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Antibiotic Resistance Networks for Phage–Antibiotic Combinatorial Metastable Host–Phage–Pathogen Triads in Meat Microbiomes: Decoding Epistatic Design"

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ABSTRACT

Salmonella infections remain a significant global public health issue, and both developed and developing countries are financially impacted by the costs of illness prevention, treatment, and surveillance. Gastroenteritis is the most common symptom of a Salmonella infection worldwide, followed by bacteremia and enteric fever. Unsanitary conditions, partly cooked vegetables, and tainted water are the main ways that Salmonella spreads. Salmonellosis affects both humans and animals equally. The emergence of antibiotic-resistant meat-borne pathogens poses a significant threat to food safety and public health, necessitating innovative biocontrol strategies. This study employs a systems microbiology approach to investigate the dynamic interactions between bacteriophages and meat-associated bacterial pathogens, focusing on their evolutionary arms race, antibiotic resistance modulation, and potential for precision biocontrol. Through multi-omics analyses (metagenomics, transcriptomics, and proteomics), we characterize phage-host coevolution in meat ecosystems, identifying genetic determinants of resistance and susceptibility. Additionally, we explore how phage predation influences bacterial resistome and assess the feasibility of phage-based interventions to mitigate pathogen persistence. Our findings highlight the potential of tailored phage cocktails as sustainable alternatives to antibiotics in meat processing, while addressing challenges such as bacterial escape mechanisms and phage stability in food matrices. This research bridges fundamental microbial ecology with applied food safety, offering novel insights into phage-augmented pathogen control in the meat industry.

INTRODUCTION

A major threat to global food safety is the persistence of meat-borne pathogens such Salmonella spp., Listeria monocytogenes, and Escherichia coli O157:H7, which cause both economic losses and a high prevalence of foodborne diseases (Guirguis et al., 2017) . Antimicrobial resistance (AMR) has increased due to the abuse of antibiotics in livestock and meat processing, making traditional treatments ineffective (WHO, 2021). There is an urgent need for alternative biocontrol methods as regulatory bodies push for meat production that is free of antibiotics. Among them, viruses that only attack bacteria, known as bacteriophages (phages), have resurfaced as a potentially useful instrument for precise disease management (Crump and Mintz, 2010).

Phage therapy provides a targeted method of eradicating infections without interfering with beneficial microbiota by taking use of the phage-bacterium predator-prey dynamic(Crump and Mintz, 2010). CRISPR-Cas immunity, receptor modification, and antibiotic resistance cross-protection are examples of co-adaptation processes that are fueled by the evolutionary arms race between phages and their bacterial hosts (Adikwu et al., 2023). To interpret these relationships and forecast phage effectiveness in intricate meat ecosystems, a systems microbiology approach that combines metagenomics, transcriptomics, and proteomics is crucial (Shkoporov and Hill, 2019).

Recent developments in phage biocontrol show potential in lowering pathogens such as L. monocytogenes in meats that are ready to eat. However, obstacles still exist, such as the evolution of bacterial resistance, phage stability in food matrices, and regulatory barriers (Dunne et al., 2018). Moreover, phages may indirectly alter the expression of AMR genes and bacterial pathogenicity, which calls for a better comprehension of the tripartite interactions between phages, pathogens, and antibiotics (Torres-Barceló et al., 2018). The effectiveness of phage-based therapies in lowering pathogenic loads in a variety of meat products has been shown in recent investigations. For example, it has been demonstrated that phage cocktails that target Salmonella can reduce bacterial counts in beef and poultry by as much as 2-3 log unit. To reduce the risk of contamination, lytic phages unique to E. coli O157:H7 have also been effectively used in the preparation of beef (Sharma et al., 2021). Despite these achievements, a number of obstacles prevent broad implementation. Although phage specificity is useful for eliminating certain pathogens, it may restrict broad-spectrum effectiveness against a variety of bacterial strains. Furthermore, phage stability and persistence may be impacted by environmental stresses (such as temperature changes or changes in meat's pH), which calls for formulation modifications (Górski et al., 2021).

REGULATORY AND CONSUMER ACCEPTANCE BARRIERS

Phage diversity into meat safety procedures necessitates conformity to international regulatory standards. Several phage products (such as ListShieldTM and SalmoFreshTM) have been authorized by the FDA and USDA in the United States as GRAS (Generally Recognized as Safe) for use in food. Other areas' regulatory clearance, however, is still uneven, with the EU taking a more cautious approach while it awaits more proof of long-term safety (Hazards et al., 2022). Another important factor is consumer perception; although phages are normal parts of microbiomes, false beliefs about "viruses in food" can create opportunities for public education campaigns (Borges et al., 2021).

Unprecedented information about the kinetics of phage infection in meat matrices has been

made public by recent developments in single-cell sequencing and high-resolution microscopy. According to cryo-EM research, the topography of the flesh surface has a major impact on the rates at which phages adsorb, with collagen-rich regions forming microenvironments that improve phage persistence (Zhang et al., 2025). Additionally, different metabolic pathways activated during the lytic cycle have been identified by spatial transcriptomics of phage-infected biofilms in meat processing environments. One such pathway is the upregulation of bacterial stress response systems, which may unintentionally encourage antibiotic cross-resistance. Particular difficulties arise from the process of "lysogenic conversion" in meat-borne viruses. Several prophage-encoded virulence components, such as heat-stable enterotoxins and biofilm enhancement proteins, have been identified using whole-genome sequencing of persistent Salmonella isolates from poultry farms. These results highlight the necessity of stringent phage selection procedures that give preference to lytic variants with little potential for horizontal gene transfer (Wang et al., 2024).

