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Prevalence of *bla*$_{SHV}$ gene in Cephalosporin Resistant *Salmonella* Isolates from Meat Samples in South India

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Abstract- Antibiotic resistant *salmonella* infections posing a serious threat global health. *bla*$_{SHV}$ genes belongs a major group of extended spectrum beta lactamases (ESBL). In the current study, Cephalosporin Resistant in *Salmonella* spp conformed by detecting *bla*$_{SHV}$ genes using PCR amplification. 28 cephalosporin resistant *Salmonella sp* (*S. typhimurium*, n= 22 and *S. enteridis* n=06) isolated from meat samples collected from various vendors in Hyderabad city, South India were subjected for phenotypic detection of ESBL production and *bla*$_{SHV}$ gene responsible for ESBL production was detected by PCR amplification. Among the 28 cephalosporin resistant *Salmonella sp (CeRSsp),* 4 isolates (14.2%) were positive for ceftazidime mediated ESBL production. Screening of these isolates indicated the presence of about 6 kbp plasmid. PCR amplification indicated the presence of *bla*$_{SHV}$ gene with an amplicon size of 755 bp. The presence *bla*$_{SHV}$ gene contributes for ESBL production which makes organisms to show reduced susceptibility against third generation cephalosporins.

***Keywords*:** *Cephalosporin resistant Salmonella sp, ESBL, bla*$_{SHV,}$ *food-borne pathogens,*

I. INTRODUCTION

Typhoid fever caused by *Salmonella* infections are of high prevalence across the globe, with particular prevalence in developing countries and where they possess a high mortality rate, economic impacts and their antibiotic resistance. The frequent use of antimicrobial agents in farm animals is a major factor for the emergence of antibiotic-resistant bacteria [1,2]. Some of the reports suggest that multidrug resistant organisms contaminate animal products and subsequently lead to serious human infections [3,4], among them antibiotic-resistant *Salmonella* serovars are the most commonly encountered instances [5,6]. These *Salmonella* sp develop antibiotic resistance when farm animals are frequently exposed high doses of antibiotics [7].

In spite of the improved technology and hygienic practices in the developed countries at all stages of poultry and beef meat production, food-borne infections remain as a continuous threat to human and animal health. *Escherichia coli* and *S. enterica* serovars are the dominant members of *Enterobacteriaceae* causing foodborne infections. The expansion of antibiotic resistance in bacteria is also an emerging public health hazard due to the compromised efficacy in the treatment of infectious diseases [8].

Salmonella enterica subsp has been recovered from environmental samples, meat samples, insects, and nearly all vertebrate species [9, 10]. Extended-spectrum cephalosporin's such as ceftazidime, ceftriaxone and ceftiofur are important therapeutic agents and are often used for invasive *Salmonella* infections [11, 12]. Thereafter, the emergence of *Salmonella* isolates which are resistant to extended-spectrum cephalosporins has been reported [13].

Even though SHV enzymes continue to remain a major extended spectrum β-lactamases (ESBLs) in bacteria from humans (14), their occurrence in bacteria from food animals are rarely recorded (15). Primarily, this broad-spectrum cephalosporin resistance has been majorly dominated by cephamycinase resistance determinants (16, 17). Nevertheless, according to recent reports *Salmonella* enterica isolates carrying *bla*SHV genes.

The frequent exposure of bacterial strains to β-lactams has steered to rapid mutation of β-lactamases [18]. In addition, ESBLs are predominantly, derivatives of point mutations in the *bla*$_{TEM-1}$ and *bla*$_{SHV-1}$ *β*-lactamase genes, which ultimately cannot hydrolyse cephamycins and are subsequently inhibited by clavulanic acid [19], while ampicillin class C *β*-lactamase (*Amp*C) enzymes are active on cephamycins as well as oxyimino cephalosporin's and monobactams [20]. ESBLs are predominantly located on mobile genetic elements (plasmids or integrons), facilitates their mobility from one bacterial species to others by horizontal gene transfer [21]. Reports in Egypt, some of *Salmonella* isolates

from chicken meat and other organs exhibited high level of resistance to different class of antibiotic [22–24].

Therefore, the principal objective of this study was to investigate the detection of *bla*$_{SHV}$ and associated plasmids from these *S. enterica isolates*, isolated from chicken and goat meat obtained from the retail shop of Hyderabad city, India

II. METHODOLOGY

Bacterial isolates

Salmonella sp (*S. typhimurium* and *S. enteridis*) were isolated from meat samples collected from various vendors in Hyderabad city and were identified by the conventional cultural and serological tests. A total of 28 isolates (*S. typhimurium, n= 22 and S. enteridis n=06)* were phenotypically confirmed as resistant to cephalosporins by antimicrobial susceptibility test (CLSI) and selected for the study.

