*Pak. J. Biotechnol. Vol. 20(1), 131-138, **2023*** http://doi.org/10.34016/pjbt.2023.20.01.783

Research Article

OPEN ACCESS

Available on http://www.pjbt.org
Pakistan Journal of Biotechnology
(PJBT)
(P-ISSN: 1812-1837 and E-ISSN: 2312-7791)

DETERMINATION OF ANTIBIOTIC RESISTANCE OF *SALMONELLA* SPP. ISOLATED FROM ICE CREAM

Maimoona Noor[1], Samreen Riaz[2*], Faiza Saleem[1]

[1]Department of Biotechnology, Lahore College for Women University, Lahore Punjab, Pakistan
[2]Institute of Microbiology and Molecular Genetics, University of Punjab, Lahore, Punjab, Pakistan
Punjab, Pakistan
*Corresponding author email: samreen.mmg@pu.edu.pk

Article Received 25-01-2023, Article Revised 08-03-2023, Article Accepted 26-04-2023.

ABSTRACT

The current study sought to determine the extent of antibiotic resistance and prevalence among *Salmonella* spp. The *Salmonella* spp. Antibiotic resistance threat and bacteriological safety of food goods like ice cream present therapeutic management problems within the public health system. *Salmonella* bacteria are Gram-negative, facultative anaerobic, flagellated bacilli containing antigens like V, Hi, and O. Nearly 1800 serovars have been found. Each of them is categorized as a different species under the current classification system. To obtain the concentration of food borne pathogens, Ice cream was gathered from various locations and put on SS and MacConkey agar to test for the presence of *Salmonella* spp. in ice cream. Azithromycin, gentamycin, cefotaxime, and ciprofloxacin were among the many classes of antibiotics present in the SS agar on which it was also dispersed. To lessen the burden of *Salmonella* spp. prevalence in milk, the study advises farmers and raw milk vendors to adopt substantial interventions in animal husbandry and milk marketing, respectively. Additionally, the enforcement and animal health divisions' active involvement in ensuring prudent antibiotic usage at the farm level may aid in limiting *Salmonella* spp. antimicrobial resistance. Antibiotic resistance might be reduced by concentrating on alternative remedies for treatment of GIT infections including the use of probiotics, organic approaches, and preventing the horizontal gene transfer of resistant genes.

