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Antibiotic Resistance and Biofilm Formation in Multidrug Resistant Bacteria

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Abstract

Antibiotic resistance (AR) and biofilm formation in multidrug-resistant (MDR) bacteria represent significant challenges in modern medicine, compromising the efficacy of antimicrobial therapies and increasing the risk of chronic infections. The rise in MDR bacteria, capable of resisting multiple antibiotic classes, exacerbates the complexity of treating infections, particularly in vulnerable population such as immunocompromised patients. Biofilm formation, a process by which bacterial communities adhere to surfaces and secrete extracellular matrix substances, provides a protective environment against both host immune responses and antibiotic treatment. These biofilms contribute to persistent infections, as the bacteria within are often more resistant to antibiotics due to altered metabolic states, limited penetration of drugs, and genetic adaptations. The synergistic relationship between AR and biofilm formation creates a formidable barrier to infection control, demanding novel therapeutic strategies such as the development of biofilm-disrupting agents, enhanced diagnostic techniques, and alternative antimicrobial approaches. Addressing the interplay between antibiotic resistance and biofilm formation is crucial for combating the growing threat of MDR bacterial infections and improving patient outcomes.

Key words: Antibiotic Resistance, Multidrug Resistant Bacteria, Biofilm.

1.INTRODUCTION

Antimicrobial resistance (AMR) expected to cause more deaths than cancer by 2030, reaching around 10 million deaths, antibiotic resistance is a developing worldwide health concern

(Maskera Jinnah et al., 2022; Kim et al., 2021). Multidrug-resistant (MDR) bacteria have emerged faster thanks to extensive usage, abuse, and improper application of antibiotics. Because these resistant bacteria may survive several drugs, treating an infection becomes more challenging. Contaminated food products—fruits, vegetables, meat, milk, dairy, and processed foods—offer a major path of bacterial transmission (Maskera Jinnah et al., 2022; Olubenga Adekunle Olowe et al.,2019).

MDR bacteria acquire resistance by means of several processes including biofilm development, horizontal gene transfer, and genetic alterations. Poor cleanliness, cross-contamination, or environmental exposure can all cause MDR bacteria to contaminate food products. Once in the food chain, these bacteria seriously compromise public health by causing foodborne diseases and treatment-resistant infections (Hennekinne et al., 2012; Chen et al., 2020).

Bacterial biofilms are structured communities of microorganisms enclosed in a protective extracellular matrix. Biofilm formation significantly enhances drug resistance by limiting antibiotic penetration, promoting genetic exchange, and enabling bacteria to evade the host immune response. Mechanisms contributing to biofilm-associated resistance include enzymatic degradation of antibiotics, reduced permeability, and stress-induced adaptation (B. Anu Monisha et al., 2022).

Unlike free-floating (planktonic) bacteria, cells within biofilms exhibit enhanced resistance to environmental stresses and antimicrobial agents. Research has demonstrated that bacteria within biofilms can endure chemical stressors, leading to persistent infections and treatment failures. Moreover, biofilms facilitate the exchange of resistance genes, accelerating the spread of AMR within microbial communities (Dinesh Kumar Bhardwaj et al., 2020; Monte et al.,2014; DP et al., 2021).

Over the past a long time, sizable studies have focused on the biofilm-forming capability of *Salmonella* species, a first-rate cause of foodborne contamination worldwide. This pathogen can survive in harsh environments, inclusive of meals processing centre and host tissues, increasing its capacity for huge transmission (Arjan Kabir et al., 2025). Due to its growing antibiotic resistance, the World Health Organization (WHO) has categorized Salmonella as a concern pathogen requiring urgent intervention.

The capability of *Salmonella* species to shape biofilms plays a critical role in its persistence and resistance to treatment. Biofilms shield bacterial cells from outside threats, including antibiotics and disinfectants, making infections difficult to eliminate. Conventional antibiotic susceptibility assessments, which include minimum inhibitory concentration (MIC) measurements, frequently fail to correctly investigate the resistance of bacteria within biofilms, leading to ineffective treatment strategies (Bermúdez-Capdevila et al., 2022; Cadena et al., 2019.

Understanding the link between the formation of biofilm and antibiotic resistance in the Salmonella species is necessary to develop better treatment strategies and control outbreaks. Since food production settings and health care are important transfer points, targeted intervention is necessary. Effective strategies include, increase hygiene and hygiene practice in food processing industries, use strict antibiotic policy to dampen resistance growth, develop new antimicrobial agents and biofilm-related strategies and improvement of clinical methods to correct and address infections related to biofilm (Xiaoxue Yan et al., 2025).

