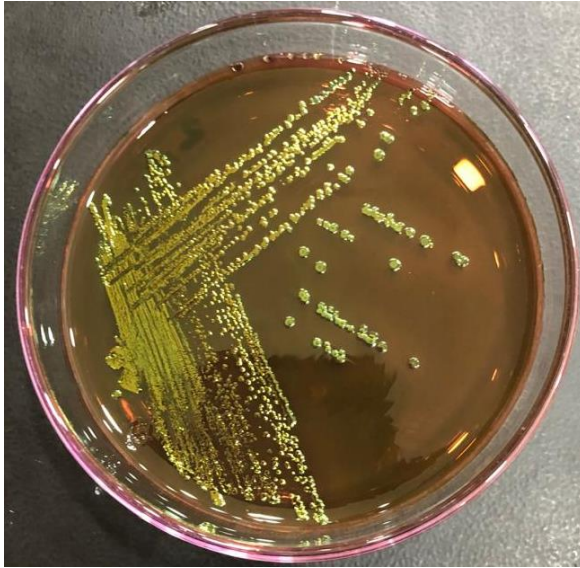


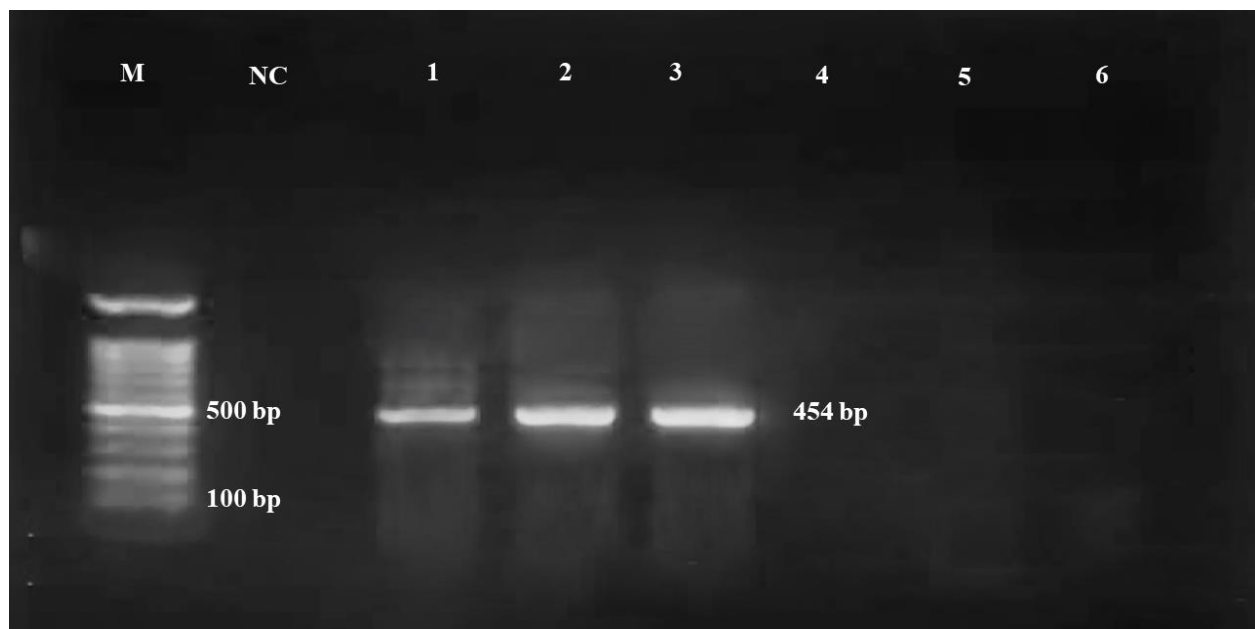
Supplementary Figures and Tables

Supplementary figure 1: Cultural morphology of *E. coli* on EMB agar



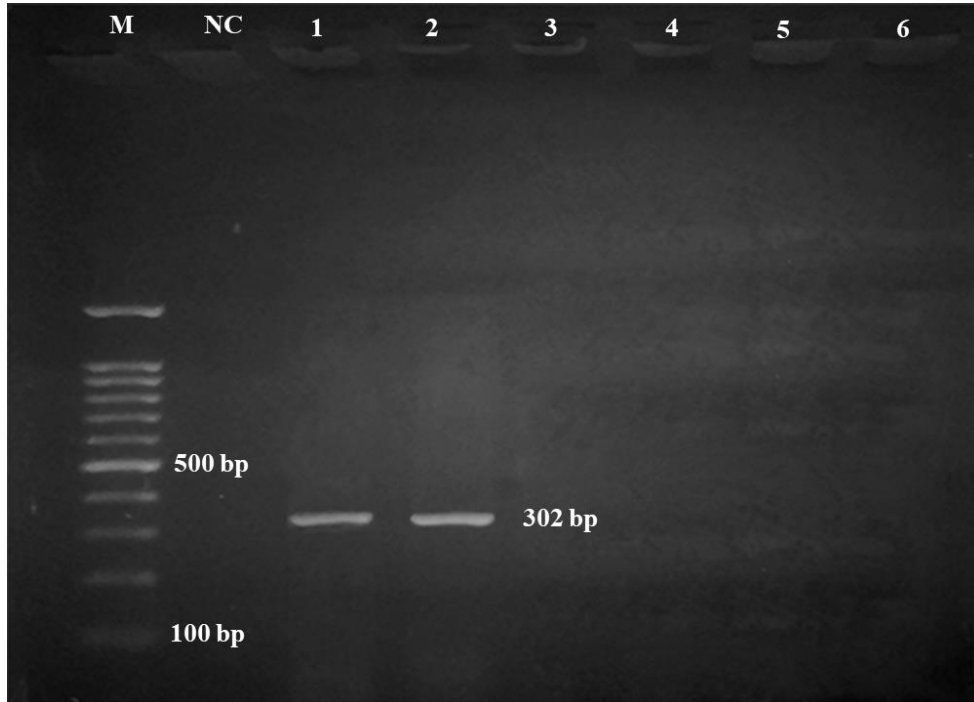
Supplementary figure 1: Characteristic green metallic sheen colonies onto the EMB agar

Supplementary figure 2: Gel image showing amplified DNA of *eaeA* gene of *E. coli* isolates



