Readers Insight
Publisher of open access books and journals

PJMLS
Online ISSN: 2707-4471. Print ISSN: 2707-448X

Research Article	Pak-Euro Journal of Medical and Life Sciences
DOI: 10.31580/pjmls.v4i3.2414	Copyright © All rights are reserved by Corresponding Author
Vol. 4 No. 3, 2021: pp. 171-178	
www.readersinsight.net/pjmls	
Submission: July 23, 2021	**Revised:** August 28, 2021 **Published Online:** September 30, 2021

DETECTION OF PATHOGENIC *SALMONELLA* SPP. FROM RAW MEAT (BEEF, MUTTON, CHICKEN) AND SEAFOOD ITEMS BY STANDARD MICROBIOLOGICAL METHODS

Qurrat-ul-ain Leghari[1], Khuzeema Tanveer[2*], Nawab Khan Panezai[3], Mir Abdul Qadir[4], Yasmeen Lashari[5], Sahrish Mariam[6], Abdul Saboor[3], Syed Zaheer Hussain Shah[3], Muhammad Yousaf[7], Muhammad Umer[7]

[1]**Department of Physiology,** University College of Medicine & Dentistry, The University of Lahore, Lahore, Pakistan

[2]**Institute of Microbiology,** University of Agriculture, Faisalabad, Pakistan

[3]**Livestock and Dairy Development Department**, Balochistan, Quetta, Pakistan

[4]**Task Group on Family Medicine,** Institute of Public Health, Quetta, Pakistan

[5]**Department of Microbiology Pathology,** Bolan Medical College, Quetta, Pakistan

[6]**Department of Dermatology,** Nishtar Hospital, Multan, Pakistan

[7]**Center for Advanced Studies in Vaccinology & Biotechnology (CASVAB),** University of Balochistan, Quetta, Pakistan

*Corresponding Author: Dr. Khuzeema Tanveer. E. mail: ktayyab23313@gmail.com

Abstract

Among food borne pathogens or those involved in food spoilage, Salmonella are considered among the leading ones. Although several modern and sophisticated techniques are available to diagnose the pathogens but in developing countries like Pakistan the availability of resources are the bottle necks to adopt those techniques. In such situation conventional and traditional biochemical tests become handy and carried out in any laboratory with minimum infrastructure and cost.

This study was designed to detect the Salmonella from raw meat (beef, mutton and chicken) and seafood (fish and prawn) items sold in Lahore city with help of standard microbiological tests. Total of 125 samples of meat and seafoods were collected from retailers and were evaluated for the contamination of Salmonella.

Conventional methods including Gram staining, specific culture media and biochemical tests were employed to identify the Salmonella.

Results revealed that out of 125 food items 23 were contaminated with Salmonella. Salmonella was isolated from 4 samples out of 25 samples of beef, while 3 from mutton, 8 from chicken, 6 from fish and 2 from prawn samples. Although the tests performed were laborious and time consuming however yielded confirmatory results with less cost and without any costly equipment.

This study concludes that conventional methods can be employed to detect food pathogens where modern equipment is not available.

Keywords: *Food pathogens, Food spoilage, Salmonella spp., Salmonellosis*

INTRODUCTION

Food-borne diseases are the leading health problems in all countries of the world. In developing countries, more than one billion individuals suffering from gastroenteritis and around five million infected individuals die annually. In 21st Century meat is a highly consumed food and gained much popularity worldwide. However, several problems like obesity, disturbance in lipid profile and food hygiene are also associated with such food items (1-3). Several out breaks have been reported after consumption of semi or uncooked meat and seafoods (4-6). Although developed countries have adopted strict measures to sustain the quality of food items sold but in developing countries like Pakistan no strict laws have been implanted

so far. Previously several studies have reported the contamination of raw meat items like chicken/beef meat, mutton, fish, prawn, vegetables and even drinking water with several bacterial pathogens (7-13).

