

Co-occurrence of extended spectrum beta-lactamase genes and mcr-1 gene in strains of *Escherichia coli* isolated from clinical cases of bovine mastitis in Greece

G. Filioussis, G. Christodouloupoulos, M. Adamopoulou, A. Tzivara, S.K. Kritas

Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, GR

Faculty of Veterinary Medicine, School of Health Sciences, University of Thessaly, GR

Microbiology-Virology Laboratory, Technological Institution of Athens, GR

Background

- What is bovine mastitis
 - Inflammation of one or more quarters of the udder



Swelling
pain
warm
redness

- Causes **significant economic losses** to the dairy industry in the EU
 - ✓ 200 Euro/infected cow/year
 - ✓ 1 billion Euro/year/EU

Background

- **What is coliform bovine mastitis**
 - **Caused by** *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae* and *Serratia marcesans*.
 - **Source:** Infected cows, environment, replacement animals
 - **Clinical signs:** Inflammation of the udder, high fever, depressed appetite, rapid weight loss, abnormal milk and decreased production
 - **Treatment :** Fluid therapy, anti-inflammatories, steroids, and systemic antibiotics with Gram-negative activity
 - **Health concerns :** Poor quality milk, antibiotic residues in milk



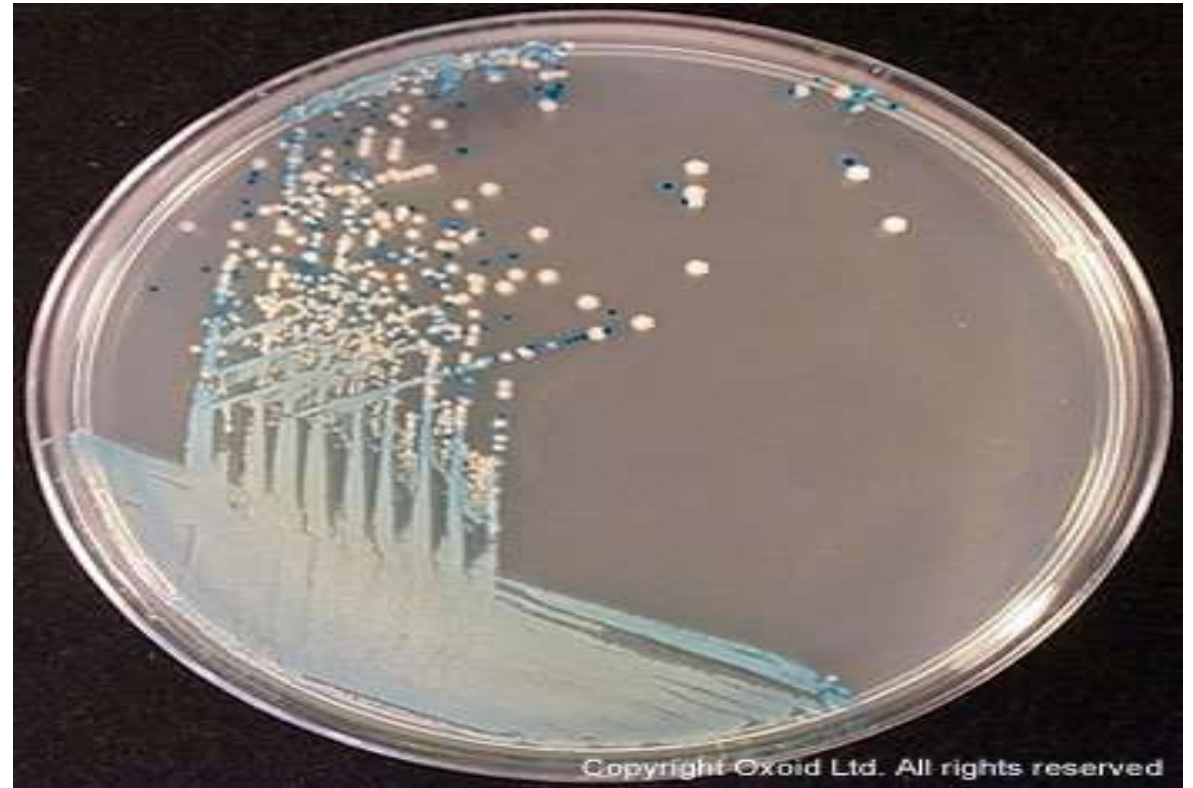
Material

- December 2015 - April 2016
- 3-9 years of age.
- 8 farms in the Prefecture of Central Macedonia.
- Average animals 350 cows/herd.
- Hygiene conditions were varying from poor to satisfactory.
- Number of workers was varying from 3-8.
- In all 8 farms we verified the use of antibiotics.
- 50 cow/herd that exposed clinical mastitis during the last three months.
- Milk samples collected in sterile Falcon tubes.
- Isolation of *E.coli*.



Isolation and Identification of *E. coli*

- Inoculation in a chromogenic agar for the detection of β -glycouronidase activity (TBX Oxoid Ltd)
