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ESBL producing ability of *Salmonella* species isolated from animals, birds and environmental source

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Abstract
Aim: The current study was aimed to know about the extended spectrum beta-lactamase producing ability of *Salmonella* species, subspecies and serovars.
Materials and Methods: The presence of ESBL in 12 *Salmonella* isolates (6 *Salmonella typhimurium*, 3 *Salmonela enterica* spp., 2 *Salmonela enterica* subspp *salamae* and 1 *Salmonela enterica* sub. spp. *diarizonae*) was determined using phenotypic (combined disc method) method and genotypic methods by targeting EBSL genes.
Results: Out of 12 *Salmonella* isolates, five isolates were confirmed as ESBL producers by both phenotypic and genotypic methods of which all are Typhimurium serotypes.
Conclusion: The current study showed that only Typhimurium isolates were able to produce extended spectrum beta-lactamase which is an important pathogenic bacterium in birds and livestock and can acts as a health hazard to humans as it will help in developing antibiotic resistance, thereby reducing the efficiency of the treatment.

Keywords: Extended-spectrum beta-lactamases, *Salmonella*

1. Introduction
Salmonella is the major pathogenic bacteria with a major social and economic impact. The fecal wastes from infected animals act as an important source of bacterial contamination of the environment and enter humans through the food chain (Ponce *et al*., 2008) [8]. The last few decades have shown emergence of antibiotic-resistant Gram-negative bacteria, particularly the extended spectrum β-lactamase (ESBL)-producing *Salmonella* strains. ESBL producing *Salmonella* carry broad range of β-lactamase enzymes against beta-lactam antibiotics that have ability to hydrolyze third generation cephalosporins and aztreonam but are inhibited by clavulanic acid. ESBLs are classified into different types, among which CTX-M, SHV and TEM are the most prevalent around the globe (Paterson and Bonomo, 2005) [7]. The extensive and improper use of antibiotics in food animals acts as a source of resistant strains for human and animal consumption.
ESBLs are plasmid-mediated and are easily transmitted among members of the *Enterobacteriaceae* family. These ESBL genes have been identified in *Salmonella* isolated from animals and food products of animal origin (Proietti *et al.*, 2020) [9] and transmitted to human beings through the food chain resulting in serious consequences in terms of treatment failure and leading to rapid outbreaks of antibiotic-resistant *Salmonellae*. The emergence and spread of antibiotic-resistant strains is due to the production of β-lactamase enzymes in Gram-negative organisms due to the acquisition of *bla* gene which show resistance against beta-lactam antibiotics and becoming an emerging world threat (Ranjbar *et al*., 2010) [10]. Considering these facts, the present study was aimed to know the ESBL spectrum of *Salmonella* and its circulation in an epidemiological unit.

2. Materials and Methods
A total of 12 different isolates comprising of 6 *Salmonella typhimurium*, 3 *Salmonela enterica* spp., 2 *Salmonela enterica* subspp *salamae* and 1 *Salmonela enterica* subspp *diarizonae* were isolated from different sources *viz*. feces of animal and bird origin, cloacal swabs, soil, and floor swabs and were confirmed using PCR and were maintained in glycerol stock at Department of Veterinary Public Health and Epidemiology, Veterinary College-

Shivamogga, Karnataka. These confirmed isolates were revived and selected for the present study.

2.1 Phenotypic detection of ESBL

Previously standardized inoculum for antimicrobial susceptibility testing was used. Combined disc method was performed according to CLSI, (2018) using Cefotaxime (CTX-30 µg), Cefotaxime + Clavulanic Acid (CAC) and Ceftazidime (CAZ-30 µg), Ceftazidime + Clavulanic Acid (CEC) antimicrobial discs on Mueller-Hinton agar. The disc was placed on MHA agar surface and in incubated at 37 °C for 24 hours. The result of the zone of inhibition was interpreted.