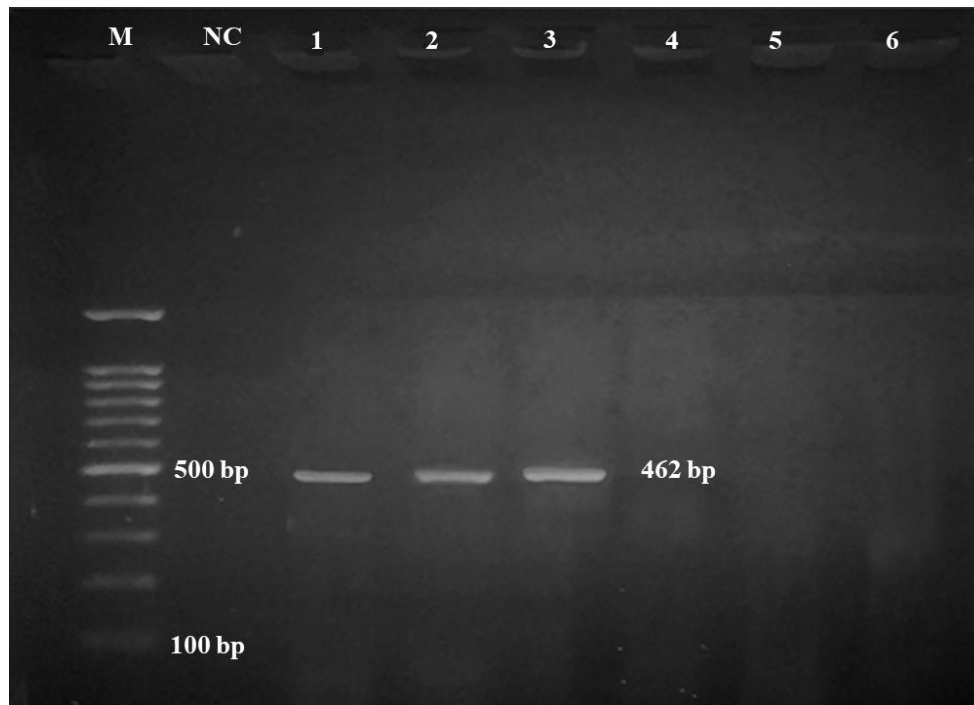
Supplementary figure 2: Amplified DNA of *eaeA* gene of *E. coli* isolates. The positive isolates amplified at 454 bp. Lane M: 100bp DNA Ladder; Lane NC: (-ve) Control; Lane 1-3: *E. coli* positive isolates

Supplementary figure 3: Gel image showing amplified DNA of *stx1* gene of *E. coli* isolates



