www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(4): 277-282
© 2019 TPI
www.thepharmajournal.com
Received: 16-02-2019
Accepted: 20-03-2019

Y Suresh
M.V.Sc., Dept. of VPE, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh, India

CH Bindu Kiranmayi
Assistant Professor, Dept. of VPE, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh, India

T Srinivasa Rao
Associate Professor & Head, Dept. of VPE, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh, India

M Srivani
Assistant Professor, Dept. of Veterinary Microbiology, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh, India

N Subhashini
Assistant Professor, Dept. of VPE, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh, India

G Chaitanya
M.V.Sc., Dept. of VPE, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh, India

B Swathi Vimala
M.V.Sc., Dept. of VPE, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh, India

B Suresh
JRF, DST Project, Dept. of VPE, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh, India

Correspondence
CH Bindu Kiranmayi
Assistant Professor, Dept. of VPE, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh, India

Multidrug resistance and ESBL profile of *Salmonella* serovars isolated from poultry birds and foods of animal origin

Y Suresh, CH Bindu Kiranmayi, T Srinivasa Rao, M Srivani, N Subhashini, G Chaitanya, B Swathi Vimala and B Suresh

Abstract
Salmonella is a foodborne pathogen having a worldwide public health concern. The present study was undertaken to study antibogram profiles of multidrug resistant *Salmonella* isolates and detection of β-lactamase (ESBL-Extended Spectrum β-Lactamases) activity by both phenotypic and genotypic methods. Out of 21 *Salmonella* spp. isolated from different sources, antibiogram revealed 100% susceptibility to co-trimoxazole and polymyxin–B, intermediate resistance against ampicillin (28.57%), cefotaxime (19.04%), gentamycin (14.28%), amikacin (9.52%), ceftriaxone (9.52%), ciprofloxacin (9.52%), tetracycline (4.76%) and streptomycin (4.76%) while, higher resistance was observed towards amikacin (61.90%) followed by ampicillin (52.30%), tetracycline (38.09%), ceftriaxone (33.33%), gentamicin, sulfamethoxazole, cefpotaxime and nalidixic acid (28.57% each), ciprofloxacin (23.80%), doxycycline hydrochloride and chloramphenicol (19.04% each) and streptomycin (9.52%). Of the 21 *Salmonella* isolates, 15 isolates were found resistant to β-lactam antibiotics like ceftriaxone (33.33%), cefotaxime (28.57%), aztreonam (23.80%) and ceftazidime (23.80%). β-lactamase genes were detected in a total of 11 isolates (11/21, 52.38%) where *bla*TEM being the predominant gene detected (9/11, 81.18%), followed by *bla*CTX-M group-2 (2/11, 18.18%), *bla*OXA (1/11, 9.09%) and *bla*CTX-M group-9 (1/11, 9.09%) and no single isolate showed *bla*CTX-M group-1 and *bla*SHV genes.

Keywords: *Salmonella* typhimurium, enteritidis, antibiogram, ESBL detection

Introduction
In the past few decades, the emergence of antibiotic resistance among different species of bacteria is on rise (Davies and Davies, 2010) [12]. This antibiotic resistance poses great threat to public health in case of zoonotically important bacteria transmitted from food animals. So, contaminated poultry products serve as an important threat to public health as it is important reservoir of *Salmonellae*. Irrational use of antibiotics as growth promoters in poultry is an important factor that has favoured the selection of resistant bacteria in faecal microflora of poultry. These resistant microflora easily pass into human beings through the food chain resulting in serious consequences in terms of treatment failure and leading to rapid outbreaks of antibiotic resistant *Salmonellae* (Van Den Boggard *et al*., 1999) [31].

In present days, *Salmonella* species showing increasing resistance to commonly used antimicrobials and those strains that have acquired multiple drug resistance (MDR) against two or more therapeutic agents have become a matter of concern. Although fluoroquinolones, such as ofloxacin, ciprofloxacin and extended-spectrum cephalosporins, such as cefotaxime and ceftriaxone, have proved to be effective alternatives but resistance to these agents also has emerged (Parry, 2003) [22].

