International Journal of Advanced Multidisciplinary Research
ISSN: 2393-8870
www.ijarm.com
DOI: 10.22192/ijamr Volume 7, Issue 3 -2020

Review Article

DOI: http://dx.doi.org/10.22192/ijamr.2020.07.03.003

Antimicrobial Susceptibility Pattern of *Escherichia coli*: A Review

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Keywords

Escherichia coli, Antimicrobial resistance.

Abstract

The study conducted to isolate and characterize *Escherichia coli* from dairy product, to determine antimicrobial susceptibility for the isolates, and to identify hygienic practice condition considering dairy farms (farm management and dairy production enterprise) along with hygienic practice status associated with pathogens. Dairy breeds (pure jersey, pure Holstein Friesian and Jersey cattle crossed and local breed) found in semi intensive production system, milk shop, and local market will be studied. The environment to be considered in the study includes feaces from cattle house floor, milk container and equipment, milkier handle. Milk and milk product Samples will be collected from dairy farm and environment to isolate and detect *Escherichia coli* and then test for antimicrobial susceptibility. Hygienic practice, sanitation and awareness status in relation to pathogens will also studied using structured questioner. The finding will have greater positive impact for the production and improvement of dairy production via preventing zoonosis and improve public health.

Introduction

Escherichia coli O157:H7 and *Staphylococcus aureus*, are the main microbiological hazards among the most pathogenic bacteria that linked to raw milk (Kousta *et al.*, 2010; Yang *et al.*, 2012; Claeys *et al.*, 2013) and raw cheese. *Escherichia coli* O157:H7 was isolated from commercially distributed raw milk, pasteurized milk and also from cheese (Mora, *et al.*, 2007). *E. coli* strains can cause a variety of diseases, including diarrhea, dysentery, and hemolytic uremic syndrome, bladder and kidney infections and death. Enterohemorrhagic *E. coli*(EHEC) or shiga toxin producing *E. coli* (STEC).The main reservoirs of Shiga toxin-producing *E. coli* are ruminants, contaminating milk through subclinical mastitis or feces, and the bacteria can persist in milking equipment (Arqués *et al*., 2015). The main route of STEC infections in humans is via consumption of contaminated food. STEC cause severe clinical syndromes in humans such as haemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS. Enterotoxigenic *E. coli* (ETEC), which induce traveller's diarrhoea, Entero pathogenic *E. coli* (EPEC), which cause a persistent diarrhoea in children living in developing countries, Enteroadherent *E. coli* (EAEC), which provoke diarrhoea in children, Entero invasive*E.coli* (EIEC) that are biochemically and genetically related to *Shigella* species and can induce diarrhoea, Diffusely adherent *E. coli*, which cause diarrhoea and are distinguished by a characteristic type of adherence to mammalian cells (Fratamico, and Smith 2005).

National Hygiene and Sanitation Strategy program (W H O, 2005) reported that about 60% of the disease burden is related to poor hygiene and sanitation in Ethiopia. Unsafe sources, contaminated raw food items, improper food storage, poor personal hygiene during food- preparation, inadequate cooling and reheating of food items and a prolonged time lapse between preparing and consuming food items were mentioned as contributing factors for outbreak of foodborne diseases. Different parts of the country showed the poor sanitary conditions of catering establishments and presence of pathogenic organisms like *Staphylococcus aureus*, *Escherichia coli*, and others. (Tefera, *et al*., 2009).

Antimicrobial resistance is a major and increasing global healthcare problem. Since the introduction of the penicillin, a large number of bacteria have responded to the use of antibiotics with their ability to evolve and transmit antimicrobial resistance to other species. Increased consumption of antimicrobial agents and their inappropriate use are among factors which further accelerated this phenomenon. Furthermore, the continuous migration of people between countries as well as international tourism and business travel play an important role in the acquisition and spread of multidrug resistant strains. Antimicrobial resistance was also observed in animals, where the antimicrobials are used for therapy and prophylaxis of infectious diseases. The therapeutic treatment of *E. coli* infections is threatened by the emergence of antimicrobial resistance and it's strains prevalence of multidrug-resistant is increasing worldwide principally due to the spread of mobile genetic elements, such as plasmids.

Insight towards isolation and identification of the possible sources of *Escherichia coli and, Staphylococcus aureus* for effective control and prevention of the disease, as well as prevention of antimicrobial resistant of the bacteria and in addition to maintain hygienic practice for dairy production.
The objectives of this study are:-

- To isolate *Escherichia coli* and *Staphylococcus aureus* in different dairy farms.
- To assess hygienic practice and awareness status in dairy farms associated with pathogens.

Review of Literature

Escherichia coli

Escherichia coli, originally known as *Bacterium coli* commune, were identified in 1885 by the German paediatrician, Theodor *Escherich*. Strains of *Escherichia coli* are a common part of normal microbial flora of animals, including humans. Most strains are harmless, but some cause diarrhea. Strains carrying particularly virulent properties have emerged as a serious hazard, with the consumption of even low numbers of these organisms bearing the risk for life threatening illness (ICMSF, 2002).

