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Prevalence and Antibiogram profiles of Shiga toxin-producing *Escherichia coli (STEC)* strains isolated from raw cow's milk in New-Valley Governorate, Egypt

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Abstract

A cross sectional study was conducted to isolate and identify Shiga toxin-producing *Escherichia coli (STEC)* from raw cow milk. A total of 267 milk samples were collected from apparently healthy cattle in 7 different dairy farms. Thirty-nine *STEC* isolate were identified out of 86-positive *E. coli* strains (56.98%). The prevalence of *E. coli* and *STEC* was 32.2% and 18.4%, respectively. Antimicrobial susceptibility of the isolated *STEC* was determined by disc diffusion method. A total of 16 antimicrobials of seven antimicrobial classes were used. Commercially available antibiotic disc namely aminoglycosides [Erythromycin (15 µg), Clindamycin (2µg, Kanamycin (30 µg, Amikacin (30 µg) , Gentamicin (10 µg), cephalosporins (Cefepime (30 µg, Cefotaxime (30µg), Cefazolin (30µg], Fluoroquinolones [Naladixic acid (30 µg), Ciprofloxacin (5 µg), Levofloxacin (5µg)], penicillin (Ampicillin 10 µg),Tetracycline (tetracycline 30 µg), Sulphonamides (Sulphamethoxazol 30 µg, and Carbapenems [Meropenem 10 µg, Imipenem 10 µg] were used. *E. coli* strain ATCC25922 was used as a control strain in the experiment. Highest resistance was observed against erythromycin (100%) followed by cefepime (97.4%) and clindamycin (82.1%), Nalidixic acid (61.5%), sulphamethazon (48.7%), tetracycline (41.0), kanamycin (33.3%), and cefotaxime (33.3%). Carapenems were found to be the most effective antimicrobial group where the examined *STEC* isolates were susceptible to Imipinem at 97.4% (38/39), followed by Meropenem at 94.9% (37/39). Results showed that multidrug resistance *STEC* isolates were 81.25% of the tested antimicrobial (13/16). In conclusion, high prevalence of STEC in raw milk indicate insufficient hygienic measures adopted during milking and handling and verify that raw milk is potential source of these multidrug resistant strains.

Keywords:

Antimicrobial susceptibility, *Escherichia coli*, Prevalence, Raw milk, STEC.

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Introduction

Shiga toxin-producing *Escherichia coli* (*STEC*) have been identified as a global cause of severe human gastrointestinal disease and life-threatening hemolytic uremic syndrome), which is the major cause of acute renal failure specially in children. *E.coli* that contain one gene encoding Shiga toxins are important human and animal's pathogen. They first came to medical attention in 1983 with two nearly concurrent reports, one of which identified *E.coli* O157:H7 in the stools of patients with bloody diarrhea who had been exposed to undercooked hamburgers (Riley et al., 1983), and other identified *E.coli* O157:H7 and Shiga toxin-producing *E.coli* (*STEC*) of different serogroups in the stools of children with hemolytic uremic syndrome (HUS) (Karmali, et al., 1989). *STEC* is considered as one of the most important recently emerged group of food-borne pathogens. Since that time, it has been implicated in a series of outbreaks primarily in Canada, Japan, the United Kingdom, and the US (Karmali, 1989; Beutin, et al., 1998; Pato et al., 1998, and Willshaw et al., 2001).

Pathogenic *E.coli* group consist of many strains, which for simplicity, can be grouped according to the virulence factors they have or diseases they cause. The intestinal pathogenic *E.coli* include enterotoxigenic *E.coli* (ETEC), enteroaggregative *E.coli* (EAEC), enteropathogenic *E.coli* (EPEC), enteroinvasive *E.coli* (EIEC), hemorrhagic *E.coli* (EHEC), diffusely adherent *E.coli* (DAEC), and shiga-toxin producing *E.coli* (O'Sullivan et al., 2007). On the other hand, extra-intestinal pathogenic *E.coli* includes uropathogenic *E.coli* (UPEC), neonatal meningitis-associated *E.coli* (NMEC), and sepsis-causing E. coli (SEPEC) (Köhler & Dodrindt (2011). More than 400 serotypes of non-*STEC* are reported; however, serogroups O26, O45, O103, O111, O121, O145, and O157 are frequently associated with severe illness and outbreaks in humans, and collectively termed the "top or big" (Hughes et al., 2006).