Abbreviations: SS agar: salmonella and Shigella agar, MDR: multi drug resistance

Introduction

Food borne disease has great impact on health of the society. The only method to recognize the cause and source of the food borne diseases and overcome this issue is controlling the cause of spread. The identification of the cause of food borne disease is very difficult and log process including bundle of various steps. This also includes eating pattern and transferring mode of the contaminated foods (Jacobs's *et al.*, 2020). *Salmonella* infection causes fever, diarrhea, and stomach cramps 1 to 3 days after the onset of first infection. Symptoms usually last 4 to 6 days, although in certain cases, the patient may have needed to admit to the hospital. Despite the fact that there are a lot of possible routes, the intake of raw meat is a source of *Salmonella* transmission, or undercooked or raw eggs or poultry are known to be of concern of great significance. *Salmonella* species have wide variety with number in thousands. Different serotypes have been identified, each with its own set of characteristics and their transmission channels. *Salmonella enteritidis*, for example, is frequently connected with eggs, but *Salmonella typhimurium* is related with a broader range of foods. Infections caused by *Salmonella* are more common in the summer because it is climate sensitive. Studies demonstrate that *Salmonella* infections are higher in warm temperature. Furthermore, since *Salmonella* may thrive in food maintained at room temperature, there is a strong biological knowledge of the processes involved. As a result, *Salmonella* infections may rise as the planet warms (Akil *et al.*, 2014). The number of infections is currently reducing across Europe as a result of successful intervention, such as better biosecurity, animal immunization, and slaughtering. *Salmonella* has a close relationship with the environment, particularly the ambient temperature. *Salmonella* reproduction is aided by higher ambient temperatures. Despite this biological process, UK infectious disease specialists believe *Salmonella* is not one of the illnesses most likely to be impacted by change in climate (Lake, 2017). Salmonellosis is regarded as one of the most serious zoonotic diseases caused by bacteria that may affect animals, humans, and fowl. There are around 2500 *Salmonella* serovars in the world (Dróżdż *et al*., 2021). *Salmonella enterica typhi* causes typhoid fever and *Salmonella enterica Paratyphi* cause paratyphoid fever in humans. Whereas, salmonellosis is an umbrella term that encompasses invasive infection with all *Salmonella* serovars as well as the normally gut-confined infections of food poisoning (Alphons *et al*, 2014). Several additional *Salmonella* serovars, rather than para*typhi* and *typhi*, cause non-typhoidal salmonellosis. *Salmonella typhimurium and S. enteritidis*, which may be transferred to humans, are widely found in poultry and animals. Human salmonellosis epidemics have been linked to animal products such as milk, chicken meat, eggs, wild, domestic and aquatic animals, pets, and rodents (Teklemariam *et al*., 2023). It has been shown that the chicken industry may be responsible for up to 50% outbreaks caused by *Salmonella*. Serovars of *Salmonella* have identified as a significant and dangerous food-borne zoonosis with public health implications. Typhoid fever outbreaks and occasional cases in humans are widespread throughout the world (Ferrari *et al.*, 2019). Although gastrointestinal symptoms are the most common sign of typhoid fever, other clinical syndromes such as pulmonary disease and disseminated intravascular coagulation can also occur. The clinical illness manifests as diarrhea with vomiting, fever, abdominal discomfort, nausea, abdominal, bradycardia, headache, and cough between 12-72 hours of infection. The disease normally lasts 4-7 days without antibiotic therapy before fully recovering. The intensity of the sickness worsens among the immunocompromised people, elderly, very young, and the diarrhea might be severe enough to require hospitalization. In such individuals, infection can spread and infiltrate the hepatobiliary system, leading to mortality if antibiotics are not administered. Cirrhosis of the liver, malignancies, acute pancreatitis, renal failure, and liver abscess are all seen in complicated instances because of the numerous risks linked with the use of various antibiotics, particularly in zoonotic illnesses, bacterial resistance in human-infecting strains has become quite prevalent (Abudabos *et al*, 2020). Human-to-human transmission happens by direct in-contact with animals, through environment, or through consumption of eggs, meat and milk, or items containing eggs. It has been reported that *Salmonella* that is resistant to cephalosporin has emerged in Egypt. The incidence of multidrug-resistant (MDR) isolates has been documented, and this has had an impact on typhoid fever in impoverished nations. *Salmonella typhi* AMR genes isolated from 29 percent of patients demonstrated resistance to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole in a research conducted in Lower Egypt. In diarrheic Egyptian individuals with *Salmonella* typhoid illness, multiple quinolone resistance has been found (Marchello *et al*., 2020). Ampicillin, trimethoprim-sulphamethoxazole tetracycline and chloramphenicol resistance was found in high numbers of individuals with non-typhoid *Salmonella* isolates (Chong *et al*, 2017). In addition, non-typhoid *Salmonella* isolates had a significant incidence of AMR genes to chloramphenicol and tetracycline. In Egypt and Algeria, a total of 76 non-typhoid *Salmonellae* were recovered from poultry and humans, with a significant rate of cephalosporin resistance among the isolates. Eissa *et al*. recently revealed that individuals with typhoid fever were resistant to combination cefotaxime and ciprofloxacin therapy. Typhoid *Salmonella* is found in only humans and could be spread by oral and fecal contact or by an in direct contact with an infected people. It is characterized by a high temperature that lasts for a long time hepatosplenomegaly, rose patches, neutropenia leukopenia, as well as relative bradycardia (Abd El-Ghany, 2020). The condition is essentially cured by in an undiagnosed patient at the conclusion of the fourth week. The symptoms include chills, a high temperature, stomach discomfort, and a headache. Typhoid is a bacterial infection caused by *S. typhi.* ecause of the existence of the resistome, *Salmonella* develops resistance to most antibiotics and multidrug resistance (MDR) develops. A resistome is a collection of bacterial genes that are resistant to drugs. here are a few therapeutic options available for salmonellosis, however they are ineffective. The parenteral nutrition commercially available Vι vaccine and live-attenuated Ty21a vaccination are only marginally effective in preventing this illness. These vaccinations also have certain drawbacks, including short-term immunity in adults and limited efficacy in children under the age of two (Kumar, 2022). arge number of bacteria are responsible for many food borne diseases. Most of them also develop resistance against antibiotics. *Salmonella* spp. is leading cause of gastrointestinal infections. With increasing use of antibiotics, antibiotic resistance has become the global issue. The aim of this study is to check prevalence, and antibiotic resistance of *Salmonella* spp. isolated from ice-cream. Objective of this study are analysis of ice cream to determine the prevalence of *Salmonella* spp. and determination of antibiotic resistance in isolated *Salmonella* spp.

