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ANTIMICROBIAL RESISTANCE AND BIOFILM FORMATION PATTERNS OF *Escherichia coli* ISOLATED FROM MARKET RAW MILK AT ZAGAZIG CITY

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ABSTRACT: In the present study, one hundred samples of raw cow milk were collected randomly from different dairy shops and markets in Zagazig city for isolation and identification of *Escherichia coli* which is considered a reliable indicator for fecal contamination and an important cause of food poisoning. Identification was done microscopically, biochemically by different biochemical tests (IMVIC) and serologically. The incidence of *E. coli* in raw milk samples was 47%. Also, the serological identification of *E. coli* isolates revealed that O26 is the most predominant serogroup by percentage of 21.3%. *E. coli* pose the greatest threat to human health because of its growing resistance to antibiotics. Antimicrobial susceptibility testing (AST) was done by disc diffusion method against 10 antimicrobials and the results revealed that *E. coli* isolates were highly resistant to amoxicillin-clavulanate, ampicillin, cefotaxime and ceftazidime with percentages of 89.4%, 89.4%, 100.0% and 100.0%, respectively. However, they were highly sensitive to chloramphenicol, ciprofloxacin and tetracycline with percentage of 100.0%, 100.0% and 93.6% respectively. In addition, 89.4% of *E. coli* isolates showed multi drug resistance (MDR). The ability of bacteria for adherence to food surfaces and biofilm formation is a source of food contamination that affect food safety and industry. Micro titer plate assay used for testing biofilm formation and represented that 78.7% of *E. coli* isolates were non-biofilm producers, 6.4% were weak biofilm producers, 14.9% were moderate biofilm producers and none of isolates was strong biofilm producers.

Key words: *E. coli*, raw milk, antimicrobial resistance, biofilm.

INTRODUCTION

Milk ranks high among other foods and is considered as the most perfect food for human from birth to senility as it is not only has good sensory properties and all nutrients required for the body for rapid growth but also could prevent or reduce risks of many nutritional deficiency diseases **(Kalkwarf *et al.*, 2003; Marshall *et al.*, 2003)**.

Raw milk is still used by large number of farm families and workers and by a growing segment of the general population who believe that the milk is not only safe but also imparts beneficial health effects that are destroyed by pasteurization **(Angulo *et al.*, 2009)**.

Due to its high nutritious content, milk allows the growth of a copious number of microorganisms. Therefore, apart from its endogenous microbiota, diverse and numerous other microorganisms originating from the teat canal, udder skin, milking machines, tanks, and containers used to store it, reflecting the farm and the pasture environment as well, might colonize the milk as soon as it has been milked **(Addis *et al.*, 2016)**.

Among all micro-organisms *Escherichia coli* is frequently contaminating organism in food and is reliable indicator of fecal contamination and generally present due to insanitary conditions of water, food, milk and other dairy products **(Jayarao and Henning, 2001)**.

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Antibiotic resistance is a seemingly growing problem in both developing and developed countries of the world. The rise in the use of antibiotics in human medicine, veterinary medicine and agriculture has been contributory to the rapid rise of antibiotic resistance among bacterial species especially and this has increased the challenge of multidrug-resistant bacteria in the environment **(Reinthaler *et al*., 2010; Xu *et al*., 2014)**.

The emergence and dissemination of multidrug-resistant (MDR) bacterial species have been associated with the extensive use of broad-spectrum antimicrobials in treating human and animal infections **(Rzewuska *et al*., 2015)**. In animal husbandry, antibiotics are used as animal growth promoters and are regularly abused for the prevention and treatment of animal infections **(Nepal and Bhatta, 2018)**. All these together contribute to the development of bacterial resistance to antibiotics. Genetic factors such as horizontal gene transfer and clonal expansion of resistant isolates also play a crucial role in the acquisition of antibiotic resistance traits in bacteria **(Peterson and Kaur, 2018)**.

A biofilm is defined as an organized collection of surface attached microbial communities of cells that are embedded into a self-produced exopolymeric matrix mainly composed of proteins, polysaccharides and sometimes DNA **(Hall-Stoodley *et al*., 2004)**. The formation of biofilm is a result of different stress condition(s) where biofilms acts as a defense mechanism enhancing the survival rate of microorganism. They play an important role in microbial pathogenesis and persistence as well as serve as grounds for genetic exchanges. It acts as shield protecting the microbial community from action of various antimicrobial agents such as antibiotics, preservatives, chemical sanitizers, thermal treatment etc. that are traditionally used in food industry, thus making them robust and hard to eradicate **(Monte *et al*., 2014)**. The biofilm formation for many bacterial species including *E. coli* occurs as early as two hours and they can survive up to ten years in food industries despite the regular cleaning and sanitation treatment **(Corcoran *et al*., 2014)**.

