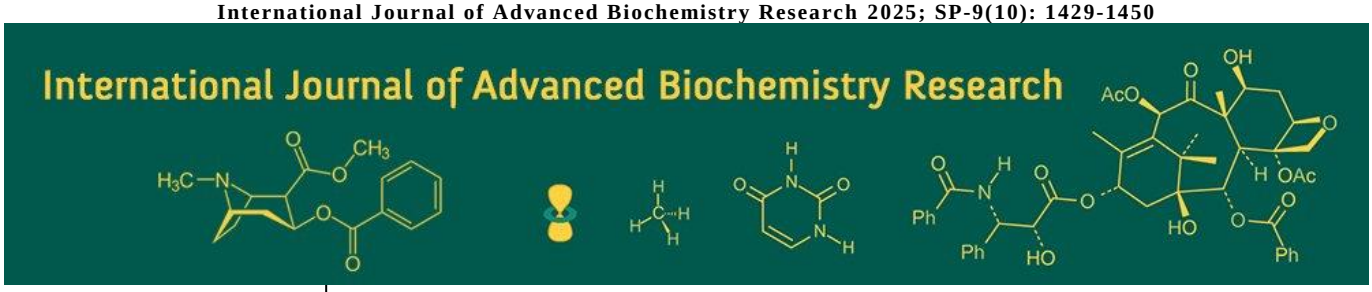

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Isolation and characterization of air microbiota from indoor and outdoor environments

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Abstract
Composition and diversity of airborne microbes vary significantly across different urban environments, influencing human health. This study was aimed to analyse the air microbiota in different environments (laboratory, garbage van, traffic congested road, hospital) which could play role on air quality and public health.
Settle-plates with Trypticase soy agar (TSA) and Potato Dextrose agar (PDA) were used for sampling air (10 min and 1 hr). Each environment harboured distinct microbial communities (both bacteria and fungi), influenced by human activity, environmental and sanitary conditions. 11 strains (SJ-1, SJ-3-7, SJ-9-10, SJ-13, SJ-15-16) were characterized based on morphology. Colonies were white, cream and yellow coloured, with mucoid or chalky consistency, and irregular or smooth margins. Fungus with white mycelia were also obtained. CFU/m^3 was estimated to be 0.0424, 0.328, 0.167 & 0.099 (10 min). All the bacterial strains were gram positive, with majority coccus and catalase positive (except SJ-3, 10). SJ-9, 15 and 16 were identified as *Staphylococcus* sp. Our tests also suggested that SJ-6, 7 belong to *Bacillus subtilis* or *Bacillus cereus*, although further tests were warranted. SJ-3, 10 were gram positive cocci, non-motile, catalase and oxidase negative, suggesting presence of *Streptococcus* sp., which is a common bacterial species in any air sample. Antibiotic susceptibility tests using Kanamycin revealed MIC 32 µg/ml for SJ-1, 7, 9, while it was above 256 µg/ml for all others. Thus, the present study sheds light on air quality in various indoor and outdoor environments, which need to be kept under check to prevent any health issues.

Keywords: Air sampling, microbiome, air microbes, settle plate, health risk, sanitation

Introduction
The study of air microbiota, which involves examining the composition and diversity of microorganisms in the atmosphere, is a critical aspect of aerobiology. Understanding these microbial communities is essential, as they represent the organisms we are exposed to through daily inhalation.
Investigating and characterizing air microbiota from both indoor and outdoor environments provides insights into microbial diversity, community dynamics, and potential health risks. Airborne microbial populations are influenced by various factors: indoor microbiota can be shaped by occupancy, ventilation, and building materials, whereas outdoor microbiota is affected by seasonal variations, pollution levels, and geographical location. Analyzing these communities enhances our understanding of microbial ecology, bioaerosol transmission, and environmental impacts on microbial dynamics.
Characterization of air microbiota employs a combination of approaches, including culture-based techniques, microscopy, and molecular methods such as polymerase chain reaction (PCR) and metagenomics. These techniques allow identification of microbial species, assessment of their abundance and diversity, and inference of their potential ecological functions. Insights gained from such studies are valuable for improving indoor air quality, monitoring environmental health, and implementing strategies to control airborne diseases.
In this study, we used the settle plate method to isolate and compare microbial diversity across different indoor and outdoor environments. The findings aim to advance our understanding of the factors shaping air microbiota composition and dynamics, and their implications for environmental and human health.

Materials and Methods

Chemicals and Media

Ethanol, Potato Dextrose Agar (PDA), Tryptone Soy Agar (TSA), kanamycin, and other analytical-grade reagents were obtained from MERCK, SRL, and HiMedia. Media were prepared following standard compositions.

Composition of PDA (g/L): Dextrose 20; Agar 15; Potato infusion 200. Composition of TSA (g/L): Pancreatic digest of casein 17; Soybean meal digest 3; NaCl 5; K_2HPO_4 2.5; Dextrose 2.5; Agar 15; pH 7.3 ± 0.2. Composition of Mueller Hinton broth (g/L): Beef extract 2; Casein hydrolysate 17.5; Starch 1.5 (Ohagim *et al*., 2017) [11].

Sampling

Airborne microbial samples were collected by settle plate method at four sites in Hazra, Kolkata: (i) Microbiology laboratory, Jogamaya Devi College; (ii) Garbage van (Hazra crossing); (iii) Traffic-congested Hazra road; (iv) Chittaranjan National Cancer Institute premises. PDA and TSA plates were exposed for 10 min and 1 h, then incubated at 37 °C for 24-48 h (Flores, *et al*., 2011) [5].

Isolation and Pure Culture

Eleven representative strains (SJ-1, SJ-3, SJ-4, SJ-5, SJ-6, SJ-7, SJ-9, SJ-10, SJ-13, SJ-15, SJ-16) were subcultured on TSA plates and purified by repeated streaking (Fakunle, *et al*., 2021) [4].

Biochemical Characterization

Gram staining was performed to differentiate Gram-positive and Gram-negative isolates. Motility was tested using TTC-supplemented semi-solid agar (Beef extract 3 g, Casein digest 10 g, NaCl 5 g, Agar 4 g/L). Mannitol Salt Agar (MSA) test assessed mannitol fermentation (NaCl 75 g, Mannitol 10 g/L, phenol red indicator). Urease test was performed on Christensen's agar (Urea 20 g/L, phenol red indicator). Oxidase test used 1% p-amino-N,N-dimethylaniline oxalate reagent on nutrient broth-grown cultures. Starch hydrolysis was examined on starch agar plates after iodine flooding (Ruokolainen, *et al*., 2017) [12].

Antibiotic Susceptibility Test

Minimum Inhibitory Concentration (MIC) of kanamycin was determined in Mueller Hinton broth using two-fold serial dilutions (4096-16 µg/ml). Isolates were adjusted to OD600 = 0.5-1.9, inoculated into test tubes, and incubated at 37 °C for 24-48 h. MIC was defined as the lowest concentration with no visible growth (Ekhaise, *et al*., 2010) [3].

Results and Discussion

Characterization of different bacterial morphologies

Settle-plates with Trypticase soy agar (TSA) and Potato Dextrose agar (PDA) were used for sampling air (10 min and 1 hr). Air samples were taken from 4 different places (laboratory, garbage van, traffic congested road, hospital) to analyse the air microbiota in different environments which could play role on air quality and public health. Many colonies were observed on each plate. Some common morphological characters were white, cream and yellow colored, with mucoid or chalky consistency, and irregular or smooth margins. Fungus with white mycelia was also obtained mainly in Potato dextrose agar (PDA) medium (Table 1, Fig. 1-13) similar as found Napoli, *et al*., 2012) [10].

Subculture and establishment of pure culture

In microbiology, streaking is a technique used to isolate a pure strain from single species of microorganisms, often bacteria. Samples can be then taken from the resulting colonies and a microbiological culture can be grown on a new plate so that the organism can be identified, studied, or tested. 11 strains (SJ-1,SJ-3,SJ-4,SJ-5,SJ-6,SJ-7,SJ-9,SJ-10,SJ-13,SJ-15,SJ-16) were chosen from the mother plates based on their striking colony morphologies and sample sites. Then, the pure cultures were established by multiple subculture by streaking on pre-prepared Tryptone soy agar (TSA) plate (Table 2). Subculturing is a common practice in microbiology that involves transferring a small amount of cells from one culture vessel to a fresh growth medium to continue their growth and maintain a healthy culture. Isolation of pure culture by streak plating technique is shown below (Fig. 5) closed to result fund by Berg, *et al*., 2014) [2].

Colony morphology and CFU/m3

A CFU stands for colony forming unit. CFU is a unit that we used as a measure of viable bacterial or fungal cells. When a microbiologist plates a sample onto a growth medium, each live cell has the potential to multiply and form a visible colony. Each colony that arises from a single cell or group of cells is counted as one CFU.

In some plates, fungus with white mycelia was also obtained. CFU/m3 was estimated to be 0.0424, 0.328, 0.167 & 0.099 (10 min). To explore the effect of haze pollutants on the concentration of culturable bacteria and fungi, we compared the numbers of CFU/m3 (Tables 12-13) (Mohamad, 2023).

Formula: CFU/m3=CFU/t*k

where, CFU-mean of colony forming unit; t- total sampling time expressed in minutes; K- a conversion factor for cubic feet to cubic meters (k=35.3) (Yang, *et al*., 2023) [19].

Gram staining

The gram staining is one of the most crucial staining techniques in microbiology to differentiation gram positive and gram negative bacteria based on their cell wall composition. The basic principle of gram staining is the properties of certain bacteria cell wall to retain the crystal violet dye. The cell walls of gram positive microorganisms have a higher peptidoglycan and lower lipid content than gram negative bacteria. The gram stain procedure distinguishes between gram positive and gram negative groups by coloring these cells red or violet. Gram positive bacteria stain violet due to the presence of thick layer of peptidiglycan in their walls, which retains the crystal violet these cells are stained with. The final step in gram staining is to use safranin to give decolorized gram-negative bacteria reddish pink color for easier identification, it is also known as counter stain (Tripathi, and Sapra, 2023) [16]. In the present study, all the bacterial strains were gram positive,

with majority coccus in shape, a few were rod (Table 5, Fig. 6-7).

Catalase test

The catalase test is performed in microbiology to differentiate bacteria based on their ability to produce the enzyme catalase, which catalyzes the breakdown of hydrogen peroxide into water and oxygen. In the present study we used catalase test for differentiating catalase positive microbes from catalase negative ones. It was also valuable in separation of certain gram positive. In case of positive reaction, oxygen was liberated as effervescence when the drop of diluted hydrogen peroxide was added to the bacterial culture taken on the slide (Aryal, *et al*., 2023) [1].

