Indian Journal of Animal Sciences **95** (2): 125–129, February 2025/Article
https://doi.org/10.56093/ijans.v95i2.154836

Genetic characterization and genotyping of β -lactamase producing *Salmonella enterica* serovars Enteritidis and Typhimurium isolated from livestock and poultry samples

C BINDU KIRANMAYI[1]✉, N SUBHASHINI[2], T SRINIVASA RAO[1] and B SURESH[1]

NTR College of Veterinary Science, Gannavaram, Krishna District – 521102, Andhra Pradesh, India

Received: 08 August 2024; Accepted: 26 March 2025

ABSTRACT

The present study was designed to detect the presence of β-lactamase producing *Salmonella enterica* Serovars Enteritidis and Typhimurium from faecal (60 cattle rectal, 108 sheep rectal, 163 pig rectal and 186 poultry cloacal swabs) and meat (55 beef, 98 mutton, 120 pork and 126 chicken) samples of different livestock and poultry. The isolates were detected by cultural isolation and further confirmed by PCR. Further these isolates were subjected to phenotypic detection and genotypic confirmation of different β-lactamases by PST and PCR, respectively. Out of 916 samples analysed, 18 Enteritidis and 14 Typhimurium were isolated, out of which β-lactamase producing *S.* Enteritidis and *S.* Typhimurium were detected in 14 (10 poultry cloacal, 2 chicken, 1 pork and 1 pig rectal) and 12 (10 chicken, 1 pork and 1 mutton) samples, respectively. Of 14 β-lactamase positive *S.* Enteritidis, 7 showed presence of *TEM,* 3 *OXA*, 2 *SHV*, one each for *CTXM*-1 and *CTXM*-9. Out of 12 β-lactamase positive *S.* Typhimurium, 8 showed presence of *TEM*, 2 for *CTXM*-9 and one each for *OXA* and *CTXM*-2. Genotyping of these Salmonella isolates by ERIC & REP-PCR has differentiated all the isolates.

Keywords: AmpC, β-lactamases, Carbapenamases, Enteritidis, ESBL, Typhimurium

Salmonellosis is caused mainly by serovars of *Salmonella enterica* subspecies *enterica*. Around 2500 serovars of *S. enterica* have been reported, of which *Salmonella* Typhimurium and *Salmonella* Enteritidis are the most common serotypes attributed to foodborne outbreaks and account for more than 75% of reported cases. Different food animal sources have been identified as reservoirs and poultry and poultry products are more frequently reported than any other animal species (USDA 1996). Non-typhoidal/ foodborne salmonellosis is the second most commonly found case next to campylobacteriosis associated with consumption of contaminated foods of animal origin and contact with infected animals. More than 75% of the annual cases of foodborne salmonellosis are believed to be the result of consumption of contaminated poultry, beef and egg products (European Food Safety Authority 2022). Recently, *Salmonella* species with increasing resistance to commonly used antimicrobials and multiple drug resistance (MDR) *Salmonella* strains are considered a matter of concern (Parry 2003). β-Lactam antibiotics are widely used for the treatment of salmonellosis in both medical and veterinary medicine. Emergence of ESBL harbouring *Salmonella* isolates has become a major concern in β-lactam therapy. Resistance to β-lactams is primarily caused by the production of β-lactamase enzymes like ESBLs, AmpC and Carbapenamases with varying degree of activity against different generations of beta-lactam antimicrobials (Bush and Jacoby 2010). Although numerous *Salmonella* spp. infections have been reported, very few studies have been reported on the β-lactamase activity of *S*. Enteritidis and *S.* Typhimurium in livestock and poultry. The present study was aimed at recovery of *S*. Enteritidis and *S.* Typhimurium isolates from livestock and poultry samples and to characterize their β-lactamase activity and genetic diversity.

MATERIALS AND METHODS

Primers and bacterial standard controls: American Type Culture Collection cultures of *S.* Enteritidis (ATCC 13076) and *S.* Typhimurium (ATCC 14028) were used as standard positive controls. The specific oligonucleotide primers used in this study were obtained from Bioserve Biotechnologies (Hyderabad, India).

