OPEN ACCESS
Advances in Animal and Veterinary Sciences
Research Article

Relationship between Phenotypic and Genotypic Antimicrobial Resistance of *Escherichia coli* Isolates from Mastitic Milk

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Abstract | Bovine mastitis is the predominant problem in dairy farms worldwide which caused mainly by *Escherichia coli* (*E. coli*) as one of the main causes of what is called "environmental mastitis". A total of 68 *E. coli* isolates from 205 raw milk samples of Holstein cows with mastitis in different dairy farms from different governorates by bacteriological isolation and 63 by PCR were investigated for the *E. coli* 16S rRNA and *rfbE*O157 encoding gene as Shiga toxin-producing *E. coli* (STEC). The occurrence of *E. coli* O157 in mastitic cows was 3% within *E. coli* isolates. Molecular investigation of extended-spectrum β-lactamases (ESBLs) and plasmid-mediated AmpC β-lactamases (PABLs) encoding genes reported in all of the isolates (100%) encoded TEM-type ESBLs, none of which (0%) encoded OXA-type ESBLs, on the other hand, CTX-M-type ESBLs and SHV-type β-lactamases were encoded in 34/63 (53.9%) and 3/63 (4.7%) of the ESBL isolates, respectively and 27% exhibited CMYII-type PABLs. Plasmid-mediated colistin resistance encoding gene (*mcr-1*) was expressed in 1.6% of *E. coli* isolates. All *E. coli* isolates exhibited antibiotic multi-resistances with higher resistance to tetracycline and Trimethoprim-Sulfamethoxazole (45.7% and 37.3%, respectively), while the lowest resistance was observed for Amoxicillin/clavulanate (10.1%). Phenotypic resistance to extended-spectrum cephalosporins (ESCs) revealed that 42.3% of these strains were resistant to (cefotaxime and cefquinome), 15% resistant to Cefoxitin, while 32.2% were resistant to ceftazidime. Conclusively, *E. coli* was found to be the major cause of bovine mastitis treatment failure due to the multidrug resistance to most newly developed cephalosporins (third and fourth generations).

Keywords | Bovine mastitis, Milk, Antimicrobial Resistance, ESBLs, PABLs and *mcr-1*.

Received | March 30, 2021; **Accepted** | April 10, 2021; **Published** | July 01, 2021
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Citation | M El-Sayed S, Soliman SM, Fayed AAA, Ahmed SAH (2021). Relationship between phenotypic and genotypic antimicrobial resistance of *escherichia coli* isolates from mastitic milk. Adv. Anim. Vet. Sci. 9(8): 1223-1232.
DOI | http://dx.doi.org/10.17582/journal.aavs/2021/9.8.1223.1232
ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

INTRODUCTION

Mastitis is a major concern for dairy producers causing significant economic losses for the dairy industry. The most frequently isolated causative agent related to bovine intramammary infection is *Escherichia coli* (*E. coli*) (Keane et al., 2013; Olde et al., 2008; Bradley et al., 2007), Because *E. coli* is a widespread environmental pathogen, can invade the udder. The decrease in the incidence of clinical mastitis has a positive impact on animal health, animal welfare, antimicrobial usage, work pleasure, and net farm return (Trevisi et al., 2014). Herd management factors, such as milking technique and hygiene standards, were associated with variations in distributions of a mastitis-caus-

ing pathogen in the herd (Barkema et al., 1999; Dufour et al., 2011; Piepers et al., 2011; Levison et al., 2016).

Clinical mastitis can be caused mainly by coliform infections (De Vliegher et al., 2012). A wide range of systemic disease severity, from mild to severe with systemic signs including dehydration, shock, and even death (Wenz et al., 2001). As well as zoonotic public health impact on human especially Shiga-toxigenic *E. coli* (STEC) strains including O157 causing bovine mastitis (Lin et al., 2011).

Many problems facing the dairy industry requires antimicrobial therapy, Mastitis is one of them (Grave et al., 1999). Which is usually employed in treating/preventing mastitis, such as β-lactams, sulphonamides, quinolones, macrolides and tetracyclines (Bengtsson et al., 2009; Mathew et al., 2007; McEwen and Fedorka-Cray, 2002).