Ruminants, mainly cattle, have been blamed as the primary reservoir of *STEC* (Blanco et al., 2001). Usually pathogenic *E.coli* is transmitted by fecal-oral route from foods including, water, milk, meat, and other processed food. *E.coli* are responsible for many diseases with different degree of illness ranging from watery, mucoid, or bloody diarrhea; abdominal cramps; urinary tract infection; and meningitis but not generally recorded in cattle (Synge 2000). The absence of illness in cattle, caused by *STEC* is well documented, and is probably due to the absence of a specific receptor for Shiga toxin (Pruimboom-Brees, 2000). Other reasons for the difference between outcomes in humans and cattle populations have been proposed by Stamm et al., (2002) as bovine lymphocytes express functional receptors for *E.coli* shiga toxin. Cow's milk and milk products have been associated with diseases caused by *STEC* (Chapman et al., 1993; Martin & Beutin, 2011). Epidemics of milk-borne diseases caused by *STEC*, including pasteurized dairy products, have been recorded world-wide emphasizing the importance of *STEC* control for public health (Seghal et al., 2008). Six outbreaks were interrelated to the ingestion of milk and dairy products in 2012–2017 (EFSA and ECDC, 2020).

Although antibiotic treatment of *STEC* infections in animals is not advisable since there are facts that treatment may worsen the disease by inducing toxin-related tissue damage and symptoms in patients (Melton-Celas, 2015). Toxin production depends on type and concentration of the antibiotic used (Galarce, et al., 2020). In addition, it is widely accepted that extensive use of antibiotics in animal production systems is a major cause of multidrug resistance (MDR) in bacteria (Galarce, et al., 2020). There are two main causes of *E.coli*

intrinsic resistance, its outer membrane, which is impervious to many molecules, and its efflux pumps, which efficiently reduces the intracellular concentration of some antibiotics (Van et al, 2008).

Antibiotic resistance is known as an international problem in human and veterinary medicine. The wide use of these antimicrobial agents has resulted in the development and spread of antibiotic multi-resistance. Bacteria showed resistant to three antibiotic classes are named multidrug-resistant (Magiorakos et al., 2012). The misuses of antibiotics as antimicrobial agents are able to acquire antibiotic resistance genes that provide protection against most antibiotics (Braoudaki & Hilton, 2003). Nowadays, due to extensive uses of various antibiotics (such as β-lactams) against infections, high levels of antibiotic resistance and extended-spectrum beta-lactamase (ESBL)-producing bacteria are being detected. Antimicrobial resistance is a universal public health problem, and growing scientific evidence indicates that it is negatively impacted by both human and animal antimicrobial usages. Therapeutic failures due to antimicrobial resistance increase morbidity and mortality rates, with serious impact at individual, social and economical levels representing significant public concern worldwide (Erb et al., 2007; Johnson et al., 2008; Rao et al., 2014; Seni et al., 2016; Xu et al., 2015). Furthermore, antimicrobial resistance restricts the selection of therapeutic agents and increases the potential for treatment failures and adverse clinical complications. Retail foods, especially milk and milk products, may be an important vehicle for community-wide dissemination of antimicrobial resistant *E.coli* and extra-intestinal pathogenic *E.coli*. The objectives of this study were to (i) isolate *STEC* in the raw cow's milk of some dairy farms in the New Valley governorate, (ii) estimate antibiogram profile of the isolates against most common available antibiotics in the veterinary field and human medicine.

