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Antibiotic Resistant Genes in Some Pathogenic Bacteria and Its Correlation with Safety of Milk and Dairy Products

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Highlights:

The article discusses the presence of antibiotic-resistant genes in pathogenic bacteria found in milk and dairy products, emphasizing the correlation with food safety.

The study investigates the prevalence of antibiotic-resistant *E. coli* and *S. aureus* in 270 samples of raw milk and dairy products, highlighting the presence of ESBL *E. coli* and MRSA strains.

The molecular characterization of selected isolates revealed the presence of various antibiotic-resistant genes.

The effectiveness of Neutral electrolyzed water (NEW) in reducing bacterial counts is assessed, showing significant bactericidal activity

Abstract

A TOTAL of 270 samples of raw milk and dairy products were investigated for the presence of ESBL *E. coli* and *S. aureus* (MRSA). Data revealed that 41(15.6%) and 45(*16.8%)* were total positive samples for *E. coli* and *S. aureus,* respectively. The examined samples of raw milk (n=100; 33% and 30%), white soft cheese (n=100; 2% and 0%), ras cheese (n=50; 12% and 30%) and ice cream (n=20; 0% and 0%) were positive for *E. coli* and *S. aureus*, respectively. The identified serotypes of *E. coli* involved O128: H2 (26.8%), O91: H21 (19.5%), O78 (9.75%), O26: H11 (17.1%), O119: H6 (12.2%), O121: H7 (4.9%), O153: H2 (4.9%), O17: H18 (2.4%), and O159 (2.4%). The averages of MDR index were 0.424 and 0.493 for *E. coli* and *S. aureus,* respectively. Molecular characterization of isolates with a MAR index ≥ 0. 2 revealed that *blaTEM*, *blaCTX-M, blaSHV*, and *blaOXA* genes in 100%, 100%, 57.1%, and 71.4% in *E. coli* isolates and *blaZ, optrA, vanA*, and *mecA* genes were detected in 85.7%, 28.6%, 42.9%, and 85.7% in *S. aureus* isolates, respectively. The effectiveness of neutral electrolyzed water (NEW) on the viability of ESBL *E. coli* and MRSA isolates was assessed in relation to time (5, 10, and 15 minutes), a significant reduction in counts compared to control samples at 15 minutes of exposure. The count of ESBL *E. coli* and MRSA decreased to 81.3±1.04 and, $1.4x10^2$±1.05 cfu/cm^2 compared to control samples that were $1.9x10^6$±1.12 and $5.2x10^6$±1.3 cfu/cm^2, respectively. NEW showed broad-spectrum bactericidal activity.

Key words: ESBL *E. coli*, Dairy products, Electrolyzed water, MRSA, Raw milk.

Introduction

The high nutritional elements in milk and dairy products make them perfect substrates for bacteria to grow. Raw milk and dairy products are widely consumed in Egypt and usually produced under unsanitary conditions, especially in rural regions. Small-scale production of these traditional goods puts customers at risk for health issues [1]. Raw milk and raw milk products are still consumed globally, which subsequently results in food safety problems related to bacterial food-borne diseases if not followed up [2].

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Milk can get spoiled from a variety of sources, including water, contaminated utensils, milkers, and teat canals. Although hygienic milk production is essential for safe and high-quality products, the handling process can make milk unfit for human consumption due to contamination [3].

Antibiotics are used to treat bacterial infections in humans and animals, nevertheless, their widespread application favors the emergence of antibiotic-resistant pathogens. These bacteria may transmit resistant genes to humans through the consumption of contaminated dairy products. Animals are potential vectors for resistant bacteria, constituting a danger to human health. Antibiotic resistance is attributed to genetic alterations and recurrent antimicrobial medication usage in the lower gastrointestinal tract [4].

All antibiotic categories including penicillins, sulfonamides, aminoglycosides, tetracyclines, and cephalosporins that are utilized in both veterinary and human medicine are affected by bacterial resistance [2].

Escherichia coli is an important contaminant in the environment and can be passed through raw milk and dairy products due to fecal contamination and lack of hygienic measures during the milking process. It can also serve as a reservoir for antibiotic resistance genes, which can be transmitted to other bacteria, particularly MDR bacteria. *E. coli* usually has associations with genes expressing Extended Spectrum β-Lactamase (ESBL) [5].

Staphylococcus aureus is a significant foodborne illness that can be spread by raw milk from mastitic cows or food workers who contracted *S. aureus* as a result of improper hygienic acts. It has the capacity to generate a massive range of heat-stable enterotoxins, which result in food poisoning if found heavily in foods [6].

Staphylococcal enterotoxins are five types that result in food poisoning in ordinary foods, including milk, cream, butter, and cheese. Antimicrobial-resistant *S. aureus* strains, such as methicillin-resistant (MRSA), are an important contributor to morbidity and mortality due to the mecA gene's mutated penicillin-binding protein. The MRSA promotes mastitis in cattle, which is prevalent in bovine milk and transfers between humans and dairy animals. Also, it has been recognized as a concerned food safety issue related to bulk tank milk, with the potential for contaminated dairy products to get into the food chain for humans [7]. Egypt is one of the regions where resistance to antibiotics is increasing, highlighting the necessity of evaluating food-borne illnesses and creating approaches to stop them from transferring to more serious human pathogens that represent a serious risk to public health [8].

Electrolyzed water (EW) is a potential sterilizing solution for pathogen control in the food sector due to its high broad-spectrum bactericidal effectiveness and synergistic impact of available chlorine concentrations, pH, and oxidation reduction potential. Commercial sanitizers such as peroxide mixtures, quaternary ammonium compounds, ozone and, chlorine compounds are currently used as sterilization methods; nevertheless, some are not completely acceptable because of their potential toxicity, chemical residues, inadequate deactivation potency, and adverse effect on food quality. The evolution of reliable and secure sanitizers for the food sector is a never-ending subject of consideration [9].

So, our study is going to highlight the prevalence of E. *coli* and *S. aureus* in raw milk and dairy products (white soft cheese, ras cheese, and ice cream) in Egyptian markets and to what extent these isolates are resistant to most common antibiotics through phenotypic identification and subsequently detection of antibiotic-resistant genes using PCR technique in isolates with MDR index equal or greater than 0.2, and measure the effect of neutral electrolyzed water (NEW) on the viability of isolated ESBL-producing *E. coli* and Methicillin-resistant *Staphylococcus aureus* (MRSA).

Material and Methods

Collection of samples

A total of 270 samples of raw milk (100), white soft cheese (100), ras cheese (50), and ice cream (20) were collected from Egyptian markets in Damietta and Ismailia governorate from December 2023 to April 2024, in an ice box with a minimum of delay to perform laboratory tests.

Preparation of samples

All samples were prepared aseptically before the examination, according to [10].

Isolation of bacteria

Isolation of S.aureus [11].

Plating 0.1 ml of incubated samples on buffer peptone water at 37 ˚C onto the surface of Baird-Parker agar (Lab M, UK), which was supplied with egg yolk tellurite emulsion (50 ml/L), followed by incubation at 37 ºC for 48 hours. The resultant coagulase-positive colonies typically exhibit a black or grey coloration, possess a shining and convex morphology, and are surrounded by a distinct clear zone.