The misuse of antibiotics caused drug resistance and treatment failures in many cases, especially for multidrug-resistant bacteria (Suojala et al., 2013; Sweeney et al., 2018). Carattoli (2008) has announced the antimicrobial resistant *E. coli* strains increase within animals and claimed these animals to be a reservoir of such strains for humans and the environment. Potential transmission of resistant *E. coli* within animals and humans can occur through various pathways, as the food chain (Poirel et al., 2018).

Extended-spectrum β-lactamases (ESBLs) producing *E. coli*, which shows resistance to penicillins, aminopenicillins, and cephalosporins, including the third (ceftiofur) and fourth (cefquinome) generations, has been commonly isolated from food-producing animals with global veterinary and public health issues (Seiffert et al., 2013; Poirel et al., 2018). ESBLs that inactivates ESCs were graded as class A (TEM, SHV and CTXM) and class D (OXA) β-lactamases, While plasmid AmpC β-lactamases (PABLs) belonged to class C (CMYII) confer resistance to a wide variety of β-lactams, primarily 7-a-methoxy-cephalosporins (Cephamycins) such as cefoxitin (Livermore and Woodford, 2006; Jacoby, 2009). Antibiotics used for humans and animals are closely related, abuse of these drugs resulted in the development of multidrug-resistant bacteria (Cantas et al., 2013; Walther et al., 2017). So, for efficient control and treatment of mastitis; the causative agents of IMI in dairy herds need to be well-identified. Antimicrobial susceptibility determined in vitro has been considered as a pre-requisite for treatment. However, in vitro activity does not guarantee in vivo effectiveness in bovine mastitis treatment (Pyörälä, 2009).

Colistin, a member of polymyxins (polymyxin E), is the main drug for *E. coli* (Kempf et al., 2013; Poirel et al., 2017). But, Colistin resistance was identified due to the emergence of highly transmissible plasmid-mediated colistin-resistant (*mcr-1*) gene in *E. coli* strains obtained from animals, food, and patients from China (Liu et al., 2016). This resistance has created global issues due to the high transmission rate of the *mcr-1* gene to epidemic strains of *Enterobacteriaceae* and thus hinders the effectiveness of colistin in humans (Rebelo et al., 2018).

The objectives of this study were to identify the impact of multidrug resistance development of *E. coli* strains isolated from mastitic dairy cow's milk, evaluate phenotypic antibiotic resistance profile of isolated strains and their association to genotypic antimicrobial resistance to provide efficient treatment.

MATERIALS AND METHODS

Sample Collection

205 pooled milk samples were collected using the California mastitis test (CMT) from 205 mastitic dairy cows from five dairy farms located in Fayoum, Ismailia, El-sharkia, Alexandria and Giza governorates between November 2019 and October 2020. Milk samples (approximately 15 ml) were aseptically drawn from each cow immediately according to the National Mastitis Council, 1990 then samples were transferred to the laboratory for further examination.

Phenotypic Identification

Milk samples were cultured in Eosin Methylene Blue agar media (EMB) (Oxoid). Agar plates were incubated at 37°C, and the bacterial growth was evaluated after 24 and 48 hrs. Using phenotypic differentiation of bacterial species presumptively based on colony morphology and Gram's staining (David, 2011).

Genotypic Identification

The genomic DNA of all *E. coli* strains was extracted (Kang *et al.*, 2004) and stored at -20°C for detection of genes encoding for 16srRNA, *rfbE*O157 encoding virulence gene and antibiotic resistance genes of *E. coli* strains isolated from mastitic milk samples (Table 1).

PCR amplification of 16srRNA encoding gene was performed according to Wang et al. (2002) as illustrated in Table 1. The reaction was performed in a volume of 25 μl containing 12.5 μl of 2X Qiagen Multiplex PCR Master Mix (Qiagen GmbH, Hilden, Germany), 0.5 µl (10pmol/µl) concentrations of each primer, and 3 μl of DNA template. The amplified PCR products were subjected to electrophoresis using 1.5% agarose gel.

Table 1: Oligonucleotide primers used for conventional PCR assay.