Collection of samples and processing: A total of 916 samples comprising of faecal (60 cattle rectal, 108 sheep rectal, 163 pig rectal and 186 poultry cloacal swabs) and meat (55 beef, 98 mutton, 120 pork and 126 chicken) samples of different livestock and poultry were collected from different regions of Krishna District, Andhra Pradesh, India (Table 1). Ten grams of each homogenized meat

Present address: [1]NTR College of Veterinary Science, Gannavaram -521102, Krishna District, Andhra Pradesh, India, [2]College of Veterinary Science, Proddatur, Kadapa Dist., Andhra Pradesh. ✉Corresponding author email: bindukiranmayi@gmail.com

Table 1. No. of β-lactamase positive *Salmonella* serovars isolated from different samples

Sample	Total no. of respective samples	No. of positive Enteritidis isolates (%)	No. of positive Typhimurium isolates (%)
Cattle rectal swabs	60	-	-
Sheep rectal swabs	108	-	-
Pig rectal swabs	163	2 (1.23)	-
Poultry cloacal swabs	186	12 (6.45)	-
Beef	55	-	-
Mutton	98	-	1 (1.02)
Pork	120	1 (0.83)	1 (0.83)
Chicken	126	3 (2.38)	12 (9.52)
Total	916	18 (1.96)	14 (1.53)

sample was inoculated into 90 mL of trypticase soya broth (TSB; Himedia, Mumbai) the cloacal/ rectal swabs were collected from animals/birds using a sterile cotton swab (Himedia) and inoculated directly into 10 mL of TSB and incubated at 37°C for 24 h. The inocula were simultaneously streaked on MacConkey agar and Xylose Lysine Deoxycholate agar (XLD) plates (Himedia). The presumptive colonies of *Salmonella* spp. were selected based on colony morphology (red colonies with black centre on XLD and pale colonies on MacConkey agar) and biochemical properties and were enriched in Tryptic Soy Broth (TSB) for further DNA extraction and identification of *Salmonella* serovars using species-specific multiple PCR assay.

Confirmation of Salmonella serovars by serovar-specific PCR assay: DNA was extracted by boiling and snap chilling (Bindu Kiranmayi *et al.* 2021). Molecular confirmation of *Salmonella* serovars was done by m-PCR using standardized PCR mixture and cycling conditions as recommended by Soumet *et. al.* (1999) (Supplementary Fig-1). PCR products were subjected to gel electrophoresis in 2% agarose at 100 V for 1 h in 1X TAE buffer (1 mM EDTA, 40 mM Tris-HCl, 2 mM acetate and pH 8.3) with 0.05 mg/L ethidium bromide and visualized using a Gel Documentation unit (Bio-Rad).

Detection of β-lactamase producing by Salmonella isolates by phenotypic method, PST: PST (phenotypic screening test) was used for screening of *Salmonella* Enteritidis and Typhimurium isolates for β-lactamase production. All the PST positive isolates were further subjected to different m-PCR assays for genotypic confirmation of β-lactamase genes (Drieux *et al.,* 2008).

Detection of β-lactamase genes in Salmonella isolates: The DNA of all the PST-positive *Salmonella* serovar isolates were subjected to m-PCR assays (Supplementary Fig-2) using β-lactamase specific primers under standardized conditions for the detection of ESBL (Dallene *et al.* 2010), ACBL (Manoharan *et al*. 2012) and carbapenemase (Dallene *et al.* 2010) genes (Supplementary Fig. 2).

Genotyping of β-lactamase producing Enteritidis and Typhimurium by ERIC-PCR and REP-PCR assays: All β-lactamase positive *Salmonella* serovar isolates were subjected to genetic fingerprinting by ERIC-PCR and REP-PCR assays (Mohapatra *et al*. 2007). DNA of respective ATCC cultures and autoclaved distilled water were used as positive and negative controls, respectively (Fig. 1 and 2 and Fig. 3 and 4). Dendrogram/ cluster analysis was done using the DOLLOP program of PHYLIP v. 3.6 (https:// evolution.gs.washington. edu/phylip. html).

