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Growth Dynamics of *Salmonella*, Isolated from Different Sources, at different Temperature and pH

M.A. Malik[1], S.K. Kotwal[1], M. Rashid[1], H.K. Sharma[1], M. Singh[1] and Neelesh Sharma[2]*

[1]Division of Veterinary Public Health & Epidemiology, F.V.Sc. & A.H., SKUAST-J, R.S. Pura, Jammu, INDIA
[2]Division of Veterinary Medicine, F.V.Sc. & A.H., SKUAST-J, R.S. Pura, Jammu, INDIA

**Corresponding author: N. Sharma; Email: drneelesh_sharma@yahoo.co.in*

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ABSTRACT

A total of 350 samples 50 each from raw poultry meat, poultry cloacal swabs and human diarrheic cases, besides 200 eggs were processed for the isolation of *Salmonella.* Effect of varying temperature and pH on growth of *Salmonella* isolates was evaluated by growing the organism in Brain Heart Infusion Broth at 4, 10, and 30°C with pH values of 4.5, 5.5 and 6.5. At 10°C, the mean generation time of the isolates at pH values of 4.5, 5.5 and 6.5 was 44.23 ± 0.44, 15.51 ± 0.08 and 10.23 ± 0.10 hrs, respectively, while at 30°C, the generation time of the isolates at pH values of 4.5, 5.5 and 6.5 was 126.10 ± 0.68, 59.96 ± 0.14 and 36.22 ± 0.07 min, respectively. No growth observed at 4°C, at any given pH value. As the temperature and pH were lowered, significant increase in generation time of the organism was observed.

Keywords: Salmonella, growth, temperature, pH.

The genus *Salmonella* is a typical member of the family Enterobacteriaceae and consists of Gram -ve, oxidase -ve, straight sided rod shaped bacteria which are castalase +ve, and have both a respiratory and a fermentative metabolism of carbohydrates. Most Salmonellae are motile by peritrichous flagella. More than 2,500 *Salmonella* serovars have been identified, most belonging to the species *Salmonella bongori* and *Salmonella enterica*. Based on pathogenesis, *S. enterica* can be divided into two broad groups, Group 1 consists of a large number of serovars, including *Salmonella enterica* serovars Typhimurium and Enteritidis, which can cause paratyphoid in infected animals. This group colonizes the alimentary tract of food animals, and leads to gastrointestinal disease in a broad range of hosts, including humans. Group 2 comprises a small number of serovars that cause systemic typhoid-like disease in a restricted range of host species. A wide variety of domestic and wild animals viz. cattle, swine, sheep, goat, horses, and fowl could be the reservoirs of zoonotic Salmonellae. Practically, any food of animal origin can be a source of infection for man. The most common vehicles are contaminated poultry, pork, beef, eggs and milk (Acha and Szyfres, 2003).

Salmonellae can grow over a wide range of temperature, pH, and a_w in good culture medium rather in a poor one. For example, minimal temperatures for growth in food range from 6.7 to 7.8°C in chicken to over 10°C in custard and ham salad. They grow well at room temperature, but optimum temperature for their growth is about 37°C and maximal temperature at which they can grow is about 45.6°C. The pH range for their growth is 4.1 to 9.0, growing in low-acid foods, however, salad at pH 5.5 to 5.7 has been found unfavourable for growth. The lowest a_w for growth varies with the food but is about 0.93 to 0.95. The species and strains of *Salmonella* differ too, in heat resistance and effect of environmental factors on growth (Frazier and Westhoff, 2003). Several years ago, hurdle technology was developed as a new concept for the realization of safe, stable, nutritious, tasty, and economical foods (McMeekin *et al*., 2000). Several environmental factors such as temperature, pH, water activity (aw), atmosphere, and presence or absence of preservatives

affect the growth of microorganisms in foods. It has been known that combinations of inhibitory factors can give synergistic effects. It is thought that a multifactorial approach for food preservation may be more successful than a more extreme use of any single treatment (Thomas *et al.,* 1991).

MATERIALS AND METHODS

Isolation of Salmonella

Collection of samples

Samples of raw poultry meat, and egg were collected from market of Jammu city and adjoining areas. Poultry cloacal swabs: These were collected from organized and unorganized poultry farms of Jammu region. Human Diarrheic Cases: Stool samples from patients with diarrhea were collected from Sub- District hospital, R. S. Pura, Jammu, India.

