

Prevalence of extended spectrum beta-lactamase-producing *Escherichia coli* in chicken meat

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ABSTRACT

Extended spectrum β -lactamase (ESBL) producing *Escherichia coli* (*E. coli*) have emerged as a global threat to both human and veterinary medicine and the prevalence has increased throughout the world. The objective of the present study was to determine the prevalence of ESBL-producing *E. coli* in chicken meat collected from retail shops in Chennai, India. Around thirty one *E. coli* strains were isolated and screened for ESBL production by double disc diffusion method. Further, ESBL-positive isolates were analyzed for the presence of CTX-M, TEM, and SHV genes. Sixteen *E. coli* isolates (51.6%) were found to be ESBL positive in the initial screening. All the 16 isolates were found to harbour one or more ESBL genes and 10 isolates (62.5 %) were found to harbour all the three genes. The results of this study clearly indicated the emergence of ESBL-producing *E. coli* in chicken and the need to curb the nontherapeutic usage of antimicrobials in poultry production.

Key Words: *Escherichia coli* – Poultry – ESBL – Beta-lactamase - Resistance

INTRODUCTION

Antibiotics of the β -lactam group including penicillins, cephalosporins, monobactams and carbapenems are used commonly in livestock and poultry owing to their high efficacy and broad spectrum of activity. Due to continuous use of these antibiotics in veterinary practice, resistance has been reported (Kong *et al.*, 2010). The most common mechanism by which the microorganisms such as *Escherichia coli* acquire resistance to these β -lactam antibiotics is by the production of β -lactamase.

Development of oxyimino cephalosporins (third generation cephalosporins) in early 1980s was a major breakthrough to combat β -lactamase-mediated bacterial resistance (Paterson and Bonomo, 2005). However, resistance to these cephalosporins emerged soon in *Klebsiella pneumoniae*, *K. ozaenae* and *Serratia marcescens* and it was first reported in 1983 (Rawat and Nair, 2010). The resistance is due to the production of enzymes by microorganisms which are evolved as mutations outside the active site of β -lactamase and they are referred to as extended-spectrum β -lactamase (ESBL) because of their increased spectrum of activity, especially against oxyimino

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cephalosporins. ESBLs can hydrolyze and mediate resistance to all β -lactam antibiotics including penicillins, cephalosporins and monobactams (e.g., aztreonam) but do not affect cephamycins or carbapenems and they are inhibited by β -lactamase inhibitors like clavulanic acid (Rawat and Nair, 2010). Production of ESBL is one of the most common methods of acquiring resistance to third and fourth generation cephalosporins among Enterobacteriaceae such as *E. coli* and *Klebsiella*. Chicken and other animal produce are frequently incriminated in the spread of resistance to human pathogens. However, there is paucity of information regarding the prevalence of ESBL-producing *E. coli* in chicken meat in India. Hence, the present work was undertaken with the aim to study the prevalence of ESBL-producing *E. coli* in chicken meat collected from retail outlets of Chennai.

MATERIALS AND METHODS

Sample Collection

A total of 105 chicken meat samples were collected from the retail outlets in Chennai, Tamilnadu, India from May 2016 to July 2016. About 5 grams of single meat samples were collected from each bird following Food Safety and Standards Authority of India guidelines. Fresh meat samples were collected immediately after slaughter in sterile plastic envelopes. They were stored at 4° C until processed.

Isolation and Identification of *E. coli*

One gram of minced meat samples was inoculated into 9 ml of sterile nutrient broth and incubated overnight at 37° C. The nutrient broth samples which showed

growth as indicated by the turbidity of the growth medium were then inoculated into MacConkey agar plates and Eosin Methylene Blue agar plates for selective isolation of *E. coli*. The isolated strains were confirmed by the following biochemical tests: Oxidase, TSI, Urease, Motility, Catalase, Indole, Simmons Citrate, Methyl Red and Voges Proskauer (Thanigaivel and Anandhan, 2015).

Phenotypic Screening for ESBL

Isolated organisms were screened for extended-spectrum β -lactamase production by double disc diffusion method as per CLSI guidelines (CLSI, 2016). For this purpose, following four antibiotic disc were used: cefotaxime (30 μ g), ceftazidime (30 μ g), cefotaxime-clavulanic acid (30/10 μ g) and ceftazidime-clavulanic acid (30 μ g/10 μ g). Bacterial inoculums were prepared from single colonies and the turbidity of the inoculum was adjusted to the 0.5 McFarland standard. Using a sterile cotton swab, the organisms were inoculated into Mueller Hinton agar plates by lawn culture. Using a sterile forceps, antibiotic discs were placed on Mueller Hinton agar plates inoculated with the *E. coli* isolates organism. Plates were incubated at 37°C for 18 h. After incubation, the zone of inhibition around disc was measured. The zone of cephalosporin and the respective cephalosporin/clavulanic acid combination were compared and a difference of ≥ 5 mm was considered as positive for phenotypic confirmatory test for ESBL production.

