Int.J.Curr.Microbiol.App.Sci (2018) *7*(4): 1732-1737

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 04 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.704.197

Prevalence and Study of Antibiotic Resistant Pattern of *Salmonella* in Raw Milk

R.M. Dhingani*, B.H. Joshi and R.V. Prasad

Department of Food Quality Assurance, College of Food Processing Technology and Bio-Energy, Anand Agricultural University, Anand-388110, Gujarat, India

**Corresponding author*

Keywords

Raw milk, *Salmonellosis*, RT-PCR, Antibiotic susceptibility and pasteurization

Article Info

Accepted:
12 March 2018
Available Online:
10 April 2018

A B S T R A C T

Salmonellosis is one of the most commonly reported food borne disease all over the world and developing countries. This study was conducted to determine the prevalence of *Salmonella* spp. in raw milk. Isolation and identification of *Salmonella* was carried out using real time polymerase chain reaction (RT-PCR) technique and sensitivity tests were done by the Kirby-Bauer disc diffusion method. Total of 70 raw milk samples were collected from the 5 different dairy farms. The prevalence of *Salmonella* spp. in raw milk of the study area was 8.57%. This study reveals that collected *Salmonella* spp. were susceptible to antibiotics and also heat sensitive at pasteurization process which ensures the safety of pasteurized milk against *Salmonella* spp.

Introduction

Milk is an essential part of daily diet for the growing children. It has been described as a nearly perfect food because it contains the essential nutrients required by the body in appropriate proportions.

However, the safety of milk and dairy products with respect to food-borne diseases is a major global issue especially in the developing countries where production of milk and milk product takes place under poor hygienic, sanitary and agricultural practices (Jordan, 2007).

Raw milk and milk products are increasingly becoming important sources of human infection with *Salmonella.*

It is major pathogenic bacteria that cause salmonellosis on human being and other organisms in the world (Mrema *et al.*, 2006).

Salmonellosis is one of the major zoonotic diseases all over the world with annual estimates of 22 million cases and 200,000 deaths due to typhoid fever (Crump, *et al.*, 2004) and 93.8 million cases of gastroenteritis and 155 000 deaths due to non-typhoidal Salmonellae (NTS) (Majowicz, *et al.*, 2010).

Salmonella includes more than 2500 different serotypes represents a leading cause of foodborne infections worldwide. The majority of the infections are associated with ingestion of contaminated foods such as poultry, beef, egg, milk, cheese, seafood, fruits, juice and vegetables (Brands *et al.*, 2005; Zhao *et al.*, 2008). The distribution of *Salmonella* can vary greatly depending on the Serovars. Species such as *Salmonella enterica serotype Enteritidis* and *Salmonella enterica serotype Typhimurium* have established global niches (Ellermeier and Slauch, 2006). *Salmonella* infection is generally self-limiting illness, but severe cases in immuno-compromised individuals, elderly persons or neonates, and systemic infections may require effective chemotherapy.

Traditional microbiological methods offered standardized procedures for microbial detection. However, they are time consuming, laborious and not always compatible with short-time-to-result demand. Therefore, food microbiology aims for supplementation of classical methods with molecular techniques based on detection of the nucleic acids, which shorten the analysis time and lower the limit of detection (Kusar *et al.*, 2010). Since *Salmonella* was closely related to both public and animal health, more rapid and sensitive methods for the identification of these bacteria are required. RT-PCR technology offers several advantages compared with classical bacteriology in terms of speed, detection limit, potential for automation and cost.

The development of antimicrobial resistance among bacteria (AMR) is currently one of the world's most pressing public health problems. The World Health Organization (WHO) has reported as increase in the incidence of antibiotic resistant strains of *Salmonella* due to the use of antibiotics as treatment and prophylaxis frequently around the world WHO (2010). Misuse of antimicrobial agents both in humans and animals has narrowed the potential use of antibiotics for the treatment of infections in humans. To monitor the evolution of AMR and to develop control measures, some countries, have set up national integrated monitoring systems.

Similarly, with *Salmonella* being an important cause of food-borne diarrheal disease in human beings, the reduction in the number of antibiotics available for effective treatment of *Salmonella* related infectious diseases in humans and animals has become a serious concern. The frequency and extent of the resistance to antimicrobials by *Salmonella* vary based on the antimicrobial usage in humans and animals and the ecological differences in the epidemiology of *Salmonella* infections.

Thus, the aim of this project was to study prevalence and antimicrobial resistance pattern of *Salmonella* from the raw milk.

Materials and Methods

Sample collection

The study was conducted in region of Anand and its surrounding area of Gujarat, India. Samples of raw milk were collected aseptically in sterilized 500 ml screw capped bottle from five different dairies at different time interval. Collected samples were transported to laboratory under cooling condition and temporarily kept under refrigerator at 4°C until processed further.