Materials and methods

Study animals and sample collection

Seven dairy farms in the New Valley governorate were selected for the current study. Two hundred-sixty seven samples were collected. The milk samples were aseptically collected directly from the teats to sterilized containers (bottles) and were directly transported to the labs. of Faculty of Veterinary Medicine, Assiut University using an icebox and stored at 4°C until the laboratory work was started.

Study design and sample size

A cross sectional study was conducted to isolate, identify and testing the antimicrobial susceptibility of the isolated *STEC* strains from seven selected dairy farms in the New Valley governorate. A total of 267 raw milk samples were collected from seven dairy farms (Table 1).

Table 1: Prevalence of Shiga toxin-producing *E. coli (STEC)* in raw milk samples.

Farm	No. of examined samples	Positive for *E.coli*		Positive for *STEC*	
		No.	%	No.	%
El-Kharja	42	12	28.6	5	11.9
El-Mawhoob west	30	9	30	4	13.3
Al-Maasra	35	12	34.3	5	14.3
Al-Moshia	54	23	42.6	20	37.8
Moot	40	12	30	2	5
Al-Gadida	30	8	26.7	6	20
Al-Kasr	36	10	27.8	7	19.4
Total	**267**	**86**	**32.21**	**49**	**18.4**

Isolation and identification of *STEC*

Protocol for *E.coli* isolation of Quin et al. (2002) was followed with some modification. Ten ml was thoroughly mixed with 90 ml of MacConkey broth (HiMedia, India) for enrichment and incubated at 37°C for 24 h. A loop full of enriched samples was streaked on MacConkey Agar (MCA) (HiMedia, India) and the inoculated plates were incubated at 37°C for 24 h. Pink-colored (lactose fermenting) colonies were considered presumptive of *E.coli*. Single well-isolated colonies were picked from MCA and streaked on Eosin Methylene Blue Agar (EMB) (HiMedia, India) and incubated at 37°C for 24 h. The characteristic green metallic sheen growth of colonies is a presumptive identification for *E.coli*. Colony morphology and colour on MCA and EMB agar plates together with the Gram stain characters were used as an initial identification of *E.coli* (Merchant & Packer, 1969; Eaton et al., 1995). The typical colonies (pinkish color appearance on MacConkey agar and metallic sheen on Eosin Methylene Blue agar) were then sub-cultured onto nutrient agar slopes (Huanki, Ltd., Guangdong, China) and Brain heart infusion agar slopes (BHIA) were incubated at 37°C for 24 hours kept at 4°C for further biochemical examination. Standard biochemical tests (Catalase test, Indole, Methyl red, Voges-Proskauer test, Nitrate reduction, Citrate utilization, and Urease production, Triple sugar iron agar, and sugar fermentation tests) were used as confirmation of identification (Cheeobrough, 1985; Vanderzant and Splittstresser, 1992; Jarvis et al., 1994; MacFaddin, 2000, and Brenner et al., 2005). The detected *E.coli* was stored in a seed preservation tube containing 20-30% glycerol in brain heart infusion broth and stored in deep freezing at -80°C. For reproducibility, the test was performed in triplicate.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done on Mueller-Hinton agar (MHA) (Merck biolab, Gauteng) by the standard disc diffusion method recommended by the Clinical and Laboratory Standards Institute, CLSI (2011). Prior to use, MHA were visually examined to ensure that plates are free of visible contamination before poured to a uniform depth of approximately 4 mm. Fresh cultures (about 22 h old) were transferred into test tubes containing 5 mL sterile normal saline. The turbidity of the suspension was adjusted to 0.5 McFarland standards (equivalent to 1.5×10^8 CFU/100 mL). Sterile swabs were soaked into the bacterial suspensions and used to inoculate the MHA plates by spreading uniformly on the surface of the agar, after which five antibiotic discs were placed equidistant from each other on the agar surface and the plates were incubated at 35 ± 2.0 °C for 18 to 24 h. After incubation, diameter of the zone of microbial growth inhibition around antimicrobial disk was measured in millimeters. A total of 39 well-identified *STEC* isolates were used for antimicrobial susceptibility testing against the panel of 10 test antibiotics. The antimicrobial susceptibility testing was applied according to the guidelines stipulated by Clinical and laboratory standards institute CLSI (2011). Accordingly, the antimicrobial discs and their concentrations as well as the diameters zones of inhibition for the tested strains are demonstrated in Table 2. The tested strains were evaluated as susceptible, intermediate and resistant. Multiple Antibiotic Resistance (MAR) index for each strain was determined according to the formula stipulated by Singh et al. (2010) as follow:

MAR index =
No. of resistance / Total No. of tested antibiotics.