The increase in antibiotic resistance, especially *Salmonella* species as a biofilm formation bacteria, emphasizes the need for immediate control measures. By improving our understanding of resistance mechanisms associated with biofilm, researchers and decision makers can develop better strategies for dealing with AMR and protecting global public health (Afrina Haque et al., 2024).

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This research provides evidence for a new strain of multidrug-resistant (MDR) bacteria and describes a different antibiotic resistance and biofilm formation mechanism. It reveals a novel and previously uncharacterized gene or gene product that mediates antibiotic resistance and biofilm formation: furthermore, it describes a new relationship between antibiotic resistance and biofilm-related genes, which can translate to alternative therapeutic targets. Using a new in vitro model for biofilm formation that more accurately replicates in vivo conditions, the study provides insights into the behaviours of MDR pathogens that would have otherwise been unreasonable with standard laboratory conditions. The research also serves as initial evidence for a novel therapeutic strategy with enhanced efficacy, comprised of combining antibiotics with a biofilm-disrupting agent, for MDR infections, which should be further explored.

2. Review of Literature

Fruits and vegetables represent a large segment of the bulk of human nutrition and consumption has increased substantially because of the immunological benefits of vitamins, minerals and dietary fibre **(Abadias *et al*., 2008).** There are some fruits/vegetables which can be consumed raw or they are rarely processed **(Zhu *et al*., 2017).** The contaminated fruits and vegetables may be the cause for transmission of diseases.

Pomegranate has bioactive compounds which include pantyagines, alasinic acid and flavonoids, all of these contain antibacterial properties. When conditions are poor, some metabolites create antimicrobial affect.

Identifying MDR bacteria will also help to understand natural microbial density resistance **(Ahmed Saeed Kabbashi *et al*., 2024).**

The fruit cucumber belongs to the cucurbitaceae family and it exhibits multidrug resistance.

(Maskera Jinnah *et al*., 2022).

The multidrug resistance pattern was determined by the disk diffusion method and the susceptibility of the species was evaluated in respect to given 5-10 antibiotics. The present study aims to detect Salmonella species isolated from leafy green vegetables (spinach) from coastal India, using as rapid cultureindependent method **(Rajesh P. Shastry *et al*., 2021).**

Enterococcus species are found in fish, shellfish, and other aquatic animals because they are able to adapt to freshwater and marine environments **(Paganelli *et al*., 2017).** Enterococcus spp. constitutes an emerging fish disease with implications for aquaculture globally **(Paul *et al*., 2021)** and the disease is referred to as "pop-eye" in tilapia, is a fish disease that can lead to high mortality in aquariums and lost revenue **(Anshary *et al*., 2014).** E. faecalis could be highly or weakly pathogenic to tilapia. Enterococcus spp. can be used as probiotics, bio preservatives, and provide evidence of faecal contamination in food or water **(Ben Braiek and Smaoui 2019).** Enterococcus spp. is also a confirmation of antimicrobial resistance (AMR) monitoring systems in humans and animals **(Yang *et al*., 2023).**

Dairy products, especially cheese and yogurt, can facilitate the growth of microorganisms, because most dairy foods have moisture, nutrition, and a fermentation process **(Zhang *et al*., 2020).**

Spearheads are developed by contamination with pathogenic or opportunistic bacteria, some of which could be MDR tribes **(Karami *et al*., 2022).** Dairy products are popular for pollution, and they have the capacity to generate strong biofilms **(Khan *et al*., 2021).** Biofilm acts like an insulator, reducing the effectiveness of antibiotics, disinfectant and heat treatments **(Symes *et al*., 2019).** Bacterial species like enterococcus species in large amounts are abundant in milk and milk products.

