Prevalence, Antibiotic Resistance, and Implications for Public Health due to *Salmonella*Contamination in Food Products

Abstract:

In this researchwe investigated the prevalence of *Salmonella* species in meat, chicken, fish, prawn, and milk samples, and their resistance to antibiotics was examined. The study findings demonstrated varying levels of *Salmonella* contamination in different food types, with meat and chicken samples showing higher prevalence rates compared to fish, prawn, and milk. Notably, the isolated *Salmonella* strains exhibited resistance to multiple antibiotics, raising concerns about the potential dissemination of antibiotic-resistant strains through the food chain and its implications for public health.The study underscores the critical importance of continuous surveillance in monitoring *Salmonella* prevalence and antibiotic resistance in food products. It also highlights the significance of promoting responsible antibiotic usage in both human and veterinary medicine to safeguard food safety and public health.

Keywords:

Enterobacteriaceae, Oxygen tolerance, *Salmonella enterica*, *Salmonella bongori*, Serogroups, Serovars, *S.pullorum*, *S.Gallinarum*, Salmonellosis, Bacteraemia.

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Introduction:

Salmonella a member of the family *Enterobacteriaceae* is a gram-negative, rod-shaped motile bacterium. *Salmonella* can survive with or without oxygen. *Salmonella* is comprised of two known species: *Salmonella* enterica and *Salmonella bongori*. Salmonella enterica consists of six subspecies: *enterica, salamae, arizonae, diarizonae, houtenae*, and *indica*. The subspecies are categorized into serogroups and serovars. Serogroups are based on O antigens while serovars are based on H antigens. Different strains' identification depends on O and H antigens (1,2). *Salmonella* enterica is the most common infection reported in warm-blooded animals (2).*Salmonella* as a source of infection poses a significant threat to food-producing animals, poultry in particular, and has direct implications for their global market and food products derived from them. *Salmonella* serovars like *S.pullorum* and *S.Gallinarum* cause high mortality in young and older birds. Poultry carries serovars of *Salmonella* in their gastrointestinal tracts without showing any signs of illness, increasing the risk of contamination in raw products from animals during slaughtering and processing(3).Salmonellosis can be classified as a minor or major disease. Minor Salmonellosis arises from non-typhoidal strains of *Salmonella* and is characterised by self-limiting diarrhoea and rarely progresses to bacteraemia. Whereas Major Salmonellosis causes typhoid fever (1). The bloodstream is primarily affected by typhoid fever. Non-typhoidal strains of *Salmonella* cause gastroenteritis in individuals with healthy immune systems (2).

Salmonella enters poultry flocks by various means such as from environment, feed, vectors or primarily due to inadequate biosecurity measures (5). *The salmonella* problem has intensified due to the enormous animal and human food production and the rapid international trade of livestock. Detecting *Salmonella* in live animals, animal-derived food and environment is crucial for developing effective control and prevention strategies(6,5).Antibiotic resistance in *Salmonella* is a global concern. The prevalence of antibiotic-resistant *Salmonella* species is largely attributed to the use of antibiotics in animal farms (5). The Centres for Disease Control and Prevention (CDC) has classified antibiotic-resistant *S.typhi* as a serious disease and stresses the importance of monitoring and preventive measures to control the spread of resistant strains(9).

The increased utilization of fluoroquinolone antibiotics, like ciprofloxacin, has led to resistant *Salmonella* strains or less susceptible strains for this particular antibiotic(7,9). The various mechanisms like PMQR elements, mutations in target genes

and drug efflux systems, contribute to the development of fluoroquinolone resistance in *Salmonella*(7). The emergence of resistant strains is not only limited to humans but to animals as well. Reports indicate that extensive use of antibiotics in treating animal diseases or as growth promoters have led to drug-resistant bacteria dissemination and occurrence (9).
This study aimed to assess the prevalence of multi-drug resistant *Salmonella* strains from various sources such as meat from various sources, fish, eggs, etc. This study aimed to determine drug resistance patterns and the extent of spread of multi-drug resistant strain of *Salmonella*.

Methods and Materials:

Sample Collection: For this study, we targeted consumers from three main regions in Mumbai: the western suburbs, central, and harbour areas. To ensure a diverse and representative sample, we randomly selected four towns from each of the three areas. The chosen towns for sample collection were Vasai, Borivali, Santacruz, Lower Parel, Matunga, Sion, Ghatkopar, Kurla, Chembur, Wadala, Vashi, and Panvel. We collected 34 meat samples,29 fish samples,16 chicken samples,10 prawn samples and 9 milk samples, from various shops across Mumbai.

