Study of antimicrobial resistance due to extended spectrum beta-lactamase-producing *Escherichia coli* in healthy broilers of Jabalpur

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doi: 10.14202/vetworld.2016.1259-1263

How to cite this article: Shrivastav A, Sharma RK, Sahni YP, Shrivastav N, Gautam V, Jain S (2016) Study of antimicrobial resistance due to extended spectrum beta-lactamase-producing *Escherichia coli* in healthy broilers of Jabalpur, *Veterinary World*, 9(11): 1259-1263.

Abstract

**Aim:** To study the prevalence of antimicrobial resistance due to extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in samples collected from the ceca of healthy broilers of poultry sale outlets (PSOs) Jabalpur.

**Materials and Methods:** A total of 400 cecal swab samples were taken randomly from freshly slaughtered poultry of 39 PSOs located at four different zones or areas of Jabalpur and were screened for the presence of ESBL-producing *E. coli* using standard methods. Further they were characterized phenotypically by standard methods.

**Results:** All the 400 samples were screened for *E. coli* producing ESBL enzyme. Among the samples positive for *E. coli* 135 were positive for ESBL producing *E. coli* giving an overall prevalence of 33.5%.

**Conclusion:** This study relates to the prevalence of ESBL-producing *E. coli* in healthy broilers in Jabalpur is indicative of antibiotic resistance prevalent in the healthy birds which are used for human consumption as well. It also signifies resistance prevalent against beta-lactam antibiotics including third and fourth generations of cephalosporins.

**Keywords:** cecal swab, *Escherichia coli*, extended spectrum beta-lactamase, healthy broilers, Jabalpur.

Introduction

Antimicrobial resistance, within a large range of infectious agents, is a rising health risk of broad concern to countries and multiple sectors. It not only menaces the effective prevention and treatment of an ever-increasing range of infections but also results in reduced efficacy of antibacterial drugs. In intensively reared poultry, antibiotics are administered to whole flocks rather than individual animals. In addition to this poultry farmer also use low doses of antibiotics as growth-promoting substances, which result in the high antibiotic selection pressure for resistance with relatively high proportion of resistant bacteria in poultry fecal flora.

Most resistant phenotypes present in animal populations are present in *Escherichia coli*, therefore commensal *E. coli* can be used as indicators of the Gram-negative species. During the passage through the intestine, these bacteria may transfer their resistance genes to host-adapted bacteria or to pathogens.

All animals generally carry such indicator bacteria this is why trends in the occurrence of resistance, can be studied more accurately in indicator bacteria [1].

Beta-lactams (penicillins, cephalosporins, carbapenems, and monobactams) constitute the therapy of choice for some well-established practices and infections in veterinary medicine [2]. The third generation of cephalosporins has been associated with the emergence of beta-lactamases mediated bacterial resistance, which subsequently led to the development of extended spectrum beta-lactamase (ESBL)-producing bacteria.

ESBLs have been defined as plasmid-encoded enzymes found in the Enterobacteriaceae [3], frequently in *E. coli* and *Klebsiella pneumoniae*, that confer resistance to a variety of beta-lactam antibiotics by catalyzing the hydrolysis of the beta-lactam ring of antibiotic specially oxyimino-cephalosporins, which can be inhibited by beta-lactamase inhibitors [4].

ESBL-producing organisms are frequently co- or multi-resistant, exhibiting resistance to other antimicrobial classes such as fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole due to associated resistance mechanisms, which may be either chromosomally- or plasmid-encoded [5,4].

During the last two decades, ESBL-producing Gram-negative bacilli have emerged as a major problem mainly due to the clonal expansion of producer
organisms, the horizontal transfer of ESBL genes on plasmids [6]. Apart from therapy and prophylaxis, antibiotics are consumed to increase growth and feed efficiencies. There was a limited number of drugs sensitivity for these bacteria only and drug of choice is imipenem, followed by amikacin in injectable form. However, most probably in the near future, if this irrational use is not stopped, infection with that Gram-negative bacteria increase the rate of resistant to drugs that are now sensitive, resulting increase morbidity and mortality. Looking into the severity of the problem present study was undertaken for the prevalence and characterization of ESBL-producing E. coli in healthy broilers.

Materials and Methods

Ethical approval
No ethical approval was required as no live animals were used in this study. However, samples were collected as per standard sample collection methods following all aseptic precautions.

Study site
The study was conducted at Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Jabalpur, during January 2015 to January 2016.