Tomato sauce is rich in water and nutrients and is a favourable place for microbes to grow in **(Zhao *et al*., 2021).** In the general context, the growing microorganisms are pseudomonas spp., bacillus spp., enterobacteria, listeria monocytogenes and fungi **(Silva *et al*., 2019).** Some of the bacteria are MDR and are capable of formation of biofilm and can contribute to food -borne

infection **(Liu *et al*., 2022).**

Chicken and red meat are very poor because of their high protein and moisture content and are poor candidates for microbial malfunctions **(Nychas *et al*., 2020).** The meat can create strong biofilms in many foodborne pathogens which help them to endure in a processing environment **(Shi *et al*., 2021).**

Peptone allows for salt resolution in the homogenization and dissemination of bacterial cells, thereby permitting suitable recuperation on selective and nonselective media for the separation of bacteria from poor food samples **(Mandal *et al*., 2021).** PCA allows for the proper assessment of bacterial contamination in meat and dairy products for a combination of peptone salt resolution to produce a sample and for calculation purposes.

In microbiological studies, the use of peptone-based deceleration enhances the existence of bacteria during the processing of food samples and can be useful for the detection of antibioticresistant bacteria in food samples **(Schrader *et al*., 2020).** *Salmonella* species was detected in poor food samples using traditional cultural methods followed by biochemical characterization and confirmation molecular technology. The identification of *Salmonella* species was performed through a selection of biochemical tests that included Indole, Triple Sugar Iron (TSI), Urease and Citrate, which are traditionally used to separate ancient bacteria **(D'Aoust, 2020).**

The biochemical characteristics for *salmonella* species typically indicate a negative response for Indole and Urease, negative H -sitter production in the TSI, and positive citrate usage would help it be separated from other Enterobacteriaceae **(Andino and Hanning, 2019).**

These multidrug-resistant (MDR) bacterial species are emerging and disseminating because of the use of broad-spectrum antimicrobials to treat infections in both humans and animals. In low- and middle-income countries, antibiotics are used indiscriminately and largely unregulated; antibiotics are even provided for coughs and sinus disorders—symptoms that are self-limiting. All over the world, antibiotics are either incorrectly or illegally prescribed due to the demands of the patients. With animal husbandry, in many instances, antibiotics are used as animal growth promoters and thereby routinely misused as preventive care and treatment for animal infections. All of these activities encourage bacterial resistance to antibiotics. **(Olugbenga Adekunle Olowe *et al*., 2019).**

It can be resistant, intermediate and sensitive to several antibiotics including, but not limited to, Ciprofloxacin, penicillin -G, streptomycin, vancomycin etc. Salmonella species are resistant to Ciprofloxacin. It can also be sensitive to some antibiotics **(Adeoye John Kayode *et al*., 2022)**.

E. coli biofilm formation is a significant risk factor for 60% of all infection severity in humans and antimicrobial resistance since the bacterial extracellular matrix provides a protective environment from antimicrobial agents and it may also induce chronic infections and treatment challenges based on species and environmental factors. One part of the biofilm matrix that is well established are the adhering fimbrial structures known as curli, which are thin, tightly coiled, highly aggregative fiber(s) of varying lengths, that extend beyond the E. coli bacterial surface(s), allow E. coli to adhere to a multitude of proteins, colonize animal tissue, elicit immune responses, and allow for biofilm development in animals and on inanimate surfaces. **(Adeoye John Kayode *et al*., 2022)** and **(Sathish J.V *et al*., 2017).**

A microtiter plate facilitated the development of the biofilm. The suspension of the organism was prepared in Mueller Hinton broth (MHB) medium and incubated overnight at 37°C.

(Lakshmi Krishnasamy and Jai Ganeshan Muttiah Velmurugan 2019).

3.MATERIALS AND METHODS

3.1Study Population

Ten different food samples—pomegranate, spinach, cucumber, fish, prawn, yoghurt, cheese, chicken, red meat, and tomato sauce—were acquired from various local markets and retail stores across different regions in Chennai, Tamil Nadu, India, relative to June and August 2024. Chennai lies in the tropical region of South India and is located approximately at 13.08°N latitude and 80.27°E longitude. These food items were selected due to their widespread consumption and capacity for contamination with multidrug-resistant germs. Prior to sample package, verbal accept was possessed from local vendors and shopkeepers. (Olugbenga Adekunle Olowe et al., 2019).

3.2Sample collection, isolation and identification of *Salmonella* species:

Ten samples were taken from various areas and five primary sources: fruits, vegetables, meat, milk products, and processed foods. The samples were routinely cultured in both peptone salt solution and Plate Count Agar and incubated at 37 °C for 24–48 hours. Further subcultures were made on the respective media. The identification step included biochemical tests and Gram staining. The identified bacterial species might be *Salmonella spp.* (Adeoye John Kayode et al., 2022).

