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Molecular Isolation and Identification of Multidrug-resistant *Escherichia coli* from Milk, Meat, and Product Samples

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Abstract

Pathogens can acquire resistance to antimicrobials used in veterinary and medical fields. Such pathogens can be found in several dietary and environmental sources. As Gram-negative infections in humans are most frequently caused by *Escherichia coli* (*E. coli*), antibiotic resistance in this organism is particularly concerning. This investigation was carried out to ascertain the antibiotic sensitivity profile of *E. coli* isolated from various food products randomly gathered from Egypt. To extract *E. coli* and examine its pattern of antibiotic susceptibility, 100 samples of raw milk, karish cheese, ground beef, and beef were bacteriologically processed. In the current study, *E. coli* strains were detected at a high frequency of 40% in raw milk, 28% in Karish cheese, 16% in ground beef, and 8% in beef. *E. coli* was isolated from 23% of milk, meat, and product samples. The 16S rRNA gene was detected using polymerase chain reaction (PCR) to confirm *E. coli* strains. The isolates of *E. coli* with the greatest percentages of multidrug-resistant (MDR) were tetracycline (26%), ampicillin (21.7%), streptomycin and sulfamethoxazole-trimethoprim (17.3%), cefotaxime, kanamycin and ceftazidime (13 %). The total occurrence of MDR *E. coli* was 34.7%. Pathogen cycling in food is common and may endanger the consumer's health. To avoid this entirely, good hygiene practices for dairy farms and abattoirs are essential for preventing contamination of milk, meat, and product samples.

KEYWORDS
Multidrug-resistant, *Escherichia coli*, Milk, Meat, Molecular

INTRODUCTION

Public health has increasingly become concerned about antibiotic resistance. The World Health Organization (WHO) has suggested a global surveillance system in veterinary and human medicine as a solution to the issue (Rahman *et al*., 2020).

The most prevalent species of facultative anaerobic bacteria in both animal and human gastrointestinal systems is *E. coli*. It is normally a benign germ, but it can potentially be hazardous bacteria that can lead to several debilitating conditions (Friedman *et al*., 2002). *E. coli* is one of the considerable causes of foodborne infectious disease, Inadequate sanitary techniques applied during the handling and processing of meat, dairy, and their products could be one probable entry site for different microorganisms (Kiranmayi *et al*., 2011)

Microorganism concentrations are more likely to be high in foodstuffs, especially that from animal origin especially milk, meat, and product samples, which are due to their nature- one of the most fertile environments for the growth of *E. coli* bacteria, and are exposed to contamination from several ways during production, distribution, and storage. Contamination of milk, meat, and product samples with *E. coli* is a direct threat to consumer health especially multi-drug resistance species (Ombarak *et al*., 2016).

Identical *E. coli* was discovered in both the food and the patients who consumed it (Molbak, 2004). When bacteria enter the colon after passing through the digestive system, the local flora usually serves as a barrier to keep the outside bacteria out. Drug resistance can still spread throughout the GI tract. But if our meal contains a significant amount of resistant microorganisms, it might be a significant contributor to the fecal flora's resistance. Antibiotic resistance is thought to be primarily caused by antibiotic overuse. Horizontal gene transfer or gene modifications are used to gain this resistance (Hughes and Andersson, 2015). Multidrug-resistant (MDR) bacteria typically have multiple drug-resistance genes. Human morbidity and mortality have increased due to the extremely rapid occurrence of MDR *E. coli* strains (de-Been *et al*., 2014).

Bacterial enzymes known as beta-lactamases hydrolyze the beta-lactam ring to confer resistance to beta-lactam antibiotics such as penicillin and cephalosporin. AmpC beta-lactamases and Extended-spectrum beta-lactamases (ESBLs) are two novel varieties of beta-lactamase enzymes that have appeared in recent years (Babic *et al*., 2006). On mobile genetic elements, ESBLs and AmpCs are primarily found. By horizontal gene transfer processes including transduction, transformation, and conjugation, these mobile genetic components are transmitted to other bacterial cells. A source of AmpC- and ESBL-developing *E. coli* in meat and retail animals raised for food. (Maleki, *et al*., 2015)

Egypt is a developing country with poor sanitary raw food

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processing technology and inadequate antimicrobial drug resistance management. The possibility of the formation of resistant bacterial strains grows with the unchecked use of antibiotics for the diagnosis, treatment, and control of diseases in food animals. In this investigation, we found and isolated strains of *E. coli* in milk, meat, and product samples from popular stores in Egypt. Furthermore, tetracycline and other regularly used antibiotics were examined for resistance by these isolates.