Sample enrichment

Within 2 to 6 h of collection, 25 ml of collected samples were homogenized with 225 ml of non-enrichment buffered peptone medium (peptone: 10 g; sodium chloride: 5 g; disodium hydrogen orthophosphate. $12H_2O$: 9 g; potassium dihydrogen orthophosphate:

1.5g; deionized water to: 1000ml; pH: 7 ± 0.2). In stomacher bag and incubated at 37°C±1 for 24 h.

DNA extraction and purification

DNA was extracted from the pre-enrichment broth using iQ-Check *Salmonella* II kit (Bio-Rad) following the manufacturer instruction; One ml of enriched samples were collected into sterile tubes from the pre-enrichment broth and centrifuged at 10,000 rpm for 5 min. Supernatant discarded and 200 µl of lysis reagent added in to the pellet and mixed it well. For incubation it was placed in water bath at 95°C for 15 min. After incubation period it was centrifuged at 10,000 rpm for 5 min. After centrifugation, supernatant (5 µl) were used as a sample.

Sample preparation for PCR

Total 50 µl of PCR mixture were prepared; 5 µl of sample were mixed with 40 µl of amplification mix (containing taq polymerase, buffer, dNTPs, $MgCl_2$ and primers) and 5 µl of fluorescent probe (Fig. 2).

PCR setup

PCR mix were amplified and analyzed along with the positive and negative control by CFX 96 Real Time System with C1000 Touch Thermal Cycler.

Antibiotic resistance pattern of *Salmonella*

The antimicrobial susceptibility testing for *Salmonella* isolates were carried out following the Kirby- Bauer's disc diffusion method as described in the guidelines of National Committee for Clinical Laboratory Standards (NCCLS). The *Salmonella* isolates were tested against following antibiotics; ampicillin (10 µg), gentamicin (10 µg), nalidixic acid (30 µg), chloramphenicol (30 µg), cefalexin (30 µg) and co-trimoxazole (25 µg). A standard suspension of active culture prepared in sterile N-saline and it was spread on nutrient agar plate. The antibiotic hexavalent disc was dispensed on the medium and incubated at 35°C for 18 h and zone of inhibition was measured.

Heat resistant profile of *Salmonella*

Heat resistant profile of the selected *Salmonella* isolates was studied by exposing all the isolates at 62.8 ºC for 30 min.

Results and Discussion

Sample collection

Total of 70 raw milk samples were collected from the different places for the prevalence study of *Salmonella* as depicted in Figure 1.

Identification of *Salmonella*

Real Time Polymerase Chain Reaction techniques were used for the identification *Salmonella* by using CFX 96 real time system with C1000 touch thermal cycler (Fig. 3).

From the five different regions 70 samples of raw milk were collected. Out of it 6 raw milk samples were found to be contaminated with *Salmonella* (Figure 4). Isolates collected from the contaminated samples were further tested for antibiotic and heat resistance profile.

Antibiotic resistance profile of *Salmonella*

Salmonella isolated from contaminated raw milk were subjected for antibacterial resistance profile studies as per the guidelines of National Committee for Clinical Laboratory Standards (NCCLS). The study reveals that all selected isolates were sensitive to selected antibiotics as shown in Figure 5.

