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RESEARCH ARTICLE

PREVALENCE OF ANTIMICROBIAL RESISTANT *ESCHERICHIA COLI* STRAINS ISOLATED FROM POULTRY, MEGHALAYA, INDIA

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ABSTRACT

The frequent use of antimicrobial has rendered the risk of increase in resistance against *E. coli*. The objective of this study was to observe the resistance pattern against most commonly used drugs. A total of 92 *Escherichia coli* were isolated from tissues of diseased poultry birds. The antibiotic resistance-susceptibility profiles were conducted by standardized disc diffusion following CLSI method. Test for resistance to 14 antimicrobial agents were conducted. The most frequently encountered form of resistance in all the samples were to sulphafurazole (98.91%) trimethoprim-sulfamethoxazole (96.73%), colistin (88.04%), tetracycline (86.95%), co-trimoxazole (76.08%), enrofloxacin (75%) and ampicillin (70.65%). 67 (72.82%) of the isolates showed resistance to more than three antimicrobial classes. The high percentage of multi-drug resistance prevailing among the samples was mainly against sulphonamides, tetracycline and fluoroquinolones. Therefore, the increase in resistance against colistin, sulphonamides and fluoroquinolones which were considered highly effective against *E. coli* was highly notable.

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INTRODUCTION

Antimicrobial resistance (AMR) is one of the most burning concern and thus considered as a global problem in human as well as veterinary medicine (Boerlin *et al.*, 2005). The widespread risks developed can be attributed to the frequent use and misuse of the antimicrobials. The presence of antibiotic–resistant *Escherichia coli* in domestic poultry, beef and swine has been reported (Sandvang *et al.*, 1997; Madsen *et al.*, 2000; Guerra *et al.*, 2003). *Escherichia coli* play a crucial role either as commensal bacteria or as potential foodborne pathogens. It is ubiquitous in nature and can enter the food chain through contamination of faecal matter or food animal slaughter. Poultry is one of the most important farm animals as it serves as an integral food item for the meat eaters all over the world. Therefore, antimicrobials are often used as growth promoters for production purpose, particularly in poultry industry. Colibacillosis, a common disease in poultry is caused by *E. coli*. It not only renders huge economic losses but also can infect human with diseases such as haemorrhagic colitis and haemolytic uremic syndrome (Chansiripornchai, 2009; Ferens and Hovde, 2011). The selection pressure rendered upon using antimicrobials as feed additives or therapy has resulted in the emergence of multiple antimicrobial resistant bacteria thus making treatment difficult (Witte, 1998; van den Bogaard *et al*., 2000). The emergence and dissemination of antibiotic resistant genes among bacterial strains leading to the ineffectiveness of broad spectrum antibiotic treatment is an increasing problem (Maynard *et al.*, 2003). In recent times, many outbreaks of E. coli infection have been seen all around the world. ESBL (Extended Spectrum Beta Lactamases) producing bacteria which are resistant to the third and fourth generations of cephalosporins, penicillins and monobactams possess challenging infection control issues (Rupp and Fey, 2003). World Health Organization (WHO) has listed several antimicrobials belonging to ESC (Extended spectrum cephalosporins) used in the treatment of human and livestock as important since they are the sole therapeutic option available for curbing life-threatening diseases (WHO, 2007). Antimicrobial resistance is typically more frequent and higher among pathogens than commensal bacteria (DANMAP, 2004). Therefore, the main aim of this study was to observe the prevalence trends of the various classes of antimicrobials in the

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diseased poultry samples collected from the farms of Meghalaya, India, and also to investigate the antimicrobial resistant patterns.

MATERIALS AND METHODS

Collection of samples

The samples of poultry were randomly collected from organised and unorganised farms in and around Meghalaya, India. A total of 106 post-mortem tissue samples (heart, liver, spleen, intestine and kidney) were collected aseptically using disposable gloves and sterile vials. The samples were kept in ice-box and transported. They were then processed within 6h from the time of collection. Proper care was taken during collection and transportation for the viability of the samples.

Isolation and identification procedure

The tissue samples were cut into small pieces and weighed around 1g approximately. It was inoculated in MacConkey broth (HiMedia) and then incubated at 37^0C for 18 to 24h. A loopful of the broth culture was taken and streaked onto MacConkey Lactose Agar plate (MLA, HiMedia) and incubated for 18 to 24h at 37^0C. A suspected pink colony was selected and streaked on Eosin Methylene Blue Agar (EMB, HiMedia). Colonies showing green metallic sheen were then subjected to biochemical tests for further confirmation. The tests include IMViC (indole, methyl red, Voges Proskauer and citrate) as well as oxidase and urease tests. The confirmed isolates were then stored in BHI (Brain Heart Infusion) broth containing 15% glycerol at -80^0C.