After performing the catalase test aseptically, data was recorded for each of the colonies. Rate of effervescence (fast, delayed and no effervescence) was noted and shown in Table 6 and Fig. 8. Majority of strains were catalase positive except SJ-3 & SJ-10 (Tankeshwar, 2022) [5].

Mannitol salt agar (MSA) test

Mannitol salt agar (MSA) is a selective and differential medium used in microbiology to isolate and identify Staphylococcus aureus from other Staphylococci. Staphylococci that can ferment mannitol produce acidic byproducts, causing the phenol red indicator in the medium to turn yellow. The medium mainly used for this purpose was Mannitol salt agar medium (Kateete, *et al*., 2010) [7].

In this study, after performing the MSA test based on morphology and color, we have selected only 4 strains and performed the test. By this test we have 3 (SJ-9, 15, 16) positive and SJ-10 was negative result. Data were recorded (Table-16, Figure- 9) similar as result found Kumurya, 2017) [8].

Motility test

The motility test is performed in microbiology to determine if a microorganism is capable of movement, which can be important for identifying certain species of bacteria. The test involves inoculating a semisolid agar medium with the microorganism and observing for the presence of growth away from the point of inoculation, indicating motility. This test is particularly useful in distinguishing between motile and non-motile species, which can be relevant for clinical diagnosis, environmental monitoring, and research purposes (Shields, and Cathcart, 2011) [13].

After performing the test, all the bacterial strains were motility positive after 24 & 48 h. except SJ-10 & SJ-13 were negative after 24 h only (Tables 9-10 & Fig. 10-11).

Urease test

Hydrolysis of urea produces ammonia and CO2. The formation of ammonia alkalinizes the medium, and the pH shift is detected by the color change of phenol red from light orange at pH 6.8 to magenta (pink) at pH 8.1. Rapid urease-positive organisms turn the entire medium pink within 24 to 72 hours. Christensen's agar medium was used to detect urease activity in a variety of microorganisms similar as Uotani,and Graham, 2015) [17].

This test was primarily used to differentiate organisms based on their ability to produce urease and the positive result was given by SJ-4, SJ-7, SJ-15, SJ-16 another left strains were negative for this result. Data was recorded in Table 19 and Fig. 12.

Oxidase test

The oxidase test is performed in microbiology to quickly identify bacteria that produce cytochrome c oxidase, an enzyme involved in the electron transport chain. In presence of the enzyme cytochrome oxidase (gram-negative bacteria), the N, N-dimethyl-p-phenylenediamine oxalate and α-naphthol react to indophenol blue. The test involves applying a drop of oxidase reagent to a bacterial colony, and if the colony turns purple within seconds, it indicates the presence of the oxidase enzyme. I have used Nutrient broth medium to study oxidase test (Shields, and Cathcart, 2011) [13].

So I was performed this test properly, and results the majority of strains were negative for this test except SJ-4 & 13 (Table 11, Fig. 13).

Starch hydrolysis test

Starch hydrolysis test is use to determine if a bacterium can break down starch using the enzyme amylase. In this test, bacteria were grown on a starch - containing medium and after incubation, iodine was added. According to test, if the starch is hydrolyzed using the enzyme amylase then the medium around the bacterial growth will remain clean and make a zone showing the starch is absent if the starch is not hydrolyzed, then the iodine will turn the medium blue-black colour (Sigmon, 2008) [14].

In this study, after adding the iodine solution I found that some strains were positive for this test and some were negative. And the positive strains are SJ-1, 4, 6, 7 & 9. (Table 12).

Antibiotic susceptibility test by Minimum Inhibitory Concentration (MIC) method

The Antibiotic Susceptibility Test by Minimum Inhibitory Concentration (MIC) method is performed to determine the lowest concentration of an antibiotic that inhibits the growth of a specific bacterium. This information is crucial for selecting the most effective antibiotic treatment for bacterial infections. By determining the MIC, healthcare providers can identify the most appropriate antibiotic and dosage regimen to achieve optimal therapeutic outcomes while minimizing the risk of antibiotic resistance.

Firstly, primary culture must be performed to do this test (Table 13). I used the kanamycin for this test and done by MIC method. I have choosen the range of concentration between 2048 to 16 цg/ml to check in which concentration the bacteria will inhibited and I was observed that the MIC value for some strains were 32 цg/ml and some strains have the MIC value between 128-256 цg/ml except one strain that was SJ-5, the MIC value for this was greater than 2048 цg/ml. The final results data are given below (Table 14, Fig. 16-26) similar as found by Kadeřábková, *et al*., 2024).

Table 1: Characterization of morphology of colonies for different places

10A.Microbiology laboratory:

Media	Time	No.of colonies	Morphology
TSA	10 min	02	White;round;smooth;big;mucoid
		01	Creamy yellow;round;smooth;big;mucoid
		06	White;round;smooth;medium;mucoid
		06	White;round;smooth;small;mucoid
	1 h	01	White;round;smooth;brittle;big;elevated
		04	White;round;smooth;brittle;medium;elevated
		01	White;irregular shape;irregular margin;brittle;small;elevated
		01	Fungus with white mycellia
		01	White;brittle;medium;smooth margin;round;elevated
		08	White;round;smooth;big;mucoid
		12	White;round;smooth;medium;mucoid
PDA	10 min	01	White;round;smooth;small;mucoid
	1 h	02	White;round;smooth;small;mucoid

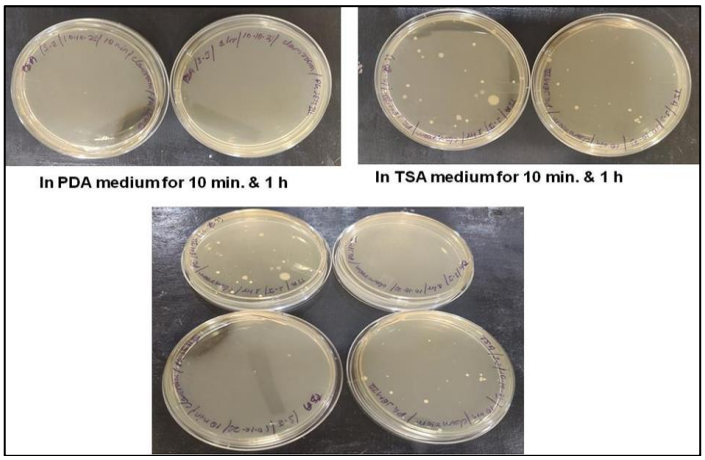


Fig 1: Observed colonies of sample in different medium taken from laboratory classroom

10B.Traffic cogested road:

Media	Time	No.of colonies	Morphology
TSA	1 hr.	5	White; round; smooth; big; brittle; elevated.
		19	White; irregular shape; smooth; medium; brittle; elevated.
		1	white; irregular shape; smooth; small; brittle; elevated.
		4	White; irregular shape; big; flat
		7	White; round; smooth; big; mucoid.
		29	White; round; smooth; medium; mucoid.
		75	White; round; smooth; small; mucoid.
		2	Yellowish; round; medium; mucoid; smooth.
		5	Creamy white; round; medium; mucoid; smooth.
		8	Creamy white; round; small; mucoid; smooth.
	10min.	2	Round; hollow; medium; smooth.
		12	Round; white; smooth; big; mucoid
		5	Round; white; smooth; medium; mucoid
		14	Round; white; smooth; small; mucoid
		2	Round; white; smooth; big; brittle; elevated.
		2	White; irregular; smooth; big; brittle; elevated.
		1	White; irregular; smooth; medium; brittle; elevated.
		1	White; irregular; smooth; big; chalky; elevated.
		3	White; irregular; smooth; medium; chalky; elevated.
		1	fungus with white mycelllia
		1	Irregular; white; smooth; medium; chalky.
		1	Round; creamy yellow; smooth; big; mucoid.
		3	Round; creamy yellow; smooth; medium; mucoid.
		68	Round; creamy yellow; smooth; small; mucoid.
PDA	1 hr.	11	fungus with white mycelllia.
		2	White; round; medium; smooth; mucoid.
		2	White; round; small; smooth; mucoid.
	10min	4	Fungus with white mycellia.

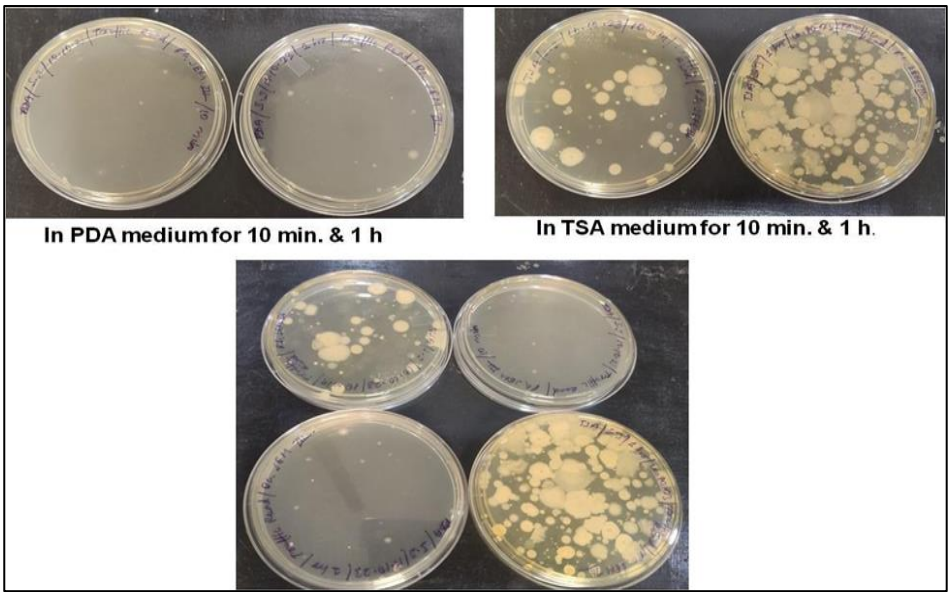


Fig 2: Observed colonies of sample in different medium taken from traffic congested road

10C .Garbage van :

Media	Time	No.of colonies	Morphology
TSA	1hr	57	Irregular; white; medium; smooth; brittle; elevated.
		7	Irregular; white; small; smooth; brittle; elevated.
		1	Irregular shape; white; big; serrated; chalky.
		25	Round; white; smooth; medium; mucoid.
		43	Round; white; smooth; small; mucoid.
	10min	21	Fungus with white mycellia.
		1	Ovate; white; medium; irregular; brittle; elevated
		8	White; round; medium; smooth; mucoid.
		25	White; round; small; smooth; mucoid.
		3	Yellowish; round; medium; smooth; mucoid.
		22	Yellowish; round; small; smooth; mucoid.
PDA	1hr.	1	Ovate; white; big; irregular; mucoid; elevated.
		12	Round; white; smooth; medium; mucoid.
		28	Round; white; smooth; big; mucoid.
		9	Round; white; smooth; small; mucoid.
	10min	1	Ovate; white; irregular; medium; mucoid.
		13	Round; white; smooth; big; mucoid.
		40	Round; white; smooth; medium; mucoid.
		17	Round; white; smooth; small; mucoid.