Isolation of E. coli [12]

Using surface plate technique, 0.1 ml of incubated samples on buffer peptone water at 37 ˚C was plated on TBX agar media (Chromogenic medium for detection and enumeration of *E. coli* (Liofilchem), for the recovery of sub-lethally injured *E. coli*, incubate plates at 37°C or 30°C for 4 hours then continue incubation at 44°C for additional 18-20 hours. β-glucuronidase-positive *Escherichia coli* is blue to blue-green.

Identification of isolated bacteria

Identification of S. aureus

Morphological examination of S. aureus [13]

From each sample, two isolates were selected, exhibiting the typical *S. aureus* morphology which is gram-positive cocci in the form of clusters resembling grape.

Biochemical identification of S. aureus [14]

Biochemical assays were screened, including rabbit plasma coagulation, catalase positivity, oxidase negativity, DNase positivity, absence of indole production, and the ability to ferment lactose, sucrose, glucose, and mannitol.

Identification of E. Coli

Biochemical Identification

Suspected isolates of *E. coli* were identified biochemically, according to MacFaddin [14] indicating indole positive, Simmon's Citrate negative, acidic reaction on TSI with production of gas, positive for methyl red and negative for Voges-Proskauer.

Serological Identification of E. Coli

Typical *E. coli* reaction was identified serologically as recorded by Akinjogunla et al [15] through using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) for the diagnosis of enteropathogenic types.

Antibiotic Resistance of Isolated Pathogens (Antibiogram):

Antimicrobial susceptibility was tested by the single diffusion method according to Mary and Usha [16] for *E. coli* and according to Deresse et al [17] for *S. aureus*. The susceptibility of the isolated strains was determined using antibiotic sensitivity discs with different concentrations (Oxoid Limited, Basingstoke, Hampshire, United Kingdom). As a consequence, an antimicrobial sensitivity test was done to comply with the requirements established by the NCCLS [18]. Antimicrobial resistant test included the following antibiotics; daptomycin (30 ug), gentamicin (10 ug), cefotaxime (30 ug), kanamycin (30 ug), azithromycin (15 ug), vancomycin (5 ug), oxacillin (1 ug), linezolid (30 ug), meropenem (10 ug), ciprofloxacin (5 ug), clindamycin (10 ug), erythromycin (15 ug), amikacin (30 ug), sulphamethoxazol (25 ug), and tetracycline (30 ug). The MAR index of each isolate was determined using the method described by Singh et al [19].

Detection of antibiotic-resistant genes by using multiplex PCR assay

DNA extraction: Isolates were extracted using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH), with modifications to the manufacturer's protocols. For 10 minutes, incubate 200 µl of specimen suspension with 10 µl of proteinase K and 200 µl of lysis buffer at 56^0 After the incubation process, add 200 µl of 100% ethanol to the lysate. The sample was rinsed and centrifuged in accordance with manufacturer guidelines. Nucleic acid was obtained using 100 µl of buffer for elution from the kit.

Oligonucleotide Primer

Primers utilized were supplied from Metabion (Germany), the reference of each primer is listed in Table (1).

PCR amplification: Primers were used in a 25-µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix **(Takara, Japan)**, 1 µl of each primer of 20 pmol concentration, 5.5 µl of water, and 5 µl of DNA template. The reaction took place in an Applied Biosystems 2720 thermal cycler.

Analysis of the PCR Products: The PCR products were electrophoresed on a 1.5% agarose gel **(Applichem, Germany, GmbH)** in 1x TBE buffer at room temperature with slops of 5 V/cm. Gel screening included putting 15 µl of products into each slot. The individual fragment sizes were determined using a generuler 100 bp ladder (Fermentas, Germany) and the Gelpilot 100 bp plus ladder **(Qiagen, Gmbh, Germany)**. The gel was photographed using a system for documenting gel **(Alpha Innotech, Biometra)**, and the findings were assessed by computer software.

Effect of NEW on the viability of ESBL producing E. coli and MRSA on a simulated industrial model (stainless steel coupons) [20]

Preparation of bacterial cultures

Stored ESBL *E. coli* and (MRSA) *S. aureus* were inoculated into 9 ml of buffered peptone water (Lab M), and reconstituted cultures were incubated at 37 °C for 24 h. One ml of the overnight incubated cultures was inoculated into a test tube containing nine ml of tryptic soy broth, diluted 1:10 (Tryptic soy broth, Lab M).

Preparation of stainless-steel coupons:

The surface was scrubbed for 1 minute with a cleansing solution and then rinsed twice with deionized water. The surface was then sterilized by autoclaving at 121 °C for 15 minutes before use. Stainless steel (type 304, no. 4. finish) coupon materials were prepared; the diameter of the coupon was 10×10 cm. A template was used in the sampling procedure of one cc square area, and the preparation of coupons was performed according to Maharjan et al [21].

Method of infection

To determine the initial bacterial count, bacterial cells were counted using the method stated by Hoa et al [22]. In summary, coupons were aseptically removed from the strain culture and placed independently in a sterile glass petri dish, and then washed twice using 10 mL of distilled water to remove non-attached bacteria [23]. Cells sticking to each coupon were eliminated using the swab method; both sides of each coupon were attentively swabbed with a pair of cotton swabs priorly dipped in sterile saline solution to eradicate as many cells as possible from the surfaces. The heads of the cotton swabs were cut off and placed into tubes containing 5ml sterile saline. The bacteria present on swabs were resuspended by manual shaking for detachment of bacteria. The cell solution was then diluted in series, with 0.1 mL of each dilution being plated on selective media for *E. coli* and *S. aureus.*

Applying the Neutral electrolyzed water (NEW)

The characteristics of NEW were: pH 7.5± 1.5, ORP 780 ± 100 mV, and C. active 500mg/1±50 NEW is taken from the original factory-sealed bottles vol. 1L (Envirolyte W.P.C. Ltd.). The contaminated coupons were dipped in a sterile baker containing NEW that was used without dilution at different times (5, 10, and 15 minutes).

Surface sampling:

Swabs are taken according to Microbiology of the Food Chain Horizontal Methods for Surface Sampling [24]. The plates were incubated for 72 hours at 37 °C, and the number of bacterial colonies was counted (cfu/cm^2). All analyses were repeated five times, and the results obtained were transformed into logarithmic values (log $_{10}$).

Statistical analysis

The statistical analysis was performed to determine the significance of the NEW effect on the viability of targeted bacteria, which consisted of mean comparison tests. The normality of the data was determined by the Shapiro test. If data was normal, univariate analysis of variance (ANOVA) was performed, followed by Tukey's post-hoc test ($P < 0.05$). If data was non-normal, non- parametric test (Kruskal-Wallis's) was performed. Both tests evaluate the significant difference in contact time between treated groups and control samples. Statistical procedures were done using SPSS computer software (version 22), IBM software, USA, Chicago.

