



## Assessment of the Chemical and Microbiological Quality of Some Cheese Assortment in Egypt: Highlighting the incidence of multidrug-resistant *Staphylococcus* Species

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### ABSTRACT

Hard and semi-hard cheeses are popular dairy products in Egypt, but they may convey a health risk for consumers due to their poor hygienic quality and safety. One of the common problems that consumers face when buying these cheeses from the Egyptian market is the bad odour that shortly develops after purchase even though they are refrigerated. This problem may indicate a high bacterial count in the cheese or the presence of undesirable microorganisms. Therefore, the current study aimed to assess the hygienic quality and safety of some retailed cheese sold in Egyptian markets. One hundred hard and semi-hard cheese samples locally manufactured (including 34 Ras, 33 Cheddar, and 33 Gouda) were analyzed for their microbiological and chemical quality. The results indicated that the Ras cheese samples had the highest bacterial counts, salt, and fat content, while the Cheddar cheese samples had the highest acidity, moisture content and the lowest microbial load. The Gouda cheese samples had the lowest fat/total solids ratio and a variable bacterial count. Forty-five *Staphylococcus aureus* strains were isolated from the cheese samples; 25 of them were coagulase-positive. The antibiotic susceptibility of twenty isolates was phenotypically and genotypically evaluated. All isolates were resistant to Cefixime, Oxacillin, and Metronidazole, while sensitive to Amoxicillin/Clavulanic Acid and Linezolid. The resistance to other antibiotics varied among the isolates. The antibiotic resistance genes were detected by polymerase chain reaction (PCR). The obtained results indicate that the hard and semi-hard cheeses sold in many markets were poor in hygienic quality and safety and posed a health risk for consumers due to the presence of multidrug-resistant *Staphylococcus* species. Ras cheese was the worst cheese type in most quality and safety parameters.

**Keywords:** Antibiotic resistance genes, Cheddar, Gouda, Ras, *Staphylococcus*.

### Original Article:

DOI:<https://dx.doi.org/10.21608/javs.2023.243014.1285>

Received : 16 October, 2023.

Accepted : 03 December, 2023.

Published in January, 2024.

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*J. Appl. Vet. Sci.*, 9(1) : 85-93.

### INTRODUCTION

Cheese is a dairy product that results from the coagulation of milk proteins and fats. It has a long history and a diverse range of types, each with its own features and characteristics. Cheese can have different textures, flavour and aromas depending on production methods and ripening conditions. Cheese production and consumption in Egypt have ancient origins (Todaro *et al.*, 2013). Egyptian cheese is considered truly important for the nutritional, economic and health aspects. One of the top

manufacturers and consumers of cheese in Africa and the Middle East is Egypt (Owusu-Apenten *et al.*, 2022). Some of the most common types of cheese in Egypt are Ras cheese (which is mostly named Romy cheese), Gouda cheese, and Cheddar cheese (Helal and Tagliazucchi, 2023).

In Egypt, Ras is usually produced from raw milk, so there are different groups of microorganisms in this kind of cheese; some of them are beneficial, giving cheese characteristic flavour and body texture, while others are harmful and/or spoilage

microorganisms (Salama et al., 2021). Furthermore, it may be stored uncovered in unprotected rooms under humid and unregulated sanitary conditions. This promotes the growth of mould and yeast. The traditional Ras cheese does not meet the Egyptian standard because of its high fungal growth and other undesirable microorganisms. This affects its quality, ripening, storage and marketing (Amer et al., 2023).

Raw milk has diverse and dynamic microflora that may affect its quality and safety (Ali et al., 2021). The microbial communities of cheese can include starter cultures, non-starter lactic acid bacteria (NSLAB), yeasts, moulds, pathogenic bacteria and spoilage bacteria (Quigley et al., 2013). The presence of these microorganisms directly influences the sensory characteristics, nutritional composition and health benefits of cheese. Additionally, they can lead to spoilage or contamination, resulting in defects in the final product (D'amico and Donnelly, 2010). Some of them can also produce heat-resistant toxins that cause food poisoning, such as *staphylococcal* enterotoxins (Le Loir et al., 2003). Moreover, some bacteria can acquire antibiotic resistance genes (Hegab et al., 2020). This characteristic makes them more challenging to manage and poses greater risks to public health.