Phenotypic test for ESBL detection

Phenotypic ESBL production was confirmed by double disk synergy test (DDST) and combined disk diffusion test according to the Clinical and Laboratory Standards Institute (CLSI) criteria for ESBL screening [25]. Synergy test was was carried out as per the method originally described by Jarlier *et al* [26] using a disc of amoxicillin-clavulanate (20 µg/10 µg) (augmentin) at the centre and a 30-µg disc of each third-generation cephalosporin placed at a distance of 20 mm from center to center on a Mueller-Hinton Agar (MHA) plate swabbed with the test isolate. Clear extension of the edge of the inhibition zone of cephalosporin toward the augmentin disc was interpreted as positive for ESBL production. Combined disc diffusion test was conducted using a disc of ceftazidime (30 µg) alone and ceftazidime + clavulanic acid (30 µg/10 µg)placed at a distance of 25 mm, center to center, on a MHA plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards and incubated overnight at 37°C. An increase in the inhibition zone diameter of 5 mm or more for a combination disc versus ceftazidime disc alone confirms ESBL production.

Plasmid DNA extraction.

Plasmid DNA was extracted from bacterial isolates by alkaline lysis method. The DNA bands were observed after agarose gel electrophoresis under UV- transilluminator. The extracted DNA was stored at -20^{0}C in TE buffer [27].

PCR amplification of *bla*$_{SHV}$ gene

All reactions were performed with a Master Cycler Gradient 5331 PCR device (Eppendorf). The amplification was carried out with *bla*$_{SHV}$ specific PCR primers 5′TGCTTTGTTCGGGCCAA3′. The PCR master mix (50 µl) which includes 4 µl DNA (100 ng), 2 µl DNTPs (10 mM), 10X taq buffer with $MgCl_2$, 2 µl each of forward and reverse primer (0.4 mM), and 3U taq polymerase. The thermocycling parameters were as follows: an initial denaturation step of 94°C for 10 mins, 30 cycles of denaturation at 94°C for 1 min, primer annealing at 60°C for 1 min, and extension at 72°C for 1 min and final extension at 72 °C for 7 mins. The PCR amplicons were separated on 2 % agarose gel electrophoresis along with 1 kb DNA ladder [28]

III. RESULTS AND DISCUSSION

Selection of Bacterial Strains

Among the 75 Salmonella sp. Isolated from meat samples from Hyderabad, 28 (*S. typhimurium, n= 22 and S. enteridis n=06)* were found to be resistant to cephalosporin and were screened for ESBL production phenotypically and *bla*SHV was detected by PCR amplification**.** Among selected cephalosporin resistant *Salmonella sp* (CeRSsp), 14.2% (n=04) isolates were positive for ceftazidime mediated ESBL production as observed by enhancement of zone in the direction of Clavulinic acid from ceftazidime in the double disc synergy test (Figure 1 and Figure 2). The results are comparatively similar to the previous studies conducted by Drieux *et al.,* 2008 [26]; and also by Ziech *et al.,* 2016 [29].

Extraction of Plasmid DNA and PCR amplification of *bla*TEM gene

All the four ESBL positive CeRSsp isolates showed the presence of Plasmid DNA, of the size of about 6kb (Figure 3), PCR amplification of *bla*SHV gene codes for ceftazidime mediated ESBL production yielded amplified *blaSHV* gene product with amplicon size of 755 bp (Figure 4). The previous studies conducted by Ehlers *et al.,* 2009 [28] showed comparatively similar results.