2.2 Genotypic confirmation of ESBL producing genes

ESBL producing genes *viz*. bla_{TEM}, bla_{SHV} and bla_{CTX-M} were targeted by performing multiplex-PCR according to Monstein *et al.* (2007) [6]. Stock solutions were prepared according to manufacturer's instruction to get 100 µM/µl concentration. The following primers were used *viz*. forward primer 5'-TCG CCG CAT ACA CTA TTC TCA GAA TG-3' and reverse primer 5'-ACG CTC ACC GGC TCC AGA TTT AT-3' for bla_{TEM} gene, forward primer 5'-ATG CGT TAT ATT CGC CTG TG-3' and reverse primer 5'-TGC TTT GTT CGG GCC AA-3' for bla_{SHV} gene and forward primer 5'-ATG TGC AGY ACC AGT AAR GTK ATG GC-3' and reverse primer 5'-TGG GTR AAR TAR GTS ACC AGA AYC AGC G-3' for bla_{CTX-M} gene. The working solution was then prepared by diluting at 1:10 ratio using nuclease-free water to make a final concentration of 10 picomoles/µl concentration per PCR reaction.

PCR master mix was prepared in 2 ml Eppendorf tube for a total of 25 µl genes that contained 7.5 µl of sterile water each, 12.5 µl of PCR master mix each, 0.5 µl of forward and 0.5 µl of reverse primer of bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes and 2 µl of template DNA. The PCR cycling conditions include initial denaturation at 94 °C for 10 min, denaturation at 94 °C for 60 sec, annealing at 60 °C for 60 sec, and extension at 72 °C for 1 min with 30 cycles.

3. Results

3.1 Phenotypic detection of ESBL production

Phenotypic detection of ESBL producing *Salmonella* isolates using combined disc method. Among 12 *Salmonella* isolates, 5 were ESBL-producing isolates that belonged to the *Salmonella typhimurium* serotype (Table 1; Fig. 1).

3.2 Genotypic detection of ESBL-producing genes

Five isolates that were confirmed as ESBL producing *Salmonella* were subjected for multiplex-PCR by targeting bla_{TEM} (445 bp), bla_{SHV} (747 bp) and bla_{CTX-M} (593 bp) genes. All the five isolates showed amplification for only bla_{TEM} at 445 bp. None of the five isolates showed amplification for either bla_{SHV} (747 bp) nor bla_{CTX-M} (593 bp) (Table 1; Fig. 2).

Table 1: Phenotypic and genotypic analysis of AMR of *Salmonella typhimurium* by ESBL production

Sl. No.	Source of sample	*Salmonella* serotypes	Phenotypic analysis: Zone of inhibition (mm)				Genotypic analysis: ESBL genes targeted			ESBL production
			Cefotaxime (CTX-30 µg)	Cefotaxime + Clavulanic Acid (CAC)	Ceftazidime (CAZ-30 µg)	Ceftazidime + Clavulanic Acid (CEC)	bla_{TEM} (445 bp)	bla_{SHV} (747 bp)	bla_{CTX-M} (593 bp)	
1.	Chicken cloacal swab	*S. typhimurium*	26	32	22	30	+	-	-	+
2.	Chicken cloacal swab	*S. typhimurium*	26	32	21	29	+	-	-	+
3.	Soil	*S. typhimurium*	30	32	28	30	-	-	-	-
4.	Cattle feces	*S. typhimurium*	27	32	20	28	+	-	-	+
5.	Chicken cloacal swab	*S. typhimurium*	25	30	22	28	+	-	-	+
6.	Sheep floor swab	*S. typhimurium*	25	31	21	26	+	-	-	+
7.	Sheep feces	*Salmonella enterica*	26	28	24	26	-	-	-	-
8.	Soil	*Salmonella enterica*	28	30	25	26	-	-	-	-
9.	Chicken cloacal swab	*Salmonella enterica*	27	28	24	25	-	-	-	-
10.	Floor swab	*S. enterica* sub spp. *salamae*	28	29	25	27	-	-	-	-
11.	Sheep feces	*S. enterica* sub. spp. *diarizonae*	29	30	25	25	-	-	-	-
12.	Cattle feces	*S. enterica* sub. spp. *salamae*	30	31	26	28	-	-	-	-

Fig 1: Phenotypic analysis of AMR of *Salmonella typhimurium* by ESBL production by combined disc method
Plate showing zone of inhibition [Disk diffusion zone for Ceftazidime (≤ 22 mm) and Cefotaxime zone (≤ 27 mm). A ≥ 5-mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanate vs the zone diameter of the agent when tested alone = ESBL]