Sample collection
A total of 400 cecal swab samples were collected randomly from 38 poultry sale outlets (PSOs) located at the various parts of Jabalpur. Sample collection area was divided into four zones east, west north and south and five areas were selected randomly in each zone area (Table-1). Samples were taken from the freshly slaughtered healthy broilers in an ice pack and taken to the lab. The properly labeled interlocked polythene bags containing the ceca were brought to the laboratory of the Department of Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Jabalpur for further study.

Table-1: List of different sample collection areas of Jabalpur.

| Name of the area | Number of samples |
|------------------|-------------------|
| North Zone 1     | 12                |
| North Zone 2     | 31                |
| North Zone 3     | 12                |
| North Zone 4     | 10                |
| North Zone 5     | 58                |
| South Zone 1     | 8                 |
| South Zone 2     | 13                |
| South Zone 3     | 17                |
| South Zone 4     | 34                |
| South Zone 5     | 5                 |
| East Zone 1      | 11                |
| East Zone 2      | 17                |
| East Zone 3      | 23                |
| East Zone 4      | 17                |
| East Zone 5      | 10                |
| West Zone 1      | 11                |
| West Zone 2      | 5                 |
| West Zone 3      | 95                |
| West Zone 4      | 7                 |
| West Zone 5      | 4                 |
| Total samples    | 400               |

Results and Discussion

This study revealed the presence of ESBL-producing E. coli in the healthy broilers of Jabalpur. Out of the total 400 cecal swab samples screened, 135 samples were found to be positive for ESBL giving an isolation prevalence percent of 33.5% as given in Table-1. Previously, different workers have reported the prevalence of similar ESBL-producing E. coli in healthy boilers.

In the present investigation initial screening in the buffered peptone water and M.H. broth and later in chromogenic medium specific for E. coli enriched with cefotaxime (2 μg/ml), cefpodoxime (2 μg/ml), and aztreonam (4 μg/ml) showed 135 samples positive out of 400 samples and here resistance to cefotaxime and aztreonam in the above-mentioned concentration for further selection of desired organisms. For further confirmation, the phenotypic characterization of ESBL producing E. coli was undertaken using standard methods combined disc diffusion test (DDST), double disc synergy test method (DDST), and Ezy MIC strip method.

Sample processing
Taking all, the standard aseptic measures directly cecal material were collected by incising the intact ceca with the help of sterile B.P.blade, later sterile swab swirled around and immediately transferred into the enrichment medium containing buffered peptone water 25 ml/5 g of sample for increasing the sensitivity and clonal expansion of the ESBL producing E. coli. Further, it was transferred into the M.H. broth supplemented with cefotaximes and cefpodoxime (2 μg/ml) and aztreonam (4 μg/ml) for the selective enrichment. Overnight enriched samples were streaked into the tryptone bile X-glucuronic agar plate supplemented with cefotaxime and aztreonam in the above-mentioned concentration for further selection of desired organisms. For further confirmation, the phenotypic characterization of ESBL producing E. coli was undertaken using standard methods combined disc diffusion test (DDST), double disc synergy test method (DDST), and Ezy MIC strip method.

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Available at www.veterinaryworld.org/Vol.9/November-2016/15.pdf

Veterinary World, EISSN: 2231-0916 1260
Table-2: Percent prevalence of ESBL E. coli from different collection areas of Jabalpur.

| Name of the area | Number of samples | Positive samples | Negative samples | Percent prevalence |
|------------------|-------------------|------------------|------------------|--------------------|
| North Zone 1     | 12                | 7                | 5                | 58.3               |
| North Zone 2     | 31                | 2                | 29               | 6.5                |
| North Zone 3     | 12                | 8                | 4                | 66.7               |
| North Zone 4     | 10                | 9                | 1                | 90.0               |
| North Zone 5     | 58                | 9                | 49               | 15.5               |
| South Zone 1     | 8                 | 8                | 0                | 100.0              |
| South Zone 2     | 13                | 4                | 9                | 30.8               |
| South Zone 3     | 17                | 7                | 10               | 41.2               |
| South Zone 4     | 34                | 0                | 34               | 0.0                |
| South Zone 5     | 5                 | 1                | 4                | 20.0               |
| East Zone 1      | 11                | 7                | 10               | 41.2               |
| East Zone 2      | 17                | 7                | 10               | 41.2               |
| East Zone 3      | 23                | 5                | 18               | 21.7               |
| East Zone 4      | 17                | 0                | 17               | 0.0                |
| East Zone 5      | 10                | 8                | 2                | 80.0               |
| West Zone 1      | 11                | 6                | 5                | 54.5               |
| West Zone 2      | 5                 | 0                | 5                | 0.0                |
| West Zone 3      | 95                | 53               | 42               | 55.8               |
| West Zone 4      | 7                 | 0                | 7                | 0.0                |
| West Zone 5      | 4                 | 0                | 4                | 0.0                |
| Total samples    | 400               | 135              | 265              |                     |

