Indian Journal of Poultry Science (2024) 59(1): 81–84; Research Article
https://doi.org/10.56093/IJPS.v59i1.154966

Identification of the infective dose of recovered multi-drug resistant non-typhoidal *Salmonella* spp. and the intestinal histomorphometric changes in broiler chicken

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(Received on August 23, 2023; accepted for publication on September 20, 2023)

ABSTRACT

Reshma, M.M., Aswathi, P.B., Jess, V., Stella, C., Surjith, K.P., Patki, H.S., Beena, J.C., Arun, K. and Bibin, M. 2024. Identification of the infective dose of recovered multi-drug resistant non-typhoidal Salmonella *spp. and the intestinal histomorphometric changes in broiler chicken. Indian Journal of Poultry Science, 59(1): 81-84.*

The study is aimed to determine the ID_{50} of the two recovered *Salmonella* isolates from various broiler farms in Wayanad district, Kerala. The isolates were confirmed as *Salmonella* Typhimurium through cultural and molecular characterisation. On antibiotic susceptibility testing both the isolates were found to be resistant to more than six classes of antibiotics. Vencobb 430Y chicks (n=24) were equally distributed into four groups (T1, T2, T3 and T4) with six birds in each group. All the birds tested negative for *Salmonella* on the fifth day. On the seventh day, the birds of treatment T2, T3 and T4 were orally inoculated with 10^8,10^9 and 10^{10} cfu/mL of MDR-*S*. Typhimurium. The birds were examined closely for clinical symptoms and changes in faecal matter consistency. The body weight of the birds was taken before infection and also on weekly basis post-infection. The clinical symptoms started showing from the fifth-day post-infection in T4 and the seventh day post-infection in T2 and T3. Fifty percentage of the birds from T4 exhibited progressive weakness with diarrhoea after the first-week post-infection without causing mortality. The body weight difference of birds from different treatment was non-significant during pre-infection and during the first and second week post-infection body weight was in the order T1>T2>T3>T4. The caecal *Salmonella* load was highest in T4 and it was found to increase with time. When the histomorphometry of the different segements of small intestine were compared, a significantly lower villi-to-crypt ratio was exhibited by T4, indicating impaired intestinal function. The ID_{50} of recovered*S*. Typhimurium was established as 10^{10} cfu/mL based on the clinical findings, faecal matter consistency pattern, body weight variations and intestinal histomorphometry parameters.

Keywords: Broiler chicken, MDR-NTS; ID_{50}, Intestinal histomorphometry, *Salmonella*

INTRODUCTION

Poultry is one of the most crucial elements of the agricultural sector worldwide with a growth rate of 16.81 percent in its population in India alone from 2012 to 2019 (BAHS, 2022). The fastest-growing segment of worldwide meat production and export is poultrymeat (Aswathi *et al*., 2013). Poultry, constituting approximately 50 percent of India's total meat production, stands as the predominant species among meat producers (Varunkkumar *et al.*, 2022). The poultry sector is affected regularly by the introduction of bacterial infections. *Salmonella* infection is one of the most significant bacterial illnesses in chickens, resulting in significant economic loss due to death and decreased production (Mouttotou *et al*., 2017; Jubeda *et al.*, 2022). The genus *Salmonella* is a member of the bacterial family Enterobacteriaceae and consists of more than 2500 serologically distinguishable variants. Among them, non-typhoidal *Salmonella* serotypes (*S. enterica* subspecies *enterica* serovar Enteritidis and *S. enterica* subspecies *enterica* serovar Typhimurium) are virtually invariably present in both wild and domestic animals, are primarily of concern as a source of foodborne illness in humans (Saif, 2009). Poultry products are frequently identified as important sources of *Salmonellae* that cause human illness, although most non-typhoidal *Salmonella* infections are associated with self-limiting gastroenteritis, they have the potential to cause fatal infections among infants, young children, older adults and immunocompromised individuals (Pandey and Saxena, 2015; Wen *et al*., 2017; Balasubramanian *et al*., 2019).