Results

Isolation of E. coli and S. aureus

The prevalence of pathogenic *E. coli* and *S. aureus* in raw milk (n = 100), white soft cheese (n = 100), ras cheese (n = 50), and ice cream (n = 20) was, respectively, 33% (33), 2% (2), 12% (6), and zero for *E. coli,* and for *S. aureus,* 30% (30), 0%(zero), 30% (15), and 0%(zero), respectively Fig. (1)**.** *Escherichia coli* **serotypes** incidence **and** strain characterization of each serotype were as follows: Enteropathogenic *E. coli*: O119:H6 (**12.2%**), O153:H2 (4.9**%**), and O17:H18 (**2.4%**); Enterohaemorrhagic *E. coli*: O91:H21 (19.5**%**), O121:H7 (4.9**%**), and O26:H11 (17.1**%**); Enterotoxigenic *E. coli*: O128:H2 (**26.8%**); and O78 (**9.75%**); and Enteroinvasive *E. coli*: O159 (**2.4%**). The most prevalent serotype is O128: H2, followed by O91: H21 Fig. (2).

Phenotypic antimicrobial resistance patterns of E. coli *and* S. aureus

Table (2) showed phenotypic antimicrobial resistance patterns of *E. coli* (n = 41) and *S. aureus* (n = 45). The resistance of *E. coli* isolates was as follows: erythromycin 100%, oxacillin and vancomycin 95.1%, penicillin-g 73.1%, sulphamethoxazol 65.8%, kanamycin 51%, daptomycin 41.5%, linezolid and clindamycin were 31.7%, 17% for tetracycline, amikacin, cefotaxime and ciprofloxacin 12.2%, 7.3% for gentamycin, 4.2% for meropenem and 4.8% for azithromycin, while kanamycin, clindamycin, tetracycline, cefotaxime, azithromycin, erythromycin, penicillin-g, gentamicin, sulphamethoxazol, ciprofloxacin, meropenem, oxacillin, amikacin, vancomycin, linezolid, daptomycin were resisted by 45 (100%), 42 (93.3%), 37 (82.2%), 34 (75.5%), 28 (62.2%), 28 (62.2%), 28 (62.2%), 23 (51.1%), 23 (51.1%), 20 (44.4%), 14 (31.1%), 11(24.4%), 11 (24.4%), 8 (17.7%), 6 (13.3%) and 3 (6.7%), respectively for *S. aureus* isolates.

Table (3) showed the antimicrobial resistance profile of *E. coli* strains, MAR index in isolated strains ranged from 0.1 to 1 (average 0.422); the highest MAR index was 1 related to O128:H2 isolated from Ras cheese. *E. coli* isolates were subdivided into groups that have the same MAR index (from A to M) for molecular characterization. Table (4) demonstrated

that the MAR index of *S. aureus* ranged from 0.0625 to 1, with an average of 0.493. The molecular characterization of detected *E. coli* and *S. aureus* was based on the phenotypic characterization of isolates with an MAR index ≥ 0.2.

Molecular characterization of S. aureus and E. coli antibiotic-resistant genes

Results revealed that all isolates of *E. coli* had *blaTEM* and *blaCTX-M* genes Fig. 3: D and A, respectively, while *blaSHV* and *blaOXA* were expressed as 4 (57.1%) and 5 (71.4%), Fig. 3: C and B, respectively**.** Isolates from ras cheese were free from *blaOXA* gene Fig. 3; B lane 6 and 7, while soft cheese isolate exhibited all investigated genes Fig. 3: lane 5. Seven isolates of *S. aureus* were selected randomly; six were from raw milk and one was from ras cheese, for the detection of antibiotic-resistant genes *blaZ, mecA, optrA,* and *vanA* that showed prevalence as follows: 85.7% (6 raw milk), 85.7% (5 raw milk and one ras cheese), 28.6% (one in raw milk and one in ras cheese), and 42.9% (2 raw milk and one ras cheese), respectively. Fig. 4**.** **T**wo isolates (28.6%) have *mecA* and *optrA* genes (Fig. 4: B, C Lane no. 6,7), they were detected in one raw milk and in one ras cheese samples, while the *vanA* gene was detected in 42.9% of *S. aureus* isolates (two raw milk and one ras cheese).

The effect of Neutral Electrolyzed Water (NEW) against the viability of E. coli *and* S. aureus

Table (5) showed the effect of NEW against the viability of *E. coli* and *S. aureus* in relation to exposure time (5, 10, 15 minutes) and compared it with control, expressing the results by $\log_{10}$ (mean± SE). Table (5) was subdivided into two parts; experiments 1 and 2 that related to NEW against *E. coli* and *S. aureus*, respectively. Data revealed that the bacterial counts expressed as Mean ± SE in relation to time 5, 10, and 15 minutes were $1.4X10^6$±1.10, $4.7X10^2$±1.14 and 81.3±1.04 with $\log_{10}$ reduction 0.13, 3.6, and 4.47 for *E. coli* and $2.5X10^5$±1.25, $1.5X10^3$±1.18 and $1.4X10^2$±1.05 with $\log_{10}$ reduction 1.3, 3.5, and 4.6 for *S. aureus* in accordance to the initial counts (control) that were $1.9X10^6$± 1.12 and $5.2x10^6$±1.3 for *E. coli* and *S. aureus*, respectively. By comparing means using ANOVA for experiment 1 and Kruscall-Wallis' test for experiment 2**,** Table (5) revealed that different superscript letters at the same column are significantly different from each other at the level of p value<0.05, while the same superscript letters at the same column are non-significantly different at the level of p value>0.05.

Discussion

Nutritious and secure food is crucial for maintaining efficiency and productivity. The prevalence of pathogenic *E. coli* and *S. aureus* in raw milk and dairy products is a crucial public health matter for the dairy market organization in Egypt. Our findings are revealed in Fig. (1). Many previous studies in different countries explored the prevalence of *E. coli* in raw milk as follows: 76.4%, 32% and 10% in Egypt **[**25, 26, 27], 25.4%, 90%, 40.5%, and 25% in Kenya, Iran, Pakistan, and Ethiopia, respectively [28, 29, 30, 31]. Gaffer et al **[**26**]** agreed with our results, while the results of Sombie et al; Yihunie et al and Younis et al **[**28, 31, 27**]** were lower than our results. Furthermore, Madani et al [28] detected results higher than our records**.**

Contamination of raw milk with pathogenic *E. coli* could be from direct faecal contact, contaminated water, or other sources [30]. These studies indicated that the prevalence of *E. coli* in raw milk is a great risk.

The incidence of *E. coli* in dairy products was investigated in many studies as follows; 21.7% in ras cheese; 20, 32, and 36% in ice cream, domiatti, and Karish cheese; 4.4% in white soft cheese; 19.05% and 4.76% in kariesh cheese and ice cream; 32, 48, and 38.8% in domiatti, tallaga, and ras cheese; and 1% in white soft cheese in Egypt [25, 26, 33, 34, 35, 36]. Moreover, a study in Iran showed 8.33% and 11.54% in cheese and ice cream, respectively [32]. According to the Egyptian Standard (2005), cheese must be free from *E. coli* [35].