MATERIALS AND METHODS

Samples

From 50 different locations around Egypt, 100 samples total of 25 numbers each of raw milk, karish cheese, ground beef, and beef were chosen at random. Within two hours of the time of purchase, The samples were taken in a sterile manner, wrapped in aseptic polythene zip bags, and transported to the lab in sterile circumstances. When possible, duplicate samples were acquired. Within 2h of the samples' incoming at the research lab, each sample was examined. Samples were cut from surfaces in sterile plates using a sharp sterile knife.

Isolation and identification of E. coli.

A 25 g of the samples were mixed with 225 mL of sterile tryptic soy broth (TSB) (Sigma-Aldrich, Germany) and left there for 6–8 hours at 37°C to isolate bacteria. The TSB culture was streaked onto MacConkey plates (oxide, UK) and incubated for 24 hours at 37°C (Ethelberg *et al*., 2009). Gram stain, motility, and common biochemical tests, such as catalase, oxidase, production of indole, fermentation of lactose and glucose using triple sugar iron agar, Voges Proskauer test, methyl red test, urease test, and citrate utilization, were used to select and identify lactose-fermenting colonies (Quinn *et al*., 2002). *E. coli* isolates that had undergone biochemical confirmation were tested for antimicrobial sensitivity.

Molecular recognition of E. coli

PCR assays were utilized to further identify the recovered *E. coli* strains. Table 1 contains a list of the amplification primers (Eurofins, Yokohama, Japan). A 585 bp amplicon produced by a species-specific PCR encoding the 16S rRNA genes was used to validate the occurrence of isolated *E. coli*. Moreover, the reaction mixture was divided into five-microliter aliquots, and the samples were electrophoresed on 1.5% agarose gels from Sigma-Aldrich in the United States, stained with ethidium bromide, and examined under UV light afterward.

Antibiotic susceptibility testing

Each of the 23 *E. coli* isolates was examined for antimicrobial susceptibility. As per the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2016), the procedure of disc diffusion was used to test twelve different antimicrobials. As a quality check, *E. coli* strain ATCC 25922 was utilized. The following antimicrobials were employed (Oxoid Ltd.): ciprofloxacin (CIP; 5 µg), ampicillin (AMP; 10 µg), streptomycin (STR; 10 µg), sulfamethoxazole-trimethoprim (SXT; 1.25 µg/23.75 µg), cefotaxime (CTX; 30 µg), ceftazidime (CAZ; 30 µg), kanamycin (KAN; 30 µg), imipenem (IPM; 30 µg), tetracycline (TET; 30 µg), chloramphenicol (CHL; 30 µg), nalidixic acid (NAL; 30 µg), and fosfomycin (FOS; 50 µg). As stated by the Clinical and Laboratory Standards Institute's zone diameter interpretative standards recommendations.

RESULTS

Prevalence of E. coli strains in milk, meat, and their products

Among the 100 samples, 23% (23) *E. coli* bacteria were returned from milk, meat, and product samples and were verified by PCR analysis (Fig. 1). In terms of sample type, raw milk had the highest incidence of *E. coli* strains at 40% (10/25) followed by 28% (7/25) Karish cheese, 16% (4/25) ground beef, and 8% (2/25) beef (Table 3).

Antibiotic susceptibility testing

The antimicrobial susceptibility of 23 samples of *E. coli* recovered from raw milk, Karish cheese, ground beef, and beef was assessed. As shown in Table 4 the isolates of *E. coli* were resistant to tetracycline (26%), ampicillin (21.7%), streptomycin, and sulfamethoxazole-trimethoprim (17.3%), cefotaxime, kanamycin and

Table 1. Specific gene and primer sequences of *E. coli*.

Target genes	Primer Sequences	Sequences (5′–3′)	Amplified segments	References
Gen *16s rRNA*	ECO-f ECO-r	GAC CTC GGT TTA GTT CAC AGA CAC ACG CTG ACG CTG ACC A	585 bp	Seidavi *et al. (*2010)

Table 2. Amplification cycle of Gene 16s rRNA in *E. coli*.

