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Isolation, Serotyping and Prevalence of Salmonellosis from Humans Diarrheic Samples in Jammu Region

Palneez Kour[1*], M.A. Malik[1], Navjot Singh Resum[2], Maninder Singh[1] and P.K. Verma[3]

[1]Division of Veterinary Public Health and Epidemiology, F.V.Sc. & A.H., SKUAST-J, R.S. Pura Jammu, INDIA
[2]Veterinary Officer, Military Farm, Jammu, INDIA
[3]Division of Veterinary Pharmacology and Toxicology, F.V.Sc. & A.H., SKUAST-J, R.S.Pura Jammu, INDIA

**Corresponding author: P Kour; Email: palneezkour@yahoo.com*

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ABSTRACT

The present investigation was carried out to study the prevalence of *Salmonella* species in human diarrheic cases in different areas of Jammu district. A total of 200 human diarrheic samples were processed for the detection of *Salmonella* out of which 5 samples were found positive for *Salmonella* with an overall prevalence of 2.5 per cent. The prevalence was higher in females (3%) as compared to males (2 %). The patients of age group of <1-19 years (3.12 %) showed the highest prevalence, followed by patients of age group of 19-49 years (2.85 %). The prevalence was higher in farmers (4%) followed by students (2.5%). Diarrhoea and fever were present in all the 5 patients found positive for salmonella. The isolates were confirmed at National *Salmonella* Centre, IVRI Bareilly as *Salmonella* Typhimurium. Alcoholic leaf extract of *Alstonia scholaris* at the concentration of 100µg was the most effective against *Salmonella* Typhimurium and the activity of alcoholic leaf extract decreased as the concentration decreased. Aqueous leaf extract of *Alstonia scholaris* showed no antibacterial activity against *Salmonella* Typhimurium.

Keywords: *Salmonella,* prevalence, diarrheic samples

Salmonella infection is a serious medical and veterinary problem worldwide causing concern in the food industry. Salmonellae are widely distributed in nature and cause a spectrum of diseases in man and animals. In India, salmonellosis is hyperendemic (Kumar *et al*., 1997) and causes heavy economic losses every year. Of more than 2500 serovars of *Salmonella*, 209 have been reported from India and *Salmonella* Typhimurium was found to be one of the most common serovars prevalent both in man and animals (Verma *et al*., 2001). Conventional Kauffmann-White scheme still remains the standard and only reliable method for serotyping of *Salmonella* isolates (Bottledoorn *et al*., 2004; Johnson *et al*., 2001) and classifies *Salmonella* according to three major antigenic determinants composed of flagellar H antigens, somatic O antigens and virulence (Vi) capsular K antigens.

Plants have been always a treasure of medicines. The plant of *Alstonia scholaris* belongs to family Apocynaceae and is also known as Devil's tree or Dita Bark tree in English, *Datyuni* and *Chatiun* in Hindi and *Saptaparna* in Sanskriti. It grows throughout India, in deciduous and evergreen forests and also in plains. It is known to possess a lot of medicinal properties in folk medicine (Mukherjee *et al*., 2012). It contains various iridoids, alkaloids, coumarins, flavonoids, leucoanthocyanins, reducing sugar, simple phenolics, steroids, saponins (Kaushik *et al*., 2011). It is known to possess *in vitro* antioxidant, antimalarial, anti free radical scavenging (Arulmozhi *et al*., 2007), analgesic, anti-inflammatory and anti-ulcerogenic activities (Arulmozhi *et al*., 2012a). Besides, it also possesses anti-anxiety and anti-depressant activities (Arulmozhi *et al*., 2012b). The bitter milky juice of the plant is applied on wounds, ulcers and rheumatic pains. The bark extracts of *Alstonia scholaris* possess immunostimulating effect (Iwo *et al*., 2000), it also possess anticancer activity on skin carcinogenesis (Jahan *et al*., 2009). Leaf extract of *Alstonia scholaris* possesses broncho-vasodilatory activity (Channa *et al*., 2005).

Methanolic crude extract of *Alstonia scholaris* possesses anti-diarrhoeal and spasmolytic activity (Shah *et al*., 2010). The alkaloid fraction of the leaf shows anti-tussive, anti-asthmatic and expectorant activities and is proved to be a valuable lead molecule developed for respiratory diseases drug development (Shang *et al*., 2010).