RESULTS AND DISCUSSION

Out of 916 samples analysed, *S.* Enteritidis and *S.* Typhimurium were detected in 18 (12 poultry cloacal, 3 chicken, 2 pig rectal and 1 pork) and 14 (12 chicken, 1 pork and 1 mutton) samples, respectively with highest prevalence of *S.* Enteritidis in poultry cloacal swabs (6.45%, 12/186) followed by chicken (2.38%, 3/126), pig rectal swabs (1.23%, 2/163) and pork (0.83%, 1/120) whereas highest prevalence of *S.* Typhimurium was found in chicken samples (9.52%, 12/126) followed by mutton (1.02% 1/98) and pork (0.83%, 1/120). Enteritidis isolates were not found in beef, mutton and rectal swabs of cattle and sheep whereas Typhimurium isolates were not found in any of the rectal/ cloacal swabs and beef samples (Table 1). Almost all the Enteritidis and Typhimurium were isolated from poultry cloacal and swine samples except one Typhimurium isolated from mutton indicating that poultry serves as the main reservoir for various non-typhoidal *Salmonella* (NTS) serotypes especially Enteritidis and Typhimurium (Syamily *et al.* 2023) and pigs being major zoonotic reservoir among different food animals, pigs and pork products often been a source of human infection with these serovars (Kirkwood *et al.* 2021). Further it was also reported that Salmonella Enteritidis and Salmonella Typhimurium were commonly isolated from human salmonellosis out-breaks associated with chicken (Finstad *et al.* 2012).

β-Lactam antibiotics are commonly used in human and veterinary medicine and poultry industry for both preventive and therapeutic purposes against several gram-positive, gram-negative and anaerobic organisms (Jung *et al.* 2023). Salmonella serovars are important pathogens that cause foodborne and waterborne diseases. β-lactam antibiotics (particularly third and fourth generation cephalosporins) are generally used for treating such conditions. β-Lactamase production is an important

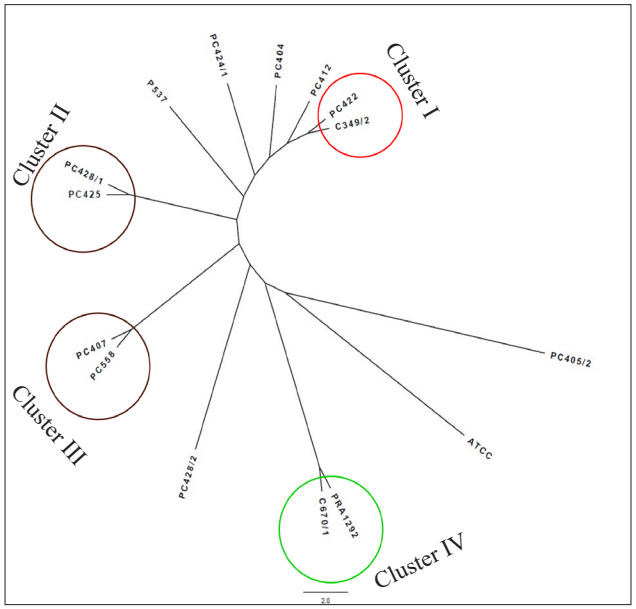

Fig. 1. Cluster analysis of ERIC-PCR fingerprints of ESBL producing Enteritidis isolated from different livestock sources