This study was undertaken to determine the occurrence and characteristics of *E.coli* strains in market raw cow milk at Zagazig city and to estimate the potential of these sources acting as vehicles of antimicrobial resistance.

MATERIALS AND METHODS

Samples Collection and Preparation

One hundred random samples of raw milk were collected from different dairy shops and markets in Zagazig city, Sharkia Governorate, Egypt under hygienic condition during the period from February to August 2020. Approximately 500 ml of the samples were transferred to the laboratory of Food Control Department, Faculty of Veterinary Medicine, Zagazig University in an insulated icebox with a minimum of delay to be examined microbiologically.

Isolation and Identification of *E. coli*

Eleven ml of well-mixed samples were aseptically transferred into a sterile bottle containing 99 ml of sterile buffered peptone water (BPW) to make a dilution of 1:10 and incubated at 37°C for 24hrs. A loopful of the BPW enrichment was streaked on Eosin Methylene Blue agar (EMBA; Oxoid) and then incubated at 37°C for 24hrs. The agar plates were examined for growth of *E. coli*. To get pure cultures, a single colony was further sub-cultured on EMBA according to **(Ngaywa *et al*., 2019)**

Films of pure suspected cultures were stained with Gram's stain and examined microscopically. Cultures analyzed by using the following biochemical tests:

Indole Motility Test, Methyl Red, Voges-Proskauer Tests and Simmons Citrate Agar Test according to **APHA. (2004)**

Serological Identification of Isolated *E. coli*

The isolates were serologically identified by slide agglutination test according to **Kok *et al*. (1996)** using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) to identify O antigen.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing (AST) was done using the Kirby-Bauer disc diffusion

method on Mueller-Hinton agar (Oxoid, Hampshire, England) as described by the Clinical and Laboratory Standards Institute **(CLSI, 2017)**. with an inoculum equivalent to 0.5 McFarland standards. Incubation was done at 35±2°C, ambient air, for 16–18 hrs. The following 10 antimicrobials were tested:

Amoxicillin/clavulanic acid, ampicillin, cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, kanamycin, Nalidixic acid, streptomycin and tetracycline

The sensitivity/resistance was interpreted based on the diameter zone of inhibition, inclusive of margins, following the Clinical Laboratory Standards Institute guidelines **(CLSI, 2017)**. The isolates were classified as intermediate, susceptible or resistant **(CLSI, 2017)**. In addition, we calculated the number of *E. coli* isolates that were considered multidrug resistant (MDR), that is, resistant to three or more antimicrobials of different classes **(Frye and Fedorka-Cray, 2007)** and the Multiple Antimicrobials Resistance index (MAR) for each *E. coli* isolate according to the methodology described by **(Krumperman, 1983)**. This index shows the relationship between the number of resistant *E. coli* to each antimicrobial and the total number of classes tested.

Biofilm Formation

The biofilm formation test was performed as described by **Stepanovic *et al*. (2000)**, where *E. coli* strains were incubated in TSB broth at 37 ± 1°C for 24 hrs. Then aliquots were diluted in a new tube until reaching the turbidity of one on the McFarland scale. Subsequently, 200 µL of each suspension in triplicate were inoculated into a 96-well sterile polystyrene micro plates. In the first three wells, only sterile broth was added as a negative control. The plates were incubated without air circulation at 37 ± 1°C for 24 h. Next, the bacterial suspensions were aspirated from each well and washed three times with 250 µL of sterile 0.9% sodium chloride solution. The bacterial cells were then fixed with 200 µL of methanol (PA) for 15 min and dried at room temperature. Later, they were stained with 200 µL of 2% Hucker crystal violet for 5 min, washed in running water and dried at room temperature. Re-solubilization was performed with 160 µL of 33% glacial acetic acid followed by reading using a spectrophotometer (Thermo Scientific® Multiskan GO) at 570 nm. After reading, the optical density value of each strain (OD) was obtained by calculating the arithmetic mean of the absorbance of the three wells, and this value was compared to three standard deviations above the mean of the absorbance of the negative control (ODC). The strains were classified in four different categories as follows: A) non-biofilm producer ($OD \leq ODC$); B) weak biofilm producer ($ODC < OD \leq 2 \times ODC$); C) moderate biofilm producer ($2 \times ODC < OD \leq 4 \times ODC$); and D) strong biofilm producer ($4 \times ODC < OD$) **(Stepanovic *et al*., 2000)**.

RESULTS AND DISCUSSION

Incidence of *E.coli* in Market Raw Milk and Serological Identification of Isolates

Milk, a perishable complete nutritious food is considered a good medium of growth for many of the microorganisms **(Khayal and Ragia, 2013)**.