*Isolates classified as intermediate were considered sensitive for MAR index.

For reproducibility, the test was performed in triplicate. *E.coli* strain ATCC25922 was used as a control strain in the experiment. Isolates showing resistance to ≥ 5 antimicrobial agents or more are considered multidrug resistant (MDR) (Magiorakos et al., 2012). The results were recorded on the basis of the zone-size interpretative chart supplied by the manufacturer.

Table 2: Antimicrobial resistance patterns of E.coli isolates from New-Valley dairy farms

Groups	Agent	Disc conc.	Result interpretation						MARI	Overall susceptibility
			Sensitive		Interm.		Resistant			
			NO	%	NO	%	NO.	%		
Aminoglycosides	**Erythromycin** (E)	15 µg	-	-	-	-	39.0	100	1.0	0.0%(0.0/39)*
	Clindamycin (CL)	2 µg	3	7.7	4	10.2	32.0	82.1	0.82	17.9 (7/39)*
	Kanamycin (K)	30 µg	25	64.1	1	2.6	13.0	33.3	0.33	66.7 (26/39)*
	Amikacin (AK)	30 µg	31	79.5	1	2.6	7.0	17.9	0.18	82.1 (32/39)
	Gentamycin (G)	10 µg	34	87.1	1	2.6	4.0	10.3	0.10	89.7 (35/39)
Cephalosporins	**Cefepime** (FEP)	30 µg	-	-	1	2.6	38	97.4	0.97	2.6 (1.0/39)
	Cefotaxime (CF)	30 µg	23	59.0	3	7.7	13.0	33.3	0.33	66.7 (26/39)*
	Cefazolin (CZ)	30 µg	29	74.4	2	5.1	8.0	20.5	0.21	79.5 (31l39)*
Quinolones	**Ciprofloxacin** (CP)	5 µg	32	82.1	2	5.1	5.0	12.8	0.13	87.2 (34/39)*
	Levofloxacin (L)	5 µg	36	92.3	1	2.6	2.0	5.1	0.05	94.9 (37/39)*
	Nalidixicacid (NA)	30 µg	11	28.2	4	10.3	24.0	61.5	0.615	38.5 (15/39)*
Penicillins	**Ampicillin** (AM)	10 µg	28	71.8	-	-	11.0	28.2	0.28	71.8 (28/39)*
Tetracyclines	**Tetracycline** (T)	30 µg	21	53.9	2	5.1	16.0	41.0	0.41	59 (23/39)*
Sulphonamides	**Sulphamethoxazol** (SXT)	30 µg	15	38.5	5	12.8	19.0	48.7	0.49	51.3 (20/39)*
Carbapenems	**Meropenem** (M)	10 µg	34	87.2	-	-	5.0	12.8	0.13	87.2 (34/39)

Results

Results illustrated in the Table 1 and Fig. 1 indicated that the prevalence percentages of STEC in raw milk were ranged from 5-37.8%. The highest prevalence percentage was in Al-Moshia dairy farm (37.8%), followed by Al_gadida (20%), Al-Kasr (19.4%), Al-Maasra (14.3%), El-Mawhoob (13.3%), El-Kharja (11.9%), and Moot (5.0%).