The infective Dose 50 (ID_{50}) is a quantitative measurement to describe the number of microorganisms or particles required to cause an infection in 50 percent of the individuals or experimental subjects exposed to the pathogen (Ramesh *et al*., 2020). The infective dose of *Salmonella* in broiler chickens can be influenced by a range of factors including, strain variability, host susceptibility, gut microbiota composition, stress and many other factors (Cosby *et al*., 2015). The present study was conducted to estimate the ID_{50} of MDR-*S*. Typhimurium in broiler chicken of one week age.

MATERIALS AND METHODS

Confirmation of Salmonella isolates

The study utilised the two *Salmonella* isolates obtained from distinct broiler farms in Wayanad district, Kerala**.** Isolates were selectively plated on Xylose Lysine

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Deoxycholate (XLD) agar, and molecular confirmation was achieved through polymerase chain reaction employing the *invA* and *spy* genes (Nair *et al.*, 2015). Antibiotic susceptibility test was done by disc diffusion method using commonly used antibiotic discs as per guidelines (Bauer *et al.*, 1966). One of the multi-drug resistant non-typhoidal*Salmonella* isolates among the tested samples was used further in the study.

Experimental design

Day-old Vencobb-430 Y broiler chicks (n=24) were distributed equally on body weight (43 g) basis into four groups (T1-T4) each having 6 birds. All birds were fed commercial broiler chicken feed. On the fifth day, cloacal swabs were obtained from each group and tested for the existence of *Salmonella* by streaking onto XLD agar. The birds of treatments T2, T3 and T4 which tested negative for *Salmonella* were orally inoculated with multi-drug resistant *S*. Typhimurium at the concentration of 10^8, 10^9 and 10^{10} cfu/ml respectively on the seventh day. Faecal matter consistency and other clinical changes were monitored on daily basis and cloacal swab examination was done on weekly basis.Thecaecal and liver samples were collected in sterile PBS broth to ascertain the existence of the organism by spread plate method on XLD agar plates supplemented with 100 ìg of ampicillin.The body weights of each bird were measured at weekly intervals. All the birds were slaughtered humanely on the third week and the duodenal, jejunal and ileal segments were collected in 10 percent formalin for histomorphometric study.

Intestinal histomorphometry

The representative pieces of the duodenum, jejunum and ileum kept in 10 per cent neutral buffered formalin were used for histomorphometric examination. After seven days the 0.5 cm long tissue was cut from the duodenum, jejunum and ileum part and placed in a cassette and processing was done by standard tissue processing protocols (Luna, 1968). Sections were taken at 5ìm thickness by using a semi-automatic M-TECH microtome. Haematoxylin and Eosin staining method was used for histological studies. The histomorphometric measurements were taken at 40x magnification for intestinal villus and crypt depth using image analysing software (MicapsMicroview).

RESULTS AND DISCUSSION

On cultural characterisation, red colonies with black centre confirmative for *Salmonella* were obtained and these isolates were tested positive for the *invA* and *spy* gene on PCR. On antibiotic susceptibility testing both the isolates were resistant to more than six classes of antibiotics and hence they were multidrug-resistant *S*. Typhimurium. The study reports published by Salehi *et al*. (2005), Alcaine *et al.* (2007), McDermott *et al*. (2016), Liljebjelke *et al.* (2017), Sharma *et al.* (2019), Jayaweera *et al.* (2020), Gayathridevi *et al*. (2021) and Raghavendra (2022) also stated that the isolated NTS strains from broilers, carcass and poultry farm environment exhibited resistance to more than three classes of antibiotics. Michael and Schwarz (2016) reviewed the existence of resistance genes, mutations, and genetic modifications associated with *Salmonella* spp. leading to the emergence of MDR conditions.

