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Occurrence and risk factors associated with antimicrobial resistance of *E. coli*

***Corresponding Author:** Tsion Bilata*

Email: tsi.bilata@gmail.com

Tsion Bilata[2]*; Ephrem Shimelis[1]; Hika Waktole[1]

[1]Addis Ababa University, College of Veterinary Medicine, Department of Veterinary Microbiology, Immunology and Public Health. Bishoftu Ethiopia.

[2]Animal Health Institute, Sebeta, Ethiopia.

Abstract

Antimicrobial resistance is current world wide One Health issues in the areas of human, animal, and environmental fields due to the extensive use and misuse of antimicrobials in the human and animal fields as for growth promotion, prophylaxis and therapeutics. The transfer of antibiotic resistance *E. coli* from animals to humans is of concern in recent times. The b-lactam antibiotics have been used successfully to treat infections caused by pathogenic *E. coli*. However, currently, the utility of b-lactams is being challenged severely by a large number of hydrolytic enzymes the b-lactamases expressed by bacteria. The b-lactam antibiotics have been used successfully to treat infections caused by pathogenic *E. coli*. However, currently, the utility of b-lactams is being challenged severely by a large number of hydrolytic enzymes the b-lactamases expressed by bacteria. The handling and consumption of poultry or poultry products is the risk factors repeatedly associated with the transmission of bacterial pathogens to the human population. The control and prevention of such treats require the knowledge, attitude and practices of using antimicrobials in poultry farm as recommended by different scholars. Successful management of infections caused by such resistant strains requires an understanding of the diversity of b-lactamases, their unambiguous detection, and molecular mechanisms underlying their expression and spread with regard to the most relevant information about individual bacterial species. Properly using the antibiotics as its recommended time intervals and dosages, using specific spectrum rather than broad spectrum, educe the using of antibiotic as prophylaxis and growth promoters in Animal feeds and training of farmers and other community on the public health importance and risk factors associated with the antimicrobial resistant bacterial infections.

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Introduction

Currently the significant failures in treatments of bacteria in field of Veterinary and medical medicine is caused by the emergence of antibiotic-resistant zoonotic bacterial infection in feed of Animal origin [1-30]. The multi-drug resistance is usually emerged from unrecommended rules to use of antibiotics in animal and human health sector [11]. Zoonotic Infectious diseases are rooted in unsustainable and unjust human-animal relationships [20]. The handling and consumption of poultry or poultry products is the risk factors repeatedly associated with the transmission of bacterial pathogens to the human population [14]. Globally, the inappropriate dispensing and use of antibiotics to treat such zoonotic bacteria in animals and the utilization of antibiotic growth promotor in poultry feed has contributed to established and the development of bacterial antimicrobial resistance [8,23]. According to the report of Rahman et al. (2020) antibiotics resistant *Escherichia coli* (*E. coli*) was common in poultry products with the prevalence of 12.8% broiler chicken and 7.61% in the layer chicken in association with presence of the AmpC beta-lactamase producing gene (CITM) in 4.56% and 3.26% of broiler and layer chicken, respectively.

The transfer of antibiotic resistance from animals to humans is of concern in recent times [19]. Extended-spectrum beta-lactamase-producing (ESBL) Escherichia coli, has emerged as a global health threat [5,25]. The control and prevention of such treats require the knowledge, attitude and practices of using antimicrobials in poultry farm as recommended by different scholars [26]. despite, the information, the knowledge and attitude toward the prevalence of antimicrobial resistance E.coli in the animal farms in Ethiopia is rare [12]. The b-lactam antibiotics have been used successfully to treat infections caused by pathogenic *E. coli*. However, currently, the utility of b-lactams is being challenged severely by a large number of hydrolytic enzymes the b-lactamases expressed by bacteria [4]. The b-lactam antibiotics have been used successfully to treat infections caused by pathogenic *E. coli*. However, currently, the utility of b-lactams is being challenged severely by a large number of hydrolytic enzymes the b-lactamases expressed by bacteria [16].