Contamination of food items with *Salmonella* can lead to Salmonellosis which can be mild and self limiting to life threatening depending on the bacterial strain pathogenicity and the dose consumed. The symptoms occur within 36 hours after consumption of contaminated food. Vomiting, abdominal cramps with diarrheal and increase in body temperature are the most common signs and symptoms of Salmonellosis (14). The extreme age group and immune- compromised persons can exhibit more severity of disease, in such cases diarrhea and vomiting can lead to dehydration which can be life threatening in some cases (15-16). The common source of *Salmonella* infection is egg, meat and milk or any other food item contaminated with pathogen. The other neglected source of contamination is the food handler, the carrier of *Salmonella* may contaminate the utensils used to serve food or food itself while preparation and serving. To prevent or minimize the chances of disease spread, special attention should be given to hygienic measures but in developing countries like Pakistan these lack the most. The meat vendors place their food stalls at road side having no shelter or covering to prevent the food contamination from environmental bugs. The utensils are consciously washed in same water stored in one big tub. The food handler himself never gets checked for any certification or for any disease. The situation becomes worse when it come to treatment and diagnosis. Usually, with onset of symptoms, people start self medication which is unfortunately most common in Pakistan. As the patient's condition gets worse they consult a doctor where again symptomatic treatment is prescribed with no proper diagnosis (17-19).

MATERIALS AND METHODS

SAMPLE COLLECTION, PROCESSING AND INCUBATION

Total of 125 samples of meat items; beef, mutton, chicken, fish and prawn (n=25 each) were collected randomly from local meat vendors. ISO 6579:2002 method was employed to analyze the samples. Briefly, 225ml (1:10, w/v) of BPW-ISO was used to dissolve the 25g of food samples for primary enrichment of microbes. The collected samples were incubated at optimum temperature (37°C for 24 hours) for suspected microbial growth. For selective enrichment of microbial growth Rappaport-Vassiliadis broth (RV) and Mueller Kauffman Tetrathionate Novobiocin (MKTT) broth was used.

DIFFERENTIAL PLATTING FOR *SALMONELLA*

After selective enrichment, the bacterial colonies were streaked on XLD and SS agar plates. These selective media yields the typical black *Salmonella* colonies.

GRAM STANING AND MICROSCOPY

The bacterial growth from XLD and SS agar were subjected to Gram staining and later examined under microscope to visualize Gram negative rods.

STANDARD MICROBIOLOGICAL TESTS

The bacterial colonies visualized under microscope and suspected as *Salmonella* were further confirmed with series of sugar fermentation and biochemical tests. The tests performed were Indole, MR, VP, Urease, Gelatin, Nitrate, Lysine, Ornithine, TSI and Oxidase.

CARBOHYDRATE FERMENTATION TEST

Fermentation capability of microorganisms is checked by using Glucose, Manitol, Dulcitol, Sorbitol and Arabinose in growth media. Monitoring the acid production during fermentation process is the key which is usually checked by pH meter. *Salmonella* can ferment Glucose, Manitol and Sorbitol but not the Rabinose and Dulcitol.

RESULTS AND DISCUSSION

Randomly collected 125 meat samples were subjected to detect the *Salmonella* by traditional and conventional methods. After having the colonies in enrichment media, the bacterial growth was streaked on *Salmonella Shigella* SS agar and Xylose Lysin deoxycholate XLD agar. Typical black *Salmonella* colonies were evident for the contamination of food samples with pathogen as shown in Fig. 1.