MATERIAL AND METHODS

Collection of samples

Stool samples of patients with the history of diarrhea were collected from different hospitals and laboratories in Jammu region.

Sample size

For isolation of *Salmonella* species, a total of 200 diarrheic samples were collected and processed. The details of various samples collected from patients along with their source are given in Table 1.

Table 1: Details of samples collected from patients

S. No.	Place of Collection	No. of Samples
1	GMC, Jammu	30
2	SMGS, Jammu	50
3	Sub-District Hospital, R.S.PURA	60
4	Sub-District Hospital, Gandhi Nagar	30
5	Clinics, Diagnostic Centres	30

Methodology

The detection of *Salmonella* in diarrheic samples was done according to the method of Addis *et al.* (2011). Fecal samples were collected from different hospitals and laboratories and transported to the laboratory over ice. Pre-enrichment of the samples were done by incubating them in 1% buffered peptone water (Hi-Media, Mumbai) at 37ºC for 24 hours. After 24 hours of incubation, selective enrichment of the samples was done by transferring 0.1 ml of pre-enriched culture into 10 ml of Selenite F broth (Hi-Media) and incubating at 37ºC for 24 hours. One loopful from the broth was streaked on to Brilliant Green Agar (Hi-Media, Mumbai) followed by incubation at 37ºC for 24 hours. After the incubation, the plates were examined and pink coloured colonies were picked and streaked on to MacConkey Agar and incubated at 37ºC for 24 hours. Non lactose fermenting pale colonies were streaked on to XLD and incubated at 37ºC for 24 hours. The black head colonies, presumptive of *Salmonella* were subjected to morphological and biochemical tests for confirmation.

The salmonella isolates were send for serotyping confirmation at National *Salmonella* Centre, Central Research Institute, IVRI, Bareilly.

Preparation of alcoholic and aqueous leaf extracts of *Alstonia scholaris*

The leaves of *Alstonia Scholaris* were collected. After collecting, sufficient fresh leaf were cleaned and air-dried in shade (temperature not exceeding 40ºC) for 3-4 weeks. After air drying, leaves were pre-crushed and later pulverised into fine powder using electric blender. Aqueous extract was prepared by soaking dry powder in 1:10 ratio in distilled water for 72 hrs with intermittent shaking. After 72 hrs of soaking, the content was filtered through filter papers (Whatman filter paper) and filterate was then concentrated and dried under reduced pressure using rotatory evaporator. Alcoholic extract was prepared by using ethanol as solvent in extract container of soxhlet apparatus according to method described by Harborne (1984). The alcoholic and aqueous extracts were preserved at 5°C in an airtight bottle until required for further use.

Preparation of plant extracts as test samples

A single concentration of 200 µg of different test extracts dissolved in 100 µl PBS (pH 7.4) were used for the entire test.

Agar well diffusion

Agar-well diffusion method as described in European pharmacopeia with slight modification was used for antimicrobial testing (Misra *et al*., 2011). Wells were cut using sterile well bore of 6 mm diameter. The plates were swabbed uniformly using a sterile swab and allowed to dry for 5 minutes and different concentrations (100 µg, 70 µg, 5o µg, 25 µg) of test extract were dissolved in PBS and transferred to each wells on the agar plate. The

antibacterial activities were observed after incubating the plates for 24 hours at 37°C as evidenced by the zone of inhibition surrounding the well.

RESULT AND DISCUSSION

Out of these 200 samples examined, 5 samples were found positive for *Salmonella* with an overall prevalence of 2.5 per cent. The prevalence was higher in females (3%) compared to males (2%) (Table 2). The prevalence was 4 per cent, 2.5 per cent, 1.66 per cent, 2 per cent in farmers/ workers, students, teachers and housewives respectively. The highest prevalence was recorded in farmers/workers being 4 per cent (Table 3). The clinical signs exhibited by these patients were nausea present in 2 (40%) patients, whereas, 3 (60%) patients showed signs of vomiting and abdominal cramps. Diarrhea and fever were reported in all 5 (100%) positive cases, but only 1 (20%) patient showed the signs of headache. Chills were reported in 4 (80%) patients. All the isolates were gram negative and positive for methyl red, citrate utilization, TSI and catalase tests, while they were negative for indole production, Voges-Proskauer and oxidase tests. Moreover, All the isolates fermented glucose, fructose, mannitol, sorbitol but did not ferment lactose, arabinose and sucrose. The isolates were confirmed as *Salmonella* Typhimurium at the National *Salmonella* Centre, Indian Veterinary Research Institute, Izatnagar, Bareilly, India. The results of antibiogram of *Salmonella* isolate are given in Table 4.