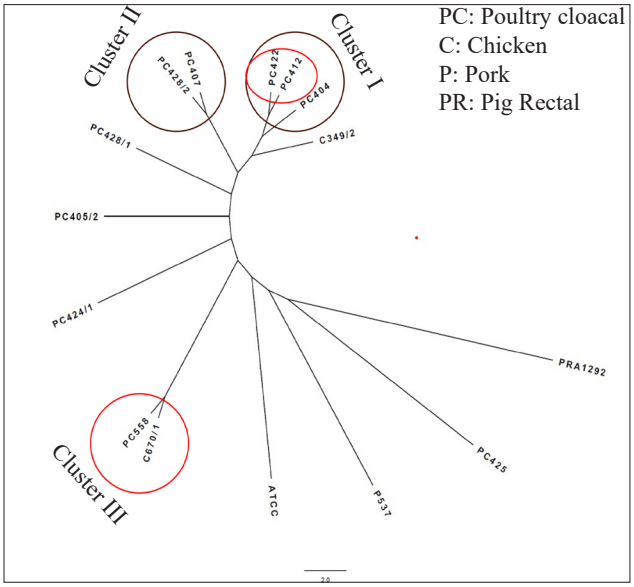

Fig. 2. Cluster analysis of REP-PCR fingerprints of ESBL producing Enteritidis isolated from different livestock sources

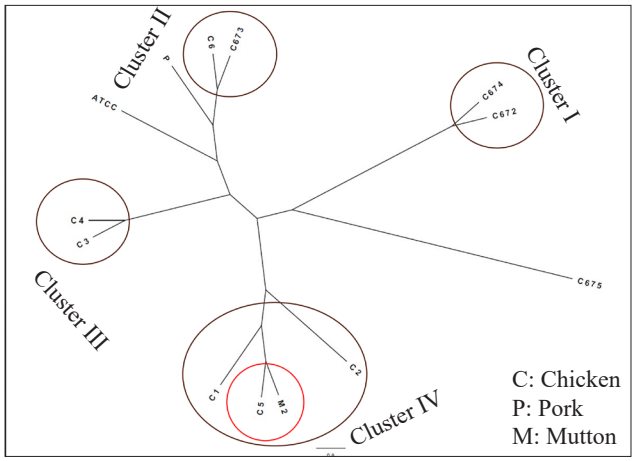

Fig. 3. Cluster analysis of ERIC-PCR fingerprints of ESBL producing Typhimurium isolated from different livestock sources

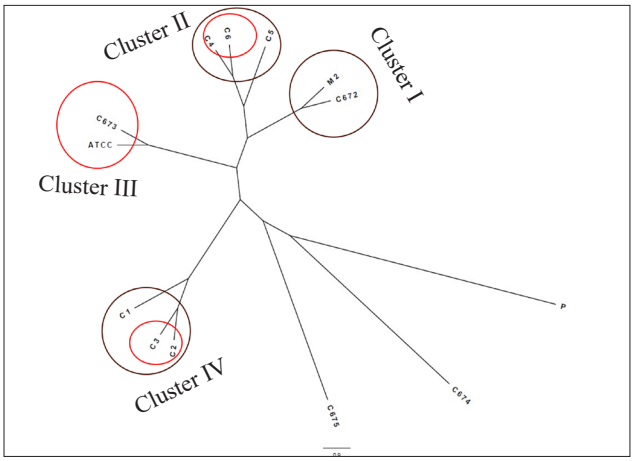

Fig. 4. Cluster analysis of REP-PCR fingerprints of ESBL producing Typhimurium isolated from different livestock sources

mechanism of resistance developed by many microbes against β-lactam antibiotics (Adak *et al*. 2002).

All the 32 *Salmonella* serovars (18 Enteritidis and 14 Typhimurium) were found to be PST positive indicating possible β-lactamase production, and were further subjected to m-PCR assays for genotypic characterization of β-lactamase production and only 26 (14 Enteritidis and 12 Typhimurium) were found to be carrying any one of the selected β-lacatmase genes (Table 2). Highest prevalence of β-lactamase positive Enteritidis were found in poultry cloacal swabs (5.38%, 10/186) followed by chicken (1.59%, 2/126), pork (0.83%, 1/120) and pig rectal swabs (0.61%, 1/163) and whereas that of Typhimurium were found in chicken (7.94%, 10/126) followed by mutton (1.02%, 1/98) and pork (0.83%, 1/120).

