INTERNATIONAL JOURNAL OF CURRENT ADVANCED RESEARCH
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 11; Issue 10(A); December 2022; Page No. 1705-1709
DOI: http://dx.doi.org/10.24327/ijcar.2022.1709.0382

Research Article

QUICK DETECT OF *Salmonella* spp. IN AN INDIAN STREET SNACK PANIPURI WATER AND GENOTYPIC INCIDENCE OF CHLOREMPHENICOL RESISTANCE USING INVA AND CMLA GENE

Mamta Meena[1], Rajnadani Katariya[3] and Mayank Tenguria[2]*

[1]Department of Microbiology, Gandhi Medical College, Bhopal, M.P. India
[2]Biopharmaceutical & Biomedical Division, Lenience Biotech Lab, Bhopal, M.P. India
[3] Departments of Biosciences, Indiana State University, Indiana Polis, United States of America

A R T I C L E I N F O

Article History:

Received 29th October, 2022
Received in revised form 15th November, 2022
Accepted 16th December, 2022
Published online 28th December, 2022

Key words:

salmonellosis, chloremphenicol resistance, *inv*A gene, *cml*Agene and Panipuri

A B S T R A C T

The primary reason of global spread is microbial contamination and its transfer through tainted water and food sources. Salmonella species are important infectious pathogens that are members of the enteriobacteriacea family and can infect both humans and animals with illnesses. Due to the use of contaminated drinking water in preparations, street meals in India are frequently the cause of contracting *Salmonella* spp. infection. Flavored water of Panipuri (Indian Street Snack) is one of the most well-known examples. Molecular identification of harmful microorganisms is now a quick and reliable assay technique. Therefore, the goal of the current investigation was to identify Salmonella spp. using the *inv*A gene and the prevalence of chloremphenicol resistance within using the *cml*A gene in flavoured water samples of Panipuri that were afterwards randomly collected from food vendors on the streets of Bhopal. For culture of bacteria Salmonella Selective Enrichment broth supplemented with novobiocin was used. After incubation, the water culture was centrifuged, and the DNA was isolated and submitted to PCR to determine the incidence of the cmlA gene and the existence of the invA gene using a standard PCR methodology. On a 1% agarose gel, the target gene was found in the PCR result. Based on the presence of the *inv*A gene unique to *Salmonella* spp., all 30 of the samples were found to be positive for the presence of *Salmonella* spp. However, based on the existence of *cml*A genes, it was shown that 53% of the samples had a genotypically observed incidence of chloremphenicol resistance. The results of this brief investigation point to an alarming situation regarding the incidence of probable drug-resistant salmonellosis and diarrhoea dissemination.

INTRODUCTION

Due to the lack of knowledge about the scope of infectious diseases and their origins, microbial infections are the major cause of death worldwide. It is important to raise knowledge about microbial infections and how they might spread through contaminated food and water sources. As a significant cause of infections that has been well-documented in countries at all economic development levels, virus transmission through water continues to be a problem. More than 13 million people perish each year as a result of drinking dirty water.

In the cities of emerging nations like India, a variety of street foods are quite popular. Millions of people in all small and large cities eat street cuisine like panipuri, snacks, and drinks that are sold by vendors. One of the biggest sources of foodborne and waterborne infections is typically these types of foods, which are made using regular water without any sort of treatment. Street foods are frequently linked to diarrheal diseases because they are handled improperly, served in unhygienic conditions, and are not protected from flies (Mensah, *et al*., 2002; Dardano, 2003; Hanoshiro, *et al*., 2004; Barro *et al*., 2006; Bryan, 1998), which increases the risk of contamination in street foods. However, these types of food items are prepared either at home or on the street (Bhaskar *et al*., 2004; Tambekar, *et al*., 2009).

For the analysis of the risks, several elements must be taken into account, from the initial contamination of raw foods with pathogenic bacteria to the subsequent contamination by vendors during the production of street foods (Dawson and Canet, 1991; Mankee, *et al*., 2003). Many bacteria, such as *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, *Proteus* species, and *Pseudomonas* species, are directly linked to food contamination (Hanoshiro, *et al*., 2004). *Salmonella* species are important infectious pathogens that are members of the enteriobacteriacea family and can infect both humans and animals with illnesses. In India, street food is frequently a source of contraction Infection with Salmonella spp. brought on by the use of tainted drinking water in recipes; flavoured water used in Panipuri (an Indian street snack) is a common example. Molecular identification of harmful microorganisms is now a quick and reliable assay technique. Therefore, the goal of the current investigation was to identify *Salmonella*

Corresponding author:* **MAYANK TENGURIA
Biopharmaceutical & Biomedical Division, Lenience Biotech Lab, Bhopal, M.P. India

spp. using the *inv*A gene and the prevalence of chloremphenicol resistance within using the *cml*A gene in flavoured water samples of Panipuri that were afterwards randomly collected from food vendors on the streets of Bhopal.

