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Antimicrobial resistance and molecular genotyping of *Escherichia coli* and *Staphylococcus aureus* isolated from some Egyptian cheeses

Nahed Gomaa Kasem, Maha Al-Ashmawy, Mohammed Elsherbin, Adel Abdelkhalek

Department of Food Control and Hygiene, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt.

ABSTRACT

Objective: This work investigated the antimicrobial resistance (AMR) and virulence of *Escherichia coli* and *Staphylococcus aureus* in communally consumed cheeses in Egypt.

Materials and Methods: This study examined 100 samples of Domiat, Tallaga, Cheddar, and Ras cheese collected from several shops and supermarkets. Samples were spread on selective media to isolate bacterial strains. Molecular characterization of bacterial isolates was carried out using polymerase chain reaction to determine Shiga toxin 1 (stx1), Shiga toxin 2 (stx2), eaeA, and nuc genes. The isolates were tested for susceptibility to 14 antibiotics by disk diffusion assay.

Results: In this study, several *E. coli* serotypes were identified. *E. coli* O26:H11, O103:H2, and O111:H2 expressed stx1/2. *E. coli* O114:H4 expressed stx1, *E. coli* O17:H18, O21:H7 and O146:H21 expressed stx2, while only *E. coli* O26:H11 and O111:H2 expressed eaeA. The *E. coli* isolates were resistant to at least one antibiotic, while most isolates (82.4%) showed multidrug resistance (MDR). AMR to erythromycin was the highest (100%), followed by nalidixic acid (94.1%), cefotaxime (82.4%), vancomycin and cephalexin (64.7%), penicillin G (52.9%), sulfamethoxazole (47.1%), amikacin and kanamycin (35.3%), ampicillin (29.4%), tetracycline and ciprofloxacin (23.5%), and doxycycline (11.8%), while gentamicin showed the least resistance (5.9%). The multiple antibiotic resistance (MAR) index of the isolated *E. coli* ranged from 0.071 to 1 (mean = 0.478). All *S. aureus* isolates expressed the nuc gene and demonstrated resistance to at least one antibiotic, and 90% of isolates were MDR. AMR to kanamycin and cephalexin was the highest (100%), followed by penicillin (90%), doxycycline (70%), nalidixic acid and sulfamethoxazole (60%), erythromycin (50%), tetracycline, cefotaxime, and gentamicin (40%), ciprofloxacin and ampicillin (30%), and amikacin (20%). In comparison, vancomycin showed the least resistance (10%). MAR index of isolated *S. aureus* ranged from 0.143 to 1 (mean = 0.529).

Conclusion: The antimicrobial-resistant *E. coli* and *S. aureus* are potential risks for public health and may have a role in disseminating AMR to other pathogenic and non-pathogenic microbes.

Introduction

Currently, antimicrobial resistance (AMR) is a challenge that faces public health. It negatively influences the treatment of bacterial infections, resulting in increased death rates, morbidities, treatment costs, and decreased animals’ productivity [1]. In 2020 and beyond, AMR cannot be overlooked. At the global level, bacterial infections which are not effectively managed as a result of AMR influence approximately 700,000 individuals every year and probably result in 10 million deaths over 30 years, at the cost of US$100 trillion [2]. AMR, the silent worldwide pandemic, can worsen the coronavirus disease 19 (COVID-19) pandemic, which can exacerbate the AMR [2]. Data from five countries advocated that 6.9% of COVID-19-infected individuals had infections caused by bacteria (3.5% associated and 14.3% post-COVID-19) [3].

The antibiotics can be abused by healthcare personnel and the population resulting in a quick spreading of bacterial strains that resist antibiotics. Most bacterial strains that frequently result in infections in humans and animals have a high resistance degree to the first-line antibiotics [1].

Correspondence Nahed Gomaa Kasem nahedkasem92@gmail.com Department of Food Control and Hygiene, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt.

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The term AMR means the absence of response to a standard dose of an antibiotic. Bacterial strains show resistance to the antagonistic properties of antibiotic agents. They had former sensitivity, causing bacterial strains to survive despite using a standard dose of a particular antibiotic [4].