All mentioned previous investigated studies had results higher than our data in ras cheese [25, 35] and ice cream [25, 31, 33]. Our investigation in white soft cheese is higher than Nadi et al [36] while Gaffer et al [26], Chaleshtori et al [32], Mohamed et al [33], Aal et al [34] and Kasem et al [35] showed higher results.

This study demonstrated a distinct variation in the incidence of *E. coli* in dairy products compared to previous studies. Such variation due to variability in preparation processes, storage, kind of cheese, and raw or pasteurized milk may be accountable for these contradictions. Additionally, this is probably due to the unsanitary conditions applied where cheeses are manufactured and workers engaged in the procedure [35].

Our findings were shown in Fig. (2), illustrating the prevalence of *E. coli* serotypes in raw milk and dairy products. By comparing with other studies in Egypt that found O146: H21, O17: H18, O119: H6, O114: H4, O26: H11, O111: H2, O103: H2, and O121: H7 were prevalent in karish, tallaga, and ras cheese, in addition to that, O128: H2 was the most prevalent serotype [35] that resembling our findings**,** another study exhibited O111, O27, O114, O125, and untypeable serotypes in raw milk [27], while

serotypes O128: H2, O91:H21, O55:H7, O121:H7, O146:H21, O111:H2, O114:H4, O78, O124, O126:H21, O127:H6, O146:H21, and O26:H11 were detected in milk, domiatti, and karish cheese **[**26**]**. Contamination of dairy products with *E. coli* is frequently related to fecal contamination or improper hygienic practices during the manufacturing process [35].

Our investigation revealed that AMR and MDR bacteria were prevalent in isolated *E. coli* strains from raw milk, white soft cheese, and ras cheese. All *E. coli* isolates were resistant to erythromycin, while meropenem showed 97.5% sensitivity, followed by gentamycin at 92.7%, and intermediate-resistant isolates at 24.3% to amikacin. All isolates were AMR (anti-microbial resistant), while 95.1% were MDR (resisting more than one antibiotic) Table (2).

Previous studies showed that AMR (antimicrobial resistant) and MDR (multi-drug resistant) were prevalent in *E. coli* isolated from cheese. AMR to gentamicin was the least (5.9%), followed by doxycycline (11.8%), tetracycline and ciprofloxacin (23.5%), ampicillin (29.4%), kanamycin (35.3%), amikacin (35.3%), sulfamethoxazole (47.1%), penicillin g (52.9%), cephalothin (64.7%), vancomycin (64.7%), cefotaxime (82.4%), nalidixic acid (94.1%), and erythromycin was the highest (100%). The MAR index ranged from 0.071 to 1 (average 0.478) [35], another investigation showed that the antimicrobial susceptibility of isolated strains was 100%, 98.44%, 92.19%, 71.87%, 65.63%, and 64.06% against tetracycline, oxacillin, erythromycin, nalidixic acid, sulphamethoxazol, and ampicillin, respectively. These strains were isolated from white soft cheese, milk, and ice cream (Egypt) [37]. A study from Pakistan revealed that *E. coli* strains were highly resistant to penicillin, amoxicillin, clavulanic acid, cefotaxime, gentamicin, sulphamethoxazole, and streptomycin, while antimicrobial sensitivity was expressed as follows: norfloxacin (59%), followed by enrofloxacin (54%), florefenical 50%, and the least sensitivity was recorded for oxytetracycline (36%) [30]. Variations in MDR-*E. coli* among studies may be attributed to geographical variances in antimicrobial use.

We subdivided *E. coli* isolates into groups according to their antimicrobial resistance profile Table (3). The molecular characterization of *E. coli* isolates is performed according to phenotypic characterization to detect antimicrobial genes of extended-spectrum beta-lactamase (ESBL).

We selected one isolate from each serotype with a MAR index of 0.2 or more; only seven isolates were selected randomly. Results revealed that all isolates had *blaTEM* and *blaCTX-M* genes, while *blaSHV* and *blaOXA* were expressed as 4 (57.1%) and 5 (71.4%), respectively. Phenotypically, serotype O153:H2 in group L Table (3) was sensitive to penicillin-g, cefotaxime, and meropenem (beta-lactams), while it was resistant to oxacillin with a MAR index of 0.2. The molecular characterization of the isolate revealed that it exhibited all the investigated genes Fig. (2) lane 5. One explanation for this finding is that phenotypic drug resistance may be controlled more by intrinsic bacterial mechanisms for resistance than by genetic acquisition, this agreed with Wang et al [38], as they found *E. coli* isolates (100%) were sensitive to quinolones despite a *qnrB* resistance gene being isolated.

Many studies explored the investigated genes; *blaTEM* gene was detected in 15.4% of cheese samples (Kazakhstan) [39]. In Egypt, an investigation reported that the prevalence of the *blaTEM* gene was 55.6%, 42.8%, 66.7%, and 100% in raw buffalo and cow milk, karish cheese, and talaga cheese, respectively**,** while *blaCTX-M1, blaTEM,* and *blaSHV* genes were present in 48%, 44%, and 14.8% of raw milk and dairy products (ice cream, kareish, and domiati cheese), respectively [40].

In Pakistan, authors found that 1.37% of STEC isolates produced extended-spectrum beta-lactamase (ESBL); *blaCTX-M* gene, and no *blaTEM* or *blaSHV* [30]. Another study in China reported that 20.6% and 17.3% of *E. coli* isolated from mastitic cows carried *blaTEM* and *blaCTX-M*, respectively, and none of them carried *blaOXA* [41].

These findings shed insights on the crucial role of continued control, strict preventive procedures, and region-specific measures necessary to minimize the potential hazards of bacterial contamination in Raw milk [30]. Finally, the MAR index is accessible, fast, and accurate. Furthermore, it is easy to use and does not need any particular experience or costly supplies. Detecting resistant *E. coli* is essential since it raises the number of bacteria able to persist against antibiotics [35].

Staphylococcus aureus was isolated from raw milk samples, as revealed in Fig. (1), many studies showed the prevalence of *S. aureus* in different countries as follows: 42% in Egypt [42], 36.66%,11.2%, and 10.8% in Turkey [43, 46, 47], 61.7% in China [44] and 18.4% in Iran [45].

Results of Ahmed et al [42], Keyvan et al [43] and Kou et al [44] were higher than our results while, Shariatifar et al [45], Muș et al [46] and Kizanlik et al [47] showed lower results.

Variations in the incidence of *S. aureus* isolates in different countries refer to the conditions of

milking and the quality of hygiene of bulk tank milk [43].

The prevalence of *S. aureus* in dairy products has been detected in many studies as follows: 14%, 38%, 24%, and 74% in domiatti, karish, ras cheese, and small-scale ice cream; 48%, 60%, and 72% in domiatti, tallaga, and ras cheese in Egypt [42, 35], 15.7% and 10.1% in ice cream and cheese; 60% in fresh cheese in Turkey [46, 47] and 21.96% in cheese in Croatia [48], respectively.

Our results were lower than the mentioned studies in white soft cheese [42, 35, 47, 48], ras cheese [42, 35] and ice cream [42, 46].

The observed differences in the incidence of *S. aureus* between investigations reflect multiple factors like time of sampling, post-harvest method, geographical location, and isolation and identification techniques [45].

