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**RESEARCH ARTICLE**

# Chemical and Bacteriological Assessment of Raw Milk Collected from Some Dairy Farms in El-Behera Governorate, Egypt.

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**Abstract**

Milk is an essential food for humans and is considered as a good medium for microbial growth. Therefore, 120 raw milk samples from some dairy farms were obtained for bacteriological and chemical evaluation. Chemical evaluation of the examined raw cow's milk samples showed mean values of fat, protein, casein, solid not fat, lactose, galactose, glucose, urea contents were (4.25±0.11), (3.06±0.06), (2.27±0.03), (8.38±0.10), (4.51±0.04), (0.29±0.06), (0.72±0.08), and (27.90±1.45), respectively. The determination of acidity of examined samples showed that the mean values of acidity degree, lactic acid and citric acid were (19.85±1.22), (0.19±0.01), and (0.12±0.01), respectively. The freezing point was (-0.46±0.01). Moreover, Mean values of aerobic plate count, coliform count and Staphylococcus aureus count were (2.9x105±0.16x105),(3. 8x103±0.13x103), and (3.1x103 ± 0.12x103), respectively .Escherichia coli prevalence was20%(24/120), while the prevalence of Mycobacterium bovis was 3.33% (4/120). E. coli isolates were serogrouped into O111, O26, O91, O44, O128, and untyped serogroups. The molecular characterization of five E.coli isolates revealed that; all tested strains showed ompA gene and only two showed stx1 gene. While resistance genes (bla TEM and aac(3)-IV) were detected in all tested strains. All tested S. aureus isolates showed virulence genes (Spa gene and PVL gene) and resistance genes (mecA and aac gene). Antibiotic sensitivity testing revealed the presence of multidrug resistant (MDR) isolates. The public health importance of isolated microorganisms was discussed.

**Key words:** Raw cow's milk, Chemical evaluation, Microbial load, Dairy farms, Antibiotic sensitivity, PCR, E. coli, S. aureus.

## Introduction

Milk is an essential source of fat, proteins, carbohydrates, vitamins, minerals, and other water soluble components [1]. Milk should be free from any pathogenic microorganisms that could be transmitted from animals to humans and affect public health [2]. Good quality milk is a product with a unique color, taste, and composition and does not contain bacteria, pathogens, antibiotic residues or toxic substances in excess of legal limits. It is produced from healthy animals under clean and hygienic conditions [3].

The bacterial count in milk is important for determination of milk quality and is considered as an indicator for poor hygienic production condition or ineffective pasteurization of milk [4]. Coliforms bacteria including *Escherichia coli*, *Enterobacter*, *Klebsiella spp.*, *Serratia*, and *Citrobacter* contaminate raw milk through several environmental

sources, including water, soil and garbage. *E. coli* is a common contaminant of raw milk and milk products. Their presence indicates possible environmental and/or fecal contamination. *E. coli* isolated from milk and dairy products harbored high levels of toxins; Vero or Shiga toxins that allows bacterial adhesion and penetration to epithelial cells of intestine leading to severe damage (A/E) [5]. Shiga toxin-producing *Escherichia coli* (STEC) strains are among the most important pathogens causing foodborne illness worldwide. Human infection with these pathogens results in clinical illness ranging from self-limiting diarrhea to life-threatening hemolytic uremic syndrome (HUS). Cattle are incriminated as the most cause of zoonotic human STEC worldwide[6].The presence of middlemen or traders makes milk traceability difficult and leads to cross-contamination and microbial overload due to poor handling of milk by transporters and adulterated milk[7].

*Methicillin-resistant Staphylococcus aureus* (MRSA) are opportunistic pathogens that are associated with a significant disease burden through nosocomial infections, particularly in the healthcare sector. *Methicillin-resistant S. aureus* has been identified in a variety of livestock animals, with the highest prevalence observed in pigs, fattening calves, and turkeys as well as dairy cattle herds, where they pose an additional threat to animal health by causing subclinical and clinical mastitis [8,9].

In 2019, there were an estimated 10 million cases of active human tuberculosis worldwide; an estimated 140,000 (range 69,800-235,000) were new cases of zoonotic tuberculosis (1.4%), of which approximately 11,400 (8.1%, range 4,470-21,600) died. The incidence of zoonotic tuberculosis is higher in some regions and countries than in others, particularly where there is a close relationship between the number of cattle and the population and where milk and dairy products are often consumed unpasteurized[10,11].

Therefore, the aim of this study was to evaluate the chemical and bacteriological status of raw milk from different dairy farms in different localities of El Behera province. In addition, studying antibiotic susceptibility testing, some virulence and antibiotic resistance genes.