Table 2 showed that the antimicrobial susceptibility of STEC isolated were highly variable. Sixteen antimicrobial of 7 antimicrobial groups were tested in the current study against 39 STEC isolates. The results illustrated that the resistance was ranged from 2.6-100%. Table 3 indicated that three antibacterial agents showed resistance ranged from 80-100%. All tested isolates showed complete resistance to erythromycin (100%), followed by cefepime (97.4%), and clindamycin (82.1%). There was another antibacterial agent showed resistance ranged from 50-80% (Nalidixic acid). The third group showed resistance ranged from 30-50% including Kanamycin (33.3%), cefotaxime (33.3%), tetracycline (41.0%), and sulphamethoxzol (48.7%). The fourth group showed resistance ranged from 0-30% including eight antibacterial agents as amikacin 17.9%), gentamycin (10.3%), cefazolin (20.5%), ciprofloxacin (12.8%), levofloxacin (5.1%), ampicillin (28.2%), meropenem (12.8%), and imipenem (2.6%). STEC isolates showing resistance to three antimicrobial agents is considered multi-drug resistant strain. Concerning the multi-drug resistance, table 2 indicated that the tested STEC isolates showed resistance to 11 antimicrobial agents from the total sixteen agents tested (68.75%).

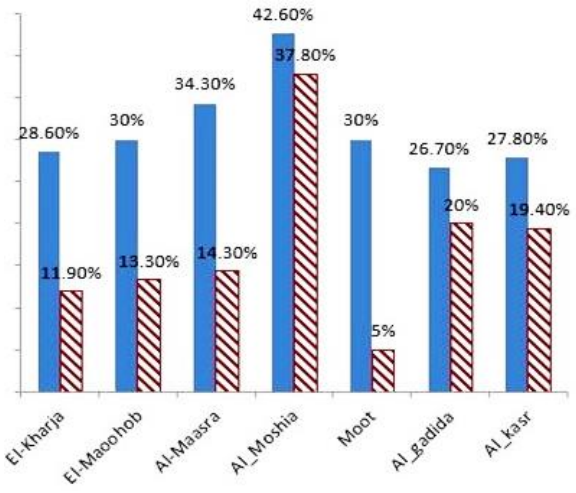

Fig. 1. Prevalence of *E. coli* (light blue) and STEC (red lined) in raw milk of dairy farms

Discussion

STEC prevalence in cattle

A total of 267 raw milk samples were collected from five dairy farms in the new valley governorate (Table 1). A total of 86 *E .coli* isolates were recorded (32.2%). Prevalence of *STEC* was 18.4% (49/267). This observation is not surprising when comparing to similar prevalence studies. In Iran, a study was conducted by Mohammadi et al. (2013) who found that the prevalence of *STEC* in raw milk was 17.47 % (36 out of 206 samples). More or less similar results were reported in previous studies by Abadu et al. (2020) and Sethulekshmi et al. (2018) who found that the prevalence of *STEC* in raw milk samples were, 19% and 19.26%, respectively. Moreover, similar results were recorded in Germany by Zschock et al., (2000) who stated that *STEC* in raw milk was 18%. In Egypt, the prevalence rates of *STEC* in raw milk samples were reported by Elhadidy and Mohammed (2012), Elmonir et al. (2018), and Younis et al. (2018) who found that *STEC* in raw milk samples was 11.3; 13.2%, and 14.7%, respectively%. Different prevalence rates ranging from 3.7- 14.3 were reported in France, Switzerland, US, Iran, Ethiopia, and Egypt (Vernozy-Rozand et al. ,2005; Jayarao et al., 2006; Stephan et al., 2008; Venegas-Vergas et al., 2016; Ranjbar et al. 2018; Weldeselassie et al. 2021, and Elmonir et al., 2021)). On the other hand, higher prevalence rates were recorded in other study in Brazil (31.1%) by Vendramin et. al. (2014), France (34%) by Pradol, et al. (2000), Egypt (28.8%) by Elafify et al., (2020).