Molecular Confirmation &Antibiotic Resistant Detection

Total genomic DNA samples from each sample culture were subjected to screening for the *inv*A gene and *clm*A gene, which stand for the conserved region of DNA specific to *Salmonella* spp. and the chloramphenicol resistance gene, respectively. Previously reported primer sequences were used for this purpose whose sequence details & annealing conditions are mentioned in table 1. The PCR reaction was performed in 25 µl volume which contains 12.5 µl PCR TaqMixture (HiMedia, India), 0.5 µl each of forward and reverse primers (*Eurofins Genomic* India Pvt. Ltd and Bioserve Biotechnologies, India) and 5 µl template DNA and 6.5 µl molecular grade distilled water (HiMedia). The Sterile distilled water was used in place of DNA as negative control

Table 1 List of Primer sets used for the detection of *Salmonella* spp. and incidence of antibiotic resistance gene in cultures of flavoured water samples in singlex PCR.

S.N.	Detection	Target Gene	Primer Sequences	Annealing Temperature	References
1.	*Salmonella* Spp.	*inv*A	Forwards:TCATCGCACCGTCAAAGGAACC Reverse:GTGAAATTATCGCCACGTTCGGGCAA	61^{o}C	Kadry, *et al.,* (2019).
2.	Chloramphenicol Resistance	*cml*A	Forwards: CCGCCACGGTGTTGTTGTTATC Reverse:CACCTTGCCTGCCCATCATTAG	58^{o}C	Van, *et al.,* (2008); Rahman, *et al*., (2020)

MATERIALS AND METHODS

Sample Collection

As samples, flavor-infused water from the Indian panipuri street food were chosen at random from several Bhopal City locations. For the purpose of screening for Salmonella spp. between August 2020 and January 2021, 100 ml of panipuri water sample were collected from the 30 sites using clean, dry, and sterilized glass bottles.

Table 2 PCR protocols followed for amplification of target genes in isolated DNA samples whole bacterial cultures

S.N.	Gene	Initial Denaturation	PCR Cycles			
			Denaturation	Annealing	Extension	Final Extension
1.	*inv*A	94°C for 5 min	94°C for 30 S,	55 °C for 30 S	72°C for 1 min	72°C for 5 min.
2.	*cml*A		94°C for 30 S,	56 °C for 30 S	72°C for 1 min	72°C for 10 min.

Culture & Enrichment of Salmonella spp

In order to culture bacteria in samples of flavoured water, the Salmonella Selective Enrichment broth (M1843, HiMedia Laboratories India Pvt Ltd) was employed, which facilitates the selective growth of the *Salmonella spp*. Following all aseptic procedures, 0.1 ml of the panipuri water samples that had been obtained was inoculated in each individual broth tube. Each tube was then incubated at 37°C for 24 hours. The samples were centrifuged to extract the collective pellet of bacteria after monitoring the turbidity in the broth cultures, and the bacteria were then exposed to DNA isolation.

DNA *Extraction*

By using the traditional boiling procedure, total genomic DNA was extracted from the collected bacterial pellets from each growth tube independently. The bacterial pellet from the growth tube is transferred to a 1.5 ml microfuge tube after being diluted with 1 ml of sterile distilled water. After centrifuging these microfuge tubes at 10000 rpm for 7 minutes, the supernatant was discarded, and the pellet rinsed with 500 µl of sterile distilled water before being centrifuged yet again at 12000 rpm for 5 minutes. After once more discarding the supernatant, 200 µl of sterile distilled water was used to homogenise the particle. This mixture was then heated for 10 minutes at 100°C in a digital water bath (Navyug, India) before cooling for 7 minutes in an ice bath. The microfuges were then centrifuged for 5 minutes at 10,000 rpm. The acquired supernatants were then kept in new sterile 1.5 ml microfuges and refrigerated at −20°C for later use.

in one tube. The PCR was carried out on a Prima96 Thermocyler (HiMedia, India) using the parameters listed in Table 2 with an initial denaturation time of 5 minutes at 94°C, followed by 30 cycles of denaturation, annealing, and extension, and a final extension time of 10 minutes at 72°C.