3.3Antibiotic Susceptibility Testing:

Antibiotic susceptibility testing was conducted using the disc diffusion method according to standard methods on Mueller-Hinton Agar for all *Salmonella* isolates. A total of eight antibiotics were involved. The zone diameters of inhibition were recorded for each antibiotic disc. Strains were classified as resistant, intermediate, or sensitive according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Multidrug resistance was defined as resistance to two or greater classes of antibiotics. (Olugbenga Adekunle Olowe et al., 2019; Adeoye John Kayode et al., 2022).

3.4Biofilm Formation:

The biofilm-forming capability of the strains used in the study was determined using a modified crystal violet assay. The biofilm assay involved adding 100 µL of the bacterial suspension consisting of 1% (v/v) glucose in Tryptic Soy Broth (TSB) at 1×10^6 cfu/mL to each well of a 24-well microtiter plate. The plates were incubated for 24-48 hours at 37 °C. Following incubation, planktonic cells were obtained by aspiration, and each well was gently washed with PBS (pH 7.5) twice to remove remaining suspended bacteria. The wells were then air dried for 15-20 min, followed by 5 min incubation with 0.1% crystal violet solution to stain the wells. Excess dye was removed using 90 % ethanol. The absorbance was measured at 595 nm. This experiment was conducted for the *salmonella spp* from six samples. (Maskera Jinnah et al., 2022; Lakshmi Krishnasamy et al., 2024; B. Anu Monisha et al., 2022).

The OD values were used to determine the level of biofilm formation, Weak biofilm producer (OD control< OD 2 * OD control), Moderate biofilm producer (2 * OD control < OD <= 4 * OD control) and Strong (OD > 4 * OD Control).

3.5 Statistical analysis:

All experiments were completed in triplicate based on standard procedures and the results were recorded.

3 RESULTS AND DISCUSSION

4.1Isolation and Enumeration of the bacteria:

Microorganisms were isolated using the dilution plating method from ten food items (P13, C23, S33, F44, P54, Y64, C73, T83, C93, and M103). Each food sample was homogenized under sterile conditions for uniform microbial distribution throughout the sample. Each of the homogenized samples underwent serial dilutions to decrease the microbial load prior to the isolation of individual colonies. The dilutions were placed on selected selective and differential agars to enhance the growth of specific bacterial groups and inhibit the growth of unwanted flora. Once selected selective and differential agars were identified, the plates were inoculated using standard microbiological methods and incubated under optimal conditions to allow for bacterial growth. (See Fig. 1 to Fig. 3 for the visual images of colony morphology and growth patterns.) The culture results yielded numerous bacterial species associated with the ten food items sampled, indicating variability in microbial contaminations. This continues to demonstrate the need for proper food handling, hygiene, and preparation practices to reduce the likelihood of foodborne illness. The selective and differential media provided an efficient media choice in the recovery of bacteria and provided additional information on microbial quality and possible pathogenicity of organisms recovered from the food samples. These findings emphasize the need for continuous monitoring of food safety, particularly in environments where cross-contamination is likely to occur.

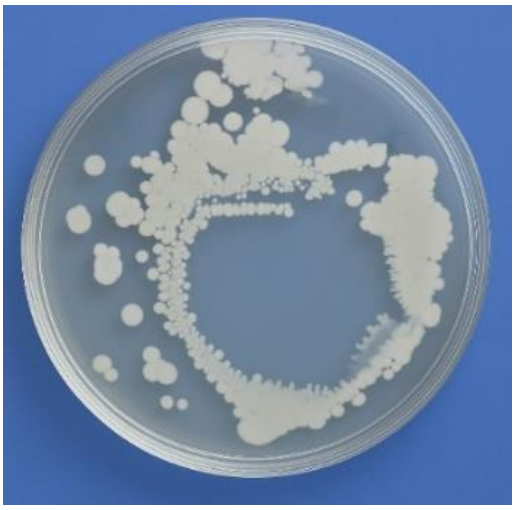

Fig1: The above picture shows the isolation of bacteria from a Prawn sample (P54).

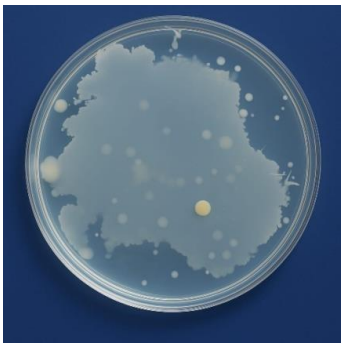

Fig2: The above picture shows the isolation of bacteria from a Yoghurt sample (Y64).

