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Antibiotic Resistance Profile of Amoxicillin-Resistant Bacteria Isolated from Chicken Liver

Saneesh Kumar[1], Priya Mitra[2], Dr. Rupak Roy[3*]

[1]Department of Biotechnology, Shanti Prasad Jain College (A Constituent Unit of Veer Kunwar Singh University), Bihar, India.
[2]SHRM Biotechnologies Pvt. Ltd. West Bengal.
[3]Research Scientist, SHRM Biotechnologies Pvt. Ltd. Email: rupak@shrmbio.com
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ABSTRACT:

Antibiotic resistance results from the genetic capacity of bacteria to encode resistance genes, neutralizing the inhibitory effects of potential antibiotics crucial for their survival. This issue arises when microorganisms, including bacteria and fungi, acquire the ability to withstand drugs designed to eradicate them. The emergence of drug-resistant bacteria, particularly concerning Amoxicillin resistance in bacteria derived from food sources like chicken liver, poses a substantial global health risk. Amoxicillin, extensively used in human and veterinary medicine, is central to this concern. The rapid escalation of antibiotic-resistant bacterial pathogens globally constitutes a pressing health epidemic, with an estimated 700,000 individuals succumbing to the consequences of antimicrobial resistance, a number projected to reach ten million by 2050 worldwide. This surpasses the toll of cancer and road traffic accidents combined. This research investigates the prevalence and characteristics of Amoxicillin-resistant bacteria isolated from chicken liver, analyzing their growth inhibition patterns through antibiotic resistance tests. Six bacteria—*Proteus vulgaris, Vibrio cholerae, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, and Klebsiella oxytoca*—were identified via biochemical tests. Antibiotic susceptibility tests revealed varied efficacy, guiding antibiotic prescription strategies for related diseases.

KEYWORDS: Amoxicillin, Chicken liver, Biochemical identification, MSA, EMB Agar, Antibiotic susceptibility test.

1. INTRODUCTION:

Over the past two decades, the world has grappled with numerous critical challenges, including the energy crisis and environmental pollution (Roy, Debnath, and Ray 2022; Roy and Ray 2022; Roy and Ray 2023). One of the gravest concerns is antibiotic resistance, a phenomenon documented by various researchers (Amber et al. 2024; Parveen et al. 2023). Antibiotics, vital in combating bacterial infections, have a rich historical application, dating back to ancient civilizations like the Egyptians who used molds and plant extracts for medicinal purposes. However, it wasn't until the late 19th century that scientists like Paul Ehrlich observed antibacterial chemicals in action, marking the inception of modern antibiotics (Banik et al. 2023; Roy et al. 2023).

The accidental discovery of penicillin by Alexander Fleming in 1928 revolutionized medicine, earning it the moniker "the wonder drug." Antibiotics play a crucial role in treating bacterial infections, extending to preventive measures for specific populations at risk, such as those with weakened immune systems or undergoing surgery (Li and Roy 2023; Roy 2022). Despite their significant contributions, the misuse and overuse of antibiotics in poultry have contributed to the emergence of antibiotic-resistant bacteria, posing serious threats to both animal and human health (Ghosal et al. 2022).

Antibiotic resistance is now a global public health crisis affecting various sectors, including healthcare, veterinary, and agriculture industries. The misuse of antibiotics in poultry, for instance, has led to the development of resistant bacteria, jeopardizing the effectiveness of these medications and impacting medical procedures (Bashar et al. 2022). Recognizing the gravity of this issue, there is a pressing need to understand the resistance profiles of bacteria, particularly those in food sources like chicken liver. This study aims to investigate the prevalence and characteristics of Amoxicillin-resistant bacteria in chicken liver, emphasizing the potential health risks associated with consuming contaminated poultry products (Vipparla et al. 2022). The research underscores the importance of ongoing efforts to address antibiotic resistance, considering its global implications on public health.

2. MATERIALS AND METHODS:

2.1 Isolation of Bacteria:

Bacterial isolation serves as a fundamental technique for distinguishing various microorganism groups. This method enables the differentiation of bacterial groups by their distinct growth patterns. The isolation of bacteria plays a crucial role in the subsequent identification and classification processes. The

procedure generally encompasses specimen collection, preservation, culturing, and microscopic examination. The process of bacterial isolation can be executed through the utilization of a broad-spectrum medium, facilitating the growth of diverse bacteria, or selective media designed to foster the growth of specific genera. This step is integral in unveiling the diversity within bacterial populations and is essential for comprehensive microbial analysis (Nannipieri 2003).

- ***Materials Required:*** The following list of materials was required to carry out this study. Chicken liver was obtained from local markets. All the glassware was procured from Borosil® (Maharashtra, India). The required chemicals were sourced from Nice® Chemicals Pvt. Ltd. (Kerala, India) which were of analytical grades: Sample (Chicken liver), Petri dishes, Test tubes, Peptone water, Amoxicillin, Stomacher bag, Motor mixture, Selective media, Autoclave, Laminar airflow, Incubator.

2.1.1 Various Media used in this study and their composition:

Table 1: Composition of peptone water.

Components	**Amount (gm/lit.)**
Peptone	10
NaCl	5

Table 2: Composition of EMB Agar.

Components	**Amount (gm/lit.)**
Peptone	10
Lactose	5
Sucrose	5
Dipotassium hydrogen phosphate	2
Eosin Y	0.4
Methylene Blue	0.065
Agar	13.5

Footnote: Final pH (at 25°C): 7.2±0.2

Table 3: Composition of MacConkey Agar.

Components	**Amount (gm/lit.)**
Peptone	17
Protease peptone	3
Lactose monohydrate	10
Bile Salt	1.5
NaCl	5
Neutral Red	0.03
Crystal Violet	0.001
Agar	13.5

Footnote: Final pH (at 25°C): 7.1±0.2

Table 4: Composition of SS Agar.

Components	**Amount (gm/lit.)**
Peptone	5
Beef extract	5

Lactose	10
Bile Salt	8.5
Sodium Citrate	10
Sodium thiosulphate	8.5
Ferric citrate	1
Brilliant green	0.00033
Neutral red	0.025
Agar	15

Footnote: Final pH (at 25°C): 7.0±0.2

Table 5: Composition of Mannitol Salt Agar.

Components	**Amount (gm/lit.)**
Peptone	5
Tryptone	5
Beef extract	1
NaCl	75
D-Mannitol	10
Phenol red	0.025
Agar	20

Footnote: Final pH (at 25°C): 7.4±0.2

Table 6: Composition of CLED Agar.

Components	**Amount (gm/lit.)**
Lactose	10
Peptone	4
Tryptone	4
Beef extract	3
L – cysteine	0.128
Bromothymol blue	0.02
Agar	20

Footnote: Final pH (at 25°C): 7.3±0.2

Table 7: Composition of TCBS Agar.

Components	**Amount (gm/lit.)**
Protease peptone	10
Yeast Extract	5
Sodium thiosulphate	10
Sodium citrate	10
Oxbile (Oxgall)	8
Sucrose	20

NaCl	10
Ferric citrate	1
Bromothymol blue	0.04
Thymol blue	0.04
Agar	15

Footnote: Final pH (at 25°C): 8.6±0.2

Table 8: Composition of Cetrimide Agar.

Components	**Amount (gm/lit.)**
Peptone	10
Magnesium chloride	1.4
Dipotassium sulphate	10
Cetrimide	0.3
Agar	13.6

Footnote: Final pH (at 25°C): 7.2±0.2

Table 9: Composition of Blood agar.

Components	**Amount (gm/lit.)**
Peptone	5
Beef extract	3
NaCl	5
Agar	15

Footnote: Final pH (at 25°C): 7.4±0.2

2.1.2 Procedure:

For the experiment (Sraboni et al. 2021), a 25 ml peptone water solution was autoclaved. 1g of the sample was then added and crushed in a stomacher bag with a motor mixer. After mixing, 250 µl of Amoxicillin (10mg/ml stock, 100 µg/ml working) was added. The mixture was incubated at 37°C overnight, following the formula C1V1 = C2V2 for precise measurements. Various selective media were prepared, and the sample was serially diluted (up to 10^-5), spread on media, and incubated. Colony morphology was observed post-incubation, and streaking on nutrient agar was done for further analysis.

2.2 Biochemical identification of bacteria

2.2.1 Gram Staining

Gram staining is a staining technique employed for the categorization of bacterial species into two major groups: Gram-positive bacteria and Gram-negative bacteria. Originally developed by Danish bacteriologist Hans Christian Gram in 1884, this method distinguishes bacteria based on the unique chemical and physical characteristics of their cell walls (Brown et al. 1973).