The emergence and spread of antibiotic resistant strains particularly the detection of the Extended Spectrum Beta-lactamase (ESBL) *Salmonella* species, is fast becoming an emerging world threat (Ranjbar *et al.*, 2010) [24]. ESBLs are enzymes capable of hydrolyzing penicillin, cephalosporin and oxyiminino-β-lactam compounds (i.e. cefuroxime, third generation cephalosporin and aztreonam) with cephamycins and carbapenems as exceptions. Most ESBLs belong to the Ambler class "A" β-lactamases and are inhibited by β-lactamase inhibitors (Clavulanate, sulbactam and tazobactam). ESBLs are plasmid-mediated and as a result, are easily transmitted among members of the Enterobacteriaceae family. This potential exacerbates the spread of resistance against β-lactams and other commonly used antibiotics including quinolones and aminoglycosides (Kocagoz *et al*., 2006) [18].

This has further limited the therapeutic options available and hence complicated the treatment and management of infections by such organisms. Hence the present study was undertaken to detect the multidrug resistant *Salmonella* spp. and their β-lactamase activity.

Materials and Methods

A total of 21 different *Salmonella spp.* (7 *S. Typhimurium*, 7 belonging to *Salmonella* group II, 3 belonging to group G, 2 *S. enteritidis*, 1 each of *S*. Daytona and *S.* Linderburg) which were isolated (from different sources like Poultry cloacal swabs, chicken, mutton and pork) at Department of Veterinary Public Health and Epidemiology, NTR C.V. Sc, Gannavaram were selected for present study.

Antibiogram and β-lactamase production

About 14 antibiotics from different classes were used for the study of antibiogram profiles and determination of MDR *Salmonella*. The antibiotics used were Penicillins: ampicillin, Aminoglycosides: amikacin, gentamicin, streptomycin, tetracyclines: doxycycline hydrochloride, tetracycline, Sulfonamides: co-trimoxazole, sulamethazole, Quinolones: ciprofloxacin, nalidixic acid, Phenicols: chloramphenicol, Polymyxins: polymyxin, Cephalosporins: ceftriaxone and cefpotaxime by Kirby Bauer disc diffusion method on Muller Hinton agar (Bauer *et al*., 1966) [3]. Resistance to atleast 3 antibiotic classes was taken as MDR (Minimum one in each class). Direct colony suspension of each isolate was made in PBS (pH 7.4) and the turbidity was adjusted to 0.5 McFarland units (equivalent to an approximate cell density of 1.5 x 108 CFU/ml). The diameter of inhibition zones was measured and susceptibility patterns of *Salmonella* species were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2018).

Detection of ESBL production was done phenotypically by Phenotypic Screening Test (PST) and Phenotypic Confirmation Test (PCT) as recommended by CLSI (2018) guidelines. PST was carried out using four indicator β-lactam antibiotics: cefotaxime (CTX, 30 µg), ceftazidime (CAZ, 30 µg), ceftriaxone (CTR, 30 µg) and aztreonam (AT, 30 µg). Resistance to at least one of the four antibiotics was considered to be positive PST for ESBL production. The positive PST isolates were then subjected to PCT by combination disc method using three pairs of antibiotic discs: ceftazidime (CAZ, 30 µg), ceftazidime plus clavulanic acid (CAC, 30/10 µg), cefotaxime (CTX, 30 µg), cefotaxime plus clavulanic acid (CEC, 30/10 µg) and ceftriaxone (CTR, 30 µg), ceftriaxone plus tazobactum (CIT, 30/10 µg). ESBL production was confirmed when zone diameter around the combination discs was more than or equal to 5 mm when compared to discs containing respective cephalosporin alone (Drieux *et al.,* 2008) [13].

Multiplex PCR for ESBL genes

mPCR assay was used to detect different classes of β-lactamase genes. In the present study, *bla*TEM, *bla*SHV and *bla*OXA gene (Fig. 1) primers have been used for the detection of broad spectrum as well as extended spectrum TEM, SHV and OXA β-lactamases, *bla*CTX-M group 1, 2 and 9 gene (Fig. 2) primers for CTX-M ESBLs.

DNA from all the PCT positive *Salmonella* isolates were subjected to two mPCR assays for detection of ESBL genes (Table 1). PCR assays were optimized in 25 µl reaction mixture containing 2 µl of DNA template, 12.5 µl of 2x master mix (Go Taq Green Master Mix, Promega), 0.5µl each of forward and reverse primers (10 pmol/µl) and the rest of the volume is made by adding nuclease free water, under standardized cycling conditions: initial denaturation at 94^0C for 10 min; 30 cycles of 94^0C for 40 s, 60^0C for 40 s and 72^0C for 1 min and a final elongation step at 72^0C for 7 min.