- Incubation in 37°C/4h and later in 44°C/18h
- The presumptive colonies in the chromogenic agar were:
 - Further identified as *E. coli* by API 20E identification strips.
 - Species identification of the isolates was confirmed by PCR that detects *usp(A)* gene



Copyright Oxoid Ltd. All rights reserved

Antibiotic Resistance of *E. coli*

- Disk Diffusion Method (Kirby-Baeur) , MICs of colistin were obtained on Mueller-Hinton agar by the agar dilution method
- Quality Control *Escherichia coli* ATCC 25922
- 16 antibiotics were used
- For the interpretation of the results, guidelines of the CLSI(M100-24/2014) were applied.

nalidixic acid NAL 30	ciprofloxacin CIP 5	doxycycline DOX 30	tetracycline TET 30	gentamycin GMN 10	Trim.Sulfom. SXT(1.75/23.75)
streptomycin SMN 10	ceftriaxone CRO 30	cefotaxime CTX 30	ceftazidime CAZ 30	amoxicillin clavulanate AMC 10/20	aztreonam ATM 30
chloramphenicol CHL 30	ampicillin AMP 10	piperacillin PIR100	Colistin COL: ranging from 0.25 to 128 µg/ml and inoculum of 10 ⁴ colony forming units per spot		

Detection of *bla* genes and *mcr-1* gene

Amplicon	Primer sequence	Size (bp)	Reference
<i>bla</i> SHV	CTT TAT CGG CCC TCA CTC AA	237	Fang,et al,2004. J. Clin. Microbiol. 42:5917–5920.
	AGG TGC TCA TCA TGG GAA AG		
<i>bla</i> TEM	CGC CGC ATA CAC TAT TCT CAG AAT GA	445	Monstein, et al, 2007. APMIS 115:1400–1408.
	ACG CTC ACC GGC TCC AGA TTT AT		
<i>bla</i> CTX-M	ATG TGC AGY ACC AGT AAR GTK ATG GC	593	Boyd, et al, 2004. Antimicrob. Agents Chemother. 48:3758–376
	TGG GTR AAR TAR GTS ACC AGA AYC AGC GG		
<i>mcr-1</i>	GCAACCAAGCCTGATATGCG	521	The present study
	CGCTTAAAATACGCAGGCC		

Isolation rate of *E. coli* from milk samples

	Herd.1	Herd.2	Herd.3	Herd.4	Herd.5	Herd.6	Herd.7	Herd.8	Total
<i>E. coli</i> isolates	12	7	23	11	9	10	4	13	89
Samples tested	50	50	50	50	50	50	50	50	400
Isolation rate	24%	14%	46%	22%	18%	20%	8%	26%	22,25%