Materials required:

Bacterial culture; Glass slide; Distilled water; Inoculation loop; Bunsen burner; Crystal violet; Gram's iodine; Acetone (or alcohol); Safranin (0.5% w/v); Microscope.

Table 10: Composition of Crystal Violet Dye.

Components	**Amount**
Crystal violet	2 gm
95% Ethyl alcohol	20 ml

Ammonium oxalate	0.8 gm
Distilled water	80 ml

Table 11: Composition of Gram's iodine.

Components	**Amount**
Iodine	1 gm
Potassium iodide	2 gm
Distilled water	300 ml

Table 12: Composition of Safranin dye.

Components	**Amount**
Safranin powder	5 gm
Ethyl alcohol	1 litre

Procedure:

Glass slides were thoroughly cleaned, and bacterial smears were air-dried before gentle heat fixation. Crystal violet was applied for 30 seconds, followed by Gram's iodine. After a 1-minute iodine act, slides were rinsed with tap water and alcohol. Safranin counterstain was applied for 30 seconds, followed by another tap water rinse. Prepared slides were examined under a 1000X magnification compound microscope using immersion oil.

2.2.2 Indole test:

The indole test distinguishes Enterobacteriaceae by evaluating their ability to break down tryptophan enzymatically, producing indole. Detection is achieved with Kovacs' or Ehrlich's reagent, resulting in a red-colored compound (Carter et al. 1990).

Materials required:

Bacterial culture; Test tubes; Test tube rack; Conical flask; Tryptone broth; Kovacs' reagent; Pipette; Bunsen burner; Inoculation loop; Autoclave; Incubator; Laminar air flow

Media composition:

Table 13: Composition of Tryptone Broth.

Components	**Amount (gm/ lit)**
Tryptone	10
NaCl	5

Table 14: Composition of Kovac's reagent.

Components	**Amount**
Para-dimethylaminobenzaldehyde	5 gm
Amyl alcohol	75 ml
HCl	25 ml

Procedure:

Tryptone broth was prepared, dispensed into 5 ml test tubes, and autoclaved. After cooling, bacterial strains were inoculated, with one tube serving as a control. After 24 hours of incubation at 37°C, Kovacs' reagent was added, and positive results were identified by a purple-colored ring formation.

2.2.3 Methyl Red and Voges Proskauer (MR-VP) Test:

The test distinguishes between mixed acid and 2,3-butanediol fermentation pathways for glucose utilization. Methyl red detects organic acids in the mixed acid pathway, while Voges-Proskauer, using alpha-naphthol and KOH, identifies acetoin, a precursor of 2,3-butanediol (Kennes et al. 1991).

Materials required:

MR-VP broth; Test tubes; Test tube rack; Conical flask; Inoculation loop; Bunsen burner; Barritt's reagent A (5% alpha- naphthol dissolved in absolute alcohol); Barritt's reagent B (40% KOH dissolved in distilled water); Methyl red indicator (0.02%); Autoclave; Incubator; Laminar air flow.

Media composition:

Table 15: Composition of MR-VP Broth.

Components	**Amount (gm/ lit)**
Peptone	7
Dextrose	5
Dipotassium phosphate	5

Footnote: Final pH set at 6.9

Table 16: Composition of MR indicator:

Components	**Amount**
Methyl red	0.1 gm
Ethyl alcohol	300 ml
Dipotassium phosphate	200 ml

Procedure:

MR-VP broth was autoclaved, inoculated with bacteria, and incubated at 37°C for 24 hours. The MR test used methyl red, and the VP test used Barritt's reagents A and B. Positive results showed a red color.

2.2.4 Citrate test:

Citrate agar evaluates an organism's ability to use citrate as an energy source. Growth indicates citrate utilization in the Krebs cycle, causing a pH shift that turns the bromothymol blue indicator from green to blue. (Atlas et al. 2011).

Materials required:

Bacterial culture; Simmons citrate agar; Test tubes; Conical flask; Inoculation loop; Bunsen burner; Autoclave; Incubator; Laminar air flow.

Media composition:

Table 17: Composition of Simmons Citrate Media.

Components	**Amount (gm/lit)**
Magnesium Sulphate	0.2
Ammonium dihydrogen phosphate	1
Dipotassium phosphate	1
Sodium citrate	2
Sodium chloride	5
Bromothymol blue	0.8
Agar	15

Procedure:

A Simmons citrate agar solution was autoclaved, poured into test tubes, and inoculated with bacterial strains. After 24 hours of incubation at 37°C, a color change from green to blue indicated a positive result.

2.2.5 Catalase test:

Certain bacteria generate hydrogen peroxide and superoxide as byproducts of oxygen reduction, posing a threat to cellular components. Survival requires defense mechanisms, often involving catalase enzymes that break down hydrogen peroxide. Catalase production is assessed by introducing H_2O_2 to a culture incubated for 18-24 hours (Mishra et al. 2012).

Materials required:

Bacterial culture; Glass slides; 3% H_2O_2; Inoculation loop; Bunsen burner; Pipette.

2.2.6 Oxidase test:

The oxidase test is vital for distinguishing Pseudomonadaceae from Enterobacteriaceae, identifying bacteria relying on oxygen for respiration. Gordon-McLeod reagent detects oxidase enzyme, turning dark purple upon oxygen interaction.

Materials required:

Bacterial culture; Gordon-McLeod reagent (oxidase reagent); Filter paper; Pipette; Inoculation loop; Bunsen burner.

Procedure:

1-2 drops of the oxidase reagent were applied to the filter paper. Bacterial culture straight lines were drawn using an inoculation loop. Observe the color change; a positive result is indicated by the appearance of a purple color, while a negative result is denoted by the absence of the color change.

2.2.7 Gelatin hydrolysis test:

Gelatin hydrolysis test identifies gelatinase presence in organisms, causing liquefaction of nutrient gelatin medium (Liu et al. 2015).

Materials required:

Bacterial culture; Gelatin media; Test tubes; Conical flask; Inoculation loop; Bunsen burner; Autoclave; Incubator; Lamina air flow.

Media composition:

Table 18: Composition of gelatin media.

Components	**Amount (gm/lit)**
Gelatin	120
Peptone	5
Yeast extract	3

Procedure:

Gelatin media were autoclaved, poured into test tubes, and inoculated with bacteria. After 72 hours of incubation at 37°C, refrigeration differentiated positive (liquid) and negative (solid) results.

2.2.8 Urea hydrolysis test:

Urea hydrolysis produces ammonia and CO2, increasing medium alkalinity. Phenol red color shift, from orange (pH 6.8) to pink (pH 8.1), identifies rapid urease-positive organisms turning the medium pink within 24 hours. (Mehdi et al. 2019).

Materials required:

Bacterial culture; Urease media; Test tubes; Conical flask; Inoculation loop; Bunsen burner; Autoclave; Incubator; Laminar air flow.

Media composition:

Table 19: Composition of urease media.

Components	**Amount (gm/lit)**
Peptone	1
Dextrose	1

NaCl	5
Potassium dihydrogen phosphate (monobasic)	2
Urea	20
Phenol red	0.012
Agar	20

Footnote: Final pH was adjusted to 6.8±0.2.

Procedure:

Urease media, autoclaved and containing urea, were inoculated with bacteria and incubated. After 24 hours at 37°C, a color change from yellow to pink indicated a positive result.

2.2.9 Starch hydrolysis test (Amylase test):

The starch hydrolysis test assesses the organism's ability to break down starch into maltose via the action of the extracellular alpha-amylase enzyme. Starch, a crucial carbohydrate source for humans, constitutes a polysaccharide mixture comprising two polymers, amylose and amylopectin (Sudan et al. 2018).

Materials required:

Bacterial culture; Petri plates; Conical flask; Starch media; Inoculation loop; Bunsen burner; Iodine solution; Autoclave; Incubator; Laminar air flow

Media composition:

Table 20: Composition of starch media.

Components	**Amount (gm/lit)**
Starch	2
Peptone	5
Yeast extract	3
Agar	20

Procedure:

Starch media, autoclaved and poured onto petri plates, were inoculated and incubated at 37°C for 24 hours. Transparent colonies after iodine application indicated a positive result in the starch hydrolysis test.

2.2.10 Casein hydrolysis:

The casein hydrolysis test identifies milk-thriving microorganisms and distinguishes Bacillaceae, Enterobacteriaceae, and other bacterial families. It aids in identifying aerobic actinomycetes using casein, the primary milk protein (Kannan et al. 2012).

Materials required:

Bacterial culture; Casein media; Petri plates; Conical flask; Inoculation loop; Bunsen burner; Autoclave; Incubator; Laminar air flow.

Media composition:

Table 21: Composition of casein media.