Target gene	Primer sequence	Amplicon size (bps)	Source
Detection of *E. coli* isolates			
16S rRNA	CCCCCTGGACGAAGACTGAC ACCGCTGGCAACAAAGGATA	401	Wang *et al.* (2002)
β- lactamase genes			
O157 (*rfbEo157)*	CGG ACA TCC ATG TGA TAT GG TTG CCT ATG TAC AGC TAA TCC	259	Possé *et al.* (2007)
***E. coli* O157 gene**			
bla SHV	CTT TAT CGG CCC TCA CTC AA AGG TGC TCA TCA TGG GAA AG	237	Fang *et al.* (2014)
bla TEM	CGC CGC ATA CAC TAT TCT CAG AAT GA ACG CTC ACC GGC TCC AGA TTT AT	455	Monstein *et al.* (2007)
bla CTX-M	ATG TGC AGY ACC AGT AAR GTK ATG GC TGG GTR AAR TAR GTS ACC AGA AYC AGC GG	593	Boyd *et al.* (2004)
bla OXA	ACA CAA TAC ATA TCA ACT TCG C AGT GTG TTT AGA ATG GTG ATC	813	Ouellette*et al.* (1987)
PABLs encoding gene			
CMY II	AGCGATCCGGTCACGAAATA CCCGTTTTATG CACCCATGA	695	Junyoung *et al.* (2009)
Colistin resistance encoding gene			
mcr1	AGTCCGTTTGTTCTTGTGGC AGATCCTTGGTCTCGGCTTG	320	Ana Rita Rebelo *et al.* (2018)

Esc Resistant *E. Coli* Isolates Identification

ESC E. coli isolates were determined by resistance to one or more third and fourth generation cephalosporins (CDC, 2020).

Antimicrobial Susceptibility Test

Antibiotic susceptibility test of *E. coli* isolates against nine different antibiotics was performed according to the Kirby-Bauer disc diffusion method using Mueller-Hinton agar (Bauer et al., 1966). The susceptibility of the *E. coli* isolates against each antimicrobial agent was measured and readings have been noted and compared with the Clinical and Laboratory Standards Institute guidelines (CLSI, 2020) (Table 2).

Table 2: Different antimicrobials used in disc diffusion method.

Antibiotic	Concentration	Abbreviation
Tetracycline	30 µg	TE
Cefoxitin	30 µg	CX/FOX
Cefotaxime	30 µg	CTX
Trimethoprim Sulfamethoxazole	1.25/23.75 µg	SXT
amoxycillin clavulanate	20/10 µg	AMC
Cefquinome	30 µg	CEQ
Cetazidime	30 µg	CAZ

RESULTS AND DISCUSSION

A high phenotypic prevalence (using EMB) of *E. coli* intramammary infection from mastitic dairy cows (68 out of 205) at the percent of 33%, where the genotypic prevalence (PCR to detect 16S rRNA gene) revealed 30.7% (63 out of 205) of dairy cows contract *E. coli* infection. The prevalence rate of bovine mastitis caused by *E. coli* was 33% of the overall milk samples. Most infections of the cows with *E. coli are* from their environment, as faces and straw as hypothesized by Lipman et al. (1995).

The proved 63 *E. coli* strains were then subjected for detection of *E. coli* O157 virulence gene, where only two STEC strains having *rfbE*O157 encoding gene had been detected using uniplex PCR at a percentage of 3.2% (Figure 1 & 2). Shiga toxin-producing *E. coli* (STEC) strains considered to be the most important pathogens of a recently emerged group of food-borne strains in the milk of infected cows. This type of strain has been associated with outbreaks of diarrhoea, gastroenteritis and hemorrhagic colitis (HC) or the hemolytic uremic syndrome (HUS) in humans (Karmali, 1989; Paton and Paton, 1998; Beutin et al., 2004). It is agreed with Hassan et al. (2012) who recorded that STEC strains can induce bovine mastitis and reduce milk quality for human consumption because some of the mastitis cases are subclinical and the diagnosis is based solely on accurate diagnostic tests.