Antimicrobials must be understood as any kind of agent with inhibitory or killing properties to a microorganism [2]. In order to prevent itself from such challenges, the bacteria can use different mechanism including hydrolyze the β-lactam core of the antibiotic by producing β-lactamases enzymes that affect the effective uses of such antibiotics in treatment of infection disease and growth promotor in animal feeds as it develops antibiotics resistance human and animals [23]. The ESBL genes are frequently encoded on transferable plasmids that encode resistance genes, and the acquisition of these resistant genes by commensal or fecal isolates leads, in turn, to Multidrug Resistant (MDR) pathogens [30]. The occurrence of Extended-Spectrum Beta-Lactamase (ESBL)-producing bacteria due to extensive cephalosporin use in the human and food animal is of particular concern. ESBL-producing bacteria was firstly isolated from human clinical practice, but, in recent years, they have been detected in food-producing animals and becoming the food safety issues [10]. One of the groups of antibiotics used to treat the pathogenic bacteria in the world is Beta lactams, however its efficacy is significantly affected by Extended-Spectrum Lactamases (ESBL) enzyme produced by multidrug resistant strains of gram-negative bacilli [3]. The b-lactam antibiotics have been used successfully to treat infections caused by pathogenic *E. coli*. However, currently, the utility of b-lactams is being challenged severely by a large number of hydrolytic enzymes-the b-lactamases expressed by bacteria [4]. The study conducted on the Antibiotic use in Poultry Production in central Ethiopia from Antibiotic Stewardship Perspective by Meazene et al. (2020) clearly demonstrated lack of awareness about antibiotic use and also lack of polices and regulatory system on antibiotic use, in the country as a whole lead to the propagation of antibiotic resistant bacteria and foster accumulation of antibiotic residues in poultry products in the study area, however, it playing an important roles in treatments to reduce mortality and morbidity of pathogenic bacterial infection, antimicrobials treatment have been hindered by the development of drug resistant bacterial infections [30]. Therefore, the aim of current review is to elaborate information concerned about the occurrence and risk factors associated with antimicrobial resistant *E. coli*.

Use of antibiotics

Now days, production animals are exposed to antimicrobial not only during the treatments of infected animals, but also animals can be accessed to antimicrobial during consumption of commercial feed which supplemented with antibiotics as disease prevention and as growth promoters in livestock [11]. For more than fifty years antibiotics (streptomycin, sulfasuxidine, and streptothricin) were used as animal growth promoters in United state (USA) and other developing countries and it shown a significant production result particularly to pig and chicken feed [22]. One main risk factors in antimicrobial resistant was the using non-specific broad spectrum antibiotic in treatments of animals or Homans infection so that in order to reduce this challenges, novel antimicrobial therapies are needed with increased specificity for the site of infection. Photo pharmacology could enable such specificity by allowing for the control of antibiotic activity with light, as exemplified by Trans/Cis-Tetra-Orthochloroazobenzene-Trimethoprim (TCAT) conjugates [15]. For at least 50 years, farms in the United States and other developed countries have used antibiotics as Antimicrobial Growth Promoters (AGPs). AGP was first introduced in the mid-1950s. The use of antibiotics as AGP were for first reported. beta-lactamase (ESBL) has significantly influenced antibiotic choices for controlling severe *E. coli* infections [28].

Causes of Drug resistance

Antimicrobial resistance is current world wide One Health issues in the areas of human, animal, and environmental fields due to the extensive use and misuse of antimicrobials in the human and animal fields as for growth promotion, prophylaxis and therapeutics [21]. Antibiotics are commonly used to treat infections caused by *E. coli* and can reduce morbidity and mortality rates. Unfortunately, as a result of self-medication and the overuse of antibiotics in the poultry business to increase the population, these harmful bacteria are becoming resistant to various first line antibiotics, rendering them ineffective [16]. *Escherichia coli* has been widely used to monitor AMR in livestock and food of animal origin. This is because *Escherichia coli* can be found in the digestive tracts of warm-blooded animals [11]. cephalosporins and monobactams are third generation beta lactam antibiotics that usually hydrolyzed by enzyme generated by bacteria called extended-spectrum beta-lactamase. The gene encoding for this enzyme can easily transferred between bacteria as it encoded in plasmid as plasmid is horizontally