Of the 14 β-lactamase positive Enteritidis isolates, 7 showed presence of *TEM,* 3 *OXA*, 2 *SHV*, and one each for *CTXM*-1 and *CTXM*-9. Of the 12 β-lactamase positive Typhimurium isolates, 8 showed presence of *TEM*, 2 for *CTXM*-9 and 1 each for *OXA* and *CTXM*-2. None of the isolates carried ACBLs or carbapenamases. Amongst the 26 *Salmonella* isolates, the most prevalent beta-lactamase gene was *bla*TEM (57.69%) followed by *bla*OXA (15.38%), *bla*CTX-M9 (11.54%), *bla*SHV (7.69%) and *bla*CTX-M1 and *bla*CTX-M2 (3.84% each). Suresh *et al.* (2019) also reported *bla*TEM (81.18%) to be the most prevalent gene among *Salmonella* serovars (Enteritidis and Typhimurium together) followed by *bla*CTX-M2 (18.18%), *bla*OXA (9.09%) and *bla*CTX-M9 (9.09%).

Salmonella spp. is widely distributed in the environment, but their main habitat is the intestinal tract of animals. Salmonellosis occurs mainly through the consumption of contaminated foods of animal origin: milk, egg and poultry meat (Afshari *et al.* 2018). Indiscriminate use of antimicrobials as growth promoters in poultry industry is an important factor that favoured the selection of resistant

Table 2. β-lactamase producing *Salmonella* Enteritidis and Typhimurium isolated from different samples

Sample positive for *Salmonella* Typhimurium and Enteritidis (No.)	No. of positive isolates	No. of β-lactamase positive isolates	ESBL					
			TEM	*SHV*	*OXA*	*CTX*M1	*CTX*M2	*CTX*M9
Salmonella Enteritidis								
Pig rectal swabs (163)	2	1	1	-	-	-	-	-
Poultry cloacal swabs (186)	12	10	5	2	2	-	-	1
Pork (120)	1	1	-	-	-	1	-	-
Chicken (126)	3	2	1	-	1	-	-	-
Total	18	14	7	2	3	1	-	1
Salmonella Typhimurium								
Mutton (98)	1	1	1	-	-	-	-	-
Pork (120)	1	1	1	-	-	-	-	-
Chicken (126)	12	10	6	-	1	-	1	2
Total	14	12	8	-	1	-	1	2
Grand Total	32	26	15	2	4	1	1	3

bacteria in faecal microflora of poultry. These resistant bacteria gain access into the food chain resulting in rapid outbreaks of antibiotic-resistant *Salmonella* infections and treatment failure (Van Denn Bogaard and Stobberingh 1999).

Molecular epidemiology is a discipline where molecular or genetic markers are used to trace the source of a disease in a population and to understand transmission along with the population structure and evolution of bacterial pathogens. Phylogenetic analysis of molecular markers allows the determination of the genetic relatedness of strains from different sources, geographic locations and/ or even different time periods and inferring evolutionary relationships (Wang *et al.* 2015). Several DNA/ PCR-based techniques have been employed to assess the genetic diversity of different microorganisms. But the ERIC and REP-PCR approach is a rapid and highly reproducible method that target two different sets of repetitive elements (Mehta *et al.* 2002). Genetic fingerprinting of β-lactamase producing Enteritidis and Typhimurium isolates using ERIC-PCR and REP-PCR differentiated them into 14 and 12 strains, respectively including standards (ATCC culture) with discriminatory power of 1 (Simpson's index of diversity, Hunter and Gaston 1988). ERIC fingerprinting grouped β-lactamase positive Enteritidis them into four different clusters (Fig 1), each comprised of two isolates each with >60% similarity in band pattern. Cluster II and III comprised of poultry cloacal swab isolates (Fig 1). The chicken isolates (C349/2; C670/1) of cluster I and IV were found to be closely related to the isolates of poultry cloacal swab (PC 422) and pig rectal swab (PRA1292), respectively. REP fingerprinting grouped β-lactamase positive Enteritidis into three different clusters where cluster I consisted of 3 poultry cloacal swab isolates of which 2 isolates were sub-clustered (PC 422 and PC 412) with > 80% similarity in band pattern indicating close genetic relatedness (Fig 2). The cluster II is comprised of two poultry cloacal isolates (PC 407 and PC 428/2) whereas cluster III had a chicken isolate (C670/1) and poultry cloacal swab isolate (PC558).