## Material and methods

### *Sample collection*

One hundred and twenty raw milk samples were randomly collected from different dairy farms in El Behera province, Egypt. Samples were aseptically collected from bulk milk in sterile plastic tubes, labeled, packaged, transferred to a laboratory, and then examined chemically and bacteriologically.

### *Chemical evaluation of examined raw milk samples*

*Determination of milk components*

Determination of fat, protein, solids-not-fat, lactose, acid content, as well as freezing point and adulteration parameters were carried out using Milko scan FT1 (FOSS).

*Determination of heavy metals (lead)*

The lead contents in collected samples was determined according to Ahmad *et al.* [12]

### *Bacteriological examination of Cow's raw milk samples*

*Samples preparation*

One mL of the well-mixed milk sample was transferred to 9mLof sterile peptone water solution (1%) and mixed thoroughly to have a1:10 dilution from which serial decimal dilutions as

recommended by American Public Health Association (APHA)[13]

*Aerobic plate count determination*

Aerobic plate count has been done using standard plate count agar media according to American Public Health Association [14].

*Coliform count*

Violet Red Bile (VRB) Agar medium was used for detection of lactose fermenting coliforms. After 24 hours of incubation at 37°C, the typical pink to red colonies surrounded by a reddish area of precipitated bile[15].

*Isolation and identification of E. coli*

Samples were inoculated into buffered peptone water and incubated for18–24hrs at 37°C.A loopful from enriched broth was placed on Eosin-Methylene Blue and MacConkey Agar plates and incubated for 24hrs at37°C. Morphological and biochemical identification of the suspected colonies were done according to Quinn *et al.*[16].

*Serotyping of E. coli isolates*

According to Quinn *et al.* [16] *E.coli* isolates were selected and identified using polyvalent and monovalent antisera of *E. coli*.(Denka Seiken Co. LTD, Tokyo, Japan for antisera) .

*Determination of S. aureus count*

*S. aureus* was determined using Baird Parker agar according to De Vos *et al.* [17]**.**

*Isolation and identification of Mycobacterium spp.*

*a. Sample preparation*

About 100 mL of well mixed raw milk sample were centrifuged at 3000 rpm for 30min.The sediments were then subjected to Ziehl-Neelsen staining and culture [16].

*b. Ziehl-Neelsen staining*

Sediments from previously prepared samples were spread onto slides, allowed to air dry, heat fixed, then flooded with carbol fuchsin and heated on stainless-steel racks. Slides were thoroughly washed and decolorized with an acid-alcohol, followed by water washing and then Loffler's methylene blue was used as a counter stain. Each slide is examined for shape, arrangement and acid-fast characteristics [16].

*c. Culture of milk samples*

The sediments were mixed with an equal volume of 1.8% HCL and incubated for 30 min at 37°C., then centrifuged at 3000 rpm for 30 min. Neutralization with 2% NaOH solution using phenol red indicator and then centrifugation were done .A loopful from decontaminated sediment was inoculated into two tubes containing Löwenstein-Jensen medium with and without sodium pyruvate, and Middle Brook 7H10 agar medium .Inoculated Löwenstein-Jensen medium tubes were incubated at 37°C for 90 days at least and observed daily then weekly. Middle Brook 7H10 agar plates were incubated at 37°C for a maximum of 24 days. All isolates were biochemically identified according to Quinn*et al.* [16].

***Antibiotic sensitivity testing of E. coli and S. aureus isolates***

Antibiotic sensitivity pattern of the *E. coli* and *S. aureus* strains were studied using standard disc diffusion method according to CLSI [18] principles. The antibiotics tested were purchased from Himedia® and included Levofloxacin (LEV,5µg), Amikacin (AK, 30µg), Gentamicin (GEN, 10µg), Amoxicillin (AML, 25µg), Oxytetracyclin (OT, 30µg), Imipenem (IMP, 10µg), Cefotaxime (CTX, 30µg), ampicillin (AMP,10µg), Enrofloxacin (ENF,5µg),Cotrimoxazole (SXT,25µg), and Penicillin G (P,10µg).

***Molecular characterization of E.coli isolates***

Biochemically and serologically confirmed *E. coli* isolates were subjected to DNA extraction using the QIAamp DNA Mini Kit(Qiagen, Germany, GmbH). About 200 µL of the sample suspension was incubated for 10 min at 56°C with 10 µL of proteinase K and 200 µL of lysis buffer. Two hundred microliters of 100% ethanol were added to the lysate after incubation. Washing and centrifugation were done according to the manufacturer's recommendations. Then elution with 100 µL of elution buffer. The extracted DNA was then subjected to Polymerase chain reaction using oligonucleotide primers supplied from Metabion (Germany) as displayed in Table (1). The PCR reaction volume was 25µL consisted of 12.5 µL of Emerald Amp Max PCR Master Mix (Takara, Japan), 1µL of each used primer of 20bpm.A concentration of 5.5µL water and 5µl of DNA template. The reaction was done in Applied Biosystem 2720 thermal cycler. PCR products were separated by agarose gel electrophoresis 1.5%.