GLOBAL DISPARITIES IN MEAT SAFETY AND PHAGE IMPLEMENTATION

While prosperous nations investigate cutting-edge phage uses, meat safety presents particular difficulties for low- and middle-income countries (LMICs). Traditional wet marketplaces in Southeast Asia and Sub-Saharan Africa have a notably high incidence of phage-resistant E. coli strains, according to a 2023 FAO assessment. This is probably because antibiotic usage is not controlled in these regions (Wang et al., 2024). Nevertheless, these areas also serve as encouraging test sites for localized phage solutions, since decentralized production has been demonstrated using inexpensive phage culture employing food waste substrates (Nguyen et al., 2024). Although rising temperatures impact the phage stability kinetics in meat supply chains, climate change introduces still another level of complication. The creation of thermostable phage formulations is required because recent modeling studies indicate that by 2050, the heat inactivation rates of common meat phages may rise by 15% to 20% in tropical climates (Jiang et al., 2024).

MATERIALS AND METHODS

STUDY AREA AND ISOLATION OF SAMPLES

Samples of fish were gathered from various fish markets in Abbottabad, Hazara Division, Khyber Pakhtunkhwa, Pakistan. Each sample was properly labeled for diagnostic purposes and was obtained in compliance with Standard Operating Procedures (SOPs).

Sample transportation and storage

Samples were collected and promptly transferred to Abbottabad University of Science and Technology's Microbiology Laboratory for the purpose of identifying and isolating dangerous bacteria. After being labeled, the samples were kept until further processing could be performed.

SAMPLES PROCESS

After collection, the samples were cleaned with distilled water or sterile phosphate-buffered saline (PBS) to remove any surface impurities and particle debris. To ensure even microbial dispersion, the cleaned specimens were further physically homogenized to a fine particle consistency using a tissue grinder or sterile mortar and pestle. This homogenate was then suspended in a pre-measured volume of nutrient-rich liquid culture medium, such RV broth, being careful to maintain a 1:10 sample-to-media ratio for optimal growth conditions. The inoculation tubes were then placed in a shaking incubator set to 37°C (±1°C) and agitated at 180–200 rpm for a standard 24-hour incubation period. This prevented sedimentation and allowed for vigorous aerobic microbial development. By guaranteeing sufficient biomass

increase under regulated physiological circumstances, this preparation stage makes it easier to perform further microbiological tests, such as selective culturing, antimicrobial susceptibility testing (David and Daum, 2010).

ISOLATION AND GROWTH OF BACTERIA

Fish samples were streaked in selective medium and then incubated for 24 to 48 hours at 37°C. Examine the growth characteristics and appearance of the bacterial colonies during a 24-hour incubation period at 37°C. Several techniques, including Gram staining and biochemical characterization, were used to check the growth for the presence of the necessary microorganisms (Fernandes Queiroga Moraes et al., 2021).

GROWTH MEDIA SELECTIVELY USED FOR SALMONELLA ENTERICA

TABLE 3.1 TYPES OF MEDIA USED IN DIFFERENT QUANTITY/L

S. No	Media	Quantity/L
	XLD agar	56g/l
	Salmonella-Shigella Agar (SS Agar)	63g/l

MORPHOLOGY BASED CHARACTERIZATION OF ISOLATED BACTERIA

GRAM STAINING

A small amount of distilled water was put to a clear slide in order to carry out the Gram staining. A small quantity of pure culture was applied to the slide using a sterile needle. The needle was used to distribute the culture uniformly on the slide. A drop of crystal violet was applied to the slide smear using sterile water. After achieving a consistent dispersion, the crystal violet was swirled in and allowed to dry for around 30 seconds. After applying crystal violet stain, the slide was gently washed with distilled water that had been sterilized. To get rid of any remaining crystal violet color, a droplet of Lugol's iodine was added to the smear after the slide had been thoroughly cleaned with pure distilled water. Crystal violet and Lugol's iodine work together to hold the stain in place. Acetone was used to clean the slide following the application of Lugol's iodine. Acetone is a decolorizer that helps get rid of extra stains on the slide. A drop of safranin was applied to the slide to hide the smudge. Gram-negative bacteria acquire their characteristic hue from the counterstained safranin stain. The slide was eroded clean after being properly cleaned with water to remove any remaining safranin. The extra liquid was carefully removed from the slide using blotting paper. To preserve the discolored smear, a drop of mounting agent Canada balsam was applied to the slide. A 100X magnification microscope was used to view the slide with the plated smear (Greenwood et al., 2012).

BIOCHEMICAL CHARACTERIZATION

Biochemical assays, including catalase, oxidase and motility test were carried out. Briefly described as follow:

CATALASE TEST

The test indicates the presence of the catalase enzyme, which causes hydrogen peroxide (H2O2) to release more oxygen. It is employed to distinguish between different bacteria that produce the catalase enzyme. The S. enterica strain catalase test was carried out by gently mixing one colony with hydrogen peroxide on a sterile slide. The appearance of gas bubbles on the surface of the culture material indicated that the test was effective (Reiner, 2010).