Components	**Amount (gm/lit)**
Skim milk powder	28
Tryptone	5
Yeast extract	2.5
Dextrose	1
Agar	20

Procedure:

Casein media, autoclaved and poured onto petri plates, were inoculated and incubated at 37°C for 24 hours. Clear zones around growth indicated a positive casein hydrolysis test; otherwise, it was negative.

2.2.11 H_2S production and motility test:

Hydrogen sulfide is essential for bacteria reducing sulfur compounds. Bacterial motility, observed in semi-solid agar like SIM medium, is assessed for sulfur reaction, indole production, and motility (Fors et al. 2008).

Materials required:

Bacterial culture; Test tubes; Conical flask; SIM media; Inoculation loop; Bunsen burner; Autoclave; Incubator; Laminar air flow.

Media composition:

Table 22: Composition of SIM Media.

Components	**Amount (gm/lit)**
Peptone	20
Beef extract	6
Sodium thiosulphate	0.3
Ferric ammonium citrate	0.2
Agar	3.5

Procedure:

SIM media, autoclaved and inoculated with bacteria, were incubated at 37°C for 24 hours. Results were observed, with H2S production indicated by black colonies and motility by bacterial spread.

2.2.12 Carbohydrate fermentation:

The carbohydrate fermentation test assesses a bacterium's ability to ferment a specific carbohydrate, producing organic acids that lower the medium's pH (Gibson et al. 2004).

Materials required:

Bacterial culture; Carbohydrate fermentation broth; Test tubes; Conical flask; Inoculation loop; Bunsen burner; Autoclave; Incubator; Laminar air flow.

Media composition:

Table 23: Composition of carbohydrate fermentation broth.

Components	**Amount (gm/lit)**
Peptone	10
Beef extract	1
NaCl	5
Phenol red	0.025
Carbohydrate	10

Procedure:

Fermentation broths, autoclaved and inoculated with bacteria, were incubated at 37°C for 24 hours. A positive result was indicated by a red-to-yellow color change in the media.

2.3 Antibiotic susceptibility test:

Susceptibility testing determines effective antimicrobial agents against bacteria or fungi causing an infection. The widely adopted 'Disk diffusion technique' involves placing antibiotic disks on inoculated agar plates for assessment. (Berkow et al. 2020).

Materials required:

Bacterial culture broth; Petri plates; Conical flask; Nutrient agar media; Cotton swab; Antibiotic disk; Autoclave; Incubator; Laminar air flow.

Media composition:

Table 24: Composition of nutrient agar media.

Components	**Amount (gm/lit)**
Peptone	5
Beef extract	3
NaCl	5
Agar	20

Procedure:

Nutrient agar media were autoclaved and dispensed onto Petri plates. Bacterial strains were inoculated, and antibiotic disks were placed on the media. After 24 hours of incubation at 37°C, the zone of inhibition was observed.

4. RESULTS:

4.1 Bacterial Isolation:

Figure 1: Bacterial growth on MacConkey Agar

Figure 2: Bacterial growth on Mannitol Salt Agar (MSA)

Figure 5: Bacterial growth on blood Agar

Figure 6: Bacterial growth on Cetrimide Agar

Figure 3: Bacterial growth on SS Agar

Figure 4: Bacterial growth on TCBS Agar (MSA)

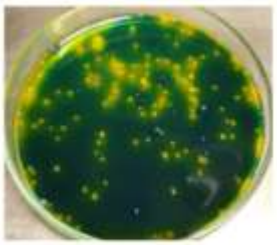


Figure 7: Bacterial growth on CLED Agar

Figure 8: Bacterial growth on EMB Agar

Based on morphology of bacterial cells grown on the SS agar, two different types of colonies were inoculated namely- SS1 & SS2.

Table 1: Biochemical identification of bacteria.

2. Biochemical identification of bacteria

Table 25: Biochemical tests (V = Variable result)

Tests \ Plates	SS1	SS2	EMB	Mac Conkey	Cetrimide	TCBS	CLED	MSA	Blood
G-Staining	-	-	-	-	-	-	-	+	-
Shape	Short rod	Short rod	Short rod	Short rod	Rod	Comma	Short rod	Round	Short rod
Indole	+	+	+	+	-	+	+	-	+
Citrate	-	+	-	+	+	+	V -	+	-
Mr	+	-	+	-	-	-	+	+	+
Vp	-	+	-	+	-	V +	-	+	-
Catalase	+	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	+	+	-	-	-
Gelatin	-	-	-	-	+	+	+	+	-
Urease	-	+	-	+	-	-	+	+	-
Amylase	-	-	-	-	+	+	-	-	-
Protease	-	-	-	-	+	+	-	-	-
H_2S	-	-	-	-	-	-	+	-	-
Motility	+	-	+	-	+	+	+	-	+
Dextrose	+	+	+	+	-	+	+	+	+
Lactose	+	+	+	+	-	V +	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	-	+	+	+	+
Sucrose	+	+	+	+	-	+	+	+	+

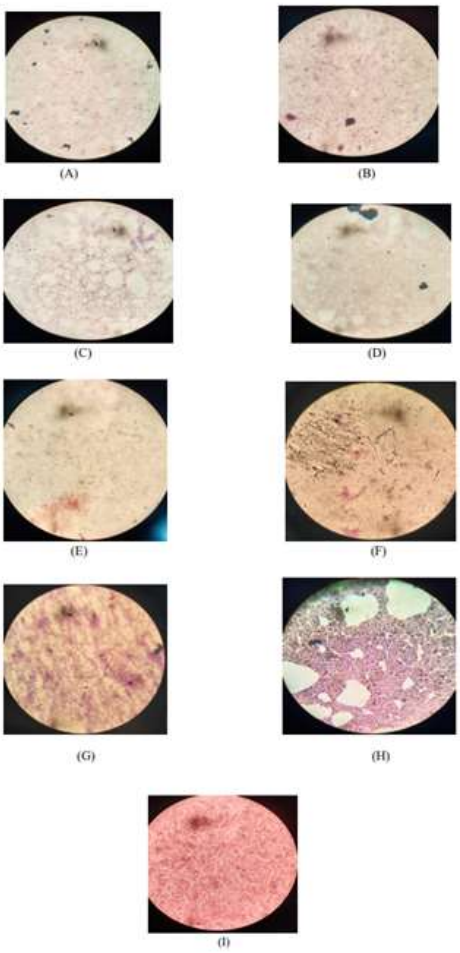


Figure 9: Gram staining of bacteria grown on different selective media (A = SS1, B = SS2, C = EMB, D = Mac Conkey, E = Cetrimide, F = TCBS, G = CLED, H = MSA & I = Blood). For biochemical test two different colonies from SS agar were taken, which were represented by SS1 & SS2.

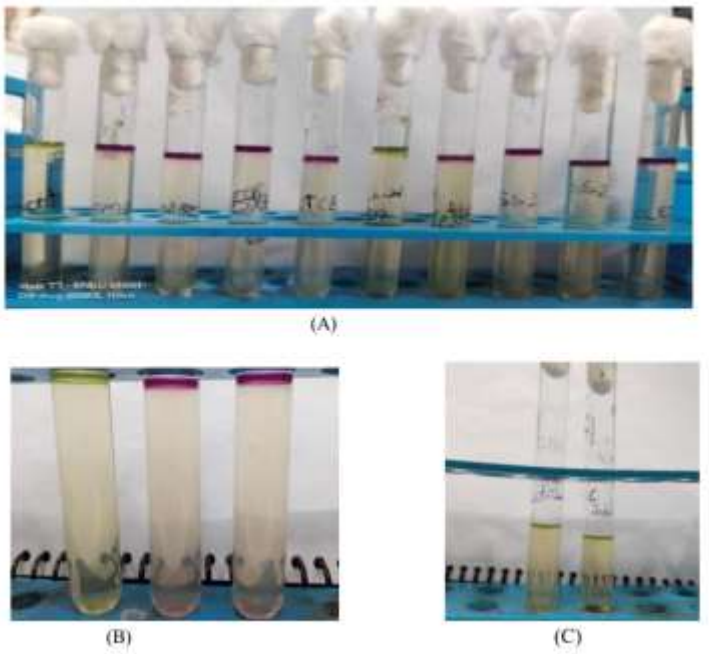


Figure 10: Indole test of bacteria (B & C tests are repeated for SS2 and Mac Conkey & Cetrimide respectively).