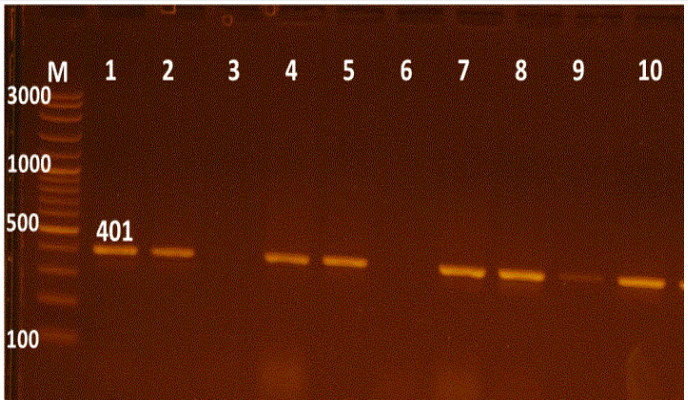

Figure 1: Uniplex PCR for 16s rRNA detection of *E. coli*, Lane M:100-3000bpDNA marker; Lanes 1-10 were positive *E. coli* isolates.

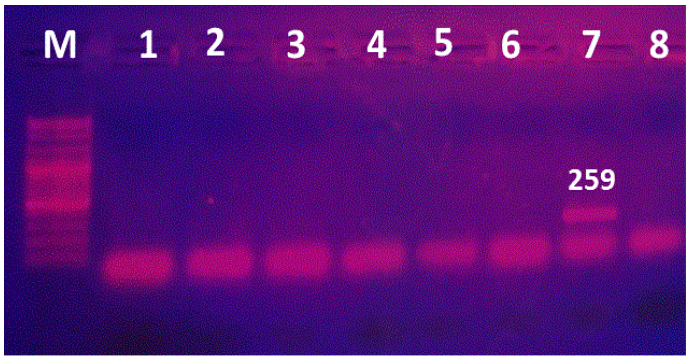

Figure 2: PCR for detection of *rfbEO157* encoding gene. Lane M:100-3000bpDNA marker; Lane 1, 2: positive isolate for *rfbEO157* gene; Lanes 3-7: negative strains.

Domestic ruminants, especially cattle, sheep and goats, are the principal reservoirs of STEC strains that cause human infections (Zschock et al., 2000; Chapman et al., 2001).

Regarding resistance genes, all of the isolates (100%) encoded TEM-type ESBLs, while none of which (0%) encoded OXA-type ESBLs. But, both CTX-M-type ESBLs and SHV-type β -lactamases were encoded in 53.9% (34 out of 63) and 4.7% (3 out of 63) of the ESBL isolates, respectively. Also, 27% exhibited CMYII-type PABLs. For plasmid-mediated colistin resistance encoding gene (mcr-*1*) was expressed in only one *E. coli* isolate at a percentage of 1.6% (1 out of 63). Regarding phenotypic non-β-lactams antimicrobial resistance, about 45.7% of *E. coli* isolates showed resistance to tetracycline, while 37.3% exhibited resistance to Trimethoprim-Sulfamethoxazole. This finding is similar to Sobhy et al. (2020) who associated higher resistance to Tetracyclines and Sulfamethoxazole/Trimethoprim with the prolonged use of these cheap antibiotics in the Egyptian dairy farms. In the same regard, Okubo et al. (2019) reported about 47.8% of bovine *E. coli* strains were co-resistant to Ampicillin, Tetracycline and Sulfamethoxazole/Trimethoprim due to extensive use of these antimicrobials in Ugandan livestock.

Concerning molecular detection of the *mcr-1* gene in ESC *E. coli* isolates were about 3%. This finding is in contrast to Umpiérrez et al. (2017) who recorded the absence of the *mcr-1* gene in bovine *E. coli* strains, while Haenni et al. (2016) who detected an increase in the proportion of *mcr-1* within ESBL-producing *E. coli* strains ranged from 4.76% in 2006 to 21.28% in 2014, prompting reducing colistin exposure.

