacy of antibiotics, which have revolutionized medicine and saved countless lives⁵⁻¹⁰. Decades after antibiotics revolutionized healthcare, bacterial infections are once again an approaching threat¹¹. This emphasizes the urgent need to address antibiotic resistance. Different mechanisms or their combination are used by bacteria in developing resistance to antibiotics; however, a particular mechanism may be dominant and can be identifiable¹². Antibiotic resistance can be reduced but not stopped since bacteria evolve. The high rates of antimicrobial resistance occurrence have attracted the attention of international bodies such as the World Health Organization (WHO), Food and Agriculture Organization (FAO) and the World Organization for Animal Health who have now united to combat it as a common force¹³. It is estimated that worldwide, 700,000 patients die annually as a result of resistant infections and if nothing is done to combat antimicrobial resistance, the death rate is estimated to escalate to 10 million by the year 2050¹⁴. Hence the present study has been taken up where we have analysed the prevalence of potential pathogenic bacteria from the soil sample in Durgapur, West Bengal region and to determine the antibiotic resistance profiles of the isolated bacteria.

MATERIALS AND METHODS: While conducting the present study, n = 10 samples were collected from different regions of Durgapur, West Bengal, India during January–February 2025. The sampling sites included areas representing different environmental conditions such as agricultural land, roadside soil, garden soil, residential areas and open fields. The selection of multiple locations was intended to represent different environmental niches of Durgapur city in order to assess the diversity of soil bacteria and their antibiotic resistance profiles. All soil sampling procedures were performed using aseptic techniques. At each location, soil samples were collected from a depth of 5–10 cm below the surface using a sterile spatula to avoid surface contamination. Approximately 4 g of soil from each site was transferred into sterile polythene bags and transported to the laboratory in ice boxes for microbiological analysis. As a precautionary control, sterile blank samples (sterile swabs exposed to the sampling environment and processed similarly in the laboratory) were included to monitor possible contamination during

sampling and handling. No microbial growth was observed in these control samples.

The moisture content and the pH of all the soil samples were determined. Furthermore soil samples were inoculated using appropriate culture media, including nutrient agar, Tryptic Soy broth with 5% yeast extract and GYM medium for obtaining bacterial isolates. After serial dilution in sterile normal saline and vortexing, the aliquots (100 μ L) from each dilution were spread on the media and incubated overnight at 37 °C. This incubation temperature was selected to facilitate the growth of mesophilic bacteria including potential human-associated microorganisms, which were of particular interest in the present study due to their possible clinical relevance. However, it is acknowledged that incubation at 37 °C may preferentially select mesophilic organisms and may under-represent soil bacteria that grow optimally at lower temperatures (25–30 °C). After incubation, multiple colonies (approximately 5–10 colonies per plate) showing distinct morphological characteristics were observed. From these, colonies displaying clearly different morphological features (size, shape, colour, margin and elevation) were selected for preliminary screening. To simplify comparative antibiotic resistance analysis, one representative morphologically distinct isolate from each soil sample was selected for further characterization and antibiotic susceptibility testing. The isolated bacterial cultures were purified by streaking on Mueller-Hinton agar plates. For short-term maintenance, the isolates were preserved on cystinetryptone agar slants and stored at 4 °C. For long-term preservation, bacterial cultures were maintained as 20 % glycerol stocks at –80 °C, which ensures genetic stability of the isolates for subsequent analysis.

The isolated bacteria were characterized following phenotypic techniques (colony morphology study, gram-staining and a range of biochemical tests including indole production, methyl red, Voges–Proskauer, citrate utilization, catalase, oxidase, cellulose degradation, triple sugar iron, nitrate reduction, gelatin hydrolysis, casein hydrolysis test and sugar fermentation) for identification, according to Halt¹⁵ and Forbes., *et al.*¹⁶. These tests help to identify and differentiate bacterial species based on their metabolic properties.

These identifications should be considered presumptive, as molecular confirmation such as 16S rRNA gene sequencing was not performed in the present study. Antibiotic susceptibility testing of the isolates was performed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar plates following the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Bacterial inoculum was prepared from fresh cultures and adjusted to 0.5 McFarland turbidity standard (approximately 1.5×10^8 CFU/mL). The standardized inoculum was evenly spread onto Mueller–Hinton agar plates of approximately 4 mm depth using a sterile swab. In the present study, a set of commonly used antibiotics (ampicillin, chloramphenicol, gentamicin, methicillin and piperacillin) was selected in order to obtain a preliminary screening of resistance patterns among environmental bacterial isolates.