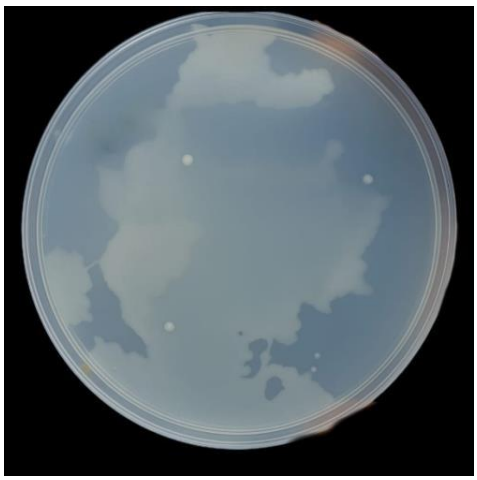

Fig3: The above picture shows the isolation of bacteria from a Red Meat sample (M103).

4.2 Identification of the bacteria:

The results emphasize the significance of persistent food safety monitoring, especially in the presence of cross-contamination. We conducted biochemical tests to further characterize the isolated bacteria. The Indole and Urease tests were negative, indicating that the isolate cannot produce indole from tryptophan and does not produce the urease enzyme. The negative Indole and Urease tests suggest the isolate can be differentiated from other indole- and urease-positive enteric species. The Triple Sugar Iron (TSI) test showed an alkaline slant (red) and an acid butt (yellow) along with a black precipitate, indicating hydrogen sulfide (H_2S) production. The results indicate fermentation of glucose and the release of H_2S gas, which is a biochemical profile matching *Salmonella species*.

The Citrate utilization test was positive, indicating that the organism can utilize citrate as its only source of carbon and identifies characteristic of *Salmonella.* The Gram stain indicated that the organism is Gram-negative which supports the classification of the organism as part of the Enterobacteriaceae family.

The biochemical and microscopic findings indicate that *Salmonella* species were present in the food samples analyzed. The negative Indole and Urease tests are consistent with the characteristics of *Salmonella*, as they do not produce indole or urease like some enteric bacteria such as Proteus and E. coli. The outcome of the TSI test - an alkaline slant and acid butt with black precipitate - indicates glucose fermentation and hydrogen sulfide production, which are typical for *Salmonella*. The citrate test was positive, denoting the bacterial species maybe *salmonella,* has the the ability to utilize citrate as a sole source of carbon. The Gram-negative test was also negative, indicating salmonella species. These findings indicate likely contamination by improper handling or

cooking of food items, indicating the need for strict hygiene procedures in the handling and preparation of food. There is a need for continued microbiological monitoring and surveillance to reduce the incidence of foodborne illness and protect consumer safety.

4.3 Antibiotic Resistance Profile:

The isolates of *Salmonella* species obtained from food samples demonstrated a multidrug resistant (MDR) profile that was resistant to at least four or more antibiotics. The results of whole antibiotic susceptibility testing, as depicted in Figures 4-6, demonstrated a broad spectrum of resistance to several commonly-used antimicrobial agents. Resistance was highest against vancomycin (VAN), penicillin-G (PCN), and clindamycin (CD), suggestive that these antibiotics are likely ineffective against infections caused by the tested *Salmonella* strains, with penicillin-G being the antibiotic exhibiting the highest resistance level of all tested antibiotics. Despite high levels of resistance, isolates demonstrated somewhat sensitivity to ciprofloxacin

(CIP) and gentamicin (GEN) with inhibition zone diameters of 22 mm - 35 mm further suggesting these antibiotics may still be effective against *Salmonella* Infection. Furthermore, we observed intermediate susceptibility to some isolates against rifampicin (RIF), and chloramphenicol (C), with inhibition zones of 14 mm - 17 mm. These results indicated the isolates demonstrated the potential development of full resistance over time. In general, the multidrug-resistant nature of the *Salmonella* isolates highlighted considerable public health concerns in regards to treatment foodborne infections, while also supporting the importance of continued surveillance and stage for antibiotic for antibiotic use.