Conditions for contamination of raw milk at different critical points are due to less hygienic practices in pre-milking udder preparation, sub-optimal hygiene of milk handlers, and poor sanitation practices associated with milking and storage equipment, higher environmental contamination during transportation or contamination during waiting along the roadside **(Garedew *et al*., 2012)**

There is an increasing in number of people whose consuming raw unpasteurized milk due to enhancing nutritional quality, taste and health benefits in spite of several documented milk born disease outbreaks occurred from consumption of raw unpasteurized milk and dairy products manufactured using raw milk **(Leedom, 2006; Oliver *et al*., 2009)**.

In recent years, much attention has been paid toward *E. coli* because of its importance as an organism of true faecal origin with the possible existence of associated enteric pathogens**.** Commensal *E. coli* plays a dynamic role in the ecology of intestinal tract. The *E. coli* genome exhibits a high degree of heterogeneity; therefore, these bacteria can be a commensal organism. On the other hand, it can be also a dangerous pathogen causing intestinal or extra-intestinal infections. It is of major public health

significance related to risk of introducing these bacteria to the food chain **(Newell *et al*., 2010)**.

Illness caused by entero-pathogenic *E. coli* can range from self-limited watery diarrhea to life threatening manifestations such as hemorrhagic colitis, hemolytic uremic syndrome, and thrombocytopenic purpura and may lead to death **(Alexander and Prado, 2003)**.

The results presented in Table 1 revealed that, the incidence of *E.coli* in raw milk were 47%. **Meshref (2013)** reported nearly similar results where he found *E.coli* by (52.6%). The contamination rate in raw milk samples was extremely lower than the findings of **Sobeih *et al*. (2002), Soomro *et al*. (2002), Chye *et al.* (2004) and Altalhi and Hassan (2009)** as they found 88, 65, 65 and 66% of their samples were contaminated by *E. coli*, respectively, but higher than the rate of 32, 27.5,10 and 3.3% reported by **Ahmed and Sallam (1991), Mezyed *et al*.(2008), Tasci (2011) and El-Prince *et al*. (2010)**, respectively.

Table 2 illustrated the serological identification of isolated *E. coli*. The strains of *E. coli* isolated from examined raw milk samples were O25, O26,O44, O55, O78, O86, O114, O119, O125, O127, O158 and O168 by percentage of (10.6%), (21.3%), (8.5%), (14.9%), (4.3%), (4.3%), (6.4%), (6.4%), (8.5%), (4.3%), (4.3%) and (6.4%) respectively. While nearly similar results were obtained by **El-Nahas *et al.* (2015)** who found O114, O26, and O127 in raw milk samples. Also **El-Zamkan *et al*. (2018)** isolated *E. coli* O26, O55 & O119 from raw milk. **Khafagy *et al*. (2017)** isolated O158, O55, and O86 from raw milk samples obtained from dairy farms and shops. **Rashid *et al.* (2013)** reported the presence of one milk sample serologically identified as O86. **El-bagory *et al*. (2016)** and other researchers as; **Lamey *et al*. (2013) and Abike *et al*. (2015)** recorded for the identification of *E.coli* strain that belongs to the serogroup O55. Others as **Wenz *et al*. (2006) and Koraney (2016)** recorded O158, which is one of the frequent serogroups that isolated from raw milk samples. In addition, **AbdEl-Maabud (2014) and Shalaby *et al*. (2019)** isolated *E. coli* O26 from raw milk samples.

Globally, the unsupervised use of antimicrobial agents in the treatment of animal and human infections have been contributed to the emergence of antimicrobial resistance **(Van Boeckel *et al*., 2015)**. The antimicrobial resistance mainly originates from the transfer of resistance genes across microbes enabling them to survive in the presence of antimicrobial agents that eventually resulted in failure of antibiotic therapeutic protocols **(Blair *et al*., 2015)**. Furthermore, the overuse of antibiotics in animal husbandry as growth promoters could be a potential source of bacterial resistance through dissemination of resistant microbes from intestinal microbiota of livestock that contaminate the surrounding environment and enhance the transmission of resistant genes to autochthonous bacteria (resident microbes) **(McEwen and Collignon, 2018)**.