**Table (1): Primers sequences, target genes, amplicon sizes and cycling conditions for conventional PCR.**

| Bacteria | Target gene | Primers sequences | Amplified segment (bp) | Primary denaturation | Amplification (35 cycles) | | | Final extension | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Secondary denaturation | Annealing | Extension | | |
| ***S. aureus*** | ***Pvl*** | **ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A** **GCA TCA AST GTA TTG GAT** **AGC AAA AGC** | **433** | **94˚C 5 min.** | **94˚C 30 sec.** | **55˚C 40 sec.** | **72˚C 45 sec.** | **72˚C 10 min.** | **(19)** |
| | ***mecA*** | **GTA GAA ATG ACT GAA CGT CCG ATA A** **CCA ATT CCA CAT TGT TTC GGT CTA A** | **310** | **94˚C 5 min.** | **94˚C 30 sec.** | **50˚C 30 sec.** | **72˚C 30 sec.** | **72˚C 7 min.** | **[20]** |
| | ***Spa*** | **TCA ACA AAG AAC AAC AAA ATG C** **GCT TTC GGT GCT TGA GAT TC** | **226** | **94˚C 5min.** | **94˚C 30S.** | **55˚C 30S.** | **72˚C 30S.** | **72˚C 7 min.** | **[21]** |
| | ***aac(6')aph(2'')*** | **GAAGTACGCAGAAGA GA** **ACATGGCAAGCTCTA GGA** | **491** | **94˚C 5min.** | **94˚C 30S.** | **54˚C 40S.** | **72˚C 45S.** | **72˚C 10 min.** | **[22]** |
| ***E. coli*** | ***ompA*** | **AGCTATCGCGATTGC AGTG** **GGTGTTGCCAGTAAC CGG** | **919** | **94˚C 5min.** | **94˚C 30S.** | **58˚C 40S.** | **72˚C 1 min.** | **72˚C 10 min.** | **[23]** |
| | ***Stx1*** | **ACACTGGATGATCTC AGTGG** **CTGAATCCCCCTCCA TTATG** | **614** | **94˚C 5min.** | **94˚C 30S** | **58˚C 40S.** | **72˚C 45S.** | **72˚C 10 min.** | **[24]** |
| | ***blaTE*** | **ATCAGCAATAAACCA GC** | **516** | **94˚C 5min.** | **94˚C 30S.** | **54˚C 40S.** | **72˚C 45S.** | **72˚C 10** | **[25]** |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***M*** | **CCCCGAAGAACGTTTTC** | | | | | | **min.** | |
| ***aacC*** | **GGCGCGATCAACGAATTTATCCGACCATTCGATGCCGAAGGAAACGAT** | **448** | **94˚C 5 min.** | **94˚C 30S.** | **60˚C 40S.** | **72˚C 45S.** | **72˚C 10 min.** | **[26]** |

***Statistical Analysis***

Data was expressed as mean ± SEM using SAS software according to SAS[27].

## Results and discussion

Microorganisms can contaminate milk during handling, transportation and distribution. Poor health conditions of dairy cows, poorly cleaned and disinfected milking equipment and workers can be potential sources of bacterial contamination [28]. Milk quality depends on its composition and varies according to the stage of lactation, milking method (manual or automatic), environment, season, and feeding system [29]. The presence of pathogenic bacteria in the analyzed samples is considered assign of poor hygiene during and after milking and it canals oberelated to pollution from cow dung, soil and water used [30].

The mean values of fat, non-fat solids, protein, casein, lactose, galactose, glucose, and urea contents were; (4,25 ± 0.11), (8.38 ± 0.10), (3.06 ± 0.06), (2.27 ± 0.03), (4.51 ± 0.04), (0.29 ± 0.06), (0.72 ± 0.08), and(27.90 ± 1.45) as presented in Table 2. Similar fat ratio was detected in Turkey as 4.26 [31]. Also, in Turkey, Similar protein content was detected by Yurt [32]as2.79 in raw cow's milk. In Turkey, similar lactose content of raw cow's milk was detected and ranged from 3.60% to 5.50% [33]and similar solid nonfat percent as 8.39 detected in the examined raw cow's milk samples [34]. Lower results of SNF percent were detected in Bangladesh in raw milk as 7.91[35].