Antimicrobial Sensitivity testing

Antimicrobial sensitivity test was performed on all the 92 *E. coli* isolates as per recommendation of Clinical Laboratory Standards Institute (CLSI, 2014) (Figure 1). Mueller-Hinton Agar (HiMedia, Mumbai, India) was used for the purpose. The following antimicrobial discs belonging to nine different classes were commercially procured (HiMedia, India) and screened: tetracycline (30µg), neomycin (30µg), ciprofloxacin (5 µg), colistin (30µg), enrofloxacin (5 µg), ampicillin (10 µg), chloramphenicol (10 µg), streptomycin (25 µg), ceftriaxone (30µg), ceftaxidime (30µg), trimethoprim (30µg), gentamycin (120 µg), co-trimoxazole (25 µg), sulphafurazole (300 µg).

The results were interpreted according to CLSI (2014) and EUCAST (2014). The standard strain of *Escherichia coli* (ATCC 29522) was used as a quality control. An isolate was regarded as 'multi-resistant' if it exhibited resistance to drugs of ≥3 antimicrobial types (Tenover, 2006).

RESULTS

Out of the 106 post-mortem tissue samples collected from diseased poultry birds, 92 (86.79%) were biochemically tested and confirmed as *E. coli* isolates. The 92 *E. coli* isolates were subjected to antimicrobial susceptibility tests to 14 antimicrobials belonging to 9 different classes. One strain from each positive sample was used for further analysis. The highest resistance was registered in sulfafurazole. 98.91% isolates showed resistant to it followed by trimethoprim-sulfamethoxazole (96.73%), colistin (88.04%), tetracycline (86.95%), co-trimoxazole (76.08%), enrofloxacin (75%) and ampicillin (70.65%) (Table 1). Combined resistance to sulfafurazole, trimethoprim-sulfamethoxazole, colistin, tetracycline, co-trimoxazole, and ampicillin was found in 54.34% isolates. Ceftazidime and ceftriaxone, the third generation antibiotics belonging to the class Cephalosporins showed resistance up to 10.86% and 6.52% respectively. None of the isolates were found resistant to gentamicin, eventually making it the most sensitive with 91.30% as shown in Figure 1. 79.34% of the isolates were sensitive to ceftriaxone followed by chloramphenicol (77.17%).

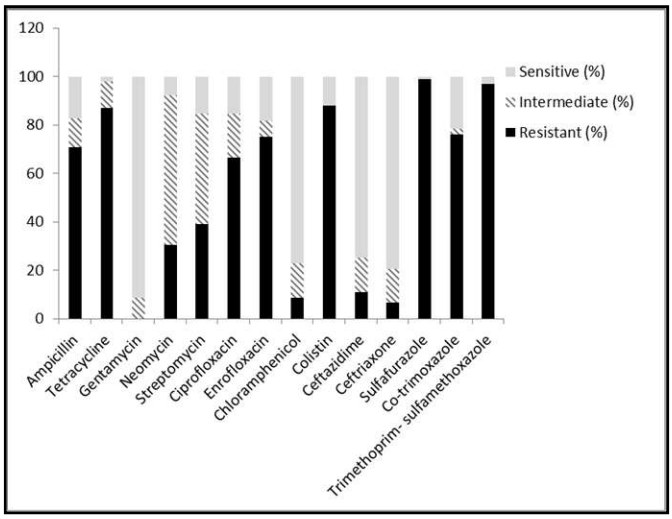

Figure 1. Graphical representation of the sensitivity pattern of the *E. coli* isolates

Table 1. Numbers (%) of *Escherichia coli* isolates from diseased poultry sample depicting antimicrobial resistance profile

Antimicrobial Class	Antimicrobials	Disc content (µg)	Resistance breakpoint (mm)	*E. coli* isolates (n=92)		
				S (%)	I (%)	R (%)
Penicillin	Ampicillin	10	≤13	16(17.39)	11(11.95)	65(70.65)
Cephalosporins	Ceftazidime	30	≤14	69(75)	13(14.13)	10(10.86)
	Ceftriaxone	30	≤13	73(79.34)	13(14.31)	6(6.5)
Aminoglycosides	Gentamycin	120	≤12	84(91.3)	8(8.69)	0(0)
	Neomycin	30	≤12	7(7.6)	57(61.95)	28(30.43)
	Streptomycin	25	≤11	14(15.21)	42(45.65)	36(39.13)
Fluoroquinolones	Ciprofloxacin	5	≤15	14(15.21)	17(18.47)	61(66.3)
	Enrofloxacin	5	≤16	17(18.47)	6(6.52)	69(75)
Phenicols	Chloramphenicol	10	≤12	71(77.17)	13(14.13)	8(8.69)
Tetracycline	Tetracycline	30	≤14	2(2.17)	10(10.86)	80(86.95)
Polypeptides	Colistin	10	≤10	11(11.95)	0(0)	81(88.04)
Sulphonamides	Sulfafurazole	300	≤12	1(1.08)	0(0)	91(98.91)
	Co-trimoxazole	25	≤10	20(21.73)	2(2.17)	70(76.08)
Trimethoprim-sulfamethoxazole		30	≤10	3(3.26)	0(0)	89(96.73)