In light of these concerns, it is important to evaluate the quality and safety of some popular cheese varieties in the Egyptian market, such as Ras, Cheddar and Gouda. Moreover, the antimicrobial resistance of the isolated staphylococcus strains was tested. It was also discussed the possible sources of contamination and the suggested recommendations for improving cheese production and handling practices. This study contributes to the existing literature on cheese quality and safety in Egypt by providing updated data and insights on some popular cheese varieties.

## MATERIALS AND METHODS

### 1. Samples collection

A total of one hundred locally manufactured Ras, Gouda and cheddar cheese samples (34 Ras, 33 Gouda, and 33 Cheddar) (250 g each) were obtained randomly from Cairo and Giza markets. The samples were identified, labelled and promptly transported in an insulated ice box to be analyzed immediately.

### 2. Chemical analysis

The moisture, titratable acidity and fat% were determined according to AOAC (2000), while salt% was estimated according to APHA (2004).

### 3. Microbiological examination

Eleven grams of representative cheese samples were placed in a sterile stomacher bag that

had been preheated to (40–45) °C and contained 99 ml of 2% sodium citrate dilution buffer. To achieve the homogenate (1/10 dilution), the mixture was homogenized in a stomacher (Labstomacher, 400) for 2 minutes. Then, one milliliter of the preceding homogenate was mixed with nine milliliters of sterile 0.1% peptone water.

### 3.1. Total psychrotrophic bacterial count: (APHA, 2004)

Plate count agar was used in duplicate plates with one milliliter of the previously produced dilution. Then the plates were incubated 10 days at 7 °C followed the inoculation.

### 3.2. Total Lipolytic bacterial count: (APHA, 2004)

0.1 ml of the previously made serial dilutions were used to inoculate duplicate Spirit blue agar plates, and the plates were then incubated at 32 °C for 48 hours. Around and underneath each colony, lipolytic bacteria produce a clear zone and/or a deep blue color.

### 3.3. Total Proteolytic bacterial count: (APHA, 2004)

0.1 ml of the previously made serial dilutions were used to inoculate duplicate plates of standard caseinate agar, and the inoculated plates were incubated at 32 ±1 °C for 48–72 hours. Proteolytic colonies are those are encircled by a white or off-white zone of para-casein precipitate.

### 3.4. Staphylococci count: (APHA, 2004)

A Baird Parker agar plate was inoculated with one ml of each of the prepared serial dilutions, which was then incubated at 37°C for 48 hours. The overall staphylococcal count per gram was estimated after counting the suspected colonies (shiny black colonies). *S. aureus* strains were molecularly characterized and enterotoxin genes were found. A total of six *S. aureus* genes (five enterotoxins and one 23S rRNA) and six pairs of PCR reactions (five multiplex and one uniplex) were used.

### 3.5. Total Yeast count and Mold count

Total fungal count was estimated according to the method described by (APHA, 2004), Inoculated duplicate plates of Malt Extract Agar were incubated at 25 °C for 5 days. The yeast and mold count per gram was reported.

### 4. Antibiotic susceptibility testing (AST) of selected *S. aureus* strains

#### 4.1. Phenotypic analysis (Disc Diffusion Assay)

All the Staphylococcus strains (20 isolates) were evaluated for antibiotic susceptibility to fourteen different antibiotics. (Oxoid, UK): amoxicillin/clavulanic (30 µg/disk), linezolid (30

µg/disk), levofloxacin (5 µg/disk), ciprofloxacin (5 µg/disk), gentamicin (10 µg/disk), clindamycin (15 µg/disk), streptomycin (10 µg/disk), chloramphenicol (30 µg/disk), azithromycin (15 µg/disk), amoxicillin (25 µg/disk), cefixime (5 µg/disk), oxacillin (5 µg/disk), metronidazole (5 µg/disk) and vancomycin (30 µg/disk) discs. Using the typical disk diffusion technique on Mueller-Hinton Agar (Oxoid, UK), findings were classified as "Susceptible," "intermediate," or "resistant" based on the size of the inhibition zone in accordance with CLSI (Clinical and Laboratory Standards Institute) recommendations ,2013 (Berhilevych *et al.*, 2017).