The mean values of acidity degree, lactic acid percent, citric acid percent, and freezing point was (19.85±1.22), (0.19±0.01), (0.12±0.01), and (-0.46±0.01), respectively (Table 2). These results were similar to Akin *et al.* [36] in Turkey as 0.161% and 0.220%. While El-Leboudy *et al.* [37] in Egypt reported acidity mean values in raw cow's milk as0.16± 0.04. Similar freezing point was detected in Bangladeshas-0.46 in raw cow' milk [35]. Also, Ahmad *et al.* [38] detected similar freezing point in raw buffalo's milk as -0.526 in Pakistan.

**Table 2: Statistical analytical results of chemical composition, acidity, freezing points, and heavy metals (lead) in examined cows' raw milk samples.**

**Chemical composition of examined cows' raw milk samples**

| Parameters | Minimum | Maximum | Mean ±SEM |
|---|---|---|---|
| **Fat** | 3.50 | 4.90 | 4.25±0.11 |
| **Protein** | 2.60 | 3.30 | 3.06±0.06 |
| **Casein** | 2.10 | 2.50 | 2.27±0.03 |
| **SNF%** | 7.70 | 8.90 | 8.38±0.10 |
| **Lactose** | 4.20 | 4.80 | 4.51±0.04 |
| **Galactose** | 0.07 | 0.86 | 0.29±0.06 |
| **Glucose** | 0.06 | 1.36 | 0.72±0.08 |
| **Urea** | 20.10 | 39.10 | 27.90±1.45 |

**Acidity, freezing points and heavy metals (lead) in examined cows' raw milk samples.**

| Parameters | Minimum | Maximum | Mean ±SEM |
|---|---|---|---|
| **Acidity degree** | 16.20 | 32.0 | 19.85±1.22 |
| **Lactic acid** | 0.16 | 0.30 | 0.19±0.01 |
| **Citric acid** | 0.07 | 0.16 | 0.12±0.01 |
| **Freezing point** | -0.42 | -0.53 | -0.46±0.01 |
| **Heavy metal** | Permissible limit of lead is 0.5 mg/kg) | | |
| **Lead** | 0.001 | | 0.001 |

The aerobic plate counts mean values of coliforms count, and *S.aureus* count were as follow; ($2.9x10^5$±$0.16x10^5$),($3.8x10^3$±$0.13x10^3$), and ($3.1x10^3$±$0.12x10^3$), respectively (Table 3). Similar results of aerobic plate count were recorded in Namibia and ranged from $7.8\times 10^4$ to $1.3\times10^6$ (cfu/ml) in raw cow's milk collected from dairy farms[39]. In addition, El-Leboudy *et al.* [37] recorded TBC as $2.6x10^5$± $0.2x10^5$ in raw Cow's milk.

Higher Standard Plate Count (SPC) was recorded in Bangladesh as $38.1\times10^6$(cfu/ml) in raw cow's milk from different dairy farms[40]. In addition, Oladipo*et al.* [41] recorded aerobic plate count ranged from $0.2\times 10^6$ to 4.2 x $10^6$ (cfu/ml) from raw cow's milk samples collected from dairy farms in Nigeria. In Ethiopia, aerobic plate count was $3.4\times 10^8$ in raw cow's milk from storage area in dairy farm while $5.96\times 10^8$ from milk container in distribution center [42]. In addition, Abuelnaga *et al.* [2] in Egypt recorded aerobic count in raw Cow's milk as$1.6x10^6$.

Similar results of coliforms count were reported in Nigeria by Mirabeau *et al.* [43] and ranged from $2.87\times10^3$ to $3.3\times10^3$(cfu/ml). Higher values reported in Bangladesh as $4.5\times0^3$ to $2.03\times10^6$(cfu/ml) [44]. In addition, the coliforms count in raw cow's milk samples collected from dairy farm in Bangladesh were $1.0\times10^4$ to $2.0\times10^5$ (cfu/ml) and from $0.6\times10^6$ to $7.8\times10^6$ (cfu/ml) as recorded by Banik *et al.* [45]and Chowdhury *et al.* [46], respectively. While in Namibia lower coliforms count reported in raw cow's milk from dairy farm was $2.4\times 10^2$ to 2.3

$\times 10^3$ (cfu/ml) by Bille *et al.* [39]and $1.05x10^1$ (cfu/ml) by Hussaini *et al.* [47]. Unsanitary milking practices, contaminated water, poor flock hygiene as well as poorly washed and maintained equipment can all lead to increased level of coliforms in raw milk [48].

Regarding *S. aureus* count, lower results were obtained in Egypt by Abuelnaga *et al.* [2] as $1.7\ x10^3$. Higher results were reported by Khan and Abdul [49] as $4.7\times10^6$ (cfu/ml). In Bangladesh, *S. aureus* count in raw milk samples ranged from $5.7\times10^4$ to $1.48\times10^6$ (cfu/ml) [44].