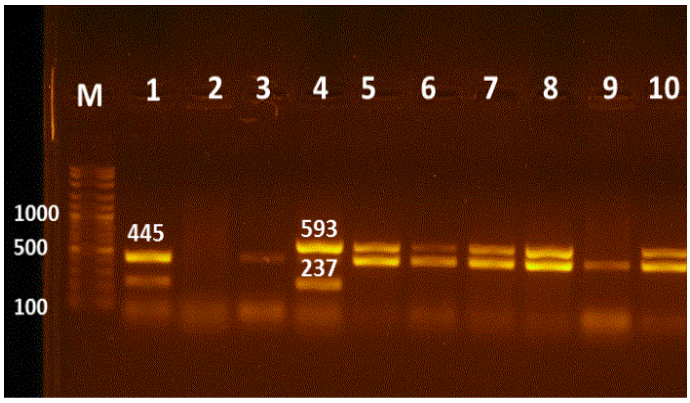

Figure 3: Multiplex PCR for detection of bla_{TEM}, bla_{CTXM}, bla_{SHV} and bla_{OXA} genes in *E. coli* isolates. Lane M:100-3000bpDNA marker; Lane 1 positive bla_{TEM}& bla_{SHV} at 445, 237bp, respectively; Lanes 3,9 positive bla_{TEM} at 445bp; Lane 4 positive bla_{CTXM}, bla_{SHV} *at* 593, 237bp, respectively; Lanes 5-8&10 positive strains for bla_{TEM} & bla_{CTXM} at 445 and 593bp; Lane 2 negative sample.

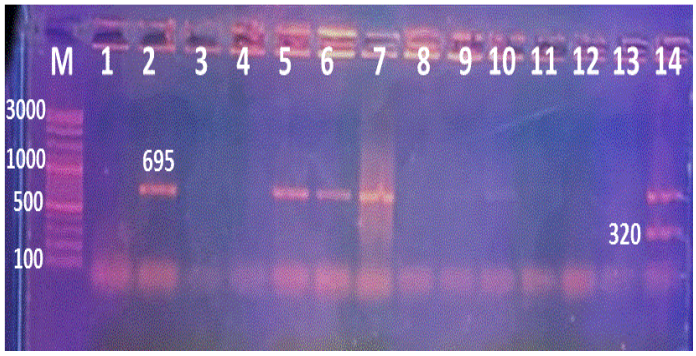

Figure 4: Multiplex PCR for *mcr1* and bla_{cmyII} genes detection, Lane M:100-3000bpDNA marker; Lanes 2,5, 6 and 7 were positive bla_{cmyII} at 695bp, while Lane 14 positive *mcr1* and bla_{cmyII} at 320, 695bp, respectively. Lanes 1,3, 4 and 8-13 are negative samples.

21 *E. coli* isolates demonstrated phenotypic resistance to cefotaxime (CTX) and were encoding for the bla_{CTX} gene. But, only 4 isolates showed phenotypic resistance although they lack bla_{CTX} resistance gene (Table 3 & 4) & (Figure 3 & 4). The phenotypic resistance to ESCs antibiotics as (Cefotaxime, Cefquinome and Ceftazidime) was increased, due to their extensive and widespread use in veterinary medicine as mentioned by Ahmed and Shimamoto, (2015) when they declared that ESCs (3rd and 4th generation Cephalosporins) are necessary antibiotics used in vet

Table 3: Comparison between phenotypic and genotypic antimicrobial resistance pattern of *E. coli isolates* and their relation to O157 virulence gene.

Isolate code	O157	Resistance phenotype							ESBLs genes				Colistin resistance *mcr1*	PABLs bla_{cmyII}
		B-lactams					Non B-lactams							
		CX	CAZ	CTX	CEQ	AMC	SXT	TE	bla_{TEM}	bla_{CTX}	bla_{SHV}	bla_{OXA}		
2, 18				+	+			+	+	+				
11, 57									+					+
14	+	+	+						+	+				
15			+	+	+				+	+				
16				+					+	+				
17				+	+		+		+	+				
20, 175, 177, 178									+	+				+
22, 50, 35				+	+		+	+	+	+				
23				+					+					
28, 41, 187, 204									+	+				
34, 46							+		+	+				
42			+	+	+			+	+	+				
45			+	+				+	+		+			
48, 188				+	+				+	+				
81			+	+	+		+	+	+	+	+			
103, 104, 109, 111							+	+	+					
110, 202							+	+	+					+
130			+	+	+	+	+	+	+	+				
148							+		+					
149, 150, 163		+	+						+					
151		+					+		+					
155			+						+					
157						+		+	+					
176			+	+	+		+	+	+					+
179					+				+	+				+
181				+	+			+	+	+				+
182					+			+	+	+				+
183					+				+	+				
184									+	+				+
185				+	+				+	+				+
186			+	+	+	+		+	+	+				+
189	+	+	+	+	+			+	+	+			+	+
191			+	+	+	+	+	+	+					
192		+	+	+	+	+	+	+	+	+				+
194		+	+	+	+				+					
195			+	+	+	+	+	+	+	+				+
196		+	+	+	+	+	+	+	+	+				