ERIC fingerprinting of β-lactamase positive Typhimurium resulted in four different clusters. Cluster I, II and III comprised of two chicken isolates each whereas cluster IV had four isolates with a sub-cluster comprising of isolates from mutton and chicken (Fig 3). REP fingerprinting of β-lactamase positive Typhimurium showed four different clusters. Cluster I and III had two isolates each (Fig 4). In cluster-I, isolate from mutton (M2) was found closely related to that from the (C672) whereas in cluster-III, chicken isolate (C673) was found to have close association with ATCC standard culture. Cluster II & IV had three chicken isolates each with one sub-cluster each (Fig 4). The ERIC and REP clustering of mutton isolate with chicken isolates revealed the possibility of cross-contamination since mutton sample and chicken samples were collected from slaughter cum meat retail shops where both the meat are sold together. Both the ERIC and REP dendrogram analysis revealed a wide genetic diversity among the β-lactamase producing *Salmonella* serovars isolated and differentiated them into 14 and 12 different Enteritidis and Typhimurium strains, respectively.

In conclusion, the growing concern on emergence of ESBL producing bacterial pathogens in foods of livestock origin emphasizes the importance of continuous monitoring. In the current study, 83.33% (15/18) of the Enteritidis isolates and 85.71% (12/14) of the Typhimurium were isolated from poultry origin. Around 81% (26/32) of the isolates were found to be β-lactamase producing of which 84.62% (22/26) were of poultry origin highlighting the importance of monitoring genotypes of *Salmonella* in chicken and also Enteritidis and Typhimurium have been commonly isolated from human salmonellosis out-breaks associated with chicken (Diarra *et al.* 2014). These findings emphasize the importance of monitoring the genotypes of Salmonella isolates in chicken for designing effective control strategies to enhance food protection and to mitigate multidrug resistance and β-lactamase producing pathogens with in the food chain.

CONFLICT OF INTEREST

The authors hereby declare no potential conflicts of interest with respect to research, authorship and/or publication of this article

ACKNOWLEDGEMENT

The authors are thankful to DST-SERB, Govt. of India [Department of Science and Technology (DST) – Science and Engineering Research Board (SERB)] for funding the research project and we thank Sri Venkateswara Veterinary University, Tirupati for providing the facilities to carry out the current study.

REFERENCES

Adak G K, Long S M and O'Brien S J. 2002. Trends in indigenous foodborne disease and deaths, England and Wales, 1992 to 2000. *Gut* **51**(6):832–41.

Afshari A, Baratpour A, Khanzade S and Jamshidi,\ A. 2018. Salmonella Enteritidis and Salmonella Typhimurium identification in poultry carcasses. *Iran Journal of Microbiology* **10**(1): 45-50.

Bindu Kiranmayi Ch, Subhashini N, Srinivasa Rao T, Suresh B, Venkata Chaitanya P and Bhavana B. 2021. Detection of β-lactamase-producing *Proteus mirabilis* strains of animal origin in Andhra Pradesh, India and their genetic diversity. *Journal of Food Protection* **84**(8): 1374-1379. doi: 10.4315/JFP-20-399.

Bush K and Jacoby G A. 2010. Updated functional classification of β-lactamases. *Antimicrobial Agents Chemotherapy* **54**(3):969–76.

Dallene CA, Da Costa D, Decre C, Favier and Arlet G. 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. *The Journal of Antimicrobial Chemotherapy* **65**(3): 490–95. https://doi.org/10.1093/jac/dkp498.

Diarra S, Delaquis P, Rempel H, Bach S, Harlton C, Aslam M, Pritchard J and Topp E. 2014. Antibiotic Resistance and Diversity of Salmonella enterica Serovars Associated with Broiler Chickens. *Journal of Food Protection* 77(1): 40-49. https://doi.org/10.4315/0362-028.JFP-13-251.

Drieux L, Brossier F, Sougakoff W and Jarlier V. 2008. Phenotypic detection of extended spectrum beta-lactamase production in Enterobacteriaceae: review and bench guide. *Clinical Microbiology and Infections* 14: 90-103.