This significant resistance pattern is especially concerning from a public health perspective. *Salmonella* species is a well-established cause of foodborne illness, and the presence of multidrug resistance will complicate treatment, especially in severe or invasive disease. The identified resistance to key antibiotics, specifically fluoroquinolones and aminoglycosides, could mean a longer illness, increased health costs, and a higher chance of complications or treatment failure. These results emphasize the clear necessity for judicious and careful use of antibiotics in both the clinical setting and in agriculture, which may drive resistance patterns due to misuse. Furthermore, the findings support the need for active antimicrobial stewardship programs, ongoing monitoring of resistance patterns, and strict adherence to food safety practices to minimize the risk of MDR pathogens entering the food chain.

Fig 4: The above picture shows the zones of multidrug resistance using various antibiotics for the sample S33.

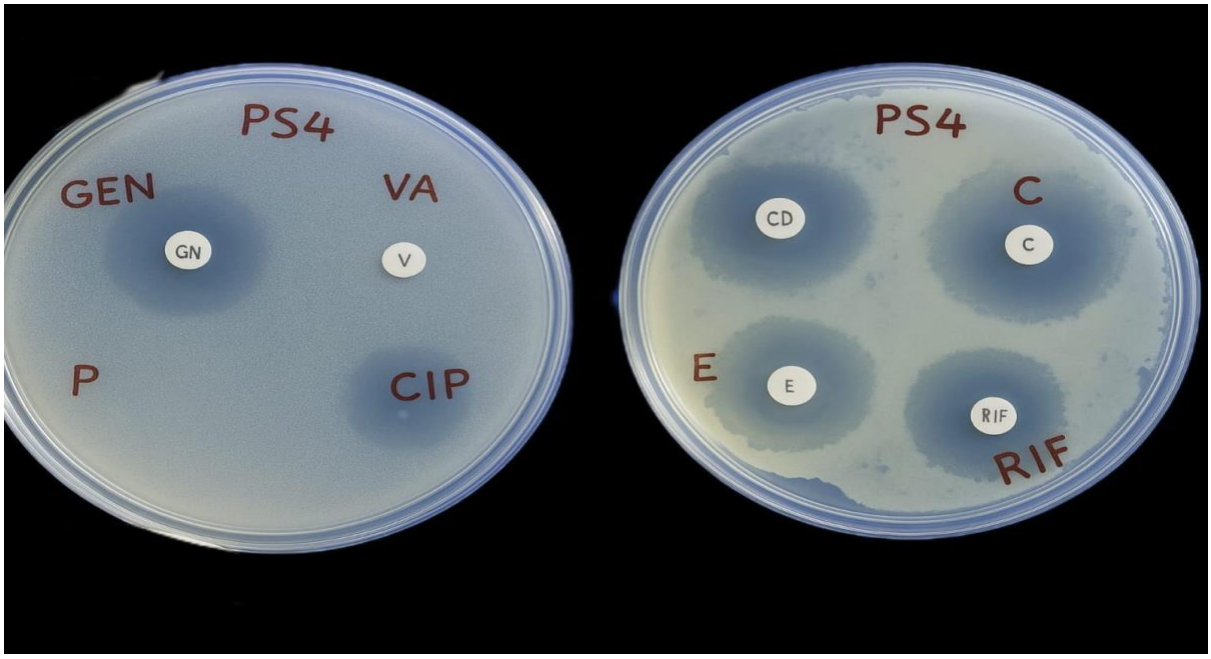

Fig 5: The above picture shows the zones of multidrug resistance using various antibiotics for the sample P54.