Data Analysis

The PCR result was put through electrophoresis on 1% Agarose gel in 1X TAE buffer running at 100 volt for 30 minutes after the PCR process was finished. The gel was then viewed on the E-Gel Imager gel documentation system to validate the presence of target genes as a band on the gel (Thermo Fischer Scientific, US). *Salmonella* spp. prevalence and the prevalence of the gene for antibiotic resistance to chloramphenicol was assessed using a percentage correlation.

RESULTS AND DISCUSSION

Microbial Contamination in Samples

A total of 30 samples of Panipuri-flavored water were randomly chosen from street food sellers in Bhopal City and taken to the sites listed in table 3. It was reported that all of the samples were contaminated with different types of bacteria, particularly any *Salmonella spp*., when they were subjected to culture in Salmonella Selective Enrichment broth. This was supported by the turbidity in liquid culture media that appeared after the period of incubation. This shows that it is certain to claim that the water used to prepare street foods or street snacks is significantly contaminated, whether as a result of pre-existing pollution in water supplies or unclean handling and preparation techniques.

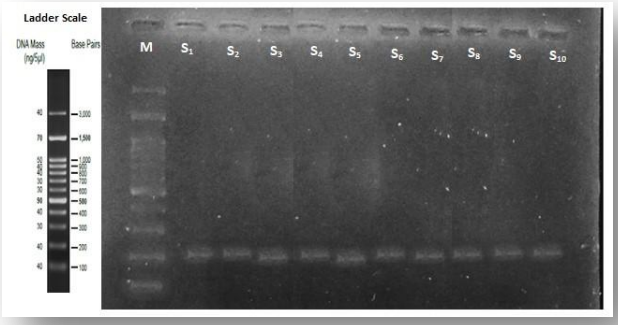

Image 1 Agarose gel image showing *inv*A gene detected in prospected *Salmonella* spp. isolates from S_1 to S_{10} at the range of 200 bp on marker scale.

Molecular Detection of Salmonella spp. & Resistant Gene

The technique for bacterial DNA extraction has been modified in accordance with the suggestions made by earlier researchers (Lee, *et al*., 2009; Ahmed and Dablool, (2017). The DNA was produced in such a way from the full culture of panipuri-flavored water sample that it functioned well with the following PCR amplification of the desired fraction within the DNA.

In accordance with the procedure recommended by Kadry, *et al*., (2019) the *inv*A gene primer set was utilized to produce an amplified PCR product with a size range of around 284 bp, confirming the detection of *Salmonella* spp. (Kaushik, *et al*., 2014). Based on the presence of the *Salmonella* spp. unique *inv*A gene at the range of 200 bp on marker scale, the amplified PCR result showed all 30 samples were positive for the presence of *Salmonella* spp. when run on a 1% agarose gel (see image 1).

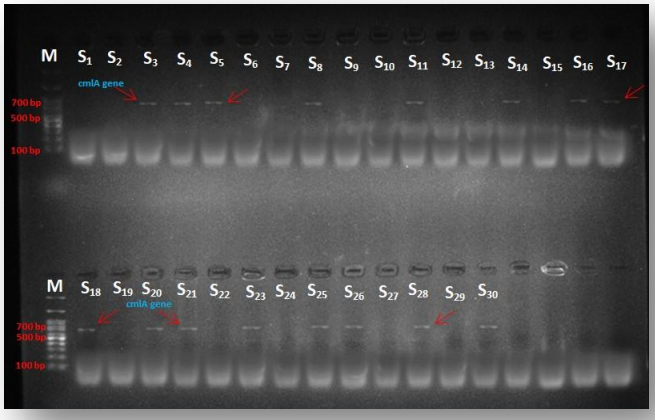

Image2 The presence of band on gel confirms the presence cmlA gene Salmonella spp. isolates from S 1 to S 10 at the range of 700 bp on marker scale.

Despite being promoted as an efficient, quick, and accurate method for identifying Salmonella in food and water samples, the *inv*A gene is viewed as a characteristic for the identification of salmonellosis around the world (Malorny, *et al*., (2003). The ability of the invA gene to infiltrate and persist in macrophages is referred to as the ability to invade cells (Gole, *et al*., 2013).