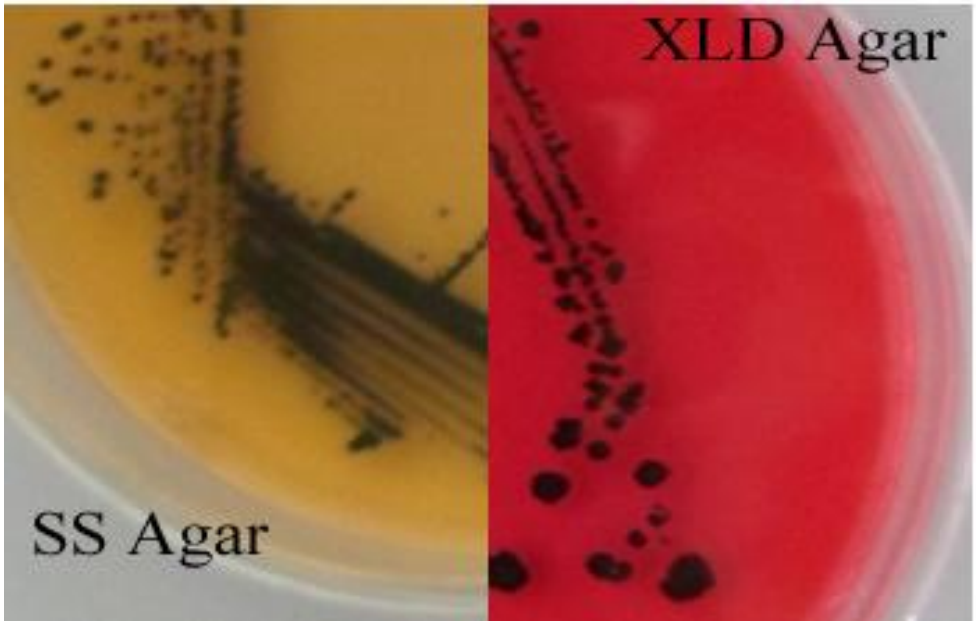

Fig. 1. Typical black colonies of *Salmonella* on SS Agar and XLD Agar

These black colonies were Gram stained for further confirmation. The images of microscopy demonstrated the Gram negative rods as shown in Fig. 2. These results further support the evidence of food contamination with *Salmonella*.

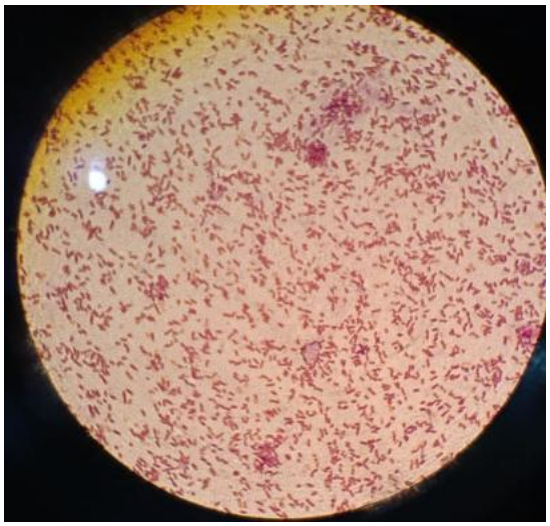

Fig. 2. Clearly visible Gram negative rods

For further confirmation a series of biochemical tests were performed like Triple Sugar Iron test (TSI) SIM agar test, IMViC Tests, Oxidase, Catalase, Urease, Nitrate reduction Tests and sugar fermentation tests, as shown in Fig. 3-5. *Salmonella* shows characteristics black colouration in butt and alkaline pink coloured slant in TSI test tube. In SIM agar *Salmonella* shows black colouration which is due to the production of H_2S gas. In Citrate utilization test *Salmonella* is not capable to utilize Citrate sugar. *Salmonella* belongs to oxidase negative Enterobacteriacea family of microbes thats why it is facultative anaerobe with oxidase negative property and catalase positive. Salmonella is capable to reduce nitrates to nitrites as it has nitrate reduction enzyme so it is presents nitrate positive tests. Salmonella does not have urease and

gelatinase enzymes which help any microorganism to break gelatine and urea. So *Salmonella* gave urease and gelatinase negative tests. Different bacterial genera have different unique patterns of sugar fermentation in a series of positive and negative sugar fermentation results. On the basis of these patterns many genera even upto species level can be identified. In current scenario *Salmonella* has been tested with six sugars viz, Glucose, Arabinose, Rhamnose, Sorbitol, Mannitol and Dulcitol as shown in Table I.