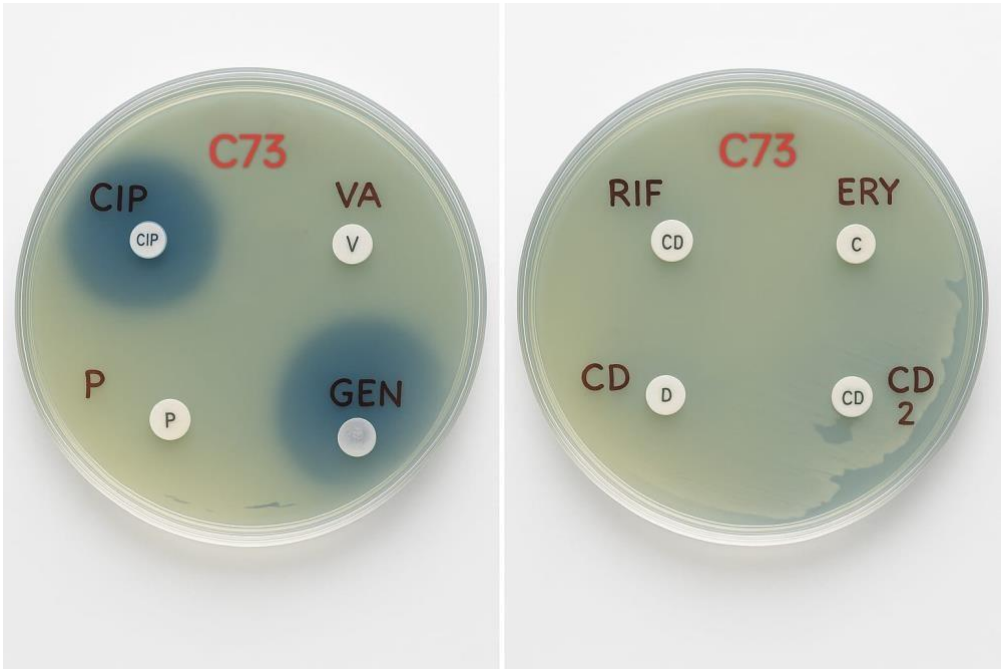

Fig 6: The above picture shows the zones of multidrug resistance using various antibiotics for the sample C73.

4.4 Biofilm formation by *salmonella* species isolates:

The *Salmonella* species isolates tested varied in their ability to form biofilm, demonstrated by the microtiter plate assay and test tube method. Of the tested isolates, Sample M103 was a strong biofilm producer, as it had high optical density (OD) values, indicating high biofilm biomass. Sample Y64 formed moderate biofilms, while Sample C73 formed weak biofilms based on lower absorbance readings associated with biofilm development. The images and tables provide visual and numeric representations of these findings. Tables 4 represent biofilm formation and biomass. Fig 7 illustrates structural characteristics and estimated differences in biomass

development between isolates. Differences in biofilm formation may mean the *Salmonella* species isolates differed in virulence, and thus their abilities to persist in food processing facilities or resist antimicrobial agents.

From the findings of the microscopic and structural assessment, biofilms were found to consist of thick, multilayered assemblages of bacterial cells that are produced and protected by an extracellular matrix. The matrix is assumed to provide additional resistance to environmental stress and antimicrobials, thereby enhancing the bacterial community's survival characteristics.

The biofilm-forming ability of *Salmonella* species on food contact surfaces is a serious concern due to its role in food processing and raw food product contamination. Biofilms are associated with the protection of bacteria from sanitizers and cleaning chemicals, which would enhance the risk of infection transmission to consumers. Cells that are found in biofilms also exhibit enhanced tolerance to antibiotics, thereby complicating treatment of infected animals and humans. The research indicates the need for better hygienic practices to be applied to food processing and handling, as well as the need for targeted approaches to disrupt or prevent biofilm construction. The formation of biofilms, in conjunction with antibiotic resistance, represent a combined concern for food safety and public health, and should command increased focus both as research study objective and in regulatory guidelines.

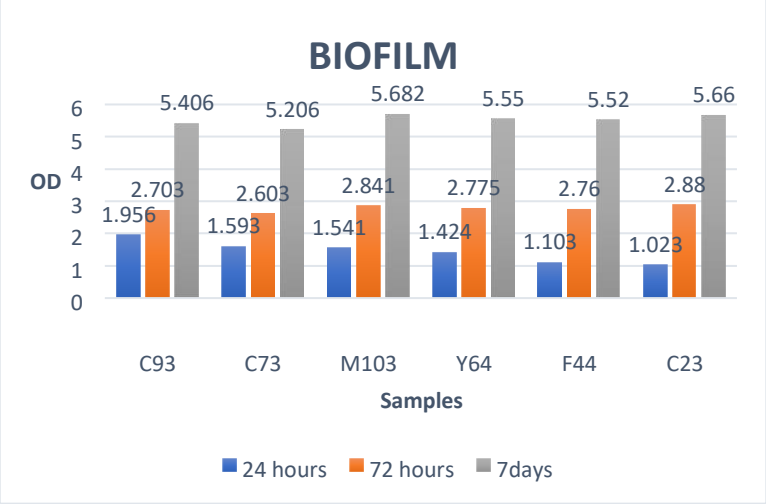

Fig 7: The above graph illustrates the biofilm formation of resistant samples after 24 hours,72 hours and 7 days using the microtiter plate method.