Table 1: Oligonucleotide primers used for the detection of different β-lactamase genes (Dallene *et al.,* 2010)

Primer	Target	Nucleotide sequence	Amplicon size (bp)
m-PCR I			
MultiTSO-T	*bla*TEM gene	CATTTCCGTGTCGCCCTTATTC	800
		CGTTCATCCATAGTTGCCTGAC	
MultiTSO-S	*bla*SHV gene	AGCCGCTTGAGCAAATTAAAC	713
		ATCCCGCAGATAAATCACCAC	
MultiTSO-O	*bla*OXA gene	GGCACCAGATTCAACTTTCAAG	564
		GACCCCAAGTTTCCTGTAAGTG	
m-PCR II			
MultiCTXM-Gp1	blaCTX-M group 1 gene	TTAGGAAATGTGCCGCTGTA	688
		CGATATCGTTGGTGGTACCAT	
MultiCTXM- Gp2	BlaCTX-M group 2 gene	CGTTAACGGCACGATGAC	404
		CGATATCGTTGGTGGTACCAT	
MultiCTXM- Gp9	BlaCTX-M group 9 gene	TCAAGCCTGCCGATCTGGT	561
		TGATTCTCGCCGCTAAG	

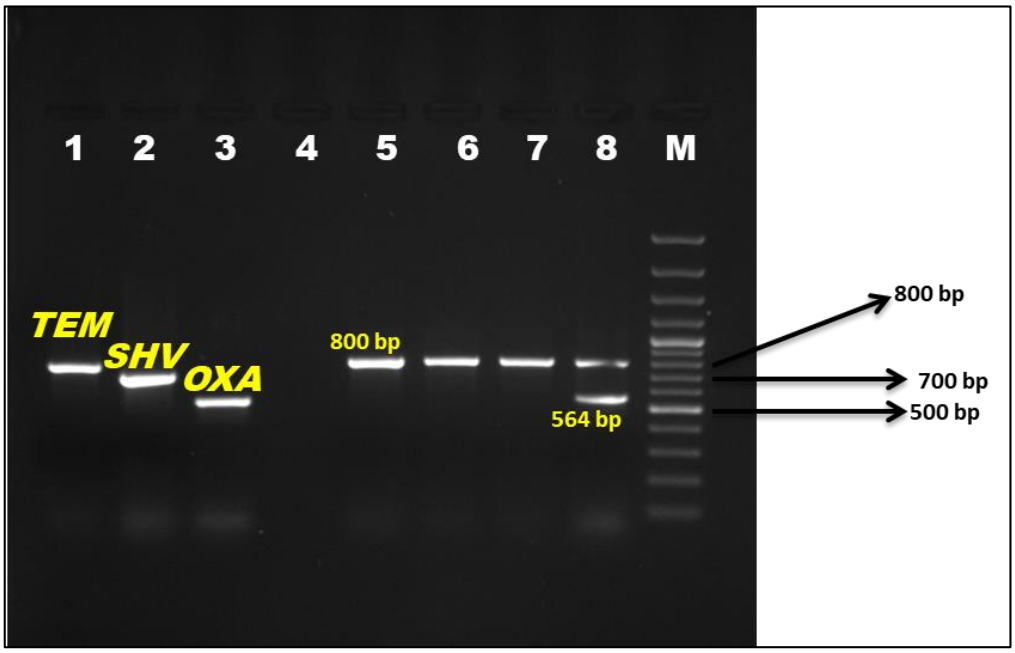

Fig 1: Gel photograph of m PCR I targeting *bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{OXA}$ genes in ESBL producing *Salmonella* isolates.

Lane 1	Known DNA standard for *bla*$_{TEM}$ (800 bp)
Lane 2	Known DNA standard of *Klebsiellapneumoniae*(ATCC 700603) for *bla*$_{SHV}$ (713 bp)
Lane 3	Known DNA standard for *bla*$_{OXA}$ (564 bp)
Lane 4	Negative control
Lane 5 & 6	*Salmonella*Typhimurium isolate carrying *bla*$_{TEM}$gene
Lane 7	*Salmonella*Enteritidis isolate carrying *bla*$_{TEM}$gene
Lane 8	*Salmonella* Typhimurium isolate carrying *bla*$_{TEM}$ and *bla*$_{OXA}$gene
Lane M	Molecular weight marker (100bp)