Antibiotic discs (Hi-Media, India) containing ampicillin (10 µg), chloramphenicol (30 µg), gentamicin (10 µg), methicillin (5 µg) and piperacillin (100 µg) were placed on the inoculated plates¹⁷. Methicillin was included as a β-lactam resistance indicator, although current CLSI guidelines recommend cefoxitin as a surrogate marker for *mecA*-mediated methicillin resistance in *Staphylococcus aureus*. Piperacillin was included in the antibiotic panel as a representative broad-spectrum β-lactam antibiotic for evaluating resistance patterns among environmental isolates. Although piperacillin–tazobactam combinations are commonly used in clinical susceptibility testing, piperacillin alone was selected in this study to obtain a preliminary assessment of β-lactam resistance among soil bacteria. The plates were incubated at 37 °C for 18–24 hours, after which the zones of inhibition were measured in millimeters and interpreted according to CLSI guidelines¹⁸. Quality control of the antibiotic susceptibility testing was performed using standard reference strains such as *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923, and the obtained zone diameter values were found to be within the acceptable CLSI ranges.

The MAR indices for the isolated soil bacteria were calculated and the results were explained following the criteria of Krumperman¹⁹. The antibiotic resistance phenotypes were determined, for the

isolates and the isolates with resistance to ≥ 3 test antibiotics expressed multiple antibiotic resistance phenotypes²⁰.

In the present study, the selected antibiotics were applied uniformly across all isolates to provide a comparative overview of resistance patterns in environmental bacteria. However, it is recognized that certain antibiotic–organism combinations (for example, β-lactams tested against non-fermenting Gram-negative rods such as *Pseudomonas* species) may not correspond to standard clinical testing panels.

RESULTS AND DISCUSSION: Increased and unwise use of antibiotics accelerates the emergence of multiple antibiotic resistant (MAR) bacteria, which restricts the efficacies of the currently available antibiotics in curing bacterial infections of different human ailments worldwide. Obviously, the antibiotic resistances are not limited in clinical settings, rather reported in agricultural as well as various environmental settings and foods^{19, 20}. The bacterial isolates obtained in the present study were recovered from soils collected from different environments within the Durgapur region, including residential areas, roadside soils and agricultural locations. These environments are frequently exposed to anthropogenic activities such as disposal of domestic waste, agricultural use of fertilizers and antibiotics, and industrial emissions. Such environmental conditions may contribute to the selection and persistence of antibiotic-resistant microorganisms in soil ecosystems.

The moisture content and the pH of all the soil samples were depicted in **Table 1**. A total of 10 soil bacteria isolated from the sample S1, S2, S3, S4, S5, S6, S7, S8, S9 and S10 respectively displayed varied morphological features of their colonies developed on nutrient agar plates **Table 2**. Among the isolated bacteria **Table 3, 4** were gram negative rods (n=4; S5, S7, S8 and S9) and the other 6 isolates were either gram-positive cocci (n=5; S1, S2, S4, S6, S10) or gram-positive rods (n=1; S3). The biochemical and sugar fermentation test results of the isolated bacteria have been represented in **Table 4** and **Table 5**. The presumptive identities of the isolated bacteria based on phenotypic and biochemical characterization are presented in **Table 6**.

TABLE 1: SOIL PHYSICOCHEMICAL PROPERTIES

Sample	pH	Moisture (%)
S1	6.8	12
S2	7.1	10
S3	6.5	15
S4	7.2	11
S5	6.9	13
S6	7.0	12
S7	6.7	14
S8	6.8	11
S9	7.3	9
S10	6.6	13

TABLE 2: COLONY CHARACTERISTICS OF BACTERIA ISOLATED FROM SOIL SAMPLES

Sl. no.	Isolate	Form	Size	Elevation	Colour	Margin	Surface	Opacity	Organism
1	S1	Circular	Small	Raised	Cream	Entire	Smooth	Opaque	<i>Staphylococcus sp.</i>
2	S2	Circular	Small	Flat	Yellow	Entire	Smooth	Opaque	<i>Micrococcus sp.</i>
3	S3	Circular	Small	Raised	White	Entire	Smooth	Opaque	<i>Bacillus sp.</i>
4	S4	Circular	Small	Flat	Cream	Entire	Smooth	Opaque	<i>Streptococcus sp.</i>
5	S5	Irregular	Small	Raised	Cream	Serrate	Smooth	Opaque	<i>Proteus sp.</i>
6	S6	Circular	Small	Raised	Cream	Entire	Smooth	Opaque	<i>Staphylococcus sp.</i>
7	S7	Circular	Small	Raised	Light green	Entire	Wrinkled	Opaque	<i>Pseudomonas sp.</i>
8	S8	Irregular	Small	Raised	Cream	Serrate	Smooth	Opaque	<i>Proteus sp.</i>
9	S9	Irregular	Small	Raised	White	Entire	Smooth	Opaque	<i>Alcaligenes sp.</i>
10	S10	Circular	Small	Flat	Yellow	Entire	Smooth	Opaque	<i>Micrococcus sp.</i>