Sample size

A total of 350 samples were processed for the isolation of *Salmonella.* The samples comprised of 50 each from raw poultry meat, poultry cloacal swabs and human diarrheic cases, besides 200 eggs. The details of various samples collected along with their source are given in Table 1.

Table 1. Details of samples processed for isolation of *Salmonella*

S.No.	Type of sample	No.	Source
1	Raw poultry meat	50	Local markets of Jammu
2	Eggs	200	Local markets of Jammu
3	Poultry cloacal swabs	50	Organized and unorganized poultry farms of R.S.Pura
4	Human diarrheic cases	50	Sub- District hospital, R.S.Pura
	Total	350	

Methodology

The isolation of *Salmonella* from various samples was done as per the ISO 6579/2002 method**.** The media were obtained from HiMedia Laboratories Pvt. Ltd., Bombay, India. and were prepared as per the instructions of the manufacture.

Effect of varying Temperature and pH on Growth of *Salmonella*

The growth of all the isolates at varying temperature and pH was evaluated. The study was carried out as per method of Juneja and Marks (2006) with some modifications. The thermal inactivation of the organism was not done, instead growth of the organism was studied at different temperature and pH combinations. Medium used: Brain Heart Infusion Broth (HiMedia, M210I).

The medium was dispensed into 20 ml proportions in 30 ml test tubes and sterilized by autoclaving at 15 lbs pressure for 15 minutes and sterility was tested by incubating at 37°C for 24 hours. For each isolate two sets I and II consisting of 9 test tubes each were prepared and each set was further divided into 3 subsets a, b and c consisting of 3 test tubes with pH values of 4.5, 5.5 and 6.5, respectively. In the test tubes of set II, no organism was inoculated and these were kept as negative control. After inoculation of the test tubes in set-I with the organism, one test tube from each subset of both the sets was incubated at 4, 10, and 30°C. Total sampling time ranged from 14- 21 days at 4, 10°C, and 48- 60 hrs at 30°C. Sampling frequency was every 24 hrs at 4, 10°C, and every 3 hrs at 30°C.

The bacterial populations were determined at different time intervals by using spread plate method (Collee *et al.,* 1989). Bacterial count was carried on TSA (3. XIV).

Generation time of the isolates at varying temperature and pH

It was calculated as per formula of Brock (1979):

Generation time = 1/k

Where $K = \frac{\log X - \log X0}{(0.301)t}$

X_0 = Bacterial population at the beginning of log phase.

X_t = Population at the peak of log phase.

t = Time taken for reaching Xt from X0.

0.301 = Constant

RESULTS AND DISCUSSION

Isolation of *Salmonella* from various sources

Isolation of *Salmonella* was carried out from raw meat of poultry (n = 50), poultry cloacal swabs (n = 50), eggs (n = 200) and human diarrheic cases (n = 50).

Isolation and identification of *Salmonella* isolates from raw meat of poultry, poultry cloacal swabs, eggs and human diarrheic cases

Of the 50 meat samples collected from freshly slaughtered poultry, salmonellae were isolated from three samples, the incidence being 6 per cent. Similarly, out of fifty poultry cloacal swabs that were screened for *Salmonella,* only four samples were positive and the prevalence being 8 per cent. Likewise, 1 and 3 isolates of *Salmonella* could be recovered from 200 eggs and 50 human diarrheic cases, respectively, thus showing the prevalence of 0.5 per cent in eggs and 6 per cent in human diarrheic cases. The results are presented in Table 2.

Table 2. Isolation of *Salmonella* from various sources

Type of sample	No. of samples	No. of isolates	Per cent isolation
Raw poultry meat	50	3 (S_1 , S_2 , S_3)	6
Poultry cloacal swabs	50	4 (S_4, S_5, S_6, S_7)	8
Eggs	200	1 (S_8)	0.5
Human diarrheic cases	50	3 (S_9, S_{10}, S_{11},)	6

Growth of the isolates at 30 °C at pH 6.5

Growth of isolates, obtained from raw poultry meat, at 30°C at pH 6.5

Isolate S_1 : The initial bacterial count of the isolate was 2.8 x10^2 CFU/ml. Lag phase recorded was of 6 hrs duration and log phase lasted for 15 hrs. Maximum bacterial population of 9.4 x10^9 CFU/ml was attained after 21 hrs. The generation time for S_1 obtained was 36.5 min.

