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Phenotypic characterization of *Salmonella* serovars isolated in farm and backyard chicken samples

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Abstract
Poultry sector in India has undergone a paradigm shift in structure and operation from a mere backyard activity into a major commercial Agri-based industry over a period of four decades. The genus *Salmonella* represents the most common zoonotic pathogens isolated from food-producing animals including chicken that pose a serious concern to public health, animals and food industry worldwide. A total of 480 samples comprised of cloacal swabs and caecal contents were collected aseptically from commercial farms and backyard chicken flocks located in and around Jaipur region. The samples were pre enriched in Rappaport Vassiliadis, Fluid Selenite Cystine and Tetrathionate Brilliant Green broths and selective plating was done on Xylose Lysine Deoxycholate and Hektoen Enteric agar. The typical black centred colonies were selected for isolation and identification by using biochemical test kit of *Salmonella* identification.

Keywords: *Salmonella*, isolation, farm chicken, backyard chicken, phenotype, biochemical characteristics

Introduction
Poultry farming in India has gained much importance as a subsidiary occupation of the farmers. It could play an effective role in improving the socio-economic status of the rural people by increasing their income besides providing nutritious food through meat and egg. Poultry egg and meat, in recent years, have become important food for 68 percent of the non-vegetarian population of the country. Development of high yielding layer (310-340 eggs) and broiler (2.4 - 2.6 kg at 6 wks) varieties together with standardized package of practices on nutrition, housing, management and disease control have contributed to spectacular growth rates in egg (4-6% per annum) and broiler production (8-10% per annum) both in southern and western parts of India (Chatterjee and Rajkumar, 2015) [3].

Salmonellosis is a bacterial disease caused by various strains of *Salmonella* and affects diverse number of hosts worldwide (Baumler *et al.*, 1998) [2]. *Salmonella* also contributes to negative economic impacts due to the cost of surveillance, investigation, treatment and prevention of illness (Pui *et al.*, 2011) [7].

It has also been referred as endemic disease in poultry industry of India and its prevalence in other animals acts as a continuous threat to men (Arora *et al.*, 2013) [1].

Salmonella enterica represents the most pathogenic species and includes more than 2600 serovars. *Salmonella* can be transmitted to humans along the farm-to-fork continuum, commonly through contaminated foods of animal origin including poultry and poultry-related products. Some *Salmonella* serovars are restricted to one specific host commonly referred to as "host-restricted" whereas others have broad host spectrum known as "host-adapted" serovars (Jajere 2019) [6].

Identification, surveillance and antibiotic sensitivity of the *Salmonella* serotypes prevalent in India would help devise suitable prevention and control program for this important poultry pathogen. Conventional bacterial culture methods are being used routinely not only to isolate but also to identify phenotypic characteristics of the *Salmonella* that require at least 3-11 days.

This study was aimed to investigate the biochemical characteristics of *Salmonella* prevalent in cloacal swabs and caecal contents samples of commercial and backyard chicken flocks, located in and around Jaipur region. The typical black centred colonies were selected for isolation and identification as well as for phenotypic characterization

of *Salmonella* by using biochemical test kit of *Salmonella* identification.

Materials and Methods

Sample collection

In present study, a total of 480 cloacal swabs and caecal contents samples were collected aseptically from farm and backyard chicken flocks located in and around Jaipur region. All samples were collected randomly from apparently healthy chicken as well as from sick birds showing signs of diarrhoea.

Isolation and identification of *Salmonella*

Isolation and identification of *Salmonella* was conducted according to Standard guide lines of Global Salm-Surv, laboratory protocols as per *Salmonella* ISO 6579:2002(E); Hendriksen (2003) [5].

The collected samples were pre enriched in Rappaport Vassiliadis, Fluid Selenite Cystine and Tetrathionate Brilliant Green broths and selective plating was done on Xylose Lysine Deoxycholate and Hektoen Enteric agar.