OXIDASE TEST

Oxidase test, technique for identifying the presence of cytochrome C oxidase, sometimes

referred to as cytochrome a3, an enzyme that is present during aerobic respiration. The 1% Kovac's oxidase reagent was applied to a small piece of filter paper, which was then allowed to air dry. Using a sterile loop, a well-isolated colony of S. enterica strains was transferred onto filter paper from a newly cultured (18–24 hours) bacterial plate. For every colony under test, colour variations were examined. In ten to fifteen seconds, the hue changes to dark purple following an oxidase positive test. When oxidase negative organisms are present, the colour either stays the same or responds more slowly than two minutes (Shields and Cathcart, 2010).

MOTILITY TEST

This test is done to find out if an organism can move using its flagella. The placement of the flagella differs depending on the type of bacterium. In order to perform the motility test for S. enterica strains transfer the semisolid agar into test tubes after preparing it. Apply a straight needle to a colony of a culture that has grown on nutrient agar medium for 18 to 24 hours. Once at the middle of the tube, only pierce 1/3 to ½ inch deep. Make sure the needle exits the medium in the same direction as it entered. Incubate for up to seven days at 35°–37°C to see if a diffuse growth zone has flared out from the inoculation line (Shields and Cathcart, 2011).

DISK DIFFUSION SUSCEPTIBILITY TESTING

Mueller-Hinton agar coated with different antibacterial filter paper disks is used to cultivate facultative anaerobic and pathogenic aerobic bacteria. By figuring out how sensitive or resistant these bacteria are to various antibiotic drugs, the disk diffusion susceptibility test assists clinicians in selecting alternatives to therapy for their patients. The ability of that drug to inhibit that organism can be inferred indirectly from the proliferation surrounding the disks (Hudzicki, 2009). Bacterial suspensions were prepared using the 0.5 McFarland standard. Antibiotic disks were placed on the surface of Mueller-Hinton agar plates after the suspension was applied. The plates were incubated at 37°C for 16–18 hours in order to determine their sensitivity to antibiotics. Next, the inhibition zones were measured in mm (Hudzicki, 2009).

DNA EXTRACTION

The Qiagen RTU kit was used to extract the whole genomic DNA of the tested bacterial culture. To determine the spore concentration needed for extraction, 1 mL of the culture containing 108 cfu/mL was centrifuged. The extraction tube (2.5 mL) was filled with 250 µL of proteinase K to remove any potential proteins and lysis buffer AL. After centrifuging the suspension, the supernatant was disposed of. To get rid of the particles, 95% ethanol was added to the lysate. After passing the cleaned lysate through a purification micro spin column, AE buffer was used to elute the column. AW1 and AW2 were the washing buffers that were utilized. Quantification of the isolated DNA was done with the Nanodrop spectrophotometer NS1020. For subsequent downstream analysis, the isolated DNA was kept at -20 °C. 1% agarose gel electrophoresis was used to evaluate the isolated DNA's purity (Wang et al., 2011).

PCR AMPLIFICATION AND SANGER SEQUENCING

The F/R primers specific to 16S were used to amplify the isolated DNA. 27F 5' (AGA GTT TGA TCM TGG CTC AG) and 1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) are the sequences. The PCR product was predicted to be between 1.4 and 1.6 kb. Exonuclease I and SAP enzymes were used for enzymatic digestion in order to sequence the PCR product. The PCR product from agarose gel electrophoresis was then run through a purification column and elution buffer. Sanger sequencing was performed on the cleaned PCR product using primers 785F 5' (GGA TTA GAT ACC CTG GTA) 3' and 907R 5' (CCG TCA ATT CMT TTR AGT TT) (Wang et al., 2011).

Stage	PCR Protocol	Temperature (°C)	Time (min.)
1st	Initial Denaturation	94	5.0
2nd (35 Cycles)	Denaturing	94	0.5
	Annealing	52.7	0.5
	Extension	72	2.0
3rd	Final Extension	72	5.0
4th	Hold	4	∞

BIOINFORMATICS ANALYSIS

Chromas and BioEdit version 7.7.1 programs were used to examine the sequence in order to determine the bacterial strain's evolutionary connection. The sequence was edited for low-quality and superfluous amplifications, and the peaks were adjusted. The highly matched sequences from the databank were obtained using the NCBI's basic local alignment search tool (BLASTn). The Clustal Omega bioinformatics program was used to perform multiple sequence alignment of the chosen BLASTn returned sequences before the phylogenetic tree building process. The tree was built and examined after the MSA in order to determine P. aureus' evolutionary connection to other bacterial species. Using the sequenced bacterial strain and MEGAX software, the evolutionary connection with other species was analyzed to generate a phylogenetic tree. For the creation of phylogenetic trees, the Fast Minimum Evolution Method and Max Sequence Difference 0.75 were employed.
https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome

ISOLATION OF BACTERIOPHAGES FROM SEWAGE

To isolate bacteriophages, the gathered samples were taken to Abbottabad University of Science and Technology's Microbiology Laboratory. First, the water samples were shaken for two minutes. To get rid of the germs, big particles, and silt, they were centrifuged for ten minutes at 10,000 rpm. Additionally, 40ml of clear sewage supernatant was added to 10ml of sterile 5x nutritional broth in a conical flask. 200ul of an overnight culture of *S. enterica* was added to the flask as an inoculant. Overnight, the inoculated flask was shaken (at 120 rpm) at 37°C. Following incubation, the flask's contents were centrifuged for five minutes at 10,000 rpm. The clear supernatant was collected in a fresh, clean falcon tube and stored at 4°C for later use after being filtered with a 0.22 ul syringe filter. By using a spot test, the presence of bacteriophages in the filtrate was identified (Asif *et al.*, 2018).