Antimicrobial Resistance and Biofilm Formation of *E. coli* Isolates

The development of antimicrobial resistance among the pathogenic bacteria poses a problem of high concern. Table 3 shows that *E. coli* isolates were highly resistant to amoxicillin-clavulanate, ampicillin, cefotaxime and ceftazidime with percentages of 89.4%, 89.4%, 100.0% and 100.0% respectively. In addition, the isolates were highly sensitive to chloramphenicol, ciprofloxacin and tetracycline with percentage of 100.0%, 100.0% and 93.6% respectively. Nearly similar results were obtained by **Nobili *et al*. (2016)** where he reported a significantly higher percentage (100%) resistance to amoxicillin-clavulanic acid. **Thaker *et al*. (2012)** recorded higher resistance rate to ampicillin (100 %). While **Bhardwaj *et al*. (2021)** found complete resistance against amoxicillin and ampicillin. **Shalaby *et al*. (2019)** found that all *E.coli* isolates exhibited susceptibility to chloramphenicol, ciprofloxacin, and tetracycline (except for one isolate that was resistant to ciprofloxacin). In addition, the results reported by **Tadesse *et al*. (2018)** were relatively similar to this study where the in vitro growth *E. coli* was restrained by ciprofloxacin and tetracycline.

The multiple antibiotic resistance (MAR) index, an index describing the resistance of isolates to different antibiotics, was calculated for each isolate as described in the materials and methods section. Values for the MAR index were 0.2 (*i.e.*, an isolate being resistant to two

Table 1. Incidence of *E. coli* in examined market raw milk samples

No. of samples	Positive samples	
	No.	% of total
100	47	(47.0%)

Table 2. Serological identification of *E. coli* strains isolated from market raw milk samples

Serogroup	No.	% of total
O25	5	(10.6%)
O26	10	(21.3%)
O44	4	(8.5%)
O55	7	(14.9%)
O78	2	(4.3%)
O86	2	(4.3%)
O114	3	(6.4%)
O119	3	(6.4%)
O125	4	(8.5%)
O127	2	(4.3%)
O158	2	(4.3%)
O168	3	(6.4%)
Total	47	(100.0%)

Table 3. Antibiogram pattern of identified *E. coli* isolates (n=47)

Antimicrobial	Concentration (µg)	No. of isolates (%)		
		Resistant	Intermediate	Susceptible
Amoxicillin-clavulanate	**20/10 µg**	42(89.4%)	0(0.0%)	5(10.6%)
Ampicillin	**10 µg**	42(89.4%)	0(0.0%)	5(10.6%)
Cefotaxime	**30 µg**	47(100.0%)	0(0.0%)	0(0.0%)
Ceftazidime	**30 µg**	47(100.0%)	0(0.0%)	0(0.0%)
Chloramphenicol	**30 µg**	0(0.0%)	0(0.0%)	47(100.0%)
Ciprofloxacin	**5 µg**	0(0.0%)	0(0.0%)	47(100.0%)
Kanamycin	**30 µg**	9(19.1%)	25(53.2%)	13(27.7%)
Nalidixic acid	**30 µg**	0(0.0%)	29(61.7%)	18(38.3%)
Streptomycin	**10 µg**	20(42.6%)	20(42.6%)	7(14.9%)
Tetracycline	**30 µg**	3(6.4%)	0(0.0%)	44(93.6%)

out of the 10 antibiotics tested), 0.4 (resistance to 4 out of the 10 antibiotics tested), 0.5 (resistance to 5 out of the 10 antibiotics tested) and 0.6 (resistance to 6 out of the 10 antibiotics tested) with percentages of 10.6%, 53.2%, 23.4% and 12.8%, respectively.

E. coli has also been shown to be a significant reservoir of genes coding for antimicrobial drug resistance and therefore is a useful indicator for resistance in bacterial communities **(Ars`ene-Ploetze *et al*., 2018; Katakweba *et al*., 2018)**.

In the present study, 89.4% of *E. coli* isolates showed resistance to more than three classes of antimicrobials (Table 4).

Biofilm formation is one of the most important virulence factors that protect microbes from antimicrobial drugs and treatment **(Olsen, 2015)**. The ability of spoilage and pathogenic bacteria to adhere onto food surfaces and form biofilms serve as a persistent source of food contamination that threatens food safety and causes huge losses to the food industry **(Tezel and Şanlıbaba, 2018)**.

Table 5 revealed that 78.7% of *E. coli* isolates were non-biofilm producers, 6.4% were weak biofilm producers, 14.9% were moderate biofilm producers and none of isolates was strong biofilm producers. Nearly similar studies of **Milanov *et al*. (2015)** who recorded that 19 (76%) isolates of *E.coli* did not produce biofilm and 6 (24%) were classified as weak biofilm producers.

Musa *et al*. (2019) found that 6 out of 15 (40%) of *E. coli* were strong biofilm producers, 2 out of 15 were moderate biofilm producers (13.3%), 4 out of 15 (26.7%) were weak biofilm producers and only three isolates (20%) were non-biofilm producers.

Bhardwaj *et al*. (2021) reported that out of 32 *E.coli* isolates tested, 4 were strong formers, 11 were moderate, 15 were weak producers and 2 non-producers.