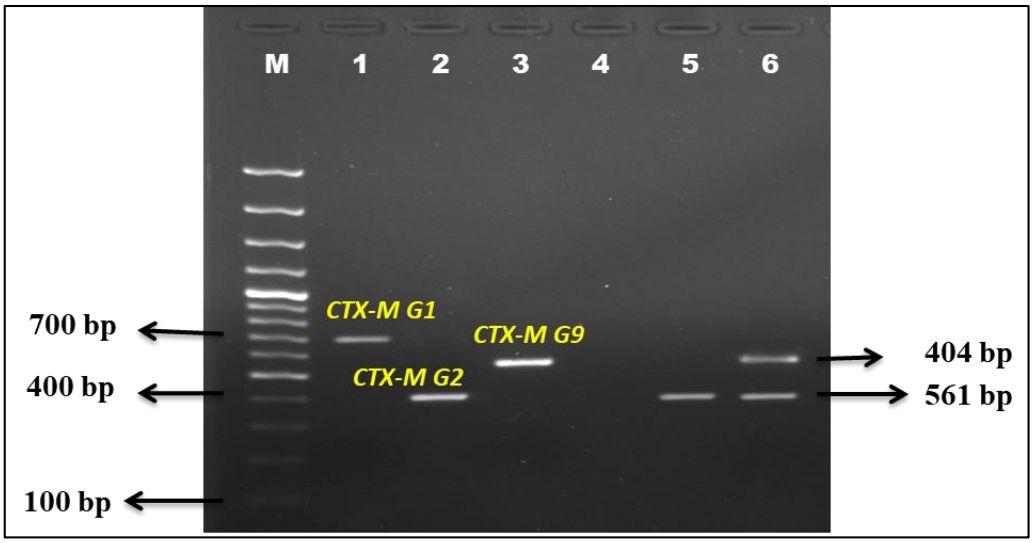

Fig 2: Gel photograph of m-PCR targeting *bla*$_{CTX\text{-}M}$ group 1, group 2 and group 9

Lane M	Molecular weight marker (100bp)
Lane 1	Known DNA standard of for *bla*$_{CTX\text{-}M}$ group 1 (688 bp)gene
Lane 2	Known DNA standard for *bla*$_{CTX\text{-}M}$ group 2 (404 bp)gene
Lane 3	Known DNA standard for *bla*$_{CTX\text{-}M}$ group 9 (561 bp) gene
Lane 4	Negative control
Lane 5	*Salmonella* Typhimurium isolate carrying *bla*$_{CTX\text{-}M}$ group 2 (404 bp) gene
Lane 6	*Salmonella*Typhimurium carrying both *bla*$_{CTX\text{-}M}$ group 2 and grop 9 (404bp & 561 bp)genes

Results and Discussion

Among *Salmonella* serotypes, *S. Typhimurium* and *S. Enteritidis* are the most important agents of foodborne salmonellosis in humans (Baay and Huisin'tveld, 1993 and Tan and Shelef, 1999). *Salmonella* group II, considered as *Salmonella enterica* subspp. *salamae*, comprising of *Salmonella* Sofia, *Salmonella* Tranoroa, *Salmonella* Hagenbeck, *Salmonella* Nairobi serovars will be commonly associated with cold blooded animals but can be an occasional pathogen in man and other animals (Wuthe, 1969) [34].

Salmonella group G includes serovars like *S.* Poona, *S.* Worthington, *S.* Mississippi, *S.* Grumpensis, *S.* Atlanta, *S.* Cubana, *S.* Wichita etc. (Bridges and Scott, 1935) [5].

The emergence of multidrug resistance (MDR) among foodborne pathogens is a cause of grave concern to public health (Tiwari *et al*., 2013) [29]. In this context, an *in vitro* antibiotic sensitivity test was performed for 21 *Salmonella* isolates obtained from different sources using 14 different antibiotics belonging to different classes, to know the multidrug resistance among *Salmonella* isolates (Table-2 & 3). It was found that all the isolates were sensitive to co-trimaxazole (100%) and polymyxin–B (100%) followed by higher susceptability towards streptomycin (85.71%) chloramphenicol (80.95%), doxycycline hydrochloride (80.95%), nalidixic acid (71.42%), sulphamethoxyzole (71.42%), ciprofloxacin (66.66%), tetracycline (57.14%), gentamycin (57.14%), ceftriaxone (57.14%), cepfotaxime (52.38%), amikacin (28.57%) and ampicillin (19.05%).