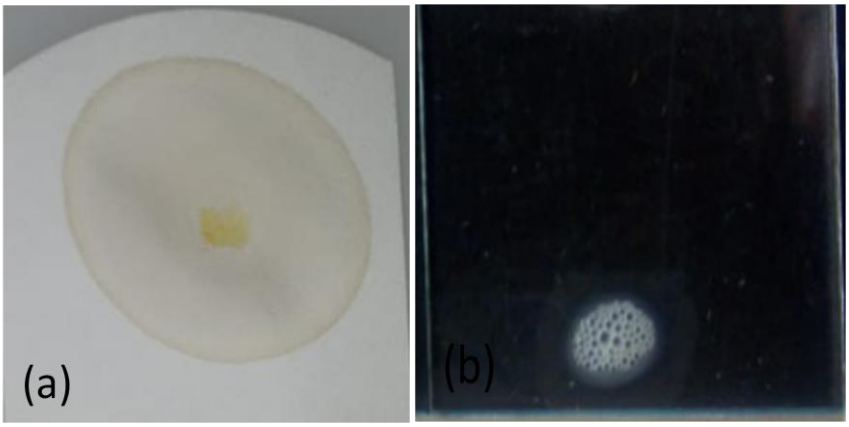

Fig. 3. Oxidase –ve and Catalase+ve test

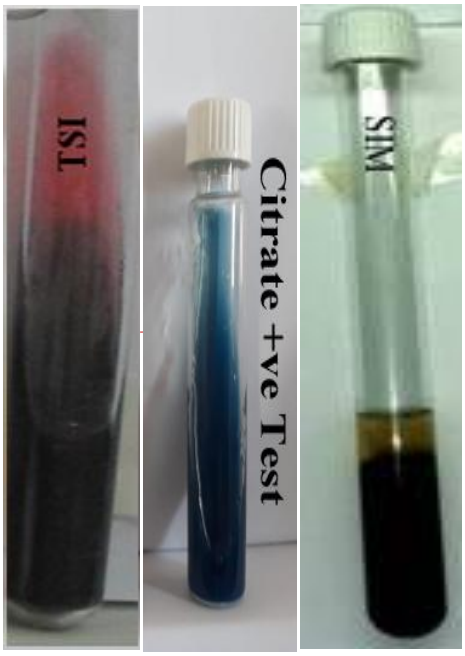

Fig. 2. TSI (K/A with black butt), SIM and Citrate tests used for *Salmonella*

Fig. 5. Biochemical test and Sugar fermentation results for *Salmunella*

The outcome of the biochemical tests performed and shown above is detailed in Table I. These biochemical tests also confirmed the contamination of food samples with *Salmonella*. For example *Salmonella* is negative for indole, MR and VP tests and our biochemical tests also confirmed the same as shown in figure 3 and mentioned in table 1. Those suspected colonies isolated from food samples were positive for the tests which have been previously documented the same for *Salmonella*.

Table 1: List of biochemical tests performed for identification of *Salmonella*

Test Name	Outcome
Indole	-ve
MR	+ve
VP	-ve
Urease	-ve
Gelatin	-ve
Nitrate	+ve
Lysine	+ve
Ornithine	+ve
Dulcitol	+ve
Manitol	+ve
Sorbitol	+ve
Glucose	+ve
Rhaminose	+ve
Arabinose	+ve
TSI	+ve
Oxidase	-ve

In last we quantified the results obtained after performing biochemical tests as shown in Table II, fish was the most contaminated food item while mutton was the least contaminated raw meat item (Table II).

Table II: *Salmonella* isolated from the collected raw meat samples

Raw meat items	Number of samples	*Salmonella* positive %
Beef	25	16% (n=4)
Mutton	25	08% (n=2)
Chicken	25	32% (n=8)
Fish	25	24% (n=6)
Prawn	25	12% (n=3)
Total	125	18.4% (n=23)