The clinical symptoms started showing from the fifth day post-infection in T4 and the seventh day post-infection in T2 and T3. The clinical symptoms included dullness, depression and reduced feed intake. Fifty percentage of the birds from T4 exhibited progressive weakness with diarrhoea after first- weekpost -infection without causing mortality. The results obtained were in agreement with Dra *et al.* (2019), who reported the development of clinical symptoms from the first week in broiler chicken infected with *S*. Typhimurium at the dose of 2×10^8 cfu/ml. On caecal and liver sample examination the *Salmonella* load was found to be higher in T4, the birds that were infected with 10^{10} cfu/ml. Based on these findings and through the application of the probit regression model the ID_{50} for the recovered MDR-*Salmonella* spp. challenge was conclusively set at 1×10^{10} CFU/ml.Akil and Ahmad (2019) reported that the consumption of at least 1.46×10^4 CFU/g for *Salmonellaenterica*serovarEnteritidis or 6.4×10^3 CFU/g for *S*. Typhimurium is required to develop an infection in 50 per cent of the population. The obtained infective dose 50 was different from the previous studies and that might be attributed to the changes in the *Salmonella*'s virulence pattern and other factors which affect the colonization of bacteria such as survival of *Salmonella* through the gastric barrier and genetic background of the chicks (Cosby *et al.*, 2015).

From the third day post-infection,the body weight of birds up tothe third week was analysed and a significant difference ($P<0.001$) could be observed during the second and third weeks (Table 1). Significantly higher body weight observed in T1 and T4 exhibited lower body weight.The body weight of the different treatments after post-infection was as follows T1>T2>T3>T4. *Salmonella* infection in poultry is linked to severe morbidity, malabsorption, slowed development, and ineffective feed utilisation, especially in young birds.

The villi height, crypt depth and villi-to-crypt ratio showed significant differences between the four treatments. In duodenum, villi height and crypt depth were significantly higher in T1 ($P<0.001$) and villi to crypt ratio was significantly lower in T4 ($P<0.001$). The villi height and villi to crypt depth were significantly higher in T1 ($P<0.001$) and the crypt depth was significantly higher in T4. The ileal villi to crypt ratio was significantly higher in T1 and the villi height was lower in T4 ($P<0.001$). The villi height to crypt depth

Table 1: Body weight upto third week (g)

Week	T1	T2	T3	T4	P-value
Week 1	211.50 ± 1.54	208.83 ± 0.60	209.83 ± 0.60	207.50 ± 0.76	0.051^{ns}
Week 2	321.33 ± 1.05^{a}	305.33 ± 1.11^{b}	290.83 ± 0.94^{c}	282.66 ± 0.66^{d}	$< 0.001^{**}$
Week 3	752.33 ± 0.98^{a}	730.33 ± 0.66^{b}	712.50 ± 0.99^{c}	706.33 ± 0.76^{d}	$< 0.001^{**}$

Table 2: Histomorphometric parameters of duodenum, jejunum and ileum

Group	Duodenum			Jejunum			Ileum		
	Villi height (µm)	Crypt depth (µm)	Villi: Crypt	Villi height (µm)	Crypt depth (µm)	Villi: Crypt	Villi height (µm)	Crypt depth (µm)	Villi: Crypt
T1	1398 ± 14^{a}	164 ± 3^{a}	8.51 ± 0.17^{b}	833 ± 8^{a}	110 ± 5^{b}	7.68 ± 0.41^{a}	738 ± 17^{ab}	104.1 ± 1.13^{b}	7.09 ± 0.18^{a}
T2	788 ± 16^{c}	109 ± 2^{c}	7.22 ± 0.22^{c}	565 ± 16^{c}	110 ± 1^{b}	5.15 ± 0.17^{b}	765 ± 14^{a}	130.2 ± 6.60^{a}	3.08 ± 0.14^{c}
T3	964 ± 4^{b}	101 ± 3^{d}	9.55 ± 0.28^{a}	591 ± 31^{c}	112 ± 2^{b}	5.27 ± 0.25^{b}	697 ± 36^{b}	105.3 ± 4.69^{b}	6.61 ± 0.19^{ab}
T4	708 ± 4^{d}	151 ± 3^{b}	4.71 ± 0.07^{d}	702 ± 15^{b}	131 ± 6^{a}	5.37 ± 0.20^{b}	398 ± 5^{c}	129.0 ± 10.25^{a}	6.06 ± 0.42^{b}
P-value	<0.001	<0.001	<0.001	<0.001	0.004	<0.001	<0.001	0.014	<0.001

ratio is utilised as a predictor of the small intestine's potential capability for digestion. This ratio rises in proportion to increased digestion and absorption as per Montagne*et al*. (2003). Consistent with the present investigation, Sieo *et al.* (2005) observed that an enhanced intestinal villi height and an increased ratio of villi height and crypt depth indicate a larger surface area for nutrient absorption and improved absorption function. .The findings are indicative of the impaired nutrient absorption and utilization in MDR-NTS infected birds especially in birds orally infected at a dose of 10^{10}cfu/ml.

