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Isolation and Antimicrobial Susceptibility Testing of E. *Coli*

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Abstract:

In this work, we isolate Escherichia coli from chicken in Nanded. Isolated Escherichia coli tested for antimicrobial susceptibility. In the planned investigation selected food sample like chicken were tested for isolation of *E. Coli*. Isolated *E. coli* strains were checked for the occurrence of antibiotic resistant genes by Kirby- Bauer disc diffusion method using Mueller – Hinton agar, which is fast, appropriate and accurate .Isolation of *E.coli* from chicken confirm on EMB agar and biochemical test. Twenty antibiotics were screened for resistance out of that nine antibiotic show positive result.

Introduction

Escherichia coli is commonly found in human and animal intestinal tracts. This microbe is usefully harmless, but it is also a medically important bacterium causing a number of significant infections. Recently, many strains of *Escherichia coli* have been found to be resistant to multiple, structurally unrelated antimicrobial classes (H. Momtaz et. al., 2012) A number of E. coli strains are recognized as important pathogen of colibacillosis in poultry and some of them can cause severe human disease such as haemorrhagic colitis and haemolytic uremic syndrome (Riley et.al 1983; Chansiripornchai, 2009;Ferens and Hovde 2011). Various uses of antimicrobial agents in medicine, production of food animals, and crop protection are some of the reasons for increasing resistance to those agents (American Society of Microbiology, 2007). Today the development of antibiotic resistance and lack of discoveries of new antibiotics have created a serious public health concern.If bacteria come into contact with antibiotic but are not killed by antibiotic they may adapt their cell structure and/ or metabolism to make themselves resistant to that antibiotic. Once antibiotic resistance is acquired, they can share this information with other bacteria via vertical gene transfer. The veterinary practitioners have a limited choice of antibiotics for the treatment of animals, due to antimicrobial resistance issues and human health concerns. In view of this they use same antibiotics repeatedly, which leads to an increasing rate of antimicrobial resistance in bacteria (Mooljuntee et al. 2010). This resistance is not only limited to pathogenic bacteria but also spreads in the endogenous flora of exposed animals. There are several reports of the presence of antibiotic resistant bacteria in poultry and meat products. Researchers have reported high proportion of antibiotic resistant bacteria in the faecal flora of poultry (Piddock, 1996; Bogaard and Stobberingh 1999). Momtaz et al (2012) had carried out a study to detect the distribution of antibiotic-resistant genes in *Escherichia coli* isolates from slaughtered commercial chickens in Iran. Similar studies have also been carried out in pigs during Metaphylactic Trimethoprim and Sulfamethoxazole treatment and in the Post-Exposure Period (Mazurek et al 2015). In some of the previous studies, transfer of antimicrobial-resistant bacteria from animal products to humans has been

reported (Sanchez et al. 2002; Swartz 2002). In the last few years, many strains of *E.coli* have been reported to be resistant to multiple, structurally unrelated antimicrobial classes, like quinolones, cephalosporins, and aminoglycosides (Orden et al., 2001; Donaldson et al., 2006). Resistance among microorganisms can generally be detected either phenotypically or genotypically. The phenotypic approach is the usual method when testing bacteria for clinical purposes.

Method

Chicken sample were collected for microbial analysis from Nanded city. All the sample were collected aseptically, transported to the laboratory under chilled conditions and processed for microbiological analysis within 24 hrs of collection. The samples were inoculated into 0.1% peptone salt solution and incubate at 37^0c for 24 hrs. A loopful inoculums from 0.1 % peptone salt solution was streaked onto MacConkey agar and plates were incubated at 37^0c for 24 hrs and observed for pink colony on MacConkey's agar. The well separated pure colonies were subculture on EMB agar and incubate at 37^0c for 24 hrs. Pure colonies were picked up on nutrient slant as pure culture and subjected for biochemical test.