Materials and methods

Sample collection and transportation: Since the sample being tested is ice cream, it was collected in an ice-chest to prevent melting and facilitate handling. 10 samples were collected from nearby ice cream point and bakeries that located such as jay bees, gourmet, and the cafeteria of Lahore College for Women University Punjab, Pakistan. The prevalence of *Salmonella* is the most common in dairy products. So, all collected ice creams samples were dairy ice creams. For the collection of the sample, first containers were sterilized by autoclaving them then used them for the sample collection purpose. In the table 1 sample name were given just for convince and prevent them from sample mixing. Here ICD is for dairy ice cream and ICND is for non-dairy ice cream

Table No 1: Sample collection summary

Sr. No.	Name of sample	Location
1	ICD-GS	Gourmet Shadman Lahore. Punjab Pakistan
2	ICND-GS	Gourmet Shadman Lahore. Punjab Pakistan
3	ICD-MFS	MF shadman Lahore. Punjab Pakistan
4	ICD-BG	Qulfi from begum kot Lahore. Punjab Pakistan
5	ICD-BS	Baba qulfi from Shadra Lahore. Punjab Pakistan
6	ICND-I	Ichra Lahore. Punjab Pakistan
7	ICD-YK	Yummy kulfa Lahore. Punjab Pakistan
8	ICD-JBS	Jay bees shadman Lahore. Punjab Pakistan
9	ICD-BJ	Baba qulfi, johar town Lahore. Punjab Pakistan
10	ICD-BJ	Baba qulfi, johar town Lahore. Punjab Pakistan

Media selection: SS agar is a differential selective medium that is mostly used for the isolation of *Salmonella* among a variety of bacterial species. The growth-promoting capabilities and selectivity of 11 commercially available media recommended for the isolation of Salmonella and Shigella were evaluated. The following media were examined: Mueller-Hinton, an unselective medium, Endo, Poskiriew, MacConkey, DC (Deoxycholate Citrate Agar), SS (*Salmonella-Shigella* Agar), and Eosin Methylene Blue (EMB) Agar (Vadlamudi *et al.*, 2022).

Preparation of SS Agar: Medium was prepared according to instructions of manufacturer (Biotrend Wilhelm-Mauser-Str. 41-4350827 Köln – Germany) According to the instructions, 52.0 grams of SS Agar were added to one liter of distilled water. The mixture was then boiled for complete dissolution, without autoclaving the medium. For the preparation of 100ml of the medium, 5.2 grams of SS Agar were added to the distilled water, which was subsequently boiled for complete dissolution (Vadlamudi *et al.,* 2022).

Preparation of MacConkey Agar: MacConkey agar has been prepared as per the instructions of the manufacturer (Labmal No.7, 2nd Floor, Jalan Puteri 1/7, Bandar Puteri, 47100 Puchong, Selangor, Malaysia Email: info@labmal.com.) According to the manufacturer's instructions, 51.5 grams of MacConkey Agar were added in distilled water. Then it was boiled to dissolve completely. After boiling, it was sterilized by autoclaving (Salman *et al.*, 2021).

Sterilization of materials: Micro tips have been autoclaved at 180 to 220°C for 20 minutes with prepared MacConkey media and petri plates. The UV light was turned on for ten to twelve minutes. After that, the laminar flow was held on for a little while, followed by sterilizing it with an ethanol spray and drying it with a cotton plug. Subsequently, the spirit lamp was ignited. Gloves were worn as a precaution, and hands had been sterilized (Sastry and Bhat, 2018).

Media pouring: The temperature of the media was reduced to a certain extent, following which it was introduced into the laminar airflow. Each plate was filled with 20ml of the media and allowed to dry for a period of time. Subsequently, the plates were covered with their respective lids and appropriately labeled. (Sastry and Bhat, 2018).

Spreading of sample: The work was conducted within the laminar airflow. For each sample, a volume of 200-300µl was extracted using a micro pipette. The sample was then poured onto the media, while the spreader was sterilized with alcohol. Subsequently, the spreader was heated on the spirit lamp and sterilized again using the alcohol present in the petri plate within the laminar airflow area. The spreader was rotated in both clockwise and counterclockwise directions to ensure complete absorption of the sample onto the media. The petri plates were covered with lids after the procedure was repeated for the remaining six plates. Any remaining sample was discarded. (Siddique *et al.*, 2021).

Incubation: The set of plates were placed in incubator for overnight at 27°C (Leng *et al*., 2019).

Data analysis: After 24 hours, the plates were removed from incubator and count CFU of bacterial species for data analysis.