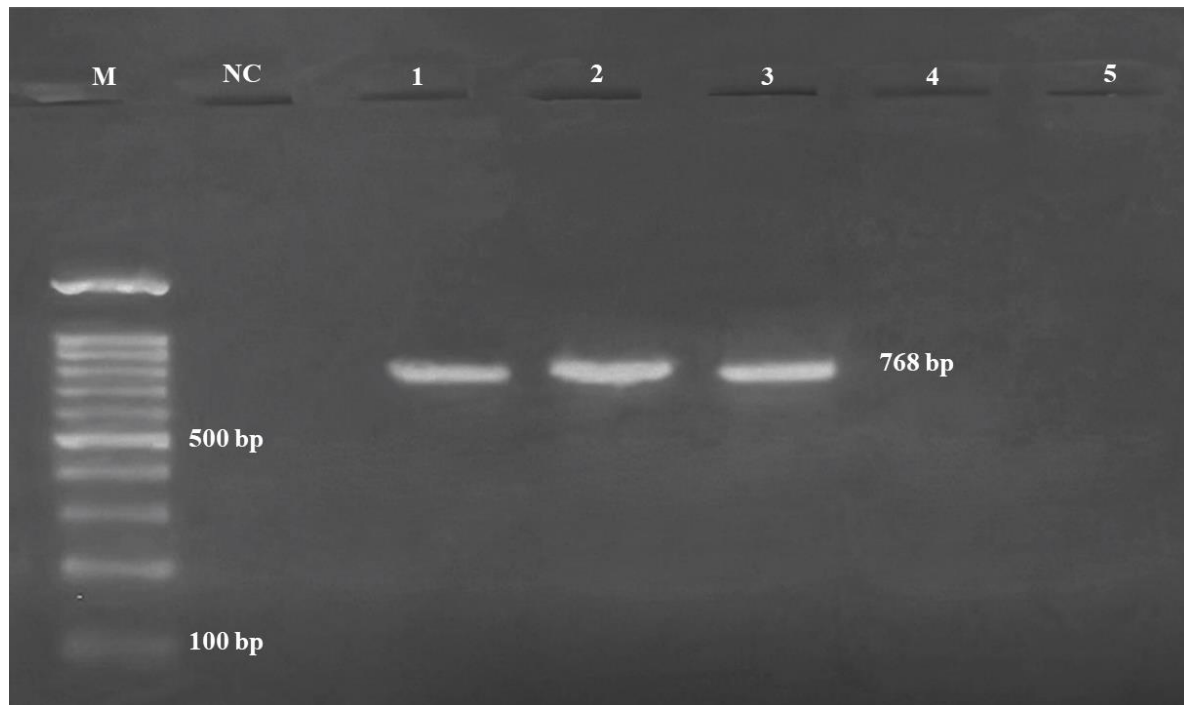
Supplementary figure 3: Amplified DNA of *stx1* gene of *E. coli* isolates. The positive isolates amplified at 302 bp. Lane M: 100bp DNA Ladder; Lane NC: (-ve) Control; Lane 1-2: *E. coli* positive isolates

Supplementary figure 4: Gel image showing amplified DNA of bla_{CITM} gene of *E. coli* isolates



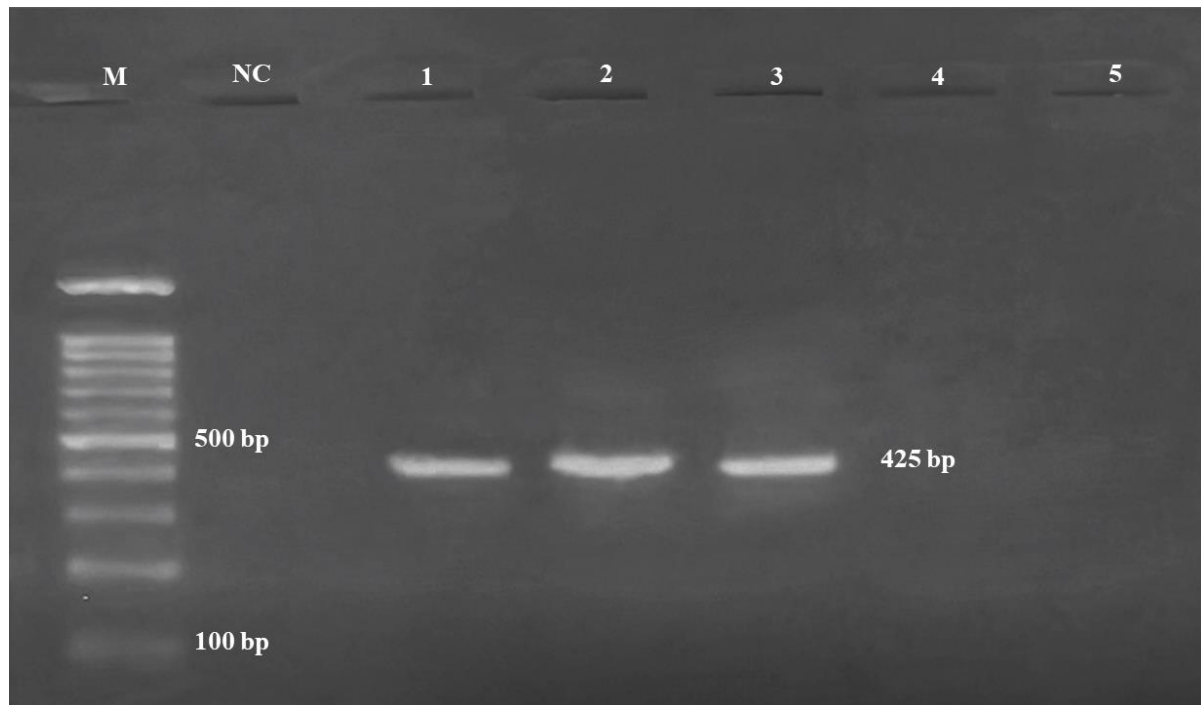
Supplementary figure 4: Amplified DNA of bla_{CITM} gene of *E. coli* isolates. The positive isolates amplified at 462 bp. Lane M: 100bp DNA Ladder; Lane NC: (-ve) Control; Lane 1-3: *E. coli* positive isolates