Among different *Salmonella* strains, the highest percentage of resistance was observed against amikacin (61.90%), followed by ampicillin (52.30%), tetracycline (38.09), ceftriaxone (33.33%), cefotaxime, gentamicin, nalidixic acid and sulfamethoxazole (28.57% each),ciprofloxacin (23.80%), doxycycline hydrochloride and chloramphenicol (19.04% each) and streptomycin (9.52%). Notable percentage of isolates were intermediately resistant against ampicillin (28.57%) followed by cefotaxime (19.04%), gentamycin (14.28%), amikacin (9.52%), ceftriaxone (9.52%), ciprofloxacin (9.52%), tetracycline (4.76%) and streptomycin (4.76%).

Table 2: Antibiotic resistance/sensitivity pattern among *Salmonella* isolates

Antimicrobial Agent (dose)	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Amikacin-AK (30µg)	6/21	(28.57%)	2/21	(9.52%)	13/21	(61.90%)
Ampicillin- A (10µg)	4/21	(19.05%)	6/21	(28.57%)	11/21	(52.30%)
Cefpotaxime-CTX (30µg)	11/21	(52.38%)	4/21	(19.04%)	6/21	(28.57%)
Ceftriaxone-CTR (30µg)	12/21	(57.14%)	2/21	(9.52%)	7/21	(33.33%)
Chloramphenicol-C (30µg)	17/21	(80.95%)	0	0	4/21	(19.04)
Ciprofloxacin-CIP (5µg)	14/21	(66.66%)	2/21	(9.52%)	5/21	(23.80%)
Co-Trimoxazole-COT (25µg)	21/21	(100%)	0	0	0	0
Doxycycline-DO (30µg)	17/21	(80.95%)	0	0	4/21	(19.04%)
Gentamicin- GE (10µg)	12/21	(57.14%)	3/21	(14.28%)	6/21	(28.57%)
Nalidixic acid-NA (30µg)	15/21	(71.42%)	0	0	6/21	(28.57%)
Polymyxin B- PB (300 U)	21/21	(100%)	0	0	0	0
Streptomycin-S (10µg)	18/21	(85.71%)	1/21	(4.76%)	2/21	(9.52%)
Sulfamethoxazole- SM (300U)	15/21	(71.42%)	0	0	6/21	(28.57%)
Tetracycline-T (30µg)	12/21	(57.14%)	1/21	(4.76%)	8/21	(38.09%)

Table 3: Antibiotic resistance patterns of different *Salmonella* serovars

Antibiotic	Typhimurium	Group II	Enteritidis	Group G	Daytona	Linderburg	Total
	Resistant strains/no.of strains examined						
AK	5/7	3/7	2/2	1/3	1/1	1/1	13/21
A	4/7	5/7	1/2	1/3	0/1	0/1	11/21
CTX	3/7	2/7	1/2	0/3	0/1	0/1	6/21
CTR	3/7	3/7	0/2	1/3	0/1	0/1	7/21
C	1/7	2/7	1/2	0/3	0/1	0/1	4/21
CIP	2/7	2/7	0/2	1/3	0/1	0/1	5/21
COT	0/7	1/7	0/2	0/3	0/1	0/1	1/21
DO	2/7	1/7	0/2	0/3	0/1	1/1	4/21
GE	2/7	2/7	1/2	0/3	0/1	1/1	6/21
NA	2/7	1/7	1/2	1/3	1/1	0/1	6/21
PB	0/7	0/7	0/2	0/3	0/1	0/1	0/21
S	2/7	1/7	0/2	1/3	0/1	1/1	5/21
SM	2/7	1/7	0/2	1/3	0/1	1/1	5/21
T	4/7	2/7	1/2	0/3	1/1	0/1	8/21

Murugukar *et al.* (2005) [21] in their study reported that 44.21% (95/654) of the *Salmonella* isolates were resistant to the tetracycline, 17.9% to nalidixic acid and 15.79% to chloramphenicol which were almost in accordance with the present findings of resistance towards tetracycline (38.09%), nalidixic acid (28.57%) and chloramphenicol (19.09%).

The findings of the present study revealed that 28.57% & 33.33% of *Salmonella* isolates were resistant to cefotaxime and ceftriaxone, while 100% sensitivity to cefotaxime was reported by both Mir *et al.* (2009) [19] and Smith *et al.* (2010) [27]. In contrast, 100% sensitivity to ceftriaxone was reported by Akter *et al.* (2012) [1], Hasan *et al*. (2011) [16] and Begum *et al*. (2010) [4]. However, a lower level of 6% sensitivity to ceftriaxone was recorded by Chen *et al.* (2004) [7]. The study revealed 100% sensitivity of *Salmonella* isolates towards polymyxin B and co-trimoxazole while, Chandane *et al.* (2017) [6] reported 93.1% sensitivity to polymyxin B. As that of present study, higher sensitivity reports of 95.8%, 97% & 75% to co-trimoxazole were given by of Sharvani *et al.* (2016) [26], Ziech *et al*. (2016) [36] and Samanta *et al.* (2014) [25], respectively.