Results

The results are analyzed by counting the colony-forming units on the petri plates. These species of bacteria could be differentiated by color and morphology of bacteria. *Salmonella* and *Shigella* gives pink or sometimes reddish brown (medium-colored) colonies on the media. On SS Agar *Salmonella* produced black-centered colored colonies. *Shigella* produced colorless colonies on the SS Agar. On the other hand, *E. coli* gives pink to red color on the SS

Agar. Table 2 indicates that *Salmonella* is prevalent in the dairy ice creams. This tabular data shows that there is not only *Salmonella* but also the prevalence of *Shigella* and *E. coli* also observed. Fig 1shows the number of *Shigella* is in excess but *Salmonella* is in very small quantity. Figure 2 shows that on MacConkey agar the number of *E. coli* is in excess. There are some resistant colonies that grow on SS Agar containing a different class of antibiotics such as gentamycin, ciprofloxacin, cefotaxime, and azithromycin as shown in table no 2 and 3. These results indicate bacterial colonies are less resistant to ciprofloxacin and cefotaxime while gentamycin and azithromycin are strong antibiotics.

Discussion

The prevalence of bacteria such as Salmonella, Shigella, and E. coli, which are responsible for food poisoning and foodborne diseases, is alarmingly high. Antibiotics are commonly employed as therapeutic agents to combat these bacterial infections. Regrettably, our bodies are progressively developing resistance to these medications. It is important to note that foodborne illnesses are not solely limited to typhoid; Salmonella poses a significant global public health concern. In the United States, laboratory-confirmed cases of salmonellosis among infants reached 121.6 infections per 100,000 infants, which is approximately eight times higher than the incidence observed in other age groups. It is worth mentioning that there is a scarcity of data on this topic in numerous other countries, particularly in developing nations (Yang *et al*., 2014).

Third-generation antibiotics such as gentamycin, azithromycin, cefotaxime, and ciprofloxacin are commonly utilized in clinical practice. SS Agar, a culture medium, consists of various constituents including pancreatic casein digestion, beef extract, sodium thiosulphate, bile salts, brilliant green, agar, peptic digestion of animal tissues, lactose, sodium citrate, ferric ammonium citrate, and neutral red. The medium maintains a pH of approximately 7. Its selectivity is primarily based on its ability to inhibit the growth of Gram-positive bacteria. The inclusion of lactose in the medium allows for the detection of acid production by lactose-fermenting microorganisms, which results in the formation of red colonies when reacting with the neutral red indicator. On the other hand, lactose non-fermenters produce colorless colonies. Salmonella Shigella (SS) Agar, serving as a moderately selective and differential medium, is employed for the isolation, cultivation, and differentiation of Salmonella spp. In contrast to lactose fermentation, Salmonella bacteria produce hydrogen sulphide (H2S) gas. Consequently, the resulting bacterial colonies exhibit a white color with black centers (Meteab and Abed, 2018).

Dairy-based ice cream predominantly comprises milk, eggs, and egg products, which provide favorable conditions for the growth of Salmonella bacteria. These ingredients serve as nutrient-rich substrates, facilitating the proliferation of Salmonella within the ice cream. Analysis of the tabular data presented in Tables 1 and 2 suggests that local vendors and their handling practices of ice cream, as well as the storage compartments or containers utilized by them, contribute to the creation of an environment conducive to the flourishing and propagation of these bacteria.

We do not consume food in microliters, despite the fact that all of the bacteria in the sample were put into microliters and their prevalence in food items was close to 200 CFU. We often calculate our food intake in milliliters and liters. So the incidence of the bacteria will rise, reaching a maximum of 200 x 5 = 1000 CFU, which will undoubtedly be the cause of food poisoning. As shown by the data analysis in the mentioned tables 1 and 2 which shows the concentration of bacteria, particularly *Salmonella*, in each serving, our consumption of bacteria present in food is excessive, which is what causes food related illness. *Salmonella* CFU concentrations are frequently excessive in ice cream purchased from local vendors or produced in unsanitary circumstances. Under conditions where stringent hygiene standards are maintained and samples are collected in a controlled environment, the presence of bacteria is minimal, and no colonies are observed on antibiotic-containing media. The study area under investigation represents one of the most socioeconomically disadvantaged regions in Pakistan, characterized by prevailing poverty and a higher burden of illness. In this context, the current study demonstrated elevated prevalence rates of Salmonella spp. across various areas in Southern Punjab, indicating a heightened frequency of salmonellosis. Notably, in the Multan area, the overall incidence of Salmonella spp. was found to be 25.89%, while comparable findings revealed 21.89% prevalence in other sampled locations. Previous research also reported prevalence levels ranging from 7.61% to 11.9% for Salmonella spp., attributing these variations to factors such as sanitation practices, training of food handling personnel, and overall hygiene conditions (Qamar *et al*., 2020).