Our results revealed that white soft cheese and ice cream samples were free from *S. aureus*, which may be due to adequate hygiene during the milking process and strict measures during production and storage.

Our findings demonstrated the phenotypic antimicrobial resistance patterns of *S. aureus* strains, as shown in Table (2).

A study by Wang and his colleagues [38] showed that 71.4% of *S. aureus* strains were resistant to trimethoprim-sulfamethoxazole and, penicillin G followed by 57.1% of strains resistant to gentamicin, kanamycin, clindamycin, and amikacin. All examined strains were sensitive to tetracycline, chloramphenicol, and ciprofloxacin, followed by cefoxitin and erythromycin (57.1%).

Four strains of *S. aureus* were MDR to at least five drugs within three classes**.** An investigation in Brazil reported that 20% of *S. aureus* strains isolated from raw milk cheese were resistant to sulfazotrim, trimethoprim, and erythromycin at the same resistance rates, 5% (only one) were resistant to ciprofloxacin and enrofloxacin; and gentamicin, streptomycin, and broad-spectrum amino-glycosides were efficient against all isolates evaluated in this study [49].

Kizanlik and Goksoy [47] showed that 63.9%, 24.4%, 19.7%, 12.8%, 10.4%, 10.4%, 10.4%, 8.1%, 6.9%, 6.9%, 4.6%, 3.5%, 3.5%, 3.5%, 1.1%, 0.0% of *S. aureus* strains were resistant to penicillin, oxacillin, clindamycin, erythromycin, cefoxitin, ciprofloxacin, fusidic acid, daptomycin, fosfomycin, tetracycline, linezolid, teicoplanin, trimethoprim-sulfamethoxazole, vancomycin, gentamicin and tigecycline, respectively.

All isolates in our study were resistant to kanamycin; the least resistant antibiotic was daptomycin. The MAR index ranged from 0.0625 to 1, with average 0.493. In other studies, the authors showed that all *S. aureus* isolates were resistant to kanamycin, but the least resistant antibiotic was vancomycin (10%), with MAR index ranging from 0.143 to 1 (mean = 0.529), this agreed with our results [35].

The MAR index values of *S. aureus* in another investigation ranged from 0.05 to 0.64; additionally, 89.4% have a MAR index value > 0.2, indicating that these strains were isolated from a high-risk source of contamination where growth promoters or many antibiotics are used [50].

Methicillin-resistant *S. aureus* (MRSA) evolved as a result of adaptation to b-lactam antibiotics, including methicillin that is mediated by the *mecA* gene [51]. Overuse of β-lactam antibiotics in dairy cows for preventative measures and mastitis treatment can introduce MRSA to milk and dairy products that do not induce any change in milk, the spread of MRSA to humans via mastitic milk or close contact with dairy cows considers significant threats to food safety [52].

The molecular characterization of detected *S. aureus* was based on the phenotypic characterization of isolates with a MAR index ≥ 0.2. Seven isolates were selected randomly; six were from raw milk and one was from ras cheese, for the detection of antibiotic-resistant genes *blaZ, mecA, optrA,* and *vanA* that showed prevalence as follows: 85.7% (6 raw milk), 85.7% (5 raw milk and one ras cheese), 28.6% (one in raw milk and one in ras cheese), and 42.9% (2 raw milk and one ras cheese), respectively. Resistance to penicillin is mainly caused by the *blaZ* gene encoding beta-lactamase production, which hydrolytically destroys beta-lactams **[50].** Methicillin resistant *S. aureus* (*mecA)* was detected in two samples of Minas Frescal cheese (raw milk cheese in Brazil) [49], In China, *blaZ* and *mecA* were detected in 42.9% and 28.6%, respectively, in *S. aureus* isolated from fresh milk [38]. A study from Turkey showed that the mecA gene was found in 75.4% of *S. aureus* isolated from raw milk [43], while another study could not detect the methicillin resistance (*mec*A) gene in isolated *S. aureus* from milk and dairy products (cheese and ice cream) [46]. According to Egyptian standards, soft cheeses and hard cheeses (as ras cheese) must be free from MRSA and their toxins [42]. The *optrA* gene encodes resistance to oxazolidinone, like linezolid, as well as nonfluorinated and fluorinated phenols, like chloramphenicol [53].

The two examined isolates that have *mecA* and *optrA* genes (Fig. 4: B, C lane no. 6,7) were detected

in 28.6% (one in raw milk and one in ras cheese) of samples.

Oxazolidinones have high antibacterial activity against MDR Gram-positive bacteria, including MRSA. Unfortunately, transferable oxazolidinone resistance genes, including *optrA,* located on chromosomal DNA or plasmids, were described in many gram-positive bacteria, including *Staphylococcus,* that may indicate the impossibility of treatment [53]. None of the 16 isolates of MRSA isolated from mastitic cows did not harbor *the optrA* gene [54].

Vancomycin is the first glycopeptide antibiotic identified and one of the most commonly used initial drugs for treating MRSA infections. Nevertheless, the scenario altered in July 2002, when the Centers for Disease Control and Prevention (CDC) in the USA established the first case of Vancomycin-resistant *S. aureus* (VRSA) in a patient with diabetes in the USA [50]. Our study showed that 42.9% of examined *S. aureus* have *the VanA* gene (Fig. 4, D**)**. By comparing with other studies, it revealed that 4% of isolated *S. aureus* carry *vanA* gene from clinical mastitis [55], while authors in another study could not detect *vanA* gene in 65 milk samples [50].

Electrolyzed water is used in food safety industrial applications, including cleaning and disinfection in dairy processing systems. Its antibacterial activity is influenced by pH, chlorine concentration, and oxidation-reduction potential. HOCl is a superior biocide, and neutral electrolyzed water (NEW, pH 7) has been shown to be beneficial in eliminating bacterial infections [56]. We studied the effect of neutral electrolyzed water (pH 7.5± 1.5, ORP 780 ± 100 mV, and C. active 500 ppm/1±50) on the viability of ESBL *E. coli* (O153:H2, Fig. 3, Lane 4) and *S. aureus (*MRSA, Fig. 4, Lane7) at different contact times (5, 10, 15 min.). Aniyyah et al **[**57] revealed that 50–200 ppm of HOCl produced from 10 grams of NaCl is acceptable for sanitizing purposes, while 500–800 ppm from 30 grams of NaCl is effective for disinfection, highlighting the various harmful amounts of HOCl produced through electrolysis. NEW can be used as an alternative to clean and disinfect stainless steel plate surfaces, particularly for the dairy industry [58]. Our findings showed that NEW is effective against the viability of *E. coli* and *S. aureus.* Table (5) revealed that the application of NEW for 15 minutes had a significant decrease in *E. coli* and *S. aureus* counts compared to control groups. The inhibitory activity of NEW was increased by increasing the duration of exposure from 5 to 10 minutes, with a significant decrease at 15 minutes. There was no significant difference (p-value > 0.05) between the control (base line) and 5-minute exposure with log10 reductions (1.3 and 0.13) for *S. aureus* and *E. coli*, respectively. Increasing exposure time to 10 minutes decreased the bacterial log to 1.5X103±1.18 cfu/cm^2 and 4.7X102±1.14 cfu/cm^2, with log10 reductions of 3.6 and 3.5 for *E. coli* and *S. aureus*, respectively, with no significant difference between contact time of 5 min. and 10 min. (p-value > 0.05). Increasing the contact time to 15 minutes resulted in a log10 reduction to 4.47 and 4.6 for *E. coli* and *S. aureus*, respectively Table (5), this is consistent with the findings of Moradi et al [59] who found that increasing the contact time of NEW resulted in bacteriostatic activity when it was given 30 seconds, but it had bactericidal activity when it was given 60 seconds or more, as NEW is capable of penetrating and effectively killing bacteria incorporated in biofilms in the initial phases of development.