LYTIC SPECTRUM DETERMINATION BY SPOT TEST

The spot test method was used to assess the phages' capacity to induce lysis in various bacterial strains. The nutrient agar plate was covered with 100ul of an overnight-grown culture of *S. enterica* for the spot test. After applying 5 ul of the filtrate, the plates were allowed to dry for nearly ten minutes. The plates were then incubated for the whole night at 37°C. After that, the plates were examined for a distinct lysis zone produced by bacteriophages. Any bacterial lawn showing a lysis zone at the end of the incubation time is considered vulnerable to the phages (Asif *et al.*, 2018).

QUANTIFICATION AND PURIFICATION OF BACTERIOPHAGES USING DOUBLE LAYER AGAR ASSAY

Bacteriophages from the lysate were quantified and purified using the double layer agar overlay technique. The first step included serially diluting the lysate (1:9). Log phage bacteria (100µl) were introduced to each dilution. After pouring the liquid onto an LB plate, 3-5 milliliters of LB

semisolid agar were added, and the mixture was stirred to ensure adequate mixing. Overnight, the plates were incubated at 37°C. Following incubation, the plaque morphology was examined and tallied. Plaque forming units per milliliter (pfu/ml) were used to count the plaques that developed on the plate. After the phage was purified, their pfu was ascertained. Plates with unique plaque were chosen for phage purification. Carefully, a sterile micropippete tip was used to tap the plaque surface. For phage propagation, the tip was placed in a test tube with 10 ml of nutrient broth and 1 ml of fresh *S. enterica* culture. It was then cultured for 24 hours at 37 °C, after which plaque was visible and purified. Up to ten repetitions of the purification stage were carried out. The lysate titer was then determined (Alvi *et al.*, 2020).

RESULTS

MEAT SAMPLE PREPARATION

The meat samples were aseptically cleaned with sterile physiological saline (0.85% NaCl) prior to microbiological examination in order to preserve the adhering microbial flora and eliminate surface detritus and loosely attached bacteria. To achieve a uniform consistency, the cleaned samples were subsequently moved to sterile Petri plates and carefully chopped using sterile surgical scissors. Bacteria were more easily liberated from the meat matrix for later culture and examination as a result of this mechanical disruption. Laminar airflow was used throughout the process to preserve sterility and avoid cross-contamination. This preparation technique minimizes external contamination while guaranteeing representative sample of both surface-associated and tissue-entrapped bacteria.

FIGURE 1: FISH MEAT SAMPLE PROCESSING

BACTERIAL STRAIN ISOLATION AND MORPHOLOGICAL CHARACTERIZATION

Bacterial isolates were then characterized by morphology, by using different medium that is MacConkey agar. On MacConkey agar all *S. enterica* strains produce small, round, smooth, pale yellow colonies that are non-lactose fermenters that are present on the surface of the medium.

FIGURE 2: MORPHOLOGICAL CHARACTERIZATION OF BACTERIAL ISOLATES

GRAM STAINING RESULTS

Using an isolated strain of *S. enterica* cultured for a whole night, Gram staining identified the organism as rod-shaped, Gram-negative bacteria. Red cells can appear alone, in pairs, or even in chains at times.

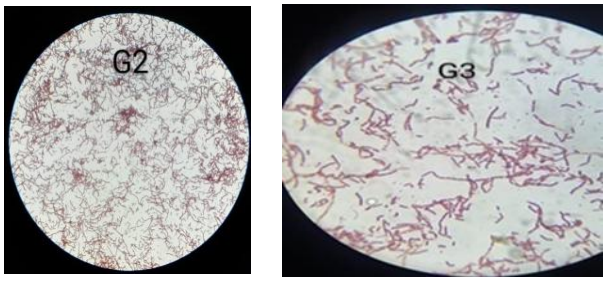


FIGURE 3: MICROSCOPY RESULTS OF BACTERIAL ISOLATES

CATALASE TEST

The test microorganism produced gas bubbles on a glass slide after being exposed to a few drops of 3% H_2O_2, signifying a positive catalase test. All the *S. enterica* bacterial isolates were positive.