In accordance with the PCR protocol recommended by Van, *et al*., (2008) and Rahman, *et al*., (2020) with appropriate modifications as per the requirement of the current investigation, the amplification of *cml*A genes in DNA samples confirmed for incidence of *Salmonella* spp. in panipuri-flavored water samples was also carried out. According to the literature (Pourtaghi and Sodagari, 2016), a single band measuring 698 base pairs (bp) in size was found to contain the *cml*A genes in the PCR result (see image 2). Out of the 30 DNA samples from bacterial cultures, it was found that 16 samples had *cml*A genes. Based on the presence of *cml*A a gene, the genotypic incidence of chloremphenicol resistance in these samples was reported to be 53% (see table 3). The results of this short study show that there is a concerning scenario regarding the occurrence of probable drug-resistant salmonellosis and diarrhoea, however a large number of random samples would assist to clarify the data. One of the most significant water and food borne organisms that poses a risk to food safety for the food industry and the public health sector is *Salmonella*. Worldwide, salmonellosis is a serious medical problem and a big challenge, with developing nations bearing the brunt of its impact (Adel El-Sebay, *et al*., 2017).The risk of *Salmonella* may differ amongst food production systems due to variations in the pathogen's level of resistance or factors affecting illness onset and shedding (Zheng, *et al*., (2007)

Table 3: The prevalence of the antibiotic resistance gene to Chloremphenicol in 30 samples of *Salmonella* spp. found in samples of flavoured panipuri water

S.N.	Sample Locations	Sample Code	*cml*Agene Detected	Total Incidence Percentage
1.	6 no. Market	S_1	0	
2.	6 no. Market	S_2	0	
3.	6 no. Market	S_3	1	
4.	M.P. Nagar	S_4	1	
5.	M.P. Nagar	S_5	1	
6.	M.P. Nagar	S_6	0	
7.	Nehru Nagar	S_7	0	
8.	Nehru Nagar	S_8	1	
9.	Nehru Nagar	S_9	0	
10.	10 no. Market	S_{10}	0	
11.	10 no. Market	S_{11}	1	
12.	10 no. Market	S_{12}	0	
13.	10 no. Market	S_{13}	0	
14.	New Market	S_{14}	1	
15.	New Market	S_{15}	0	
16.	New Market	S_{16}	1	
17.	New Market	S_{17}	1	
18.	Bitton Market	S_{18}	1	
19.	Bitton Market	S_{19}	0	
20.	Indrapuri	S_{20}	1	
21.	Indrapuri	S_{21}	1	53%
22	JK Road	S_{22}	0	
23	Chowk Bazar	S_{23}	1	
24	GhoraNakkas	S_{24}	0	
25	Jai BhimNarar	S_{25}	1	
26	Baghmugaliya	S_{26}	1	
27	BimaKunj	S_{27}	0	
28	BimaKunj	S_{28}	1	
29	BimaKunj	S_{29}	0	
30	BimaKunj	S_{30}	1	

Note: *1= presence of target gene and 0 = absence of target gene*

Foodborne illnesses brought on by eating tainted prepared food are a common occurrence that not only have negative effects on human health but also have social and economic repercussions. Fresh produce's association with foodborne illnesses drives researchers to review and develop the microbiological safety of food and water in terms of evaluation techniques and pathways for bacteria access to produce (Alegbeleye, *et al*., 2018). Salmonellosis is intended to have an impact on the world economy because it is a significant public health issue that causes significant morbidity

and mortality, and because food and water borne Salmonella spp. pose a substantial hazard when they grow resistant to some antibiotics (Adamu, *et al*., 2020; Adzitey, *et al*., 2016).
Due to the labour and time requirements of several standard foodborne detection approaches, better tactics have been developed and optimised as alternatives to be utilised in conjunction with them. Numerous of those are quick, delicate, dependable, and standardised. They might be classified as relying on nucleic acids, biosensors, or immunological methods (Croci, *et al*., 2008; Adzitey *et al*., 2013; Law *et al*., 2014). Because of their ease of use and low cost, culture techniques are still widely used and advantageous in the detection of pathogens in food and clinical prognosis of microbial pathogens Adamu, *et al*., (2020). However, PCR-based studies are quick, specific, sensitive, and safe. However, when antibiotic-resistant bacteria are found, the genotypic and phenotypic profiles of the isolates no longer generally match.

CONCLUSIONS

According to the findings of this brief study, it is evident that popular Indian street food and snacks are the most contaminated with diseases that are spread through food and water. Furthermore, if those infections are found to be multi-antibiotic resistant, the situation becomes even more serious. The results of this brief investigation show that there is a serious public health concern over the occurrence of probable drug-resistant salmonellosis and diarrhoea disseminated by the eating of such unsanitary street snacks. However, a quick and effective strategy to monitor not only the presence of food-borne pathogens but also any occurrence of antibiotic resistance in food and water samples would be to conduct a quality check using molecular technologies.