Multiple antibiotic resistance (MAR) index is an effective and cost-effective method for source tracking of bacterial strains having AMR. MAR index is the ratio between antibiotics’ number that a bacterial strain shows resistance to and the total antibiotics’ number the bacterial strain is exposed to. An MAR index exceeding 0.2 indicates an increased risk source of contamination where antibiotic agents are frequently used [5].

Milk and dairy products are rich sources of nutrients for humans worldwide. Various cheese types are formed worldwide. Cheeses are significantly consumed due to their high nutritional value [6]. The cheese quality is affected by equipment and environmental hygienic measures during manufacturing, packaging, and handling [7]. During cheese production, particularly during ripening, cheeses are exposed to unsterile environmental conditions where many opportunistic organisms, such as Staphylococci, Escherichia coli, and others, are reported [8].

Foodborne illness that might be associated with consuming cheese was reported in several regions worldwide. For instance, Honish et al. [9] concluded that E. coli caused a cheese-associated outbreak among 13 persons in Canada, resulting in two cases of hemolytic uremic syndrome. Additionally, and according to Delbes et al. [10], Staphylococcus aureus infection has been associated with the utilization of unpasteurized milk or with contamination related to unhygienic handling since these bacteria, when exceeding 5 Log colony-forming unit ml⁻¹, release heat-resistant enterotoxin.

Studies carried out in the last 10 years revealed both the likelihood of AMR transmission via food chains and the significance of the food-handling environment as a possible hot spot for AMR development and dissemination [11]. Thus, investigating AMR in humans and animals is significant for detecting altering resistance patterns, applying control measures on antimicrobials misuse, and avoiding the spread of multidrug-resistant pathogens [12]. This work was conducted to detect the occurrence and molecular identification of E. coli and S. aureus in some Egyptian cheeses and determine the AMR of the bacterial isolates.

**Material and Methods**

**Sample collection**

The current work included 100 samples of Domiati, Tallaga, Cheddar, and Ras cheese (25 each) collected from different supermarkets between July 2019 and May 2020 in Egypt. All the samples were stored in pre-sterilized aseptic plastic containers with caps and were preserved in an ice-box at 4°C till they reached the laboratory.

**Isolation of E. coli and S. aureus**

Based on the methodology described by Soomro et al. [13], E. coli were isolated. In brief, 25 g from every sample was mixed with 225 ml of buffered peptone water, and homogenization was carried out for 3 min. Then, 0.1 ml of the suitable dilutions of each sample was distributed onto MacConkey Agar plates (Oxoid, CM 0115) and incubated at 37°C for 24 h. Then, each plate was examined for the presence of viable E. coli. Five typical suspected colonies (round pink) were picked up for streaking onto MacConkey Agar. Incubation was carried out at 37°C for 24 h. To identify the E. coli, Gram-stain followed by microscopic examination and biochemical tests (indole, methyl-red, Voges–Proskauer, and citrate utilization) were carried out.

For the isolation of S. aureus, 0.1 ml of prepared dilutions of each sample was spread onto Baird–Parker plate and then distributed by surface plating method till complete absorption [14]. The plates were incubated at 37°C for 1–2 days and evaluated for S. aureus colonies.

**Serological identification of E. coli serotypes**

Serotyping of E. coli was carried out using E. coli antisera sets (DENKA SEIKEN Co., Tokyo, Japan) [15].

**Bacterial deoxyribonucleic acid (DNA) extraction**

DNA samples were extracted from the isolated bacteria using Fermentas GeneJET genomic DNA purification kit (Thermo Scientific, Australia), as stated by the manufacturer. DNA was preserved at −20°C till polymerase chain reaction (PCR) assay was carried out.