A significant trend observed in the field of antibiotics is their ongoing transition and diminishing efficacy against emerging and re-emerging microorganisms. The widespread and indiscriminate use of antibiotics for both animal and human diseases has resulted in genetic modifications within bacterial strains, leading to the development of resistance. Antibiotic resistance in bacteria poses a substantial and escalating threat to human health, carrying significant economic burdens. Foodborne transmission is often considered the primary route for disseminating resistance between humans and animals. Among the 69 isolates analyzed in this study, 47 (68.1%) exhibited multidrug resistance, indicating their simultaneous resistance to at least three different classes of antibiotics (Ahmed *et al*., 2014).

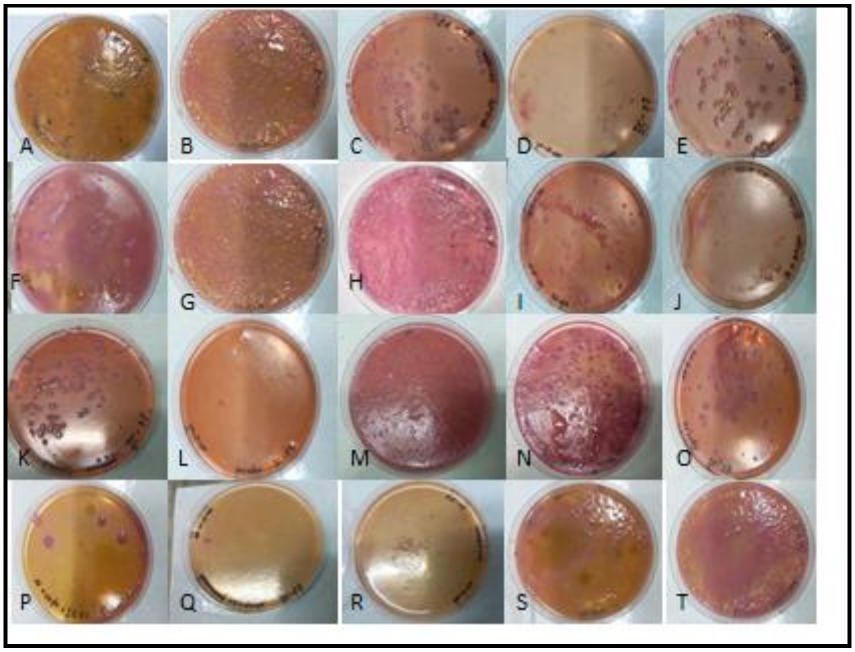

Figure 1 Appearance of colonies of *Salmonella* and *Shigella* from 100µl of ice cream all samples spread on SS Agar and MacConkey Agar after overnight incubation at 37°C.

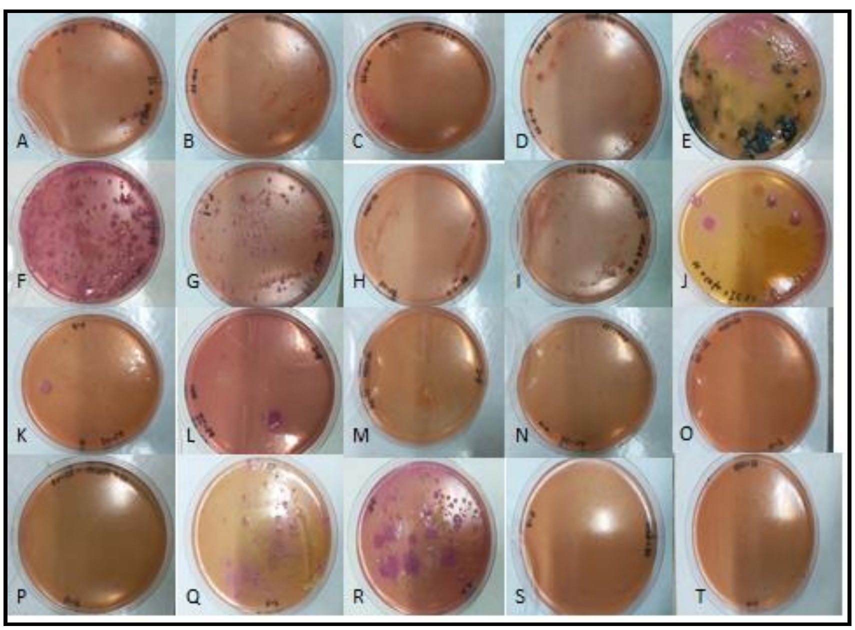

Figure 2 Appearance of colonies of *Salmonella* and *Shigella* from 100µl of ice cream of all samples spread on SS Agar containing antibiotics after overnight incubation at 37°C.