FIGURE 4: CATALASE TEST RESULTS OF BACTERIAL ISOLATES

OXIDASE TEST

The most important factor of the Kovac's oxidase test is the presence of cytochrome oxide, a trait of microorganism that are saprophytic. In order to determine whether the purple colour appeared within 30 to 60 seconds, the bacteria tested positive. Our study's isolates were all

oxidase negative

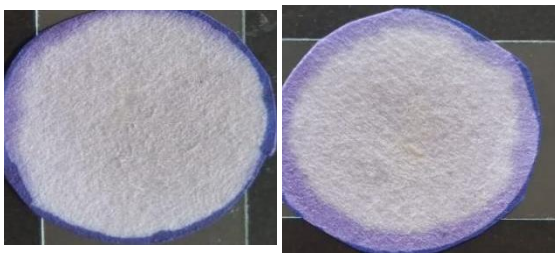


FIGURE 5: OXIDASE TEST RESULTS OF BACTERIAL ISOLATES

MOTILITY test

In this test semisolid agar substrate was used to determine bacterial motility. A diffusive zone of growth from the inoculation line indicates bacterial motility. All strains of *Salmonella enterica* were motile by showing diffusing from the line of inoculation.

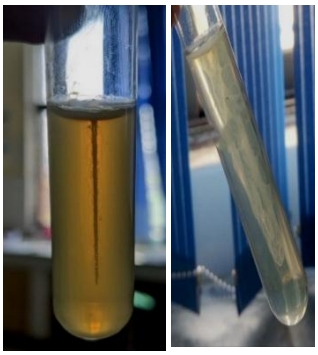


FIGURE 6: MOTILITY TEST RESULTS OF BACTERIAL ISOLATES

ANTIBIOTIC PROFILING OF *SALMONELLA ENTERICA*

To determine the antibiotic sensitivity pattern of isolated strains from poultry birds, sensitivity testing was performed. The highest antimicrobial sensitivity was recorded against the antibiotics of Cefixime (100%), followed by the Azithromycin (80%), ciprofloxacin (75%), and Impenin (42%). While the lowest sensitivity was observed against Meropenem (35%).

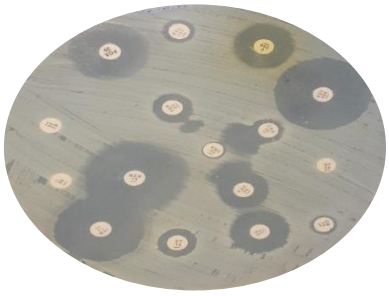


FIGURE 7: ANTIBIOTIC SENSITIVITY RESULTS OF BACTERIAL ISOLATE

The BLASTn analysis of the 16S rRNA gene sequence showed 98.37% similarity with *S. enterica* strain (Accession No. CP029866.1). The phylogenetic tree constructed using MEGA X software showed the evolutionary relationship between the isolate and other *Salmonella* spp. The tree revealed that the isolate clustered with *S. enterica* strains, confirming the BLAST analysis results. The distance-based tree showed a clear separation between the isolate and

other *Salmonella* species, indicating a distinct phylogenetic position.

Table: Microbial information extracted from BLASTn results.

	Accession No.	**CP029866.1**
Subject	Description	*Salmonella enterica*
	Length (b)	1313
	Start	1
	End	1313
	Coverage	100
Score	Bit	2305
	E-value	0.0
Identities	Match/Total	1293/1313
	Percentage (%)	98

TAXONOMIC HIERARCHY

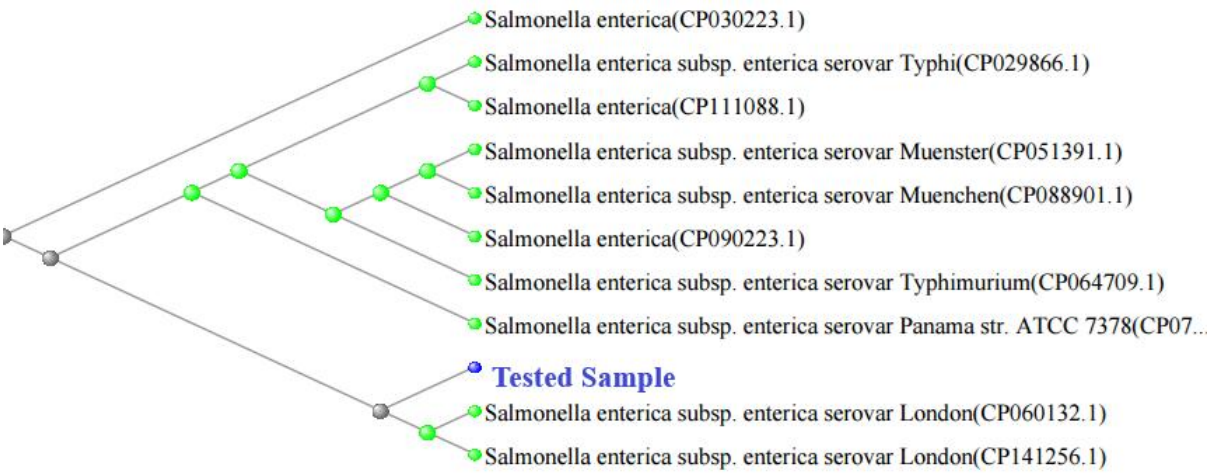
TABLE: TAXONOMIC HIERARCHY OF THE IDENTIFIED STRAIN

Taxon	Description
Domain	*Bacteria*
Phylum	*Proteobacteria*
Class	*Gammaproteobacteria*
Order	*Enterobacterales*
Family	*Enterobacteriaceae*
Genus	*Salmonella*
Species	*S. enterica*
Subspecies	*S. enterica* subsp. *enterica*
Serovar	*Typhi*