exchanged among bacterial strains. The frequent administering of antibiotics in the treatment of poultry diseases may contribute to emergence of antimicrobial resistant strains [8]. Beta-lactam antibiotics can prevent the multiplication of fast-growing bacteria including E.coli by inhibiting biosynthesis of the enzymes used in cell wall synthesis through the interruption the terminal transpeptidation process and induces, loss of viability and lysis, also through autolytic processes [7,18]. The results also suggest the inadequacy of antimicrobials with a single mode of action to curtail AMR bacteria with multiple mechanisms of resistance and virulence factors. There are four basic mechanisms by which resistance to drug may occur in bacteria: decrease amount of antimicrobial reaches the target by decreasing due to porin mutation or by an exit increase caused by the pumping out by an efflux transporter; the presence of an enzymatic mechanism that totally or partially destroys the antimicrobial molecules; and the development of an alternative metabolic pathway involving precursors. Microbes can simultaneously be resistant to several groups of antibiotics. This event is called multidrug-resistant, i.e., when the microbes are resistant to at least 3 types of antibiotics. This incident is associated with the use of antibiotics that are not according to the rules. Such as excessive use of antibiotics and errors in diagnosing diseases. Not only because of its use in humans but also on farm animals [11]. Numerous genetic mechanisms are concerned in the gaining and distribution of *E. coli* AMR through variable region (as gene cassettes) plasmids, transposons, insertion sequences, and integrons (intI) that previously detected in poultry farms in different studies [21].

Detection of resistant gene

Conventionally antimicrobial resistant test can be conducted by using the disc diffusion method in Mueller-Hinton; while ESBL screening can be done by a double-disc interaction test between the two of 3rd generation cephalosporins (CAZ and CTX) as it interpreted as positive for ESBL- beta-lactams were determined by microdilution using an automatic system (MicroScan® Walkaway system, Beckman Coulter, Inc., Brea, CA, USA). When resistance to at least three families of antimicrobial agents was detected, the isolates were considered Multidrug Resistant (MDR) (21). PCR method can detect the resistant gene in *E. coli* after isolation of *e. coli* from sample, and extract specific gene DNA from E.coli isolate using the boiling method and PCR reactions for specific antibiotics genes primer sequences (Table 1) [27,28].

Table 1: Prime targeting specific resistance gene of different antibiotics.

Antimicrobial agent	Target resistance gene	Primers Sequence	Amplicon size (base pair)	Annealing temperature (oC)	Reference
Streptomycin	*aad* (A1)	F: 5′- TATCCAGCTAAGCGCGAACT- 3′ R: 5′-ATTTGCCGACTACCTTGGTC- 3	447	58	(Puno-Sarmiento *et al* 2013
Tetracycline	*tet* (A)	F: 5′- CCTCAGCTTCTCAACGCGTG- 3′ R: 5′-GCACCTTGCTGATGACTCTT- 3′	634	56	(Puno-Sarmiento *et al* 2013)
	tet (B)	F: 5′- GGTTCACTCGAACGACGTCA- 3′ R: 5′-CTGTCCGACAAGTTGCATGA- 3	577	37	(Puno-Sarmiento *et al* 2013))
Trimethoprim	*dfr* (A1)	F: 5′- GGAGTGCCAAAGGTGAACAGC- 3′ R: 5′GAGGCGAAGTCTTGGGTAAAAAC-3	367	45	Torkan *et al (*2015)
Fluoroquinolone	qnr	F:5′- GGGTATGGATATTATTGATAAAG-3 R: 5′-CTAATCCGGCAGCACTATTTA- 3′	670	50	(Li 2005)
Gentamicin	*aac (3)*-(IV)	F: 5′- CTTCAGGATGGCAAGTTGGT- 3′ R: 5′-TCATCTCGTTCTCCGCTCAT- 3′	286	55	(Van *et al* 2008)
Sulfonamide	*sul* (1)	F: 5′- TTCGGCATTCTGAATCTCAC- 3′ R: 5′-ATGATCTAACCCTCGGTCTC- 3′	822	47	(Van *et al* 2008
Cephalothin	*bla* (SHV)	F: 5′- TCGCCTGTGTATTATCTCCC- 3′ R: 5′-CGCAGATAAATCACCACAATG- 3′	768	52	Van *et al* 2008)
Ampicillin	CITM	F: 5′- TGGCCAGAACTGACAGGCAAA- 3′ R: 5′-TTTCTCCTGAACGTGGCTGGC- 3′	462	47	(Van *et al* 2008)
Erythromycin	ere	F: 5′- GCCGGTGCTCATGAACTTGAG- 3′ R: 5′-CGACTCTATTCGATCAGAGGC- 3′	419	52	(Van *et al* 2008)
Chloramphenicol	*cat* (1)	F: 5′- AGTTGCTCAATGTACCTATAACC-3 R 5′: TTGTAATTCATTAAGCATTCTGCC3	547	55	(Van *et al* 2008)
	cml (A)	F: 5′- CCGCCACGGTGTTGTTGTTATC- 3′ R: 5′-CACCTTGCCTGCCCATCATTAG- 3′	698	55	