Supplementary figure 5: Gel image showing amplified DNA of blas_{SHV} gene of *E. coli* isolates



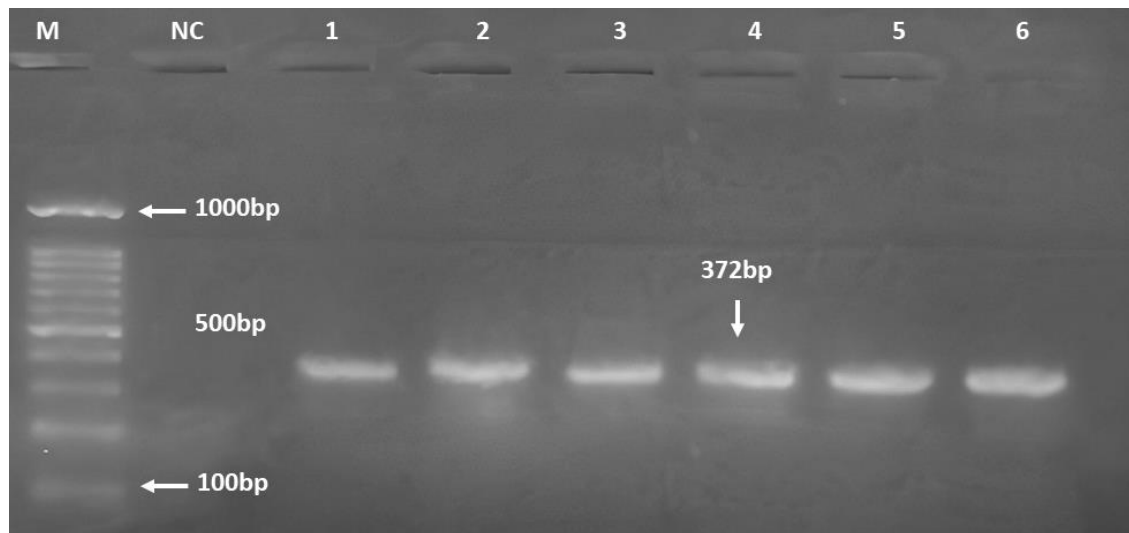
Supplementary figure 5: Amplified DNA of blas_{SHV} gene of *E. coli* isolates. The positive isolates amplified at 768 bp. Lane M: 100bp DNA Ladder; Lane NC: (-ve) Control; Lane 1-3: *E. coli* positive isolates

Supplementary figure 6: Gel image showing amplified DNA of bla_{TEM} gene of *E. coli* isolates