#### 4.2.Genotypic analysis (Identification of antibiotic resistant genes)

According to Darwish and Asfour (2013), a quick boiling process was utilized to produce crude DNA from bacterial strains. The Staphylococcus isolates were taken off the mannitol salt agar plate and suspended in 200 µl of lysis buffer (1% Triton X-100, 0.5% Tween 20, 10 mM Tris-HCl (pH 8.0), and 1 mM EDTA). To remove bacterial debris, the solution was centrifuged for 5 minutes after boiling for 10 minutes. 5 µl of the supernatant was immediately utilized for PCR amplification after being aspirated.

By amplification of the existing gene, selected strains were tested for the presence of the following AR genes: blaZ, mecA, aacA-aphD, erm (ermA, ermC), tetM, and vanB. Table 1 shows the primers for the various genes. All PCR reactions were carried out in a 20-µl reaction volume containing 5 µl of template DNA, 20 picomol of each primer, and 1X of PCR master mix (Dream Taq Green PCR Master Mix, Fermentas Life Science). Initial denaturation was performed at 94°C for 4 minutes, followed by 35 cycles of 94°C for 60 seconds, 55°C for 60 seconds, and 72°C for 60 seconds. A final extension step of 10 minutes at 72°C was completed. Amplification products were electrophoresed at 70 volts for 70 minutes in a 1.5% agarose gel containing 0.5X TBE and examined under ultraviolet light.

#### Statistical analysis

The statistical analysis for three independent experiments was conducted using IBM SPSS Statistics (version 27.0) for Windows. The results were presented as mean ± SE. A one-way analysis of variance (ANOVA) was employed to analyze the differing outcomes. Multiple comparisons of the means for all tested parameters were performed using Post Hoc analysis, specifically the least square difference test (LSD). Significance was determined at the P < 0.05 level.

Table 1: primers used in the genotypic analysis

	Primer name	Target gene	Associated with resistance	Primer sequence 5'-3'	PCR product size	References
mPCR I	16srRNA F 16srRNA R	16S rDNA	Staphylococcus Spp	GTA GGT GGC AAG CGTTAT CC CGC ACA TCA GCG TCA G	228	Monday and Bohach, (1999)
	Nuc FNuc R	S. aureus-specific sequence	S. aureus-specific sequence	GCGATTGATGGT GATACGGTT AGCCAAGCCTTGACGAACTAAAGC	279	Asfour and Darwish, (2014)
mPCR II	BlaZ F BlaZ R	blaZ gene	β-Lactamase p.staphylococci	AAG AGA TTT GCC TAT GCT TC GCT TGA CCA CTT TTA TCA GC	517	Vesterholm-Nielsen <i>et al.</i> , (1999)
	mecAF mecA R	mecA gene	mecithilin resistant staphylococci	GTG AAG ATA TAC CAA GTG ATT ATG CGC TAT AGA TTG AAA GGA T	147	(Zhang <i>et al.</i> , 2005)
mPCR III	vatB F vatB R	van(B)	vancomycin resistant staphylococci	GCT GCG AAT TCA GTT GTT ACA CTG ACC AAT CCC ACC ATT TTA	136	Strommenger <i>et al.</i> , (2003)
	ermA F ermA R	erm(A)	erythromycin resistant staphylococci	AAG CGG TAA ACC CCT CTG A TTC GCA AAT CCC TTC TCA AC	190	(Strommenger <i>et al.</i> , 2003)
mPCR IV	ermC F ermC R	erm(C)	erythromycin resistant staphylococci	AAT CGT CAA TTC CTG CAT GT TAA TCG TGG AAT ACG GGT TTG	299	Strommenger <i>et al.</i> , (2003)
	Saaac F Saaac R	aacA-aphD	gentamycin resistant staphylococci	TAA TCC AAG AGC AAT AAG GGC GCC ACA CTA TCA TAA CCA CTA	227	Strommenger <i>et al.</i> , (2003)
	Tet F Tet R	tet(M)	tetracycline resistant staphylococci	AGT GGA GCG ATT ACA GAA CAT ATG TCC TGG CGT GTC TA	158	Strommenger <i>et al.</i> , (2003)