Acknowledgment

The authors would like to pay their sincere thanks to the management and colleagues at Lenience Biotech Lab, Bhopal and Gandhi Medical College, Bhopal, for necessary facilitations and moral support to conduct this small piece of significant investigations.

Conflict of Interest
All the authors confirm that they have no conflict of interest regarding the present work.

Authors Contribution

MT contributed to the conception, design, supervision, administration of the study and writing the original version of manuscript. All authors contributed their expertise in data acquisition, analysis, and revising the manuscript.

Funding Statement

No funding available. The research work was supported by Lenience Biotech Lab, Bhopal for laboratory facility and cost of experiments.

Data Availability

All datasets generated experimentally or analysed during this study are included in the manuscript.

Ethics Statement

Since there were no animal or human subjects involved in the experimental design and all the experiments performed were *in vitro*, numerical and in laboratory conditions only, hence no special ethical permission required in experimental work.

References

1. Adamu, M.O., Ishaleku, D., Ngwai, Y.B., Obiekezie, S.O., and Owuna, J.E., (2020). Antibiotic Susceptibility Profile and Molecular Detection of *tetA* Genes in Tetracycline Resistant *Salmonella* Species Isolated from Different Water Sources in Keffi, Nasarawa State, Nigeria. *South Asian Journal of Research in Microbiology*; 6(4): 27-41.
2. Adel El-Sebay, N., Abu Shady, H.M., El-Zeedy, S.A. and Samy, A.A., (2017) *InvA* gene Sequencing of *Salmonella* Typhimurium Isolated from Egyptian Poultry. *Asian Journal of Science*; 10(3): 194-202.
3. Adzitey, F, Ashiagbor, CKN, and Abu, H., (2016). Prevalence and Antibiotic Susceptibility of *Salmonella* spp. from Water Sources in Tamale, Ghana. *International Journal of One Health*; 2: 24-28.
4. Adzitey, F., Huda N., and Ali G.R.R., (2013). Molecular Techniques for Detecting and Typing of Bacteria, Advantages and Application to Foodborne Pathogens Isolated from Ducks. *3 Biotech*.;3(2):97–107.
5. Ahmed, O.B., and Dablool, A.S., (2017). Quality Improvement of the DNA Extracted by Boiling Method in Gram Negative Bacteria. *International Journal of Bioassays*; 6(4): 5347-5349
6. Alegbeleye, O.O., Singleton, I., and Sant'Ana, A.S., (2018). Sources and Contamination Routes of Microbial Pathogens to Fresh Produce during Field Cultivation: A Review. *Food microbiology*, *73*, 177-208. https://doi.or g /10.1016/j.fm.2018.01.003
7. Barro, N., Bello, A.R., Savadogo, A., Ouattara, CAT., Ilboudo, A.J., and Traore, A.S., (2006). Hygienic Status Assessment of Dish Washing Waters, Utensils, Hands and Pieces of Money from Street Food Processing Sites in Ouagadougou (Burkina Faso). *African Journal of Biotechnology*; 5(11): 1107-1112.
8. Bhaskar J, Usman M, Smitha S, and Bhat GK. (2004).. Bacteriological Profile of Street Foods in Mangalore. *Indian Journal of Medical Microbiology*; 22: 97-197.
9. Bryan, F.L., (1998). Risks Associated with Practices, Procedures, and Processes that Lead to Outbreaks of Food Borne Diseases. *Journal of Food Protection*; 51: 663-763.
10. Croci, L., Dubois, E., Cook, N., de Medici, D., Schultz, A.C., China, B., and Van der Poel, W.H., (2008). Current Methods for Extraction and Concentration of Enteric Viruses from Fresh Fruit and Vegetables: Towards International Standards. *Food Anal. Meth.* ; 1(2):73–84.
11. Dardano, C., (2003). Carribbean Regional Working Group on Street Food Vendors. Report of FAO, PAHO and BNSI. (Online) Available: ftp:ftp.fao.org/es/esn/ food/carribean_report.pdf.
12. Dawson, R.J, and Canet, C., (1991). International Activities in Street Foods. *Food Control*; 2: 135-139.

13. Gole, V.C., Chousalkar, K.K. and Roberts, J.R. (2013). Survey of *Enterobacteriaceae* Contamination of Table Eggs Collected from Layer Flocks in Australia. *Int. J. Food Microbiol*., 164(2-3): 161-165.