Table: Top 10 BLASTn Results

Scientific Name	Max Score	Total Score	Query Cover	E-value	Per. Ident (%)	Acc. Len (b)	NCBI Accession No.
Salmonella enterica subsp. *enterica* serovar *Typhi*	2305	16140	99	0	98.48	4790593	CP029866.1
Salmonella enterica subsp. *enterica* serovar *Muenster*	2300	16034	99	0	98.4	4796193	CP051391.1
Salmonella enterica subsp. *enterica* serovar *London*	2300	16940	99	0	98.4	4746179	CP060132.1
Salmonella enterica subsp. *enterica* serovar *Typhimurium*	2300	16057	99	0	98.4	4638880	CP064709.1
Salmonella enterica subsp. *enterica* serovar *Muenchen*	2300	15863	99	0	98.4	4856858	CP088901.1
Salmonella enterica subsp. *enterica* serovar *Panama str. ATCC 7378*	2300	15968	99	0	98.4	4555899	CP074210.1
Salmonella enterica subsp. Enterica serovar London	200	15951	99	0	98.4	4708192	CP141256.1

Sa*lmonella enterica*	2300	15935	99	0	98.4	4573982	CP111088.1
Sa*lmonella enterica*	2300	15358	99	0	98.4	4688830	CP030223.1
Sa*lmonella enterica*	2300	16068	99	0	98.4	4780334	CP090223.1



Based on the 16S rRNA gene sequence analysis and phylogenetic tree construction, the isolate was identified as a strain of *Salmonella enterica* subsp. *enterica* serovar *Typhi*. The results suggest a close evolutionary relationship between the isolate and other *S. enterica* strains. These findings provide valuable insights into the genetic diversity and phylogenetic relationships among *Salmonella* species. More importantly, the bacterial strain was isolated from meat sample, so there are chances that the population may be affected this type of zoonotic typhoid fever by consuming the affected meat.

The BLAST result reveals a strong match between the query sequence and the *Salmonella enterica* serovar *Typhi* genome, with a score of 2305 bits (1248) and an expect value of 0.0, indicating a highly significant alignment. The query sequence shares 98% identity with the subject sequence, with 1293 out of 1313 bases matching, and only 14 gaps (1%) observed in the alignment. The alignment occurs on the minus strand of the subject sequence, as indicated by the Plus/Minus strand orientation (https://blast.ncbi.nlm.nih.gov/Blast.cgi). These results suggest that the query sequence is likely a part of the *S. typhi* genome, and the high degree of similarity implies that it may be a conserved region or a gene of interest. The small number of gaps further supports the robustness and reliability of the alignment. Overall, this BLAST result provides valuable insights into the genomic characteristics of *S. typhi* and can inform further research on this pathogen.

Salmonella enterica is a gram-negative, rod-shaped bacterium belonging to the *Enterobacteriaceae* family. It's a significant human pathogen, causing salmonellosis and typhoid fever. It is a rod-shaped (*Bacilli*) with dimensions of 0.7-1.5 µm in diameter and 2-5 µm in length. Most of the species are motile due to peritrichous flagella. It is a non-spore-forming and non-capsulated (except for *S. typhi* and *S. paratyphi*). Some serotypes have fimbriae, aiding in

adhesion to host surfaces. It is a facultative anaerobe, capable of producing energy through aerobic respiration or fermentation. This strain is positive for catalase, H_2S production, and lysine decarboxylase and is reported negative for oxidase and indole production biochemical tests. Besides its enzymatic bioassays, it ferments glucose, mannitol and sorbitol producing organic acids and gases. It is mostly found inhabiting human and animal intestines and contaminating the biosphere for human population. If it established its colony, it propagates through fecal-oral route from infected person to others and also through contaminated food-stuff. It is responsible for causing inflammation in intestinal epithelial tissue layer in humans. This bacterium is primarily responsible for food poisoning (gastroenteritis), systemic infections (typhoid fever), and bloodstream infectious disease (bacteremia). It is comprised of single circular chromosome and over 2,500 serotypes have been identified through O, H and Vi antigens.

Score:2305
bits (1248),
Expect:0.0,
Identities:1293/1313(98%),
Gaps:14/1313(1%),
Strand: Plus/Minus

Query 1
AAGTGGTAAGCGCCCTCCCGAAGGTTAAGCTACCTACTTCTTTTGCAACCCACTCC
CATG 60
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 288944
AAGTGGTAAGCGCCCTCCCGAAGGTTAAGCTACCTACTTCTTTTGCAACCCACTCC
CATG 288885

Query 61
GTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGGCATTCTGA
TCCACG 120
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 288884
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TCCACG 288825

Query 121
ATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTAC
GACGC 180
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 288824
ATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTAC
GACGC 288765

Query 181
ACTTTATGAGGTCCGCTTGCTCTCGCGAGGTCGCTTCTCTTTGTATGCGCCATTG
TAGCA 240
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 288764
ACTTTATGAGGTCCGCTTGCTCTCGCGAGGTCGCTTCTCTTTGTATGCGCCATTG