EFSA, European Food Safety Authority. 2022. The European Union One Health 2022 Zoonoses Report. https://doi.org/10.2903/j.efsa.2023.8442.

Finstad S, O'Bryan C A, Marcy J A, Crandall P G and Ricke S C. 2012. *Salmonella* and broiler processing in the United States: relationship to foodborne salmonellosis. *Food Research International* **45**: 789-94.

Hunter P R and Gaston M A. 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. *Journal of Clinical Microbiology* **26**: 2465-6.

Jung HR, Lee Y J, Hong S, Yoon S, Lim S K and Lee Y J. 2023. Current status of β-lactam antibiotic use and characterization of β-lactam-resistant *Escherichia coli* from commercial farms by integrated broiler chicken operations in Korea. *Poultry Science* **102**(12):1-7. https://doi.org/10.1016/j.psj.2023.103091.

Kirkwood M, Vohra P, Bawn M, Thilliez G, Pye H, Tanner J, Chintoan-Uta C, Branchu P, Petrovska L, Dallman T, Hall N, Stevens MP and Kingsley RA. 2021. Ecological niche adaptation of *Salmonella* Typhimurium U288 is associated with altered pathogenicity and reduced zoonotic potential. *Communications Biology* **4**: 498. https://doi.org/10.1038/s42003-021-02013-4.

Manoharan A, Sugumar M, Kumar A, Jose H, Mathai D and ICMR-ESBL study group. 2012. Phenotypic and molecular characterization of AmpC β-lactamases among *E. coli, Klebsiella* spp. and *Enterobacter* spp. form five Indian medical centres. *Indian Journal of Medical Research* **135**(3): 359.

Mehta A, Mehta YR and Rosato YB. 2002. ERIC- and REP-PCR amplify non-repetitive fragments from the genome of *Drechslera avenae* and *Stemphylium solani*. *FEMS Microbiology Letters* **211**: 51-5. https://doi.org/10.1111/j.1574-6968.2002.tb11202.x.

Mohapatra BR, Broersma K and Mazumder A. 2007. Fecal Comparison of five rep-PCR genomic fingerprinting methods for differentiation of *Escherichia coli* from humans, poultry and wild birds. *FEMS Microbiology Letters* **277**(1): 98-106. doi: 10.1111/j.1574-6968.2007.00948.x.

Parry CM. 2003. Antimicrobial drug resistance in *Salmonella enterica. Current Opinion in Infectious Diseases* 16: 467-72.

Soumet C, Ermel G, Rose V, Rose N, Drouin P, Salvat G and Colin P. 1999. Identification by a multiplex PCR-based assay of *Salmonella* Typhimurium and *Salmonella* Enteritidis stains from environmental swabs of poultry houses. *Letters in Applied Microbiology* **29**:1-6.

Suresh Y, Bindu Kiranmayi CH, Srinivasa Rao T, Srivani M, Subhashini N, Chaitanya G, Swathi Vimala B and Suresh B. 2019. Multidrug resistance and ESBL profile of *Salmonella* serovars isolated from poultry birds and foods of animal origin. *The Pharma Innovation Journal* **8**(4): 277-82.

Syamily S, Ramesh KS and Revathi S. 2023. *Salmonella* infection in poultry: A review on the pathogen and control strategies. *Microorganisms* **11**(11): 2814. https://doi.org/10.3390/microorganisms11112814.

USDA. 1996. Tracking food borne pathogens from farm to table: data needs to evaluate control points. Conference proceedings. 9-10.

Van Denn Bogaard AE and Stobberingh E.E. 1999. Antibiotic usage in animals: impact on bacterial resistance and public health. *Drugs* **58**: 589-607.

Wang X, Jordan IK and Mayer LW. 2015. A Phylogenetic Perspective on molecular epidemiology (Ch. 29). *Molecular Medical Microbiology* (Second Edition). Vol I: 517-536. https://doi.org/10.1016/B978-0-12-397169-2.00029-9.