**RESULTS**

**Physicochemical parameters**

The results presented in Table 2 showed the mean ± SE for the analyzed physicochemical parameters in the three types of cheeses under investigation: Ras, Cheddar, and Gouda. There is a significant difference (P value less than 0.05%) between the means of salt% in all cheese types; moreover, there is a significant difference in the means of fat% between Ras and Gouda at a P value < 0.05. There is a significant difference in means of moisture% between Ras and the other two types (Cheddar and Gouda) at a P value < 0.05, but no significant differences between Gouda and cheddar. Moreover, there is a significant difference in the mean fat percentage between Ras and Gouda. Interestingly, there is a significant difference between the means of salt% in all cheese types. The acidity levels were fairly similar among the three cheese types. Ras cheese has the highest salt content (2.82 ± 0.87 g/100 g cheese) and the lowest acidity (1.50 ± 0.50%), in contrast to cheddar cheese samples which recorded the lowest salt content (1.51 ± 0.60 g/100 g cheese) and the highest acidity (1.73 ± 0.54%). Gouda cheese was in between (2.14 ± 0.60 g/100 g cheese for salt and 1.65 ± 0.52% for acidity).

Regarding the moisture contents, Ras cheese had the least (25.46% ± 1.48), while cheddar recorded the highest percent (29.78% ± 1.49). Ras cheese also presented the highest fat percentage among the tested samples (43.79% ± 10.99), while Gouda had the lowest fat content (36.74% ± 5.32).

Table 2: Statistical analytical results of physicochemical parameters in the examined cheese samples

Cheese type No. of samples	Ras	Cheddar	Gouda
	34	33	33
Mean salt (%) ± S.E.M.	2.82 ± 0.87 <sup>*a</sup>	1.51 ± 0.60 <sup>*b</sup>	2.14 ± 0.60 <sup>*c</sup>
Mean acidity (%) ± S.E.M.	1.50 ± 0.50 <sup>*a</sup>	1.73 ± 0.54 <sup>*a</sup>	1.65 ± 0.52 <sup>*a</sup>
Mean moisture (%) ± S.E.M.	25.46 ± 1.48 <sup>*a</sup>	29.78 ± 1.49 <sup>*b</sup>	28.48 ± 1.48 <sup>*b</sup>
Mean fat (%) ± S.E.M.	43.79 ± 10.99 <sup>*a</sup>	41.87 ± 10.69 <sup>*a</sup>	36.74 ± 5.32 <sup>*b</sup>
Mean fat/TS (%) ± S.E.M.	59.13 ± 15.17 <sup>*c</sup>	41.87 ± 10.69 <sup>*a</sup>	51.41 ± 13.38 <sup>*b</sup>

<sup>a,b,c</sup> The difference between values in the same raw that have different superscripts is significant (P < 0.05). The data reflect the mean of three independent readings with standard error of mean (SEM)

**The microbiological assessment**

Fig.1 demonstrates the microbiological assessment of the examined cheese samples (mean log/g cheese ± S.E). There was a significant difference in the means of proteolytic activity between Ras and Gouda, while a significant difference was clearly presented in the means of lipolytic, Staphylococcal, and yeast counts between Ras and the other two types (Cheddar and Gouda), but no significant differences in psychrophilic and mold counts in all cheese samples.