With regard to multidrug resistance profiles, out of the 12 antimicrobials tested, all isolates were resistant to at least one antimicrobial agent, 73.91% were resistant to more than five and 59.78% were resistant to more than seven. Two particular isolates were resistant to 11 antimicrobial agents and intermediately resistant to 3 antimicrobials. 67 (72.82%) of the isolates showed total resistance to more than three antimicrobial classes.

DISCUSSION

The higher resistance rate in tetracycline, ampicillin and trimethoprim-sulfamethoxazole has been in accordance with previous studies (Mooljuntee S. *et al*., 2010; Jiang *et al*., 2011; Momtaz *et al*., 2012; Rasheed *et al*., 2014). Also a zero percentage of resistance was monitored in gentamycin among the poultry isolates (Mooljuntee *et al*., 2010). The finding of the present work somewhat corresponds with the study done by Hamisi *et al.* (2014) on tropical free-range chickens from Tanzania. According to their study, 83.1% isolates has shown resistance to at least one antimicrobial agent. The increase in frequency of antibiotic resistance obtained is also in accord with that stated by Van *et al*. (2007, 2008).

The presence of multidrug resistant *E coli* in chicken has been previously reported from different countries (Kolar *et al*., 2001; Schroeder *et al*., 2003). Such alarming rates of multi resistance might be due to the frequent administration and over-usage of the antimicrobials (Moniri and Dastehgoli, 2007; Miranda *et al*., 2008). Moreover, during slaughter, resistant strains from the gut can easily contaminate poultry carcasses, therefore, contaminating the poultry meats with resistant *E. coli* (Van den Bogaard *et al*., 2001; Vaidya *et al*., 2005; Akond *et al*., 2009; Altekruse *et al*., 2009). According to Overdevest *et al.* (2011), close relationship has been established between ESBL-producing *E. coli* strains of poultry and humans. Such links can be alarming for the poultry industry as well as humans. Third generation cephalosporin is considered a major drug for human disease treatments. The occurrence of cephalosporin resistant *E. coli* in retail meat is of great concern. The intestinal tract of many food producing animals has been known to possess cephalosporin resistant *E. coli* (Riano *et al*., 2006; Cloeckaert *et al*., 2007; Liu *et al*., 2007). Moreover, 35% of *E coli* strains isolated from live broilers were found to be resistant to third generation cephalosporin (Depoorter *et al*., 2012). In many European countries, the genetic background for cephalosporin resistance in *E.coli* isolates, from broilers and retail chicken meat, is heterogeneous including genes of the CTX-M, TEM and SHV groups (Ewers *et al*., 2012). Therefore, an attempt should be made to keep the prevalence of cephalosporin-resistant *E. coli* in retail meat low, thus, somewhat curbing the probability of zoonotic transmission.

In conclusion, the results of this study indicate that *E. coli* strains isolated from post-mortem tissue samples of diseased poultry showed a higher resistance rate for important antimicrobials including fluoroquinolones and colistin. The presence of high percentage multi-drug resistance (MDR) in the isolates is quite a concern. A proper monitoring is required while prescribing or giving feed additives.

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REFERENCES

Akond, M.A., Alam, S., Hassan, S.M.R. and Shirin, M. 2009. Antibiotic resistance of Escherichia coli isolated from poultry and poultry environment of Bangladesh. *Internet Journal of food safety*, *11*, 19-23.

Altekruse, S.F., Berrang, M.E., Marks, H., Patel, B., Shaw, W.K., Saini, P., Bennet, P.A. and Bailey, J.S. 2009. Enumeration of Escherichia coli cells on chicken carcasses as a potential measure of microbial process control in a random selection of slaughter establishments in the United States. *Applied and Environmental Microbiology*, *75*(11), 3522-3527.