TABLE 3: GRAM STAINING PROPERTY OF BACTERIA ISOLATED FROM SOIL SAMPLES

Sl. no.	Isolate	Gram stain	Shape
1	S1	Gram positive	Cocci in cluster
2	S2	Gram positive	Cocci in irregular cluster
3	S3	Gram positive	Rod shaped single cell or in pair
4	S4	Gram positive	Cocci existing in pairs and chains
5	S5	Gram Negative	Rod shaped
6	S6	Gram positive	Cocci in cluster
7	S7	Gram Negative	Rod shaped
8	S8	Gram Negative	Rod shaped
9	S9	Gram Negative	Rod shaped
10	S10	Gram positive	Cocci in irregular cluster

TABLE 4: BIOCHEMICAL TEST RESULTS FOR BACTERIA ISOLATED FROM SOIL SAMPLE

Strain code	TSI	Citrate	Indole	Catalase	Oxidase	MR	VP	DNase
S1	Y/Y	-	-	+	-	-	+	+
S2	Y/Y	-	-	+	+	-	-	-
S3	Y/Y	+	-	+	-	-	+	-
S4	Y/Y	-	-	-	-	+	-	-
S5	K/A	+	+	+	-	+	-	-
S6	Y/Y	-	-	+	-	-	+	+
S7	K/K	+	-	+	+	-	-	-
S8	K/A	+	+	+	-	+	-	-
S9	K/K	+	-	+	+	-	-	-
S10	Y/Y	-	-	+	+	-	-	-

MR: Methyl red; VP: Voges-Proskauer; TSI: Triple sugar iron, P: Pink; Y: Yellow; -: Negative; + : Positive.

TABLE 5: SUGAR FERMENTATION TEST RESULTS FOR BACTERIA ISOLATED FROM SOIL SAMPLES

Strain Code	Sugars				
	Sorbitol	Sucrose	Lactose	Maltose	Mannitol
S1	-	+	+	+	+
S2	+	+	+	+	+

S3	-	W	-	-	-
S4	-	+	+	+	-
S5	-	+	-	+	-
S6	-	+	+	+	+
S7	-	-	-	-	-
S8	-	+	-	+	-
S9	-	-	-	-	-
S10	+	+	+	+	+

W: Weakly positive; -: Negative; +: Positive.

TABLE 6: PRESUMPTIVE IDENTIFICATION OF BACTERIA ISOLATED FROM SOIL SAMPLES

Strain Code	Bacterial identity
S1	<i>Staphylococcus aureus</i> (presumptive)
S2	<i>Micrococcus luteus</i> (presumptive)
S3	<i>Bacillus subtilis</i> (presumptive)
S4	<i>Streptococcus pyogenes</i> (presumptive)
S5	<i>Proteus vulgaris</i> (presumptive)
S6	<i>Staphylococcus aureus</i> (presumptive)
S7	<i>Pseudomonas aeruginosa</i> (presumptive)
S8	<i>Proteus vulgaris</i> (presumptive)
S9	<i>Alcaligenesfaecalis</i> (presumptive)
S10	<i>Micrococcus luteus</i> (presumptive)

The zone diameters of inhibition (mm) for each isolate–antibiotic combination are presented in **Table 7**. These measurements were interpreted according to CLSI criteria to determine susceptibility or resistance. The antibiotic resistance profiles observed are depicted in **Table 8** for the isolated gram-negative and gram-positive bacteria. Modern CLSI guidelines recommend the use of cefoxitin disc diffusion for detection of methicillin resistance in *Staphylococcus aureus*. In the present study methicillin discs were used as a preliminary indicator of β -lactam resistance, which represents a limitation of the methodology. In clinical microbiology laboratories, β -lactam/ β -

lactamase inhibitor combinations such as piperacillin–tazobactam are frequently used for susceptibility testing. However, the present study employed piperacillin alone as part of a simplified antibiotic panel for environmental screening.

Soil is widely recognized as a reservoir of microbial contaminants including antibiotic resistance genes (ARGs) and human bacterial pathogens. The antibiotic susceptibility test results, in terms of ZDIs, for the bacteria isolated from soil samples of Durgapur. The antibiotic resistance phenotypes and the MAR indices of the isolated bacteria are represented in **Table 8**.