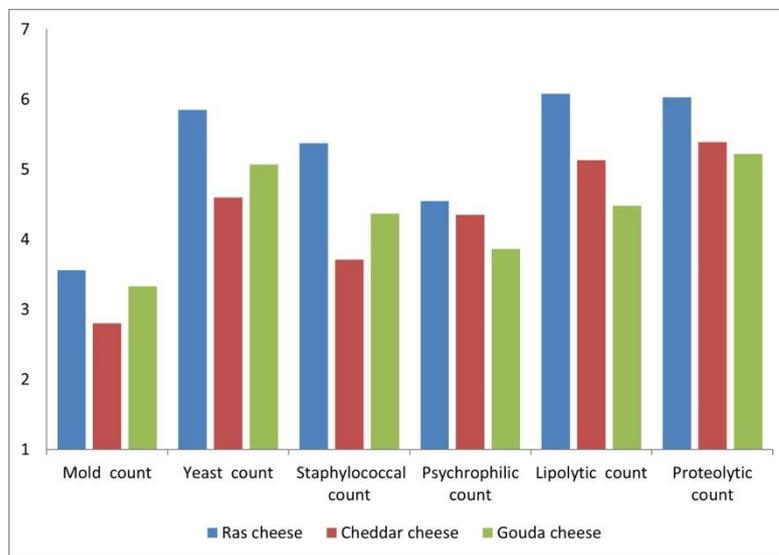


Fig. 1: Microbiological assessment of the examined cheese samples (Mean Log/g cheese Mean± S.E).

Table (3) presented the acceptability of cheese samples in accordance with Egyptian standards (**ES:1007-5/2005**) for Ras cheese, (**ES:1007-2/2020**) for Cheddar cheese and (**ES:1183-1/2020**) for Gouda cheese. Regarding Ras cheese samples, they all met the moisture content requirement; 82.35% were acceptable for fat/TS and 76.47% for coagulase-positive *Staphylococcus*. However, none of the Ras cheese samples complied with the acceptable total yeast criteria, while 20.59% met the total mould requirement. In contrast, all examined Cheddar cheese samples were compliant with the fat/TS percentage (100%), total solids (96.97%), and coagulase-positive *Staphylococcus* level (84.85%). Similarly, all Gouda cheese samples adhered to the total solids standard, while 78.88% was acceptable for Fat/TS and 84.85% for coagulase-positive *Staphylococcus*.

Table 3: Overall acceptability of the examined samples according to the Egyptian standards – 2005 for Ras & ES 2020 for Cheddar and Gouda cheese and the National Food Safety authority (NFSA) microbiological standards in food

Products	Fat/TS No (%)	Moisture No (%)	Total solids No (%)	Coagulase positive staph No (%)	Total yeast count No (%)	Total mold count No (%)
Ras cheese	28 (82.35%)	34(100%)	34(100%)	26 (76.47%)	0 (0%)	7 (20.59%)
Cheddar cheese	33 (100%)	32 (96.97%)	32 (96.97%)	28 (84.85%)	NM*	NM*
Gouda cheese	29 (78.88%)	33 (100%)	33 (100%)	28 (84.85%)		

\*: Not Mentioned

### Antimicrobial resistance (AMR)

Fig.2 showed the results of testing the susceptibility of *Staphylococcus* isolates from Ras, Cheddar, and Gouda cheeses to 14 antibiotics using the disk diffusion method. The results are expressed as the percentage of isolates that were susceptible (S), intermediate (I), or resistant (R) to each antibiotic.

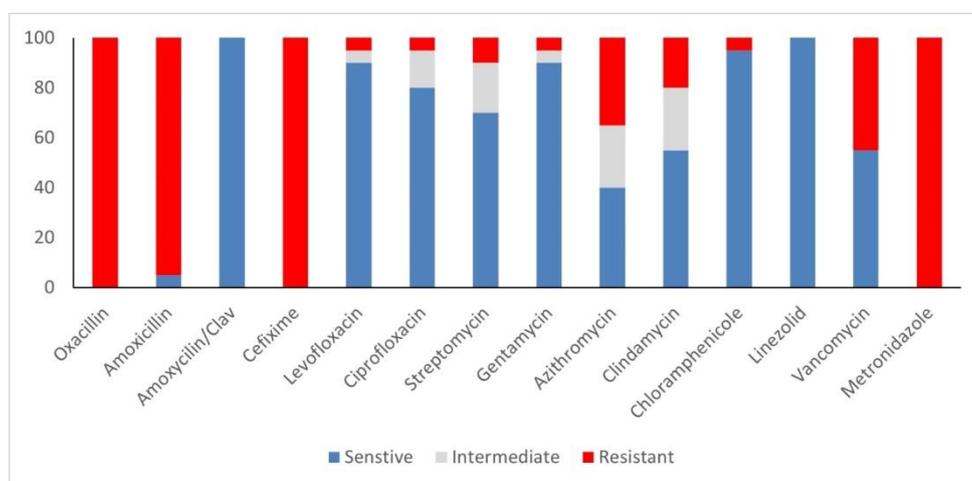


Fig. 2: Phenotypic antibiotic resistance of *Staphylococcus* isolates (n=20) isolated from cheese samples (9 Ras cheese, 6 Cheddar and 5 Gouda) using Disc Diffusion assay.