**Primers and multiplex PCR**

The multiplex PCR was utilized to determine Shiga toxin 1 (stx1), Shiga toxin 2 (stx2), and eaeA in 17 E. coli isolates using the primers (Pharmacia Biotech) mentioned in Table 1. The procedure was carried out according to Paton and Paton [16]. A thermal cycler (Hamburg, Germany) was used to amplify 20 ng of DNA, and amplification was carried out

| Gene | Primer (5′ → 3′) | Size | References |
|------|-----------------|------|------------|
| stx1-F | 5′-ATAATCGCATGCTGTTGACTAC-3′ | 180 bp | [16] |
| stx1-R | 5′-AGAACGCACCGTAGATCAT-3′ | | |
| stx2-F | 5′-GCCACGTCGAACTCCTG-3′ | 255 bp | |
| stx2-R | 5′-CGGATTATTTCAGACCTTG-3′ | | |
| eaeA-F | 5′-GACCGCGCAACAAGTAAGC-3′ | 384 bp | [15] |
| eaeA-R | 5′-CCACCTTGCGAAACAGGG-3′ | | |

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in 25 µl of buffer solution, which contained 3 µM of oligonucleotides, 200 µM of each deoxynucleoside triphosphate (dNTP), 3.5 mM magnesium chloride, and 2.5 U of DNA Taq polymerase. A total of 35 cycles of PCR were carried out. In every cycle (for the initial 10 cycles), DNA was denatured at 95°C for 1 min, annealed at 65°C for 2 min, denatured to 60°C at cycle number 15, elongated at 72°C for 90 sec, and incremented for 2.5 min from cycle 25 to cycle 35. The entire PCR amplification products were separated on 1.5% agarose gel and were stained using ethidium bromide to visualize using an ultraviolet light transilluminator.

The primers of nuc utilized for the detection and identification of S. aureus are shown in Table 2. The procedure was carried out according to the method described by Chu et al. [17]. The amplification was carried out on the thermal cycler utilizing 25 μl of PCR mix that contained 3 μl of boiled cell lysate, 200 µM of dNTP, 1.4 U of Taq DNA polymerase (Biotools, Spain), buffer (20 mM Tris-hydrochloride pH 8.4, 50 mM potassium chloride and 3 mM magnesium chloride), and 20 µM of each primer (nuc). The amplification program included denaturation for 5 min at 94°C. Denaturation was carried out for 25 cycles at 94°C for 45 sec, followed by annealing at 55°C for another 45 sec, and eventually extension at 72°C for 10 min.

**Antimicrobial susceptibility of E. coli and S. aureus**

This was carried out using Mueller Hinton agar-based agar disk-diffusion testing. Various concentrations of sensitivity disks (Oxoid Limited, Basingstoke, Hampshire, United Kingdom) were used. Antibiotic classes comprised tetracycline (tetracycline, doxycycline), penicillin (ampicillin, penicillin G), macrolide (erythromycin), sulfonamide (sulfamethoxazole), cephalosporin (ceftazidime, cephalothin), aminoglycoside (kanamycin, amikacin, and gentamicin), fluoroquinolones (nalidixic acid, ciprofloxacin), and glycopeptide (vancomycin) (Table 3). Inhibition zones on plates were measured depending on the Clinical and Laboratory Standard Institute’s guidelines [18]. Multiple drug resistance was reported as resistance to ≥3 antibiotics [19].

**Determination of MAR index**

MAR index was calculated as follows: Number of antimicrobials showing resistance divided by the number of utilized antimicrobials [5].

**Results and Discussion**

Cheeses are widely consumed dairy products in Egypt. It supplies protein, fat, vitamins, and minerals to the consumer. However, the cheese might be contaminated during its manufacture, distribution, and/or storage [20]. Due to their unique composition and properties, these may act as rich growth media for pathogens. *Staphylococcus aureus* and *E. coli* are the most commonly occurring potential microbes in the milk or dairy industry. They are thus the major bacteriological hazards associated with milk and cheese consumption [21].