Conclusion

In conclusion, the study highlighted the potential hazards of ESBL-*E. coli* and MRSA strain transmission through the food chain. Preventive measures are needed to control the transfer of anti-biotic-resistant genes through food. Neutral Electrolyzed Water (NEW) is a promising agent used as a sterilizing solution for controlling microorganisms in the food industry. Electrolyzed water (EW) is a practical sterilizing solution for the food industry. Additional studies are needed to determine its long-term efficacy, safety, and effects on food quality. Standardized guidelines for using EW in the milk production and processing stages should be created.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

TABLE 1. Oligonucleotide Primer

Target bacteria	Target gene	Primers sequences	Amplified segment (bp)	Amplification (35 cycles)				Final extension	Reference
				Primary denaturation	Secondary denaturation	Annealing	Extension		
E. coli	*bla*$_{TEM}$	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	C˚72 10 min.	
	blaSHV	AGGATTGACTGCCTTTTTG ATTTGCTGATTTCGCTCG	392	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 40 sec	˚72C 10min.	[60]
	BlaOXA-1	ATATCTCTACTGTTGCATCTCC AAACCCTTCAAACCATCC	619	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	72˚C 10 min	
	BlaCTX-M	ATG TGC AGY ACC AGT AAR GTK ATG GC TGG GTR AAR TAR GTS ACC AGA AYC AGC GG	593	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	[61]
S.aureus	*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 10 min.	[62]
	vanA	CATGACGTATCGGTAAAATC ACCGGGCAGRGTATTGAC	885	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 50 sec.	72˚C 10 min.	[63]
	blaZ	TACAACTGTAATATCGGAGGG CATTACACTCTTGGCGGTTTC	833	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 50 sec.	72˚C 10 min.	[64]
	optrA	AGGTGGTCAGCGAACTAA ATCAACTGTTCCCATTCA	1395	94˚C 5 min.	94˚C 30 sec.	53˚C 1 min.	72˚C 1.2 min.	72˚C 12 min.	[65]

TABLE 2. Phenotypic antimicrobial resistance patterns of *E. coli* and *S. aureus* strains

Antimicrobial classes	Antimicrobial agents	*E. coli* (n=41)			*S. aureus* (n=45)		
		S	I	R	S	I	R
aminoglycoside	Kanamycin (K)	13(31.7%)	7(17.1%)	21(51%)	-	-	45(100%)
lincosamide	Clindamycin (CL)	21(51%)	7(17%)	13(31.7)	-	3(6.7%)	42(93.3)
Tetracycline	Tetracycline (T)	25(60.9%)	9(21.9%)	7(17%)	-	8(17.8%)	37(82.2%)
beta-lactam	Cefotaxime (CF)	30(73.2%)	6(14.6%)	5(12.2%)	3(6.73%)	8(17.8%)	34(75.5)
macrolides	Azithromycin (AZ)	34(82.9%)	5 (12.2%)	2 (4.8%)	11(24.44)	6(13.33%)	28(62.2%)
macrolides	Erythromycin (E)	-	-	41(100%)	14(31.1%)	3 (6.7%)	28(62.2%)
beta-lactam	Penicillin-G (P)	4(9.7%)	7(17%)	30(73.1%)	14(31.1%)	3 (6.7%)	28(62.2%)
aminoglycoside	Gentamicin (G)	38(92.7%)	-	3 (7.3%)	16(35.5%)	6(13.3%)	23(51.1%)
sulfonamide	Sulphamethoxazol (SXT)	10(24.4%)	4(9.8%)	27(65.8%)	20(44.4%)	2(4.4%)	23(51.1%)
fluoroquinolones	Ciprofloxacin (CP)	27(65.8%)	7(17%)	7(17%)	25(55.55%)	-	20(44.4%)
beta-lactam	Meropenem (M)	40(97.5%)	-	1(4.2%)	25(55.55%)	6(13.3%)	14(31.1%)
beta-lactam	Oxacillin (OX)	-	2(4.9%)	39(95.1%)	31(68.8%)	3(6.7%)	11(24.4%)
aminoglycoside	Amikacin (AK)	23(56%)	10(24.3%)	7(17%)	34(75.5%)	-	11(24.4%)
glycopeptide	Vancomycin (V)	2(4.9%)	-	39(95.1%)	34(75.5%)	3(6.7%)	8(17.7%)
oxazolidinone	Linezolid (LZ)	28(68.2%)	-	13(31.7%)	36(82.2%)	6(13.3%)	6(13.3%)
lipopeptide	Daptomycin (DA)	18(43.9%)	6(14.6%)	17(41.5%)	39(86.6%)	3(6.7%)	3(6.7%)

TABLE 3. Antimicrobial resistance profile of *E. coli* strains (n=41)

Antimicrobial resistance profile	MAR index	Source of Serotype				Isolates group
		Raw milk	Soft cheese	Ras cheese	Total No.	
E, OX, V, P, SXT, K, DA, CL, LZ, AK, T, CP, CF, G, AZ, M	1	-	-	O128: H2	1	A
E, OX, V, P, SXT, K, DA, CL, LZ, AK, T, CP, CF, G, AZ	0.937	O91: H21	-		1	B
E, OX, V, P, SXT, K, DA, CL, LZ, AK, T, CP, CF, G	0.875	O128: H2	-	O78	2	C
E, OX, V, P, SXT, K, DA, CL, LZ, AK, T, CP, CF	0.812	O26 : H11			1	D
E, OX, V, P, SXT, K, DA, CL, LZ, AK, T, CP	0.750	O119: H6		O91: H21	2	E
E, OX, V, P, SXT, K, DA, CL, LZ, AK	0.625		O128:H2		1	F
E, OX, V, P, SXT, K, DA, CL, LZ	0.562	O128: H2, O91:21(2), O78	O26 :H11		5	G
E, OX, V, P, SXT, K, DA	0.438	O128: H2, O91: H21, O26 : H11, O119: H6			4	H
E, OX, V, P, SXT, K	0.375	O128: H2(2), O91: H21, O78,			4	I
E, OX, V, P, SXT	0.313	O26 : H11(2), O78, O91: H21, O128:H2,		O119: H6	6	J
E, OX, V, P	0.250	O128: H2, O26: H11,		O26 : H11	4	K
E, OX, V	0.20	O121: H7, O128: H2(2), O78, O119: H6(2), O153: H2(2)		O121: H7	8	L
E	0.10	O17 : H18, O159			2	M
Average			0.424			