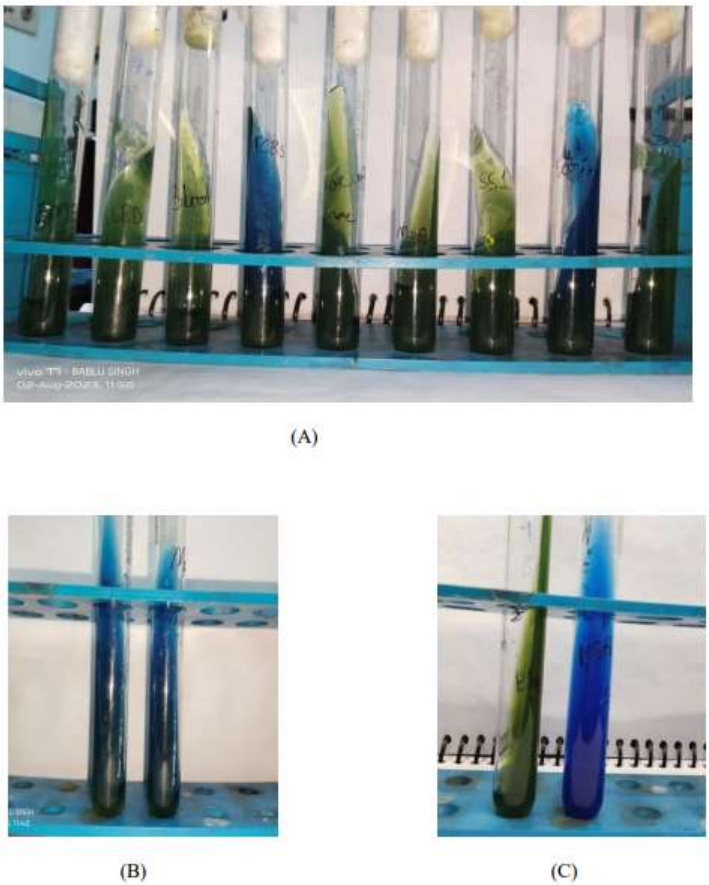


Figure 11: Citrate test of bacteria (B & C tests are repeated for SS2 and Mac Conkey & MSA respectively).

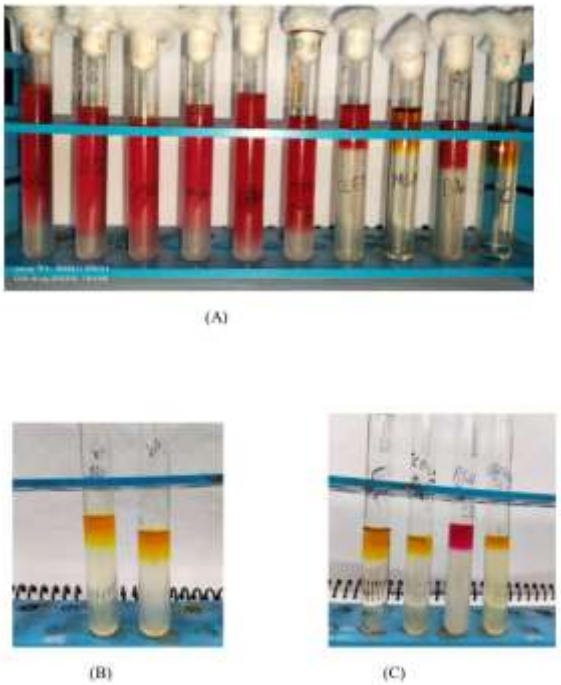


Figure 12: Methyl red test of bacteria (B & C tests are repeated for SS2 and Mac Conkey & TCBS, MSA and Cetrimide respectively).

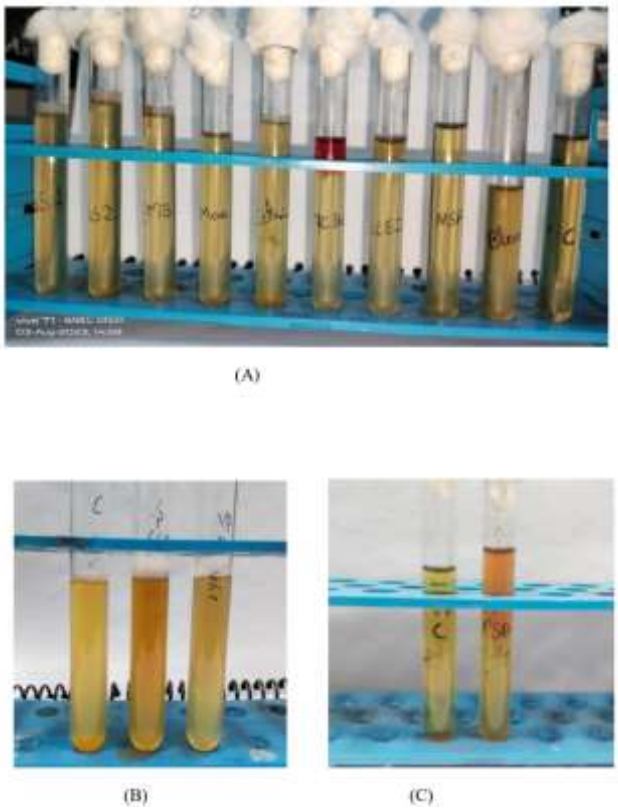


Figure 13: Voges-Proskauer test of bacteria (B & C tests are repeated for SS2 and Mac Conkey & MSA respectively).

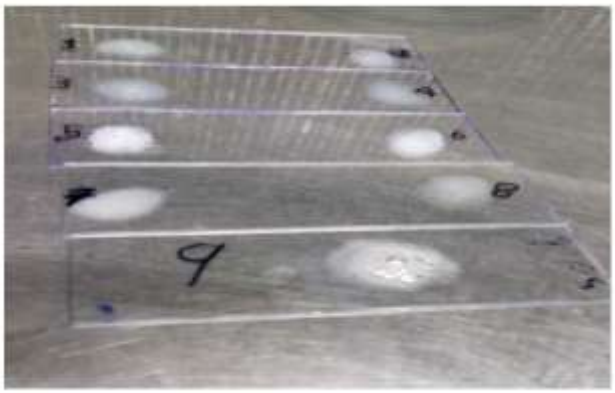
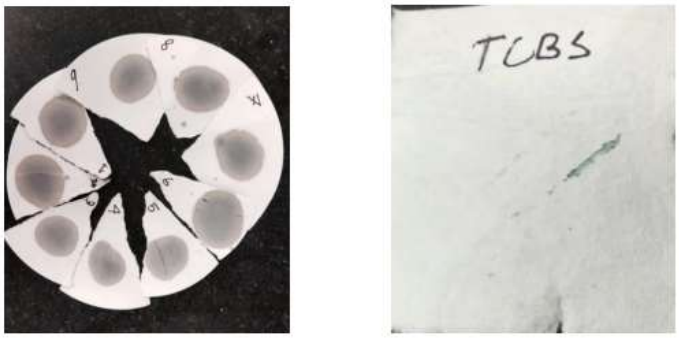
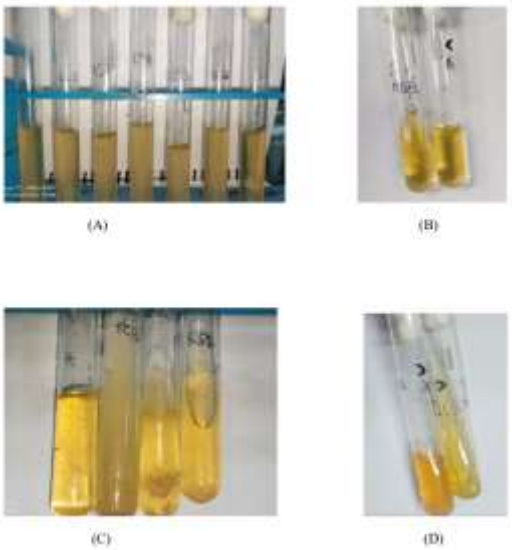


Figure 14: Catalase test of bacteria.



(A) (B)

Figure 15: Oxidase test of bacteria (B test is repeated for TCBS using TEMED).



(A) (B)

(C) (D)

Figure 16: Gelatin test of bacteria (A - negative result, C - positive result, and B & D tests are repeated for MSA and SS1 & CLED respectively).