Pre-enrichment, enrichment and isolation on media:
The egg shells were first wiped and sterilized with 70% alcohol and then cracked open under laminar airflow. The yolks and whites were separated carefully and the yolks were individually inoculated into 50mL of sterile Buffered Peptone Water (BPW). The chicken, meat, fish and prawn samples were minced well using sterile scalpels and then inoculated in 50mL BPW. After 24hrs pre-enrichment at 37^{o}C on shaker conditions,5mL from each of the 24 samples were re-inoculated into 45mL of *Salmonella* Selection Broth (HiMedia) and kept at 37^{o}C for 24hrs. Brilliant Green (0.07g/L) was added into SS broth to inhibit Gram-positive organisms' growth.
The milk samples were inoculated into selective enrichment broth like Buffered Peptone Water(BPW) and incubated at 37 ^{o}C for 24 hrs. Selective enrichment was done by transferring a portion of the pre-enriched broth into a selective enrichment medium like Rappaport Vassiliadis(RV) broth and incubating at 41.5^{o}C for 24 hrs.
After enrichment, a loopful of samples were streaked on Xylose Lysine Deoxycholate media (HiMedia) by hexagonal method and kept at 37^{o}C for 24hrs. This process was done in triplicates for all 24 samples. Colonies were repeatedly streaked on fresh medium until pure (axenic) isolates were obtained.

Identification of Selected Isolates:

We subjected presumptive Salmonella isolates to a battery of identification tests, including oxidase, catalase, sugar fermentation, indole, methyl red, Voges Proskauer, citrate utilization, Triple Sugar Iron (TSI), urease, nitrate reduction, lysine decarboxylase, ornithine decarboxylase, malonate, and gelatinase. We performed sugar fermentation tests using various sugars, such as glucose, lactose, maltose, sucrose, xylose, and mannitol. After 24hrs incubation at 37^{o}C, only the confirmed Salmonella isolates were subjected to antibiotic resistance screening.

Antibiotic resistance screening:
As with any antimicrobial susceptibility testing, we utilized Mueller Hinton broth for all isolates. The antibiotics were procured from pharmacies/distributors after written approval by a practising doctor since antibiotics are not given without a prescription. Gentamycin was available as a sterile injectable, the rest were available as sterile powders. 1mg/ml was prepared as stock for all and kept under storage at 4^{o} C as per the manufacturer's instructions. A combination of guidelines with modifications was used. MDR phenotype was estimated to be resistant to 3 or more 3 antibiotics at 10 µg/mL. 100µL of 106 cfu/mL of all the confirmed Salmonella isolates were inoculated into sterile 96 well microtiter plates containing 100µL antibiotics at a final concentration of 10µg/mL. For this screening, 10 antibiotics were employed namely Gentamycin, Nalidixic acid, trimethoprim, tetracycline, Ciprofloxacin, Chloramphenicol, Erythromycin, ampicillin, ofloxacin and Cefoperazone. The plates were incubated at 37^{o}C for 24hrs following which growth was checked using a Resazurin assay.

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5µL of Resazurin dye was added into each well and positive growth was indicated by a change in Resazurin dye colour from blue to bright pink.

Resistance-tolerance-sensitivity profiles:

Variations or changes in Resazurin dye colour after incubation were also recorded as it indicated varying degrees of resistance-tolerance-sensitivity pattern. Optical densities were compared with positive (media with respective isolate, without antibiotics) and negative controls (media with saline) to assess each pattern. Prominent blue was considered as sensitive, light purple-pink as higher tolerance, dark purple as lower tolerance and bright pink-pinkish red as resistant.

Multiple Antibiotic Resistance (MAR) indices: Multiple antibiotic resistance index or MAR index was calculated as a/b, where a is the number of antibiotics against which resistance was observed and b is the total number of antibiotics employed for the present study. The MAR index of more than 0.2 is considered a health hazard due to the possibility of high contamination from multiple sources.

Results and Discussion:

Salmonella is often found in contaminated food, particularly in raw or undercooked meat, poultry, eggs, and unpasteurized dairy products. Screening in food production facilities plays a vital role in identifying tainted products, thus preventing widespread foodborne illnesses. Salmonella infections can lead to gastroenteritis, manifesting as symptoms like diarrhoea, cramps, and fever, which poses significant risks to vulnerable populations. By conducting screenings, outbreaks can be detected and controlled, safeguarding public health and lessening the burden on healthcare systems. Additionally, the screening process yields essential data that allows health authorities to track trends, pinpoint potential contamination sources, and develop targeted interventions. Adhering to food safety regulations is imperative for businesses to ensure consumer safety and uphold their reputation. Regular screening serves as a preventive measure, averting economic losses resulting from outbreaks, which may include recalls and damage to brand reputation.