The current work identified *E. coli* in 86.6% of Tallaga samples, 85.7% of Domiati samples, 52.1% of Cheddar samples, and 38.8% of Ras cheese samples (Table 4). Soft cheeses were highly contaminated with *E. coli* than hard cheeses (Ras cheese), which might be due to the high moisture of soft cheese than that of hard cheese and its

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**Table 2.** Primers utilized to identify *S. aureus* gene.

| Gene  | Primer (5’→3’)               | Size | References |
|-------|------------------------------|------|------------|
| nuc-F | 5’-GCGATGATGGTGATACGGTT-3’   | 270 bp | [37]       |
| nuc-R | 5’-AGCCAAGCTGGACGAATACACC-3’ |      |            |

**Table 3.** Antibiotic disks, concentrations, and action on pathogens.

| Antibiotic        | Sensitivity disc content (ug) | Resistant (mm) | Intermediate (mm) | Susceptible (mm) |
|-------------------|------------------------------|---------------|-----------------|-----------------|
| Amikacin          | 30                           | ≤12           | 13–15           | ≥16             |
| Penicillin G      | 10 IU                        | ≤20           | 21–28           | ≥29             |
| Gentamicin        | 10                           | ≤12           | 13–14           | ≥15             |
| Doxycycline       | 30                           | ≤14           | 15–18           | ≥19             |
| Kanamycin         | 30                           | ≤13           | 14–17           | ≥18             |
| Vancomycin        | 15                           | ≤15           | 16–21           | ≥22             |
| Nalidixic acid    | 30                           | ≤13           | 14–18           | ≥19             |
| Ciprofloxacin     | 5                            | ≤15           | 15–19           | ≥20             |
| Tetracycline      | 30                           | ≤14           | 15–18           | ≥19             |
| Erythromycin      | 15                           | ≤13           | 14–22           | ≥23             |
| Cefotaxime        | 30                           | ≤17           | 18–22           | ≥23             |
| Ampicillin        | 10                           | ≤13           | 14–17           | ≥18             |
| Cephalothin       | 30                           | ≤14           | 15–17           | ≥18             |
| Sulphamethoxazole | 25                           | ≤10           | 11–15           | ≥16             |
shorter shelf-life due to bacterial spoilage. It was demonstrated that most soft and unripened cheeses are bacteriologically unstable because of the metabolic activities of bacterial strains [22]. It should be noted that according to Egyptian Standard (2005), cheese must be free from E. coli [23]. Accordingly, the four types of cheeses used in this study did not fulfill the Egyptian standards. Regarding the incidence of S. aureus in cheeses, 60% of Tallaga samples, 48% of Domiati samples, 48% cheddar samples, and 72% of Ras cheese samples were associated with S. aureus (Table 5).

A notable difference in prevalence was found between the results of this study and previous reports. Differences in preparation procedures, storage, type of cheese, and whether milk was raw or pasteurized might be responsible for such discrepancies. In addition, this is probably because of the unhygienic measures taken where cheeses are produced and workers involved in the process [24]. Al-Gamal et al. [25] evaluated Tallaga cheese, Ras cheese, Domiati cheese, and Feta cheeses in Egypt and reported that 26.6% had E. coli. In Iran, among 77 soft cheese samples, E. coli could be isolated in 76 (98.70%) samples, of which 15 (19.48%) isolates were Enteropathogenic E. coli (EPEC) [26]. Ombarak et al. [27] isolated E. coli in 22% of Ras cheese. In Egypt, Younis et al. [28] isolated E. coli and S. aureus in 56%, 88%, 68%, and 76% of Tallaga and Ras cheese samples, respectively. A study examined soft cheese samples in Brazil and reported that S. aureus was detected in 20% of samples, and EPEC was detected in about half of the total samples (49.1%) [29]. Abdel-Hameid Ahmed et al. [30] detected S. aureus in 14% of Domiati cheese. In Iranian research, authors detected S. aureus in 22.5% of 100 cheese samples [31]. Abulreesh and Organji [32] detected S. aureus in Ras cheese samples collected in Saudi Arabia.

In our study, 41% of the E. coli isolates were identified as EPEC (main pathotype), 29% as Enterohemorrhagic E. coli (EHEC), 24% as Enterotoxigenic E. coli (ETEC), and 6% as Enteroinvasive E. coli (EIEC) (Table 6). Consistent with our findings, a study in Iraq revealed that 40.5% of cheese samples showed contamination with EPEC [33].