Isolate S_2: S_2 had bacterial count of 3.5 x10^2 CFU/ml on zero hrs and attained maximum bacterial population of 9.2 × 10^8 CFU/ml after 30 hrs. The lag phase and log phase lasted for 6 and 24 hrs respectively and the generation time for S_2 was 36 min .

Isolate S_3: The initial bacterial count of the isolate was 3.6 × 10^2 CFU/ml. Lag phase was of 6 hrs duration and log phase lasted for 24 hrs. Maximum bacterial population of 9.1 × 10^8 CFU/ml was attained after 30 hrs. Generation time for S_3 was 36.5 min .

Growth of isolates, obtained from poultry cloacal swabs, at 30°C at pH 6.5

Isolate S_4 : The initial bacterial count of the isolate was 2.6 x10^2 CFU/ml. Lag phase was of 6 hrs duration and log phase lasted for 24 hrs. Maximum bacterial population of 8.9 × 10^8 CFU/ml was attained after 30 hrs. Generation time for S_4 was 36 min.

Isolate S_5: It had bacterial count of 2.1 × 10^3 CFU/ml on zero hrs and attained maximum population of 7.4 × 10^9 CFU/ml after 30 hrs. The lag phase and log phase lasted for 6 and 24 hrs respectively and the generation time was 36.5 min.

Isolate S_6: The bacterial count of the isolate was 3.1 × 10^3 CFU/ml on zero hrs. Lag phase was of 6 hrs duration and log phase lasted for 24 hrs. Maximum bacterial population of 8.3 × 10^9 CFU/ml was attained after 30 hrs and the generation time was 36 min.

Isolate S_7: S_7 had bacterial count of 3.2 × 10^3 CFU/ml on zero hrs and attained maximum bacterial population of 8.6x10^9 CFU/ml after 30 hrs. The lag phase and log phase lasted for 6 and 24 hrs respectively. Generation time for S_7 was 36 min.

Growth of isolates, obtained from eggs, at 30°C at pH 6.5

Isolate S_8 : The initial bacterial count of the isolate was 1.5 × 10^2 CFU/ml. Lag phase was of 6 hrs duration and log phase lasted for 24 hrs. Maximum bacterial population of 7.8 × 10^8 CFU/ml was attained after 30 hrs. Generation time for S_8 was 36.5 min.

Growth of isolates, obtained from human diarrheic cases, at 30°C at pH 6.5

Isolate S_9: The initial bacterial count of the isolate was 2.5 x10^2 CFU/ml. Lag phase was of 6 hrs duration and log phase lasted for 24 hrs. Maximum bacterial population of 8.8 × 10^8 CFU/ml was attained after 30 hrs. Generation time for S_8 was 36 min.

Isolate S_{10}: The isolate had bacterial count of 3.7 × 10^2 CFU/ml on zero hrs and attained maximum population of 8.7 × 10^8 CFU/ml after 30 hrs. The lag phase and log phase lasted for 6 and 24 hrs respectively and the generation time calculated was 36.5 min .

Isolate S_{11}. The bacterial count of the isolate was 2.1 × 10^3 CFU/ml on zero hrs. Lag phase was of 6 hrs duration and log phase lasted for 24 hrs. Maximum bacterial population of 7.4x10^9 CFU/ml was attained after 30 hrs and the generation time obtained was 36 min .

Growth of isolates at 30°C with pH of 5.5

Growth of isolates, obtained from raw poultry meat, at 30°C at pH 5.5

Isolate S_1: The initial bacterial count of the isolate was1.5 x10^2 CFU/ml. Lag phase was of 6 hrs duration and log phase lasted for 24 hrs. Maximum bacterial population of 7.8 × 10^8 CFU/ml was attained after 30 hrs and the generation time recorded was 59.4 min.