198, 200			+	+					
203				+					

Table 4: Multidrug resistance pattern and resistance gene of *E. coli isolates*

Number of isolates	Resistance phenotype	16s RNA	O157	ESBLs genes				Colistin *(mcr1)*	PABLs (bla_{cmyII})
				TEM	CTX	SHV	OXA		
17	SXT, CTX, CEQ	+	-	+	+	-	-	-	-
22, 50	SXT, TE, CTX, CEQ	+	-	+	+	-	-	-	-
35	SXT, TE, CTX, CEQ	+	-	+	+	-	-	-	-
81	SXT, TE, CAZ, CTX, CEQ	+	-	+	+	+	-	-	-
14	CX, CAZ	+	+	+	+	-	-	-	-
109, 111,104, 103, 77	SXT, TE	+	-	+	-	-	-	-	-
163, 150, 149	CX, CAZ	+	-	+	-	-	-	-	-
157	TE, AMC	+	-	+	-	-	-	-	-
151	SXT, CX	+	-	+	-	-	-	-	-
130	SXT, TE, AMC, CAZ, CTX, CEQ	+	-	+	+	-	-	-	-
110	SXT, TE	+	-	+	-	-	-	-	+
2, 18	TE, CTX, CEQ	+	-	+	+	-	-	-	-
42	TE, CAZ, CTX, CEQ	+	-	+	+	-	-	-	-
45	TE, CAZ, CTX	+	-	+	-	+	-	-	-
15	CAZ, CTX, CEQ	+	-	+	+	-	-	-	-
48, 188	CTX, CEQ	+	-	+	+	-	-	-	-
181	TE, CTX, CEQ	+	-	+	+	-	-	-	+
182	TE, CEQ	+	-	+	+	-	-	-	+
185	CTX, CEQ	+	-	+	+	-	-	-	+
186	SXT, TE, CAZ, CTX, CEQ	+	-	+	+	-	-	-	+
189	TE, CX, CAZ, CTX, CEQ	+	+	+	+	-	-	+	+
195	SXT, TE	+	-	+	-	-	-	-	-

Table 5: Extended spectrum and plasmid mediated ampicilin β-lactamases and colistin resistance genes of *E. coli* isolates from mastitic milk samples.

E. coli isolates		ESBLs				PABLs no.	Colistin no.
		bla_{TEM}	bla_{CTXM}	bla_{SHV}	bla_{OXA}	bla_{CMYII}	Mcr-1
No.	63	63	34	3	0	17	1
%	-	100	54	4.7	0	27	1.6

erinary and human medicine.

The molecular detection of resistance genes such as ESBLs, PABLs and colistin resistance genes revealed that all *E. coli* isolates harbour bla_{TEM} and about half of them bear bla_{CTXM}, while 27% of the isolated have bla_{CMYII} and only one isolate (1.6%) has *Mcr-1* (Table 5) (Figure 3 & 4). Chirila et al. (2017) and Poirel et al. (2018) declared that *E. coli* may develop resistance to antimicrobials by chromosomal genes mutation or by horizontal gene transfer of resistance genes within commensal and pathogenic *E. coli* strains, rendering *E. coli* as a major reservoir of resistant genes that could be responsible for human and veterinary treatment failure.

There was a significant increase in isolates with resistance genes and exhibit ESC resistance as isolates carried $bla_{TEM,}$ bla_{TEM}+ bla_{CTXM} *and* bla_{TEM}+ bla_{CTXM}+ bla_{CMYII}. In addition, ESC susceptible isolates also bear resistance genes such as bla_{TEM}+, bla_{TEM}+ bla_{CTXM}+ bla_{CMYII}, bla_{TEM}+ bla_{CTXM} *and* bla_{TEM}+ bla_{CMYII} (Table 6). ESC resistant *E. coli* strains were determined according to their resistance to one or more of

Table 6: ESC resistant and susceptible *E. coli* isolates and their antimicrobial resistance genes profile.