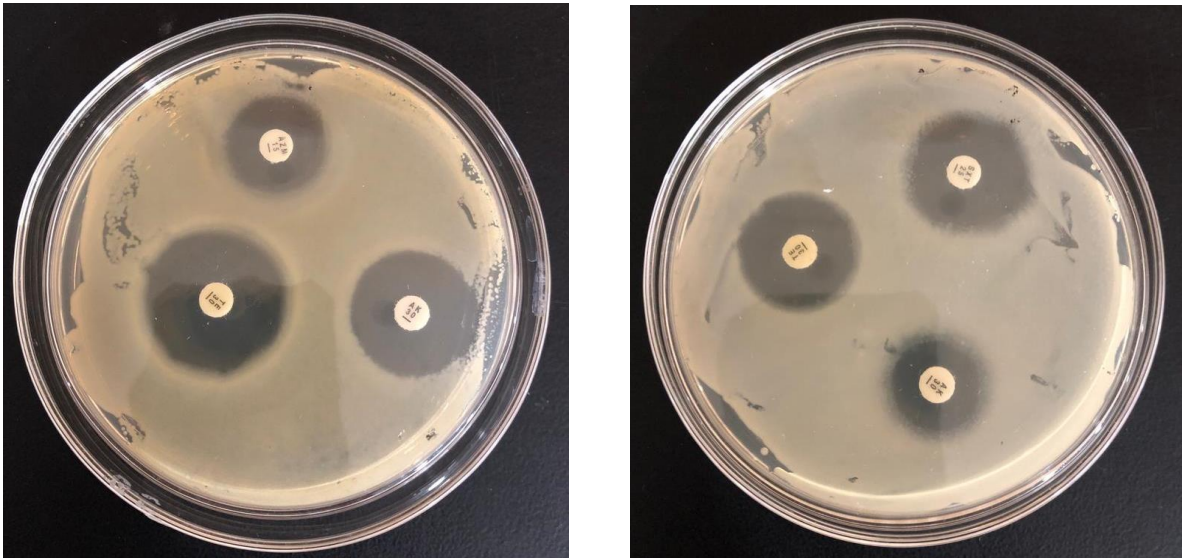
Supplementary figure 6: Amplified DNA of bla_{TEM} gene of *E. coli* isolates. The positive isolates amplified at 425 bp. Lane M: 100bp DNA Ladder; Lane NC: (-ve) Control; Lane 1-3: *E. coli* positive isolates

Supplementary figure 7: Gel image showing amplified DNA of tetA gene of *E. coli* isolates



Supplementary figure 7: Amplified DNA of tetA gene of *E. coli* isolates. The positive isolates amplified at 372 bp. Lane M: 100bp DNA Ladder; Lane NC: (-ve) Control; Lane 1-6: *E. coli* positive isolates

Supplementary figure 8: Antimicrobial resistance patterns of *E. coli* isolates



Supplementary figure 8: Phenotypic antimicrobial resistance patterns of *E. coli* isolates against antibiotics

Supplementary table 1: Primer sequences and PCR conditions

Target gene	Primer Sequences (5'-3')	Size of product	Initial denaturation	Amplification (35 cycles)			Final extension	Ref
				Denaturation	Annealing	Extension		
phoA	GGTAACGTTTCTACCGCAGAGTTG CAGGGTTGGTACACTGTCATTACG	468 bp	95°C 1 min	94°C 1 min	50°C 45 sec	70°C 90 sec	70°C 10 min	(Cengiz & Adigüz el, 2020)
stx1	CGCTGAATGTCATTCGCTCTGC CGTGGTATAGCTACTGTCACC	302 bp	94°C 2 min	94°C 1 min	55°C 2 min	72°C 1 min	72°C 10 min	(Blanco et al., 2004)
eaeA	AAACAGGTGAAACTGTTGCCCTCTGCAGATTAACCTCTGC	454 bp	95°C 1 min	94°C 1 min	55°C 90 sec	72°C 90 sec	72°C 10 min	(Kahali et al., 2003)
bla _{CITM}	TGGCCAGAACTGACAGGCAA A TTTCTCCTGAACGTGGCTGGC	462 bp	95°C 3 min	94°C 1 min	47°C 90 sec	72°C 1 min	72°C 8 min	(Momtaz et al., 2012)
bla _{SHV}	TCGCCTGTGTATTATCTCCC CGCAGATAAATCACCACAATG	768 bp	95°C 3 min	94°C 1 min	52°C 90 sec	72°C 1 min	72°C 8 min	(Momtaz et al., 2012)
bla _{TEM}	AAAGATGCTGAAGATCA TTTGGTATGGCTTCATTC	425 bp	94°C 1 min	94°C 30 sec	42°C 30 sec	72°C 1 min	72°C 10 min	(Speldoren et al., 1998)
tetA	GGCCTCAATTCCTGACG AAGCAGGATGTAGCCTGTGC	372 bp	94°C 1 min	94°C 1 min	55°C 1 min	72°C 2 min	72°C 10 min	(Guillaume et al., 2000)

Abbreviation: bp, Base pair; Ref, References

Supplementary table 2: Antimicrobial resistance patterns of *E. coli* isolated from raw milk