On the farm level, Table 1, revealed that all the examined farms (100%) were positive for *STEC*. The prevalence rate per farm was ranging from 5.0% to 37.8% with a mean value of 18.4%. In Spain, Blanco et al. (2001) found that, *STEC* found in 84% of the examined farms. On the other hand, Venegas-Vargas et al. (2016) stated that in the United States, the prevalence rates per farm were ranging from 6-54% with an overall prevalence of 13%. Many factors can be implicated in the inconsistency of prevalence rates between farms such as sensitivity and specificity of the methods used to detect *STEC* isolates, geographical area, sampling season, age of animals, water supply, herd size, slurry and manure spreading on pasture, management system used in the farm, as well as the age of animals (Blanco et al., 2001; Lundborg et al. 2005; Gun et al., 2007; Gulliksen et al. 2009; Suardana et al., 2010; Windeyer, et al. 2014; Ballem et al. 2020, and Sobhy et al. 2020). These results are more or less similar to those reported in previous studies (Kobayashi et al., 2001; and Bibbal et al., 2015).

Antibiogram of the isolated strains:

Thirty-nine strains of the *STEC* isolates were used for the antimicrobial susceptibility test against 16 antimicrobials commonly used in veterinary fields and human medicine comprising 7 antimicrobial groups (Table 2). The obtained results revealed that the highest resistance was detected against Erythromycin (100%), cefepime (97.4%) and clindamycin (82.1%) followed by nalidixic acid (61.5%); sulphamethoxazol (48.7%); tetracycline (41.0%); cefotaxime

(33.3%), kanamycin (33.3%), and ampicillin (28.2%). The lowest resistance was indicated by imipenem (2.6%); levofloxacin (5.1%) and gentamycin (10.3%). The five aminoglycosides tested were showed variable resistance profile, 100% (erythromycin), clindamycin (82.1%), kanamycin (33.3%), Amkacin (17.9%), and gentamycin (10.3%). Concerning cephalosporins, 3 antimicrobials were tested. Resistance profiles were ranged from 97.4% (cefepime), cefotaxime (33.3%), and cefazolin (20.5%). The resistance of *STEC* to quinolones was revealed in nalidixic acid (61.5%), ciprofloxacin (12.8%) and levofloxacin (5.1%). Resistance of the examined strains to Sulphamethoxazol (sulphonamides) was 48.7%, tetracycline (41.0%), and ampicillin (28.2%). Carbapenems showed the lowest resistance against *STEC* strains under test where the resistance was 2.6% for imipenem and 12.8% for meropenem. Results in Table 2 showed that resistance to erythromycin, clindamycin (aminoglycoside), and cefepime (cephalosporins group (cefepime) was the highest and almost 100%. On the other hand, imipenem (Carbapenems), levofloxacin (quinolones), gentamycin (aminoglycoside), ciprofloxacin (quinolones), and meropenem (Carbapenems) showed the lowest percentages of resistance as 2.6%, 5.1%, 10.3%, 12.8%, and 12.8%, respectively. Results of the current study concerning the antibiotic resistance are commonly higher than the results reported in many countries. This may be attributed to the uncontrollable overuse of drugs the developing countries (Lan et al., 2020). In China, 87.1% of *E. coli* isolates (61/70) showed resistant to ampicillin and kanamycin (Liu et al., 2014).

Grouping of the antimicrobials used in this study was illustrated in Table 3 and could be summarized in three groups, (i) showing almost complete resistance (80-100%) was recorded in 3 antimicrobials including, erythromycin, cefepime, and clidamycin representing 18.75% of the used antibiotics (3/16). (ii) Showing resistance of 30-80% in 31.25% of the tested antibiotics (5/16) including Nalidixic acid, sulphamethoxazol, tetracycline, cefotaxime, and kanamycin. (iii) showing resistance ≤ 30% in half of the tested antimicrobials (8/16) including imipenem, levofloxacin, gentamycin, meropenem, ciprofloxacin, amikacin, cefazolin, and ampicillin.