Antibiotic Resistance rate of *E. coli*



NAL 30	CIP 5	DOX 30	TET 30
41,5% (37/89)	34,8% (31/89)	62,9% (56/89)	57,3% (51/89)
GMN 10	SXT(1.75/23.75)	SMN 10	CRO 30
10,11% (9/89)	35,9% (32/89)	53,9% (48/89)	20,22% (18/89)
CTX 30	CAZ 30	AMC 20/10	ATM 30
20,22% (18/89)	20,22% (18/89)	20,22% (18/89)	20,22% (18/89)
CHL 30	AMP 10	PIR 100	COL *
7,86% (7/89)	51,68% (46/89)	23,59% (21/89)	6,74% (6/89)
*MIC 8 µg/ml to 32 µg/ml			

Antibiotic Resistance rate of *E. coli*



NAL 30	CIP 5	DOX 30	TET 30
41,5% (37/89)	34,8% (31/89)	62,9% (56/89)	57,3% (51/89)
GMN 10	SXT(1.75/23.75)	SMN 10	CRO 30
10,11% (9/89)	35,9% (32/89)	53,9% (48/89)	20,22% (18/89)
CTX 30	CAZ 30	AMC 20/10	ATM 30
20,22% (18/89)	20,22% (18/89)	20,22% (18/89)	20,22% (18/89)
CHL 30	AMP 10	PIR 100	COL *
7,86% (7/89)	51,68% (46/89)	23,59% (21/89)	6,74% (6/89)
*MIC 8 µg/ml to 32 µg/ml			

Antibiotic Resistance rate of *E. coli*



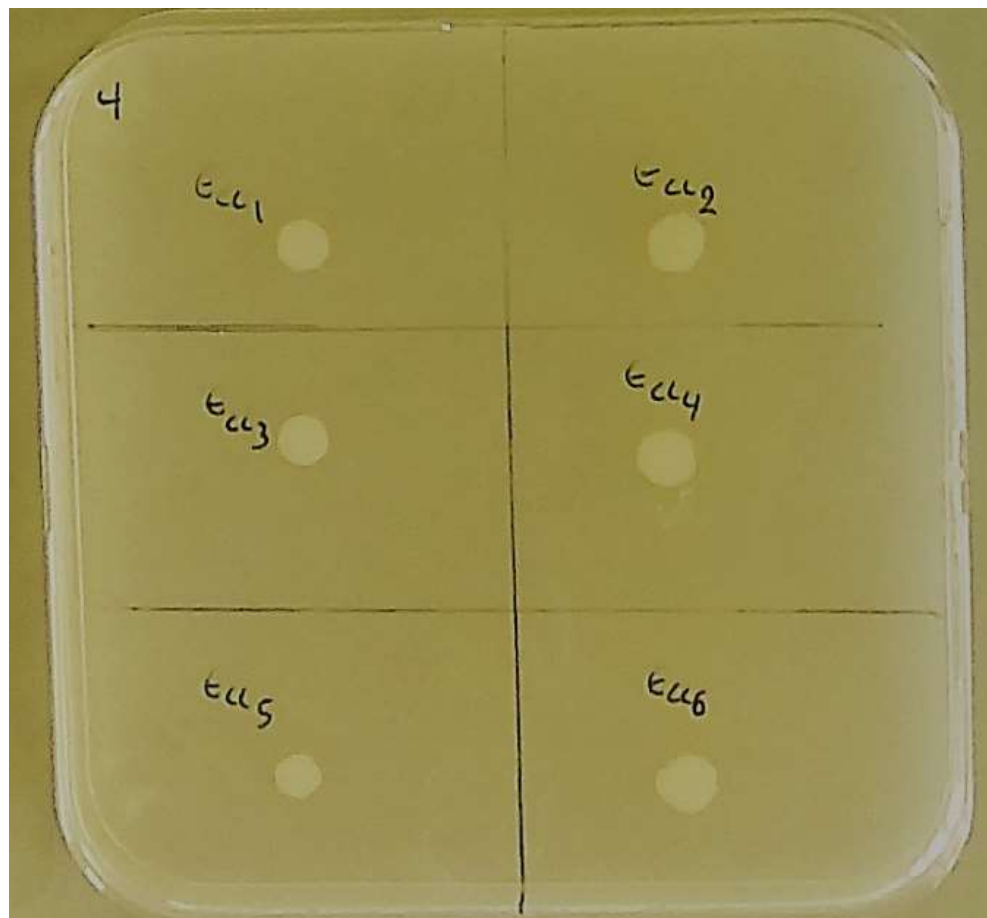
NAL 30	CIP 5	DOX 30	TET 30
41,5% (37/89)	34,8% (31/89)	62,9% (56/89)	57,3% (51/89)
GMN 10	SXT(1.75/23.75)	SMN 10	CRO 30
10,11% (9/89)	35,9% (32/89)	53,9% (48/89)	20,22% (18/89)
CTX 30	CAZ 30	AMC 20/10	ATM 30
20,22% (18/89)	20,22% (18/89)	20,22% (18/89)	20,22% (18/89)
CHL 30	AMP 10	PIR 100	COL *
7,86% (7/89)	51,68% (46/89)	23,59% (21/89)	6,74% (6/89)
*MIC 8 µg/ml to 32 µg/ml			