Approach to prevent antibiotic resistance caused by beta lactamase

One of the approaches to addressing the antibiotics resistance caused by β-lactamases is to develop β-lactamase stable β-lactam antibiotics, such as extended-spectrum cephalosporins, another method is to produces β-lactam antibiotics in combination of existing β-lactam antibiotics with β-lactamase inhibitors. The common β-lactam antibiotics include amoxicillin/clavulanic acid, ampicillin/sulbactam, and pipercillin/tazobactam [13,24].

The menace is further compounded by the highly flexible genome of *E. coli*, and propensity of resistance dissemination through horizontal gene transfer and clonal spread. Successful management of infections caused by such resistant strains requires an understanding of the diversity of b-lactamases, their unambiguous detection, and molecular mechanisms underlying their expression and spread with regard to the most relevant information about individual bacterial species [4]. β-lactam antibiotics act by inhibiting the bacterial cell wall biosynthesis; they are the most available antibiotics which treat a number of bacterial infections. A broad spectrum of bacteria can be killed by β-lactams and its toxicity to humans is very low this implies that, the resistance to β-lactam antibiotics is severe threat, 5 bacteria and other infection causing microbes are remarkably developed several ways to become resistant to antibiotics and other antimicrobial drugs. The MICs of oxyimino-b-lactams and clavulanic acid were determined at a fixed concentration of 4 mg/l. The production of *E. coli* Extended-spectrum beta-lactamases (ESBLs) was determined using the Double-Disk Synergy Test (DDST). Specifically, this was performed with cefotaxime (30 µg) and ceftazidime (30 µg) disks placed at a distance of 20mm (center to center) from the amoxicillin-clavulanic acid disk (20/10 µg).

Promoting the appropriate or prudent use of antimicrobials from the doctor and the pharmacist to the patient is the determinant in reversing the increasing rates of AMR [9]. By employing a variety of prevention strategies, including proper personal hygiene, prescreening for carrier status before hospital admission, disinfection of hospital rooms, and careful monitoring of antimicrobial prescribing, marked progress can be achieved in the control of drug-resistant pathogens, which can translate into more effective antimicrobial therapy [6]. Early and continuous access to data on antibiotic use and AMR made it possible to focus activities on areas of concern. Another factor identified was the long-term control and eradication of infectious animal diseases, including coordinated activities against endemic diseases, which reduced the need to use antibiotics. Structures and strategies for that purpose established at the national level have since proven useful in counteracting [29]. Develop awareness of the community to the Problems of AMR, the ban import and use of resistant ant developed antibiotics, continuous surveillance on antibiotic resistance patten in animals and animal product and prevention of Specific Resistance, bacteria through good hygiene [29].

Conclusion and recommendation

Antimicrobial resistance is current world wide One Health issues in the areas of human, animal, and environmental fields due to the extensive use and misuse of antimicrobials in the human and animal fields as for growth promotion, prophylaxis and therapeutics. The b-lactam antibiotics have been used successfully to treat infections caused by pathogenic *E. coli*. However, currently, the utility of b-lactams is being challenged severely by a large number of hydrolytic enzymes the b-lactamases expressed by bacteria. Successful management of infections caused by such resistant strains requires an understanding of the diversity of b-lactamases, their unambiguous detection, and molecular mechanisms underlying their expression and spread with regard to the most relevant information about individual bacterial species.

Based on the above conclusion the following Recommendation was forwarded:

• Properly using the antibiotics as its recommended time intervals and dosages.

• Using specific spectrum rather than broad spectrum.

• Reduce the using of antibiotic as prophylaxis and growth promoters in Animal feeds.

• Frequently conducting of antibiotics resistance Bacterial species surveillances on animals and food of animal origin.

• Confirming of antibiotics medication history of live animals before processing and consumption food of animal origin.

• Prevention and controls of antibiotics resistance bacteria in food animal farms.

• Training of farmers and other community on the public health importance and risk factors associated with the antimicrobial resistant bacterial infections.

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