Escherichia coli are in the family Enterobacteriaceae. The organism is a Gram- negative, non-spore-forming, straight rod (1.1 µm x 2.0-6.0 µm) arranged in pairs or singly; it is motile by means of peritrichous flagella or may be non-motile and may have capsules or microcapsules. It is a facultative anaerobic micro-organism with an optimum growth temperature of 37o C. *Escherichia coli* is oxidase-negative, catalase-positive, fermentative (glucose, lactose, D-mannitol, D-sorbitol, arabinose, maltose), reduces nitrate and is β-galactosidase-positive. Approximately 95% of the strains are indole and methyl red positive. All strains of *E. coli* are negative in the Voges-Proskauer test. Most strains do not hydrolyses urea Or produce H_2S in triple sugar iron (TSI) medium and are unable to use citrate as a sole carbon source (Simmon's citrate negative) (Wilshaw, 2000).

Cattle are the major reservoirs of E. *coli* O157:H7followed by sheep and goats. The pathogen is carried in the intestinal tract and excreted in faeces (Battisti *et al*., 2006, Chapman *et al*., 2001). Consumption of raw or undercooked foods, especially undercooked minced beef has been found to be the most common means of transmission (Chapman *et al*., 2001). Little is known about the prevalence of this serogroup and associated genes in humans, animals or in foods of animal origin in Ethiopia (Hiko *et al*., 2008). Despite being part of the normal intestinal flora, *E. coli* is found in aquatic ecosystems. The strains isolated from aquatic environments of 13 districts in Bangladesh revealed O161 to be the predominant serotype (19 %) followed by O55 and O44 (12 %) and 11 % untypable. Serotype based-

pathotyping of the *E. coli* strains revealed 47 %, 30 % and 6 % to belong to be Entero Pathogenic *E. coli* (EPEC), the Entero Toxigenic *E. coli* (ETEC) and the EnteroHaemorrhagi c*E. coli* (EHEC) pathotypes respectively (Alam*et al*., 2006). Kumar *et al*., 2005, reported that estuariesand coastal water bodies, which are the major sources of seafood in India, are often contaminated by human activities and are associated with the widespread occurrence of *E. oli* in seafood.

Food-borne outbreaks associated with Shiga toxin producing*E. coli* (STEC) have been well documented worldwide. The occurrence of the STEC has been reported in a number of food products such as beef, pork, lamb, poultry and fish, and Kumar *et al*., reported in 2001 that STEC is prevalent in seafood's in India. Dalwara *et al*., 1987, reported that shrimps destined for import and export industry tested positive for *E. coli*; these were 14 for fresh water (FW) shell-on; 5 for FW peeled deveined; 3 for Sea Water (SW) shell-on; 3 for SW peeled deveined and 3 for seawater cooked shrimps in Singapore respectively.

General Characteristics and Epidemiology:

Escherichia coli (E. coli) are bacterium which belongs to family enterobactereriae and are gram negative rod up to 3 um in length, ferment glucose and wide range of sugars. These lactase fermenters produce pink colonies on Mcconkey agar. Hemolytic activity on blood agar is characteristics of certain strain of E. coli. It's motile with peritrichous flagella and often fimbriate.The O157: H7 is the major serotype that was recognized as a cause of human illness. E. coli O157:H7 is one of the more than 60 serotypes of verotoxin producing *E. coli* that cause that a variety of human illness such as mild diarrhea, hemorrhagic colitis and hemolytic-uremic syndrome (HUS). Enterohemorrhagic *E .coli* and verocytotoxin producing *E. coli* are being recovered in humans and animals and they constitute major food borne illness. *E. coli* O157:H7 is an important of serotype and it seems to predominate in most areas. The strains producing verotoxin are shiga like toxin (SLT) which produces diarrhea in humans and animals. In most cases cattle are represents the main reservoir of *E. coli*. *E. coli* O157: H 7 is transient inhabitant of gastrointestinal tract of normally ruminant. Source of infection is contamination of food by human and animal feces. The organism can persist in manure, water trough and other farm location. The association of *E. coli* O157: H 7 with raw meat, under cooked ground beef and raw milk lead to investigation of the role of cattle as a reservoir of the pathogens (Buchanan and Doyle, 1997).

Pathogenesis and Symptoms:

Enterohemorrhagic *E .coli (*EHEC) strain may produce one or more types of cytotoxins which are collectively referred as shiga-like toxins (SLTs) since they are antigenically and functionally similar to shiga toxin produced by *Shigella dysenterica*. SLTs were previously known as verotoxin. The toxins provoke cell secretion and kill colonic epithelial cells. Enterohemorrhagic *E. coli* are characterized by presence of SLTs genes, locus for enterocyte effacement (LEE) and higher molecular-weight plasmid that encodes for a hemolysin. These three virulence factors are present in most *E. coli* associated with bloody diarrhea and hemolytic uremic crisis in humans (Radostits *et al*., 2007).