The results of the phenotypic antibiotic susceptibility test demonstrated that all isolates exhibited resistance to oxacillin, cefixime, and metronidazole. Conversely, all isolates exhibited susceptibility to amoxicillin-clavulanic acid, and linezolid. The sensitivity of the isolates varied with respect to other antibiotics, with 95% demonstrating sensitivity to amoxicillin, 45% to vancomycin, 35% to azithromycin, and 20% to clindamycin. Additionally, 5% of the isolates were sensitive to levofloxacin, ciprofloxacin, gentamycin, and chloramphenicol.

A genotypic investigation was performed using polymerase chain reaction (PCR) to confirm the phenotypic findings. Figure 3 reveals that the *blaz* gene was present in 80% of the samples. The *blaz* gene encodes the enzyme  $\beta$ -lactamase, which inactivates  $\beta$ -lactam antibiotics such as penicillins and cephalosporins. 60% of them tested positive for the *tetM* gene. Furthermore, the *aacA-aphD* gene was found in 55% of the isolates, whereas the *mec A* and *erm A* genes were found in 45%. Notably, neither the *erm C* nor *van B* genes were present in any of the isolates.

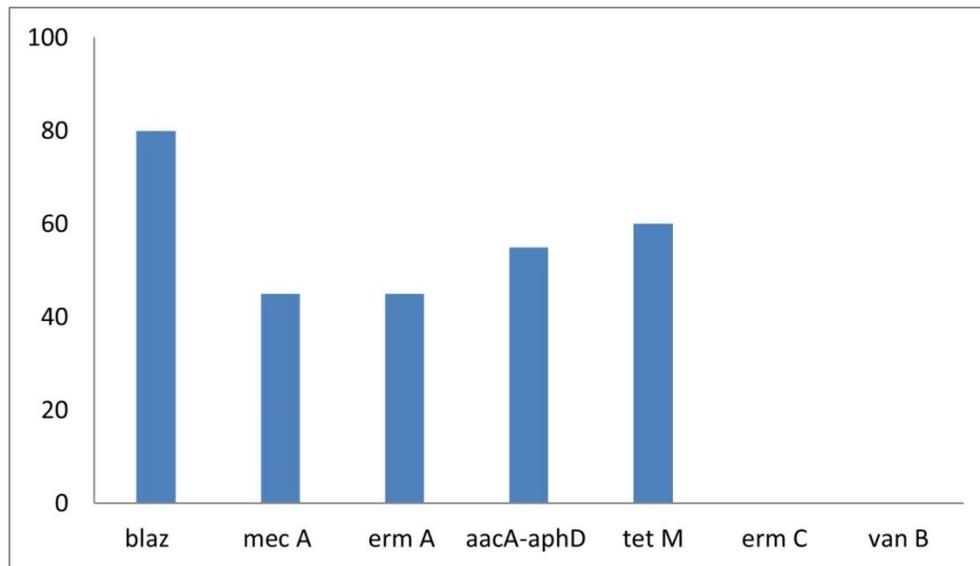


Fig. 3: Genotypic analysis for identification of antibiotic resistant genes using multiplex PCR.