Fig.1 Collection of raw milk from different region

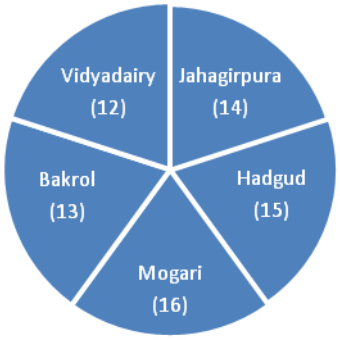


Fig.2 Amplification protocol for *Salmonella* DNA by using RT-PCR

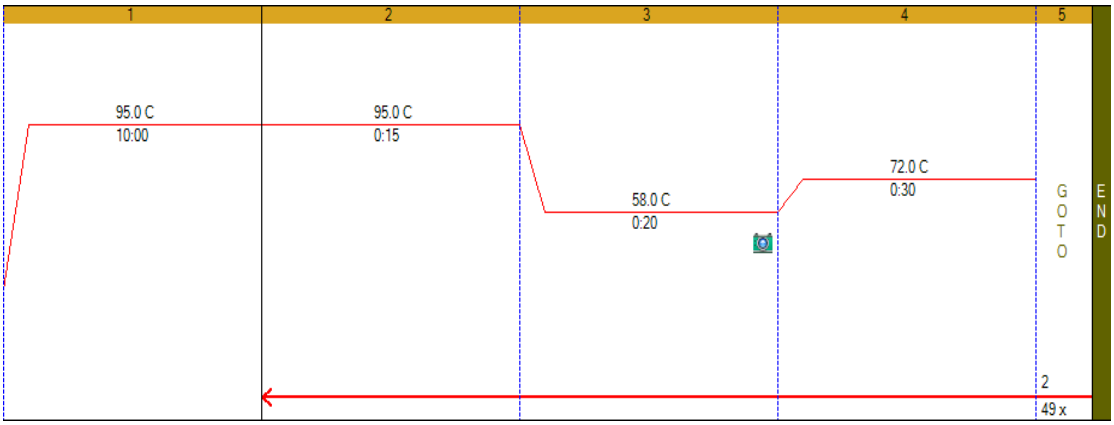


Fig.3 DNA Amplification curve with cT value of *Salmonella*

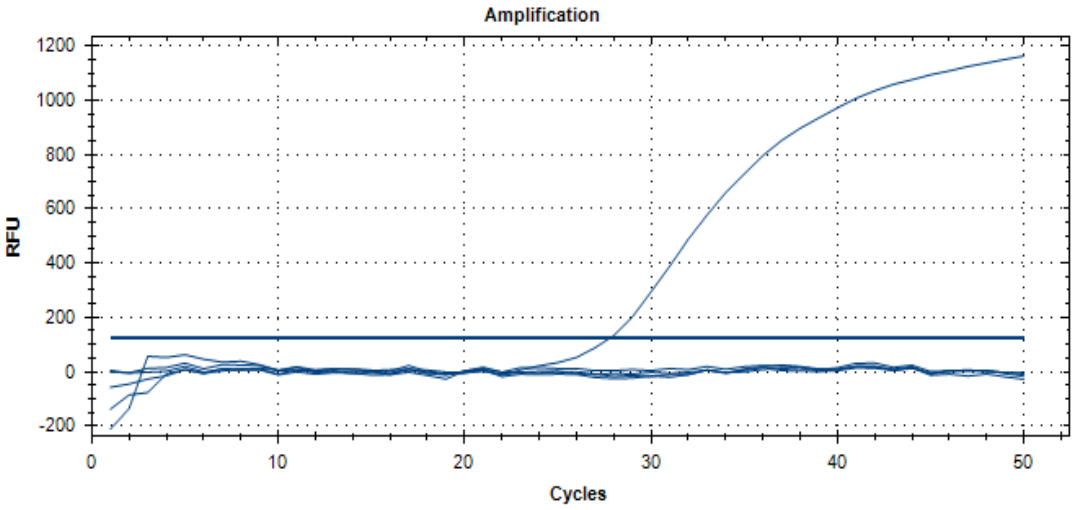


Fig.4 Distribution of *Salmonella* in raw milk

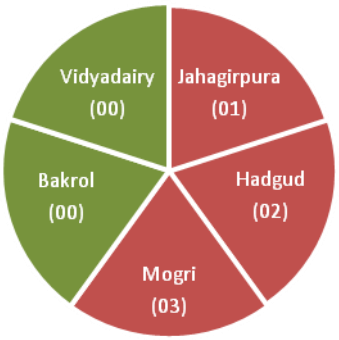
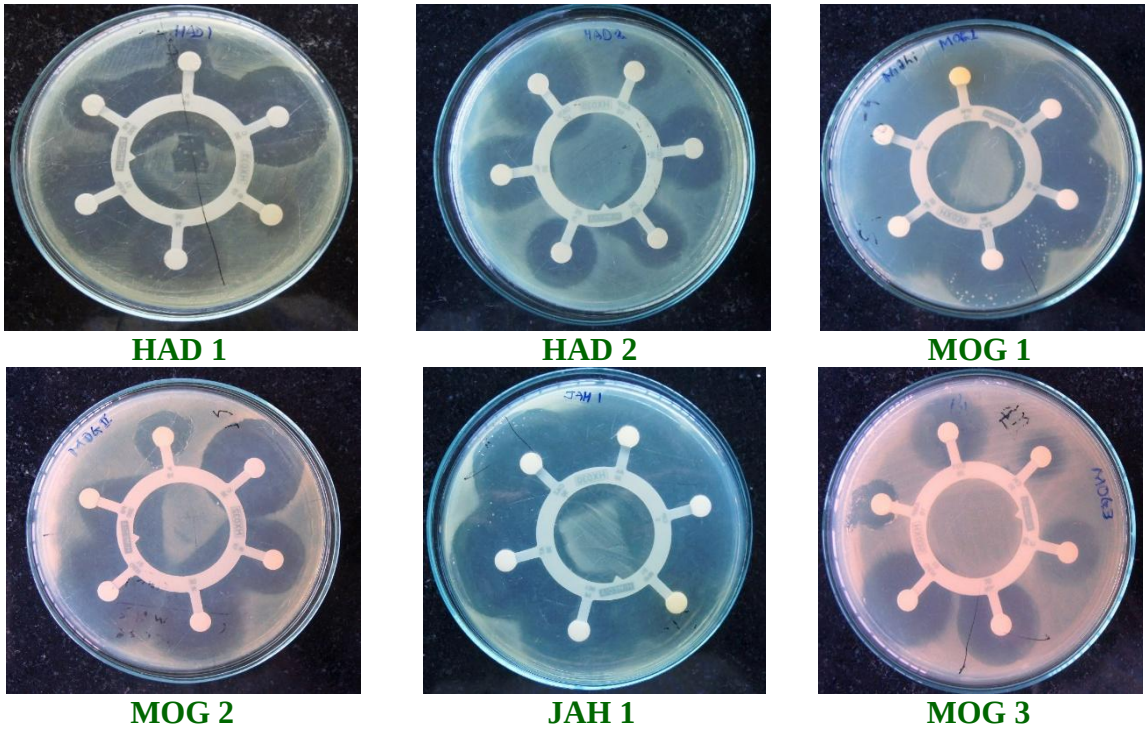