Isolate S_2: The isolate had bacterial count of 3.5 × 10^2 CFU/ml on zero hrs and attained maximum bacterial population of 9.2 × 10^8 CFU/ml after 30 hrs. The lag phase and log phase lasted for 6 and 24 hrs respectively. Generation time for S_2 was 59.6 min.

Isolate S_3. The initial bacterial count of the isolate was 3.6 × 10^2 CFU/ml. Lag phase was of 6 hrs duration and log phase lasted for 24 hrs. Maximum bacterial population of 9.1 × 10^8 CFU/ml was attained after 30 hrs. Generation time for S_3 was 60.6 min.

Growth of isolates, obtained from poultry cloacal swabs, at 30°C at pH 5.5

Isolate S_4: The initial bacterial count of the isolate was 2.6 × 10^2 CFU/ml. Lag phase was of 6 hrs duration and log phase lasted for 24 hrs. Maximum bacterial population of 8.9x10^8 CFU/ml was attained after 30 hrs. Generation time for S_4 was 60.6 min.

Isolate S_5: It had bacterial count of 2.1 × 10^3 CFU/ml on zero hrs and attained maximum population of 7.4 × 10^9 CFU/ml after 30 hrs. The lag phase and log phase lasted for 6 and 24 hrs respectively. Generation time for S_5 was 60 min.

Isolate S_6. The bacterial count of the isolate was 3.1 × 10^3 CFU/ml on zero hrs. Lag phase was of 6 hrs duration and log phase lasted for 24 hrs. Maximum bacterial population of 8.3 × 10^9 CFU/ml was attained after 30 hrs. Generation time for S_6 was 60 min.

Isolate S_7: S_7 had bacterial count of 3.2 × 10^3 CFU/ml on zero hrs and attained maximum bacterial population of 8.6x10^9 CFU/ml after 30 hrs. The lag phase and log phase lasted for 6 and 24 hrs respectively. Generation time for S_7 was 60 min.

Growth of isolates, obtained from eggs, at 30°C at pH 5.5

Isolate S_8: The initial bacterial count of the isolate was 1.5 × 10^2 CFU/ml. Lag phase was of 6 hrs duration and log phase lasted for 24 hrs. Maximum bacterial population of 7.8 × 10^8 CFU/ml was attained after 30 hrs. Generation time for S_8 was 59.4 min.

Growth of isolates, obtained from human diarrheic cases, at 30°C at pH 5.5

Isolate S_9: The initial bacterial count of the isolate was 2.5 x10^2 CFU/ml. Lag phase was of 6 hrs duration and log phase lasted for 24 hrs. Maximum bacterial population of 8.8 × 10^8 CFU/ml was attained after 30 hrs. Generation time for S_8 was 59.4 min.

Isolate S_{10}: It had an initial bacterial count of 3.7 × 10^2 CFU/ml on zero hrs and attained maximum population of 8.7 × 10^8 CFU/ml after 30 hrs. The lag phase and log phase lasted for 6 and 24 hrs respectively. The calculated generation time for S_{10} was 60.6 min.

Isolate S_{11}: The bacterial count of the isolate was 2.1 × 10^3 CFU/ml on zero hrs. Lag phase was of 6 hrs duration and log phase lasted for 24 hrs. Maximum bacterial population

of 7.4x10^9 CFU/ml was attained after 30 hrs. Generation time for S_{11} was 60 min.

Growth of the isolates at 30°C at pH 4.5

Growth of isolates, obtained from raw poultry meat, at 30°C at pH 4.5

Isolate S_1: The initial bacterial count of the isolate was 1.6 × 10^3 CFU/ml. Lag phase was of 12 hrs duration and log phase lasted for 39 hrs. Maximum bacterial population of 9.2 × 10^8 CFU/ml was attained after 51 hrs. Generation time for S_1 was 125.4 min.

Isolate S_2: S_2 had bacterial count of 1.4 × 10^3 CFU/ml on day zero and attained maximum bacterial population of 8.8 × 10^8 CFU/ml after 51 hrs. The lag phase and log phase lasted for 12 and 39 hrs respectively. Generation time for S_2 was 121.2 min.

Isolate S_3: The initial bacterial count of the isolate was 1.8 × 10^3 CFU/ml. Lag phase was of 12 hrs duration and log phase lasted for 39 hrs. Maximum bacterial population of 7.4 × 10^8 CFU/ml was attained after 27 hrs. Generation time for S_3 was 127.8 min.