	Number of Samples	Colony characteristic
Isolates from meat	34	Rod
Isolates from fish	29	Rod
Isolates from chicken	16	Rod
Isolates from prawn	10	Rod
Isolates from milk	9	Rod

Table 1: The above table shows the colony characteristics and bifurcation of *Salmonella* isolates obtained from meat, chicken, fish, prawn and milk samples.

The primary objective of the study was to identify Salmonella as the predominant bacterium and comprehend its prevalence in Mumbai. However, the study did not focus on identifying individual Salmonella species; instead, it categorized 98 confirmed Salmonella isolates into 5 groups based on variations in the samples. The identification process adhered to Bergey's Manual of Determinative Bacteriology, specifically utilizing Group 5: Family Enterobacteriaceae Lactose negative flowchart. Among the 98 Salmonella isolates, 34 (34.69%) were obtained from meat, 29 (29.59%) from fish, 16 (16.32%) from chicken, 10 (10.20%) from prawn, and 9 (9.18%) from milk samples.

Biochemical tests were utilized to verify the identity of the isolates as Salmonella. Each Salmonella isolate was then subjected to antibiotic sensitivity screening, where 12 different antibiotics were tested, including Gentamycin, Nalidixic acid, trimethoprim, tetracycline, Ciprofloxacin, Chloramphenicol, Erythromycin, ampicillin, ofloxacin, and Cefoperazone. The micro broth dilution technique was employed for this screening. To ensure consistency and comparability with other research studies, a standardized concentration of 10 µg/mL was selected for all tested antibiotics. This approach aimed to

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promote uniformity in the research and facilitate a comprehensive understanding of the antibiotic resistance patterns exhibited by the isolates.

Antibiotics	**Number of resistant organisms**	**Number of sensitive organisms**
Gentamycin	-	34(100%)
Nalidixic acid	15(44.11%)	19(55.88%)
Trimethoprim	4(11.76%)	30(88.23%)
Tetracycline	3(8.82%)	31(91.17%)
Ciprofloxacin	-	34(100%)
Chloramphenicol	-	34(100%)
Erythromycin	3(8.82%)	31(91.17%)
Ampicillin	10(29.41%)	30(88.23%)
Ofloxacin	-	34(100%)
Cefoperazone	-	34(100%)

Table 2: The above table shows the efficacy of individual antibiotics against 34 *Salmonella* isolates from meat samples.

Among the 34 meat isolates listed in Table 2, it was observed that all of them exhibited 100% resistance to five specific antibiotics. However, these isolates were generally found to be sensitive to almost all other antibiotics tested. Notably, Gentamycin, Ciprofloxacin, Chloramphenicol, Ofloxacin, and Cefoperazone demonstrated a remarkable 100% sensitivity against Salmonella derived from the meat isolates. Tetracycline and Erythromycin displayed a slightly lower sensitivity rate of 91.17%. On the other hand, Nalidixic acid, Trimethoprim, and Ampicillin exhibited sensitivity rates of 55.88%, 88.23%, and 88.23%, respectively.

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Antibiotics	**Number of resistant organisms**	**Number of sensitive organisms**

Gentamycin	-	29(100%)
Nalidixic acid	10(34.48%)	19(65.51%)
Trimethoprim	22(75.86%)	7(24.13%)
Tetracycline	23(79.31%)	6(20.68%)
Ciprofloxacin	-	29(100%)
Chloramphenicol	8(27.58%)	21(72.41%)
Erythromycin	5(17.24%)	24(82.75%)
Ampicillin	18(62.06%)	11(37.93%)
Ofloxacin	-	29(100%)
Cefoperazone	2(6.89%)	27(93.10%)

Table 3: The above table shows the efficacy of individual antibiotics against 29 *Salmonella* isolates from fish samples

Among the 29 fish isolates listed in Table 3, it was observed that all of them showed 100% resistance to two specific antibiotics. Notably, Gentamycin and Ofloxacin demonstrated an outstanding 100% sensitivity against Salmonella derived from the fish isolates. On the other hand, Chloramphenicol, Erythromycin, and Cefoperazone displayed slightly lower sensitivity rates of 72.41%, 82.75%, and 93.10%, respectively. In contrast, Nalidixic acid, Trimethoprim, Tetracycline, and Ampicillin exhibited lower sensitivity rates of 65.51%, 24.13%, 20.68%, and 37.93%, respectively.

Antibiotics	**Number of resistant organisms**	**Number of sensitive organisms**
Gentamycin	-	16(100%)
Nalidixic acid	8(50%)	8(50%)
Trimethoprim	12(75%)	4(25%)
Tetracycline	15(93.75%)	1(6.25%)
Ciprofloxacin	1(6.25%)	15(93.75%)
Chloramphenicol	1(6.25%)	15(93.75%)
Erythromycin	3(18.75%)	12(75%)
Ampicillin	14(87.5%)	2(12.5%)
Ofloxacin	-	16(100%)
Cefoperazone	1(6.25%)	15(93.75%)

Table 4: The above table shows the efficacy of individual antibiotics against 16 *Salmonella* isolates from chicken samples.