Antimicrobial susceptibility testing was performed by the Kirby- Bauer disc diffusion method using Mueller – Hinton agar and using 20 antibacterial agents: Moxifloxacin (5 mcg) , Ceftriaxore 80 mcg), Penicillin (10 mcg), Ciprofloxacin (5 mcg), Tigecycline (15 mcg), Ticarcillin (75 mcg), Azithromycin (15 mcg), Nitrofurantoin (100 mcg), Cefazolin (30 mcg), Levofloxacin (5 mcg), Ampicillin (10 mcg), Ceftriaxone (30 mcg), Minocycline (30 mcg), Doxycycline Hydrochloride (30 mcg), Methicillin (5 mcg), Imipenem (10 mcg), Gentamicin (10 mcg), Cefpodoxime (10 mcg), Sulphafurazole (300 mcg), Aztreonam (30 mcg).

The *E. coli* isolates were inoculated in nutrient broth and incubated at 37 °C for 5 hrs. The broth was diluted in normal saline solution to a density of 0.5 McFarland turbidity standard. Cotton swabs were used for streaking the diluted broth onto Mueller-Hinton agar plates. After air drying, antibiotic discs were placed . Plates were inverted and incubated aerobically at 37 °C for 16 hours. The zone of inhibition and resistance was measured, recorded, and interpreted according to the recommendation of the CLSI (NCCLS 2002).

Result

Isolation of E. coli:

E. coli show pink colonies on MacConkey's agar. Pink colonies on MacConkey's agar due to E. coli ferments lactose. On EMB agar plate E.coli growth show distinctive metallic green sheen due to the metachromatic properties of the dyes, E.coli movement using flagella and strong acid end products of fermentation.

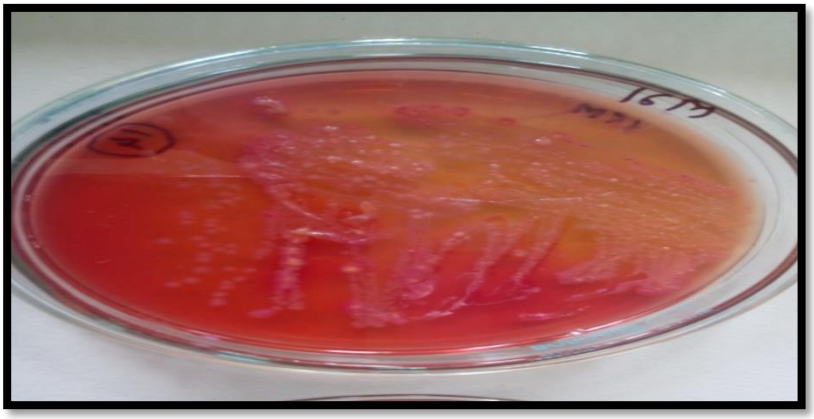

Figure: Pink colonies on MacConkey's agar

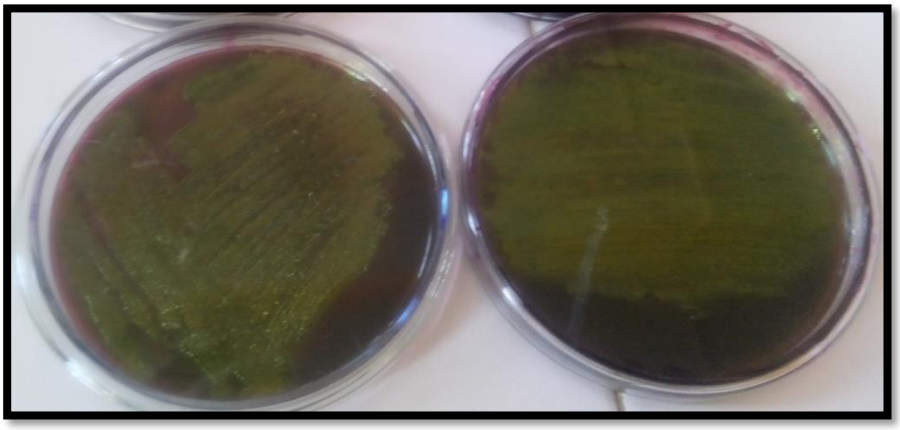

Figure: Characteristic metallic sheen of E. coli on EMB agar.