Isolate S_4: The initial bacterial count of the isolate was 1.4 × 10^2 CFU/ml. Lag phase was of 12 hrs duration and log phase lasted for 39 hrs. Maximum bacterial population of 8.7 × 10^7 CFU/ml was attained after 51 hrs. Generation time for S_4 was 123.6 min.

Isolate S_5: It had bacterial count of 1.5 x10^2 CFU/ml on zero hrs and attained maximum population of 8.2 × 10^7 CFU/ml after 51 hrs. The lag phase and log phase lasted for 12 and 39 hrs respectively. Generation time for S_5 was 128.4 min.

Isolate S_6: The bacterial count of the isolate was 1.5 × 10^2 CFU/ml on zero hrs. Lag phase was of 12 hrs duration and log phase lasted for 39 hrs. Maximum bacterial population of 8.3 × 10^7 CFU/ml was attained after 51hrs. Generation time for S_6 was 127.8 min.

Isolate S_7: S_7 had bacterial count of 1.5 × 10^3 CFU/ml on zero hrs and attained maximum bacterial population of 9.1 × 10^8 CFU/ml after 51 hrs. The lag phase and log phase lasted for 12 and 39 hrs respectively. Generation time for S_7 was 126 min.

Growth of isolates, obtained from eggs, at 30^0 C at pH 4.5

Isolate S_8: The initial bacterial count of the isolate was 1.3 × 10^3 CFU/ml. Lag phase was of 12 hrs duration and log phase lasted for 51 hrs. Maximum bacterial population of 7.4 x10^8 CFU/ml was attained after 33 hrs. Generation time for S_8 was 125.4 min.

Growth of isolates, obtained from human diarrheic cases, at 30°C at pH 4.5

Isolate S_9: The initial bacterial count of the isolate was 1.7 × 10^3 CFU/ml. Lag phase was of 12 hrs duration and log phase lasted for 39 hrs. Maximum bacterial population of 7.8 × 10^8 CFU/ml was attained after 51 hrs. Generation time for S_9 was 128.4 min.

Isolate S_{10}: It had bacterial count of 1.6 × 10^3 CFU/ml on zero hrs and attained maximum population of 9.2 × 10^8 CFU/ml after 51 hrs. The lag phase and log phase lasted for 12 and 39 hrs respectively. Generation time for S_{10} obtained was 128.4 min.

Isolate S_{11}: The bacterial count of the isolate was 2.4 × 10^3 CFU/ml on zero hrs. Lag phase was of 12 hrs duration and log phase lasted for 39 hrs. Maximum bacterial population of 9.8 × 10^8 CFU/ml was attained after 51 hrs. Generation time for S_{11} recorded was 127.8 min.

Growth of the isolates at 10°C with pH of 6.5

Growth of isolates, obtained from raw poultry meat, at 10°C at pH 6.5

Isolate S_1: The initial bacterial count of the isolate was 6.4 × 10^3 CFU/ml. Lag phase was of 48 hrs duration and log phase lasted for 168 hrs. Maximum bacterial population of 8.8 × 10^8 CFU/ml was attained on day 9 with the generation time of 10.30 hrs.

Isolate S_2: S_2 had bacterial count of 4.4 × 10^4 CFU/ml on day zero and attained maximum population of 8.8 × 10^9 CFU/ml on day 9. The lag phase and log phase lasted for 2 and 7 days respectively. Generation time for S_2 was 10.0 hrs.

Isolate S_3: The initial bacterial count of the isolate was 6.4 × 10^2 CFU/ml. Lag phase was of 2 days duration and log phase lasted for 7 days. Maximum bacterial population of

5.2×10^7 CFU/ml was attained on day 10. Generation time for S_3 was 10.52 hrs.

Growth of isolates, obtained from poultry cloacal swabs, at 10°C at pH 6.5

Isolate S_4: The initial bacterial count of the isolate was 5.6×10^3 CFU/ml. Lag phase was of 2 days duration and log phase lasted for days. Maximum bacterial population of 6.9×10^8 CFU/ml was attained on day 10. Generation time for S_1 was 10.20 hrs.