CONCLUSION

The present study investigated the ID_{50} of recovered MDR-*S*. Typhimurium in broiler chicken. The infective dose of recovered MDR-*S*. Typhimurium is determined as 10^{10} cfu/mL as per clinical symptoms, faecal matter consistency and intestinal histomorphometry.

ACKNOWLEDGEMENTS

Authors are deeply indebted to Kerala Veterinary and Animal Sciences University,Pookode, Wayanad for providing facilities and research grants to carry out the study.

REFERENCES

Akil, L. and Ahmad, H.A. 2019. Quantitative risk assessment model of human salmonellosis resulting from consumption of broiler chicken. *Diseases*,**7**: 19-29.

Alcaine, S.D., Warnick, L.D. and Wiedmann, M. 2007. Antimicrobial resistance in non-typhoidal *Salmonella*. *Journal of Food Protection*, **70**: 780-790.

Aswathi, P.B., Biswas, A.K., Beura, C.K. and Yadav, A.S. 2013. Physico-chemical andsensory characteristics of functional poultry meat sticks. *Indian Journal of Poultry Science*, **48**(2): 228-233.

Balasubramanian, R., Im, J., Lee, J.S., Jeon, H.J., Mogeni, O.D., Kim, J.H., Rakotozandrindrainy, R., Baker, S. and Marks, F. 2019. The global burden and epidemiology of invasive non-typhoidal *Salmonella* infections. *Human Vaccineand Immunotherapeutics*,**15**: 1421-1426.

Basic Animal Husbandry Statistics (BAHS). 2022. Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture, **Government of India.** Available:https://dahd.nic.in/schemes/ programmes/animal-husbandry statistics [20 Aug. 2023]

Bauer, A.W., Kirby, W.M.M., Sherries, J.C. and Tuck, M. 1966. Antibiotic susceptibility testing by a standardized disc diffusion method. *American Journal of Clinical Pathology*,**45**: 493-496.

Cosby, D.E., Cox, N.A., Harrison, M.A., Wilson, J.L., Buhr, R.J. and Fedorka-Cray, P.J. 2015. *Salmonella* and antimicrobial resistance in broilers: A review. *Journal of Applied Poultry Research*,**24**: 408-426.

Dra, L.A., Brahim, M.A.S., Boualy, B., Aghraz, A., Barakate, M., Oubaassine, S., Markouk, M. and Larhsini, M. 2017. Chemical composition, antioxidant and evidence antimicrobial synergistic effects of Periploca laevigata essential oil with conventional antibiotics. *Industrial Crops and Products*. **109***:* 746-752.

Gayathridevi, S., Prayag, P., Remiz Raja, S. and Ashifa, K.M. 2021. Inhibitory effect of garlic extract on multi-drug-resistant *Salmonella* isolated from broiler chicken meat. *Current Researchin Food Science*, **2**: 27-33.

Jayaweera, T.R., Darshanee, V., Deekshi, V.J., Kodithuwakku, S.K. andIddya, C.H. 2020. Isolation and Identification of *Salmonella* spp. from Broiler Chicken Meat in Sri Lanka and their Antibiotic Resistance. *Journal Agricultural Sciences- Sri Lanka*, **15**: 395-410.

Jubeda, B., Rajesh, K. and Nasir, A.M. 2022. Antibiotic resistance as a cause of non-responding fowl typhoid infection at a layer farm in Uttarakhand, India- A case report. *Indian Journal of Poultry Science*, **57(**1): 83-87.

Liljebjelke, K.A., Hofacre, C.L., White, D.G., Ayers, S., Lee, M.D. and Maurer, J.J. 2017. Diversity of antimicrobial resistance phenotypes in *Salmonella* isolated from commercial poultry farms. *Frontiers in Veterinary Science*, **4**: 96.

