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Multiple drug resistance in sorbitol non fermenting *E.coli* biovars isolated from Avian species in Tamil Nadu

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Abstract

E.coli is a normal intestinal flora in animals and human beings. Diarrhoea causing *E.coli* is classified as Enterotoxigenic *E.coli*, Enteroinvasive *E.coli*, Enteropathogenic *E.coli*, and Enterohaemorrhagic *E.coli* (EHEC). EHEC is an emerging pathogen causing haemorrhagic colitis and Haemolytic Uremic Syndrome.The most common EHEC is *E.coli* O157:H7.In this study, *E.coli* is isolated from 80 faecal samples of avian species in Tamilnadu. Among the 80 *E.coli* isolates,22 were sorbitol non-fermentors. These sorbitol non-fermentors were subjected to drug susceptibility to 24 different antibiotics. All the biovars were resistant to aztreonam, piperacillin and ticarcillin.95 per cent strains were resistant to carbencillin, cefazolin, ceftazidime and cefixime. Multiple drug resistance was observed.

Keywords: avian species, drug resistance, *E.coli*, *E.coli* biovars, non-fermenting *E.coli*, sorbitol

INTRODUCTION

Antibiotic usage is considered the most important factor promoting the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine (Witte, 1998; Neu, 1992).Antibiotics are used in animals as in humans for therapy and control of bacterial infections. These resistant bacteria may colonize the human intestinal tract and may also contribute resistance genes to human endogenous flora. Colonization of the intestinal tract with resistant *E. coli* from chicken has been shown in human volunteers (Linton, *et al.*,1977). Evidence that animals are a reservoir for *E. coli* found in humans was published by Cooke *et al.*, (1971), in the early 1970s.

However, the mechanism of spread of antibiotic resistance from birds to humans remains controversial. The birds apparently acquire the bacteria interacting with domestic flocks, and then transport it around the world during transcontinental migrations. Similarly, avian influenza, or bird flu, is transported around the world in wild flocks, but scientists fear domestic flocks will provide the conditions necessary for a genetic mutation that makes human-to-human transmission of the deadly virus more likely.

Spread of an antibiotic resistance plasmid, pSL222-6, in *E. coli* from chickens to human handlers was described by Levy *et al.*, (1976). Others have also presented evidence of spread of antibiotic-resistant microorganisms from poultry to humans in various countries. Linton *et al.*, (1977), found the same O serotype in chickens from a commercial rearing centre, in oven-ready birds and in humans. Ojeniyi (1989), described direct transmission of *E. coli* resistant to streptomycin, sulphonamides and tetracycline from poultry to poultry attendants in Nigeria. Chickens have also been described as a source of antibiotic resistance in humans in northern India (Singh *et al.*,1992)., Morocco (Amara *et al.*,1995) and Saudi Arabia (Al Ghamdi *et al.*, 1999). In this study the prevalence of resistance in faecal *E. coli* was analysed in free flying birds.

MATERIALS AND METHODS

Sample Collection

The night resting places of birds under investigation (crows, parrots, pigeons, ducks – 20 samples each) were located and the samples collected early in the morning between 5.30 am and 6.30 am before the birds leave the resting places. In case of crow, the freshly voided faecal samples were obtained from the bottom of the trees in which they rest. Using sterile cotton swabs, the droppings were collected and kept in labelled sterile test tubes plugged with cotton and transported to the laboratory. Pigeons being roof dwellers, samples of droppings were collected from their dwelling places. The faecal samples of domesticated birds like ducks were collected from the farm.

Eighty number of samples were used. The samples were inoculated onto Mac Conkey agar plates. Lactose fermenting colonies were selected and stored in nutrient agar slants. All the isolates were confirmed based on the standard biochemical properties.

Antibiotic resistance

Sorbitol non-fermenting *E.coli* (22) strains were subjected to drug sensitivity assay. Susceptibility to 24 antibiotics namely amikacin (30 mcg), aztreonam (30 mcg), carbencillin (100 mcg), cefazolin (30 mcg), cefdinir (5

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mcg), cefipime (30 mcg), ceftazidime (30 mcg), ceftriaxone (30 mcg), cephotaxime (30 mcg), ciprofloxacin (5 mcg), co-trimoxazole (1.25/23.75 mcg), gatifloxacin (5 mcg), gentamycin (5 mcg), kanamycin (30 mcg), levofloxacin (5 mcg), nalidixic acid (30 mcg), norfloxacin (10 mcg), trimethoprim (5 mcg), cefixime (5 mcg), piperacillin (100 mcg), ofloxacin (5 mcg), tetracycline (30 mcg), ticarcillin (75 mcg) and tobramycin (10 mcg)were tested. Drug sensitivity was assessed following the method of Bauer *et al*. (1996).

Screening for Extended Spectrum β Lactamase (ESBL) activity

Double disk synergy method (DDS)

Test strains were preincubated in brain heart infusion broth (BHIB) at 37°C to an optical density matching that of 0.5 McFarland turbidity standard. This suspension was then used to inoculate Mueller Hinton Agar (MHA) plates by swabbing them with a sterile cotton swab.30µg discs of aztreonam, ceftazidime, ceftriaxone and cefotaxime were placed 15 mm (edge to edge) from an augmentin (amoxicillin- clavulanate; 20/10 g) disc. Inoculated plates were incubated overnight at 37°C. Enhancement of the zone of inhibition between the clavulanate disc and any one of the β lactam discs indicated the presence of an ESBL (Menon *et al*., 2006).