EPEC strain was detected as O146: H21, O17: H18, O119: H6, and O114: H4. EHEC strain was detected as O26: H11, O111: H2, O103: H2, and O121: H7. ETEC strain was detected as O128: H2. EIEC strain was detected as O159. The results indicated that O128: H2 was the most prevalent serotype, followed by O119: H6 (Table 7). E. coli isolation is a major public health concern as some strains belong to enteropathogenic or toxigenic or both types [34].

The incidence of E. coli in cheeses might be related to fecal contamination or unhygienic measures in the cheese manufacturing process [35]. Many E. coli strains might result in gastrointestinal illness in humans. Among them are O157, O26, O103, O111, O145, O45, O55, O91, O113, O121, and O128 serotypes [36]. To overcome this problem, milk pasteurization is recommended during cheese production, as supported by the Egyptian Organization for Standardization and Quality Control.

The expression of stx1, stx2, and eaeA by E. coli was examined by the multiplex-PCR (Fig. 1). The results revealed that 12 (70.58%), 10 (58.82%), and 3 (17.6%) of E. coli isolates contain stx1, stx2, and eaeA singly, respectively (Table 5). Also, only 2 E. coli serovars that expressed eaeA gene were O26: H11, and O111: H2; both contained all the three virulence genes. On the other hand, serovars O17: H18, O121: H7, O146: H21, and O159 did not express stx1 gene, while O114: H4, O128: H2, and O159 did not express stx2 gene. By comparison, El-Badry and Raslan

### Table 4. Incidence of Enterobacteriaceae and E. coli in cheese samples.

| Cheese type | Total samples | Positive samples (%) | Total isolates | E. coli (%) |
|-------------|---------------|----------------------|----------------|------------|
| Tallaga     | 25            | 12 (48%)             | 30             | 26 (86.6%) |
| Domiati     | 25            | 8 (32%)              | 14             | 12 (85.7%) |
| Cheddar     | 25            | 9 (36%)              | 23             | 12 (52.1%) |
| Ras         | 25            | 18 (72%)             | 54             | 26 (48.1%) |

| Cheese type | Total samples | Positive samples (%) |
|-------------|---------------|----------------------|
| Tallaga     | 25            | 15 (60%)             |
| Domiati     | 25            | 12 (48%)             |
| Cheddar     | 25            | 12 (48%)             |
| Ras         | 25            | 18 (72%)             |

### Table 5. Incidence of S. aureus in cheese samples.

| Cheese type | Total samples | Positive samples (%) |
|-------------|---------------|----------------------|
| Tallaga     | 25            | 15 (60%)             |
| Domiati     | 25            | 12 (48%)             |
| Cheddar     | 25            | 12 (48%)             |
| Ras         | 25            | 18 (72%)             |

### Table 6. Serological characterization of E. coli isolates (n = 17).

| Strain   | No. (%) of isolates | Identified serotypes |
|----------|---------------------|----------------------|
| EPEC     | 7 (41%)             | O146:H21, O17:H18, O119:H6, O119:H6, O146:H21, O119:H6, O114:H4 |
| EHEC     | 5 (29%)             | O121:H7, O26:H11, O103:H2, O111:H2, O26:H11 |
| ETEC     | 4 (24%)             | O128:H2 |
| EIEC     | 1 (6%)              | O159 |

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study [20] reported that O127:H6 strain expressed stx1 and stx2 genes, whereas O111:H2 strain expressed stx1 only. O124: H and O55:H7 strains only expressed stx2. Besides, Fadel and Ismail [37] in Egypt detected several E. coli serovars in Ras and Kareish cheeses, which included O15:H11, O22:H5, O25:NM, O26:H11, O86:H34, O91:H10, O113:H21, O114:H2, O119:H6, O124:H7, O128:H2, O127: NM, and O145: NM. Moreover, El Bagoury et al. [38] isolated O26:H11, O111:H2, O124, O163:H2, O114, O125:H21, and O1, O15, along with a non-typed serotype in cheese samples (Tallaga, Karish, and Domiati).

Regarding the molecular characterization of S. aureus in this study, PCR was used to recognize the nuc gene in S. aureus isolates (n = 10). As shown in Figure 2, all S. aureus isolates (100%) expressed the nuc gene. Considering the findings of Brakstad et al. [39], in comparison with our study, it can be stated that PCR for nuc gene amplification has the potential for quick diagnosis and confirmation of S. aureus isolates.