**Table 3: Bacteriological evaluation of the examined raw cows' milk samples**

| | No. of positive samples | Mean ± SEM |
|---|---|---|
| **Aerobic bacterial count** | **120** | **$2.9x10^5 \pm 0.16x10^5$** |
| **Coliforms count** | **55** | **$3.8x10^3 \pm 0.13x10^3$** |
| ***S. aureus* Count** | **65** | **$3.1x10^3 \pm 0.12x10^3$** |

The prevalence of *E. coli* and *M. bovis* in the examined raw cow's milk samples revealed; 20% and 3.33%, respectively (Table 4). Similar *E. coli* prevalence was detected in Ethiopia and Egypt as 17.6 % and 18.75%, respectively [42,43]. Higher results were obtained as 57% in Pakistan [50], 35.63% in Rajasthan [51], 75% in Bangladesh [52], and 34.4 % in China [53].While lower results (12.1%) were obtained by El-Behiry *et al.* [54] from raw cow's milk in Saudi Arabia.

Moreover, In Egypt, similar prevalence of *M. bovis* in milk samples were detected as 3% and 2.5%from El-Sharkia and El –Behera Governorate [55] and [10], respectively. Lower results from Monufia Governorate (0.7%) [55]. Higher results were obtained in some private farms in Egypt as 16% by Guindi *et al.* [56] and 5% by Hossain *et al.* [44], respectively.

The serogroups of 12 representative *E. coli* isolates which were categorized as $O_{111}$,$O_{128}$, $O_{91}$, and untyped *E. coli* strains (2 strain each), $O_{26}$(3 strains), $O_{44}$ (1 strain) as displayed in Table 4.These findings agreed with Momtaz *et al.* [57]who reported that $O_{26}$, $O_{111}$, $O_{91}$ $O_{128}$ and $O_{145}$ serogroups are the most frequent *E. coli* O- serogroups detected in raw cow's milk. Additionally, Ahmed and Samer [58] reported that *E. coli*$O_{26}$, $O_{44}$, and $O_{111}$serogroups were identified from raw buffalo's milk samples in Egypt. Ranjbar *et al.* [59] found that O26, $O_{111}$, and $O_{121}$serogroups were prevalent in STEC strains detected in raw milk and milk products in Iran.

Unwise and incorrect antibiotic prescription may be the leading cause of high rates of antibiotic resistance in Shiga-toxigenic *Escherichia coli* (STEC) strains isolated from raw milk and dairy products [59].

**Table 4: Occurrence of *M. bovis* and *E. coli* in examined cows' raw milk from Dairy farms and *E. coli* serogrouping**

| Type of isolates | No. of examined samples | positive samples | |
|---|---|---|---|
| | | No. | % |
| *M. bovis* | 120 | 4 | 3.33 |
| *E. coli* | 120 | 24 | 20 |

Serogrouping of 12 representative *E. coli* isolates revealed $O_{111}$, $O_{128}$, $O_{91}$, and untyped *E. coli* strains (2 strain each), $O_{26}$ (3 strains), $O_{44}$ (1 strain).

Concerning the antibiotic sensitivity of 10 *E. coli* isolates, revealed high resistance to penicillin (10 isolates), ampicillin and oxytetracycline (9 isolates), gentamicin and amikacin (8 isolates), Amoxicillin and Cefotaxime (7 isolates), Cotrimoxazole (6 isolates), and finally Imipenem (5 isolates). On the other hand, 9 isolates were sensitive to levofloxacin and 8 isolates were sensitive to Enrofloxacin (Table 5). These results agreed with Stephan *et al.* [60] who proved that STEC strains isolated from milk products showed resistance against ampicillin, gentamicin, tetracycline and sulfamethoxazole antibiotics. In addition, Ranjbar *et al.* [59] proved that all tested STEC strains had resistance against ampicillin, gentamicin and tetracycline for 96.87%.

On the other hand, Ahmed and Samer [58] proved that *E. coli* isolates were sensitive to gentamicin, ciprofloxacin and colistin. In China, all *E. coli* strains were susceptible to gentamicin and exhibited different resistance levels to ampicillin, amoxicillin-clavulanic acid, trimethoprim-sulfamethoxazole, tetracycline, and ciprofloxacin as (46.3%), (16.4%), (13.4%), (13.4%), and (1.5%), respectively [53].