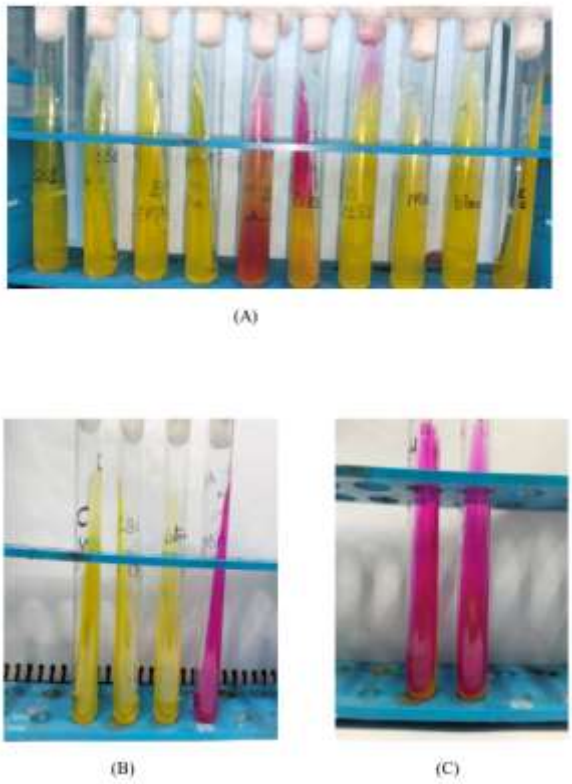


Figure 17: Urease test of bacteria (B & C tests are repeated for TCBS, Cetrimide, and MSA & SS2 and Mac Conkey).

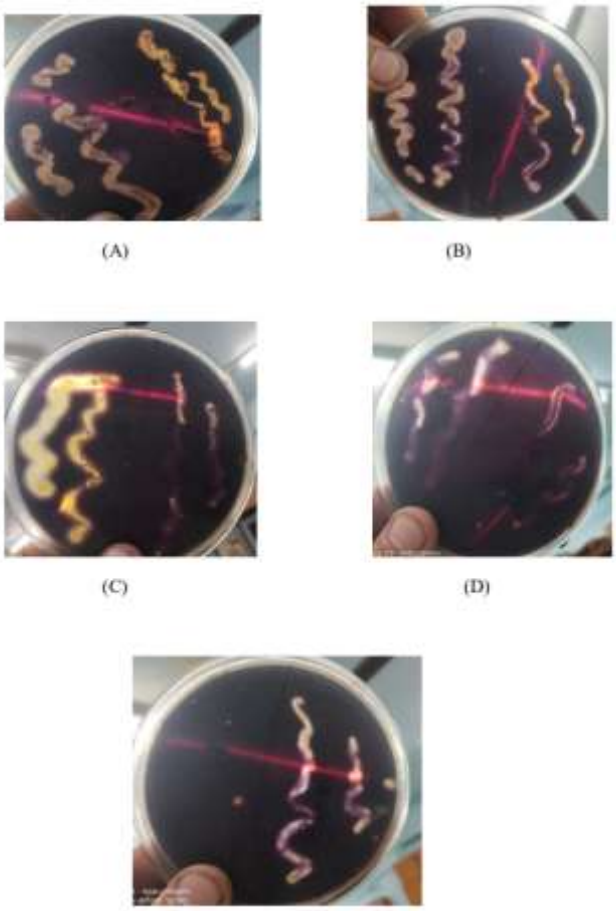
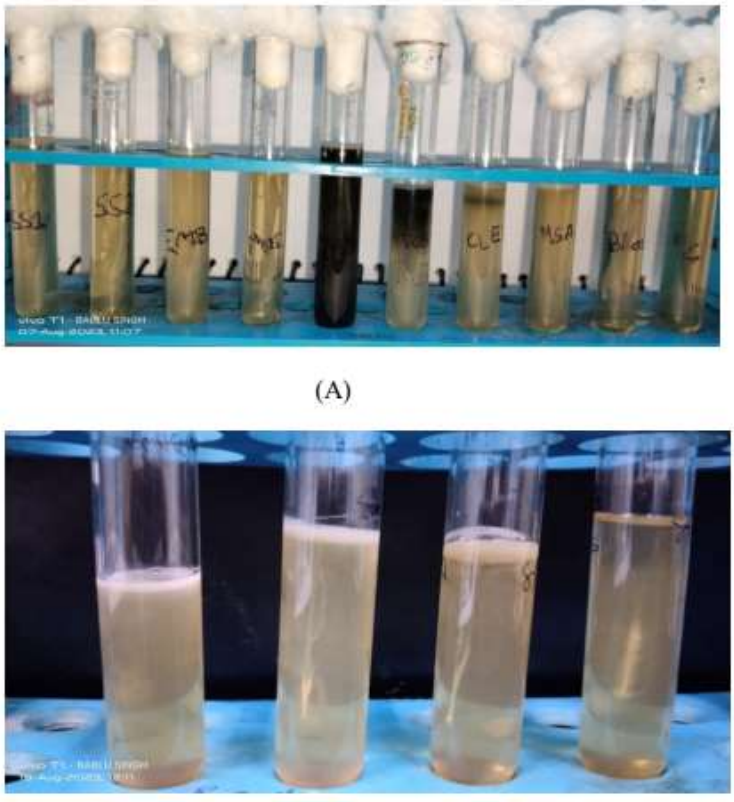


Figure 18: Amylase test of bacteria (A = SS1 & SS2, B = EMB & Mac Conkey, C = Cetrimide & TCBS, D = CLED & MSA, and E = Blood).

Figure 19: Protease test of bacteria.



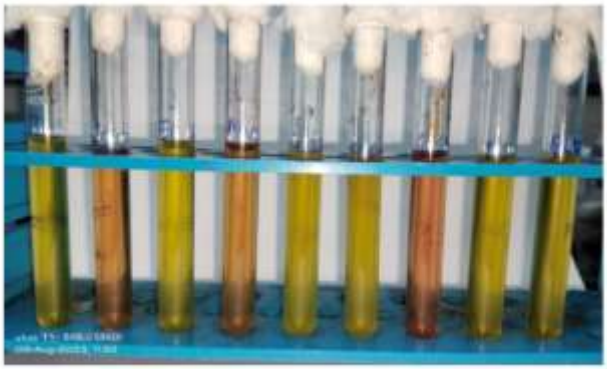
(A)

(B)

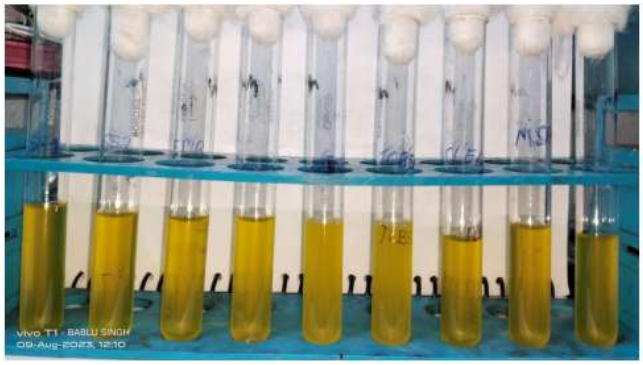
Figure 20: H_2S & Motility test (SIM Media) of bacteria (B test is repeated for Cetrimide, TCBS, MSA, and Blood).

(A)

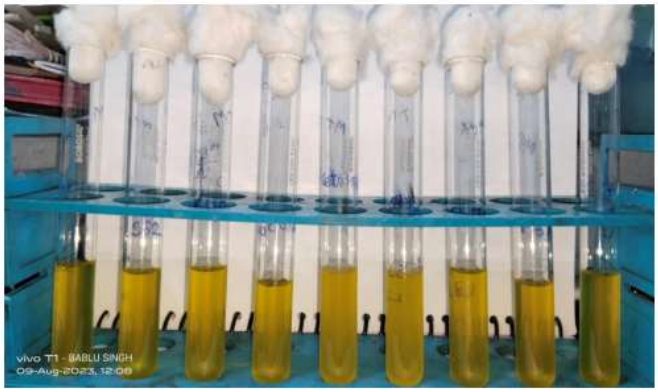
(B)



(C)


vivo T1 - BABLU SINGH
09-Aug-2023, 12:10

(D)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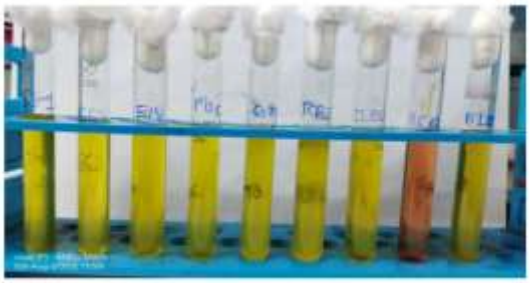
(E)



(F)

(G)

Figure 21: Carbohydrate fermentation (A - Dextrose, B – Control, C – Lactose, D – Mannitol, E – Maltose, F – Sucrose and G test is repeated for Cetrimide).

Based on these biochemical test results, total six bacteria were identified which are *Escherichia Coli, Klebsiella oxytoca, Staphylococcus aureus, Pseudomonas aeruginosa, Vibrio cholerae,* and *Proteus vulgaris*.