The AMR patterns of E. coli are shown in Figure 3. All isolates had AMR to at least one antibiotic, while 82.4% of them showed multidrug resistance (MDR) (MAR index above 0.2) (Tables 8 and 9). Elafify et al. [40] found a near similar result, and reported that 86.11% of E. coli isolates in Egyptian cheeses were MDR. Other studies detected MDR E. coli with various ratios. For instance, in Egypt, Ombarak

Table 7. Occurrence of virulence genes of E. coli isolates (n = 17) in cheese samples.

| Serotype | No. (% of isolates) | Stx1 | Stx2 | Intimin gene (eaeA) |
|----------|---------------------|------|------|---------------------|
|          | No. | %   | No. | %   | No. | %   |
| O17: H18 | 1   | 5.8%| 0   | 0   | 1   | 100 | 0   | 0   |
| O26: H11 | 2   | 11.76%| 2   | 100 | 2   | 100 | 2   | 100 |
| O103: H2 | 1   | 5.8%| 1   | 100 | 1   | 100 | 0   | 0   |
| O111: H2 | 1   | 5.8%| 1   | 100 | 1   | 100 | 1   | 100 |
| O114: H4 | 1   | 5.8%| 1   | 100 | 0   | 0   | 0   | 0   |
| O119: H6 | 3   | 17.6%| 3   | 100 | 2   | 66.7| 0   | 0   |
| O121: H7 | 1   | 5.8%| 0   | 0   | 1   | 100 | 0   | 0   |
| O128: H2 | 4   | 23.5%| 4   | 100 | 0   | 0   | 0   | 0   |
| O146: H21| 2   | 11.7%| 0   | 0   | 2   | 100 | 0   | 0   |
| O159     | 1   | 5.8%| 0   | 0   | 0   | 0   | 0   | 0   |
| **No. (%)** | **17** | **12** | **70.58%** | **10** | **58.82%** | **3** | **17.6%** |
et al. [41] stated that half of the *E. coli* isolated from Karish and Ras cheeses were MDR. In Ethiopia, Bedasa et al. [42] recorded a higher MDR of *E. coli* isolates (92.5%) in comparison with our results. These differences among MDR *E. coli* might be associated with dissimilarities in antimicrobials used at the regional level.

Our study showed that AMR and MDR are prevalent in *E. coli* isolated from cheese samples. AMR to erythromycin was the highest (100%), followed by nalidixic acid (94.1%), cefotaxime (82.4%), vancomycin (64.7%), cephalothin (64.7%), penicillin G (52.9%), sulfamethoxazole (47.1%), amikacin (35.3%), kanamycin (35.3%), ampicillin (29.4%), tetracycline (23.5%), ciprofloxacin (23.5%), doxycycline (11.8%), and gentamicin (5.9%). The MAR index ranged from 0.071 to 1 (average 0.478). Compared to other techniques like genotypic characterization, the MAR index is cost-effective, quick, and reliable. Besides, it is simple and not necessitating specific skills or costly equipment [5]. The detection of resistant *E. coli* is critical since this can increase bacteria that can resist antibiotic drugs [43].

**Figure 2.** PCR of *nuc* (270 bp) aimed at *S. aureus* identification. Lane-M: 100 bp DNA ladder; Lane-1: positive control; Lane-2: negative control; Lanes 1–10: positive for *nuc* gene.

**Figure 3.** Antibiotic susceptibility of isolated *E. coli*. 

[Graph showing antibiotic susceptibility results for *E. coli*.]
In harmony with our findings, El Bagoury et al. [38] analyzed the antibiotic susceptibility of some isolated \textit{E. coli}. They reported that \textit{E. coli} is mainly resistant to erythromycin (100%), and it was most susceptible to gentamicin (77.8%). Sulfamethoxazole and oxytetracycline demonstrated intermediate susceptibility at percentages of 55.6% and 44.4%, respectively. Also, they revealed that most \textit{E. coli} strains showing resistance were O26:H11, while \textit{E. coli} O15 was resistant to erythromycin only.