Isolate S_5: It had bacterial count of 3.8×10^4 CFU/ml on day zero and attained maximum population of 4.1×10^9 CFU/ml on day 10. The lag phase and log phase lasted for 2 and 7 days respectively. Generation time for S_5 was 10.00 hrs.

Isolate S_6: The bacterial count of the isolate was 6.4×10^2 CFU/ml on day zero. Lag phase was of 2 days duration and log phase lasted for 7 days. Maximum bacterial population of 8.3×10^7 CFU/ml was attained on day 9. Generation time for S_6 was 11.11 hrs.

Isolate S_7: The initial bacterial count of the isolate was 5.6×10^3 CFU/ml. Lag phase was of 2 days duration and log phase lasted for 7 days. Maximum bacterial population of 7.9×10^8 CFU/ml was attained on day 9. Generation time for S_7 was 10.00 hrs.

Growth of isolates, obtained from eggs, at 10°C at pH 6.5

Isolate S_8: It had bacterial count of 3.6×10^3 CFU/ml on day zero and attained maximum population of 8.1×10^8 CFU/ml on day 9. The lag phase and log phase lasted for 2 and 7 days respectively. Generation time for S_8 was 10.00 hrs.

Growth of isolates, obtained from human diarrheic cases, at 10°C at pH 6.5

Isolate S_9: The initial bacterial count of the isolate was 3.8×10^4 CFU/ml. Lag phase was of 2 days duration and log phase lasted for 7 days. Maximum bacterial population of 3.6×10^9 CFU/ml was attained on day 9. Generation time for S_9 was 10.40 hrs.

Isolate S_{10}: The initial bacterial count of the isolate was 6.4×10^2 CFU/ml. Lag phase was of 2 days duration and log phase lasted for 7 days. Maximum bacterial population of 7.9×10^7 CFU/ml was attained on day 9. Generation time for S_{10} was 10.10 hrs.

Isolate S_{11} : It had bacterial count of 5.4×10^3 CFU/ml on day zero and attained maximum population of 7.8×10^8 CFU/ml on day 9. The lag phase and log phase lasted for 2 and 7 days respectively. Generation time for S_{11} was 10.00 hrs.

Growth of *Salmonella* isolates at 10°C with pH of 5.5

Growth of isolates, obtained from raw poultry meat, at 10°C at pH 5.5

Isolate S_1: The initial bacterial count of the isolate was 4.1×10^3 CFU/ml. Lag phase was of 3 days duration and log phase lasted for 9 days. Maximum bacterial population of 6.8×10^7 CFU/ml was attained on day 12. Generation time for S_1 was 15.87 hrs.

Isolate S_2: S_2 had bacterial count of 2.9×10^4 CFU/ml on day zero and attained maximum population of 6.4×10^8 CFU/ml on day 12. The lag phase and log phase lasted for 3 and 9 days respectively. Generation time for S_2 was 15.62 hrs.

Isolate S_3: The initial bacterial count of the isolate was 2.4×10^5 CFU/ml. Lag phase was of 3 days duration and log phase lasted for 9 days. Maximum bacterial population of 5.2×10^9 CFU/ml was attained on day 12. Generation time for S_3 was 15.43 hrs.

Growth of isolates, poultry cloacal swabs, at 10^0 C at pH 5.5

Isolate S_4: The initial bacterial count of the isolate was 4.2×10^3 CFU/ml. Lag phase was of 3 days duration and log phase lasted for 9 days. Maximum bacterial population of 9.8×10^7 CFU/ml was attained on day 12. Generation time for S_4 was 15.29 hrs.

Isolate S_5: It had bacterial count of 4.6×10^4 CFU/ml on day zero and attained maximum population of 8.4×10^8 CFU/ml on day 12. The lag phase and log phase lasted for 3 and 9 days respectively. Generation time for S_5 was 15.64 hrs.

Isolate S_6: The bacterial count of the isolate was 3.4×10^3 CFU/ml on day zero. Lag phase was of 3 days duration and

log phase lasted for 9 days. Maximum bacterial population of 7.9 × 10^7 CFU/ml was attained on day 12. Generation time for S_6 was 15.15 hrs.