**Table 5: Antibiotic susceptibility of *E. coli* and *S. aureus* (10 representative isolates, each) isolated from examined cows raw milk.**

| Antimicrobial | Disc. Conc. (µg) | *E. coli* | | *S. aureus* | |
|---|---|---|---|---|---|
| | | R. | S. | R. | S. |
| Levofloxacin | 5 | 1 | 9 | 2 | 8 |
| Gentamycin | 5 | 8 | 2 | 7 | 3 |
| Imipenem | 10 | 5 | 5 | 2 | 8 |
| Cefotaxime | 30 | 7 | 3 | 6 | 4 |
| Oxytetracycline | 30 | 9 | 1 | 9 | 1 |
| Cotrimoxazole | 25 | 6 | 4 | 5 | 5 |
| Amikacine | 30 | 8 | 2 | 8 | 2 |

| | | | | | |
|---|---|---|---|---|---|
| **Enrofloxacin** | **5** | **2** | **8** | **3** | **7** |
| **Ampicillin** | **10** | **9** | **1** | **8** | **2** |
| **Amoxicillin** | **25** | **7** | **3** | **6** | **4-** |
| **Penicillin G** | **10** | **10** | **-** | **8** | **2** |

Regarding the antibiotic susceptibility profile of 10 *S. aureus* isolates, they were resistant to oxytetracycline (9 isolates), Amikacine, penicillin, and ampicillin (8 isolates), gentamycin (7 isolates), Amoxicillin and Cefotaxime (6 isolates), and Cotrimoxazole (5 isolates). While 8 isolates were susceptible to levofloxacin and Imipenem which is consistent with Zeinhom and Abed [61] who reported that *S. aureus* strains showed resistance to ampicillin and tetracycline as 72% and 60%, respectively. Our results disagreed with AbdeL-Tawab *et al.* [62] who proved that *S. aureus* isolates were sensitive to gentamycin, trimethoprim/sulfamethazole, ampicillin and cephradine. Resistance to different antibiotics indicates the presence of multidrug-resistant (MDR) strains (Figure 2 and Table 6).

The molecular characterization of five *E. coli* strains by PCR and revealed that, virulence genes *ompA* and *stx1*gene were detected in all tested and only two isolates, respectively In addition, resistance genes (*bla TEM* and *aac(3)-IV*) were found in all tested isolates.

Lower prevalence of STEC in bulk tank milk was detected in America as 3.8% [63] and 0.8 % [64]. In Pakistan, the majority of *E. coli* isolates from raw milk harbored multiple virulence genes (e.g. *Stx1*, *Stx2*, and *eae*) [65]. In Northern China *stx* genes were the most common *E.coli* virulence genes in raw milk samples [53]. El behiry *et al.* [54] recorded that out of 33 *E. coli* strains from raw cow's Milk, 30 (90.1%) and 11 (30.55%), harbored *Stx* and *Stx2* virulence genes, respectively.

Regarding the antibiotic resistance genes, results agreed with Ranjbar *et al.* [59] who detected antibiotic resistance gene *Aac(3)-IV* in all tested STEC strains from raw milk and milk products. In addition, Momtaz et al. [57] reported that *aac*(3)-*IV* gene was detected in 27.45%of *E. coli* isolates. Dehkordi *et al.* [66] detected gentamicin *aac(3)-IV* gene in 32%of STEC strains isolated from raw milk products. In China, the prevalence of β-lactamase-encoding gene as 34.3% in 67 *E. coli* strains and the prevalence of *blaTEM*, *blaCMY*, and *blaCTX-M* genes were 20.9, 10.4, and 1.5%, respectively [53].

The Molecular characterization *of* five *S. aureus* isolates revealed that all strains harbored virulence genes (Staph *PVL* and *Spa* (protein A) and resistance genes (*mecA* and *aac* gene) as presented in Figures 2 A , B and Table 6.

Lower results obtained by Abdel Tawab [65] who reported that *spa* gene detected in two (33.3%) strains of *S. aureus* isolated from raw milk. While in Uganda, PVL and *mecA* genes were detected in *S. aureus* isolates from fresh milk as (12.2%) and (50%), respectively [66].Ibrahim *et al.* [67]detected *mec*A gene in 28.57% of *S. aureus* isolated from raw buffalo's milk. Also, Zeinhom and Abed, [61] detected *mec*A gene in 66.7%

of *S. aureus* isolates of raw milk and cheese samples.