Boerlin, P., Travis, R., Gyles, C.L., Reid-Smith, R., Lim, N.J.H., Nicholson, V., McEwen, S.A., Friendship, R., & Archambault, M. 2005. Antimicrobial resistance and virulence genes of Escherichia coli isolates from swine in Ontario. *Applied and Environmental Microbiology*, *71*(11), 6753-6761.

Chansiripornchai, N. 2009. Comparative efficacy of enrofloxacin and oxytetracycline by different administration methods in broilers after experimental infection with avian pathogenic Escherichia coli. *Thai J. Vet. Med*, *39*(3), 231-236.

Clinical and Laboratory Standards Institute 2014. M100-S24. Performance standards for antimicrobial susceptibility testing: 24th informational supplement. Wayne, PA: CLSI.

Cloeckaert, A., Praud, K., Doublet, B., Bertini, A., Carattoli, A., Butaye, P., Imberechts, H., Bertrand, S., Collard J.M., Arlet, G. and Weill, F.X. 2007. Dissemination of an extended-spectrum-β-lactamase blaTEM-52 gene-carrying IncI1 plasmid in various Salmonella enterica serovars isolated from poultry and humans in Belgium and France between 2001 and 2005. *Antimicrobial Agents and Chemotherapy*, *51*(5), 1872-1875.

DANMAP. 2004. DANMAP 2003: Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark. Danish Zoonoses Center, Sørborg, Denmark.

Depoorter, P., Persoons, D., Uyttendaele, M., Butaye, P., De Zutter, L., Dierick, K., Herman, L., Imberechts H., Van Huffel, X. and Dewulf, J. 2012. Assessment of human exposure to 3rd generation cephalosporin resistant E. coli (CREC) through consumption of broiler meat in Belgium. *International Journal of Food Microbiology*, *159*(1), 30-38.

European Committee on Antimicrobial Susceptibility Testing. 2014. Breakpoint tables for interpretation of MICs and zone diameters. Version 3.1 EUCAST; 2013.

Ewers, C., Bethe, A., Semmler, T., Guenther, S. and Wieler, L. H. 2012. Extended-spectrum β-lactamase-producing and AmpC-producing Escherichia coli from livestock and companion animals, and their putative impact on public health: a global perspective. *Clinical Microbiology and Infection*, *18*(7), 646-655.

Ferens, W. A. and Hovde, C. J. 2011. Escherichia coli O157: H7: animal reservoir and sources of human infection. *Food borne Pathogens and Disease*, *8*(4), 465-487.

Guerra, B., Junker, E., Schroeter, A., Malorny, B., Lehmann, S. and Helmuth, R. 2003. Phenotypic and genotypic characterization of antimicrobial resistance in German Escherichia coli isolates from cattle, swine and poultry. *Journal of Antimicrobial Chemotherapy*, *52*(3), 489-492.

Hamisi, Z., Tuntufye, H. and Shahada, F. 2014. Antimicrobial Resistance Phenotypes of Escherichia coli Isolated from Tropical Free Range Chickens. *Int. J. Sci. Res*, *3*(9), 34-37.

Jiang, H.X., Lü, D.H., Chen, Z.L., Wang, X.M., Chen, J.R., Liu, Y.H., Liao, X.P., Liu, J.H. and Zeng, Z.L. 2011. High prevalence and widespread distribution of multi-resistant Escherichia coli isolates in pigs and poultry in China. *The Veterinary Journal*, *187*(1), 99-103.

Kolář, M., Pantůček, R., Bardoň, J., Vágnerová, I., Typovska, H., Válka, I. and Doskar, J. 2002. Occurrence of antibiotic-resistant bacterial strains isolated in poultry. *Vet Med Czech*, *47*, 52-59.

Liu, J.H., Wei, S.Y., Ma, J.Y., Zeng, Z.L., Lü, D.H., Yang, G.X. and Chen, Z.L. 2007. Detection and characterisation of CTX-M and CMY-2 β-lactamases among Escherichia coli isolates from farm animals in Guangdong Province of China. *International Journal of Antimicrobial Agents*, *29*(5), 576-581.

Madsen, L., Aarestrup, F.M. and Olsen, J.E. 2000. Characterisation of streptomycin resistance determinants in Danish isolates of Salmonella Typhimurium. *Veterinary Microbiology*, *75*(1), 73-82.

Maynard, C., Fairbrother, J.M., Bekal, S., Sanschagrin, F., Levesque, R. C., Brousseau, R., Masson, L. and Harel, J. 2003. Antimicrobial resistance genes in enterotoxigenic Escherichia coli O149: K91 isolates obtained over a 23-year period from pigs. *Antimicrobial Agents and Chemotherapy*, *47*(10), 3214-3221.