Da Silva Chagas *et al*. (2017) recorded that 11 (55%) of 20 biofilm producing strains were identified as *E.coli*, and all strains were classified as strong biofilm producers.

Cruz-Soto *et al*. (2020) found that 26 (76.5%) of the isolates formed biofilm to some degree, while the remaining 8 (23.5 %) did not form biofilm. Of the biofilm-forming isolates, 7 were classified as strong and moderate biofilm producers and 12 as a weak one.

This study provides further evidence that raw cow milk is a potential source of *E.coli*، some of which are associated with serotypes clinically significant bearing biofilm formation ability and multiple antibiotic resistance that may raise public health concern due to the potential human infection and antimicrobial resistance dissemination throughout food system.

Table 4. Multi drug resistance pattern and multiple antibiotic resistance index of identified *E. coli* isolates (n=47)

No. of isolates	Multi drug resistance (MDR)		Multiple antibiotic resistance (MAR)							
			0.2		0.4		0.5		0.6	
	No.	%	No.	%	No.	%	No.	%	No.	%
47	42	(89.4%)	5	(10.6%)	25	(53.2%)	11	(23.4%)	6	(12.8%)

Table 5. Ability of *E. coli* strains isolated from market raw milk samples to form biofilm

No. of isolates	Degree of biofilm formation							
	None		Weak		Moderate		Strong	
	No.	%	No.	%	No.	%	No.	%
47	**37**	**(78.7%)**	**3**	**(6.4%)**	**7**	**(14.9%)**	**0**	**(0.0%)**

REFERENCES

Abd El-Maaboud (2014). Studies on some food poisoning organisms in milk and some locally produced soft cheese. M.V.Sc. Thesis, Fac. Vet. Med. Sadat City Univ.

Abike, T.O., O.A. Olufunke and K.D. Oriade (2015). Prevalence of multiple diseases surveillance and response. Antibiotic resistant *Escherichia coli* serotypes in cow, raw milk samples and traditional dairy products in Osun State, Nigeria. British Microbiol. Res. J. BMR J., 5 (2):117-125.

Addis, M.F., A. Tanca, S. Uzzau, G. Oikonomou, R.C. Bicalho and P. Moroni (2016). The bovine milk microbiota: Insights and perspectives from-omics studies. Molecular Bio Systems, 12(8): 2359–2372.

Ahmed, A.M. and S.S. Sallam (1991) Prevalence of Escherichia coli serotypes in raw milk and some dairy products. Assiut Vet. Med. J., 25: 93–97.

Alexander, M. and V. Prado (2003). Detection of Shiga toxin producing *Escherichia coli* in food. Expert Rev. Mol. Diagn., 3(1):105-115.

Altalhi, A.D. and S.A. Hassan (2009). Bacterial quality of raw milk investigated by *Escherichia coli* and isolates analysis for specific virulence -gene markers. Food Control, 20 (10): 913–917.

Angulo, F., J.T. Lejeune and P.J. Rajala-Schultz (2009). Unpasteurized milk: A continued public health threat. Clin. Infect. Dis., 48 (1): 93-100.

APHA (2004) Standard Methods for the Examination of Dairy Products. 17th Ed., Ame. Public Health Assoc., Washington.

Ars`ene-Ploetze, F., O. Chiboub, D. Li`evremont, J. Farasin, K. C. Freel, S. Fouteau and V. Barbe (2018). Adaptation in toxic environments: comparative genomics of loci carrying antibiotic resistance genes derived from acid mine drainage waters. Environ. Sci. and Pollution Res., 25 (2): 1470–1483.

Bhardwaj, D.K., N.K. Taneja, D.P. Shivaprasad, A. Chakotiya, P. Patel, P. Taneja and M.G. Sanal (2021). Phenotypic and genotypic characterization of biofilm forming, antimicrobial resistant, pathogenic *Escherichia coli* isolated from Indian dairy and meat products. Int. J. Food Microbiol., 336: 108899.

Blair, J.M., M.A. Webber, A.J. Baylay, D.O. Ogbolu and L.J. Piddock (2015). Molecular mechanisms of antibiotic resistance. Nature Reviews Microbiol., 13 (1):42-51.

Chye, F.Y., A. Abdullah and M.K. Ayob (2004). Bacteriological quality and safety of raw milk in Malaysia. Food Microbiol., 21: 535–541.

CLSI (2017). Performance Standards for Antimicrobial Susceptibility Testing. 474 M100-S27. Clinical and Laboratory Standards Inst., Wayne, PA.

Corcoran, M., D. Morris, N. De Lappe, J. O'Connor, P. Lalor, P. Dockery and M. Cormican (2014). Commonly used disinfectants fail to eradicate *Salmonella enterica* biofilms from food contact surface materials. Appl. Environ. Microbiol., 80: 1507–1514.