Antibiotic class	Specific antibiotic tested	Concentration	Interpretive categories and zone diameter breakpoints (Nearest whole mm)			No. of isolates/Total isolates		
			S	I	R	S	I	R
Beta-lactams	Ampicillin	AMP (10 µg)	≥ 17	14-16	≥ 13	0/24	0/24	24/24
	Amoxicillin	AMX (25 µg)	≥ 28	14-17	≥ 13	2/24	3/24	19/24
	Aztreonam	ATM (30 µg)	≥ 21	18-20	≤ 17	12/24	6/24	6/24
	Cefoxitine	CX (30 µg)	≥ 23	15-22	≤ 14	5/24	6/24	13/24
	Ceftazidime	CAZ (30 µg)	≥ 21	18-20	≤ 17	3/24	6/24	15/24
	Ceftriaxone	CTX (30 µg)	≥ 26	23-25	≤ 22	7/24	2/24	15/24
	Meropenem	MEM (30 µg)	≥ 23	20-22	≤ 19	13/24	5/24	6/24
	Imepinem	IMP (30 µg)	≥ 23	20-22	≤ 19	9/24	6/24	9/24
Aminoglycosides	Streptomycin	S (10 µg)	≥ 15	12--14	≤ 11	6/24	4/24	14/24
	Gentamycin	GEN (10 µg)	≥ 15	13-14	≤ 12	6/24	4/24	14/24
	Amikacin	AK (30µg)	≥ 17	15-16	≤ 14	5/24	7/24	12/24
Quinolones	Ciprofloxacin	CIP (5 µg)	≥ 31	21-30	≤ 20	18/24	2/24	4/24
	Norfloxacin	NOR (10 µg)	≥ 17	13--16	≤ 12	7/24	8/24	9/24
Macrolides	Azithromycin	AZM (15 µg)	≥ 13	-	≤ 12	8/24	6/24	10/24
Tetracyclines	Tetracycline	TE (30 µg)	≥ 25	12--14	≤ 11	0/24	0/24	24/24
Sulfonamides	Sulfamethoxazole-Trimethoprim	SXT (25 µg)	≥ 16	11--15	≤ 10	12/24	5/24	7/24
Glycopeptide	Vancomycin	VA (30 µg)	≥ 17	15-16	≤ 14	19/24	3/24	2/24

Abbreviation: S, Sensitive; I, Intermediate; R, Resistant; mm, Millimeter

Supplementary table 3: Physico-chemical analysis of raw milk samples

Sample No	Fat%	Acidity%	SNF %	Protein%	Lactose%	Added water	Specific gravity	pH
1	3.31	0.24	5.56	2.4	3.62	2.1	1.023	7
2	3.46	0.22	7.19	2.01	4.69	0	1.028	6.8
3	3.46	0.24	5.56	3	3.73	0	1.024	6.3
4	3.4	0.22	7.19	2.4	3.05	0	1.03	6
5	3.46	0.15	5.56	3.39	3.75	0	1.022	6
6	3.4	0.16	8.53	3.09	5.01	2.3	1.025	6.2
7	3.46	0.21	8.87	3.23	4.87	0	1.028	6.5
8	3.31	0.24	8.85	3.25	4.72	0	1.028	6
9	3.48	0.17	8.72	3.08	5.02	0	1.029	6.2
10	3.48	0.19	8.72	3	3.75	2.1	1.024	6.6
11	5.5	0.22	8.9	2.44	3.73	9	1.025	6.5
12	5.65	0.19	9.02	2.69	3.05	3	1.023	6
13	3.15	0.21	6.58	2.48	3.73	0	1.024	6.6
14	4.05	0.22	6.58	2.69	3.62	0	1.03	6
15	4.05	0.21	8.4	3	3.05	0	1.025	6
16	3.31	0.21	8.4	2.48	3.75	2.3	1.021	6.2
17	4.26	0.21	7.4	2.69	4.06	10	1.025	6.3
18	5.5	0.22	8.9	3.39	4.87	3.46	1.026	6.9
19	3.97	0.16	7.08	2.91	5.04	3.5	1.024	6.6
20	3.82	0.21	7	2.73	4.65	0	1.023	6.5
21	3.35	0.14	8.4	3.12	4.69	0	1.026	7
22	4.33	0.19	5.56	3.6	3.73	2.1	1.03	6.6
23	5.65	0.15	9.02	3.09	4.85	2.5	1.026	6.2
24	3.97	0.24	7.08	2.49	3.62	0	1.028	6.2
25	4.12	0.22	7	2.4	4.69	2.3	1.03	6.3
26	3.2	0.19	6.58	2.01	3.62	0	1.024	6
27	3.46	0.22	6.58	2.4	3.05	3.46	1.026	6
28	3.95	0.19	6.59	2.91	3.73	0	1.03	6
29	4.12	0.19	6.46	2.73	3.05	0	1.024	6.6
30	3.31	0.21	8.4	2.01	3.89	2.5	1.03	6.2
31	4.05	0.17	5.56	3.09	4.04	3.5	1.028	6
32	4.48	0.2	9.86	3.09	5.01	0	1.03	6.5
33	3.3	0.21	8.4	2.01	3.95	2.1	1.024	6
34	3.95	0.23	6.93	3	3.89	0	1.03	6
35	4.12	0.24	6.8	2.44	4.04	2.1	1.03	6.2
36	4.48	0.19	7.39	2.69	4.06	9.89	1.021	6.3
37	4.48	0.19	6.93	2.01	3.73	0	1.024	6.4