## DISCUSSION

### Physicochemical parameters

The obtained data in Table 2 revealed that Ras cheese has the highest fat content (43.79%) and fat-to-total solids (TS) ratio (59.13%) among the three cheese types. Cheddar cheese recorded the lowest salt content (1.51%) and the highest moisture content (29.78%), while Gouda cheese recorded the lowest fat content and an intermediate level of salt and moisture. For Ras cheese, mean moisture and mean salt percents matched those of **Habliza et al., (2022)**, while the mean fat % was higher than the results of **Habliza et al., (2022)**. For Gouda cheese, the mean of the fat % matched the results recorded by **Sulieman et al., (2018)** who suggested that the high pressure technique during the manufacturing process affected the fat content. Salt is a regulatory agent to adjust the flavour, texture, water activity, and consequence the microbial growth. Salt mainly helps in decreasing the growth of some bacteria, such as lactic acid bacteria (**Fox et al., 2004**), however, some pathogenic microorganisms can tolerate the high salt content in cheese (such as Staphylococci) by adapting their behaviour to the osmotic stress (**Eissa, 2012**). Additionally, salt influences the activity of proteolytic and lipolytic enzymes generated by bacteria or rennet, which affects cheese quality and ripening (**Fox et al., 2017**). That may have contributed to the positive correlation between salt content and the microbiological parameters evaluated in our study.

### The microbiological assessment

The results mentioned in Figure 1 may indicate that there is a relationship between the chemical and microbial analysis of the cheese samples collected from the Egyptian market. We also

found a significant positive correlation between salt content in cheeses and *S. aureus*, yeast, and mould counts at the  $p < 0.05$  level. This supports the role of salt and acidity levels in determining the type and count of the microorganisms present in the cheese, as well as their enzymatic activity (**Guinee, 2004**). Salt also plays a major role as a hurdle with pH and water activity against microbial propagation. However, it may promote the growth of other microorganisms that can tolerate the high salt content (such as staphylococci and fungi) and create a selective advantage for salt-tolerant organisms over other competitors (**Medved'ová et al., 2012**).

It's important to control the levels of proteolytic bacteria in raw milk to prevent their impact on the flavour of cheese (**Paludetti et al., 2020**). It's suggested that the microbial quality of raw milk, poor hygiene and post-processing contaminants are the main reasons for the huge variance in the psychrotrophic and proteolytic counts in cheese (**Rodrigues et al., 2021**). Some sensorial and technological properties of milk and dairy products are related to the spoilage contaminant microbiota, which is mainly represented by psychrotrophic proteolytic bacteria producing thermoresistant enzymes (**Rodrigues et al., 2021**).

The results of this study showed higher mean values of microbial counts of proteolytic, lipolytic, psychrotrophic, yeast, and mould for Ras cheese than those recorded by **Hassan et al., (2019)** who recorded  $54.67 \pm 22.04$  cfu/g for total yeast and mould count,  $2.46 \times 10^6 \pm 4.50 \times 10^5$  cfu/g total psychrotrophic count,  $2.91 \times 10^5 \pm 4.13 \times 10^5$  cfu/g lipolytic count and  $5.85 \times 10^6 \pm 7.58 \times 10^5$  cfu/g for proteolytic count.

Contaminated samples are a clear indication of poor sanitary measures, beginning with the feeding of animals with mould and mycotoxins in contaminated feed, which leads to contaminated milk used in cheese processing, and continuing through poor hygiene practices during the manufacturing, handling, storage, and distribution of Ras cheese samples (Elramly *et al.*, 2019).

Ras cheese should be made from pasteurized milk or milk treated with heat comparable to pasteurization, according to the Egyptian Organization for Standardization and Quality Control (ES:1007-5/2005). It should also be free of pathogenic bacteria and their toxins, free of *E. coli*, with a total coliform count of no more than 10 cfu/g, a total mould count of no more than 10 cfu/g, and a total yeast count of no more than 100 cfu/g. (Ahlam *et al.*, 2014).

Based on the findings presented in Table 3, there was a significant difference in the means of proteolysis between Ras and Gouda. Also, a significant difference in means of lipolytic, staph, and yeast counts between Ras and the other two types (Cheddar and Gouda) was recorded, but no significant differences were recorded between psychrophilic and mould counts in the three types of cheese. Thus, it can be concluded that Ras cheese displayed the highest proportion of non-compliance according to (ES: 1007-5/2005, ES: 1007-2/2020, ES: 1183-1/2020), followed by Gouda cheese. Conversely, cheddar cheese demonstrated the highest level of acceptability among the three cheese types analyzed.

### Antimicrobial resistance (AMR)

Unpasteurized milk and dairy products, especially cheeses, have been shown to contain methicillin-resistant staphylococci (MRS). *Staphylococcus aureus* (MRSA) is the most commonly reported MRS species; however, MRS isolates of coagulase-negative staphylococci (CNS) have also been found (Bendahou *et al.*, 2008).