Isolate S_7: The initial bacterial count of the isolate was 3.9 × 10^4 CFU/ml. Lag phase was of 3 days duration and log phase lasted for 9 days. Maximum bacterial population of 6.8 x10^8 CFU/ml was attained on day 12. Generation time for S_7 was 15.72 hrs .

Growth of isolates, obtained from eggs, at 10°C at pH 5.5

Isolate S_8: It had bacterial count of 4.1 × 10^3 CFU/ml on day zero and attained maximum population of 7.6 × 10^7 CFU/ml on day 12. The lag phase and log phase lasted for 3 and 9 days respectively. Generation time for S_8 was 15.87 hrs.

Growth of isolates, obtained from human diarrheic cases, at 10°C at pH 5.5

Isolate S_9: The initial bacterial count of the isolate was 3.9 × 10^4 CFU/ml. Lag phase was of 3 days duration and log phase lasted for 9 days. Maximum bacterial population of 7.5 × 10^8 CFU/ml was attained on day 12. Generation time for S_9 was 15.64 hrs.

Isolate S_{10}: The initial bacterial count of the isolate was 2.6 × 10^3 CFU/ml. Lag phase was of 3 days duration and log phase lasted for 9 days. Maximum bacterial population of 6.6 × 10^7 CFU/ml was attained on day 12. Generation time for S_{10} was 15.10 hrs.

Isolate S_{11}: It had bacterial count of 2.6 × 10^4 CFU/ml on day zero and attained maximum population of 8.4 × 10^8 CFU/ml on day 12. The lag phase and log phase lasted for 3 and 9 days respectively. Generation time for S_{11} was 15.36 hrs.

Growth of *Salmonella* isolates at 10°C with pH of 4.5

Growth of the isolates, obtained from raw poultry meat, at 10°C with pH of 4.5

Isolate S_1: The initial bacterial count of the isolate was 6.4 × 10^4 CFU/ml. Lag phase was of 4 days duration and log phase lasted for 13 days. Maximum bacterial population of 9.1 × 10^6 CFU/ml was attained on day 17. Generation time for S_1 was 45.45 hrs.

Isolate S_2: S_2 had bacterial count of 5.2 × 10^4 CFU/ml on day zero and attained maximum population of 8.6 × 10^6 CFU/ml on day 12. The lag phase and log phase lasted for 4 and 13 days respectively. Generation time for S_2 was 45.30 hrs.

Isolate S_3: The initial bacterial count of the isolate was 3.2 × 10^4 CFU/ml. Lag phase was of 4 days duration and log phase lasted for 13 days. Maximum bacterial population of 9.5 × 10^6 CFU/ml was attained on day 17. Generation time for S_3 was 42.00 hrs.

Growth of the isolates, obtained from poultry cloacal swabs, at 10°C with pH of 4.5

Isolate S_4: The initial bacterial count of the isolate was 4.3 × 10^5 CFU/ml. Lag phase was of 4 days duration and log phase lasted for 13 days. Maximum bacterial population of 8.9 × 10^7 CFU/ml was attained on day 17. Generation time for S_4 was 43.48 hrs.

Isolate S_5: It had bacterial count of 57.4 × 10^4 CFU/ml on day zero and attained maximum population of 9.1 × 10^6 CFU/ml on day 17. The lag phase and log phase lasted for 4 and 13 days respectively. Generation time for S_5 was 45.45 hrs.

Isolate S_6. The bacterial count of the isolate was 6.5 × 10^4 CFU/ml on day zero. Lag phase was of 4 days duration and log phase lasted for 13 days. Maximum bacterial population of 9.2 x10^6 CFU/ml was attained on day 17. Generation time for S_6 was 45.45 hrs.

Isolate S_7: The initial bacterial count of the isolate was 2.2 × 10^4 CFU/ml. Lag phase was of 4 days duration and log phase lasted for 13 days. Maximum bacterial population of 9.1 × 10^6 CFU/ml was attained on day 17. Generation time for S_7 was 41.66 hrs.

Growth of the isolates, obtained from eggs, at 10°C with pH of 4.5

Isolate S_8: It had bacterial count of 4.4 × 10^4 CFU/ml on day zero and attained maximum population of 8.6 × 10^6 CFU/ml on day 17. The lag phase and log phase lasted for 4 and 13 days respectively. Generation time for S_8 was 43.47 hrs.