14. Hanoshiro, A., Morita, M., Matte, G.R., Matte, M.H., and Torres, E.A., (2004). Microbiological Quality of Selected Foods from a Restricted Area of Sao Paulo City, Brazil. *Food Control*; 16: 439-44. 5.

15. Kadry, M., Nader, S.M., Dorgham, S.M., & Kandil, M.M. (2019). Molecular Diversity of the *invA* Gene Obtained from Human and Egg Samples. *Veterinary world*; 12(7): 1033-1038. https://doi.org/10.14202/ vetworld.2019.1033-1038

16. Kaushik, P., Anjay and Dayal, S. (2014). Rapid PCR Detection of *Salmonella* from Stool Samples. *J. Vet. Public Health*; 12(2): 71-74.

17. Law, J.W.F., AbMutalib N.S., Chan K.G., and Lee L.H., (2014). Rapid Methods for the Detection of Foodborne Bacterial Pathogens: Principles, Applications, Advantages and Limitations. *Frontiers in Microbiology*; 5 doi: 10.3389/fmicb.2014.00770.

18. Lee, S.H., Jung, B.Y., Rayamahji, N., Lee, H.S., Jeon, W.J., Choi, K.S., Kweon, C.H., and Yoo, H.S. (2009). A Multiplex Real-Time PCR for Differential Detection and Quantification of *Salmonella* spp., *Salmonella entericaserovarTyphimurium* and Enteritidis in Meats. *Journal of Veterinary Science*; 10(1): 43. doi:10.4142/jvs.2009.10.1.43

19. Malorny, B., Hoorfar, J., Bunge, C and Helmuth, R. (2003). Multicenter Validation of the Analytical Accuracy of *Salmonella* PCR: Towards an International Standard. *Appl. Environ. Microbiol*., 69(1): 290-296.

20. Mankee, A., Ali, S., Chin, A., Indalsingh, R., Khan, R., Mohammad, F., Reheman, R., Sooknanan, S., Tota-Maharaj, R., Simeon, D., and Adesiyun, A.A., (2003). Bacteriological Quality of Doubles Sold by Street Vendors in Trinidad and the Attitudes, Knowledge and Perceptions of the Public about its Consumption and Health Risk. *Food Microbiology*; 20: 631-639.

21. Mensah, P., Manu, D.Y., Darko, K.O., and Ablordey, A., (2002). Streets Foods in Accra, Ghana: How Safe Are They? *Bulletin of World Health Organization*; 80(7): 546-554.

22. Pourtaghi, H., and Sodagari, H.R., (2016). Antimicrobial Resistance of Entrotoxigenic and Non-Entrotoxigenic *Escherichia coli* Isolated From Diarrheic Calves in Iran. *International Journal* of *Enteric Pathogens;* 2016 May; 4(2):e34557.

23. Rahman, M.M., Husna, A., Elshabrawy, H.A., Alam, J., Runa, N.Y., Badruzzaman, A.T.M., Banu, N.A., Mamun, M.A., Paul, B., Das, S., Rahman, M.M., Mahbub‐E‐Elahi, A.T.M., Khairalla, A.S., Ashour, H.M., (2020). Isolation and Molecular Characterization of Multidrug-Resistant *Escherichia coli* from Chicken Meat. *Scientific Reports*; 10(1). Doi: 10.1038/s41598-020-78367-2.

24. Tambekar, D.H., Murhekar, S.M., Dhanorkar, D.V., Gulhane, P.B., and Dudhane, M.N., (2009). Quality and Safety of Street Vended Fruit Juices: A Case Study of Amravati City, India. *Journal of Applied Biosciences*; 14: 782-787.

25. Van, T.T., Chin, J., Chapman, T., Tran, L.T., and Coloe, P.J., (2008). Safety of Raw Meat and Shellfish in Vietnam: An Analysis of *Escherichia coli* Isolations for Antibiotic Resistance and Virulence Genes. *International Journal of Food Microbiology*; 124(3): 217-223. doi: 10.1016/j.ijfoodmicro.2008.03.029.

26. Zheng, D.M., Bonde, M. and Sorensen, J.T. (2007). Associations between the Proportion of *Salmonella* Sero-positive Slaughter Pigs and the Presence of Herd Level Risk Factors for Introduction and Transmission of *Salmonella* in 34 Danish Organics, Outdoor (non-organic) and Indoor Finishing-Pig Farms. *Livestock Science;* 106: 189-199.

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