Out of 7 *S. Typhimurium* isolates, highest resistance was found against amikacin (71.42%) followed by ampicillin (57.14%), tetracycline (57.14%), ceftriaxone (42.85%), cefotaxime (42.85%), sulphamethoxyzole (28.57%), ciprofloxacin (28.57%), doxycycline hydrochloride (28.57%),

gentamycin (28.57%), nalidixic acid (28.57%), streptomycin (28.57%) and chloramphenicol (14.28%) and among all 7 *S. Typhimurium* isolates, 5 isolates showed MDR. The resistance pattern of *S. Typhimurium* observed towards different antibiotics like ampicillin (57.14%), ceftriaxone (42.85%), and ciprofloxacin (28.57%), were in agreement with the findings of Emmanuel *et al*. (2015) [14] who reported 43.5%, 39.5% and 29.5% resistance in *S.*Typhimurium isolates, respectively.

The two *S. Enteritidis* isolates have shown 100% resistance towards amikacin (2/2, 100%) and one isolate showed resistance towards ampicillin, cefotaxime, chloramphenicol, gentamicin, nalidixacid and tetracycline.

Strains were considered multidrug resistant if they were resistant to atleast three classes of antimicrobials (atleast one antimicrobial of each class) (Ziech *et al*., 2016). In present study, out of 21 *Salmonella* isolates, 12 (57.14%) were showing multiple drug resistance, MDR was found among 5 (41.66%, 5/12) *S. Typhimurium* isolates, 3 (25%,3/12) *Salmonella* group II, 2 (16.66%, 2/22) *Salmonella* group G, one *S. Enteritidis* (8.3%, 1/12) and one *S.* Linderburg (8.3%, 1/12) isolate. Enabulele *et al.* (2010) and Perez-montano *et al.* (2012) reported 62% and 34% of MDR *Salmonella*, respectively. However higher MDR of 86% among *Salmonella* was reported by Zeich *et al.* (2016).

In the present study, as a part of first step recommended by CLSI, *Salmonella* isolates were screened for resistance to indicator cephalosporins (cefotaxime, ceftriaxone, ceftazidime and aztreonam), which revealed resistance to aztreonam in 5 (23.80%) isolates, cefotaxime in 6 (28.57%), ceftriaxone in 7 (33.33%) and ceftazidime in 5 (23.80%) isolates. Since ESBLs vary in their hydrolysis of these cephalosporins as substrates, resistance to atleast one of them should be considered as positive (CLSI, 2018).

Of the 15 isolates that were positive in screening test, 9 isolates were phenotypically confirmed as ESBL producers by Combination Disc Method (CDM)-with an increase in inhibition zone diameter by a minimum of 5 mm), which includes *S. Typhimurium* (4), *S. Enteritidis* (1) and *Salmonella* group II (4) isolates. The overall prevalence of β-lactamase genes in *Salmonella* isolates was found to be 52.38% (11/21). Prevalence rates of β-lactamase genes among *S. Typhimurium* was 85.71% (6/7) and among 6 isolates 4 displayed *bla*TEM (57.14%, 4/7) and one isolate of *S. Enteritidis* (1/2, 50%) displayed presence of *bla*TEM gene. Among all *Salmonella* isolates, the most prevalent β- lactamase gene was *bla*TEM (9/11, 81.18%), followed by *bla*CTX-M group-2 (2/11, 18.18%), *bla*OXA (1/11, 9.09%) and *bla*CTX-M group-9 (1/11, 9.09%). *bla*TEM was found in 57.14% (4/7) of *S. Typhimurium* which was in accordance with the findings of Akiyemi *et al.* (2017) who reported 79% among *S. Typhimurium* isolates. One isolate of *S. Typhimurium* was carrying both *bla*CTX-M group-2 and *bla*CTX-M group-9 genes. Yu *et al.* (2011) also reported presence of *bla*CTX-M group-9 in *S. Typhimurium* isolates**.** One isolate of *S. Typhimurium* showed presence of both TEM and OXA genes.