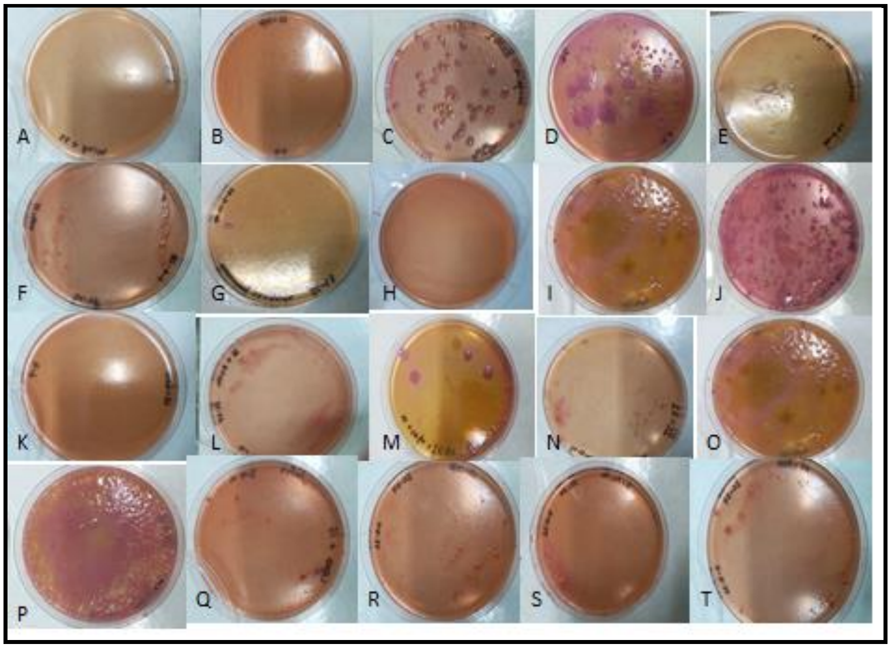

Figure 3 Appearance of colonies of *Salmonella* and *Shigella* from 100µl of ice cream of all samples spread on MaCconkey Agar containing antibiotics after overnight incubation at 37°C.

Table2. Prevalence of bacterial species on the SS Agar and MacConkey agar

Sr. No.	Name of sample	*Salmonella* (cfu)		*Shigella* (cfu)		*E. coli* (cfu)	
		Per ml	Per Serving	Per ml	Per serving	Per ml	Per serving
1	ICD-GS	210	21000	120	12000	40	4000
2	ICND-GS	120	12000	1210	121000	80	8000
3	ICD-MFS	0	0	510	51000	60	6000
4	ICD-BG	40	4000	90	9000	150	15000
5	ICD-BS	490	4900	100	10000	130	13000
6	ICND-I	0	0	10	1000	0	0
7	ICD-YK	60	6000	0	0	230	23000
8	ICD-JBS	0	0	260	26000	120	12000
9	ICD-BJ	30	3000	150	15000	200	20000
10	ICD-BJ	40	4000	90	9000	150	15000
1	ICD-GS	1320	13200	960	96000	120	12000
2	ICND-GS	700	7000	840	84000	50	5000
3	ICD-MFS	490	4900	260	26000	120	12000
4	ICD-BG	1540	15400	1940	194000	710	71000
5	ICD-BS	430	4300	890	89000	570	57000
6	ICND-I	10	100	0	0	20	2000
7	ICD-YK	830	8300	0	0	420	42000
8	ICD-JBS	0	0	10	1000	0	0
9	ICD-BJ	430	4300	890	89000	570	57000
10	ICD-BJ	1340	13400	1630	163000	710	71000

Table no 3: Results for prevalence of resistant on antibiotics mixed with media

Sr.no	Name of sample	*Salmonella* (cfu) Per serving	*Shigella* (cfu) Per serving	*E.coli* (cfu) Per serving
1	ICD-GS	1000	3200	2100
2	ICND-GS	0	0	0
3	ICD-MFS	0	2000	5
4	ICD-BG	0	0	0