This study demonstrated that raw meat sold in Lahore city is contaminated with potentially pathogenic bacteria which can cause Salmonellosis. Frequency of *Salmonella* spp. contamination was high in raw chicken meat (32%) and low in raw mutton (08%) in our observed samples. These results are in agreement to previous studies conducted in Lahore city regarding the contamination of other food items like meat, egg, salad and water. A previous study demonstrated that retail raw meat and poultry samples from markets and supermarkets in Ho Chi Minh City of Vietnam, were heavily contaminated with *Salmonella* spp. (60.8%). This very high level of contamination indicates a potential breakdown of hygiene at various stages of the food processing and distribution chain and/or a lack of refrigeration of meat (20). In the observations of Van *et al.* (2007) the levels of *Salmonella* contamination in retail beef and chicken meat samples were much higher (62.0% and 53.3% respectively) than our findings (beef 16.0% and chicken 32.0%, whereas Phan *et al.*(2005) reported 48.6% for beef samples and 21.0% for chicken meat (21). The reported rates of *Salmonella* contamination, particularly in beef, were also higher in developing countries compared to

developed countries (22-24). Different sampling procedures, sample types, and bacterial detection and isolation techniques could affect the detected prevalences of *Salmonella* spp (25-27).

Such poor hygienic conditions and contaminated food can result in disease outbreak therefore the food safety and security laws should be implemented strictly. People consuming the raw, unhygienic or moderately cooked meat are prone to develop diseases especially gastroenteritis and other GIT diseases.

CONCLUSION

The important aspect of this study is that; one can carry out the isolation and detection of bacterial pathogens with conventional methods when modern tools are not available.

References:

1. Popkin BM, Corvalan C, Grummer-Strawn LM. Dynamics of the double burden of malnutrition and the changing nutrition reality. The Lancet. 2020 Jan 4;395(10217):65-74.
2. Popkin BM, Reardon T. Obesity and the food system transformation in Latin America. Obesity Reviews. 2018;19(8):1028-64.
3. Schwartz MB, Puhl R. Childhood obesity: a societal problem to solve. Obesity reviews. 2003;4(1):57-71.
4. Taylor EV, Herman KM, Ailes EC, Fitzgerald C, Yoder JS, Mahon BE, Tauxe RV. Common source outbreaks of Campylobacter infection in the USA, 1997–2008. Epidemiology & Infection. 2013;141(5):987-96.
5. Amagliani G, Brandi G, Schiavano GF. Incidence and role of Salmonella in seafood safety. Food Research International. 2012;45(2):780-8.
6. Ali NH, Farooqui A, Khan A, Khan AY, Kazmi SU. Microbial contamination of raw meat and its environment in retail shops in Karachi, Pakistan. The Journal of Infection in Developing Countries. 2010;4(06):382-8.
7. Samad A, Abbas F, Ahmad Z, Pokryrshko O, Asmat TM. Prevalence of foodborne pathogens in food items in Quetta, Pakistan. Pakistan J. Zool. 2018 Aug 1;50(4):1-4.
8. Samad A, Abbas F, Ahmad Z, Tanveer Z, Ahmad I, Patching SG, Nawaz N, Asmat MT, Raziq A, Naeem M, Akhtar MA. Multiplex polymerase chain reaction detection of Shiga toxin genes and antibiotic sensitivity of Escherichia coli O157: H7 isolated from beef meat in Quetta, Pakistan. Journal of Food Safety. 2018;38(6):e12540.
9. Tareen AM, Ullah K, Asmat TM, Samad A, Iqbal A, Mustafa MZ, Ahmad I. Incidence of Diarrheagenic Escherichia coli Pathotypes in Children Suffering from Diarrhea in Tertiary Care Hospitals, Quetta, Pakistan. Pakistan Journal of Zoology. 2019;51(6).
10. Samad A, Abbas F, Tanveer Z, Ahmad Z, Asmat TA, Raziq AB, Zahid M. Prevalence of Salmonella spp. in chicken meat from Quetta retail outlets and typing through multiplex PCR. Rom. Biotechnol. Lett.. 2018;10:1-1.
11. Mengal H, Samad A, Asmat MT, Mustafa MZ, Abbas F, Sajjad N, Ishtiaq H, Rahim H, Ghilzai D. 3. The study to evaluate the prevalence of Yersinia species in salad sold and water supplied in Quetta city, Pakistan. Pure and Applied Biology (PAB). 2019;8(3):1902-8.
12. Asmat TM. Molecular detection of Escherichia coli (E. coli) from Diarrheal stool samples from children in Quetta, Balochistan, Pakistan. Pak-Euro Journal of Medical and Life Sciences. 2019;2(2):27-31.
13. Asmat TM. Campylobacter-Review on its Significance as a Foodborne Pathogen. Pak-Euro Journal of Medical and Life Sciences. 2020;3(3):136-42.
14. Huang C, Virk SM, Shi J, Zhou Y, Willias SP, Morsy MK, Abdelnabby HE, Liu J, Wang X, Li J. Isolation, characterization, and application of bacteriophage LPSE1 against Salmonella enterica in ready to eat (RTE) foods. Frontiers in microbiology. 2018;9:1046.
15. Carrasco E, Morales-Rueda A, García-Gimeno RM. Cross-contamination and recontamination by Salmonella in foods: a review. Food Research International. 2012;45(2):545-56.
16. Sperber SJ, Schleupner CJ. Salmonellosis during infection with human immunodeficiency virus. Reviews of infectious diseases. 1987;9(5):925-34.
17. Podolak R, Enache E, Stone W, Black DG, Elliott PH. Sources and risk factors for contamination, survival, persistence, and heat resistance of Salmonella in low-moisture foods. Journal of food protection. 2010;73(10):1919-36.
18. Damborg P, Broens EM, Chomel BB, Guenther S, Pasmans F, Wagenaar JA, Weese JS, Wieler LH, Windahl U, Vanrompay D, Guardabassi L. Bacterial zoonoses transmitted by household pets: state-of-the-art and future perspectives for targeted research and policy actions. Journal of Comparative Pathology. 2016;155(1):S27-40.