Out of the 16 chicken isolates listed in Table 4, it was found that all of them were resistant to two specific antibiotics, without exception. However, when it came to other antibiotics tested, these isolates were generally sensitive to them. It's

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worth noting that Gentamycin and Ofloxacin showed an outstanding 100% sensitivity against Salmonella obtained from the chicken isolates. On the other hand, Ciprofloxacin, Chloramphenicol, and Cefoperazone displayed a slightly lower sensitivity rate of 93.75%. In contrast, Nalidixic acid, Trimethoprim, Tetracycline, Erythromycin, and Ampicillin exhibited different sensitivity rates: 50%, 25%, 6.25%, 75%, and 12.5%, respectively.

Antibiotics	**Number of resistant organisms**	**Number of sensitive organisms**
Gentamycin	-	10(100%)
Nalidixic acid	3(30%)	7(70%)
Trimethoprim	9(90%)	1(10%)
Tetracycline	9(90%)	1(10%)
Ciprofloxacin	1(10%)	9(90%)
Chloramphenicol	-	10(100%)
Erythromycin	2(20%)	8(80%)
Ampicillin	6(60%)	4(40%)
Ofloxacin	1(10%)	9(90%)
Cefoperazone	9(90%)	1(10%)

Table 5: The above table shows the efficacy of individual antibiotics against 10 *Salmonella* isolates from prawn samples.

Among the 10 prawn isolates listed in Table 5, it was observed that all of them exhibited resistance to two specific antibiotics, without any exceptions. However, when it came to the other antibiotics tested, these isolates were generally found to be sensitive to them. Notably, both Gentamycin and Chloramphenicol demonstrated an outstanding 100% sensitivity against Salmonella obtained from the prawn isolates. Ciprofloxacin and Ofloxacin displayed a slightly lower sensitivity rate of 90%. Ampicillin, Nalidixic acid, and Erythromycin showed sensitivity rates of 40%, 70%, and 80%, respectively. In contrast, Trimethoprim, Tetracycline, and Cefoperazone exhibited a very low sensitivity of only 10%.

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Antibiotics	**Number of resistant organisms**	**Number of sensitive organisms**

Gentamycin	1(11.11%)	8(88.88%)
Nalidixic acid	-	9(100%)
Trimethoprim	3(33.33%)	6(66.66%)
Tetracycline	6(66.66%)	3(33.33%)
Ciprofloxacin	-	9(100%)
Chloramphenicol	2(22.22%)	7(77.77%)
Erythromycin	1(11.11%)	8(88.88%)
Ampicillin	8(88.88%)	1(11.11%)
Ofloxacin	-	9(100%)
Cefoperazone	-	9(100%)

Table 6: The above table shows the efficacy of individual antibiotics against 9 *Salmonella* isolates from milk samples.

Among the 9 milk isolates listed in Table 6, it was observed that all of them exhibited 100% resistance to Nalidixic acid, Ciprofloxacin, Ofloxacin, and Cefoperazone. However, there were variations in their sensitivity to other antibiotics. Both Gentamycin and Erythromycin demonstrated a moderate sensitivity rate of 88.88% each. On the other hand, Ampicillin, Tetracycline, Trimethoprim, Chloramphenicol, and Erythromycin displayed different sensitivity rates of 11.11%, 33.33%, 66.66%, 77.77%, and 88.88%, respectively.