ESC resistance	No. of isolates	bla_{TEM}+					bla_{TEM}+	bla_{TEM}+& bla_{CTXM}+		bla_{TEM}+, bla_{CTXM}+& bla_{CMYII}+
		bla_{CTXM}	bla_{SHV}	bla_{OXA}	bla_{CMYII}	Mcr-1		bla_{SHV}	bla_{CMYII}	Mcr-1
Resistant	33	15 (45.4%)	1 (3%)	-	1 (3%)	-	7 (21.2%)	1 (3%)	7 (21.2%)	1 (3%)
Susceptible	26	5 (19.2%)	1 (3.8%)	-	3 (11.5%)	-	12 (46%)	-	5 (19.2%)	-

Table 7: Antibiotic susceptibility profile of *E. coli* isolates

Antimicrobial agents		Abbreviation	Conc. (µg)	Susceptible		Intermediate		Resistance	
				No.	%	No.	%	No.	%
Non β-lactams	Trimethoprim/Sulfamethoxazole	SXT	1.25/23.75	38/59	64.4	0/59	0	22/59	37.3
	Tetracycline	TE	30	26/59	44.1	7/59	11.8	27/59	45.7
β-lactams	Cefoxitin	CX	30	48/60	80	3/60	5	9/60	15
	Amoxicillin/ clavulanate	AMC	20/10	50/59	84.7	4/59	6.7	6/59	10.1
	Ceftazidime	CAZ	30	35/59	59.3	6/59	10.2	19/59	32.2
	Cefotaxime	CTX	30	32/59	54.2	3/59	5.1	25/59	42.3
	Cefquinome	CEQ	30	29/59	49.1	6/59	10.2	25/59	42.3

the 3rd and 4th generations of cephalosporins (CDC, 2020). In this line, the percentage of ESC resistant isolates was high 56% (33 out of 59). Based on ESCs resistance pattern and the presence of antimicrobial resistance genes, it was observed that the highest percentage of ESCs resistant *E. coli* isolates had bla_{TEM}+ bla_{CTXM} followed by bla_{TEM}+ and bla_{TEM}+ bla_{CTXM}+ bla_{CMYII}. Although ESC susceptible *E. coli* isolates possess β-lactamase resistance genes but were not expressed in vitro or phenotypically. It is agreed with Ahmed et al. (2009) when declared that *E. coli* strains showed phenotypic antibiotic multi-resistance primarily against ESCs, including Cefotaxime and Ceftriaxone, and other non-β-lactams; especially Tetracycline, Sulfamethoxazole/ Trimethoprim, Nalidixic acid and Ciprofloxacin.

With regard to *E. coli* isolates, about 45.4% of ESC resistant isolates were attributed to the presence of bla_{TEM} and bla_{CTXM} genes followed by bla_{TEM} and bla_{TEM}+ bla_{CTXM}+ bla_{CMYII} combinations at a rate of 21.2% for each. Almost all ESBLs producing isolates having bla_{TEM} and two combinations including bla_{TEM} + bla_{CTXM} + bla_{CMYII} and bla_{TEM} + bla_{CTXM} + bla_{CMYII} + *mcr1*, which conferred resistance to Cefquinome as ESCs. It was contrary to Filioussis et al. (2020) who determined ESBLs producing strains from milk have bla_{TEM}+ bla_{SHV}+ *mcr-1* and bla_{TEM}+ bla_{CTXM} + *mcr-1* combinations that conferred resistance to ESCs especially cefquinome.

Most ESC resistant *E. coli* isolates 26/33 (78.8%) have more than one antimicrobial resistance gene. This was agreed with Awosile et al. (2018) who determined the existence of two or more β-lactamase genes within 44% of ESC resistant strains, illustrating the phenotypic resistance of *E. coli* isolates is highly dependent on the co-existence of two or more β-lactamase genes in such isolates. The majority of ESC *E. coli* isolates 25/33 (75.7%) have β-lactamase CTX as reported by Livermore et al. (2007) and Seiffert et al. (2013) when it was noted that the worldwide evolution of β-lactamase CTX (Cefotaximase) has been identified and is known to be the most common cause of ESC resistance in the Enterobacteriaceae.