On the contrary, Rahimi et al. [44] revealed \textit{E. coli} resistant to ampicillin (44.4%), gentamycin (44.4%), erythromycin (33.3%), amoxicillin (1.1%), nalidixic acid (1.1%), and tetracycline (1.1%). Besides, Gundogan and Avci [45] found \textit{E. coli} resistant to ampicillin (90.5%) and penicillin (82.1%). Also, they reported that the AMR was 58.4% for erythromycin, 53.7% for gentamicin, 44.2% for trimethoprim/sulfamethoxazole, and 29.4% for chloramphenicol.

The greatest MAR index for \textit{E. coli} isolates was 1 (for O128: H2) (Table 6). This indicates the high resistance of \textit{E. coli} bacteria in Egyptian cheeses. In most developing countries, like Egypt, the low cost and the wide availability of such antibiotic drugs are the primary reasons for their high utilization in treating diseases, predominantly diarrhea [46]. MDR strains can directly infect humans from the food chain or through animal contact or indirectly from environmental sources [47]. In recent times, there is a considerable increase in foodborne pathogens showing resistance to many antibiotics and as a result of extensive antibiotics’ usage in farming and through food chains which are known AMR sources [48].

The findings also demonstrated that all isolated \textit{S. aureus} had AMR to at least one antibiotic. AMR to kanamycin and cephalothin was the highest (100%), followed by penicillin (90%), doxycycline (70%), nalidixic acid (60%), sulfamethoxazole (60%), erythromycin (50%), tetracycline (40%), cefotaxime (40%), gentamicin (40%), ciprofloxacin (30%), ampicillin (30%), and amikacin (20%). In comparison, the least resistance was found to vancomycin (10%) (Fig. 4). MAR index ranged from 0.143 to 1 (mean = 0.529) (Tables 10 and 11). This came in agreement with
Arslan and Oezdemir [49], who conducted their studies on a total of 135 cheese samples. They demonstrated that S. aureus isolates had resistance to ≥1 antimicrobial agent; the greatest AMR was found to ampicillin (41%), penicillin (40.1%), and tetracycline (38.7%). On the other hand, and according to Can et al. [50], all S. aureus isolates showed susceptibility to gentamicin, oxacillin, and vancomycin. The greatest resistance was found to penicillin and ampicillin (95% and 92.5%, respectively), followed by tetracycline (30%), erythromycin (20%), and ciprofloxacin (12.5%).

MDR of S. aureus isolates was 90% in this study. By comparison, several other studies reported varying percentages of MDR. For instance, in Turkey, Kayili and Sanlibaba [51] reported MDR in 72.94% of S. aureus isolates. Also, MDR was 61.1% in China [52] and 66.67% in USA [53].

**Conclusion**

The results reveal that Tallaga, Domiati, Cheddar, and Ras cheeses in Egyptian markets show high contamination...
with <i>S. aureus</i> and <i>E. coli</i>. The existence of MDR bacteria is worrying since these bacteria may threaten public health. Thus, periodical evaluation of dairy products for ensuring consumer safety should be practiced. Good manufacturing practices and strict personal hygiene measures are mandatory for ensuring the safety and high quality of dairy products. Further studies are essential to be conducted to evaluate whether these strict hygiene measures are applied or not to protect human health, particularly during the current COVID-19 pandemic situation.

**List of Abbreviations**

AMR, antimicrobial resistance; DNA, deoxyribonucleic acid; dNTP, deoxynucleoside triphosphate; EHEC, enterohemorrhagic <i>E. coli</i>; EIEC, Enteroinvasive <i>E. coli</i>; EPEC, Enteropathogenic <i>E. coli</i>; ETEC, enterotoxigenic <i>E. coli</i>; PCR, polymerase chain reaction; stx1, Shiga toxin 1; stx2, Shiga toxin 2.

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Nothing to disclose

**Conflict of interests**

The authors declare that they have no conflict of interests.

**Authors’ contributions**

NGK and MAA were responsible for carrying out laboratory work, statistical analysis, and research writing, while AA and ME supervised the work and revised the research article.

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