Resistance rate towards ampicillin was lower than those reported by Lan et al. (2020), Bag et al., (2021), and El Seedy et al. (2019) who found that the resistance to ampicillin was 41.3%, 89.2%, and 90.9% respectively. On the other hand, the current study showed a higher resistance than those recorded in South Korea (16.6%) and some provinces of China (25.3%) (Tark et al., 2017, and Zhang et al., 2018). Concerning erythromycin, clindamycin, and nalidixic acid, Table 2, indicated that results of the current study were more or less similar to those reported by El Seedy et. al. (2019), and Rubab & Oh (2021).

Resistance toward tetracycline was 41.0% which was higher than those recorded in European Union (9.0%), Canada (14.3%), and China (34.6%) as recorded by Thomas et al., (2015); Saini et al. (2012), and Lan et al., (2020). On the other hand the results were lower than that obtained in Nigeria and Bangladesh reported by Adesoji, et al. (2015) and Bag et. al. (2021) who found that resistance toward tetracycline was 55.1% and 89.5%, respectively.

Multidrug resistant (MDR)

In the current study, out of 39 *STEC* isolates, 15 (38.5%) showed multidrug-resistant strains resistant to five or more antimicrobial drugs (Table 3). Multidrug resistant strains were reported in previous studies (Bettelheim et al., (2003), Cergole-Novella et al. (2006). Contaminating of dairy milk with multidrug-resistant *STEC*

organisms is considered a potential hazard for people consuming raw milk. In the present study, results showing a high percentage of *STEC* strains isolated from raw milk, presenting a multidrug-resistance phenotype, give rise to considerable concern and suggest that monitoring of the extent of this problem is a wise measure. A more thorough understanding of the resistance and virulence factors of *E.coli* isolates is of urgent need to aid in the achievement of proper treatments of its infection. A previous study reported that 45.8% of 48 *E.coli* strains were resistant to 13 antibiotics (Todorovic et al., 2018). The permanent use of antibiotics may cause MDR in dairy herds (Suojala et al., 2011). Therefore, it is important to control antibiotic use to prevent the risk of MDR. In Korea, researchers have concluded that decline in tetracycline resistance may be due to the decreased use of the antibiotic in cattle (Tark et al., 2017).

Reports on antimicrobial resistance are useful for understanding the pathogenesis of *E.coli* infection in bovine mastitis (Blum et al., 2008). The misuse of antimicrobials is a contributing factor, but probably not the main or single factor involved in antimicrobial resistance (Bergman et. al., 2009; Oliver, et al., 2011). Future studies should evaluate the differences between phenotype and genotype.

The abuse of antibiotics has contributed to the appearance of antimicrobial-resistant bacteria (Mia et al., 2017; VanBoeckel et al., 2015, and Zhang, et. Al., 2015). It was recorded that, 20-33% of *E.coli* isolates from bovine milk were resistant to at least one antimicrobial agent (Fairbrother et al., 2015; Suojala et al., 2011). Multidrug-resistant *E.coli*, indicate a significant global alarm (Erb et al. 2007; Rao et al., 2014; and Seni et al., 2016). In Middle East, the antimicrobial resistance and virulence genes of *E.coli* from bovine milk have been studied in Jordan and Iran (Obaidat et al., 2018 , and Tavakoli & Pourtaghi, 2017). Findings of this study indicate a potential threat of developing antimicrobial resistance in *STEC*. Occurrence of multidrug resistant *E.coli* might be responsible for the failure of antibiotic therapies in clinical cases as well as pose potential threat of transmitting and development of antibiotic resistance in human.

Susceptibility

Table 2 and 3 indicated that, the tested isolates (39 strains) *STEC* showed susceptibility (≥ 70%) to exactly 50 % of the tested antimicrobials (8/16). The tested isolates showed susceptibility to some antimicrobials including IPM (97.4%), L (94.9%), G (89.7%), CP (87.2%), M (87.2%), AK (82.1%), CZ (79.5%), and AM (71.6%). Susceptibility less than 50% was recorded in 4 antimicrobials including, E (0.00%), FEP (2.6%), CL (17.9%), and NA (38.5%). Moreover, intermediate susceptibility (≥ 50≤70%) was recorded in 4 antimicrobials including, K (66.7%), CF (66.7%), T (59.0%), and SXT (51.3%).