TABLE 7: ZONE DIAMETER OF INHIBITION (MM) FOR ANTIBIOTIC SUSCEPTIBILITY TESTING

Isolate	AMP (mm)	C (mm)	GEN (mm)	MET (mm)	PI (mm)
S1	10	11	22	9	12
S2	9	20	21	10	19
S3	11	22	23	10	12
S4	20	19	12	11	20
S5	18	20	22	10	11
S6	10	12	21	9	10
S7	11	12	20	10	18
S8	19	21	23	11	10
S9	8	9	10	8	9
S10	10	21	22	9	20

TABLE 8: ANTIBIOTIC RESISTANCE PROFILES AND MAR INDICES FOR THE ISOLATED SOIL BACTERIA (N=10)

Isolated Bacteria	Antibiotic resistance profile					MAR index
	AMP	C	GEN	MET	PI	
<i>Staphylococcus aureus</i> S1	R	R	S	R	R	0.8
<i>Micrococcus luteus</i> S2	R	S	S	R	S	0.4
<i>Bacillus subtilis</i> S3	R	S	S	R	R	0.6

<i>Streptococcus pyogenes</i> S4	S	S	R	R	S	0.4
<i>Proteus vulgaris</i> S5	S	S	S	R	R	0.4
<i>Staphylococcus aureus</i> S6	R	R	S	R	R	0.8
<i>Pseudomonas aeruginosa</i> S7	R	R	S	R	S	0.6
<i>Proteus vulgaris</i> S8	S	S	S	R	R	0.4
<i>Alcaligenes faecalis</i> S9	R	R	R	R	R	1
<i>Micrococcus luteus</i> S10	R	S	S	R	S	0.4

AMP: ampicillin; C: chloramphenicol; GEN: gentamicin; MET: methicillin; PI: piperacillin.

The bacterial isolates, in the current study, showed resistance to one or more antibiotic tested; the lowest resistance to two tested antibiotics (MAR index: 0.4) was recorded for *Micrococcus luteus*, *Streptococcus pyogenes* and *Proteus vulgaris* strain while the highest resistance to all the five antibiotics (MAR index: 1) was displayed by *Alcaligenes faecalis* strain. Among 10 isolated bacteria, 5 displayed lowest mar indices of 0.4 with resistance to two antibiotics and 1 displayed highest mar index of 1 with resistance to all five antibiotics. In between 2 displayed mar indices of 0.6 and 0.8 with resistance to three and four antibiotics respectively. No intermediate susceptibility results were observed for the tested isolates. The MAR index values obtained in the present study provide a preliminary indication of resistance patterns among the tested isolates. However, since the index was calculated using a limited panel of five antibiotics, it cannot fully represent the complexity of environmental antibiotic resistomes. Comprehensive analysis involving a larger antibiotic panel and molecular characterization would be required for a more robust assessment.

Some of the bacterial species identified in the present study are commonly associated with clinical environments. Since, identification was based on phenotypic and biochemical characteristics, the bacterial species reported in the present study should be considered presumptive identifications, and further molecular confirmation (e.g., 16S rRNA gene sequencing) would be required for definitive species-level identification.

It is acknowledged that CLSI provides organism-specific antibiotic panels and testing conditions. Some antibiotic–organism combinations evaluated in the present study are not routinely included in clinical susceptibility testing panels. These combinations were included to obtain a general overview of resistance trends among environmental

isolates and therefore the results of the present study should be interpreted primarily as comparative resistance screening rather than definitive clinical susceptibility profiling. Since, the same antibiotic panel was applied to all isolates, some organism–antibiotic combinations may not fully correspond to organism-specific CLSI recommendations.

CONCLUSION: The present study demonstrates the presence of diverse bacterial populations in soils from different locations in the Durgapur region. The soil sample of the current study supported the growth of gram-positive and gram – negative bacteria. Gram –positive bacteria are cocci and rod whereas gram-negative bacteria are mainly rod. The isolated bacteria showed phenotypic similarity to bacterial genera that are known to include clinically relevant species. However, the present study did not evaluate virulence factors or pathogenic potential, and therefore no definitive conclusions regarding pathogenicity can be drawn. The isolated presumptive bacteria possessed varied antibiotic resistances and MAR indices. These findings provide baseline information on soil microbial diversity and antibiotic susceptibility patterns in the region. Future studies incorporating molecular identification methods and broader environmental sampling are needed to better understand the ecological and public health implications of soil microbial communities. Therefore it is very much important to examine such environmental bacteria for different clinical relevance and to prepare effective guidelines for judicious use of antibiotics to intercept the bacterial multiple antibiotic resistances.

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