Antibiotic Resistance rate of *E. coli*

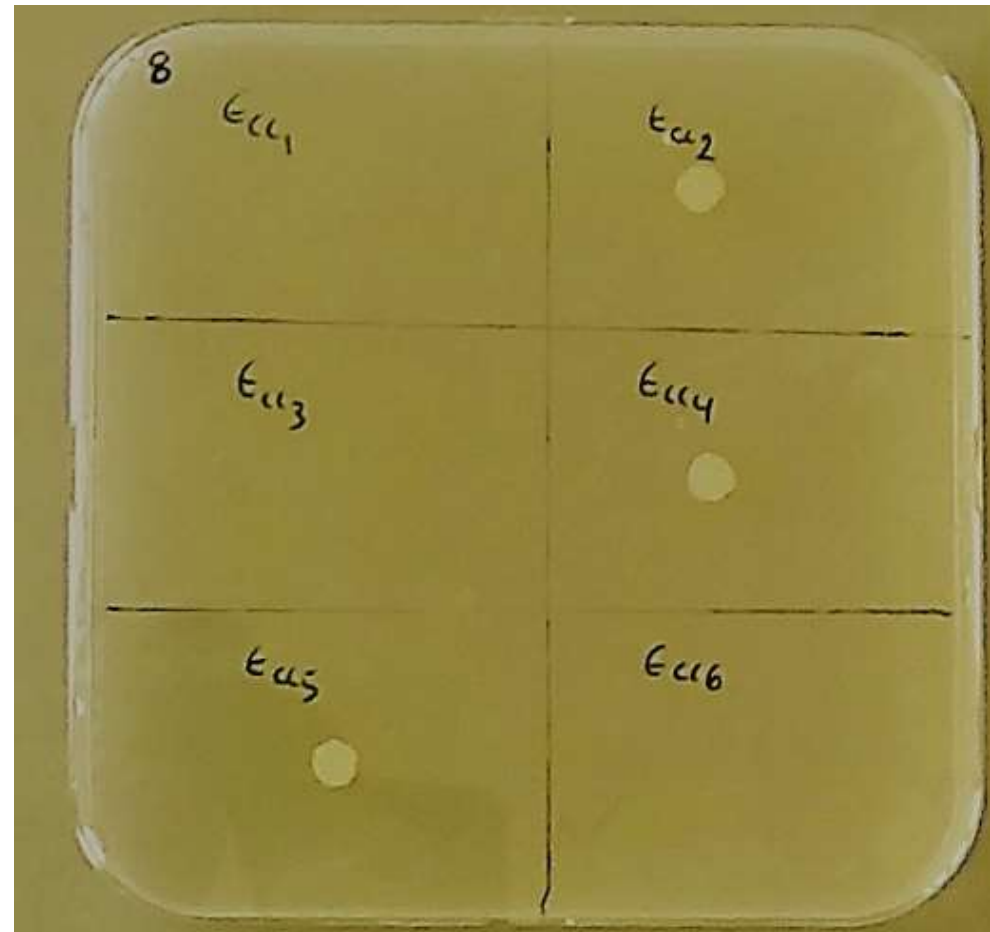


Isolate	NAL 30	CIP 5	DOX 30	TET 30	GMN 10	STX 1.75/2 3.75	SMN 10	CRO 30	CTX 30	CAZ 30	AMC 20/10	ATM 30	CHL 30	AMP 10	PIR 100
E.cc1	21	33	21	20	21	6	8	18	20	7	8	16	11	6	8
E.cc2	23	30	10	6	20	8	9	16	12	8	13	9	14	6	8
E.cc3	21	30	16	17	17	6	6	16	19	6	11	16	11	9	8
E.cc4	21	29	19	22	20	12	6	19	18	7	12	15	14	6	6
E.cc5	25	27	25	24	19	10	8	17	16	8	12	16	18	6	9
E.cc6	24	22	21	26	22	8	14	15	19	11	13	9	15	8	11
CLSI M100-S24	13	15	10	11	12	12	11	19	22	17	13	17	12	13	17