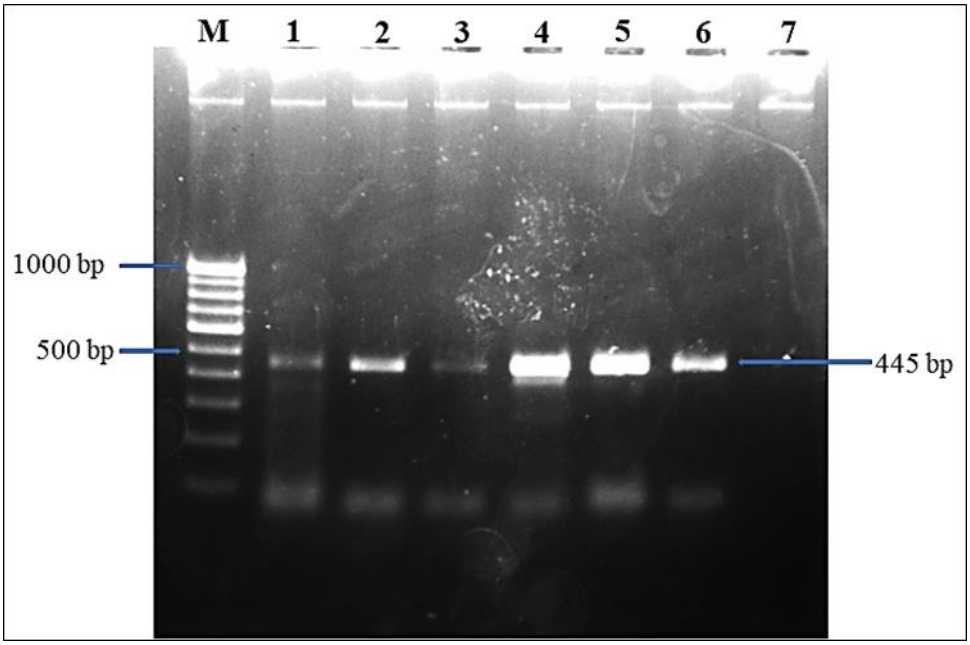

Fig 2: Agarose gel showing PCR amplification of ESBL producing genes (*bla*TEM, *bla*SHV and *bla*CTX-M) of *Salmonella typhimurium*
Lane M: 100 bp ladder
Lane 1: Positive control of *bla*TEM gene of *Salmonella*
Lane 2-6: *Salmonella typhimurium* showed amplification of *bla*TEM gene (445 bp); negative for *bla*SHV (747 bp) and *bla*CTX-M (593 bp)
Lane 7: No template control

4. Discussions

Mutation or transfer of resistance genes between *Salmonella* has led to the cause of the emergence of antimicrobial resistance (AMR) *Salmonella* (Zhang *et al*., 2006) [13]. This property of *Salmonella* has failed to respond to the many antimicrobials that resulted in MDR leading to prolonged illness and reducing the effectiveness of treatment in humans (Mitema *et al*., 2001) [5]. AMR *Salmonella* is transmitted to humans through consumption of food of animal origin, and contaminated water and emerge as a major public health issue worldwide (Marshall *et al*., 2011) [4].

The emergence of ESBL-producing *Salmonella* is a result of improper use of broad-spectrum antibiotics such as third generation cephalosporins. The increasing prevalence of multidrug resistance in *Salmonella* against clinically important antimicrobials, such as beta-lactams and fluoroquinolones, will lead to failures of the current treatment practices. ESBLs are plasmid-mediated and as a result, are easily transmitted among members of the *Enterobacteriaceae* family (Kocagoz *et al*., 2006) [3]. Interestingly, among different species of *Salmonella* isolates only Typhimurium showed ESBL production which is a major disease-causing agent in poultry and livestock. The present study is comparable with Suresh *et al*., (2019) [11] who reported that *bla*TEM gene was present in 4 out of 7 *Salmonella typhimurium* isolates with 57.14% prevalence and found that the most prevalent β-lactamase gene was *bla*TEM. While Fangyou *et al.* (2011) [2] reported presence of *bla*CTX-M group-9 in *Salmonella typhimurium* isolates. These ESBL-producing *Salmonella* can circulate within an epidemiological unit comprising different species of animals *via* contaminated water, soil and movement of animals and humans. In China, a study by Wang *et al*. (2018) [12] reported that the most common ESBL gene in *Salmonella*-resistant strains was *bla*CTX-M, followed by *bla*TEM, *bla*OXA and *bla*SHV. Another study by Elkenany *et al.* (2019) [1] in Egypt detected *bla*TEM in resistant *Salmonella* isolates. This suggests that the predominant group of ESBL enzymes varies between regions and nations, which may be caused by using various antibiotic treatment methods.