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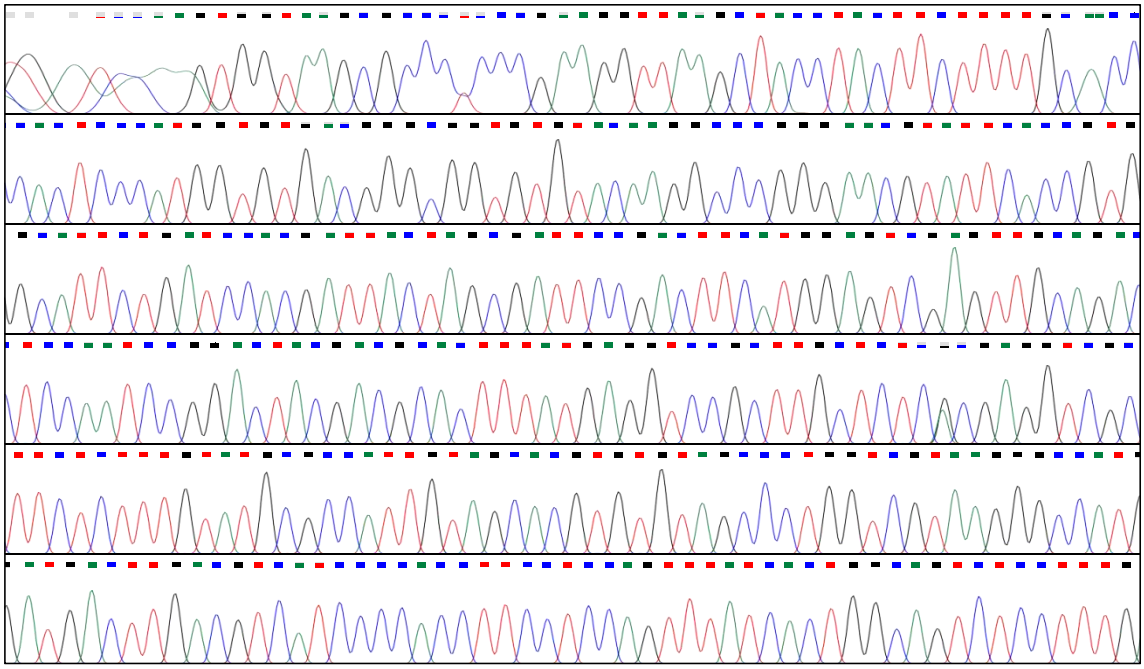

SPOT TEST

The presence of bacteriophages against *S. enterica* was detected in sewage water. Spot tests revealed infective bacteriophage, provide lytic zone.

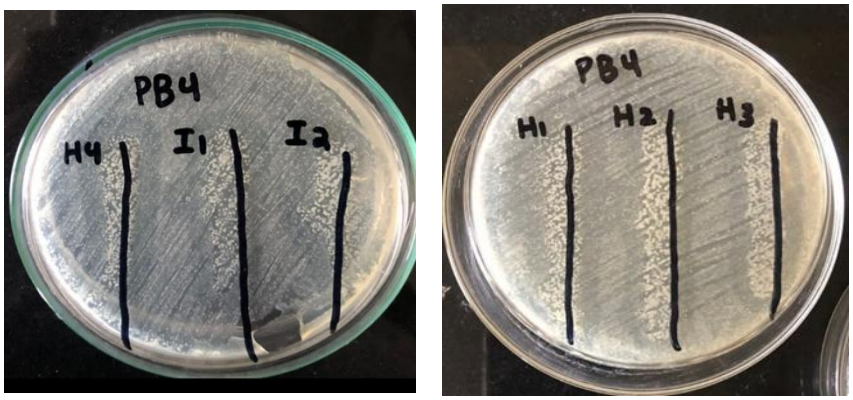

FIGURE 8: SPOT TEST RESULTS

PURIFICATION OF BACTERIOPHAGES PRODUCED CLEAR TRANSPARENT PLAQUES

On the double layer agar plate, the isolated phage produced a circular, transparent plaque against *S. enterica*. The plaque was composed of two circles, the outer circle encircling the fully transparent inner circle. A hazy layer like this surrounding plaque is a sign that bacteriophages are producing the depolymerase enzyme. The halo surrounding the plaque indicates the production of soluble enzymes by the phage, like depolymerase, which liberates the bacterial host cell from its capsule.