38	3.3	0.14	7.39	3.05	3.05	10	1.026	6.6
39	4.26	0.23	7.19	2.69	3.73	0	1.024	6.5
40	3.31	0.22	7.19	2.69	4.06	0	1.026	6.5
41	3.97	0.21	5.56	3.12	5.42	3.46	1.026	6.2
42	3.4	0.19	8.53	3.05	3.6	3	1.03	6.7
43	3.2	0.14	6.59	3.09	5.01	0	1.024	6.5
44	3.35	0.23	6.46	3.12	3.95	2.3	1.026	6.8
45	4.33	0.22	9.86	3.6	5.42	0	1.022	7.1
46	3.3	0.2	6.83	2.49	3.75	0	1.024	6.4
47	3.15	0.23	6.83	3.09	3.05	3	1.028	6.5
48	3.4	0.16	8.53	3.24	5.01	2.3	1.025	6.2
49	3.46	0.21	8.87	3.06	4.87	0	1.028	6.5
50	3.31	0.24	8.85	3.25	4.72	0	1.028	6
51	3.48	0.17	8.72	3.08	5.02	0	1.029	6.2
52	3.48	0.19	8.72	3	3.75	2.1	1.024	6.6
53	5.5	0.22	8.9	2.44	3.73	9	1.025	6.5
54	5.65	0.19	9.02	2.69	3.05	3	1.023	6
55	4.26	0.23	7.19	2.69	3.73	0	1.024	6.5
56	3.31	0.22	7.19	2.69	4.06	0	1.026	6.5
57	3.97	0.21	5.56	3.12	5.42	3.46	1.026	6.2
58	3.4	0.19	8.53	3.05	3.6	3	1.03	6.7
59	3.2	0.14	6.59	3.09	5.01	0	1.024	6.5
60	3.35	0.23	6.46	3.12	3.95	2.3	1.026	6.8
61	4.33	0.22	9.86	3.6	5.42	0	1.022	7.1
62	4.33	0.22	9.86	3.6	5.42	0	1.022	7.1
63	3.3	0.2	6.83	2.49	3.75	0	1.024	6.4
64	3.15	0.23	6.83	3.09	3.05	3	1.028	6.5
65	3.46	0.22	6.65	2.4	3.05	3.49	1.026	6
66	3.95	0.18	6.59	2.91	3.73	0	1.03	6
67	4.12	0.19	6.46	2.73	3.05	0	1.028	6.6
68	3.31	0.21	8.4	2.01	3.89	2.5	1.03	6.2
69	4.08	0.17	5.56	3.09	4.04	3.5	1.028	6
70	4.48	0.2	9.86	3.09	5.01	0	1.03	6.5
71	3.22	0.14	6.76	3.09	5.02	0	1.03	6.5
72	3.35	0.23	6.46	3.12	3.95	2.3	1.028	6.8
73	3.95	0.21	6.76	2.91	3.75	0	1.03	6
74	4.12	0.19	6.46	2.73	3.05	0	1.024	6.6
75	3.31	0.21	8.4	2.01	3.89	2.5	1.03	6.2
76	4.05	0.17	5.56	3.09	4.04	3.5	1.031	6
77	4.48	0.26	9.86	2.17	5.03	2.03	1.03	6.5
78	3.46	0.24	5.56	3.02	3.73	0	1.024	6.3

79	3.41	0.17	7.2	2.17	3.05	0	1.04	6
80	3.46	0.15	5.56	3.39	3.75	0	1.022	6

Supplementary table 4: Comparison of mean value of samples with BDS and FAO standards

	BDS	FAO	Mean value of samples
Fat	4.00	3.70	3.83
Acidity	0.15	0.16	0.20
SNF	8.20	8.50	7.47
Protein	4.10	3.50	2.83
Lactose	5.00	4.50	4.08

