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Antibiotic susceptibility pattern of resistant *Escherichia Coli* from poultry waste

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Abstract

The study was conducted in Kathmandu valley to investigate the relationship between the use of antibiotic and the development of the resistant form of *Escherichia coli* (*E.coli*), which were isolated from faecal waste of poultry farm. Out of 30 samples investigated, 20 samples were found to be *E.coli* positive. It was further investigated that the resistance pattern for the isolates of *E.coli* from poultry farm was tetracycline (100%), penicillin (100%), erythromycin (100%), amoxicillin (90%) and chloramphenicol (60%).

Keywords: Antibiotics; Resistance pattern; Tetracycline; Penicillin; Erythromycin

1. Introduction

Poultry farming is the practice of raising poultry, such as chickens, turkeys, ducks, and other fowl for the purpose of farming, meat or egg production. Nowadays, especially in poultry production antibiotics are widely used as growth promoter and in treatment of infectious disease [1]. The use of antibiotics in the poultry production industries, for the promotion of growth, largely contributes to the high resistance to antimicrobial agents in normal flora of poultry [2] and pathogenic microorganisms [3]. Besides this the antibiotics are used for the adjustment of intestinal flora, favoring useful bacteria while suppressing other bacterial population which enhances the use of antibiotics as growth promoter. Many contend that this puts humans at risk and bacterial strains develop stronger and stronger resistances [4,5].

These resistant microorganisms may act as possible source for the transfer of antimicrobial resistance to human pathogens. Plasmid and transposon mediated resistance is widely transmitted between different species and genera including human pathogens [6]. Antibiotics are used extensively to prevent or to treat microbial infections in human and veterinary medicine. Apart from their use in aquaculture, they are also employed to promote more rapid growth of livestock [7,8]. However, *E.Coli* is the causative agent of human intestinal disease which remains a major cause of morbidity and mortality worldwide. These occur primarily in the developing countries where the access to effective antibiotic therapy is limited. The increase in number of drug resistant strains has also complicated the management of disease caused by *E.coli*.

The emergence of resistance to antibiotic is a relevant matter worldwide, but the retrieval of antibiograms for *E.Coli* is severely delayed when phenotypic methods are used. Early identification of drug resistance

and particularly multidrug resistant strain (MDR) is routine in developed low prevalence countries but the methods are currently unavailable and are too costly for resource poor nations like Nepal where the burden of disease is greatest. Data on the identification of antibiotic resistant *E. Coli* is crucial in order to permit timely administration of appropriate antibiotic schedule and could potentially improve the efficacy of Multidrug Resistant treatments.

2. Materials and Methods

Sample Collection

Faecal samples of poultry were collected from different poultry farms of Kathmandu.

Bacterial isolation

Specimens were cultured on McConkey and Eosin Methyl Blue (EMB) agar and the colonies suspected to be *E. coli* were identified by various biochemical tests. The list of biochemical tests are as follows

Indole test

In this a loopful of bacteria was inoculate in a media containing Kovac's reagent containing N Amyl Alcohol 75ml, Hydrochloric acid (conc.) 25ml and p-dimethylamine benzaldehyde 5gm and observed for color change.

Methyl Red Test

In this a loop of bacteria was inoculated in a test tube having Methyl Red Voges Proskaur(MRVP) broth containing buffered peptone 7gm, dextrose 5gm, dipotassium phosphate 5gm and pH 7.4 and incubated for 24 to 48 hrs. Then methyl red was added for the color change

Voges proskaur test

In this a loop of bacteria were inoculated in a test tube having MRVP broth and incubated for 48 hours. Then 20 drops of barrits reagent containing solution A containing 5gm alpha nepthol in 100ml 95% ethanol and solution B containing 40gm potassium hydroxide in 100ml distilled water were added to the test tubes and change in the color was noted

Citrate utilization test

The bacteria were inoculated in the simmon citrate agar containing Ammonium Dihydrogen Phosphate 1gm, dipotassium Chloride 1gm, Sodium chloride 5gm, Magnesium sulphate 0.2gm, Sodium citrate 2gm, Agar 15gm, Bromothymol blue .08gm and pH 6.8 slant and incubated for 48 hours. It was then observed for any color change

Triple Sugar Iron test (TSI test)

In this the bacteria was inoculated in the TSI agar slant containing glucose 0.1gm, sucrose 1gm, lactose 1gm and distilled water 100ml and incubated for 24 hrs. Then the change in color was observed

Hydrogen Sulphide Test

In this a loopful of bacteria was inoculated in SIM media containing ammonium ferric citrate 0.2gm, casein peptone 20gm, meat peptone 6.6gm, sodium thiosulphate 0.2gm, distilled water 1000ml. The change in color was noted.

Urease test

In this the bacteria was inoculated in the urea agar containing Phenol red solution and incubated at 35⁰c for 24 hrs. The urease production was examined by observing change in color.