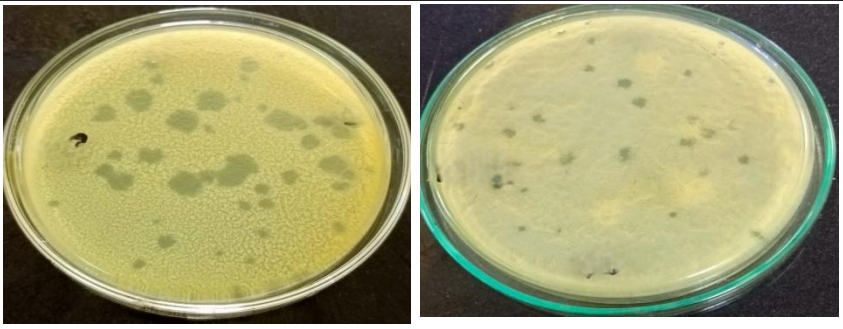

FIGURE 1: PURIFIED BACTERIOPHAGES AGAINST *S. ENTERICA*

DISCUSSION

A major worldwide health problem is antibiotic resistance, especially in foodborne bacteria found in meat microbiomes. Using bacteriophages (phages) in conjunction with antibiotics is one of the novel strategies to fight illnesses that have become necessary due to the rise of multidrug-resistant bacteria. This tactic targets resistant bacterial strains while reducing the possibility of further resistance development by utilizing the synergistic potential of phage-antibiotic combinatorial treatment. A dynamic equilibrium where phages, antibiotics, and bacterial hosts interact to reduce resistance while preserving microbial balance is introduced by the idea of metastable host-phage-pathogen triads (Jajere, 2019). Deciphering epistatic design the non-additive genetic interactions that influence viruses' evolutionary paths under combined selection pressures is necessary to comprehend these interactions. The research of these triadic relationships must concentrate on the meat microbiome as it is a vital reservoir for bacteria resistant to antibiotics. The mapping of genetic and functional relationships between resistance genes, phage predation mechanisms, and antibiotic modes of action is made possible by the application of network biology to antibiotic resistance. These networks highlight important nodes where treatments might stop the spread of resistance while maintaining beneficial bacteria (Frye and Jackson, 2013).

Sublethal antibiotic doses have been shown to increase phage infectivity by changing bacterial physiology, a phenomenon known as phage-antibiotic synergy (PAS) in a variety of bacterial species. For example, quinolones interfere with DNA repair processes, which increases the effectiveness of phage replication, whereas beta-lactam antibiotics weaken cell walls, which makes it easier to inject phage DNA. These interactions imply that choosing antibiotics that enhance rather than inhibit phage activity might maximize combinatorial therapy. The stability of host-phage-pathogen triads, in which phages coevolve with bacterial hosts without causing excessive resistance or the collapse of the microbial community, is necessary for the success of such tactics (Carattoli, 2003). By ensuring that resistance mutations have fitness costs, metastability in these triads prevents unrestrained bacterial adaptation. Because mutations that provide phage resistance may also enhance sensitivity to antibiotics, and vice versa, epistatic interactions are essential to maintaining this equilibrium. The use of antibiotics in cattle has increased the spread of resistance genes in the complex ecosystem that is the meat microbiome. Numerous resistance determinants in meat-associated bacteria have been found by metagenomic research; they are frequently connected to mobile genetic elements that promote horizontal gene transfer (Capparelli *et al.*, 2010).

These resistant strains can be targeted by phages, which are naturally occurring bacterial predators that can also operate as gene exchange vectors. Therefore, it is important to carefully

control how phages and antibiotics interact to prevent unforeseen outcomes like the expansion of virulence factors or the emergence of novel resistance mechanisms. Network-based methods assist in locating possible intervention sites and high-risk resistance routes. For instance, focusing on hub genes that facilitate phage-antibiotic cross-resistance may improve the effectiveness of therapy. Furthermore, the development of reliable combinatorial treatments can be guided by the ability of machine learning models trained on resistance network data to predict evolutionary paths (Capparelli *et al*., 2010).

Even while phage-antibiotic combinations show potential, there are still obstacles in the way of their actual use. Large-scale applications are complicated by phage specificity, regulatory barriers, and bacterial variety in food microbiomes. To make sure that phages do not compromise food safety or quality, the ecological effects of introducing them into meat production systems must also be assessed. In order to track the emergence of resistance, future studies should concentrate on high-throughput screening of phage-antibiotic pairings against meatborne pathogens in conjunction with genomic surveillance (Bao *et al*., 2011). Longitudinal research monitoring triad stability in meat microbiome simulations may shed light on potential hazards and long-term effectiveness. The integration of systems biology and synthetic ecology approaches may further refine these strategies, enabling the rational design of phage-antibiotic regimens that sustain metastability while curbing resistance (Bao *et al*., 2011).

In conclusion meat microbiome antibiotic resistance networks constitute a crucial area for public health and food safety. Using metastable host-phage-pathogen triads to investigate phage-antibiotic combinatorial therapy presents a viable way to reduce resistance. To create long-lasting antimicrobial solutions, it will be crucial to decipher the epistatic patterns that underlie these interactions. In order to destroy resistance while preserving ecological balance, researchers can pinpoint the best intervention sites by utilizing network biology, evolutionary modeling, and metagenomic monitoring. Phage treatment and antibiotic science have a lot of potential to continuously merging, but this will require multidisciplinary cooperation and thorough experimental validation.

CONCLUSION

The investigation of antibiotic resistance networks in meat microbiomes' phage-antibiotic combinatorial metastable host-phage-pathogen triads offers vital information on the intricate interactions between antibiotic pressures, bacterial pathogens, and their phage predators. We discover new ways to undermine pathogenic resilience by unraveling epistatic design, where genetic interactions influence virulence and resistance. A viable strategy to fight multidrug-resistant bacteria in food systems is the destabilization of resistance networks by phage-antibiotic synergy, according to key results. The metastable triads bring attention to dynamic evolutionary conflicts in which specific interventions may tip the scales in favor of food safety and microbiome restoration. Future studies should concentrate on translational applications in phage treatment and antibiotic stewardship, as well as predictive modeling of epistatic interactions. By utilizing these discoveries, we may create more efficient combi-treatment plans to reduce antibiotic resistance in meat microbiomes, guaranteeing safer food production and safeguarding the general public's health.

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