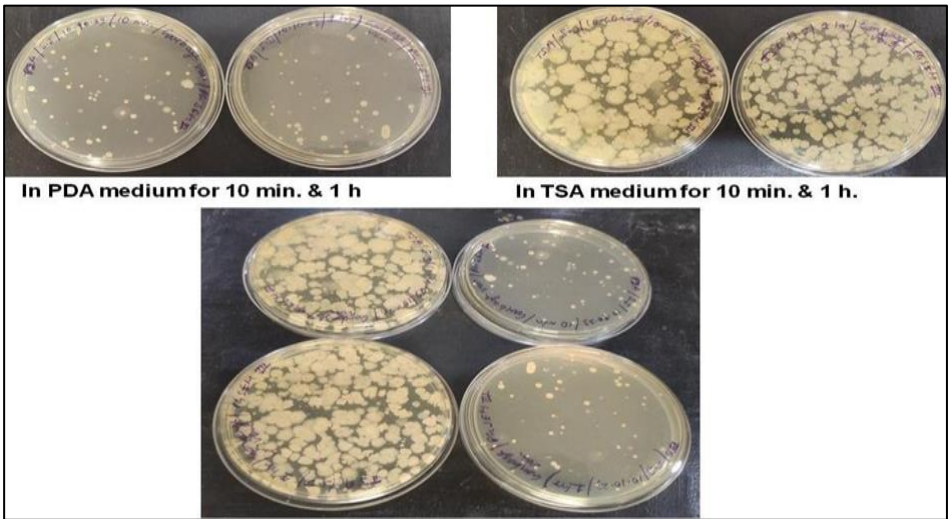


Fig 3: Observed colonies of sample in different medium taken from garbage van

10D .Hospital area:

Media	Time	No.of colonies	Morphology
TSA	1hr.	1	Round; white; smooth; large; brittle; elevated.
		1	Irregular shape; white; smooth; large; brittle; elevated.
		3	Irregular shape; white; irregular margin; large; brittle; elevated.
		2	Irregular shape; creamy white; irregular margin; large; mucoid.
		13	Round; white; smooth; large; mucoid.
		4	Round; yellowish; smooth; mucoid; medium.
		5	Round; yellowish; smooth; mucoid; small.
		3	Round; white; smooth; chalky; medium; elevated.
		4	Round; hollow; smooth; white; medium.
		1	Round; hollow; smooth; white; small.
		54	Round; white; smooth; medium; mucoid.
		58	Round; white; smooth; small; mucoid.
		1	Round; white; smooth; chalky; small; elevated.
	10min	2	Round; white; smooth; large; mucoid.
		1	Round; white; smooth; medim; mucoid.
		1	Round; white; smooth; large; brittle.
		2	Round; hollow; white; smooth; medium.
		1	Round; hollow; white; smooth; large.
		1	Round; hollow; white; smooth; small.
		7	Round; creamy white; smooth; medium; mucoid.
		1	Irregular; white; serrated; large; chalky.
		1	Ovate; white; irregular; medium; mucoid.
		3	Round; white; smooth; medium; mucoid.
		16	Round; white; smooth; small; mucoid.
PDA	1hr.	2	Round; white; smooth; brittlr; elevated;medium.
		1	Round; white; smooth; mucoid; medium.
		12	Round; white; smooth; mucoid; small.
	10min	4	Round; white; smooth; mucoid; small.

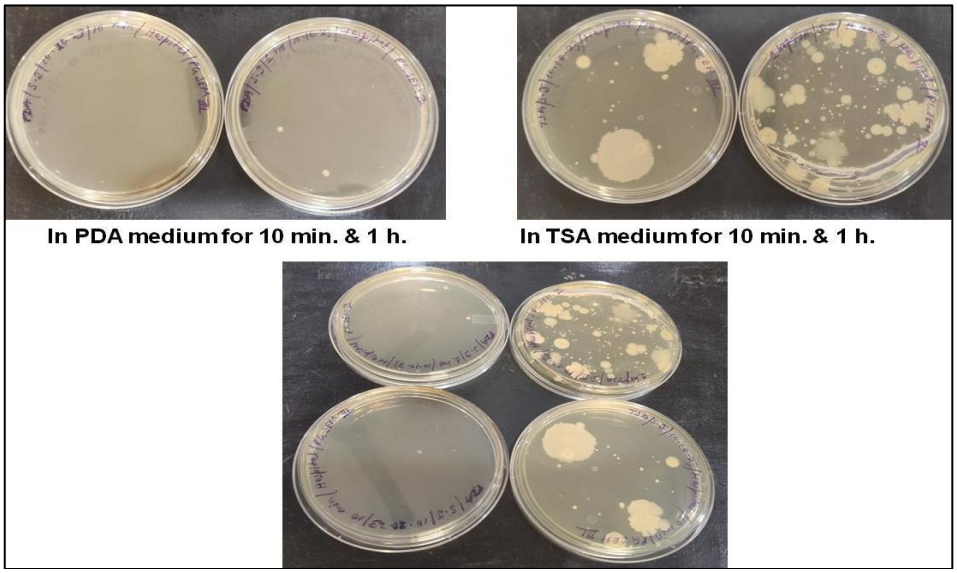


Fig 4: Observed colonies of sample in different medium taken from hospital area

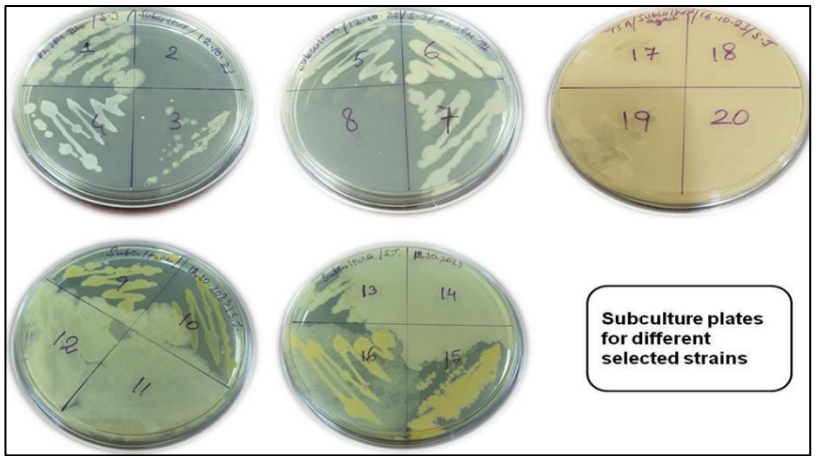


Fig 5: After 24 h incubation of subcultured plates

Table 2: Morphological characteristic of selected strains

Strain no.	Site of sample	Time	Media	Morphology of colonies
SJ-1	Microbiology laboratory	1 hr	TSA	White;round;smooth;brittle;big;elevated
SJ-3	Microbiology laboratory	1 hr	TSA	White;round;smooth;big;mucoid
SJ-4	Microbiology laboratory	1 hr	TSA	White;round;smooth;medium;mucoid
SJ-5	Microbiology laboratory	10 min	TSA	White;round;smooth;big;mucoid
SJ-6	Traffic congested road	10 min	TSA	Round ;white;smooth;big;mucoid
SJ-7	Traffic congested road	10 min	TSA	White ;irregular ;smooth;big;chalky;elevated
SJ-9	Traffic congested road	10 min	TSA	Round ;creamy yellow;smooth;medium;mucoid
SJ-10	Traffic congested road	10 min	TSA	Round ;creamy yellow;smooth;small;mucoid
SJ-13	Garbage van	1 hr	TSA	Round ;white;smooth;small;mucoid
SJ-15	Garbage van	10 min	TSA	Round,yellowish;medium;smooth;mucoid
SJ-16	Garbage van	10 min	TSA	Round ;yellowish;small;smooth;mucoid
SJ-17	CNCI Hospital	10min	TSA	Round;creamywhite;smooth;medium;mucoid
SJ-18	CNCI Hospital	10min	TSA	Irregular; white; serrated; large; chalky
SJ-19	CNCI Hospital	10min	TSA	Round; white; smooth; large; mucoid
SJ-20	CNCI Hospital	1 hr.	TSA	Round; white; smooth; small; mucoid

Table 3: Calculation for CFU/m^3 (for 10 min)

Places	Media	Bacterial colony		Fungal colony	
		CFU	CFU/m^3	CFU	CFU/m^3
Microbiology laboratory	TSA	15	0.0424	0	0
	PDA	1	0.00283	0	0
Traffic congested road	TSA	11.6	0.328	0	0
	PDA	0	0	4	0.011
Garbage van	TSA	59	0.167	21	0.059
	PDA	71	0.2011	0	0
Hospital	TSA	35	0.099	0	0
	PDA	4	0.011	0	0

Table 4: Calculation for CFU/m^3 (for 1 hr)

Places	Media	Bacterial colony		Fungal colony	
		CFU	CFU/m^3	CFU	CFU/m^3
Microbiology laboratory	TSA	27	0.0127	1	0.000472
	PDA	2	0.000944	0	0
Traffic congested road	TSA	155	0.0731	0	0
	PDA	4	0.00188	11	0.00519
Garbage van	TSA	133	0.0627	0	0
	PDA	50	0.0236	0	0
Hospital	TSA	150	0.0708	0	0
	PDA	15	0.00708	0	0

Table 5: Gram staining of the isolated strains

Strain no.	Gram +ve/-ve	Morphology
SJ-1	+ve	Single coccus
SJ-3	+ve	Coccus in chain form
SJ-4	+ve	Coccus in clump form
SJ-5	+ve	Coccus in chain form
SJ-6	+ve	Rod
SJ-7	+ve	Rod
SJ-9	+ve	Coccus
SJ-10	+ve	Coccus
SJ-13	+ve	Coccus
SJ-15	+ve	Coccus
SJ-16	+ve	Coccus