Antibiotic Resistance rate of *E. coli*



Colistin 4 µg/ml

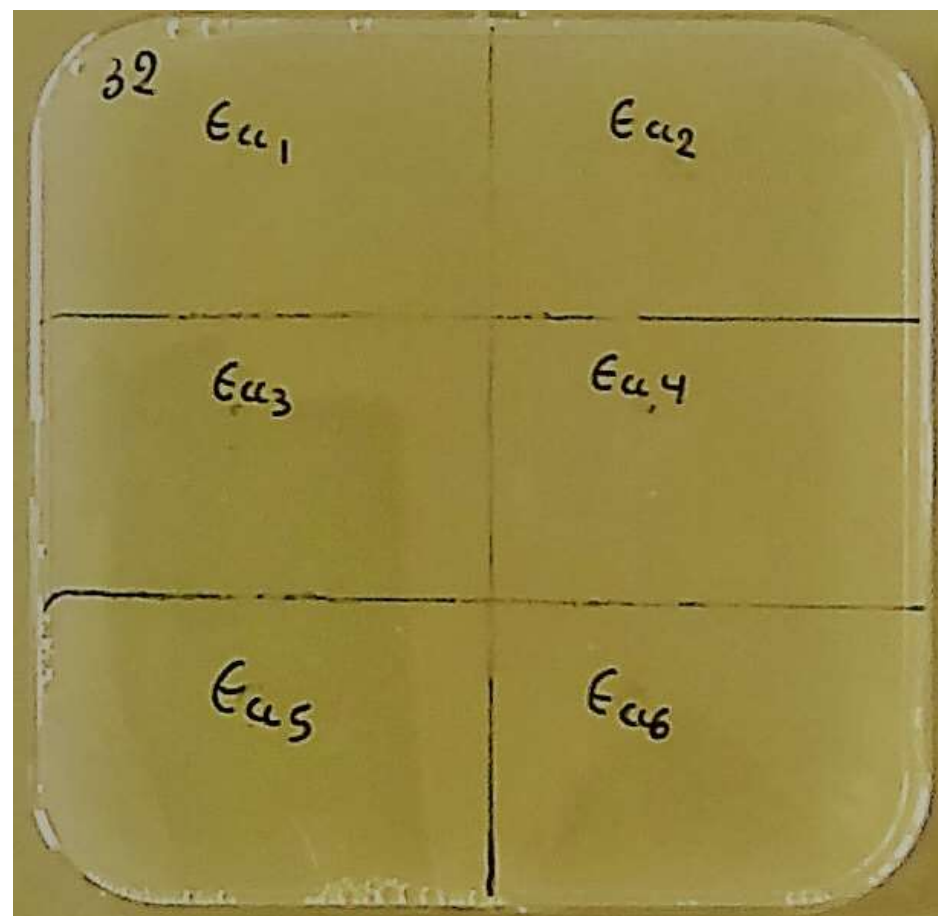


Colistin 8 µg/ml

Antibiotic Resistance rate of *E. coli*

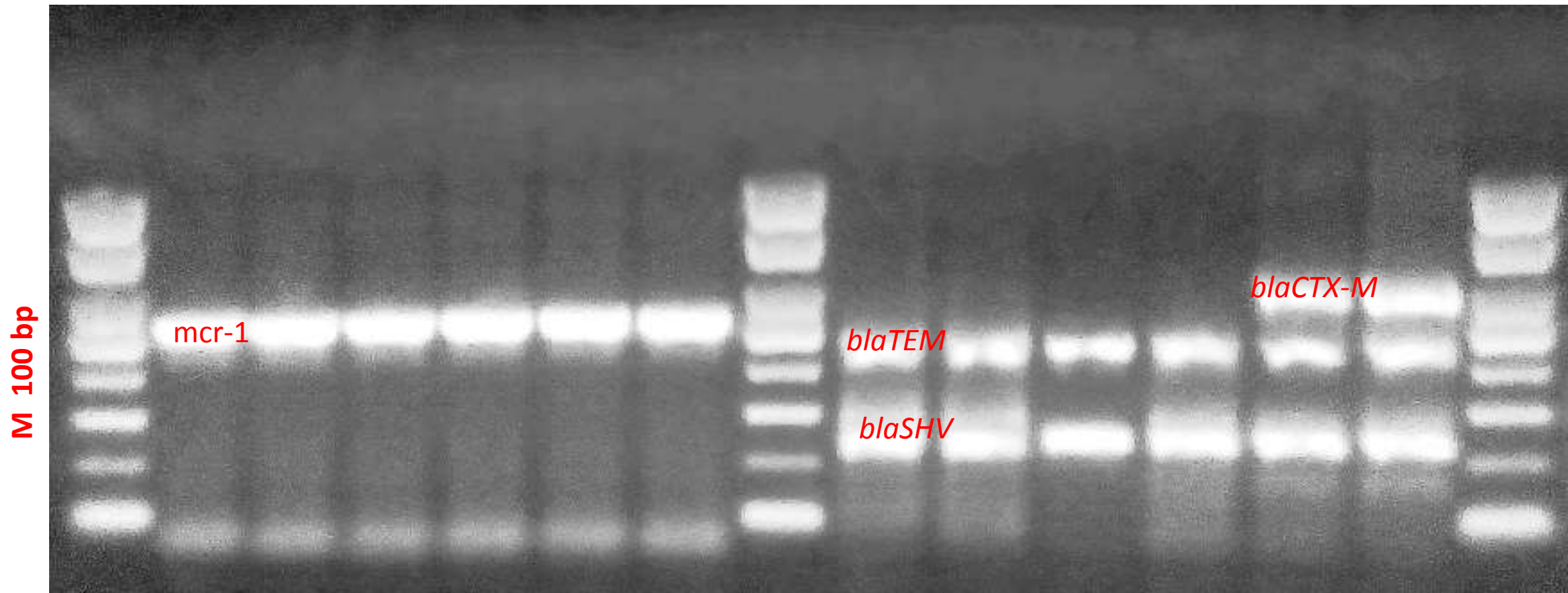


Colistin 16 µg/ml



Colistin 32 µg/ml

Detection of antibiotic resistance genes



Conclusion

- Very high resistance rates for tetracyclines, ampicillin and streptomycin.
- High resistance rates for choramphenicon and gentamycin.
- Detection of ESBL *E. coli* isolates in raw milk samples.
- Co-occurrence of ESBL and colistin resistant *E. coli* isolates a threat for public hygiene