Cruz-Soto, A.S., V. Toro-Castillo, C.O. Munguía-Magdaleno, J. E. Torres-Flores, L. E. Flores-Pantoja, P. D. Loeza-Lara and R. Jiménez-Mejía (2020). Genetic relationships, biofilm formation, motility and virulence of *Escherichia coli* isolated from bovine mastitis. Rev. Mex. Cienc. Pecu., 11(1): 167-182.

Da Silva Chagas, L.G., P. de Castro Melo, S.C. Brasão, G.B.R. Silvestre, E.C. Guimarães and A.M.C. Lima (2017). Evaluation of biofilm formation by bacterial strains isolated from milking equipment and milk samples from cows with mastitis. Semina: Ciências Agrárias, 38 (4): 1887-1895.

El-Bagory, A.M., A.M. Hammad and S.M. Alzahraa (2016). Prevalence of Coliforms, antibiotic resistant Coliforms and *E. coli* serotypes in raw milk and some varieties of raw milk cheese in Egypt. Nutr. Food Technol., 2 : 1.

El-Nahas, A.W., H.A. Mohamed, H.A. El Barbary and H.S. Mohamed (2015). Incidence of

E.coli in raw milk and its products. Benha Vet. Med. J., 29 (1): 112-117.

El-Prince, E., M. Sayed, M. Farghaly and A.M. Abdel-Rahman (2010) Investigation of milk and some dairy products for fecal pollution indicators. Assiut Vet. Med. J., 56 (127): 96–107.

El-Zamkan, M.A. and K.G.A. Hameed (2018). Molecular Characterization of Non-O157 Shiga Toxin-producing *E. coli* detected in raw milk and some dairy products. Microbiol. Res. J. Int., 1-14

Frye, J.G. and P.J. Fedorka-Cray (2007). Prevalence, distribution and characterization of ceftiofur resistance in *Salmonella enterica* isolated from animals in the USA from 1999 to 2003. Int. J. Antimicrob. Agents, 30: 134–142.

Garedew, L., A. Berhanu, D. Mengesha and G. Tsegay (2012). Identification of gram-negative bacteria from critical control points of raw and pasteurized cow milk consumed at Gondar town and its suburbs, Ethiopia. BMC Public Health, 12 (1):1-7.

Hall-Stoodley, L., J.W. Costerton and P. Stoodley (2004). Bacterial biofilms: From the natural environment to infectious diseases. Nat. Rev. Microbiol., 2: 95–108.

Jayarao, B.M. and D.R. Henning (2001). Prevalence of foodborne pathogens in bulk tank milk. J. Dairy Sci., 84: 2157–2162.

Kalkwarf H.J., J.C. Khoury and B.P. Lanphear (2003). Milk intake during childhood and adolescence, adult bone density and osteoporotic fracture in US women. Ame. J. Clin. Nutr., 77 (1): 257-265.

Katakweba, A.A., A.P. Muhairwa, A.M. Lupindu, P. Damborg, J. T. Rosenkrantz, U. M. Minga and J. E. Olsen (2018). First report on a randomized investigation of antimicrobial resistance in fecal indicator bacteria from Livestock, Poultry, and humans in Tanzania. Microbial Drug Resistance, 24 (3): 260–268

Khafagy, A., H. Eid, F. Youssif and N. E. H. Eid (2017). *Escherichia Coli* isolated from raw milk at North Sinai Governorate. Suez Canal Vet. Med. J. SCVMJ, 22(2): 121-131.

Khayal, A.A. and O.M. Ragia (2013). Biochemical and microbiological evaluation of fermented camel milk. New York Sci. J., 6: 74-79.

Kok, T., D. Worswich and E. Gowans (1996). Some serological techniques for microbial and viral infections. In Practical Medical Microbiology (Collee, J.; Fraser, A.; Marmion, B. and Simmons, A., eds.), 14th Ed., Edinburgh, Churchill Livingstone, UK.

Koraney, A.A. (2016). Molecular study on multiple antibiotic resistance of some bacteria isolated from food of animal origin in Egyptian Markets. A Thesis presented for the MVS in Vet. Med. Sci. Microbiol., Beni-Suif Univ., Fac. Vet. Med., Dept. Bact., Immunol. and Mycol**.**

Krumperman, P.H. (1983). Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. Appl. Environ. Microbiol., 46:165–170.

Lamey, A.E., A.M. Ammar, E.R. Zaki, N. Khairy, B.S. Moshref and M.K. Refai (2013). Virulence factors of *Escherichia coli* isolated from recurrent cases of clinical and subclinical mastitis in buffaloes. Int. J. Microbiol. Res., 4(1): 86-94.