Abbreviation: BDS, Bangladesh Standard; FAO, Food and Agriculture Organization Standard

Supplementary table 5: Microbiological analysis of raw milk samples

Sample No	Total Coliform Count (10^6)	Total Aerobic Mesophilic Count (10^7)	Mean of Total Coliform count (10^6)	Mean of Total Aerobic Mesophilic Count (10^7)
1	1.78	2.57	1.27	3.27
2	1.49	3.47		
3	0.94	3.6		
4	0.88	3.42		
5	1.35	3.56	1.40	3.38
6	1.1	2.9		
7	1.51	3.3		
8	1.62	3.77		
9	1.46	2.87	1.76	3.12
10	1.47	3.52		
11	2.21	3.11		
12	1.91	2.98		
13	1.9	2.45	1.20	2.21
14	0.88	1.98		
15	0.78	1.56		
16	1.23	2.85		
17	1.89	3.21	1.43	2.36
18	0.96	2.1		
19	1.89	2.89		
20	0.98	1.25		
21	2.41	3	1.74	2.57

22	1.62	2.4		
23	1.46	2.33		
24	1.47	2.56		
25	1.89	2.88	1.79	2.56
26	1.89	2.8		
27	0.95	1.68		
28	2.41	2.86		
29	1.36	2.89	1.48	2.79
30	1.62	2.87		
31	1.46	3		
32	1.49	2.4		
33	0.91	1.98	1.14	2.40
34	0.82	1.56		
35	1.35	2.85		
36	1.47	3.21		
37	2.21	3.3	1.71	3.13
38	1.89	3.12		
39	1.11	2.87		
40	1.62	3.21		
41	1.46	2.85	1.56	2.60
42	1.47	2.65		
43	0.91	1.89		
44	2.41	2.99		
45	1.36	3.13	1.27	3.14
46	1.23	3.6		
47	1.49	3.42		
48	0.99	2.41		
49	0.86	1.89	1.38	2.23
50	0.89	1.48		
51	2.41	2.97		
52	1.36	2.56		
53	1.23	2.58	1.55	2.56
54	1.62	2.87		
55	0.92	1.68		
56	2.41	3.12		
57	1.36	2.86	1.63	2.70
58	1.89	2.69		
59	0.87	1.95		
60	2.41	3.31		
61	0.96	1.82	1.49	2.53
62	2.41	3.12		

63	1.36	2.64		
64	1.23	2.53		
65	1.62	2.86	1.69	2.78
66	1.46	2.49		
67	1.47	2.67		
68	2.21	3.1		
69	1.89	2.91	1.66	2.62
70	0.97	1.93		
71	2.41	2.95		
72	1.36	2.67		
73	1.23	2.64	1.63	2.59
74	1.89	2.97		
75	0.97	1.89		
76	2.41	2.86		
77	1.46	2.94	1.76	2.89
78	1.47	2.76		
79	2.21	3.14		
80	1.89	2.73		
		Total Mean	1.53	2.72
		SD	0.20	0.34

Abbreviation: SD, Standard deviation

Supplementary table 6: Occurrence of *E. coli*, virulence factors and Antibiotic resistant genes in raw milk based on PCR results

Target gene	Total isolates/No. of positive isolates	Occurrence (%)
phoA	48/80	60
stx1	3/48	6.25
eaeA	3/48	6.25
bla _{CITM}	4/48	8.33
bla _{SHV}	3/48	6.25
bla _{TEM}	7/48	14.58
tetA	24/48	50

Supplementary table 7: Antimicrobial resistance patterns (Percentage of *E. coli* isolates)

Antibiotic class	Antibiotics	Percentage of the isolates (%)		
		Sensitive	Intermediate	Resistant
Beta-lactams	AMP (10 µg)	0	0	100
	AMX (25 µg)	8.33	12.5	79.17
	ATM (30 µg)	50	25	25
	CX (30 µg)	20.83	25	54.17
	CTX (30 µg)	29.17	8.33	62.5
	CAZ (30 µg)	12.5	25	62.5
	MEM (30 µg)	54.17	20.83	25
	IMP (30 µg)	37.5	25	37.5
Aminoglycosides	S (10 µg)	25	16.67	58.33
	GEN (10 µg)	25	16.67	60
	AK (30µg)	20.83	29.17	50
Quinolones	CIP (5 µg)	75	8.33	16.67
	NOR (10 µg)	29.17	33.33	37.5
Macrolides	AZM (15 µg)	33.33	25	41.67
Tetracyclines	TE (30 µg)	0	0	100
Sulfonamides	SXT (25 µg)	50	20.83	29.17
Glycopeptide	VA (30 µg)	79.17	12.5	8.33