The most virulent factor of *E. coli*O157: H 7 is the production of cytotoxic SLT. *E. coli*O157: H 7 likely gained ability to produce the SLT1 and SLT2 as a result of ingestion with a bacteriophage carrying SLT1 and SLT2 genes. The SLTs of *E. coli* O157: H 7 is cytotoxic to human colon and ileum cells. In animals, toxin has been shown to cause localized fluid accumulation and colonic lesion characterized by sloughing of surface and crypt epithelial cell. The incubation period is 72-120 hours. The clinical sign initially may be diarrhea with abdominal cramps, which may turn into grossly bloody diarrhea in a few days. There is however, no fever. The symptoms of *E. coli* septicemia are mainly referable to bacteremia, end toxemia and the effect of bacteria localization in a variety of tissue spaces throughout the body (Bryan, 1994).

Antimicrobial resistance of *E .coli*

Antimicrobial resistance is a major and increasing global healthcare problem. Since the introduction of the penicillin, a large number of bacteria have responded to the use of antibiotics with their ability to evolve and transmit antimicrobial resistance to other species. Increased consumption of antimicrobial agents and their inappropriate use are among factors which further accelerated this phenomenon. Furthermore, the continuous migration of people between countries as well as international tourism and business travel play an important role in the acquisition and spread of multidrug resistant strains.

Antimicrobial resistance was also observed in animals, where the antimicrobials are used for therapy and prophylaxis of infectious diseases. As in humans, the use of antimicrobials leads to an increased incidence of resistance in both pathogenic and endogenous bacteria. Resistant bacteria from animals can infect humans by direct contact as well as via food products of animal origin. Multidrug resistance is defined as resistance to three or more antimicrobial classes to which bacteria do not show intrinsic resistance. Multi-resistant strains are on the rise worldwide principally due to the spread of genes located on mobile genetic elements, including plasmids, integrons and transposons. Furthermore, the combination of these genes with chromosomally encoded resistance genes frequently results in bacteria that are resistant to all main classes of available antimicrobials (Johnson and Nolan, 2009). Antibiotic pollution promotes the fixation and mobilization of resistance genes between natural and clinical environments with world-wide spreading of resistance traits (Gillings, 2013).

Control and prevention of *E .coli*

In general, strategies for the prevention and control of the spread of E. coli should include access to safe water, good handling practices to reduce the risk of food contamination, sanitation measures, public education and vaccination (Mielke, 2010, Seib *et al*., 2012). Measures to prevent infections from food products include appropriate storage and cooking temperatures. Food irradiation technology may be used to drastically reduce bacterial load in high-risk products.(EU Directive 1999/2/EC and 2009/C 283/02)Hospital measures that limit risk of the spread of multi resistant pathogens include prevention of cross-contamination by implementing strict hygienic standard protocols as well as control over the use of antimicrobial drugs (Mielke, 2010). The main vehicles for pathogens' spread are the hands of hospital workers and medical devices. Proper hand hygiene is critical for the prevention of cross-contamination.

Antibiotics are essential for the control and treatment of *E. coli* infections in humans and animals. However, it is generally accepted that antimicrobial resistance is associated with the quantity of antibiotic consumption. Greater attention should be given to the risks associated with release of antimicrobials into environment. Probiotics could be an approach to the prophylaxis of several *E. coli* infections. Treatment of infectious diarrhea with probiotics demonstrated beneficial effects by reducing diarrhea rates. The use of Lactobacillus, which is part of the microbiota in healthy humans, in the form of probiotics reduced the risk of UTI and vaginal infections.Effective vaccine that may have a substantial impact on children's health in developing countries as well as protect travellers when visiting ETEC endemic areas. In some countries, a cholera vaccine was used against ETEC strains to stimulate anti-heat-labile toxin immunity for short term protection (Al-Abri *et al*., 2005).

Conclusion and recommendations

Escherichia coli O157:H7 are the main microbiological hazards among the most pathogenic bacteria that linked to raw milk. The study conducted to isolate and characterize *Escherichia coli* from dairy product, to determine antimicrobial susceptibility for the isolates, and to identify hygienic practice condition considering dairy farms along with hygienic practice status associated with pathogens. Dairy breeds found in semi intensive production system, milk shop, and local market will be studied.

The following recommendations forwarded:-

- The environment should be considered in the study includes feaces from cattle house floor, milk container and equipment, milkier handle.
- Milk and milk product Samples should be collected from dairy farm and environment to isolate and detect *Escherichia coli* and then test for antimicrobial susceptibility.

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Quick Response Code

DOI:10.22192/ijamr.2020.07.03.003

How to cite this article:
Tekalign Woldehana Uro. (2020). Antimicrobial Susceptibility Pattern of *Escherichia coli*: A Review. Int. J. Adv. Multidiscip. Res. 7(3): 18-22.
DOI: http://dx.doi.org/10.22192/ijamr.2020.07.03.003