RESULTS AND DISCUSSION

***E.coli* biovars**

From the 80 samples, 80 *E.coli* were isolated from Mac Conkey agar plates based on lactose fermentation (pink colonies).All the isolates were indole positive, methyl red positive, voges proskauer negative, citrate negative, acid butt and acid slant with gas production in TSI agar tests. All the 80 isolates were subjected to sorbitol fermentation. Twenty tow isolates were found not to ferment Sorbitol.3 isolates were glucuronide negative. Sorbitol non-fermenting *E.coli* with the inability to produce glucuronidase is the major identification characteristic of *E.coli* O157:H7 (Raji *et al*.,2008).But this needs further confirmation using PCR.

Prevalence of resistance

All the *E.coli* were resistant to aztreonam, piperacillin and ticarcillin. The prevalence of resistance to carbencillin, cefazolin and ceftazidime was significantly higher (95.45%). The lowest resistance rates were observed in norfloxacin, ofloxacin, tobramycin, levofloxacin, cotrimoxazole and gatifloxacin. The drug resistance pattern of these isolates are shown in table 1.

Aztreonam, Piperacillin and Ticarcillin are extended spectrum beta-lactam antibiotics. All the *E.coli* isolates

Table 1. Susceptibility pattern of sorbitol non-fermenting *E.coli* to various antibiotics.

SI.NO.	Antibiotic(mcg)	Percentage		
		Sensitive	Intermediate	Resistant
1.	Aztreonam (30)	0	0	100
2.	Piperacillin (100)	0	0	100
3.	Ticarcillin (75)	0	0	100
4.	Carbencillin (100)	0	4.5	95.45
5.	Cefazolin (30)	4.5	0	95.45
6.	Ceftazidime (30)	4.5	0	95.45
7.	Cefixime (5)	0	4.5	95.45
8.	Cefdinir (5)	4.5	4.5	90.90
9.	Tetracycline (30)	4.5	9	86.36
10.	Amikacin (30)	4.5	27.27	68.18
11.	Cefipime (30)	4.5	45.45	50
12.	Cephotaxime (30)	0	59.09	40.9
13.	Kanamycin (30)	13.6	72.72	13.6
14.	Ceftriaxone (30)	27.27	68.18	4.5
15.	Nalidixic acid (30)	0	59.09	40.9
16.	Ciprofloxacin (5)	31.81	54.54	13.63
17.	Trimethoprim (5)	36.36	45.45	18.18
18.	Gentamycin (5)	31.38	40.9	27.27
19.	Norfloxacin (10)	86.36	9	4.5
20.	Ofloxacin (5)	81.81	4.5	13.6
21.	Tobramycin (10)	81.81	9	9
22.	Levofloxacin (5)	77.2	9	13.6
23.	Co-trimoxazole (1.25/23.75)	54.54	31.81	13.63
24.	Gatifloxacin (5)	54.54	31.81	13.81

are resistant to these antibiotics showing extended spectrum beta lactamase activity. Of the 22 isolates tested, 12 (54.5%) were found to be ESBL producers in Double disk synergy method (DDS). Seven were from pigeons and five from ducks. In earlier studies, 94 per cent resistance to tetracycline and 100% resistance to tetracycline-ampicillin were reported in *E. coli* isolates of chicken origin (Nazer,1980). Indiscriminate use of antibiotics has provided selective pressure for the emergence of drug-resistant strains of bacteria associated with poultry products (Roy *et al*., 2006).

Cefixime , Cefdinir, Cefotaxime and Ceftriaxone are third generation, broad-spectrum antibiotics in the cephalosporin class. Ninety per cent of *E.coli* isolates were resistant to cefixime and cefdinir. To cefotaxime only 40 per cent resistance was observed. Tetracyclines are a group of broad-spectrum antibiotics whose general usefulness has been reduced with the onset of bacterial resistance. Despite this, they remain the treatment of choice for some specific indications. Tetracycline antibiotics are protein synthesis inhibitors, inhibiting the binding of aminoacyl-tRNA to the mRNA-ribosome complex. 86 per cent isolates were resistant to tetracycline. 100% resistance was shown in another study by Roy *et al*., (2006).

Amikacin, kanamycin, gentamicin and tobramycin are aminoglycoside antibiotics, for which 68.0, 13.6, 27.0 and 9.0 per cent resistance were observed respectively, and these drugs are also used in human medicine. Colibacillosis is mainly used for fecal- and waterborne-transmitted ailment. Effective sanitizers such as chlorine need to be used to make the water free of *E. coli*. Cefepime is a fourth-generation cephalosporin antibiotic developed in 1994. Half of the isolates were resistant to cefepime.

Nalidixic acid is the first of the synthetic quinolone. Ciprofloxacin is a second generation fluoroquinolone. Sixty per cent of isolates were moderately sensitive to quinolone antibiotics. Trimethoprim belongs to the class of chemotherapeutic agents known as . Only 18 per cent of the isolates are resistant to trimethoprim. Antibiotic usage selects for resistance not only in pathogenic bacteria but also in the endogenous flora of exposed individuals (animals and humans) or populations (Van den Boggard, 1997).

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