Antimicrobial resistance (AMR) is a global threat to public health, and the use of antibiotics in animal production is a major factor in its emergence. Dairy products can be a potential source of antibiotics that intensify bacterial resistance, so it is important to monitor the susceptibility of different strains isolated from cheese to antibiotics in order to better understand the probable risks associated with their consumption (Sharma *et al.*, 2017).

The presence of the *mecA* gene confers resistance to methicillin (MRSA). On the other hand, the phenotypic method showed that 100% of isolates were resistant to oxacillin and 95% were resistant to

amoxicillin, while no isolate was resistant to amoxicillin - clavulanic acid. As clavulanic acid acts as a  $\beta$ -lactamase inhibitor, 80% of isolates contained the *bla* gene, which is responsible for producing  $\beta$ -lactamase, so when we used a  $\beta$ -lactamase inhibitor (clavulanic acid) in conjunction with amoxicillin in a disc assay, 100% of isolates were sensitive against it. The obtained results were matched with those of Zehra *et al.*, (2017) who found that 94.6% of isolates were resistant to the penicillin group; on the other hand, only 56.7% showed resistance to ciprofloxacin, which was higher than our study, in which only 5% of isolates were resistant to ciprofloxacin.

According to Zehra *et al.*, (2017), about 10–15% of the strains were resistant to gentamicin, which agrees with the results obtained in this study. Although 55% of the isolates carried the *aacA-aphD* gene, it is associated with gentamicin-resistant *staphylococci*. Only 5% of the isolates exhibited phenotypic resistance to gentamicin. The *erm A* genes are primarily responsible for erythromycin (macrolides) resistance in methicillin-resistant *Staphylococcus* (MRSA) strains, whereas the *erm C* genes often confer erythromycin resistance in methicillin-susceptible strains (Abbas *et al.*, 2015). In our study, 45% of the isolates harboured the *ermA* gene, while none of the isolates contained the *erm C* gene. Moreover, 35% of the isolates exhibited resistance to azithromycin (macrolides), and 20% demonstrated resistance to clindamycin (lincosamides).

Overall, this study's conclusions show the need for a more comprehensive approach to antibiotic use in animal production that considers potential dangers associated with dairy consumption as well as the need to preserve antibiotics' efficacy for both human and animal health (Ferri *et al.*, 2017).

The findings indicate that *Staphylococcus* isolates exhibited resistance to multiple antibiotics, including vancomycin, tetracycline, and lincomycin, with the highest levels of resistance observed for  $\beta$ -lactam antibiotics such as penicillin and oxacillin. These results align with studies conducted by other researchers worldwide, which have consistently reported a high prevalence of resistance to  $\beta$ -lactam antibiotics. In contrast, resistance to the other tested antibiotics was relatively less common, which aligns with the overall global trend observed in antibiotic resistance patterns (Berhilevych *et al.*, 2017).

### CONCLUSION

This study was conducted to assess the quality and safety of hard and semi-hard cheese regularly consumed in Egypt. Gouda cheese was the best in most quality and safety parameters, while Ras cheese was the worst. Regarding antibiotic

susceptibility, the presence of antibiotic resistance genes detected by PCR indicates that the examined hard and semi-hard cheeses are poor in hygienic quality and safety and are considered a health threat for consumers due to the presence of multidrug-resistant *Staphylococcus* species. These results highlighted the possible need for stringent sanitary controls to be put in place throughout the manufacture, storage, and distribution of the aforementioned cheese varieties.

### Conflicts of interest

The authors declare that there is no conflict of interest regarding the research data and tools used in this review.

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**How to cite this article:**

**Mostafa A. Shawk, Eman F. Abdel-Latif, Samah F. Darwish, Adel M. Saudi and Zeinab I. Ali, 2024.** Assessment of the Chemical and Microbiological Quality of Some Cheese Assortment in Egypt: Highlighting the incidence of multidrug-resistant *Staphylococcus* Species. Journal of Applied Veterinary Sciences, 9 (1): 85-93.  
DOI:<https://dx.doi.org/10.21608/javs.2023.243014.1285>