Table 1: Result interpretation chart for biochemical tests of *Salmonella*

S. No.	Test	Method	Principle	Original Colour	Positive	Negative
1	Methyl red	1-2 drops of Methyl red reagent	Detects acid production	Colourless	Red	Yellow
2	Voges Proskauer's	1-2 drops of Baritt reagent A and 1-2 drops of Baritt reagent B	Detects acetoin production	Colourless	Pink	Colourless
3	Urease	-	Detects Urease activity	Yellow	Pink	Yellow
4	H_2S production	-	Detects H_2S production	Yellow	Black	Orange
5	Citrate utilization	-	Detects capability of organism to utilize citrate as a sole carbon source	Green	Blue	Green
6	Lysine utilization	-	Detects Lysine decarboxylation	Purple	Purple	Yellow
7	ONPG	-	Detects b-galactosidase activity	Colourless	Yellow	Colourless
8	Lactose	-	Lactose utilization	Red	Yellow	Red
9	Arabinose	-	Arabinose utilization	Red	Yellow	Red
10	Maltose	-	Maltose utilization	Red	Yellow	Red
11	Sorbitol	-	Sorbitol utilization	Red	Yellow	Red
12	Dulcitol	-	Dulcitol utilization	Red	Yellow	Red

Phenotypic and biochemical characterization of the isolates

It was done according to methods of Quinn *et al.* (1990) [8] and Cowan and Steel (1994) [4]. The typical black centred colonies were selected for different biochemical tests by using HiSalmonella Identification Kit (HiMedia, Mumbai, India). The kit contains 12 biochemical tests viz. Methyl Red, Voges Proskauer's, Urease, H_2S production, Citrate utilization, Lysine, ONPG, Lactose, Arabinose, Maltose, Sorbitol and Dulcitol. The result interpretation chart for different biochemical tests of *Salmonella* supplied with kit is presented in Table 01. In addition to these 12 tests, Indole test was also done by adding few drops of Xylene and Kovac's reagent in a sterile test tube having 5 ml of broth culture of each isolate.

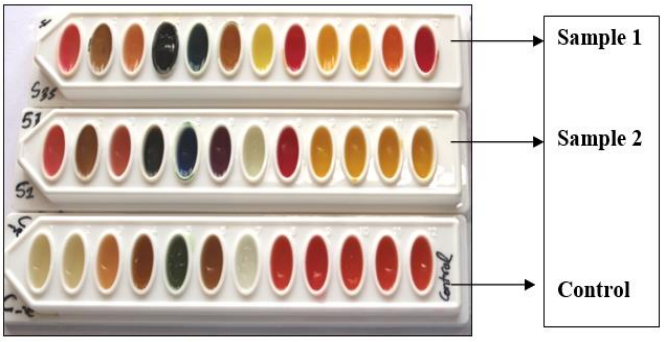

Fig 1: Biochemical tests by using HiSalmonella Identification Kit (HiMedia)

Results and Discussion

In present study, a total of 31 isolates of *Salmonella enterica* were detected in cloacal swabs and caecal contents samples of farm and backyard chicken. Molecular confirmation of all isolates was done by using PCR techniques. The serotyping report revealed the presence of 26 isolates of *S. infantis*, 3 isolates of *S. virchow* whereas 2 isolates were untypeable. The results of biochemical and phenotypic characterization of all 31 *Salmonella enterica* isolates showed similar results for all serovars with positive reaction for methyl red, H_2S production, citrate utilization, lysine decarboxylation, fermentation of arabinos, maltose and sorbitol (Figure 01). All three isolates belonged to *S. virchow* showed a negative reaction for dulcitol fermentation. Besides, considerable variability in sugar fermentation, H_2S production and citrate utilization was recorded for all Untypeable strains.

According to Hendriksen (2003) [5] *Salmonella* generally identified as a non-lactose fermenting, (NLFs) Gram negative rod shaped organism, ranged 0.7 to 1.5 x 2 to 5 *µm* in size. With the exception of *S. pullorum* and *S. gallinarum*, they were motile with peritrichous flagellae. D-glucose was fermented with the production of acid and usually gas. Other carbohydrates usually fermented were L-arabinose, maltose, D-mannitol, D-mannose, L-rhamnose, D-sorbitol (except ssp VI), trehalose, D-xylose and dulcitol. *Salmonella* was oxidase negative, catalase positive, indole and Voges Proskauer (VP) negative, methyl red and Simmons citrate positive, H_2S producing and urea negative. Some of these characteristics were used for biochemical confirmation of *Salmonella*.

For Indole test, out of all 31 isolate, one strain of *Salmonella infantis* showed positive reaction detected in caecal content sample of back yard chicken (Image 02) whereas *Salmonella* species have a negative biochemical property for Indole test (UK standards for microbiology Investigations. Identification of *Salmonella* species. https://assets.publishing.service.gov.uk › uploads › attachment_ data › file.21 November, 2019).