Antimicrobial susceptibility testing of E. *Coli*

Twenty antibiotics were screened for resistance out of that nine antibiotic show positive result.

Sr. No	Antibacterial agent	Code	Zone of inhibition (mm in diameter)	Criteria
1.	Moxifloxacin (5 mcg)	MO	35	Sensitive
2.	Ceftriaxore 80 mcg)	CIT	-	Resistant
3.	Penicillin (10 mcg)	P	-	Resistant
4.	Ciprofloxacin (5 mcg)	CIP	35	Sensitive
5.	Tigecycline (15 mcg)	TGC	18	Sensitive
6.	Ticarcillin (75 mcg)	TCC	-	Resistant
7.	Azithromycin (15 mcg)	AZM	-	Resistant

8.	Nitrofurantoin (100 mcg)	NIT	18	Sensitive
9.	Cefazolin (30 mcg)	CZ	12	Intermediate
10.	Levofloxacin (5 mcg)	LE	37	Sensitive
11.	Ampicillin (10 mcg)	AS	-	Resistant
12.	Ceftriaxone (30 mcg)	CTR	-	Resistant
13.	Minocycline (30 mcg)	MI	35	Sensitive
14.	Doxycycline Hydrochloride (30 mcg)	DO	31	Sensitive
15.	Methicillin (5 mcg)	MET	-	Resistant
16.	Imipenem (10 mcg)	IPM	38	Sensitive
17.	Gentamicin (10 mcg)	GEN	24	Sensitive
18.	Cefpodoxime (10 mcg)	CPO	-	Resistant
19.	Sulphafurazole (300 mcg)	SF	36	Sensitive
20.	Aztreonam (30 mcg)	AT	-	Resistant

Table : Assay of Antibacterial Activity

Conclusion:

In the present investigation E. coli isolated from chicken sample. Twenty antibiotics were screened for resistance out of that nine show positive result. To conclude, the primary objectives of the research were achieved by isolating E.coli and identifying presence of antiobiotic resistance gene in isolated E. coli from chicken.

References:

- American Society for Microbiology. Antimicrobial Resistance: An Ecological Perspective. 2000. Website http://www.asm.org/academy/index.asp?bid=2167. Access April, 2007.
- Mooljuntee S, Chansiripornchai P, Chansiripornchai N (2010): Prevalence of the cellular and molecular antimicrobial resistance against E. coli isolated from Thai broilers. Thai Journal of Veterinary Medicine 40, 311–315.
- Momtaz H., Rahimi1 E., Moshkelani S. (2012) Molecular detection of antimicrobial resistance genes in E. coli isolated from slaughtered commercial chickens in Iran. Veterinarni Medicina, 57, 2012 (4): 193–197.
- Mazurek J., Bok E., Stosik M., Baldy-Chudzik K. (2015) Antimicrobial Resistance in Commensal *Escherichia coli* from Pigs during Metaphylactic Trimethoprim and Sulfamethoxazole Treatment and in the Post-Exposure Period. *Int. J. Environ. Res. Public Health* 2015, *12*, 2150-2163.
- Swartz, M.N., 2002. Human Diseases Caused by Foodborne Pathogens of Animal Origin. Clin Infect Dis, S111–22.

- Orden, J.A., Ruiz-Santa-Quiteria, J.A., Cid, D., Diez, R., Martinez, S., De La Fuente, R., 2001. Quinolone Resistance in Potentially Pathogenic and Non-Pathogenic *Escherichia Coli* Strains Isolated from Healthy Ruminants. J. Antimicrob. Chemother. 48, 421-424.
- Raseed et al. (2014) Antimicrobial drug resistance in stains of Escherichaia coli isolated from food sources. Rev. Inst. Med. Trop. Sao Paulo 56(4):341-346.
- Skovgaard N. New trends in emerging pathogens. International Journal of food microbiology: 2007; 217-224.
- Suzuki S, Horinouchi T, Furusawa C. Prediction of antibiotic resistance by gene expression profiles. Nat Commun 2014; 5:5792.
- Swartz, M. N. 2002. Human Diseases Caused by Foodborne Pathogens of Animal Origin. Clin Infect Dis, S111-22.