Fig.5 Antimicrobial resistant profile of *Salmonella* isolates obtained from raw milk



Heat resistant profile of *Salmonella*

These antibiotic sensitive isolates of *Salmonella* were also evaluated for its heat resistant profile by exposing them at 62.8ºC for 30 min. None of the isolates were found heat resistant at temperature above pasteurization process.

In conclusion, total of 70 samples of raw milk were evaluated for prevalence of *Salmonella* procured from five different regions of Anand district of Gujarat. Out of that only 6 samples were found to be contaminated with *Salmonella* reveals 8.57% prevalence. All the six collected *Salmonella* species were antibiotic sensitive and none of them shown resistance at pasteurization temperature. This ensures the safety of pasteurized milk against *Salmonella* prevalent in Anand region of Gujarat.

References

Brands DA, Inman AE, Gerba CP, Mare CJ, Billington SJ, Saif LA, Levine JF, Joens LA. 2005. Prevalence of Salmonella spp. in oysters in the United States. Appl. Environ. Microbiol. 71, 893-897.

Charlotta Lofstrom, Michael Krause, Mathilde H Josefsen, Flemming Hansen and Jeffrey Hoorfar. 2009. Validation of a same day real-time PCR method for screening of meat and carcass swabs for Salmonella. BMC Microbiol. 9, 85.

Crump JA, Luby SP, Mintz ED. 2004. The global burden of typhoid fever. Bull World Health Organ. 82(5):346–353.

Ellermeier CD and Slauch JM. 2006. Genus Salmonella. In: Dworkin, M.D. The Prokaryotes: A Handbook on the Biology of Bacteria. Springer Press. New York.

Jordan D. 2007. Antimicrobial resistance in animals and impacts on food safety and public health. Infections. 28(4): 163-164.

Kusar D, Pate M, Micunovic J, Hribovsek VB, Ocepek M. 2010. Detection of Salmonella in poultry faces by molecular means in comparison to traditional bacteriological methods. Slov. Vet. Res. 47(2):45–56.

Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, Jones TF, Fazil A, Hoekstra RM. 2010. The global burden of nontyphoidal Salmonella gastro-enteritis. Clin. Infect. Dis. 50: 882–889.

Mrema N, Mpuchane S and Gashe BA. 2006. Prevalence of Salmonella in raw meat minced meat, raw fresh sausage and burger patties from retail outlets in Gaborone, Botswana. Food Control, 17: 207-212.

World Health Organization (WHO). 2010. Antimicrobial susceptibility of *Salmonella enterica* serovars in a tertiary care hospital in southern India. Indian J. Med. Res. 137: 800-802.

Zhao S, White, DG, Friedman SL, Glenn A, Blickenstaff K, Ayers SL, Abbott JW, Hall-Robinson E, McDermott PF. 2008. Antimicrobial resistance in Salmonella enteric serovar Heidelberg isolates from retail meats, including poultry. Appl. Environ. Microbiol. 74, 6656-6662.

How to cite this article:

Dhingani, R.M., B.H. Joshi and Prasad, R.V. 2018. Prevalence and Study of Antibiotic Resistant Pattern of *Salmonella* in Raw Milk. *Int.J.Curr.Microbiol.App.Sci.* 7(04): 1732-1737. doi: https://doi.org/10.20546/ijcmas.2018.704.197