**Table 6: Molecular characterization of virulence and resistance genes in *E. coli and S. aureus* isolated from cows' raw milk.**

| **Virulence and resistance genes in 5 representative *E. coli* isolates** | | | | | **Virulence and resistance genes in 5 representative *S. aureus* isolates** | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ***E. coli* isolates** | **Resistance genes** | | **Virulence genes** | | ***S. aureus* isolates** | **Resistance genes** | | **Virulence genes** | |
| | ***blaTEM*** | ***aacC*** | ***ompA*** | ***Stx1*** | | ***aac(6')aph (2'')*** | ***mecA*** | ***Spa*** | ***Pvl*** |
| 1 | + | + | + | + | 1 | + | + | + | + |
| 2 | + | + | + | + | 2 | + | + | + | + |
| 3 | + | + | + | - | 3 | + | + | + | + |
| 4 | + | + | + | - | 4 | + | + | + | + |
| 5 | + | + | + | - | 5 | + | + | + | + |

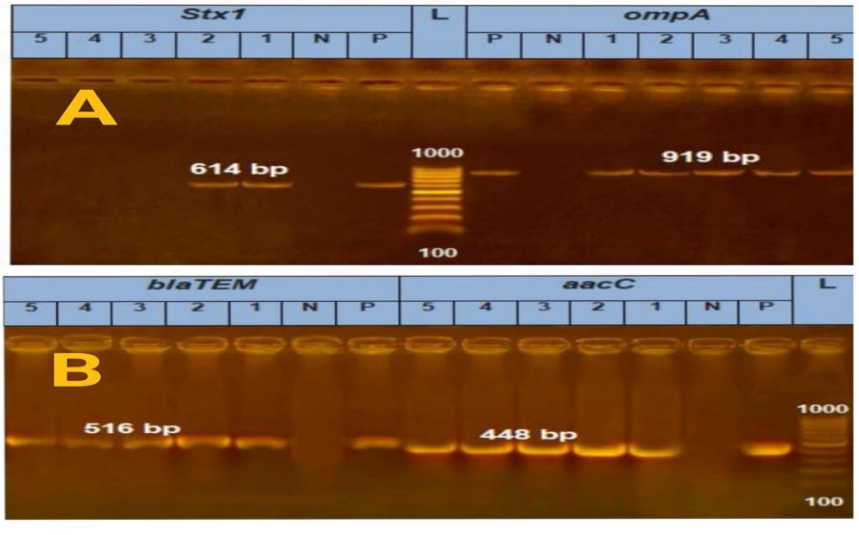


**Figure 1:A.**Agarose gel electrophoresis of PCR products showing amplification of *E. coli ompA*gene products at 919 bp and *stx1* gene at 614 bp. Lanes 1-5. Five representative *E. coli*, all of them were positive for o*mpA* gene& 2 only positive for*stx1* gene. **B.** Agarose gel electrophoresis of PCR products showing amplification of*E. coli bla TEM* gene products at 516 bp and *aacC* gene at 448bp. Lane L. 100 – 1000 bp DNA ladder, Lane P. positive control, Lane N. negative control. Lanes 1-5. Five representative *E. coli*, all of them were positive for *bla TEM* and *aacC* genes.