Isolates with MAR Index > 0.2 were 31

Isolates with MAR Index< 0.2 were 10

TABLE 4. Antimicrobial resistance profile of S. aureus strains (n=45)

Antimicrobial resistance profile	MAR index	No. of isolates	Source	
			Raw milk	Ras cheese
K, CL, T, CF, AZ, E, P, G, SXT, CP, M, OX, AK, V, LZ, DA	1	3	2	1
K, CL, T, CF, AZ, E, P, G, SXT, CP, M, OX, AK, V	0.875	5	4	1
K, CL, T, CF, AZ, E, P, G, SXT, CP, M, OX, AK	0.812	3	2	1
K, CL, T, CF, AZ, E, P, G, SXT, CP, M	0.687	3	2	1
K, CL, T, CF, AZ, E, P, G, SXT, CP	0.625	6	4	2
K, CL, T, CF, AZ, E, P, G, SXT	0.562	3	2	1
K, CL, T, CF, AZ, E, P	0.437	2	1	1
K, CL, T, CF, AZ, E	0.375	3	2	1
K, CL, T, CF	0.250	6	3	3
K, CL, T	0.187	3	2	1
K, CL	0.125	5	4	1
K	0.0625	3	2	1
Average		0.493		

Isolates with MAR Index > 0.2 were 34
Isolates with MAR Index< 0.2 were 11

TABLE 5. Effect of NEW on the viability of *E. coli* and *S.aureus* in relation to time .Population of bacteria on stainless steel surface (log10 CFU/cm2)

	Experiment (1)		Experiment (2)	
Time of contact	*E. coli* Mean/SE	Log_{10} reduction	*S.aureus* Mean/SE	Log_{10} reduction
control	$1.9X10^{6}\pm 1.12^{a}$		$5.2x10^{6}\pm1.3^{a}$	
5 min.	$1.4X10^{6}\pm1.10^{ab}$	0.13	$2.5X10^{5}\pm1.25^{ab}$	1.3
10 min.	$4.7X10^{2}\pm1.14^{bc}$	3.6	$1.5X10^{3}\pm1.18^{bc}$	3.5
15 min.	81.3 ± 1.04^{c}	4.47	$1.4X10^{2}\pm1.05^{c}$	4.6

A: Different Superscript letters at the same column are significantly different with each other at the level of p value<0.05
B: The same Superscript letters at the same column are non-significantly with each different at the level of p value<0.05
C: Experiment 1, F (DFn, DFd) value = F (3, 16) = 2710 as p value < 0.001, Experiment 2, test statistics (Kruskal-Wallis) value is18.07