Table1: The below table illustrates the results of identification of bacteria using four standard biochemical tests.

TEST TUBE/ RESULTS	P13	C23	S33	F44	P54	Y64	C73	T83	C93	M103
Indole	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Citrate	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)

Urease	(-) Growth	(+)	(+)	(+)	(+)	(-) Growth	(-) Growth	(+)	(+)	(+)
Triple Sugar Iron	Black butt, Yellow slant, No gas formation, H2S production	Blackbutt, Yellow slant, Gas Formation, H2S production	Black butt, Yellow slant, No Gas Formation, H2S production	Black butt, Yellow slant, No gas Formation, H2S production	Black butt, Yellow slant, No gas Formation, H2S Production	Pink butt, Pink slant, No gas formation, No H2S production	Black butt, Yellow slant, No gas formation, H2S Production	Black butt, Yellow slant, No gas formation, H2s production	Black butt, Pink slant, No gas formation, H2S production	Black butt, Yellow slant, Gas production, H2S production

Table 2: The above table shows the zones of multidrug resistance using different antibiotics for all the samples.

ANTIBIOTIC TEST	P13	C23	S33	F44	P54	Y64	C73	T83	C93	M103
VAN	**R**	**R**	**R**	**R**	**R**	**S** 16mm	**R**	**S** 14mm	**R** 12mm	**R**
CIP	**S** 33mm	**S** 34mm	**S** 30mm	**S** 34mm	**S** 34mm	**S** 34mm	**S** 34mm	**S** 34mm	**R** 17mm	**S** 35mm
GEN	**S** 29mm	**S** 25mm	**S** 23mm	**S** 27mm	**S** 25mm	**S** 24mm	**S** 24mm	**S** 22mm	**S** 24mm	**S** 24mm
PCN	**R**	**R**	**R**	**R** 12mm	**R** **1**1mm	**R** 12mm	**R**	**R** 11mm	**R** 16mm	**R** 14mm
RIF	**R** 13mm	**R** 11mm	**I** 17mm	**R** 12mm	**I** 18mm	**R** 14mm	**I** 17mm	**R** 12mm	**R** 7mm	**R** 14mm
E	**R** 9mm	**R**	**I** 20mm	**R** 9mm	**I** 18mm	**R** 8mm	**R** 8mm	**I** 15mm	**I** 15mm	**R** 8mm

C	**I** 15mm	**S** 28mm	**S** 25mm	**R** 11mm	**S** 25mm	**S** 28mm	**R** 12mm	**S** 24mm	**R** 11mm	**S** 13mm
CD	**S**	**R**	**R**	**R**	**S** 38mm	**R**	**R**	**S** 24mm	**R**	**R**
RESULTS	3	5	3	6	2	4	5	2	6	5

Antibiotics:

1.**VAN -** Vancomycin

2. **CIP** - Ciprofloxacin

3. **GEN -** Gentamicin

4. **PCN** - Penicillin -G

5. **RIF** - Rifampicin

6**. E** - Erythromycin

7. **C -** Chloramphenicol

8. **CD** - Clindamycin

Inhibitory Zone diameter to the nearest millimetre

R - Resistant

S - Sensitive

I - Intermediate

Table 3: The below table shows the results of resistant and sensitive samples.

Resistant	**Sensitive**
C23	S33
F44	P54
Y64	T83
C73	
C93	

M103

Table 4: The below table shows the results of biofilm formation for resistant samples after 24 hours,72 hours and 7 days using the microtiter plate method.

Sample	OD (24 hours)	OD (72hours)	OD (7days)
C93	1.956	2.703	5.406
C73	1.593	2.603	5.206
M103	1.541	2.841	**5.682**
Y64	1.424	2.775	5.55
F44	1.103	2.760	5.52
C23	1.023	2.880	5.66

4. CONCLUSION:

This study investigated bacterial contamination and antibiotic resistance in 10 different samples. The antibiotic susceptibility tests confirmed the identifiable bacterial genus and species one as Salmonella, but with resistance to several Classes of antibiotics. Additionally, the microtiter plate method was used to assess biofilm formation and revealed the presence of strong, moderate and weak biofilm producers. However, more studies will be required to determine the mechanism of action with respect to biofilm elimination and potential treatments.

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