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6. Conclusion

The present study was performed to know the ESBL producing ability of *Salmonella* isolates from animal, bird, and environmental source. The pattern of ESBL production only in Typhimurium serotypes that were isolated from animal and bird source inferred that ESBLs are prevalent only in pathogenic strains that are found commonly in birds and animals is a matter of public health concern as it can transmit to humans through the food chain. Contact with numerous vectors, such as animal feces, feeds, water supplies, etc., may result in the transmission of resistant genes to animals used for food production. This is why awareness about proper use of antibiotics, identify source of bacterial contamination, routes of transmission must be created among people.

7. Reference

1. Elkenany R, Elsayed M, Zakaria A, El-Sayed S, AbdoRizk M. Antimicrobial resistance profiles and virulence genotyping of Salmonella enterica serovars recovered from broiler chickens and chicken carcasses in Egypt. BMC Vet. Res. 2019;15:124.
2. Fangyou Yu, Qiang Chen, Xiaojun Yu, Qiaoqiao Li, Baixing Ding, Lehe Yang, *et al*. High prevalence of extended-spectrum beta lactamases among Salmonella enterica Typhimurium isolates from pediatric patients with diarrhea in China. PLoS One. 2011;6(3):1-4.
3. Kocagoz S, Budak F, Gur D. Evaluation of a chlorogenic

mediumfor rapid detection of extended spectrum beta-lactamases producing *Salmonella* spp. Indian J Med Res. 2006;124:443-446.
4. Marshall BM, Levy SB. Food animals and antimicrobials: Impacts on human health. Clin. Microbiol. Rev. 2011;24:718-733.
5. Mitema ES, Kikuvi GM, Stohr K, Wegener H. An assessment of antimicrobial consumption in food producing animals in Kenya. J Vet. Pharmacol. Ther. 2001;24:385-390.
6. Monstein HJ, Ostholm-Balkhed A, Nilsson MV, Nilsson M, Dornbusch K, Nilsson LE. Multiplex PCR amplification assay for the detection of bla_{SHV}, bla_{TEM} and bla_{CTXM} genes in Enterobacteriaceae. APMIS. 2007;115:1400-1408.
7. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: A clinical update. Clin. Microbiol. Rev. 2005;18:657-686.
8. Ponce E, Khan AA, Cheng CM, Summage WC, Cerniglia CE. Prevalence and characterization of *Salmonella* enteric serovar Weltevreden from imported seafood. Food Microbiol. 2008;25:29-35.
9. Proietti PC, Stefanetti V, Musa L, Zicavo A, Dionisi AM, Bellucci S, *et al*. Genetic Profiles and Antimicrobial Resistance Patterns of Salmonella Infantis Strains Isolated in Italy in the Food Chain of Broiler Meat Production. Antibiotics. 2020;9:1-12.
10. Ranjbar R, Glammanco GM, Aleo A, Plaro MR, Naghomi A. Characterization of the first extended spectrum beta-lactamase producing nontyphoidal *Salmonella* strains isolated in Tehran, Iran. Foodborne Pathog. Dis. 2010;7:91-95.
11. Suresh Y, Bindu Kiranmayi CH, Srinivasa Rao T, Srivani M, Subhashini N, Chaitanya G, *et al*. Multidrug resistance and ESBL profile of *Salmonella* serovars isolated from poultry birds and foods of animal origin. J Pharm. Innov. 2019;8:277-282.
12. Wang J, Li Y, Xu X, Liang B, Wu F, Yang X, *et al*. Antimicrobial Resistance of Salmonella enterica Serovar Typhimurium in Shanghai, China. Front. Microbiol. 2018;8:1-10.
13. Zhang R, Eggleston K, Rotimi V, Zeckhauser RJ. Antibiotic resistance as a global threat: evidence from China, Kuwait and the United States. Global Health 2006;2:1-14.