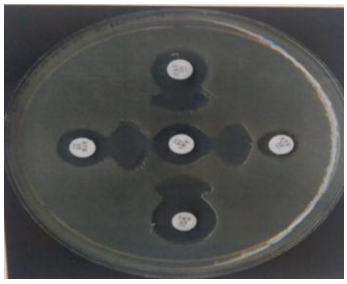

Figure 1. *Disc diffusion synergy test*

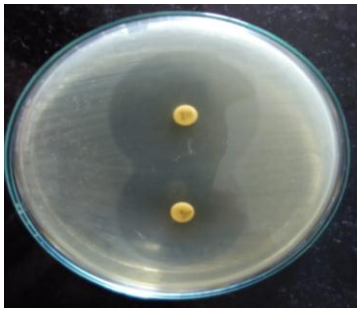

Figure 2. *Combined disc diffusion test*

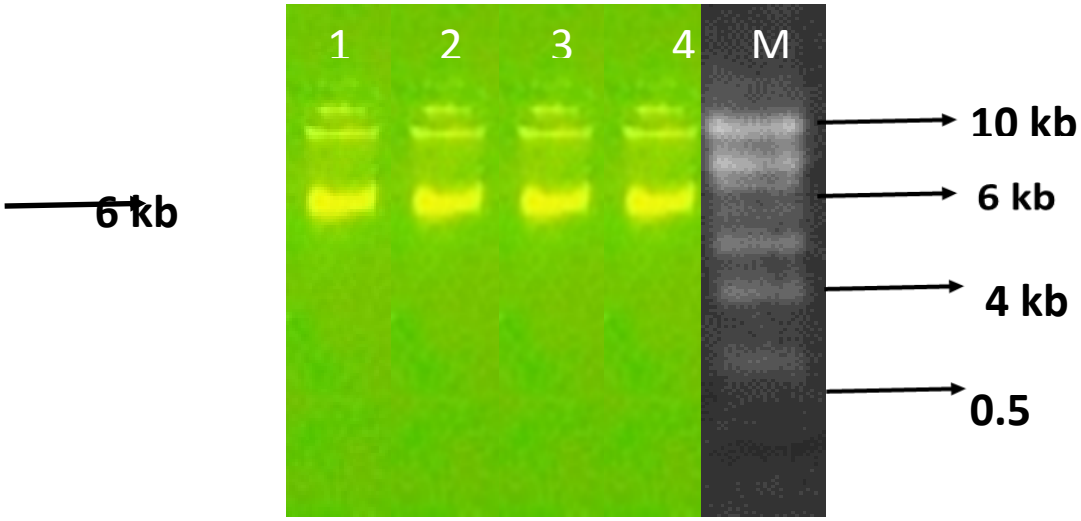

Figure 3. *Plasmid isolation of Salmonella isolates.*
Lane 1 –M (10 kb ladder) ranging from 0.5 kb – 10 kb
Lane 2- PGS 14, Lane 2- PGS 21, Lane 3- PGS 28, Lane 4 – PGS 42

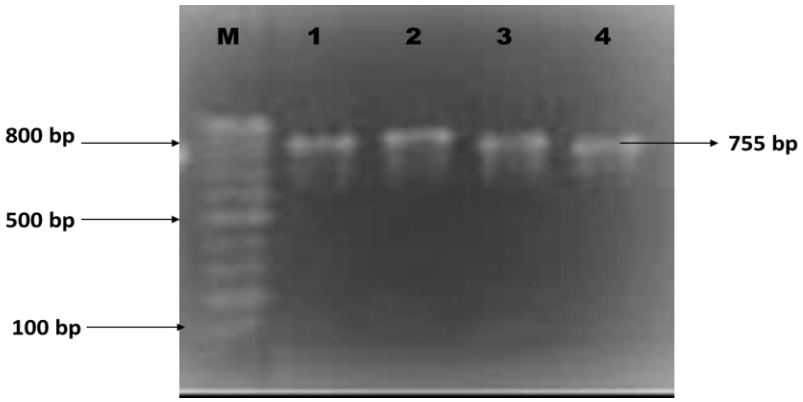

Figure 4. *bla_{SHV} gene with amplicon size of 755 bp*
Lane 1 –M (100 bp ladder) ranging from 100bp – 1kb
Lane 2- PGS 14, Lane 2- PGS 21, Lane 3- PGS 28, Lane 4 – PGS 42

Resistance to Fluoroquinolones and the third generation Cephalosporins has been reported to be the main cause of treatment failure [30, 31]. Plasmids play a major role in acquisition and dissemination of resistance genes. The evolution of MDR pathogens has been attributed to the evolution of a plasmid through the acquisition of integrons and their integration with the chromosomes Salmonella has been widely reported [32,33]. Small plasmids have been reported in about 10% of the *Salmonella* carrying genes for a wide variety of biological functions, including antibiotic resistance [34]. Small ColE1-like plasmids have been shown to carry resistance genes against kanamycin (*aph*) quinolones (*qnr*) and extended spectrum *β*-lactams (bla_{CMY}) in *Salmonella* serovars and Zioga *et al.*, 2009 have

sequenced plasmid carrying beta lactamase (*bla*) genes pA172 in serovar Newport (EU331425) [35]. Detection of the bla_{SHV} gene on plasmids isolated from the four cephalosporin resistant and ESBL positive Salmonella is in conformity with the earlier reports.

IV. CONCLUSION

Salmonella spp is one of emerging drug resistance food borne pathogen. salmonella from meat samples isolated were showing drug resistance to currently available common drugs in market. It is a serious threat. This kind of studies reveals the importance of surveillance studies in the sector of public health and control preventive methods to improve hygiene.

CONFLICT OF INTEREST

The authors declare of no conflict of interest in conducting this study.

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