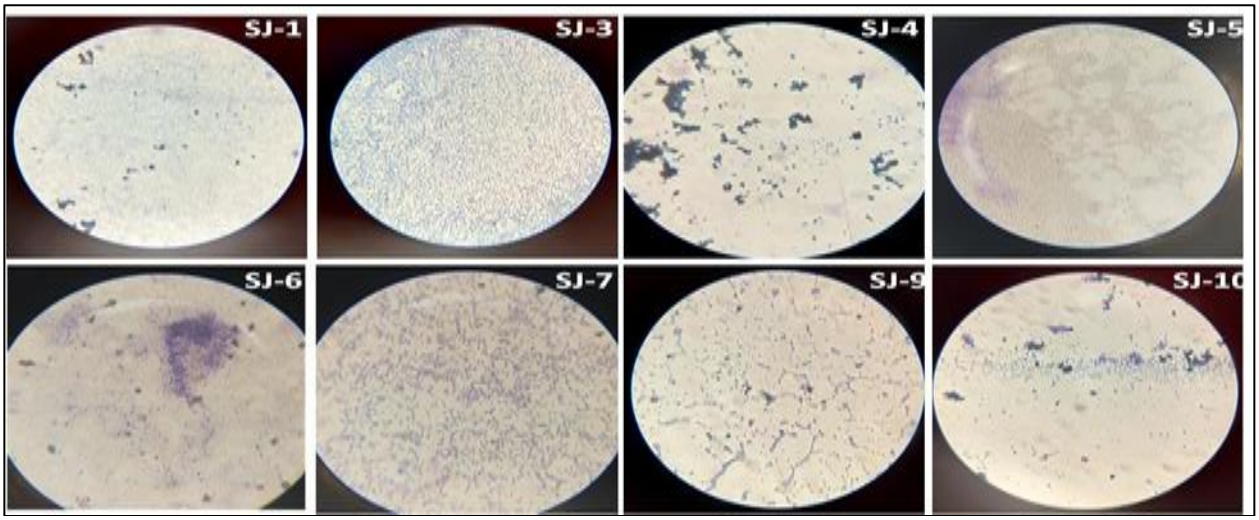


Fig 6: Pictures of gram staining of SJ-1 to SJ-10

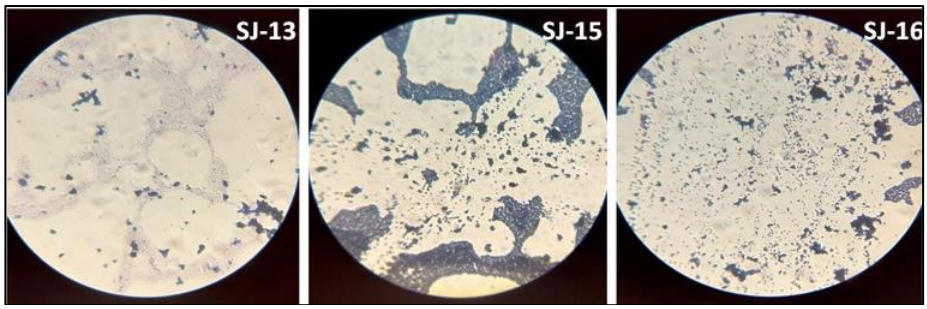


Fig 7: Pictures of gram staining of SJ-13 to SJ-16

Table 6: Catalase test

Strain no.	Effervescence (+ve/-ve)	Time
SJ-1	+ve	Delayed
SJ-3	-ve	-
SJ-4	+ve	Fast
SJ-5	+ve	Fast
SJ-6	+ve	Fast
SJ-7	+ve	Delayed
SJ-9	+ve	Fast
SJ-10	-ve	-
SJ-13	+ve	Fast
SJ-15	+ve	Fast
SJ-16	+ve	Fast

Fig 8: Observed photos of catalase test2

Table 7: Mannitol salt agar (MSA) test

Strain no.	Growth	Colour (yellow)
SJ- 9	+ve	+ve
SJ- 10	-ve	-ve
SJ- 15	+ve	+ve
SJ- 16	+ve	+ve

Fig 9: MSA plates for strains SJ-9, 10, 15 and 16

Table 8: Motility test after 24 hour incubation

Strain no.	Growth	Colour change	Inference	Motility (+ve /-ve)
control	-ve	Yellow	No change after incubation	-ve
SJ-1	+ve	Reddish pink	Colour has changed on the surface of agar and inside	+ve
SJ-3	+ve	Reddish pink	Colour has changed on the surface of agar and partially inside	+ve
SJ-4	+ve	Reddish pink	Colour has changed on the surface of agar and partially inside	+ve
SJ-5	+ve	Reddish pink	Colour has changed on the surface of agar and partially inside	+ve
SJ-6	+ve	Reddish pink	Colour has changed on the surface of agar and inside	+ve
SJ-7	+ve	Reddish pink	Colour has changed on the surface of agar and inside	+ve
SJ-9	+ve	Reddish pink	Colour has changed on the surface of agar and partially inside	+ve
SJ-10	+ve	Yellow	No change after incubation	-ve
SJ-13	-ve	yellow	No change after incubation	-ve
SJ-15	+ve	Reddish pink	Colour has changed on the surface of agar and partially inside	+ve
SJ-16	+ve	Reddish pink	Colour has changed on the surface of agar and partially inside	+ve

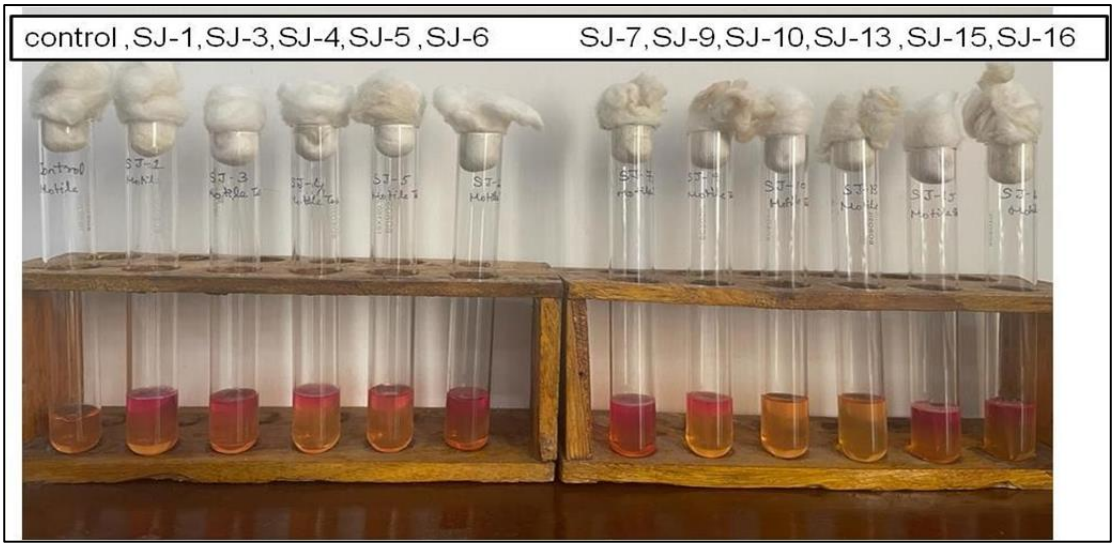


Fig 10: After 24 hour incubation of motility test

Table 9: Motility test after 48 hour incubation

Strain no.	Growth	Colour change	Inference	Motility (+ve /-ve)
Control	-ve	Yellow	No change after incubation	-ve
SJ-1	+ve	Reddish pink	Colour has changed completely	+ve
SJ-3	+ve	Reddish pink	Colour has changed completely	+ve
SJ-4	+ve	Reddish pink	Colour has changed completely	+ve
SJ-5	+ve	Reddish pink	Colour has changed on the surface of agar and inside	+ve
SJ-6	+ve	Reddish pink	Colour has changed completely	+ve
SJ-7	+ve	Reddish pink	Colour has changed completely	+ve
SJ-9	+ve	Reddish pink	Colour has changed on the surface of agar and inside	+ve
SJ-10	+ve	Reddish pink	Colour has changed on the surface of agar and inside	+ve
SJ-13	+ve	Reddish pink	Colour has changed on the surface of agar and inside	+ve
SJ-15	+ve	Reddish pink	Colour has changed completely	+ve
SJ-16	+ve	Reddish pink	Colour has changed completely	+ve

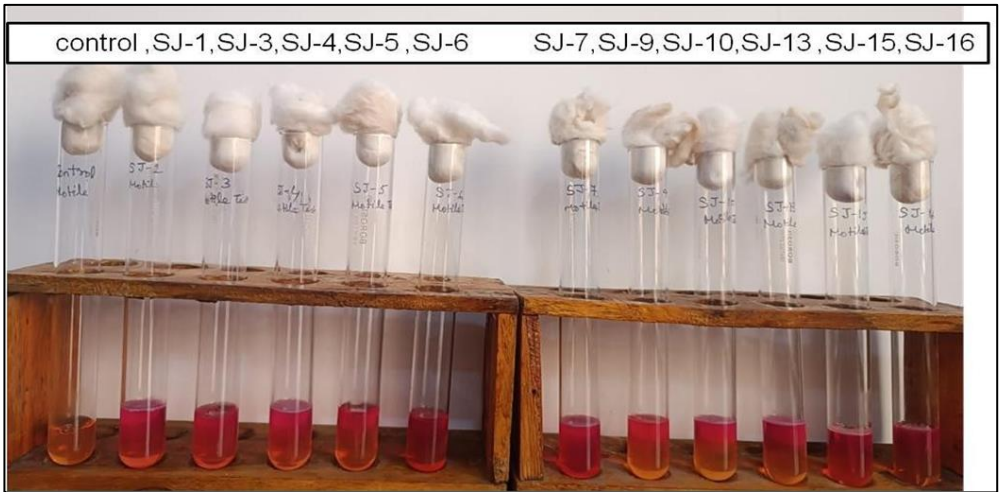


Fig 11: After 48 hour incubation of motility test

Table 10: Urease test results

Strain no.	Colour change or not		
	At 24 hour	At 48 hour	At 72 hour
Control	No colour/growth	No colour/growth	No colour/growth
SJ-1	No colour/growth	No colour/growth	No colour/growth
SJ-3	No colour/growth	No colour/growth	No colour/growth
SJ-4	Pinkish yellow with growth	Pinkish colour with growth	Pinkish colour with growth
SJ-5	Pinkish yellow with growth	Pinkish colour with growth	Pinkish colour with growth
SJ-6	Pinkish yellow with growth	Pinkish colour with growth	Pinkish colour with growth
SJ-7	Pinkish yellow & no growth	Pinkish colour with growth	Pinkish colour with growth
SJ-9	Pinkish yellow & no growth	Pinkish colour with growth	Pinkish colour with growth
SJ-10	Pinkish yellow & no growth	Pinkish colour with growth	Pinkish colour with growth
SJ-13	Pinkish yellow & no growth	Pinkish colour with growth	Pinkish colour with growth
SJ-15	Pinkish colour with growth	Pinkish colour with growth	Pinkish colour with growth
SJ-16	Pinkish colour & no growth	Pinkish colour with growth	Pinkish colour with growth