3. Antibiotic Susceptibility Determination

Antibiotic susceptibility was done by single disc diffusion method. The following antibiotic discs were applied on Mueller hinton media: Penicillin(P/10µg), chloramphenicol (C/30 µg), Tetracycline(TE/30 µg), Amoxicillin(AMX/10 µg), Erythromycin(E/10 µg). Interpretation was done according to the criteria recommended in the National Committee for clinical Laboratory Standards (NCCLS. 1993)

4. Results and Discussion

After the samples were collected from Kathmandu valley and microorganism were isolated, various biochemical tests were performed. The results of which are shown below

Table 1: showing different biochemical test

Organisms	Indole test	MR test	VP test	Citrate test	TSI(Slant/Broth)	H ₂ S test	Urease test
E.coli	Positive	positive	Negative	Negative	Yellow color / Yellow color	Negative	Negative
Shigella	Negative	Negative	Negative	Negative	Red color/Yellow color	Negative	Negative
Salmonella	Negative	Positive	Negative	Negative	Red color/Yellow color	Positive	Negative
Proteus	Positive	Positive	Negative	Negative	Red color/Yellow color	Positive	Positive

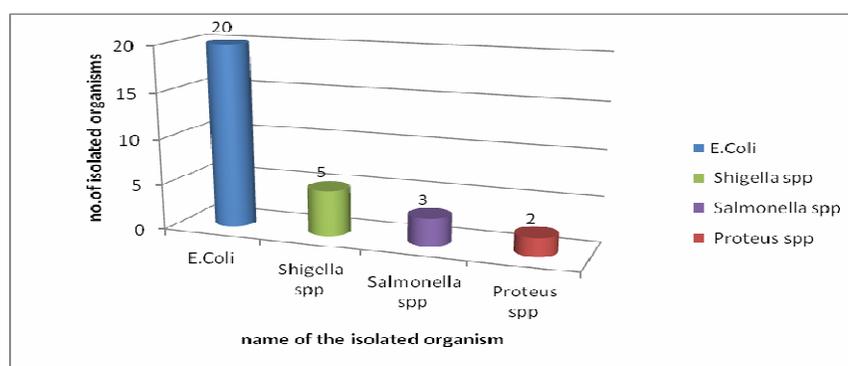


Fig 1 : Isolated Microorganism from Poultry.

From the above figure it was found that among 30, 20 (66.66%) was identified as *E.coli*. 5(16.67%) was identified as *shigella spp*. 3(10%)was *salmonella spp* and 2(6.67%)was proteus species.

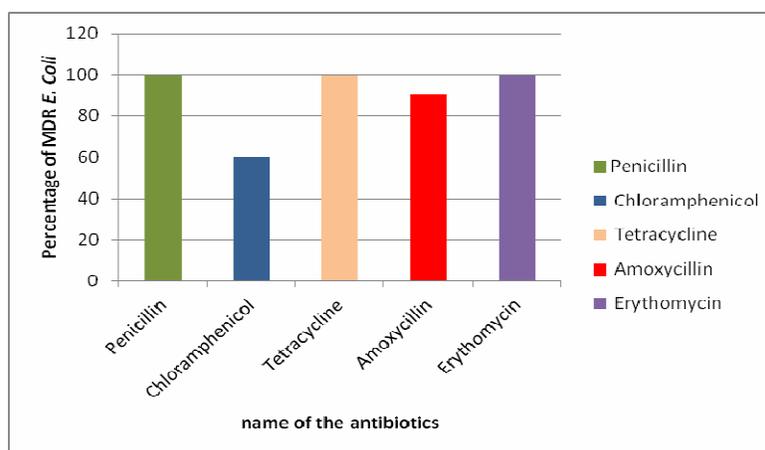


Fig 2 : Antibiotic Susceptibility Test.

From the above figure it was found that all the isolate were resistant to Penicillin(100%), Chloramphenicol(60%), Tetracycline (100%), Amoxycillin(90%) and Erythromycin(100%). Analysis of the treatment history collected from the poultry farm showed that they used antibiotics mainly for

treatment purpose. The resistance pattern of 20 isolates of *E.coli* isolated from poultry wastes clearly demonstrates the high resistance rate to all of the above tested antibiotics.

The increased antimicrobial resistance in bacteria especially pathogens, has received worldwide attention. The increasing failure of therapeutic intervention pushed the World Health Organization (WHO) to publish the “Global strategy for containment of antimicrobial resistance” in 2001[9]. This strategy was intended to minimize the increase of resistant bacterial strains while maintaining the effectiveness of the antibiotics already in use.

During recent years poultry industry has expanded very rapidly in Nepal and antibiotic resistance has emerged as one of the most severe problem having adverse effects on human health through their faecal waste and consuming their products. This has highlighted the need for taking extensive control measures to save the industry as well as humans. Worldwide data show that there is increasing resistance among urinary tract pathogens to conventional drugs especially with various strains of *E.Coli* [10]. Although new antibiotics are being developed eg. Glycylglycines [11] the level of antimicrobial resistance is not decreasing and it seems that development of resistance keeps pace with the development of new antibiotics.

Risk management of antimicrobial resistance is heavily biased towards banning the misuse of antibiotics [12]. It is also generally accepted that the high and often inappropriate use of antimicrobials and disinfectants, in both the humans and animals, is the main driver of resistance [13].

Increasingly, multiresistant bacteria are being detected in environmental compartments with a high bacterial density [14]. The source of these bacteria as well as the transfer of resistance between different environment compartments, require further investigation and should be included in risk analysis. The results obtained from this research will help in monitoring antimicrobial resistance in environment where there is a high usage antibiotic. However, healthy and preferably antimicrobial naive populations should also be monitored, to have a better overview of the spread of antimicrobial activity in a population. Besides this, it will also give a baseline data about the antibiotic susceptibility pattern of some commonly used antibiotics used in poultry farm.

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