Detection of ESBL genes by PCR

The *E. coli* isolates were inoculated into 5 ml nutrient broth and incubated

at 37°C overnight. After overnight incubation, bacterial cells were harvested by centrifugation at 5000 x g for 10 min. The supernatant was discarded and cell pellets were used for DNA extraction. DNA was extracted and purified using Qiagen DNA extraction kit as recommended by the manufacturer. The primers used for bla TEM were 5' AAAATTCTTGAAGACG 3' and 3' TTACCAATGCTTAATCA 5'; bla SHV were 5' GGGTTATTCTTATTTGTCGCT 3' and 3' TAGCGTTGCCAGTGCTCG 5' and for bla CTX-M were 5' TTTGCGATGTGCAGTACCAGTAA3' and 3' CGATATCGTTGGTGGTGCCATA 5'. The amplicon size of bla TEM, bla SHV and bla CTX-M were 1080 bp, 929 bp and 544 bp, respectively (Bora *et al.*, 2014).

Amplification of DNA was performed in a final volume of 25 µl PCR mixtures containing 12.5 µl Master mix, 1 µl forward primer, 1 µl reverse primer, 3 µl template DNA, 7.5 µl nuclease-free water with cycling parameters comprising initial denaturation at 94°C for 3 min followed by 35 cycles each of denaturation at 94°C for 30s, annealing at 54°C for 30s, amplification at 72°C for 30 s and final extension at 72°C for 10 min, for the amplification of bla TEM. For bla SHV and bla CTX-M, amplifications conditions for thermal cycling remained the same except for the annealing temperature of 53°C.

Ten µl of the amplified PCR product was electrophoresed on a 1.5% (CTX) and 0.8 % (TEM and SHV) agarose gel in 1X Tris-acetic acid-EDTA buffer (TAE) containing ethidium bromide (0.5 µg/ml) and stained DNA bands are visualized using gel documentation unit. The PCR

amplification for bla CTX, bla SHV and bla TEM genes were shown in Fig. 2 and the product sizes were of 544, 929, 1080 bp, respectively.

RESULTS AND DISCUSSION

Thirty one isolates of *E. coli* were recovered from the chicken meat samples and were screened for ESBL production by double disc diffusion method . Of the total *E. coli* isolates, 16 *E. coli* isolates (51.6%) were ESBL producers and the overall prevalence of ESBL-producing *E. coli* in chicken meat was 15.2% and it was comparatively less than the other reports. Hussain *et al* (2017) reported a prevalence rate of 46% in chicken meat collected from retail chain outlets in Karnataka, Telangana, Andhra Pradesh and Maharashtra States of India in 2015.

In order to determine the presence of ESBL genes, DNA were extracted from all the 16 isolates and analyzed by PCR (Fig 1.). All the isolates showed presence of one or more ESBL genes. Nearly 93.75% (15 isolates) of the isolates harboured CTX-M genes and 68.75% and 87.5% of the isolates were found to harbour other ESBL genes, TEM and SHV, respectively. More than one resistance gene belonging to different ESBL families was found in 87.5% of isolates (14 isolates) and 62.5% (10 isolates) of the isolates were found to harbour all the three ESBL genes. The most prevalent combination of ESBL genes were CTX/SHV (75%) followed by TEM/SHV (68.75%) and CTX/TEM (62.5%).

In our study, the prevalence of ESBL (15.2%) in chicken meat was lower

compared to reports from other countries. Overdeest *et al* (2011) reported that 79.8% of retail chicken meat samples in Netherlands contained ESBL genes. Egea *et al* (2012) reported high prevalence of ESBL genes (93.3%) in chicken meat in Spain. In France, higher prevalence of 91.7% ESBL-positive isolates (44 samples positive out of 48 samples) in chicken meat samples were reported in 2016 (Casella *et al.*, 2017).

There were only few reports on the prevalence of ESBL-producing *E. coli* in chicken meat in India. Kar *et al* (2015) conducted study on prevalence of ESBL-positive *E.coli* from food producing animals from India. They have isolated 252 *E. coli* isolates from poultry faecal samples and in which 16 isolates were reported as ESBL-producing *E. coli* (6.3%). In a study conducted in Hyderabad, 6.7% of *E. coli* isolates from raw chicken were ESBL producers (Rasheed *et al.*, 2014). Brower *et al* (2017) conducted a study on occurrence of ESBL-producing *E. coli* isolates from cloacal samples collected from poultry farms in Punjab, India. They have reported a higher prevalence of ESBL-producing *E. coli* (87%) in broiler farms.

The findings of our study clearly indicated the emergence of ESBL-producing *E. coli* in chicken. Though the incidence of ESBL-producing *E. coli* was comparatively lesser, it is still a matter of public health concern. The ESBL-producing strains are resistant to all penicillins, cephalosporins and aztreonam and can exhibit cross-resistance to trimethoprim / sulfamethoxazole and quinolones (Lenart-Boron, 2017). These genes are plasmid coded and are easily transferable to

other bacterial strains. They can also be transferred to the human through food chain and might become potential health risk. Emergence of resistance to third generation cephalosporins is considered to be most serious since this group of antibiotics are categorized by WHO as highest priority critically important antibiotics currently used to treat serious infections in human medicine (WHO, 2017).

The most important factor responsible for the emergence of drug resistant microbes is the increased and indiscriminate use of antimicrobial agents. Hence, there is a need to emphasize the rational use of antibiotics and to strictly restrict the non-therapeutic and inadvertent use of “reserve antimicrobials” in food producing animals to preserve the effectiveness of the drug in the treatment of multidrug resistant infections. In addition, regular surveillance studies covering large geographical area have to be done to assess the real status of ESBL-producing *E. coli* in India.

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Fig. 1 Gel pictures of amplified PCR products of SHV gene (929 bps), CTX-M gene (544 bps) and TEM gene (1080 bps)