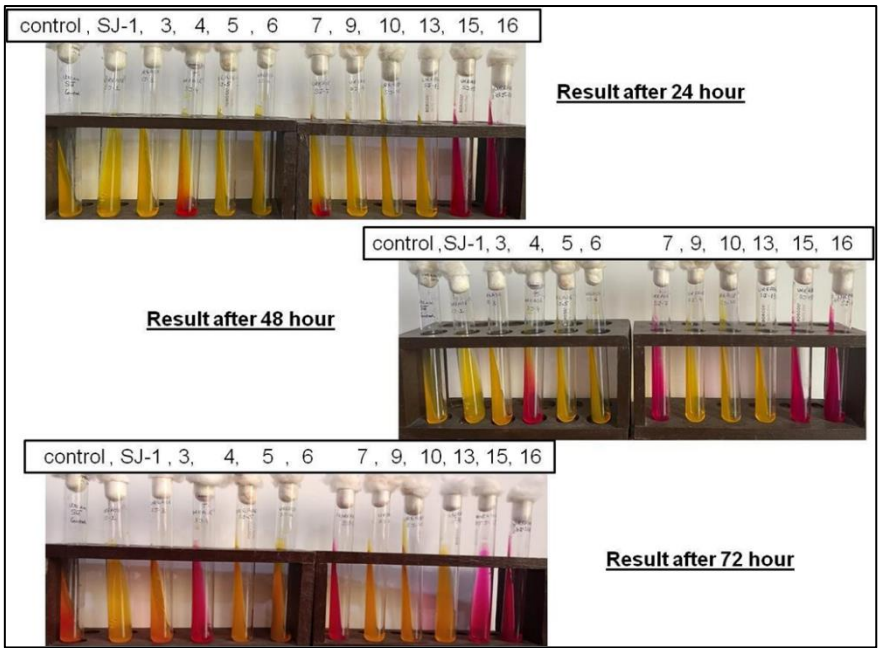


Fig 12: All observed photos for urease test after incubation period

Table 11: Oxidase test

Strain no.	Colony growth at 48 hr.	Change in colour or not	Time duration of colour change	Oxidase result (+ve/-ve)
Control	-ve	Reddish yellow	Few seconds	-ve
SJ-1	+ve	-ve	-	-ve
SJ-3	+ve	Reddish yellow	Few seconds	-ve
SJ-4	+ve	Burgundy colour	5 min.	+ve
SJ-5	+ve	-ve	-	-ve
SJ-6	+ve	Reddish yellow	Few seconds	-ve
SJ-7	+ve	-ve	Few seconds	-ve
SJ-9	+ve	-ve	Few seconds	-ve
SJ-10	+ve	-ve	Few seconds	-ve
SJ-13	+ve	Wine colour	1 min.	+ve
SJ-15	+ve	-ve	-	-ve
SJ-16	+ve	-ve	-	-ve

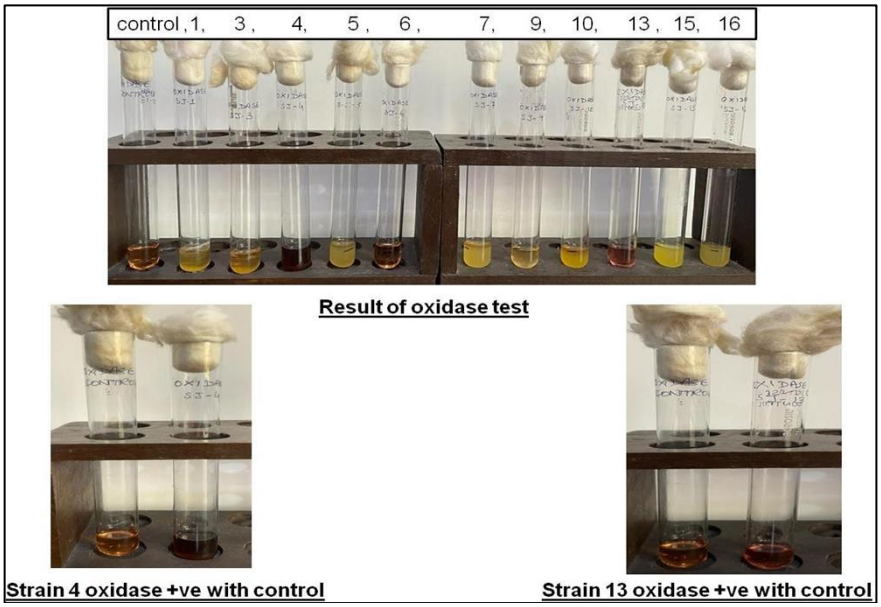


Fig 13: Observed photos of oxidase test

Table 12: Starch hydrolysis test

Strain no.	Growth	Presence of zone of hydrolysis
Control	-ve	-ve
SJ-1	+ve	+ve
SJ-3	+ve	-ve
SJ-4	+ve	+ve
SJ-5	+ve	-ve
SJ-6	+ve	+ve
SJ-7	+ve	+ve
SJ-9	+ve	+ve
SJ-10	+ve	-ve
SJ-13	-ve	-ve
SJ-15	-ve	-ve
SJ-16	+ve	-ve

Fig 14: Before starch hydrolysis test for all strains

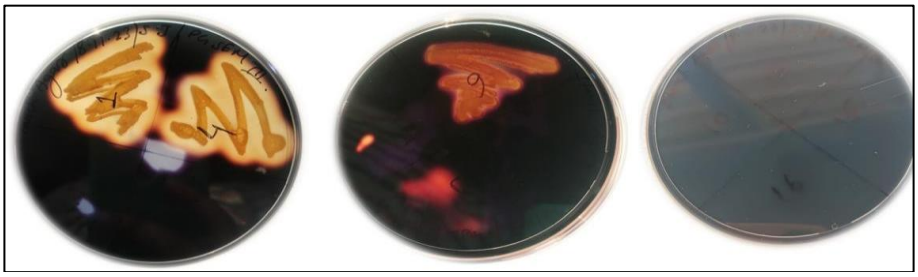


Fig 15: After adding iodine solution on all strains

Table 13: Primary culture for doing MIC test

Strain no.	OD at 600 nm	Culture taken for doing MIC (µl)
-ve control	000	000
+ve control	1.433	100
SJ-1	0.754	200
SJ-3	1.914	100
SJ-4	1.403	100
SJ-5	1.914	100
SJ-6	1.559	100
SJ-7	1.580	100
SJ-9	1.792	100
SJ-10	0.434	200
SJ-13	0.410	200
SJ-15	1.783	100
SJ-16	1.786	100

Table 14: MIC for kanamycin of individual strains

23A : For strain +ve control:		
Concentration(µg/ml)	**OD taken at 600 nm**	
	After 24 hour	After 48 hour
-ve control	000	000
16	1.345	1.758
32	1.103	1.657
64	0.596	1.424
128	0.277	0.531
256	0.252	0.259
512	0.282	0.252
1024	0.174	0.332
2048	0.243	0.343
•Value of MIC- 128 µg/ml		

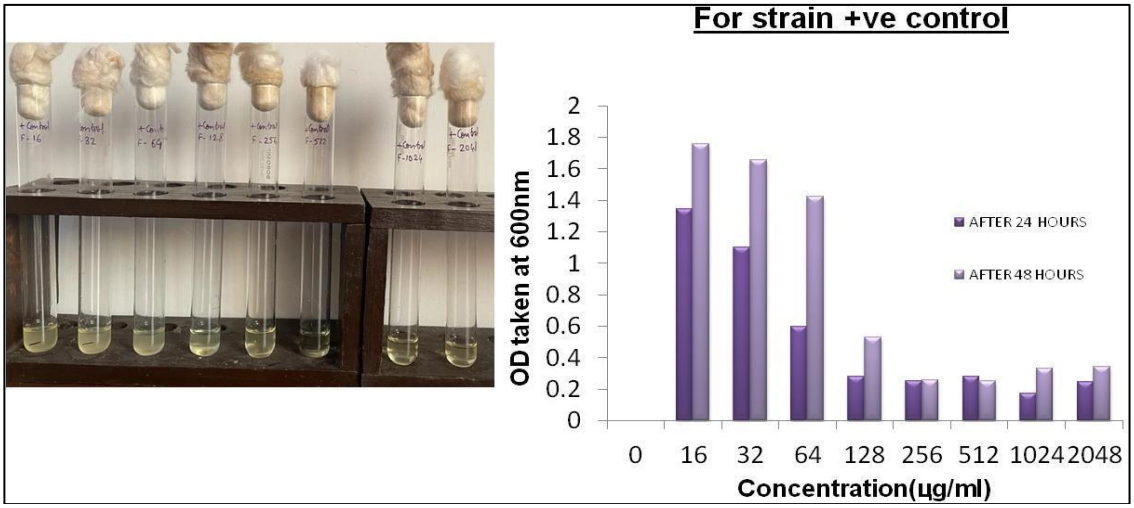


Fig 16: MIC for SJ- +ve vontrol

23B: For strain SJ-1 :		
Concentration(µg/ml)	**OD taken at 600 nm**	
	After 24 hour	After 48 hour
-ve control	000	000
16	0.170	1.727
32	0.250	0.210
64	0.193	0.184
128	0.205	0.181
256	0.154	0.214
512	0.267	0.192
1024	0.192	0.257
2048	0.242	0.290
•Value of MIC- 32 µg/ml		