Luna, L.G. 1968. *Manual of histologic staining methods of the Armed Forces Institute of Pathology*. (3rd Ed). McGraw-Hill, New York.

McDermott, P.F., Tyson, G.H., Kabera, C., Chen, Y., Li, C., Folster, J.P., Ayers, S.L., Lam, C., Tate, H.P. and Zhao, S. 2016.

Whole-genome sequencing for detecting antimicrobial resistance in nontyphoidal *Salmonella*. *Antimicrobial Agents and Chemotherapy*, **60**: 5515-5520.

Michael, G.B. and Schwarz, S. 2016. Antimicrobial resistance in zoonotic non-typhoidal *Salmonella*: an alarming trend?.*Clinical Microbiology and Infection*, **22**: 968-974.

Montagne, L., J. R. Pluske, and D. J. Hampson. 2003. A Review of Interactions between Dietary Fibre and the Intestinal Mucosa, and Their Consequences on Digestive Health in Young Non-ruminant Animals. *Animal Feed Science and Technology*, **108**: 95-117.

Mouttotou, N., Ahmad, S., Kamran, Z. and Koutoulis, K.C. 2017. Prevalence, risks and antibiotic resistance of *Salmonella* in poultry production chain.In: Mares, M. (ed.),*Current topics in Salmonella and Salmonellosis*, IntechOpen Limited, London, pp. 215-234.

Nair, A., Balasaravanan, T., Malik, S.V., Mohan, V., Kumar, M., Vergis, J. and Rawool, D.B. 2015. Isolation and identification of *Salmonella* from diarrheagenic infants and young animals, sewage waste and fresh vegetables. *Veterinary World*, **8**: 669-673.

Pandey, M. and Saxena, M.K. 2015. Cloning and immunopotential analysis of Omp 28 of Salmonella Typhimurium for the development of subunit vaccine for poultry salmonellosis. *Indian Journal of Poultry Science*, **50**(2): 138-142.

Raghavendra, 2022. Efficacy of dietary supplementation of *Kaempferia galanga* on the growth performance of broiler chicken and assessment of its antimicrobial activity against non-typhoidal *Salmonella* spp. M.V.Sc. thesis., Kerala Veterinary and Animal Sciences University, Pookode, 92p.

Ramesh, A.K., Parreño, V., Schmidt, P.J., Lei, S., Zhong, W., Jiang, X., Emelko, M.B. and Yuan, L. 2020. Evaluation of the 50% infectious dose of human Norovirus cin-2 in gnotobiotic pigs: A comparison of classical and contemporary methods for endpoint estimation. *Viruses*. **12**: 955.

Saif, Y.M. 2009. *Diseases of poultry*. (12th Ed.). John Wiley & Sons, New York, 1409p.

Salehi, T.Z., Mahzounieh, M. and Saeedzadeh, A. 2005. Detection of *invA* gene in isolated *Salmonella* from broilers by PCR method. *International Journal of Poultry Science*. **4**: 557-559.

Sharma, J., Kumar, D., Hussain, S., Pathak, A., Shukla, M., Kumar, V.P., Anisha, P.N., Rautela, R., Upadhyay, A.K. and Singh, S.P. 2019. Prevalence, antimicrobial resistance and virulence genes characterization of non-typhoidal *Salmonella* isolated from retail chicken meat shops in Northern India. *Food Control*.**102**: 104-111.

Sieo, C.C., Abdullah, N., Tan, W.S. and Ho, Y.W. 2005. Influence of â-glucanase-producing Lactobacillus strains on intestinal characteristics and feed passage rate of broiler chickens. *Poultry Science*, **84**: 734-741.

Varunkkumar, H. M., Vikas Pathak, Meena Goswami, Arun Kumar Verma and Rajkumar, V. 2022. Quality improvement of turkey meat cutlets with different enrobing materials.*Indian Journal of Poultry Science*. **57 (**1) : 69-74.

Wen, S.C., Best, E. and Nourse, C. 2017. Non typhoidal *Salmonella* infections in children: Review of literature and recommendations for management. *Journal of Paediatrics and Child Health*, **53**: 936-941.