Table 2: Prevalence of salmonellosis

S. No.	Sex	Samples Examined	Salmonella Isolated	Percent Prevalence
1	F	100	3	3
2	M	100	2	2

F=Female, M=Male

Table 3: Occupation wise prevalence of Salmonellosis

Occupation	Samples Examined	*Salmonella* Isolated	Percent Prevalence
Farmers/workers	50	2	4
Housewives	50	1	2
Teachers	60	1	1.66
Students	40	1	2.5

Table 4: Percent sensitivity of *Salmonella* isolates to various antimicrobials

Percent sensitivity	Antimicrobial agent
100	Ciprofloxacin
80	Co-trimazole, Amoxycillin/Clavulanic acid
60	Chloramphenicol, Tetracycline, Norfloxacin, Ceftriazone
40	Kanamycin, Gentamicin, Ampicillin
0	Penicillin, Streptomycin

The incidence was higher in females (3%) than in males (2%). These findings are in conformation with the findings of Khanum *et al*. (2006) and Ahmed *et al*. (1994) who reported higher incidence rate of salmonellosis in females than in males, which according to Khanum *et al*. (2006), might be because of the socio-cultural practices persistent in the society, where preference for the best health care facilities and food stuffs are intentionally preferred to boys and men. Also, in child-bearing women, there is weak immune response and hence more susceptible to infections (Khanum *et al*., 2006). All the isolates were got confirmed at National *Salmonella* centre, Bareilly as *Salmonella* Typhimurium indicating the major *Salmonella* infection in humans in Jammu region is due to *Salmonella* Typhimurium. The highest prevalence of *Salmonella* was observed in the age group of less than one to 19 years (3.12 per cent). The results are in accordance with the findings of researchers who documented that in infants and children there is greatest incidence of *Salmonella* infection (Khanum *et al*., 2006; Wain *et al*., 1998; Shimoni *et al*., 1999) which may be because of weak response of immunity to *Salmonella* infection, contaminated quality of drinking water among school going children, eating contaminated junk food items in schools canteens, open-air cafeteria and other outdoor activities (Asghar *et al*., 2002). Moreover, the highest prevalence of 4 per cent was recorded in farmers/workers followed by students (2.5 per cent). All the *Salmonella* Typhimurium isolates were tested against 12 commonly used antimicrobials and results revealed almost all isolates of *Salmonella* were resistant to penicillin-G and streptomycin but were sensitive to ciprofloxacin, co-trimazole, chloramphenicol and ceftriazone. The resistance levels are comparable to those previously reported for Salmonellae isolates by Goswami *et al*. 2003 in which *Salmonella* were resistant

to penicillin, ampicillin and tetracycline.

Alcoholic and aqueous leaf extracts of *Alstonia scholaris* were tested against *Salmonella* for their anti-bacterial properties using Agar well diffusion method. The range of zone of inhibition for alcoholic and aqueous leaf extracts have been presented in Table 5. At the concentration of 100µg the zone of inhibition of 11mm, 10mm, 8mm, 15mm, 7mm was observed for the isolates S_1, S_2, S_3, S_4 and S_5 respectively. Similarly, at the concentration of 70µg the zone of inhibition of 8mm, 6mm, 5mm, 9mm, and 5mm was observed for the isolates S_1, S_2, S_3, S_4 and S_5 respectively. Further, at the concentration of 50µg the zone of inhibition of 5mm, 4mm, 4mm, 6mm, and 4mm was observed for the isolates S_1, S_2, S_3, S_4 and S_5 respectively while minimum zone of inhibition was observed at a concentration of 25µg. However, in the present study, aqueous leaf extract showed no antibacterial activity against *Salmonella* isolates.

Table 5: The antibacterial activity of Alcoholic leaf extracts of *Alstonia scholaris* against *Salmonella* isolates

	Zone of inhibition (mm)				
Conc.(µg)	**S_1**	**S_2**	**S_3**	**S_4**	**S_5**
100	11	10	8	15	7
70	8	6	5	9	5
50	5	4	4	6	4
25	—	—	—	3	—
Control (PBS)	—	—	—	—	—

S_1 to S_5 = Samples

— = No Zone of inhibition

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