Table 2: Results of Biochemical reactions of various serotypes of *Salmonella enterica*

S. No.	Biochemical test	*S. infantis*	*S. virchow*	Untypeable
1	Methyl red	+	+	+
2	Voges Proskauer's	-	-	-
3	Urease	-	-	-
4	H_2S production	+	+	Variable
5	Citrate utilization	+	+	Variable
6	Lysine utilization	+	+	+
7	ONPG	-	-	-
8	Lactose	-	-	-
9	Arabinose	+	+	+
10	Maltose	+	+	+
11	Sorbitol	+	+	Variable
12	Dulcitol	+	-	Variable

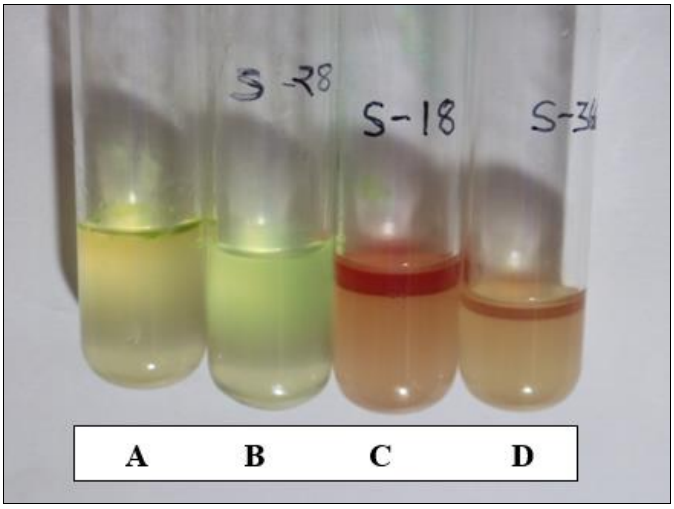

Image 2: Results of Indole test: A and B = Negative, C = Positive, D = False positive

Conclusion

In present study, a total of 31 isolates of *Salmonella enterica* were recovered in 480 cloacal swabs and caecal contents samples of farm and backyard chicken. Molecular confirmation of all isolates was done by using HiSalmonella Identification Kit for different biochemical tests along with PCR techniques. All 31 *Salmonella enterica* isolates showed similar results however a negative reaction for dulcitol fermentation was observed for *S. virchow* and the Untypeable strains showed variability in sugar fermentation, H_2S production and citrate utilization. An uncommon finding of positive reaction for Indole test was recorded in one strain of *Salmonella infantis*, belonged to caecal content sample of back yard chicken.

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References

1. Arora D, Kumar S, Singh D, Jindal N, Mahajan NK. Isolation, characterization and antibiogram pattern of *Salmonella* from poultry in parts of Haryana, India. Adv Anim Vet Sci. 2013; 1(5):161-163.
2. Baumler AJ, Tsolis RM, Ficht TA, Adams LG. Evolution of host adaptation in *Salmonella* enterica. Infect Immun. 1998; 66(10):4579-87.
3. Chatterjee RN, Rajkumar U. An overview of poultry production in India. Indian J. Anim. Hlth. 2015; 54(2):89-108.
4. Cowan ST, Steel KJ. In: Cowan and Steel's manual for the identification of medical bacteria, 2nd Ed. Cambridge University Press, Cambridge, New York. 1994, 90-93.
5. Hendriksen RS. Global Salm-Surv: A global *Salmonella* surveillance and laboratory support project of the World Health Organization. Laboratory Protocols for Isolation of *Salmonella* as per *Salmonella* ISO 6579:2002(E), 4th Edition. 2003, 1-19.
6. Jajere SM. A review of *Salmonella enterica* with particular focus on the pathogenicity and virulence factors, host specificity and antimicrobial resistance including multidrug resistance. Veterinary World. 2019; 12(4):504-521.
7. Pui CF, Wong WC, Chai LC, Tunung R, Jeyaletchumi P, Noor Hidayah MS *et al. Salmonella*: A foodborne pathogen. International Food Research Journal. 2011; 18:465-473.
8. Quinn PJ, Carter ME, Markey BK, Carter GR. Clinical Veterinary Microbiology. 1st Edn. Wolfe Publishing, Mosby-Year Book Europe Ltd. Lynton House, 1994.
9. UK Standards for Microbiology Investigations. Identification of Salmonella species. Issued by the Standards Unit, Microbiology Services, PHE 2013; https://assets.publishing.service.gov.uk › uploads › attachment_data › file. 21 November, 2019.