ESBL=Extended spectrum beta-lactamase, E. coli=Escherichia coli

Table-3: Percent prevalence of ESBL E. coli in different PSOs of Jabalpur.

| Zone   | Name of PSO | Number of samples collected | Number of positive samples | Number of negative samples | Percent prevalence |
|--------|-------------|-----------------------------|---------------------------|----------------------------|--------------------|
| North  | PSO 1       | 12                          | 7                         | 5                          | 58.3               |
| South  | PSO 1       | 12                          | 7                         | 5                          | 58.3               |
|        | PSO 2       | 1                           | 1                         | 0                          | 100.0              |
|        | PSO 3       | 9                           | 8                         | 1                          | 88.8               |
|        | PSO 4       | 8                           | 0                         | 8                          | 0.00               |
|        | PSO 5       | 16                          | 2                         | 14                         | 12.5               |
|        | PSO 6       | 7                           | 3                         | 4                          | 42.85              |
|        | PSO 7       | 28                          | 1                         | 27                         | 3.57               |
|        | PSO 8       | 29                          | 8                         | 21                         | 27.58              |
|        | PSO 9       | 7                           | 2                         | 1                          | 28.57              |
|        | PSO 10      | 3                           | 3                         | 0                          | 100.0              |
|        | PSO 11      | 2                           | 0                         | 2                          | 0.00               |
|        | PSO 1       | 17                          | 0                         | 17                         | 0.00               |
|        | PSO 2       | 10                          | 2                         | 2                          | 20.0               |
|        | PSO 3       | 3                           | 0                         | 3                          | 0.00               |
|        | PSO 4       | 5                           | 0                         | 5                          | 0.00               |
|        | PSO 5       | 6                           | 3                         | 3                          | 50.0               |
|        | PSO 6       | 11                          | 2                         | 9                          | 18.1               |
|        | PSO 7       | 34                          | 0                         | 34                         | 0.00               |
| East   | PSO 1       | 17                          | 0                         | 17                         | 0.00               |
|        | PSO 2       | 10                          | 5                         | 5                          | 50.0               |
|        | PSO 3       | 4                           | 0                         | 4                          | 0.00               |
|        | PSO 4       | 4                           | 0                         | 4                          | 100.0              |
|        | PSO 5       | 9                           | 3                         | 6                          | 33.3               |
|        | PSO 6       | 6                           | 5                         | 1                          | 83.3               |
|        | PSO 7       | 3                           | 1                         | 2                          | 33.3               |
|        | PSO 8       | 14                          | 0                         | 14                         | 0.00               |
|        | PSO 9       | 12                          | 2                         | 10                         | 16.6               |
| West   | PSO 1       | 4                           | 0                         | 4                          | 0.00               |
|        | PSO 2       | 7                           | 6                         | 1                          | 85.7               |
|        | PSO 3       | 5                           | 2                         | 3                          | 40.0               |
|        | PSO 4       | 15                          | 2                         | 13                         | 13.3               |
|        | PSO 5       | 10                          | 8                         | 2                          | 80.0               |
|        | PSO 6       | 5                           | 2                         | 3                          | 40.0               |
|        | PSO 7       | 5                           | 0                         | 5                          | 0.00               |
|        | PSO 8       | 39                          | 33                        | 6                          | 84.61              |
|        | PSO 9       | 19                          | 11                        | 8                          | 57.89              |
|        | PSO 10      | 2                           | 1                         | 1                          | 50.0               |
|        | PSO 11      | 7                           | 0                         | 7                          | 0.00               |
|        | PSO 12      | 4                           | 0                         | 4                          | 0.00               |

PSO=Poultry sale outlet, ESBL=Extended spectrum beta-lactamase, E. coli=Escherichia coli
community people, they further concluded that ESBL-producing bacterial species diversity was highest in poultry and humans were the best ESBL carriers.

A wide range of prevalence from 0% to 100% in the present investigation also revealed that occurrence of this varied range of resistant isolates does not correlate only with direct use of antibiotics, but even other species of birds and humans, can carry antibiotic resistance traits, including ESBL-producers and bring resistance in broiler birds, as these ESBL-producers have already spilled over into the environment [8]. A study performed by van den Bogaard et al. [9] indicated that transmission of resistant clones and resistance plasmids of E. coli from poultry to humans commonly occurs. In this study, the prevalence of resistance in fecal E. coli in broilers and turkeys was analyzed, and the highest prevalence of resistance was detected in turkey samples, closely followed by those from broilers.