19. Whiley H, Ross K. Salmonella and eggs: from production to plate. International journal of environmental research and public health. 2015;12(3):2543-56.
20. Van TT, Moutafis G, Istivan T, Tran LT, Coloe PJ. Detection of Salmonella spp. in retail raw food samples from Vietnam and characterization of their antibiotic resistance. Applied and environmental microbiology. 2007;73(21):6885-90.
21. Phan TT, Khai LT, Ogasawara N, Tam NT, Okatani AT, Akiba M, Hayashidani H. Contamination of Salmonella in retail meats and shrimps in the Mekong Delta, Vietnam. Journal of Food Protection. 2005;68(5):1077-80.
22. Padungtod P, Kaneene JB. Salmonella in food animals and humans in northern Thailand. International Journal of Food Microbiology. 2006;108(3):346-54.
23. Stevens A, Kaboré Y, Perrier-Gros-Claude JD, Millemann Y, Brisabois A, Catteau M, Cavin JF, Dufour B. Prevalence and antibiotic-resistance of Salmonella isolated from beef sampled from the slaughterhouse and from retailers in Dakar (Senegal). International Journal of Food Microbiology. 2006;110(2):178-86.
24. Zhao C, Ge B, De Villena J, Sudler R, Yeh E, Zhao S, White DG, Wagner D, Meng J. Prevalence of Campylobacter spp., Escherichia coli, and Salmonella serovars in retail chicken, turkey, pork, and beef from the Greater Washington, DC, area. Applied and environmental microbiology. 2001;67(12):5431-6.
25. Pangloli P, Dje Y, Oliver SP, Mathew A, Golden DA, Taylor WJ, Draughon FA. Evaluation of methods for recovery of Salmonella from dairy cattle, poultry, and swine farms. Journal of food protection. 2003;66(11):1987-95.
26. Jørgensen F, Bailey R, Williams S, Henderson P, Wareing DR, Bolton FJ, Frost JA, Ward L, Humphrey TJ. Prevalence and numbers of Salmonella and Campylobacter spp. on raw, whole chickens in relation to sampling methods. International journal of food microbiology. 2002;76(1-2):151-64.
27. Bryan FL, Doyle MP. Health risks and consequences of Salmonella and Campylobacter jejuni in raw poultry. Journal of Food Protection. 1995;58(3):326-44.