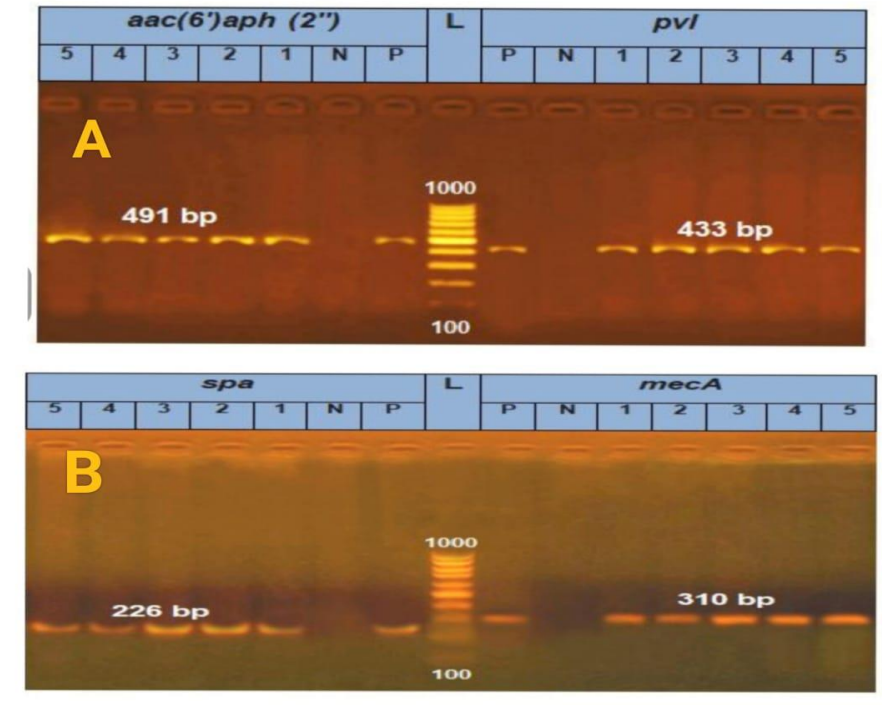


**Figure 2: A.** Agarose gel electrophoresis of PCR products showing amplification of *S.aureusPVL* gene products at 433 bp and *aac* gene at 491bp. Lanes 1-5. Five representative *E. coli*, all of them were positive for *PVL* and *aac*genes**. B.** Agarose gel electrophoresis of PCR products showing amplification of *S.aureus Spa gene* products at 226 bp and *mecA* gene at 310 bp. Lanes 1-5. Five representative *E. coli*, all of them were positive for Spa and mecA gene. Lane L. 100 – 1000 bp DNA ladder, Lane P. positive control, Lane N. negative control.

## Conclusion and recommendations

Milk is considered as a complete food for human beings as it is rich in various constituents but also support the growth of different microbes. Therefore, this study evaluated the status of raw cow's milk collected from some dairy farm bacteriologically and chemically. Moreover, detection of some virulence and antibiotic resistance genes in isolates in addition to antibiotic sensitivity testing of some isolated microorganisms. Based on our findings in this study, there were several recommendations as:

1- Cow handlers must practice good hygienic practices, such as proper handling of cows, personal hygiene, treatment of udder infections, use of sanitary processing and milking equipment, as well as properly transporting and milk storage.

2- Avoid abundant use of antibiotics which can lead to the development of multidrug resistance (MDR) strains.

3- Periodic assessments of milk quality on farms need to be conducted regularly to ensure the supply of good quality milk to consumers.

**Conflicts of Interest:** The authors declare no conflict of interest.

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## الملخص العربي

## التقييم الكيميائي والبكتريولوجي للحليب الخام المجمع من بعض مزارع الحلاب بمحافظة البحيرة ـ مصر

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**معهد بحوث الصحه الحيوانيه- مركز البحوث الزراعيه- مصر**

يعتبرالحليب غذاء أساسي للإنسان ويعمل أيضًا كوسيط جيد لنمو الميكروبات. لذلك ، تم تجميع عدد 120 عينة من الحليب من مزارع الابقار بشكل عشوائي من مواقع مختلفة للفحص الكيميائي والبكتريولوجي. أظهر التقييم الكيميائي لعينات حليب البقر الخام التي تم فحصها أن القيم المتوسطة للدهون والبروتين والكازين والمواد الصلبة غير الدهنية واللاكتوز والجلوكوز والجلاكتوز ومحتويات اليوريا كانت (4.25 ± 0.11)، (3.06 ± 0.06)، (2.27 ± 0.03)، (8.38 ± 0.10)، (4.51 ± 0.04) (0.29 ± 0.06)، (0.72 ± 0.08)، (27.90 ± 1.45) على التوالي. كما أظهرتالقياسات الصحيةلمستوىالحموضة لعينات حليب البقر الخام التي تم فحصها أن متوسط قيمة درجة الحموضة وحمض اللاكتيك و الستريك كانت (19.85 ± 1.22)، (0.19 ± 0.01)، (0.12 ± 0.01) على التوالي. كانت درجة التجمد لحليب البقر الخام هي (-0.46 ± 0.01) على التوالي. أوضحت النتائج أن نسبة انتشار الإيشيريا القولونية وميكروب السل البقري في حليب البقر الخام كانت 20.0٪ و 3.33٪ على التوالي. علاوة على ذلك ، كان متوسط عدد الميكروبات الهوائية وعدد القولونيات وعدد المكورات العنقودية الذهبية في الحليب ) (2.9x105 ± 0.16x105)، (3.8 x103 ± 0.13x103)، (3.1x103 ± 0.12x103) على التوالي. وتم التعرف على معزولات الإيشيريا القولونية المصلية على أنها O111 و O26 و O91 و O44 و O128 وأنماط مصلية غير نمطية. أظهر التوصيف الجزيئي لمعزولات الإيشيريشيا القولونية (5) أن جينات (ompAوstx1gene) تم اكتشافها في 5 معزولات (100٪) و2 (40٪) على التوالي. بينما تم الكشف عن جينات المقاومة (bla TEM و IV- (3) aac) في 5 معزولات (100٪). من ناحية أخرى كانت معزولات المكورات العنقودية (5) موجبة لجينات الضراوة (جين سبا وجين PVL) وجينات المقاومة (جين mecA و 100) (aac٪) مما يشير إلى أن هذه المعزولات كانت شديدة المقاومة للمضادات الحيوية. وأظهر اختبار حساسية المضادات الحيوية معزولات مقاومة للأدوية المتعددة (MDR). هذا وقد تمت مناقشة الأهمية الصحية للميكروبات المعزولة.