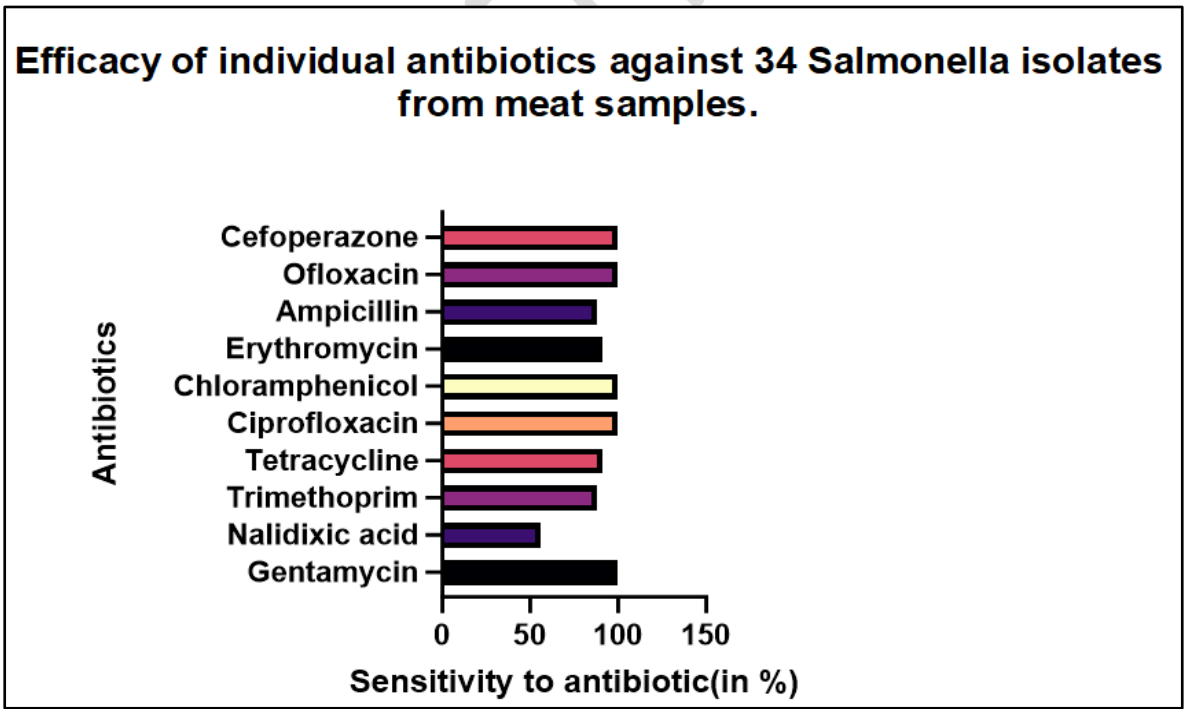

Figure 1: Graph showing the efficacy of individual antibiotics against 34 salmonella isolates from meat samples.

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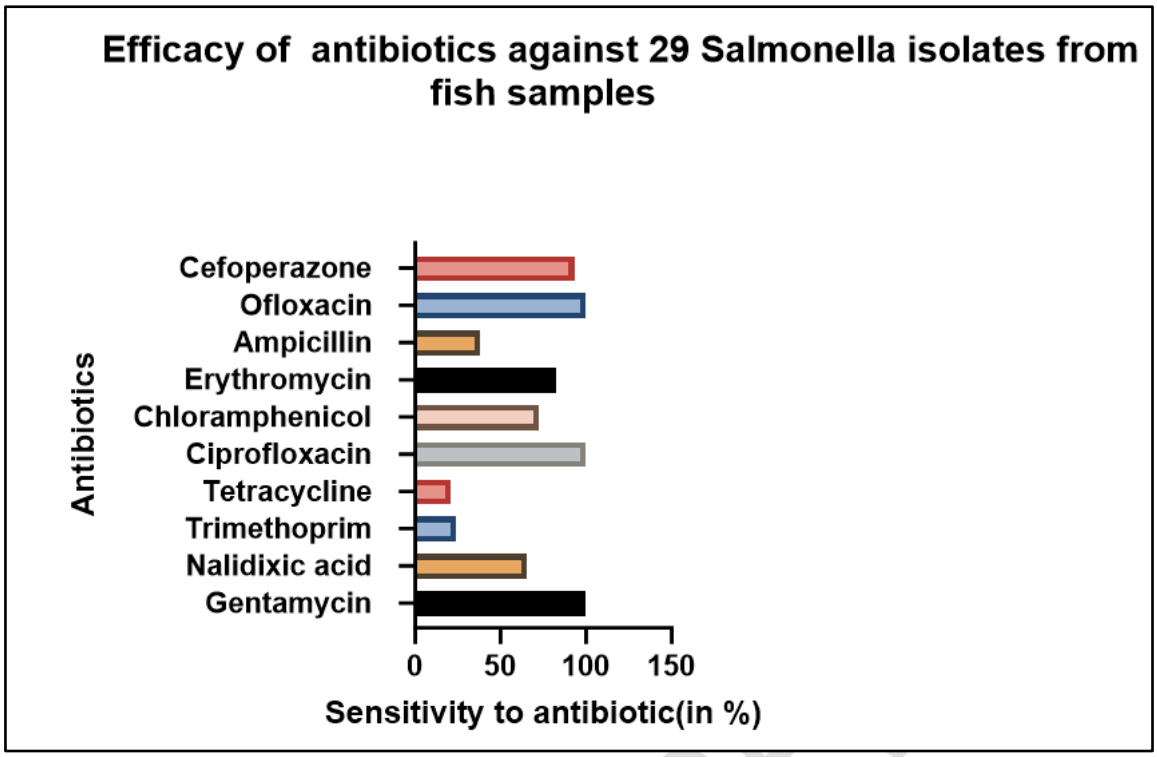

Figure 2: Graph showing the efficacy of individual antibiotics against 29 salmonella isolates from fish samples.