Conclusion

The present study indicated that the results should be taken as precautionary warning for the spread of multidrug resistance. The difference in resistance patterns to antimicrobials in different places may be due to the usage of different antibiotics for therapy and mainly emerging of MDR *Salmonella* isolates are due to the sub therapeutic dose or excessive use of commonly used antimicrobials for the treatment of livestock, poultry or in other sources. Contact with animal faeces, feeds, water sources, agriculture etc. may be cause of the transmission of resistant genes from various vectors to food producing animals. So, infections of *Salmonella* were no longer be treated by conventional therapeutic agents. Also, the emergence and spread of antibiotic resistant strains particularly the detection of the ESBL producing *Salmonella* species, is fast becoming an emerging world threat. ESBLs are frequently found in *K. pneumoniae* but recent studies have increasingly reported its occurrence in the *Salmonella* species and other Gram-negative organisms. Such *Salmonella* strains pose significant health problems worldwide by virtue of their acquisition of resistance against most beta-lactam antibiotics, which were previously used for treatment of *Salmonella* infections.

Acknowledgement

The present study is the M.V.Sc. thesis work of Dr. Y.Suresh and the authors thank Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, for providing necessary facilities and funds to carry out the work.

Reference

1. Akter L, Hassan M, Ahmed Z. Present status and antibiotic sensitivity pattern of *Salmonella* Typhi and *S.* Paratyphi In different age group hospitalized patients in Dhaka city, Bangladesh. IOSR J. Pharmacy &Biological Sci. 2012; 4(3):27-30
2. Baay MF, Huisin'tveld JH. Alternative antigens reduce cross-reactions in an ELISA for the detection of *Salmonella* Enteritidis in poultry. J Appl. Bacteriol. 1993; 74:243-247.
3. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am. J Clin. Pathol. 1966; 45(4):493-496.
4. Begum K, Reza TA, Haque M, Hossain A, Hassan FMK, Hasan SN *et al*. Isolation, identification and antibiotic resistance pattern of *Salmonella* spp. from chicken eggs, intestines and environment samples. Bang. Pharm. J. 2010; 13(1):23-27.
5. Bridges RF, Scott WM. Bact. Poonae: New type of Salmonella causing acute enteritis. J Royal Army Med. Corps. 1935; 65:221-223.
6. Chandane P, Gandhi A, Bowalekar S. Study of antibiotic susceptibility pattern of *Salmonella* Typhi in children sufferingfrom enteric fever. Ann Trop Med Public Health. 2017; 10:440-443.
7. Chen TH, Wang YC, Yang CH, Yeh KS. Serotype occurrence and antimicrobial susceptibility of *Salmonella* isolates recovered from pork carcasses in Taiwan (2000 through 2003). J. Food Prot 2004; 69(3):674-678.
8. Clayton DJ, Bowen AJ, Hulme AW, Buckley AM, Deacon VL.. Analysis of the role of 13 major fimbrial subunits in colonization of the chicken intestines by *Salmonella* enteric serovar Enteritidis reveals a role for a novel locus. BMC Microbiol. 2008; 8:228-228.
9. CLSI. Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Testing: Twenty-eight Informational Supplement. M100-S24. Wayne, PA, USA, 2018.
10. Collighan RJ, Woodward MJ. The *SEF14*fimbrial antigen of *Salmonella* entericaserovar Enteritidis is encoded within a pathogenicity islet. Vet. Microbiol. 2001;