4.2 Antibiotic Susceptibility Test:

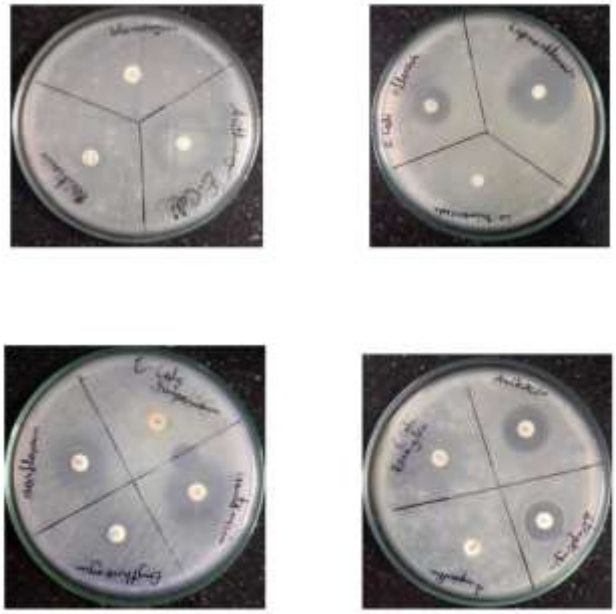


Figure 22: Antibiotic susceptibility test of *E. Coli*.

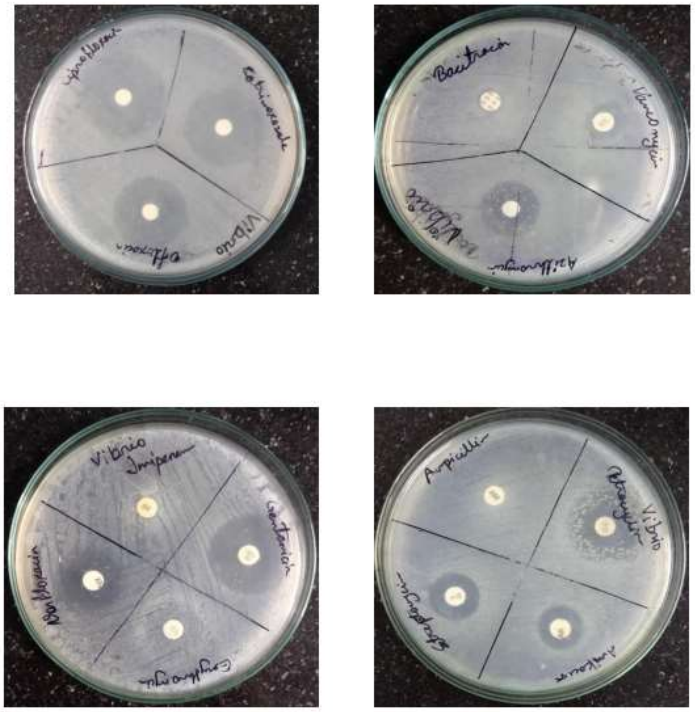


Figure 23: Antibiotic susceptibility test of *Klebsiella oxytoca*.

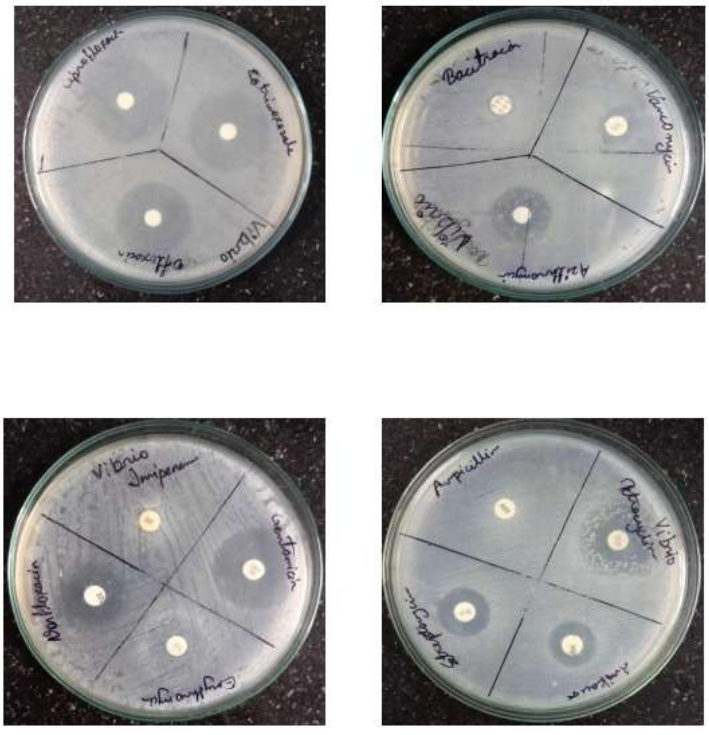


Figure 24: Antibiotic susceptibility test of *Vibrio cholerae*.

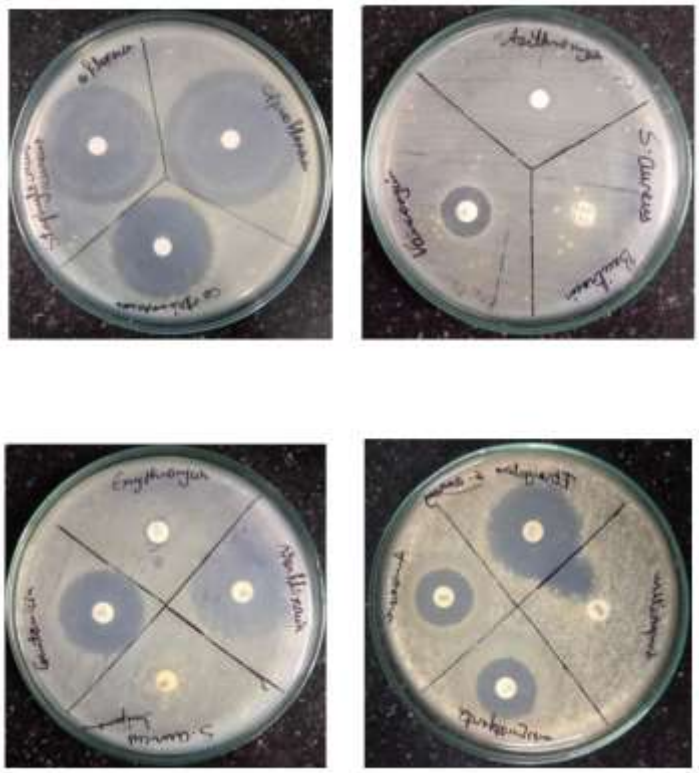


Figure 25: Antibiotic susceptibility test of *Staphylococcus aureus.*

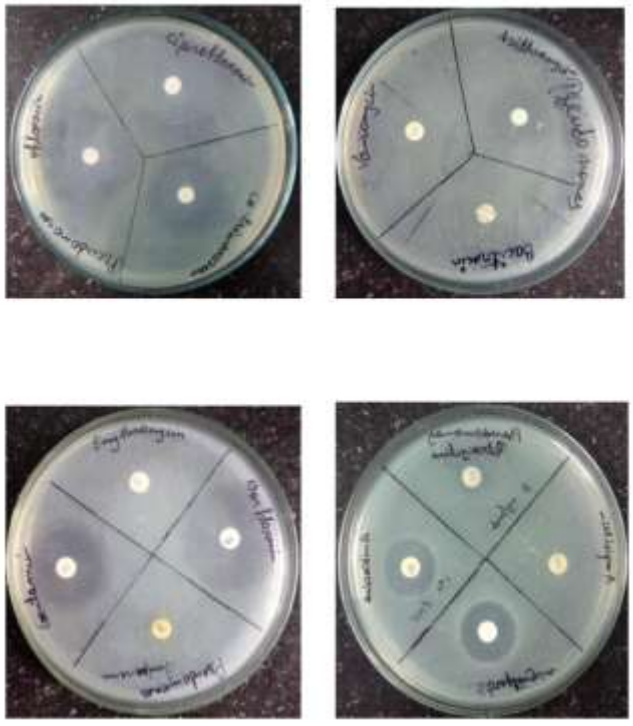


Figure 26: Antibiotic susceptibility test of *Pseudomonas aeruginosa*.