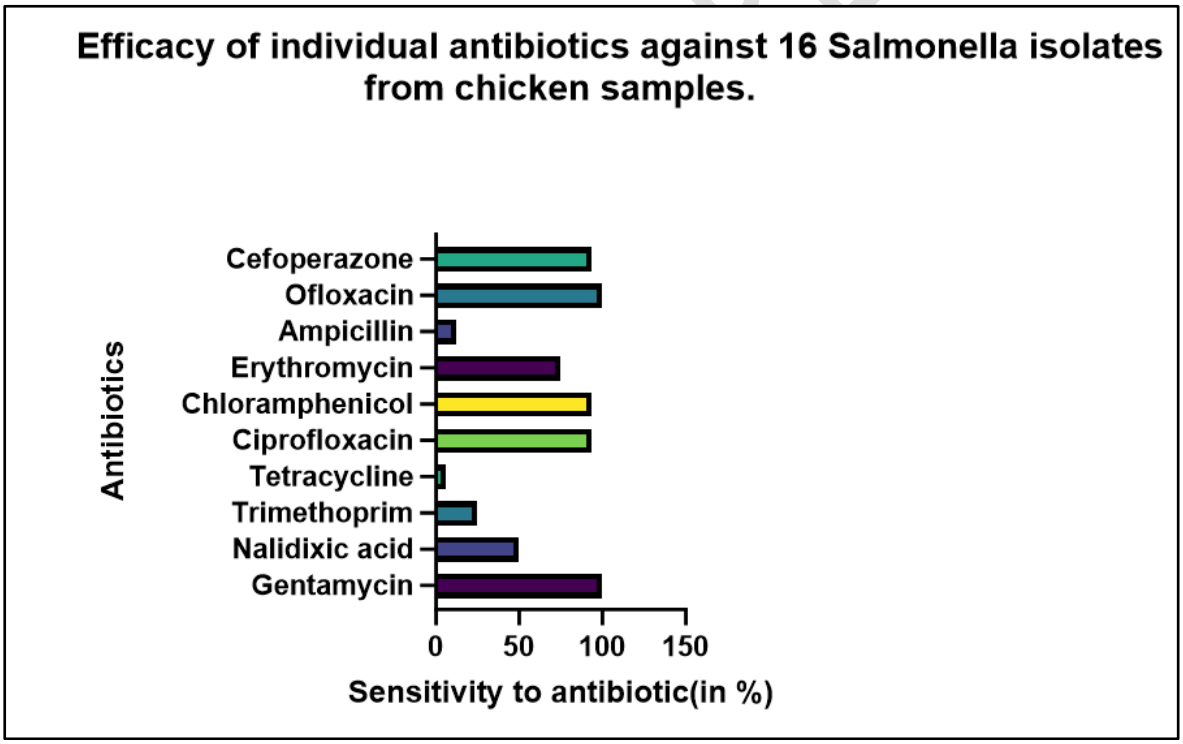

Figure 3: Graph showing the efficacy of individual antibiotics against 16 salmonella isolates from chicken samples.