In a study conducted in 14 different chicken farms in Henan Province in China 51 nonreplicate ESBL-producing E. coli were isolated. 31 of the 51 isolates were positive for an ESBL phenotype and 29 of these isolates carried one or more Bla genes [10]. Another study on Belgian broiler farms, concluded that risk factors associated with the occurrence of ESBL-producing E. coli besides the usage of any particular antimicrobial like cephalosporins, also included generic antimicrobial use [3,11] the cleanliness of the environment, the lack of acidification of drinking water, the application of more than three feed changes during the production cycle, the breed and the litter material that is used [12,13]. The aforesaid findings add another aspect to our study that, in spite of obtaining birds from same sources prevalence varied to great extent because at the level of farm management, ESBL producing bacteria may enter and proliferate in a farm through the stocking of new animals, exposure to contaminated air, through water or feed, insect or rodent vectors, human-to-animal and animal-to-animal transmission. Moreover, chemicals used in animal production - such as antiseptics, disinfectants, and metals - could play a role in the appearance of such resistant isolates [3,14-16].

In the phenotypic characterization by the CDDT method, out of 135 samples, all the samples were found to be positive and none of the samples were negative calculating a percent isolation of 100%. In DDST method out of, 135 samples screened 115 samples shown the positive results. Phenotypic characterization was also done by Ezy MIC strip for the confirmation of ESBL producers. Ceftazidime and ceftazidime + clavulanic acid containing strip was used. Out of the total samples screened for ESBL production only 84 samples depicted positive results by Ezy MIC strip (Table-4). The phenotypic characterization by CDDT method revealed most of the samples were resistant to ceftazidime, cefpodoxime and few were resistant toward ceftazidime these findings correlates with the European Union recommendations as ESBL producers are usually resistant to ceftazidime, and susceptible to cefoxitin [16]. Thus, in reference to the current recommendations [5] ceftazidime was included as marker for present investigations. Resistance to ESBL-producing isolates testing with ceftazidime would improve the ability to identify the organism and also enhance the sensitivity to identify the isolates producing certain beta-lactamases belonging to SHV and TEM families of enzymes which are ceftazidimases and have much lower activity than cefotaximases. As per the recommendation of the European Union use of specific chromogenic medium avoided the identification of the colonies belonging to the other species within Enterobacteriaceae. Enrichment at this level with cephalosporins as stated in the scientific report of EFSA, influence the bacterial conjugation, exchange of resistance plasmids and increase the sensitivity of the method [5]. This correlates with our findings as initial screening gave 135 samples positive for ESBL E. coli, and all the samples showed positive results in the phenotypic characterization by CDDT method.

**Table-4:** Comparative sensitivity of methods of phenotypic characterization of ESBL E. coli.

| Type of samples | CDDT method | DDST method | Ezy MIC strip |
|-----------------|-------------|-------------|---------------|
| Positive samples| 135         | 115         | 84            |
| Negative samples| 0           | 20          | 51            |
| Total number of samples | 135      | 135         | 135           |
| Percent sensitivity | 100      | 85          | 62            |
| Chi-square value | 67.7***    |             |               |

***p<0.01 results are highly significant. ESBL=Extended spectrum beta-lactamase, E. coli=Escherichia coli, CDDT=Combined disc diffusion test, DDST=Double disc synergy test

**Conclusion**

Animal was apparently healthy during the slaughter and was used for the human consumption; the prevalence of ESBL-producing E. coli which is a commensal bacteria indicating the problem of antibiotic resistance against beta-lactam antibiotic group which even includes third and fourth generation cephalosporins. CDDT method was found to be most sensitive among the three and most of the isolates were resistant toward cephalosporine discs and Ezy MIC strip method was the least the reason behind this was Ezy strip method is based on MIC values and the range of MIC could have been beyond the MIC range of the strip. As these organisms carry their genes on the plasmid due to horizontal gene transfer co- or multi-resistance against other antibiotics are also possible which could be an alarming sign specially for the poultry and human in contact with the birds.

**Authors’ Contributions**

AS designed and planned this research work collected the samples and executed the entire work RKS.
and YPS guided and monitored the entire research work. NS contributed in the collection of samples and also helped in the designing of work plan VG and SJ analyzed the data and were involved in the experiment. All authors contributed equally in preparation and revision of the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The authors are highly thankful to the Dean, College of Veterinary Science and Animal Husbandry, Jabalpur and Director Research Services, Nanaji Deshmukh Veterinary Science and Animal Husbandry, Jabalpur, Madhya Pradesh, India, for providing necessary financial assistance and instrumentation facilities to carry out this research work.

Competing Interests

The authors declare that they have no competing interest.

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