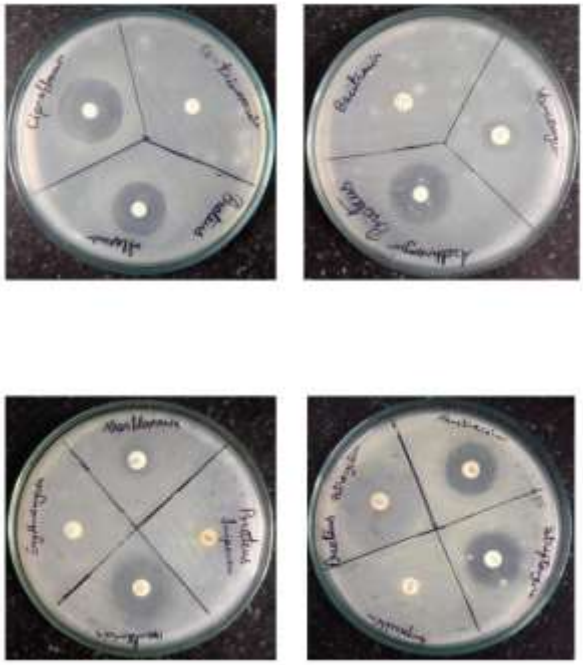


Figure 27: Antibiotic susceptibility test of *Proteus vulgaris*.

Table 2: Antibiotic susceptibility test:

Antibiotics \ Bacteria		*E. Coli*	*Klebsiella oxytoca*	*Vibrio cholerae*	*Staphylococcus aureus*	*Proteus vulgaris*	*Pseudomonas aeruginosa*
Tetracyclin (10 µg/disk)	Diameter of zone of inhibition	No zone of inhibition	No zone of inhibition	1.5 cm	3.55 cm	No zone of inhibition	No zone of inhibition
	Diameter of anti-biotic disk	0.6 cm	0.6 cm	0.6 cm	0.6 cm	0.6 cm	0.6 cm
	Final diameter	NA	NA	9 mm	29.5 mm	NA	NA
Amikacin (30 µg/disk)	Diameter of zone of inhibition	1.9 cm	1.7 cm	1.5 cm	1.9 cm	1.9 cm	1.7 cm
	Diameter of anti-biotic disk	0.6 cm	0.6 cm	0.6 cm	0.6 cm	0.6 cm	0.6 cm
	Final diameter	13 mm	11 mm	9 mm	13 mm	13 mm	11 mm
Streptomycin (25 µg/disk)	Diameter of zone of inhibition	1.6 cm	2.0 cm	1.7 cm	1.9 cm	2.0 cm	1.9 cm
	Diameter of anti-biotic disk	0.6 cm	0.6 cm	0.6 cm	0.6 cm	0.6 cm	0.6 cm
	Final diameter	10 mm	14 mm	11 mm	13 mm	14 mm	13 mm
Gentamycin (50 µg/disk)	Diameter of zone of inhibition	2.5 cm	2.2 cm	2.6 cm	2.5 cm	2.2 cm	2.5 cm
	Diameter of anti-biotic disk	0.6 cm	0.6 cm	0.6 cm	0.6 cm	0.6 cm	0.6 cm
	Final diameter	19 mm	16 mm	20 mm	1.9 mm	16 mm	19 mm
Ampicillin (10 µg/disk)		No zone of inhibition	No zone of inhibition	No zone of inhibition	No zone of inhibition	No zone of inhibition	No zone of inhibition

Table 3: Antibiotic susceptibility test.

Antibiotics / Bacteria		*E. Coli*	*Klebsiella oxytoca*	*Vibrio cholerae*	*Staphyloco-ccus aureus*	*Proteus vulgaris*	*Pseudomonas aeruginosa*
Norfloxacin (10 µg/disk)	Diameter of zone of inhibition	0.75 cm	1.6 cm	1.9 cm	2.4 cm	0.9 cm	3.4 cm
	Diameter of anti-biotic disk	0.6 cm	0.6 cm	0.6 cm	0.6 cm	0.6 cm	0.6 cm
	Final diameter	1.5 mm	10 mm	19 mm	18 mm	3 mm	28 mm
Vancomycin (30 µg/disk)	Diameter of zone of inhibition	No zone of inhibition	No zone of inhibition	1.25 cm	1.6 cm	1.4 cm	No zone of inhibition
	Diameter of anti-biotic disk	0.6 cm	0.6 cm	0.6 cm	0.6 cm	0.6 cm	0.6 cm
	Final diameter	NA	NA	6.5 mm	10 mm	8 mm	NA
Bacitracin (10 µg/disk)	Diameter of zone of inhibition	1.1 cm	No zone of inhibition	No zone of inhibition	No zone of inhibition	No zone of inhibition	No zone of inhibition
	Diameter of anti-biotic disk	0.6 cm	0.6 cm	0.6 cm	0.6 cm	0.6 cm	0.6 cm
	Final diameter	5 mm	NA	NA	NA	NA	NA
Azithromycin (30 µg/ml/disk)	Diameter of zone of inhibition	No zone of inhibition	1.8 cm	1.9 cm	No zone of inhibition	2.3 cm	1.9 cm
	Diameter of anti-biotic disk	0.6 cm	0.6 cm	0.6 cm	0.6 cm	0.6 cm	0.6 cm
	Final diameter	NA	12 mm	13 mm	NA	17 mm	13 mm
Imipenem (10 µg/disk)		No zone of inhibition	No zone of inhibition	No zone of inhibition	No zone of inhibition	No zone of inhibition	No zone of inhibition

Table 4: Antibiotic susceptibility test.

Antibiotics / Bacteria		*E. Coli*	*Klebsiella oxytoca*	*Vibrio cholerae*	*Staphyloco-ccus aureus*	*Proteus vulgaris*	*Pseudomonas aeruginosa*
Ofloxacin (20 µg/ml/disk)	Diameter of zone of inhibition	1.5 cm	3.0 cm	2.6 cm	3.6 cm	2.1 cm	3.4 cm
	Diameter of anti-biotic disk	0.6 cm	0.6 cm	0.6 cm	0.6 cm	0.6 cm	0.6 cm
	Final diameter	9 mm	24 mm	20 mm	30 mm	15 mm	2.8 mm
Ciprofloxacin (30 µg/ml/disk)	Diameter of zone of inhibition	1.9 cm	3.5 cm	2.9 cm	4.1 cm	2.5 cm	3.5 cm
	Diameter of anti-biotic disk	0.6 cm	0.6 cm	0.6 cm	0.6 cm	0.6 cm	0.6 cm
	Final diameter	13 mm	29 mm	23 mm	35mm	19 mm	29 mm
Co-trimoxazole (30 µg/ml/disk)	Diameter of zone of inhibition	No zone of inhibition	2.1 cm	2.8 cm	3.1 cm	No zone of inhibition	3.3 cm
	Diameter of anti-biotic disk	0.6 cm	0.6 cm	0.6 cm	0.6 cm	0.6 cm	0.6 cm
	Final diameter	NA	15 mm	22 mm	25 mm	NA	27 mm
Erythromycin (15 µg/disk)		No zone of inhibition	No zone of inhibition	No zone of inhibition	No zone of inhibition	No zone of inhibition	No zone of inhibition

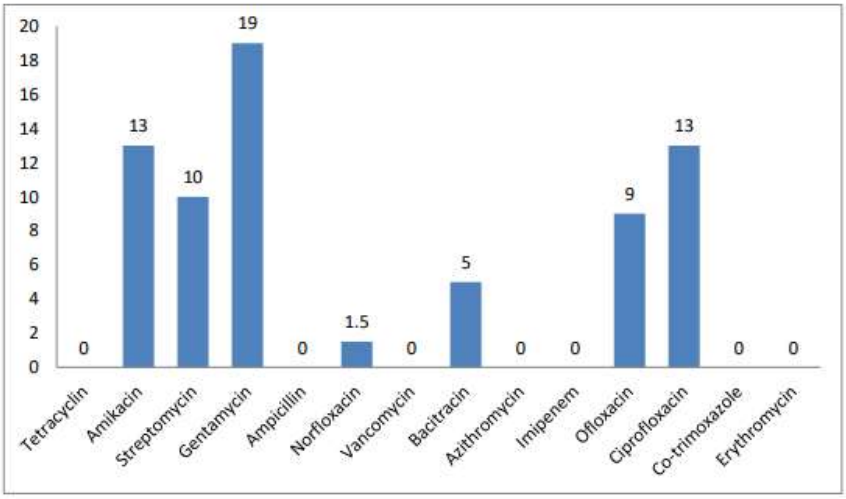


Figure 28: Antibiotic susceptibility test of *E. Coli.* (Diameter of growth inhibition in mm).