Leedom, J.M. (2006). Milk of nonhuman origin and infectious diseases in humans. Clinc. Infect. Dis., 43: 610-615.

Marshall, T.A., S.M. Levy, B. Broffit, J.J. Warren, J.M. Eichenberger- Glimore, T.L. Bruns and P.J. Stumbo (2003). Dental caries and beverage consumption in young children. Pediatrics, 112(3):184-191.

McEwen, S.A. and P.J. Collignon (2018). Antimicrobial Resistance: A one-health perspective. Microbiol. Spectrum, 6 : 2.

Meshref, A.M.S. (2013). Bacteriological quality and safety of raw cow's milk and fresh cream. Slovenian Vet. Res., 50 (1): 21-30.

Mezyed, E.M., E.M. Sharaf and N.A. Abou El-Roos (2008). Occurrence of some enteric

pathogens in raw milk and some dairy products. Vet. Med. J. Giza, 56: 29–36.

Milanov, D., B. Prunić, M. Velhner, D. Todorović and V. Polaček (2015). Investigation of biofilm formation and phylogenetic typing of *Escherichia coli* strains isolated from milk of cows with mastitis. Acta Veterinaria-Beograd, 65 (2): 202-216.

Monte, J., A. Abreu, A. Borges, L. Simões and M. Simões (2014). Antimicrobial activity of selected phytochemicals against *Escherichia coli* and *Staphylococcus aureus* and their biofilms. Pathogens, 3:473–498.

Musa, M.D., R.S. Salman and A.D. Mutar (2019). Phylogenetic Background, biofilm and antibiogram profile of *E. coli* Isolated from raw milk soled in the local markets at Al-Nasiriyah City-Iraq. Int. J. Pharma. Res., 11: 1.

Nepal, G. and S. Bhatta (2018) Self-medication with antibiotics in who Southeast Asian region: A systematic review, Cureus, 10 : 4.

Newell, D.G., M. Koopmans, L. Verhoef, E. Duizer, A. Aidara-Kane, A. Sprong and H. Kruse (2010). Food-borne diseases - the challenges of 20 years ago still persist while new ones continue to emerge. Int. J. Food Microbiol., 139: 3-15.

Ngaywa, C., G.O. Aboge, G. Obiero, I. Omwenga, N. Ngwili, G. Wamwere and B. Bett (2019). Antimicrobial resistant *Escherichia coli* isolates detected in raw milk of livestock in pastoral areas of northern Kenya. Food Control, 102:173–178.

Nobili, G., I. Franconieri, M.G. Basanisi, G. La Bella, R. Tozzoli, A. Caprioli and G. La Salandra (2016). Short communication: Isolation of shiga toxin-producing *Escherichia coli* in raw milk and mozzarella cheese in southern Italy. J. Dairy Sci., 99 (10): 7877-7880.

Oliver, S.P., K.J. Boor, S.C. Murphy and S.E. Murind (2009). Food safety hazard associated with consumption of raw milk. Food Born Pathogen Dis., 6(7): 793-806.

Olsen, I. (2015). Biofilm-specific antibiotic tolerance and resistance. Eur. J. Clin. Microbiol. Infect. Dis., 34:877–886.

Peterson, E. and P. Kaur (2018) Antibiotic resistance mechanisms in bacteria: relationships between resistance determinants of antibiotic producers, environmental bacteria, and clinical pathogens. Front. Microbiol., 9:2928

Rashid, M., S.K. Kotwal, M.A. Malik and M. Singh (2013). Prevalence, genetic profile of virulence determinants and multidrug resistance of *Escherichia coli* isolates from foods of animal origin. Vet. World, 6 (3): 139-142.

Reinthaler, F.F., G. Feierl, H. Galler, D. Haas, E. Leitner , F. Mascher and E. Marth (2010). ESBL producing *E. coli* in Austrian sewage sludge. Water Res., 44 (6): 1981–1985.

Rzewuska, M., M. Czopowicz, M. Kizerwetter-´Swida, D. Chrobak, B. Błaszczak and M. Binek (2015). Multidrug resistance in *Escherichia coli* strains isolated from infections in dogs and cats in Poland. Sci. World J., (2007–2013)

Shalaby, H.Y., K. Baraka, M.S. Ibrahim and E.M. Khalaf (2019). Characterization of bacterial pathogens associated with milk microbiota in Egypt. Afri. J. Microbiol. Res., 13(28): 580-608.