5	ICD-BS	6000	0	0
6	ICND-I	0	0	0
7	ICD-YK	2000	0	100
8	ICD-JBS	0	0	1500
9	ICD-BJ	0	0	0
10	ICD-BJ	0	0	0
1	ICD-GS	200	500	300
2	ICND-GS	0	0	0
3	ICD-MFS	0	0	0
4	ICD-BG	0	0	100
5	ICD-BS	0	0	0
6	ICND-I	0	0	0
7	ICD-YK	0	0	0
8	ICD-JBS	0	100	0
9	ICD-BJ	200	400	300
10	ICD-BJ	0	0	100
1	ICD-GS	600	1800	800
2	ICND-GS	0	0	0
3	ICD-MFS	0	600	0
4	ICD-BG	0	0	0
5	ICD-BS	0	0	1500
6	ICND-I	0	200	0
7	ICD-YK	300	0	0
8	ICD-JBS	0	0	0
9	ICD-BJ	600	800	800
10	ICD-BJ	0	0	0

References

Abd El-Ghany W. A. Salmonellosis: A food borne zoonotic and public health disease in Egypt. *Journal of Infection in Developing Countries*, **14**(7), 674-678. (2020)

Abudabos, A. M., Aljumaah, M. R., Alkhulaifi, M. M., Alabdullatif, A., Suliman, G. M. and Sulaiman, A. R. A. Comparative effects of *Bacillus subtilis* and *Bacillus licheniformis* on live performance, blood metabolites and intestinal features in broiler inoculated with *Salmonella* infection during the finisher phase. *Microbial Pathogenesis*, **139,** 103870. (2020)

Ahmed, A. M., Shimamoto, T. and Shimamoto, T. Characterization of integrons and resistance genes in multidrug-resistant *Salmonella enterica* isolated from meat and dairy products in Egypt. *International Journal of Food Microbiology*, **18**(9), 39-44. (2014)

Akil, L., Ahmad, H. A., and Reddy, R. S. Effects of climate change on *Salmonella* infections. *Foodborne Pathogens and Disease*, **11**(12), 974-980. (2014)

Alphons J.A.M. van Asten, Jaap E. van Dijk. Distribution of classic virulence factors among *Salmonella* spp., *FEMS Immunology and Medical Microbiology*, **44**(1), 251–259. (2014)

Chen, H. M., Wang, Y., Su, L. H. and Chiu, C. H. Nontyphoid *Salmonella* infection: microbiology, clinical features, and antimicrobial therapy. *Pediatrics and Neonatology*, **54**(3), 147-152. (2021)

Chong, A., Lee, S., Yang, Y. A. and Song, J. The Role of Typhoid Toxin in *Salmonella typhi* Virulence. *The Yale Journal of Biology and Medicine*, **90**(2), 283-90. (2017).

Dróżdż, M., Małaszczuk, M., Paluch, E., and Pawlak, A. Zoonotic potential and prevalence of *Salmonella* serovars isolated from pets. *Infection Ecology and Epidemiology*, **11**(1), 1975530. 2021.

Eng, S. K., Pusparajah, P., Ab Mutalib, N. S., Ser, H. L., Chan, K. G. and Lee, L. H. *Salmonella*: a review on pathogenesis, epidemiology and antibiotic resistance. *Frontiers in Life Science*, **8**(3), 284 - 293. (2015)

Hall, R. M. *Salmonella* genomic islands and antibiotic resistance in *Salmonella enterica*. *Future Microbiology*, **5**(10), 1525-1538. (2010).

Hensel, M. 2000. *Salmonella* pathogenicity island 2. *Molecular Microbiology*, **36**(5): 1015-1023. (2000).

Jacobson, A., Lam, L., Rajendram, M., Tamburini, F., Honeycutt, J., Pham, T. and Monack, D. A gut commensal-produced metabolite mediates colonization resistance to *Salmonella* infection. *Cell host and Microbe*, **24**(2), 296-307. (2018).

Kumar, A. and Kumar, A. Antibiotic resistome of *Salmonella typhi*: molecular determinants for the emergence of drug resistance. *Frontiers of Medicine*, **15**(5): 693-703. (2021).

Lake I. R. Food-borne disease and climate change in the United Kingdom. *Environmental health: A Global Access Science Source*, **16**(1): 117. (2017).

Leng, L., Xu, X., Wei, L., Fan, L., Huang, H., Li, J., and Zhou, W. Biochar stability assessment by incubation and modelling: Methods, drawbacks and recommendations. *Science of the Environment*, **664**, 11 - 23. (2019).

Li, W., Pires, S. M., Liu, Z., Ma, X., Liang, J., Jiang, Y. and Guo, Y. Surveillance of foodborne disease outbreaks in China, 2003–2017. *Food Control*, **118**, 107359. (2020).

Liu, J., Hu, D., Chen, Y., Huang, H., Zhang, H., Zhao, J.and Chen, W. Strain-specific properties of Lactobacillus plantarum for prevention of *Salmonella* infection. *Food and function*, **9**(7), 3673–3682. (2018).