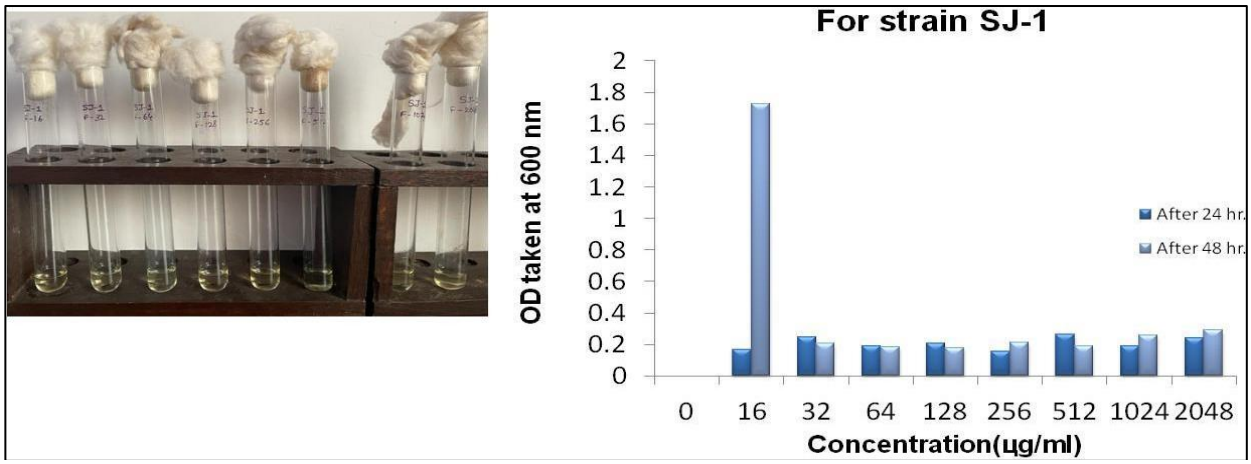


Fig 17: MIC for SJ-1

23C :For strain SJ-3 :

Concentration(µg/ml)	OD taken at 600 nm	
	After 24 hour	After 48 hour
-ve control	000	000
16	1.321	1.710
32	1.072	1.697
64	0.961	1.660
128	0.153	1.660
256	0.111	1.513
512	0.123	1.341
1024	0.166	0.358
2048	0.109	0.103

•Value of MIC- 1024 µg/ml

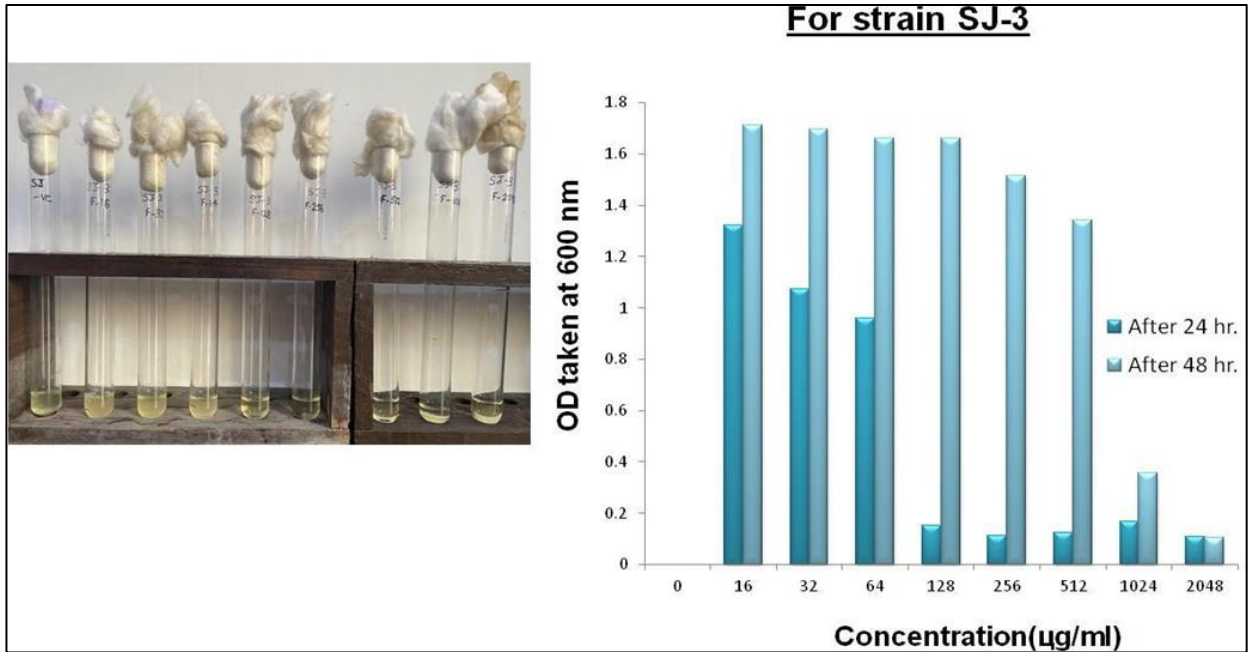


Fig 18: MIC for SJ-3

23D :For strain SJ-4 :

Concentration(µg/ml)	OD taken at 600 nm	
	After 24 hour	After 48 hour
-ve control	000	000
16	1.264	1.755
32	0.835	1.545
64	0.532	1.596
128	0.199	1.614
256	0.136	
512	0.155	1.169
1024	0.147	0.791
2048	0.114	0.082

•Value of MIC- 1024 µg/ml

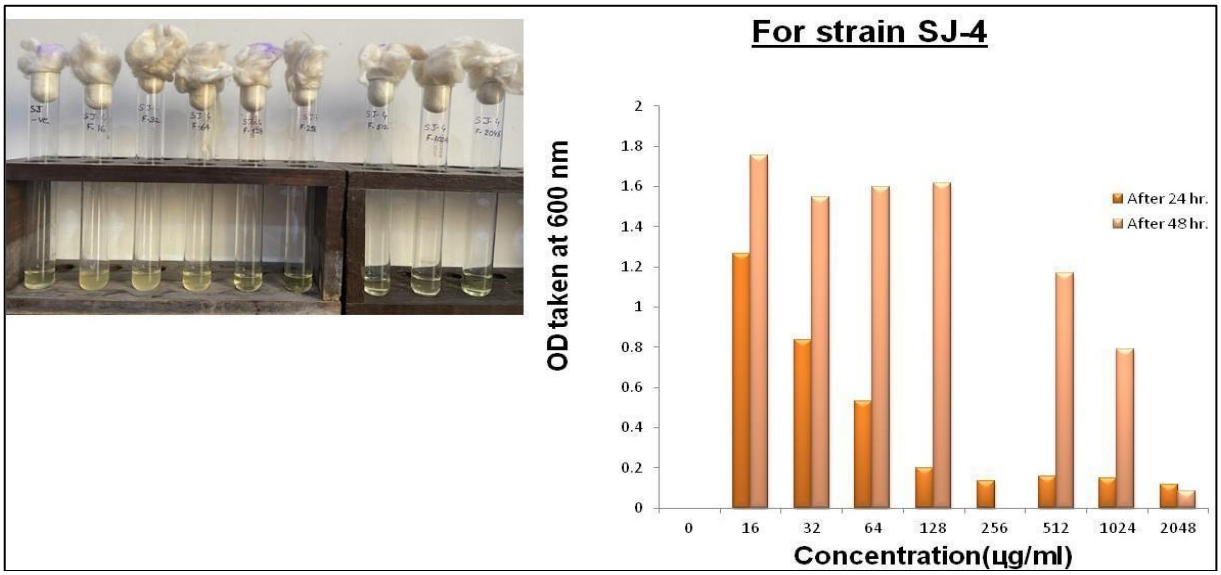


Fig 19: MIC for SJ-4

23E :For strain SJ-5 :

Concentration(µg/ml)	OD taken at 600 nm	
	After 24 hour	After 48 hour
-ve control	000	000
16	1.742	1.628
32	1.760	1.654
64	1.669	1.280
128	1.747	1.570
256	1.757	1.694
512	1.659	1.377
1024	1.761	1.540
2048	1.547	1.466

•Value of MIC- >2048 µg/ml

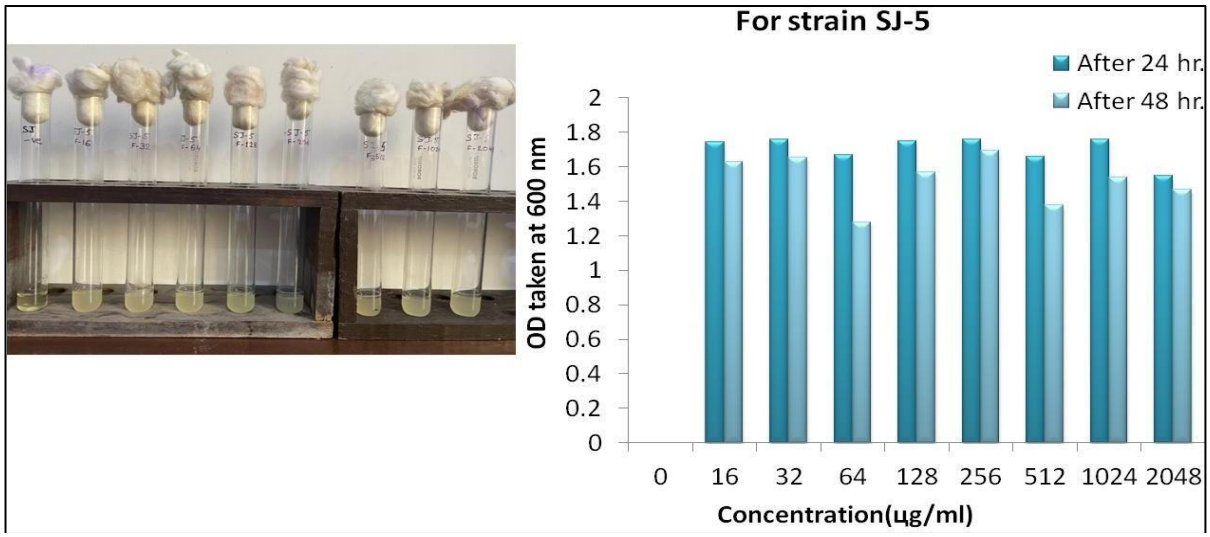
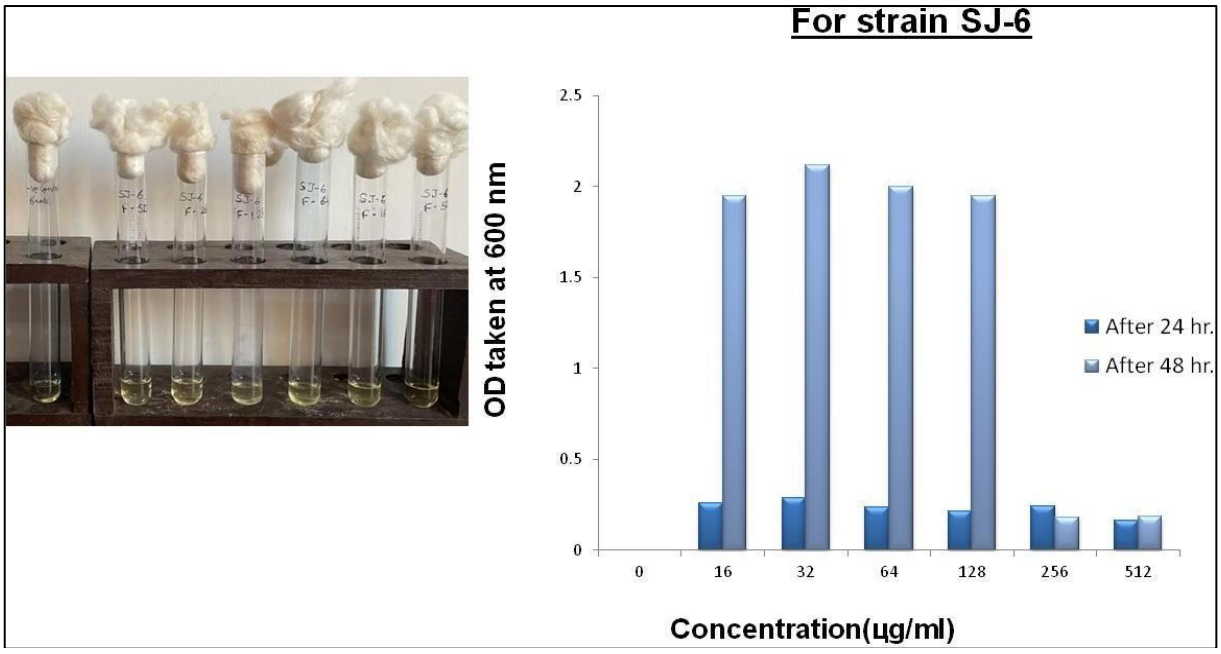