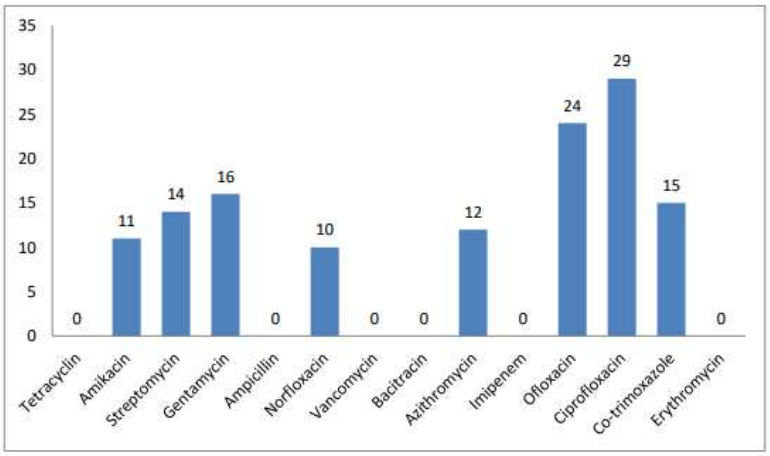


Figure 29: Antibiotic susceptibility test of *Klebsiella oxytoca* (Diameter of growth inhibition in mm)

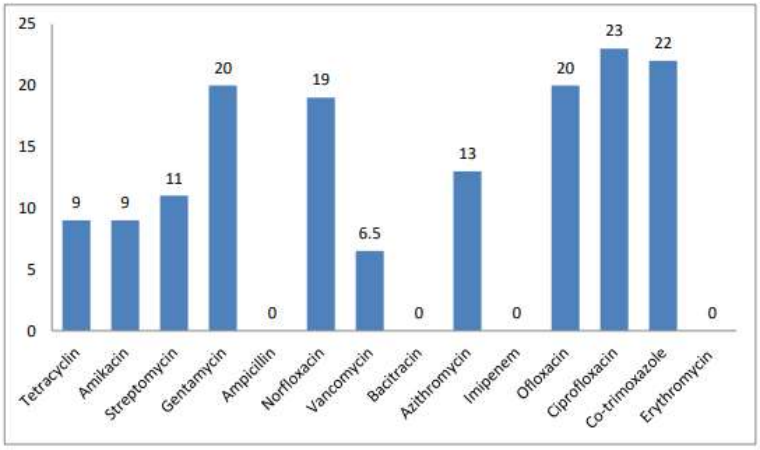


Figure 30: Antibiotic susceptibility test of *Vibrio cholerae*. (Diameter of growth inhibition in mm).

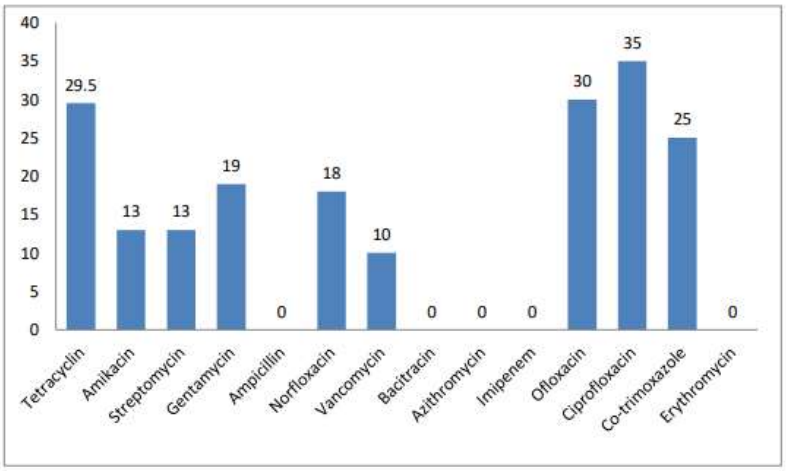


Figure 31: Antibiotic susceptibility test of *Staphylococcus aureus*. (Diameter of growth inhibition in mm).

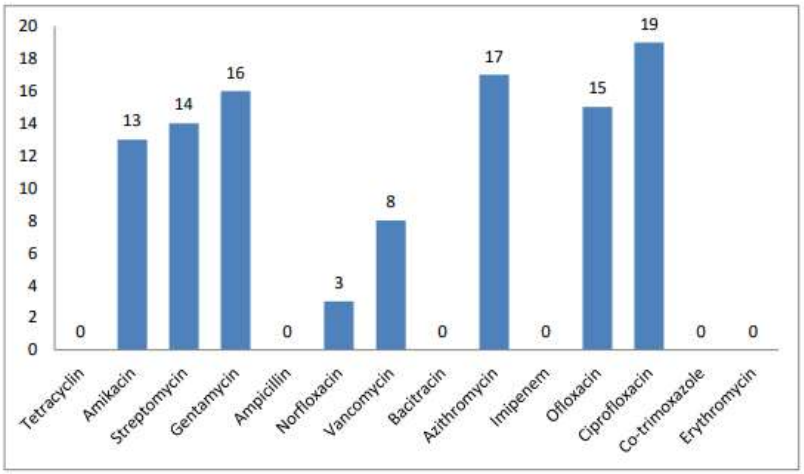


Figure 32: Antibiotic susceptibility test of Proteus vulgaris. (Diameter of growth inhibition in mm).

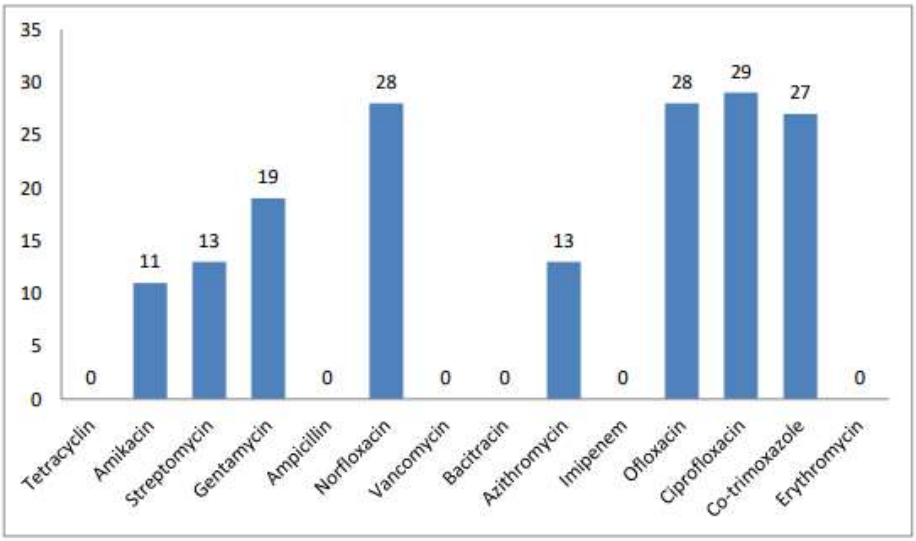


Figure 33: Antibiotic susceptibility test of Pseudomonas aeruginosa. (Diameter of growth inhibition in mm).

5. DISCUSSION:

Antibiotic resistance, a grave threat to public health, results from bacteria overcoming antibiotic effects. This study isolates Amoxicillin-resistant bacteria from chicken liver, emphasizing the growing concern over antibiotic resistance and its diverse origins. Six strains exhibit heightened resistance, with *Proteus vulgaris, Vibrio cholerae, Pseudomonas aeruginosa, E. coli, Klebsiella oxytoca,* and *Salmonella* identified through biochemical tests. Surprisingly, Salmonella shows no Amoxicillin resistance. Detailed antibiotic susceptibility testing unveils nuanced responses. Tetracycline exhibits varied efficacy, while Ampicillin, Imipenem, and Erythromycin show no impact. Bacitracin's effectiveness is poor, while Vancomycin, Azithromycin, and Co-trimoxazole demonstrate average efficacy. Notably, Amikacin, Streptomycin, Gentamycin, Norfloxacin, Ofloxacin, and Ciprofloxacin exhibit strong bactericidal effects against the resistant strains. Utilizing the disk diffusion method, the study provides comprehensive insights into antibiotic resistance patterns. The urgency to address antibiotic resistance is underscored, emphasizing the need for effective therapeutic strategies amidst evolving bacterial resistance. This research significantly contributes to understanding antibiotic susceptibility in Amoxicillin-resistant bacteria, advocating for public health protection and strategic interventions against emerging resistance.

6. CONCLUSION:

This study highlights the alarming rise in bacterial resistance to antibiotics, posing grave risks to living organisms. The diminishing efficacy of antibiotics raises significant concerns, potentially leading to severe or fatal diseases. Emphasizing the importance of antibiotic susceptibility testing, the research aims to uncover evolving resistance patterns in bacteria. It underscores the urgent need for novel, more potent antibiotics. The study's insights advance our understanding of resistance, informing prescription practices and guiding the synthesis of innovative antibiotic variants. This knowledge is crucial for effective therapeutic interventions and the development of antibiotics tailored to combat emerging bacterial challenges.

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