Concerning the antimicrobial susceptibility profile; Seven *E. coli* isolates (11.8%) were susceptible to all antimicrobials and only two isolates (3.4%) were resistant to all antimicrobials, eleven isolates (18.6%) expressed resistance to a single compound, and 36 isolates (61%) showed resistance to more than one antimicrobial agent. Whereas, twelve isolates (20.3%) were expressing resistance to 3 related compounds (i.e., Cefotaxime, Cefquinome and Ceftazidime) as extended spectrum cephalosporins (Table 7) & (Figure 5). Multidrug resistance (MDR) among *E. coli* isolates was high, where 61% of *E. coli* isolates (36/59) showed MDR against two or more antimicrobials, nine isolates (15.3%) exhibit MDR for β-lactam antibiotics, six isolates (10.2%) for non-β-lactam antibiotics, 19 isolates (32.2%) were resistant to both (β-lactams and non-β-lactams) and two isolates (3.4%) showed resistance to all antimicrobial used in this study. Our results were compatible with Pasayo et al. (2019) who declared that frequent use of antibiotic treatment leading to the production of multi-resistant strains that pose a major public health threat. But not aligned with Ahmed et al. (2009) who stated that lower multi-antibiotic resistance has been found in *E. coli*

strains are 10.4%.

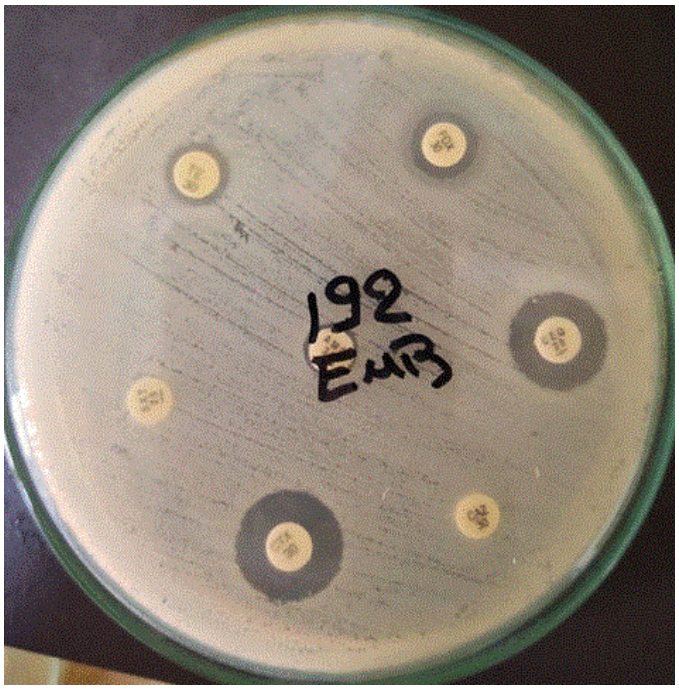

Figure 5: antibiotic sensitivity testing of *E. coli* isolates.

CONCLUSION

The emergence of antimicrobial resistance in particular to the recently introduced antimicrobials such as 3rd and 4th generations of cephalosporins in *E. coli* strains attributed to antimicrobial misuse in dairy farms for bovine mastitis therapy. *E. coli* strains acquire antimicrobial resistance through plasmid-mediated transfer leading to a widespread of multidrug resistance to ESCs, β-lactams and non-β-lactams antibiotics that can induce treatment failure in dairy farms.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Mohamed Ibrahim, farm managers, staff and all helped with this work.

CONFLICT OF INTEREST

There are no conflicts of interest in this report, according to both of the contributors.

AUTHOR CONTRIBUTIONS

Adel Abdel-Azim Fayed approved of and arranged the tests. Soliman Mohamed Soliman created the hypothesis, computed it, and analyzed the results. The experiment was carried out by Samah El-sayed Mahmoud, and the manuscript was written with input and help from all contributors. Adel Abdel-Azim Fayed and Samia Abd El-Hamid Ahmed created the model and nearly all of the technical data. All writers discussed the conclusions, offered critical input, assisted in the creation of the study, and collaborated on the final manuscript.

ETHICS STATEMENT

All research procedures were carried out in accordance with the Animal Ethics Committee of APRI, ARC, Egypt's recommendations for the treatment and use of lab animals.

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