Primary denaturation	Amplification (30 cycles)			Final extension	References
	Secondary denaturation	Annealing	Extension		
95°C 2min	95˚C 1min	57˚C 1min	72˚C 2min	72°C 5min	Seidavi *et al.* (2010)

Table 3. Prevalence of E.coli strains (n=23) in milk, meat and their products (n=100).

Types of samples	No. of samples	No. (%) of positive isolates
Raw milk	25	10 (40%)
Karish cheese	25	7 (28%)
Ground beef	25	4 (16%)
Beef	25	2 (8%)
Total	100	23 (23%)

ceftazidime (13%), chloramphenicol and nalidixic acid (8.6%), and ciprofloxacin (4.3%), respectively. None of them were resistant to fosfomycin and imipenem. The resistance phenotypic profiles of *E. coli* are shown in Table 5. Eight (34.7%) of the 23 *E. coli* isolates were MDR, or resistant to a minimum of three separate classes of antimicrobial drugs, meaning they were resistant to one or more antimicrobials.

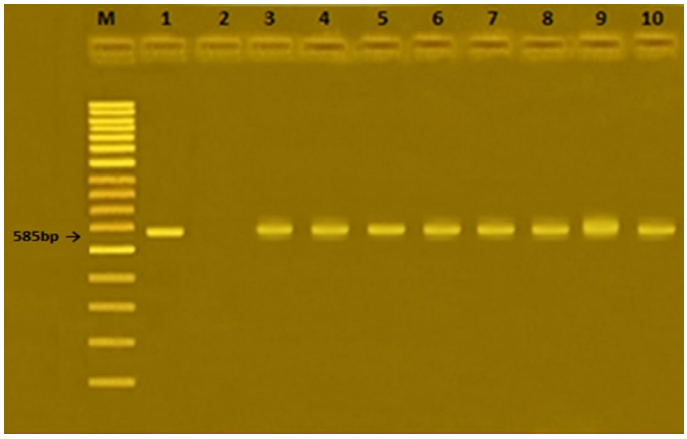

Fig. 1. Representative agarose gel electrophoresis showing amplified PCR products in *E. coli* isolated of 16s rRNA gene (585bp). Lane M: 100 bp ladder; lane 1: positive control; lane 2: negative control *E. coli*; lanes 3-10: positive samples.

DISCUSSION

One of the most significant pathogenic gastrointestinal bacteria that affect human health is *E. coli*. It is regarded as the main contributor to high morbidity and is a significant factor in several foodborne infections, including neonatal diarrhea (Rahman *et al*., 2020). Consequently, the objective of our investigation was to identify *E. coli* occurrence and antimicrobial resistance patterns from milk, meat, and product samples from different Egyptian markets.

In the current investigation, the presence of *E. coli* was checked in 100 randomly selected samples of milk, meat, and products by using a species-specific PCR encoding the 16S rRNA gene. According to sample type, raw milk had the highest incidence of *E. coli* strains at 40% (10/25) followed by 28% (7/25) Karish cheese, 16% (4/25) ground beef, and 8% (2/25) beef.

The occurrence of *E. coli* in raw milk (40%) and karish cheese (28%) was observed to be lower in this study than those obtained by other authors: 76.4 to 74.5% (Ombarak *et al*., 2016) in Egypt, 66.6% (Gran *et al*., 2003) in Zimbabwe, 57% (Singh and Prakash, 2008) in India and 50 to 65% (Soomro *et al*., 2002) in Pakistan. However, its incidence rate in raw milk and Karish cheese is more than 26% published before (Farzan *et al*., 2012) in Iran and 23.3% (Lues *et al*., 2003) in South Africa. This might be due to several factors such as climatic conditions, dryness, humidity, and hygiene of milk production and processing. The reported isolation rates of *E. coli* in ground beef (16%) and beef (8%) was less than other reported as 48.2% to 25% (Eyý and Arslan, 2012) in turkey and 73% to 100% (Thorsteinsdottir *et al*., 2010) in Iceland and more than 4.1% to 14.9% was reported by Lee *et al*. (2009) in Korea.