Sobeih, A.M.K., I.I. Al-Hawary and I.M. Aman (2002). Microbiological quality of milk and ice cream sold in Kafr El-Sheikh and El-Gharbia governorates. Minufyia Vet. J., 2(1): 79–89

Soomro, A.H., M.A. Arain, M. Khaskheli and B. Bhutto (2002). Isolation of *Escherichia coli* from raw milk and milk products in relation to public health sold under market conditions at Tandojam. Pak. J. Nutr., 1(3): 151–152

Stepanovic, S., G. Vukovic, I. Dakic, B. Savic and M. Svabic-Vlahovic (2000). A modified micro titer plate test for quantification of staphylococcal biofilm formation. J. Microbiol. Methods, 40: 175–179

Tadesse, H.A., N.B. Gidey, K. Workelule, H. Hailu, Gidey S, A. Bsrat and H. Taddele (2018). Antimicrobial resistance profile of *E.*

coli isolated from raw cow milk and fresh fruit juice in Mekelle, Tigray, Ethiopia. Vet. Med. Int.

Tasci, F. (2011). Microbiological and chemical properties of raw milk consumed in Burdur. J. Anim. Vet. Adv., 10: 635–641.

Tezel, B.U. and P. Şanlıbaba (2018). A Major Concern in Food Industry. Contamination Reservoir, Bacterial Biofilm. Science within Food: Up-to-date Advances on Research and Educational Ideas, 1-10. https://www. researchgate. net/ publication/322745857.

Thaker, H.C., M.N. Brahmbhatt, J.B. Nayak (2012). Study on occurrence and antibiogram pattern of *E. coli* from raw milk samples in Anand, Gujarat, India. Vet. World, 5(9):556.

Van Boeckel T.P., C. Brower, M. Gilbert, B.T. Grenfell, S.A. Levin, T.P. Robinson, A. Teillant and R. Laxminarayan (2015). Global trends in antimicrobial use in food animals. Proc. Nat. Acad. Sci. United States Ame., 112 (18): 5649-5654.

Wenz, J.R., G.M. Barington, F.B. Garry, R.P. Ellis and R.J. Magnuson (2006). *Escherichia coli* isolates serotypes, genotypes and virulence genes and clinical coliform mastitis severity. J. Dairy Sci., 89: 3408-3412

Xu, Z.Q., M.T. Flavin and J. Flavin (2014). Combating multidrug-resistant Gram-negative bacterial infections. Expert Opin. Invest. Drugs, 23 (2):163–182.

أنماط مقاومة المضادات الحيوية وتكوين البيوفيلم لعترات الايشريكية القولونية المعزولة من لبن السوق الخام بمدينة الزقازيق

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في هذه الدراسة، تم جمع مائة عينة من حليب البقر الخام بشكل عشوائي من متاجر وأسواق الألبان المختلفة في مدينة الزقازيق لعزل وتصنيف الإيشريكية القولونية التي تعتبر مؤشرًا موثوقًا فيه للتلوث البرازي وأحد أسباب التسمم الغذائي.وقد تم التعريف الميكروسكوبي بواسطة صبغة الجرام والتعريف الكيميائي الحيوي من خلال اختبارات كيميائية حيوية مختلفة (ايمفيك). وقد كان معدل تواجد الإيشريكية القولونية في عينات الحليب الخام 47% كما أن التعريف السيرولوجي لعزلات الإيشريكية القولونية كشف أن ع 26 هي الأكثر انتشارا بين العزلات بالنسبة المئوية 21.3%. وتشكل الإيشريكية القولونية أكبر تهديد لصحة الإنسان بسبب مقاومتها المتزايدة للمضادات الحيوية. وقد تم اختبار الحساسية لمضادات الميكروبات بطريقة انتشار الأقراص ضد 10 مضادات ميكروبات وكشفت النتائج أن عزلات الإيشريكية القولونية كانت شديدة المقاومة للأموكسيسيلين كلافولانيك والأمبيسيلين والسيفوتاكسيم والسيفتازيديم بنسبة 89.4 % و89.4 % و100.0 % و100.0 % على التوالي. وكانت شديدة الحساسية للكلورامفينيكول والسيبروفلوكساسين والتتراسيكلين بنسبة 100.0 % و100.0 % و93.6 % على التوالي. بالإضافة إلى ذلك، أظهرت 89.4 % من عزلات الإيشريكية القولونية مقاومة متعددة للمضادات الحيوية. وتعد قدرة البكتيريا على الالتصاق بأسطح الأغذية وتكوين البيوفيلم مصدرًا لتلوث الغذاء الذي يؤثر على سلامة وصناعة الأغذية. فتم استخدام أطباق المعايرة الدقيقة لاختبار تكوين البيوفيلم وأشارت النتائج أن 78.7 % من عزلات الإيشريكية القولونية كانوا غير منتجين للبيوفيلم، و6.4% كانوا ذي انتاج ضعيف للبيوفيلم، و14.9% كانوا متوسطي الإنتاج للبيوفيلم ولم يكن هناك أي من العزلات ذي انتاج قوي للبيوفيلم.