Meteab, B. K. and Abed, A. A. A. Isolation and identification of *Salmonella* serotypes in poultry. *Al-Qadisiyah Journal of Veterinary Medicine Sciences*, **17**(1): 75 - 80. (2018).

Nair, S., Patel, V., Hickey, T., Maguire, C., Greig, D. R., Lee, W., Godbole, G., Grant, K. and Chattaway, M. A. Real-Time PCR assay for differentiation of typhoidal and Nontyphoidal *Salmonella*. *Journal of Clinical Microbiology*, **57**(8): 167 - 219. (2019)

Popa, G. L. and Papa, M. I. *Salmonella* spp. infection-A continuous threat worldwide. *Germs*, **11**(1), 88. (2021)

Pradhan, D. and Devi Negi V. Stress-induced adaptations in *Salmonella*: A ground for shaping its pathogenesis. *Microbiological Research*, **22**(9): 126 - 311. (2019)

Qamar, A., Ismail, T. and Akhtar, S. Prevalence and antibiotic resistance of *Salmonella* spp. in South Punjab Pakistan. *PloS One*, **15**(11): 18 – 22. (2020)

Riaz, S. Study of protein biomarkers of diabetes mellitus type 2 and therapy with vitamin B1. *Journal of Diabetes Research*, (2015).

Riaz, S., Alam, S. S. and Akhtar, M. W. Proteomic identification of human serum biomarkers in diabetes mellitus type 2. *Journal of Pharmaceutical and Biomedical Analysis*, **51**(5), 1103 - 1107. (2010).

Salman H. A., Abdulmohsen, A. M., Falih, M. N. and Romi, Z. M. Detection of multidrug-resistant *Salmonella enterica* subsp. enterica serovar Typhi isolated from Iraqi subjects. *Veterinary World*, **14**(7), 19 - 22. (2021).

Sastry, A. S. and Bhat, S. *Essentials of Medical Microbiology*. JP Medical Ltd 2018.

Shahzad, A., Saeed, H., Iqtedar, M., Hussain, S. Z., Kaleem, A., Abdullah, R. and Chaudhary, A. Size-controlled production of silver nanoparticles by *Aspergillus fumigatus* BTCB10: likely antibacterial and cytotoxic effects. *Journal of Nanomaterials*, (2019).

Siddique, A., Azim, S., Ali, A., Andleeb, S., Ahsan, A., Imran, M. and Rahman, A. Antimicrobial resistance profiling of biofilm forming non typhoidal *Salmonellaenterica* isolates from poultry and its associated food products from Pakistan. *Antibiotics*, **10**(7), 73 - 85. (2021).

Solangi, M., Kanwal, Khan, K. M., Chigurupati, S., Saleem, F., Qureshi, U., & Bhatia, S. Isatin thiazoles as antidiabetic: Synthesis, in vitro enzyme inhibitory activities, kinetics, and in silico studies. Archiv der Pharmazie, **355**(6), 2100481. (2022).

Song, J., Gao, X. and Galán, J. E. Structure and function of the *Salmonella typhi* chimaeric A (2) B (5) typhoid toxin. *Nature*, **499**(7458), 350 –354. (2013).

Sun, H., Wan, Y., Du, P., and Bai, L. The epidemiology of monophasic *Salmonella Typhimurium*. *Foodborne Pathogens and Disease*, **17**(2), 87 - 97. (2022).

Teklemariam, A. D., Al-Hindi, R. R., Albiheyri, R. S., Alharbi, M. G., Alghamdi, M. A., Filimban, A. A., and Bhunia, A. K. Human Salmonellosis: A Continuous Global Threat in the Farm-to-Fork Food Safety Continuum. *Foods*, **12**(9), 1756. (2023).

Vadlamudi Gowthami, S. S. G., Kumar, A. V., and Krishnaiah, N. Assessment of microbial load in raw chicken at retail outlets in and around Hyderabad, India. (2022)

White, A. E., Tillman, A. R., Hedberg, C., Bruce, B. B., Batz, M., Seys, S. A. and Walter, E. S. Foodborne Illness Outbreaks Reported to National Surveillance, United States, 2009–2018. *Emerging Infectious Diseases*, **28**(6), 11 - 17. (2022).

Yang, B., Zhao, H., Cui, S., Wang, Y., Xia, X., Wang, X., Meng, J., and Ge, W. Prevalence and characterization of *Salmonella enterica* in dried milk-related infant foods in Shaanxi, China. *Journal of Dairy Science*, **97**(11), 6754 – 6760. (2014)