In human and veterinary medicine, both in developed and developing nations, antimicrobial resistance has been identified as a global issue that is on the rise. Furthermore, it is widely known that the extensive use of antibiotics in both medicine and agriculture is acknowledged as a key choosy factor in the majority of antibiotic resistance in Gram-negative bacteria. MDR Bacteria used in veterinary or human treatment can be found in a variety of dietary and environmental sources, as well as in the production of food animals (Rahman *et al*., 2020).

Table 4. Antimicrobial resistance test.

Antibiotics	Isolate (n.=23)		
	Sensitive N (%)	Intermediate N (%)	Resistant N (%)
Tetracycline (TET)	17 (73.9%)	0 (0%)	6 (26%)
Ampicillin (AMP)	17 (73.9%)	1 (4.3%)	5 (21.7%)
Streptomycin (STR)	18 (78.2%)	1(4.3%)	4 (17.3%)
Sulfamethoxazole-trimethoprim (SXT)	19 (82.6%)	0 (0%)	4 (17.3%)
Cefotaxime (CTX)	18 (78.2%)	2 (8.6%)	3 (13%)
Kanamycin (KAN)	18 (78.2%)	2 (8.6%)	3 (13%)
Ceftazidime (CAZ)	17 (73.9%)	3 (13%)	3 (13%)
Chloramphenicol (CHL)	15 (65.2%)	6 (26%)	2 (8.6%)
Nalidixic acid (NAL)	16 (69.5%)	5 (21.7%)	2 (8.6%)
Ciprofloxacin (CIP)	20 (86.9%)	2 (8.6%)	1 (4.3%)
Fosfomycin (FOS)	22 (95.6%)	1(4.3%)	0 (0%)
Imipenem (IPM)	23 (100%)	0 (0%)	0 (0%)

Table 5. Resistance pattern in isolated *E. coli*

Resistant pattern	No. and % of isolates
TET/ AMP / STR / SXT / KAN	2 (25%)
TET / AMP / STR / CTX / NAL	1 (12.5%)
TET / AMP / STR/ KAN/ CIP	1 (12.5%)
TET / AMP/ SXT/ CAZ	1 (12.5%)
SXT / CTX / CAZ	1 (12.5%)
TET / CTX / CAZ	1 (12.5%)
CHL / NAL / CHL	1 (12.5%)
Total	8 (100%)

This investigation reveals that *E. coli* in milk, meat, and product samples exhibit widespread MDR. Tetracycline (26%) and ampicillin (21.7%), streptomycin and sulfamethoxazole-trimethoprim (17.3%), cefotaxime, kanamycin, and ceftazidime (13%) were the antibiotics with the most common resistance.

These investigations are in agreement with a wide range of international investigations, including USA (Srinivasan *et al*., 2007), Germany (Meyer *et al*., 2008), Tunis (Jouini *et al*., 2009), Korea (Ryu *et al*., 2012), and Egypt (Ombarak *et al*., 2018). In contrast, 8.6% of the *E. coli* in this investigation exhibited chloramphenicol resistance. Many nations have outlawed and disapproved of the utilization of chloramphenicol in animals raised for human utilization, but Egypt continues to have chloramphenicol resistance.

Resistance to many drugs Humans can be exposed to germs either directly through direct interaction with animals or indirect contact with them via environmental channels. In the current investigation, 34.7% of isolated *E. coli* strain was antibiotic-resistant and displayed resistance to more than two classes of antibiotics. While India (Mathai *et al*., 2008) and the USA (Sahm *et al*., 2001) found a low incidence of MDR *E. coli* isolates (7.1% and 8.4%, respectively), Egypt (Moawad *et al*., 2017; Ombarak *et al*., 2018) showed a high incidence of MDR *E. coli* strains (61.9%- 50%).

To draw attention to potential horizontal gene transfer and MDR by these zoonotic organisms, more research should be done to describe *E. coli* isolates of animals and their products that come from a similar area and have similar resistance indicators.

CONCLUSION

The results of this investigation prove the value of Egyptian milk, meat, and related items as a reservoir for AMR *E. coli*. Multidrug-resistant strains are concerning because they represent a serious risk to the general health of the public. This potential risk highlights the importance of employing efficient hygienic and sanitation practices in the production of milk, meat, and a product sample as well as judicious use of antimicrobials in animals raised for food production to reduce the danger of infection with antibiotic-resistant bacteria.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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