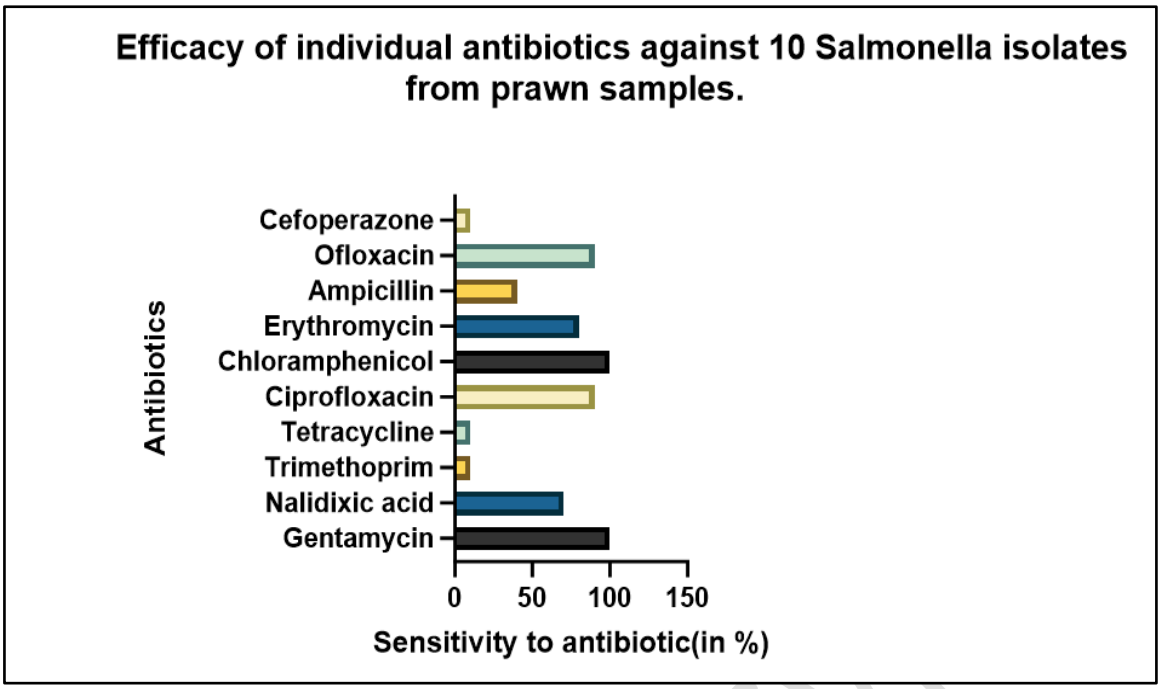

Figure 4: Graph showing the efficacy of individual antibiotics against 10 salmonella isolates from prawn samples.

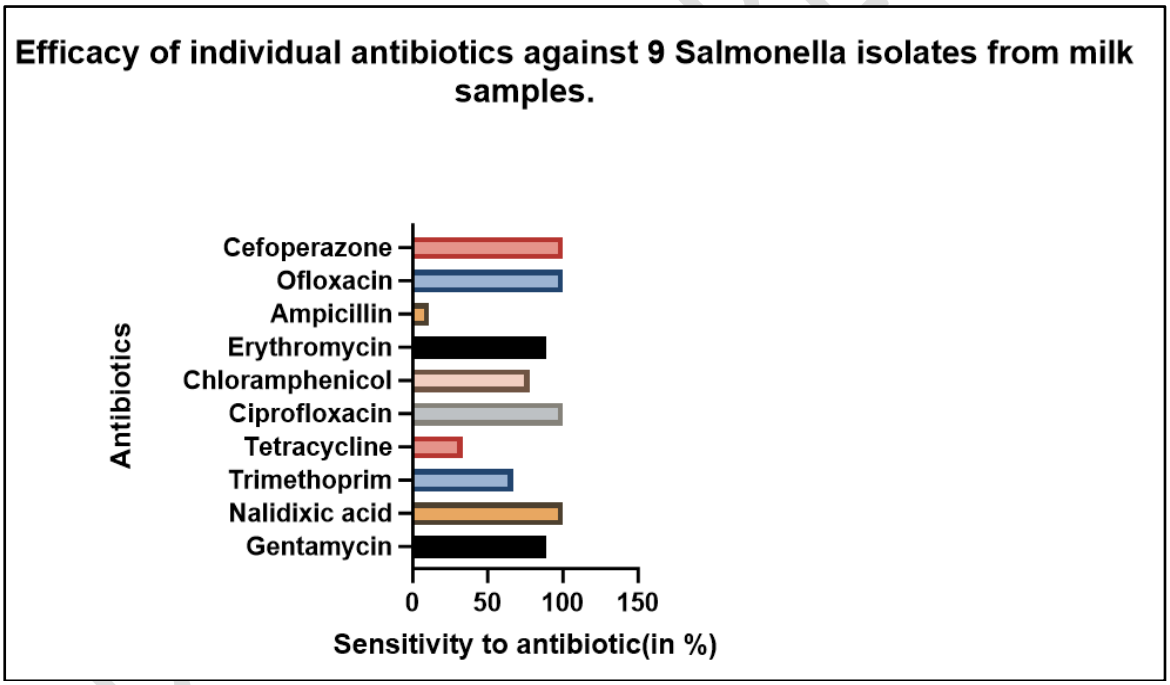

Figure 5: Graph showing the efficacy of individual antibiotics against 9 salmonella isolates from milk samples.