Conclusion

In summary, data in this work is a part of danger evaluation program for raw milk in Egypt and reinforce the idea that raw milk may be considered a possible source of transmission of pathogenic *STEC* to humans. Moreover, results of this study showed *STEC* strains have developed multi-drug resistance. More research is of urgent need to characterize mechanisms of resistance and to minimize the resistance. It is also vital to initiate a continuing antimicrobial resistance screening program of *STEC* to check any emerging antimicrobial resistance problems.

Table 3: Antimicrobial resistance of STEC strains on antibiotics used.

Resistance range	Antimicrobial agent		
	NO.	%	Agent
0-30%	8	50	AK; G; CZ; CP; L; AM; M; IPM.
30-50%	4	25	K; CF; T. SXT
50-80%	1	6.25	NA;
80-100	3	18.75	E; CL; FEP;

AK, amikacin; G, gentamycine; CZ, cefazolin; CP, ciprofloxacin; L, levofloxacin; AM, ampicilin; M, meropenem; IPM, imipenem; K, kanamycin; CF, cefotaxime, T, tetracycline; SXT, sulphamethoxazol; NA, nalidixic acid, E, erythromycin; CL, clidamycin; FEP, cefepime.

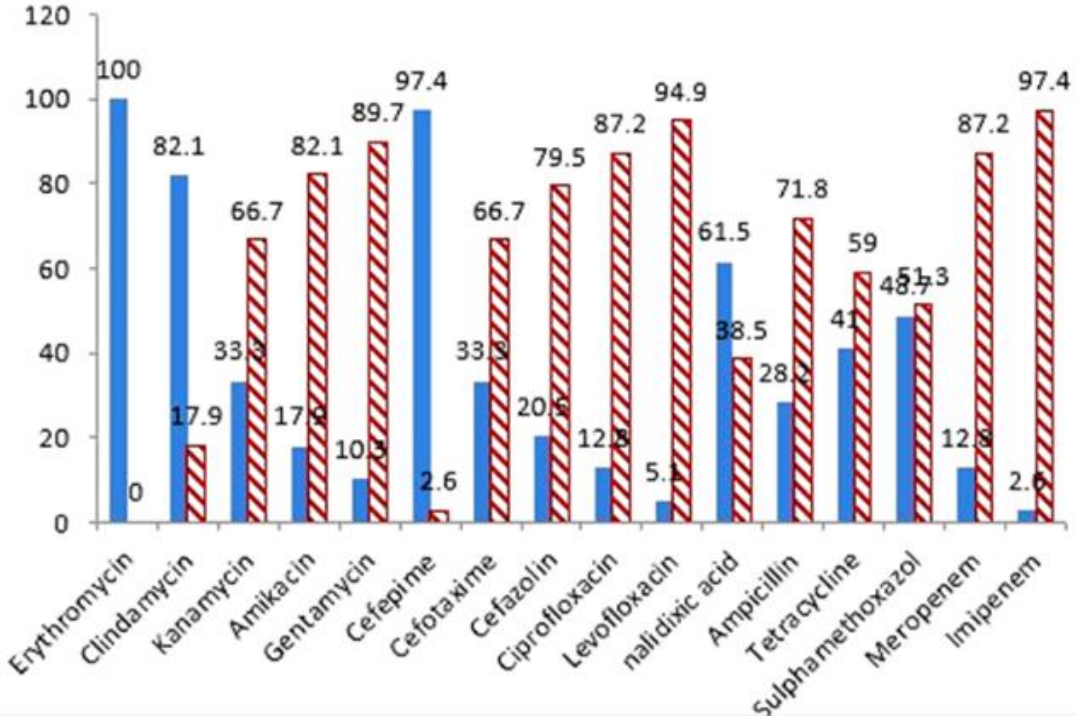

Fig. 2. Antimicrobial resistance (light blue) and Susceptibility (red lined) profile of STEC

Conflict of interest statement

The authors declare that there is no potential conflict of interest.

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