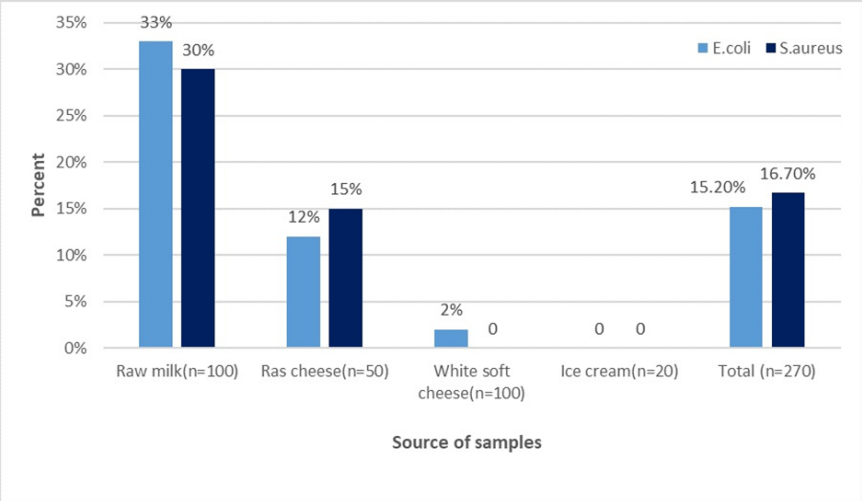

Fig. 1. Prevalence of *E. coli* and *S. aureus* in Raw milk and dairy products

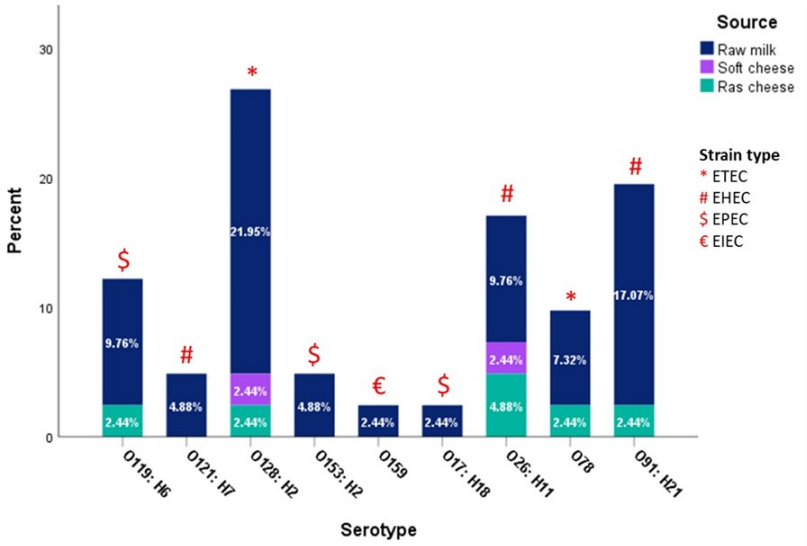

Fig. 2. Frequency distribution of *E. coli* serotypes among positive samples

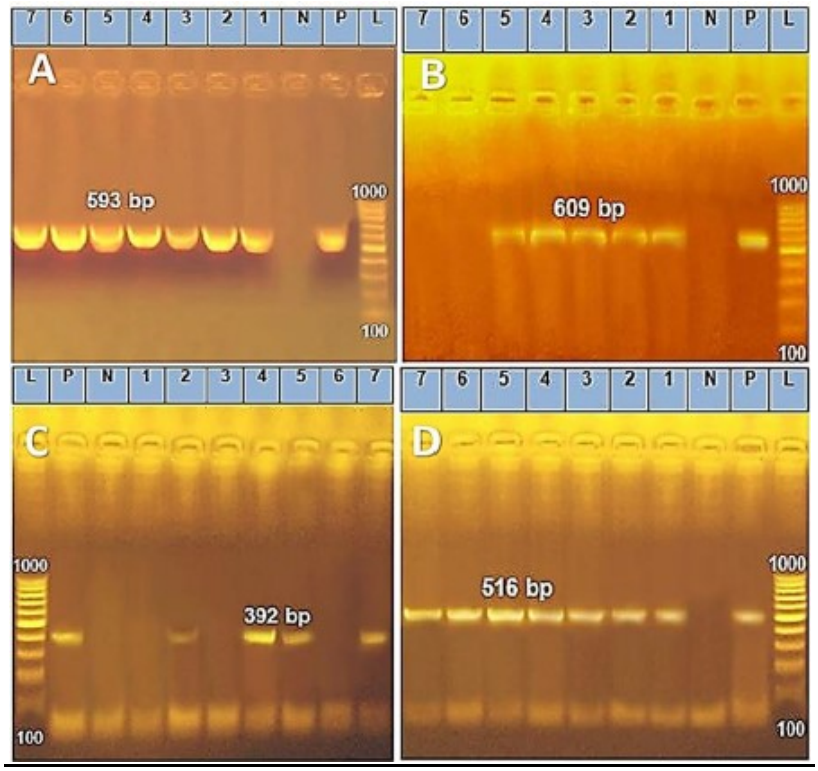

Fig. 3. Gel electrophoresis of amplified PCR products after DNA extracted from isolated *E.coli* strains using the *blaCTX-M, blaOXA, blaSHV* and *blaTEM* primers, reliable amplification of bands at 593 bp, 609pb, 392pb and 516pb that were demonestrated in A, B, C and D, respectively. Lan 1, 2,3, 4, 5, 6 and 7 represent O119: H6, O91: H21, O121: H7, O153: H2 (Raw milk), O26 : H11 (Soft cheese), O128: H2, O78 (Ras cheese), respectively.

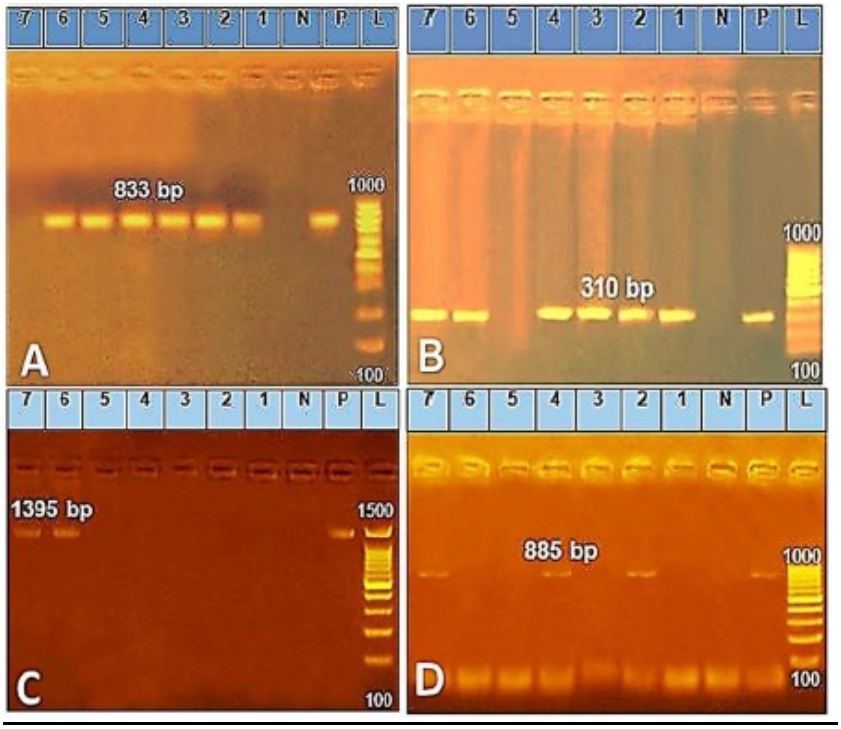

Fig. 4. Gel electrophoresis of amplified PCR products after DNA extracted from isolated *S.aureus* strains using the *blaZ, mecA, optrA* and *vanA* primers, reliable amplification of bands at 833 bp, 310 pb, 1395pb and 885pb that were demonestrated in A, B, C and D, respectively. Lan 1,2,3, 4, 5, and 6 represent *S.aureus* isolated from Raw milk, Lan 7 represents *S.aureus* was isolated from Ras cheese.

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الجينات المقاومة للمضادات الحيوية في بعض البكتيريا الممرضة وعلاقتها بسلامة الألبان ومنتجاتها

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الملخص

أجريت الدراسة على 270 عينة من الحليب الخام ومنتجات الألبان للتحقق من وجود سلالات مقاومة للمضادات الحيوية من الإشريكية القولونية والمكورات العنقودية الذهبية، وتحديدًا الإشريكية القولونية المقاومة لمضادات ESBL والمكورات العنقودية الذهبية المقاومة للميثيسيلين (الميرسا). أظهرت النتائج أن 33 (33٪) و2 (2٪) و6 (12٪) وصفر كانت إيجابية لالإشريكية القولونية بنسبة 41 (15.6٪) بينما30 (30٪) وصفر و15 (30٪) وصفر من العينات كانت إيجابية للمكورات العنقودية الذهبية بنسبة اجمالية 45 (16.8٪) في الحليب الخام والجبن الأبيض الطري والجبن الرومي والآيس كريم على التوالي. شملت الأنماط المصلية المحددة لـالإشريكية القولونية26.8) O128:H2 ٪(، و19.5) O91: H21 ٪(، و9.75) O78٪(، و O26: H11 17.1)٪(، و12.2) O119: H6٪(، و4.9)O121:H7٪(، و4.9) O153: H2٪(، و2.4) O17: H18٪(، و O159 2.4)٪ .(تم اختيار سبع عزلات من كل من الإشريكية القولونية والمكورات العنقودية الذهبية عشوائيًا للتوصيف الجزيئي بمؤشر MAR ≥ 0.02. أظهرت النتائج وجود جينات blaTEM و blaCTX-M و blaSHV و blaOXA بنسبة 100٪ و100٪ و57.1٪ و71.4٪ في عزلات الإشريكية القولونية على التوالي. بينما لوحظت جينات blaZ و optrA و vanA و mecA في 85.7% و28.6% و42.9% و85.7% في عزلات المكورات العنقودية الذهبية على التوالي. تم تقييم فاعلية الماء المحلل بالكهرباء المحايد (NEW) على قابلية بقاء عزلات ESBL E. coli و MRSAفيما يتعلق بالوقت (5 و10 و15 دقيقة). أظهرت النتائج انخفاضًا كبيرًا حيث قيمة ($p < 0.05$) في تعداد البكتيريا مقارنة بعينات التحكم عند 15 دقيقة من التعرض. كان الانخفاض في عدد الإشريكية القولونية المقاومة للبيتالاكتام واسع المفعول حتى1.04 ± 81.3 بينما كان الانخفاض في عدد المكور العنقودي الذهبي المقاوم للميثيسيلين حتى 1.4×10^2±1.05 خلية بكتيرية لكل سم^2 بالمقارنة بعينات التحكم التي كانت 1.121.9x10^6 ± و5.2 × 10^6 ± 1.3 خلية بكتيرية لكل سم^2. أظهر الماء المحايد المحلل كهربيا نشاطا واسع النطاق ضد البكتيريا التي تم تلويث سطع الاستانلس ستيل بها مما يجعلها بديلا فعالا لبعض المطهرات الخطرة على صحة الإنسان والضارة للبيئة.

الكلمات الدالة: الماء المحايد المحلل كهربيا، الإشريكية القولونية، المكور العنقودي الذهبي، الجينات المقاومة للمضادات الحيوية، الحليب الخام، منتجات الألبان.