Fig 20: MIC for SJ-5

23F :For strain SJ-6:

Concentration(µg/ml)	OD taken at 600 nm	
	After 24 hour	After 48 hour
-ve control	000	000
16	0.258	1.947
32	0.287	2.116
64	0.234	2.001
128	0.215	1.947
256	0.241	0.179
512	0.161	0.183

•Value of MIC- between 128 & 256 µg/ml



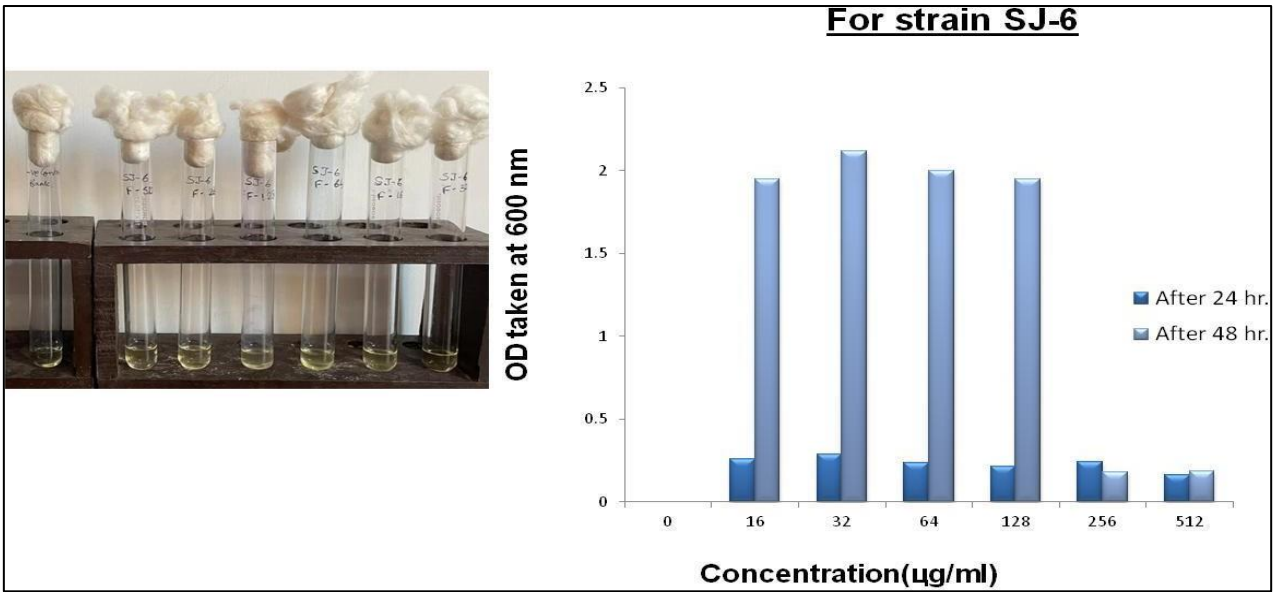


Fig 21: MIC for SJ-6

23G :For strain SJ-7 :

Concentration(µg/ml)	OD taken at 600 nm	
	After 24 hour	After 48 hour
-ve control	000	000
16	0.469	1.634
32	0.224	0.248
64	0.245	0.175
128	0.234	0.283
256	0.216	0.201
512	0.196	0.180

•Value of MIC- 32 µg/ml

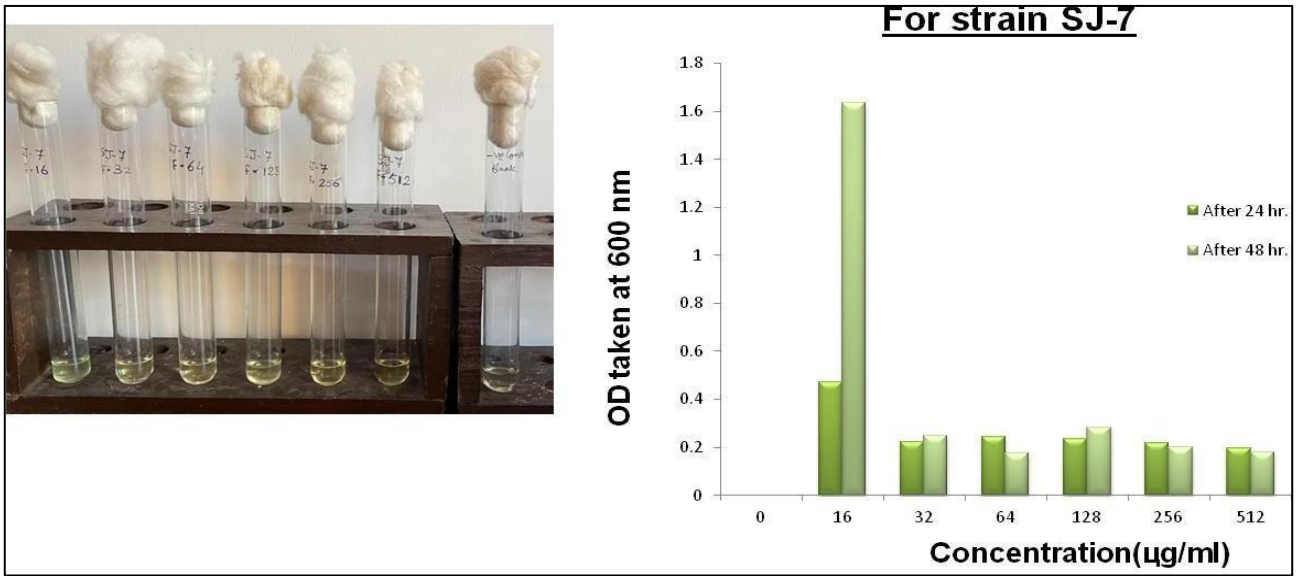


Fig 22: MIC for SJ-7

23H :For strain SJ-9 :

Concentration(µg/ml)	OD taken at 600 nm	
	After 24 hour	After 48 hour
-ve control	000	000
16	1.075	0.844
32	0.254	0.197
64	0.228	0.153
128	0.228	0.230
256	0.208	0.149
512	0.274	0.538

•Value of MIC- 32 µg/ml

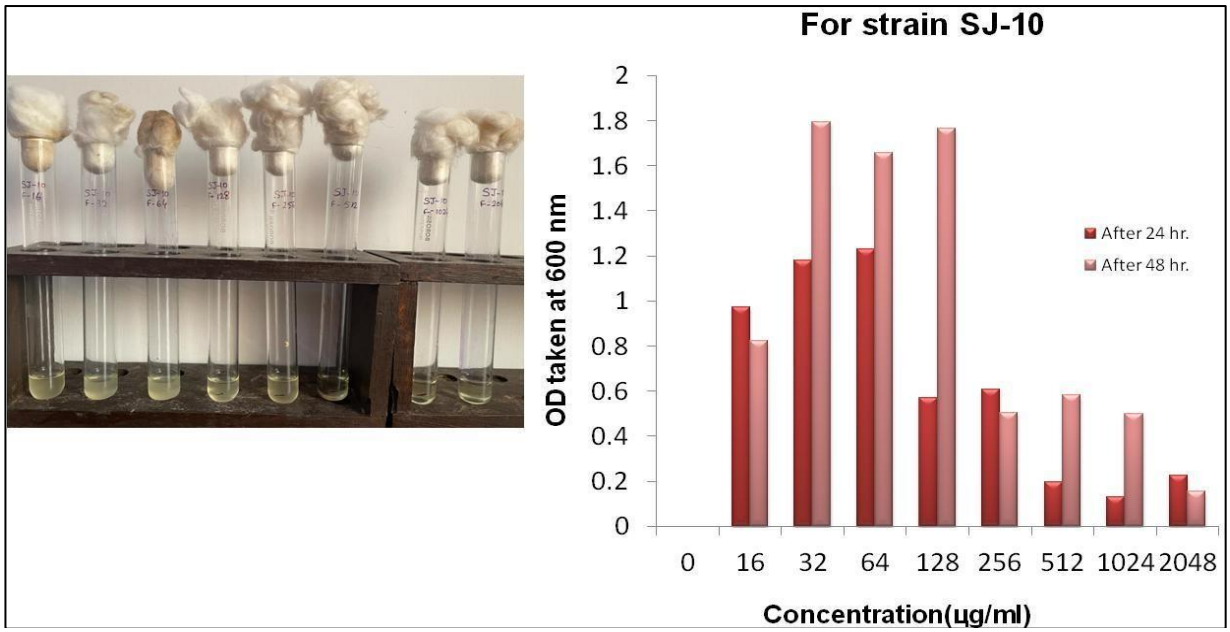


Fig 23: MIC for SJ-10

23J :For strain SJ-13 :