Growth of the isolates, obtained from human diarrheic cases, at 10°C with pH of 4.5

Isolate S_9: The initial bacterial count of the isolate was 6.3 × 10^4 CFU/ml. Lag phase was of 4 days duration and log phase lasted for 13 days. Maximum bacterial population of 9.2 × 10^6 CFU/ml was attained on day 17. Generation time for S_9 was 45.45 hrs.

Isolate S_{10}: The initial bacterial count of the isolate was 5.4 × 10^4 CFU/ml. Lag phase was of 4 days duration and log phase lasted for 13 days. Maximum bacterial population of 9.3 × 10^6 CFU/ml was attained on day 17. Generation time for S_{10} was 43.47 hrs.

Isolate S_{11}: It had bacterial count of 6.6 × 10^4 CFU/ml on day zero and attained maximum population of 9.6 × 10^6 CFU/ml on day 17. The lag phase and log phase lasted for 4 and 13 days respectively. Generation time for S_{11} was 45.45 hrs.

Growth of *Salmonella* isolates at 4°C with pH of 6.5

None of the isolates showed growth during the stipulated incubation time.

Growth of *Salmonella* isolates at 4°C with pH of 5.5

None of the isolates showed growth during the stipulated incubation time.

Growth of *Salmonella* isolates at 4°C with pH of 4.5

Growth of the isolates was not detected at the above mentioned temperature and pH.

Table 3. Mean generation time of *Salmonella* at varying temperature and pH

Isolates (n=11) with varying pH	10°C	30°C	Level of significance
pH 4.5 (n = 11)	44.23 ± 0.44 hrs	126.10 ± 0.68 mins	* *
pH 5.5 (n = 11)	15.51 ± 0.08 hrs	59.96 ± 0.14 mins	* *
pH 6.5 (n = 11)	10.23 ± 0.10 hrs	36.22 ± 0.07 mins	* *

**shows significant difference in the generation times of the isolates at pH 4.5, 5.5 and 6.5 at temperature of 10°C and 30°C, at P < 0.01 level of significance.

Mean generation time of the isolates

At 10°C, the mean generation time of the isolates at pH values of 4.5, 5.5 and 6.5 was 44.23 ± 0.44, 15.51 ± 0.08 and 10.23 ± 0.10 hrs, respectively, while at 30°C, the mean generation time of the isolates at pH values of 4.5, 5.5 and 6.5 was 126.10 ± 0.68, 59.96 ± 0.14 and 36.22 ± 0.07 min, respectively. At 4°C, none of the isolates grew at any given pH value. The results are presented in Table. 3.

It is not surprising that the growth and metabolism of micro-organisms are influenced by pH because acidity or alkalinity of an environment has a profound effect on the activity and stability of macromolecules (Adams and Moss, 2008). In our study no growth of the isolates could be observed at the temperature of 4°C, the findings are more or less in corroboration with Serraino *et al.* (2012) although they reported a decrease of *S. typhimurium* count in WBMC (Water buffalo mozzarella cheese) stored at 5°C. The mean generation time of the isolates was 44.07 hrs at 10^0C with pH of 4.5 while at 30°C with pH of 6.5, the mean generation time of the isolates was 36 minutes. In a similar study, Ferreira and Lund, (1987) reported that when *S. typhimurium* was incubated at 30°C, growth was initiated at a minimum pH of 3.8–4.0 in 1 or 2 day, at 20°C growth was initiated at a minimum pH of 3.8 – 4.0 in 3-5 d and at 10°C, incubation for >7 d was required before growth was detected. Similarly, Laub *et al.* **(**1989) who studied the growth of *Salmonella* at pH 5.2 and 7.2, reported significant increase in the generation time of the organism viz., 26 minutes at pH 5.2 to 40 minutes at pH 7.2. Lin *et al.* (1995) evaluated the growth of *S. typhimurium* in pH adjusted buffered Luria- Bertani (LB) broth and reported *S. typhimurium* to grow at pH 4.0 at 37°C with generation time of about 2.5 hrs.

The findings of the afore-mentioned workers agree with our findings which show that as the temperature and pH were lowered, there was significant increase in the generation time of the organism.

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