Miranda, J.M., Guarddon, M., Vázquez, B.I., Fente, C.A., Barros-Velazquez, J., Cepeda, A. and Franco, C.M. 2008. Antimicrobial resistance in Enterobacteriaceae strains isolated from organic chicken, conventional chicken and conventional turkey meat: A comparative survey. *Food Control*, *19*(4), 412-416.

Momtaz, H., Rahimi, E. and Moshkelani, S. 2012. Molecular detection of antimicrobial resistance genes in E. coli isolated from slaughtered commercial chickens in Iran. *Vet Med*, *57*(4), 193-7.

Moniri, R. and Dastehgoli, K. 2007. Antimicrobial resistance among Escherichia coli strains isolated from healthy and septicemic chickens. *Pakistan journal of biological sciences: PJBS*, *10*(17), 2984-2987.

Mooljuntee S., Chansiripornchai P., Chansiripornchai N. 2010. Prevalence of the cellular and molecular antimicrobial resistance against E. coli isolated from Thai broilers. *Thai J. Vet. Med*., *40*(3), 311-315.

Overdevest, I., Willemsen, I., Rijnsburger, M., Eustace, A., Xu, L., Hawkey, P., Heck, M., Savelkoul, P., Vandenbroucke-Grauls, C., vanderZwaluw, K., Huijsdens, X. and Kluytmans, J. 2011. Extended-spectrum β-lactamase genes of Escherichia coli in chicken meat and humans, The Netherlands. *Emerg Infect Dis*., *17*(7), 1216-1222.

Rasheed, M. U., Thajuddin, N., Ahamed, P., Teklemariam, Z. and Jamil, K. 2014. Antimicrobial drug resistance in strains of Escherichia coli isolated from food sources. *Revista do Instituto de Medicina Tropical de São Paulo*, *56*(4), 341-346.

Riaño, I., Moreno, M.A., Teshager, T., Sáenz, Y., Domínguez, L. and Torres, C. 2006. Detection and characterization of extended-spectrum β-lactamases in Salmonella enterica strains of healthy food animals in Spain. *Journal of Antimicrobial Chemotherapy*, *58*(4), 844-847.

Rupp, M.E. and Fey, P.D. 2003. Extended spectrum β-lactamase (ESBL)-producing Enterobacteriaceae. *Drugs*, *63*(4), 353-365.

Sandvang, D., Aarestrup, F.M. and Jensen, L.B. 1997. Characterisation of integrons and antibiotic resistance genes in Danish multiresistant Salmonella enterica Typhimurium DT104. *FEMS Microbiology Letters*, *157*(1), 177-181.

Schroeder, C. M., White, D. G., Ge, B., Zhang, Y., McDermott, P. F., Ayers, S., Zhao, S. and Meng, J. 2003. Isolation of antimicrobial-resistant Escherichia coli from retail meats purchased in Greater Washington, DC, USA. *International Journal of Food Microbiology*, *85*(1), 197-202.

Tenover, F. C. 2006. Mechanisms of antimicrobial resistance in bacteria. *The American Journal of Medicine*, 119(6), S3-S10.

Vaidya, V.M., Paturkar, A.M., Waskar, V.S., Zende, R.J. and Rawool, D.B. 2005. Detection of indicator organisms on poultry carcass sites in an organized slaughterhouse. *Journal of Muscle Foods*, *16*(4), 289-297.

Van den Bogaard, A.E. and Stobberingh, E.E. 2000. Epidemiology of resistance to antibiotics: links between animals and humans. *International Journal of Antimicrobial Agents*, *14*(4), 327-335.

Van den Bogaard, A.E., London, N., Driessen, C. and Stobberingh, E.E. 2001. Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers and poultry slaughterers. *Journal of Antimicrobial Chemotherapy*, *47*(6), 763-771.

Van, T.T.H., Chin, J., Chapman, T., Tran, L.T. and Coloe, P.J. 2008. Safety of raw meat and shellfish in Vietnam: an analysis of Escherichia coli isolations for antibiotic resistance and virulence genes. *International Journal of Food Microbiology*, *124*(3), 217-223.

Van, T.T.H., Moutafis, G., Tran, L.T. and Coloe, P.J. 2007. Antibiotic resistance in food-borne bacterial contaminants in Vietnam. *Applied and Environmental Microbiology*, *73*(24), 7906-7911.

Witte, W. 1998. Medical consequences of antibiotic use in agriculture. *Science*, *279*(5353), 996.

World Health Organization, 2007. Critically important antimicrobials for human medicine: categorization for the development of risk management strategies to contain antimicrobial resistance due to non-human antimicrobial use: report of the second WHO Expert Meeting, Copenhagen, 29-31 May 2007.

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