Concentration(µg/ml)	OD taken at 600 nm	
	After 24 hour	After 48 hour
-ve control	000	000
16	0.227	0.197
32	0.188	1.427
64	0.130	0.818
128	0.221	0.203
256	0.092	0.262
512	0.214	0.257
1024	0.151	0.181
2048	0.239	0.174

•Value of MIC- between 64 & 128 µg/ml

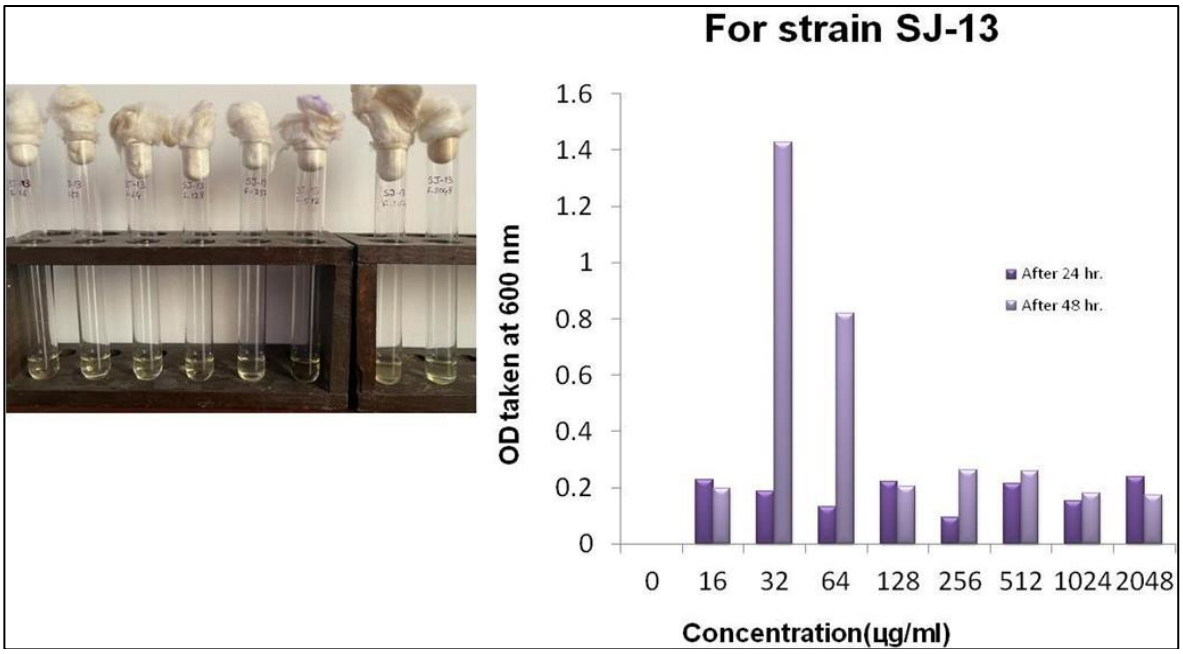


Fig 24: MIC for SJ-13

23K :For strain SJ-15 :

Concentration(µg/ml)	OD taken at 600 nm	
	After 24 hour	After 48 hour
-ve control	000	000
16	0.735	1.712
32	0.599	1.159
64	0.716	1.715
128	1.312	1.105
256	0.494	0.996
512	0.433	0.456
1024	0.250	0.220
2048	0.314	0.328

•Value of MIC- 256 µg/ml

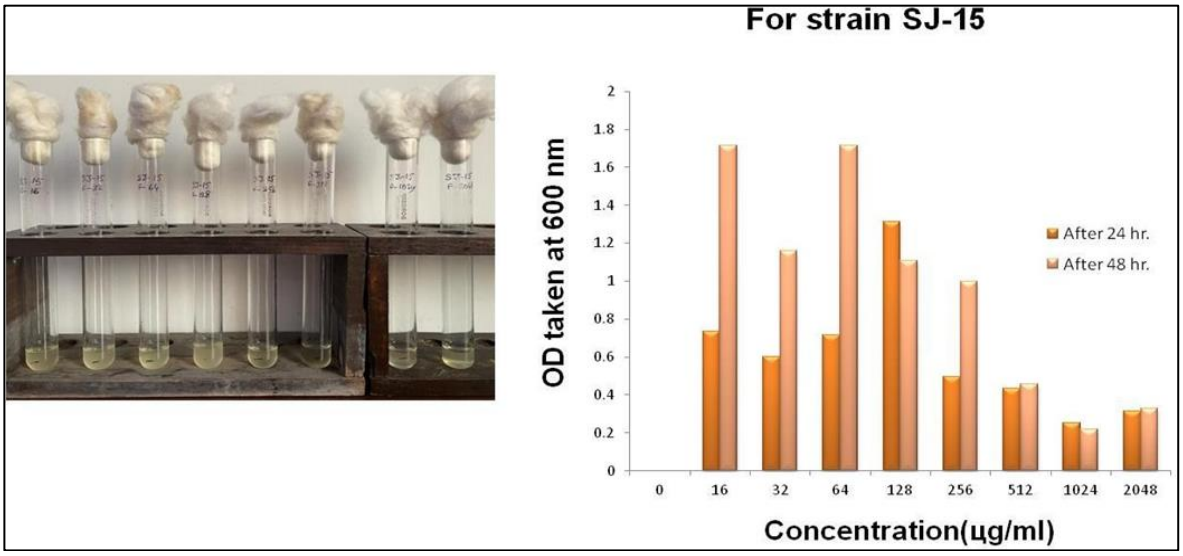


Fig 25: MIC for SJ-15

23L :For strain SJ-16 :

Concentration(µg/ml)	OD taken at 600 nm	
	After 24 hour	After 48 hour
-ve control	000	000
16	1.514	1.827
32	0.701	1.426
64	0.900	1.222
128	0.256	1.066
256	0.216	0.316
512	0.178	0.279
1024	0.285	0.482
2048	0.137	0.226

•Value of MIC- between 128 & 256 µg/ml

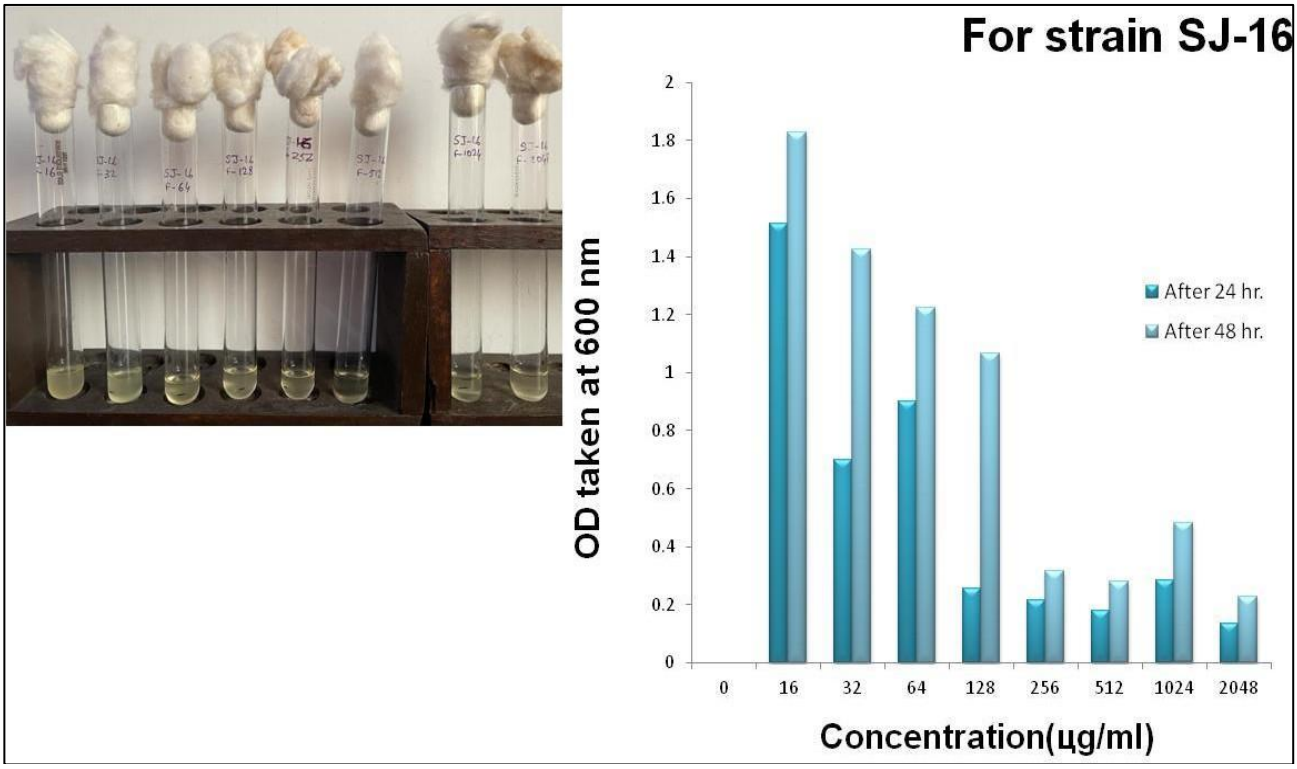


Fig 26: MIC for SJ-16

Conclusion

Air microbiota from laboratory, garbage van, traffic-congested road, and hospital environments were isolated and characterized using TSA and PDA media. Bacterial colonies displayed diverse morphologies, while fungi with white mycelia were mainly observed on PDA. Eleven representative bacterial strains were selected for pure culture establishment. All strains were Gram-positive, mostly cocci, and exhibited variable biochemical profiles: most were catalase- and motility-positive, three strains were identified as *Staphylococcus aureus* on Mannitol Salt Agar, and urease, oxidase, and starch hydrolysis tests further distinguished the isolates. Our tests also suggested that SJ-6, 7 belong to *Bacillus subtilis* or *Bacillus cereus*. SJ-3, 10 were gram positive cocci, non-motile, catalase and oxidase negative, suggesting presence of *Streptococcus sp*., which is a common bacterial species in any air sample. Antibiotic susceptibility testing via MIC revealed varying kanamycin resistance, from 32 µg/ml to >2048 µg/ml.

These results demonstrate the heterogeneity of airborne microbial communities across different environments and provide important insights into their morphological, biochemical, and antibiotic resistance traits. The findings underscore the significance of monitoring air microbiota for environmental and public health management.

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