80:235-245.
11. Dallenne C, Costa AD, Dominique D, Christine F, Guillaume A. Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae. J. Antimicrob. Chemother. 2010; 65:490-495.
12. Davies J, Davies D. Origins and evaluation of antibiotic resiatance. Microbial Mol Biol Rev. 2010; 74(3):417-33.
13. Drieux L, Brossier F, Sougakoff W, Jarlier V. Phenotypic detection of extended spectrum beta-lactamase production in *Enterobacteriaceae:* review and bench guide. ClinMicrobiol Infect. 2008; 14:90-103.
14. Emmanuel K, Hollander A, Korin B, Cymerman I, Yaron S. Adaptation of *Salmonella enterica* SerovarSenftenberg to Linalool and Its Association with Antibiotic Resistance and Environmental Persistence. Appl Environ Microbiol. 2015; 83:e03398-16.
15. Enabulele SA, Amune PO, Aborisade WT. Antibiograms of *Salmonella* isolates from poultry farms in Ovia North East local government area, Edo State, Nigeria.Agri Bio. J Nort. Ame. 2010; 1(6):1287-1290.
16. Hasan B, Gul S, Laila N, Ahmed A, Saleh A. Antimicrobial sensitivity pattern of *S.* Typhi isolated from blood culture in a referral hospital. Bangladesh J. Med. Microbio. 2011; 05(01):16-20.
17. Kent PT, Thomason BM, Morris GK. *Salmonellae* in foods and feeds. Atlanta: USA: Department of Health services. 1981, 29-30.
18. 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.
19. Mir IA, Wani SA, Hussain I, Qureshi SD, Bhat MA, Nishikawa Y. Molecular epidemiology and *in vitro* antimicrobial susceptibility of Salmonella isolated from poultry in Kashmir. Rev. sci. tech. Off. int. Epiz. 2009; 29(3):677-686.
20. Miranda JM, Mondragon AC, Martinez B, Guarddon M, Rodriguez JA. Prevalence and antimicrobial resistance patterns of *Salmonella* from different raw foods in Mexico. J Food Prot. 2009; 72(5):966-971.
21. Murugkar HV, Rahman H, Kumar A, Bhattacharya D. Isolation, phage typing &antibiogram of *Salmonella* from man & animals in northeastern India. Indian J Med Res. 2005; 122:237-242.
22. Parry CM. Antimicrobial drug resistance in *Salmonella enterica.* Curr Opin Infec Dis. 2003; 16:467-72.
23. Perez-montan JA, Gonzalez-aguilar OD, Barba J, Pacheco-gallardo C, Campos-bravo CA, Garcia S, Heredia NL, Cabrera-diaz E. Frequency and Antimicrobial Resistance of Salmonella Serotypes on Beef Carcasses at Small Abattoirs in Jalisco State, Mexico. Journal of Food Protection. 2012; 75(5):867-873.
24. 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.
25. Samanta I, Jordar SN, Das PK, Sar TK, Bandyopadhyay S, Dutta TK *et al*. Prevalence and antibiotic resistance profiles of *Salmonella* serotypes isolated from backyard poultry flocks in West Bengal, India. Journal of Applied Poultry Research. 2014; 23:536-545.
26. Sharvani V, Hemavathi, Dayanandh DK, Shenoy P, Samah P. Antibiogram of *Salmonella* isolates : Time to consider Antibiotic Salvage. J clin Diagn. Res. 2016; 10(5):06-08.
27. Smith KP, George J, Cadle KM, Kumar S, Aragon SJ, Hernandez RL *et al*. Elucidation of antimicrobial susceptibility profiles and genotyping of *Salmonella enterica* isolates from clinical cases of salmonellosis in New Mexico in 2008. World J Microbio.Biotechno. 2010; 26(6):1025-1031.
28. Tan W, Shelef LA. Automated detection of *Salmonella* spp. in foods. J Microbiol. Methods. 1999; 37:87-91.
29. Tiwari R, Chakraborty S, Dhama K, Rajagunalan S, Singh SV. Antibiotic resistance an emerging health problem: Causes, worries, challenges and solutions a review. Int. J Curr. Res. 2013; 5:1880-1892.
30. Uma B, Prabhakar K, Rajendran S, Sarayu YL. prevalance of extended sprectrum beta lactamases in *Salmonella* species isolated from patients with acute gastroenteritis. Indian J Gastroenterol. 2010; 29:201-204.
31. Van den Bogaard AE, Stobberingh EE. Antibiotic usage in animals: impact on bacterial resistance and public health. Drugs. 1999; 58:589-607.
32. Wall PG, Morgan D, Lambden K, Ryan M, Giffin M, Threlfall EJ *et al*. A case control study of infection with an epidemic strain of multi-resistant *Salmonella* Typhimurium DT104 in England and Wales. Commun Dis. Rev. 1994; 4:130.
33. Wasyl D, Hoszowski A. Antimicrobial Resistance Of*Salmonella*Isolated from Animals and Feed in Poland. Bull Vet Inst Pulawy. 2004; 48:233-240.
34. Wuthe HH. Occurrence of *Salmonella* Dubrovnik and *Salmonella* Epicrates in humans Arch Hyg Bakteriol. 1969; 153:176-178.
35. Yu F, Chen Q, Yu X, Li Q, Ding B, Yang L *et al*. High prevalence of Extended –Sprectrum, Beta Lactamases among *Salmonella enterica* Typhimurium isolates, 2011.
36. Zeich RE, Lampugnani C, Perin AP, Sereno MJS, Faciotte RAP, Soares VM *et al*. Multidrug resistance and ESBL-producing *Salmonella* spp. isolated from broiler processing plants. brazilian. Journal of microbiology. 2016; 47:191-195.