Salmonella species have earned a reputation for their capacity to develop resistance to a diverse range of antibiotics. This resistance can emerge through various mechanisms, including genetic mutations within bacterial genes and the acquisition of resistance genes from other bacteria through a process known as horizontal gene transfer.The rise of antibiotic resistance in Salmonella presents substantial challenges in managing Salmonella infections in both human and animal populations. It can result in more severe and prolonged illnesses, escalate healthcare expenditures, and lead to higher mortality rates.The excessive and improper use of antibiotics in human medicine, agriculture, and animal husbandry plays a pivotal role in fostering the development of antibiotic resistance not only in Salmonella but also in other bacterial species. When antibiotics are utilized inappropriately or discontinued prematurely, bacteria can adapt and develop resistance, rendering infections harder to treat effectively.To address antibiotic resistance in Salmonella species and other infectious agents, it is imperative

to promote responsible antibiotic usage in both human and veterinary medical settings. Additionally, implementing robust surveillance programs becomes crucial to closely monitor the prevalence and dissemination of antibiotic-resistant Salmonella strains. Such vigilance enables timely intervention and the deployment of effective control measures to mitigate the spread of resistance.

Conclusion:

Salmonella, a gram-negative bacterium, was prevalent across meat, fish, chicken, prawn, and milk samples in Mumbai, highlighting widespread food contamination. Alarmingly, many Salmonella strains showed resistance to multiple antibiotics, emphasizing the urgent global issue of antimicrobial resistance. The indiscriminate use of antibiotics in animal farming and possibly human medicine is a significant contributing factor. This resistance compromises treatment efficacy, elevates healthcare costs, and poses a severe public health threat. Enhanced surveillance and responsible antibiotic stewardship are essential to address this growing challenge and protect public health.

REFERENCES:

1:(Popa & Ioan Popa, 2021) Popa, G. L., & Papa, M. I. (2021). *Salmonella* spp. infection - a continuous threat worldwide. *Germs*, *11*(1), 88–96. https://doi.org/10.18683/germs.2021.1244

2:(Ikhimiukor et al., 2022)Ikhimiukor, O. O., Oaikhena, A. O., Afolayan, A. O., Fadeyi, A., Kehinde, A., Ogunleye, V. O., Aboderin, A. O., Oduyebo, O. O., Elikwu, C. J., Odih, E. E., Komolafe, I., Argimón, S., Egwuenu, A., Adebiyi, I., Sadare, O. A., Okwor, T., Kekre, M., Underwood, A., Ihekweazu, C., Aanensen, D. M., … Okeke, I. N. (2022). Genomic characterization of invasive typhoidal and non-typhoidal Salmonella in southwestern Nigeria. *PLoS neglected tropical diseases*, *16*(8), e0010716. https://doi.org/10.1371/journal.pntd.0010716

3:(Uro, 2019)TekalignWoldehana Uro, International Journal of Advanced Research in Biological Sciences.

DOI: http://dx.doi.org/10.22192/ijarbs.2019.06.10.008

4:(Thomas et al., 2020)Thomas, K. M., de Glanville, W. A., Barker, G. C., Benschop, J., Buza, J. J., Cleaveland, S., Davis, M. A., French, N. P., Mmbaga, B. T., Prinsen, G., Swai, E. S., Zadoks, R. N., & Crump, J. A. (2020). Prevalence of Campylobacter and Salmonella in African food animals and meat: A systematic review and meta-analysis. *International journal of food microbiology*, *315*, 108382. https://doi.org/10.1016/j.ijfoodmicro.2019.108382

5:(Wang et al., 2020) Wang, J., Li, J., Liu, F., Cheng, Y., & Su, J. (2020). Characterization of *Salmonella enterica* Isolates from Diseased Poultry in Northern China between 2014 and 2018. *Pathogens (Basel, Switzerland)*, *9*(2), 95. https://doi.org/10.3390/pathogens9020095

6:(Buytaers et al., 2021)Buytaers, F. E., Saltykova, A., Mattheus, W., Verhaegen, B., Roosens, N. H. C., Vanneste, K., Laisnez, V., Hammami, N., Pochet, B., Cantaert, V., Marchal, K., Denayer, S., & De Keersmaecker, S. C. J. (2021). Application of a strain-level shotgun metagenomics approach on food samples: resolution of the source of a *Salmonella* food-borne outbreak. *Microbial genomics*, *7*(4), 000547. https://doi.org/10.1099/mgen.0.000547

7: (Bandyopadhyay, Jha, & Kumar, 2019)Bandyopadhyay M, Jha V, Kumar B(2019). Molecular Insights into Antimicrobial Resistance in Salmonella Species. ACTA SCIENTIFIC MICROBIOLOGY (ISSN: 2581-3226).

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8: (Bandyopadhyay, Jha, Ajitkumar, et al., 2019)Bandyopadhyay, M., Jha, V., Jhangiani . A Prevalence Study and Resistance Profiles of Multi-Drug Resistant Salmonella Obtained from Poultry Across Mumbai Region

9:(Mutai et al., 2018)Multi-drug resistant Salmonella enterica serovar Typhi isolates with reduced susceptibility to ciprofloxacin in KenyaMutai W, Muigai A, Kariuki SBMC Microbiology (2018) 18(1)

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