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Occurrence, Antimicrobial Resistance, and Virulence of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* Isolated from Dairy Products

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ABSTRACT

One of the significant hurdles in the 21st century is the contamination of dairy products with pathogenic and spoilage microorganisms that convey antimicrobial-resistant genes (AMR). Therefore, this study examined the dominance of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* in 60 samples of white soft cheese and Baladi yoghurt (30 of each). Phenotypic resistance to ten antimicrobials was assessed with molecular characterization of *mecA* and bla_{IMP} genes in *S. aureus* and *P. aeruginosa*, respectively. Additionally, *slt1*, *slt2*, and *eaeA* virulence genes were ascertained in the identified *E. coli* isolates. *S. aureus*, *P. aeruginosa*, and *E. coli* were present in 19, 15%; 51, 40%; and 1, 3% of the isolated strains in white soft cheese and Baladi yoghurt, respectively. *S. aureus* (n=34) isolated strains were insensitive phenotypically to methicillin, yet only 77% carried the *mecA* gene. While the identified *P. aeruginosa* strains were unsusceptible to the studied antibiotics except for meropenem, and the bla_{IMP} gene was found in the molecularly identified strains. The molecularly identified *E. coli* (n=3) were sensitive to imipenem and meropenem. Additionally, *Slt1* and *Slt2* virulence genes were present in all *E. coli* isolates, whereas 66% of them possessed the *eaeA* gene. The application of systems of food safety and Good Manufacturing Practices (GMP) is essential to ensure the safety and quality of commercial dairy products.

Keywords: Antimicrobial resistance, Methicillin, Virulence genes, *S. aureus*, *E. coli*, *P. aeruginosa*, Dairy products.

INTRODUCTION

The livestock sector is crucial in fulfilling a rising demand for animal-derived foods while safeguarding long-term global food security. It accounts for over 40% of global agriculture. Additionally, livestock products contribute to about one-third of the worldwide protein consumption (FAO 2016; Godde et al. 2021; Khanal et al. 2022). It also supports one billion smallholder farmers' livelihoods in developing countries and employs at least 1.3 billion people worldwide (Godde et al. 2021).

Among livestock products: dairy products such as white soft cheese and yoghurt which are staples in many people's diets around the world. Despite their nutritional value, these products may harbor both food-borne pathogens and spoilage microorganisms (Majoie et al. 2020). However, thermal pasteurization can effectively eliminate those in raw milk, but there is still a danger of post-pasteurization contamination during cheese and yoghurt manufacture, likely resulting from unsanitary conditions in the production facilities (Halim et al. 2022). *S. aureus* and *E. coli* which are two of the most prevalent foodborne pathogens in addition to Pseudomonas species (one of the major spoiling microorganisms) could be isolated from several dairy products (Mohamed et al. 2020; Hassani et al. 2022). Regrettably, the development of antimicrobial resistance in such organisms increased their public health concern. The overuse and/or misuse of antibiotics by farmers and veterinarians in dairy farms to promote the growth of animals or treatment infection resulted in the presence of antibiotic residues. Consequently, these residues could develop bacteria with antimicrobial-resistant genes in the animals which discharged these bacteria into its products (Rahman et al. 2021). Antimicrobial resistance results in 700,000 deaths a year and is predicted to cause 10 million fatalities and US$ 100 trillion in economic loss annually by 2050 if immediate action is not done (Pokharel et al. 2020; Ahmed et al. 2022; Dalal et al. 2023). Therefore, periodical evaluation of antimicrobial-resistant strains in dairy products should be practiced to prevent their spread and their associated risks.

Recent studies identified staphylococcal food poisoning as the third leading cause of foodborne illness worldwide (Ahmed et al. 2022a). *S. aureus* causes around

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241,000 instances of food poisoning annually in the United States (Girmay et al. 2020). Additionally, *S. aureus* is resistant to antibiotics due to it either produces β -lactamase enzyme or has a gene that modifies the site of action of β-lactams. This led to the synthesis of the penicillin-binding protein PBP2a, which is the cause of antibiotic resistance. The produced PBP2a gene is expressed by *mecA* which is found on a genetic component called SCCmec (staphylococcal cassette chromosome mec) (Lim et al. 2002; Springer et al. 2009; Qayyum et al., 2016). Methicillin-resistant *S. aureus* (MRSA) is regarded as a significant global reason of illness and mortality (CDC 2014; Castro et al. 2020). Vancomycin was the preferred antibiotic for treating such illnesses until CDC in 2002 reported the first *S. aureus*-resistant strains to both vancomycin and methicillin (CDC 2002).

Shiga toxin-producing *E. coli* (STEC) is among the most hazardous bacteria that may be found in milk (Fahim et al. 2023). The adhesion factor intimin *(eaeA)*, and shiga toxins (*slt1* and *slt2*) are considered the main virulence genes expressed in STEC. The exponential increase of multidrug-resistant (MDR) STEC has become a major global worry (Taher et al. 2020; Zeinhom et al. 2021; Ray and Singh 2022; Mwafy et al. 2023). Therefore, antibiotic treatment is typically required for STEC infections, since this bacterium's antibiotic-resistant variant tend to produce more serious and prolonged illnesses than their sensitive counterparts (Dehkordi et al. 2014). Accordingly, Ombarak et al. (2018) stated that 50% of the identified *E. coli* strains from cheese samples were MDR. Moreover, 82.4% of the identified *E. coli* was MDR in a study stated by Kasem et al. (2021).

P. aeruginosa is not only a significant spoiling reason in the dairy sector, but it is also an important opportunistic pathogen that belongs to the ESKAPE microorganisms (Eleboudy et al. 2015). The existence of carbapenem-resistant *P. aeruginosa* raised the global concern because carbapenem is the last choice for therapy (Rezaloo et al. 2022; Saedii et al. 2022). Consequently, World Health Organization (WHO) categorized carbapenem-resistant *P. aeruginosa* as a critical priority pathogen (Catho et al. 2021).

Therefore, this study investigated the microbial quality of white soft cheese and Baladi yoghurt sold in the Egyptian markets by assessing the prevalence of *S. aureus*, *E. coli* and *P. aeruginosa*. Additionally, their antimicrobial resistance patterns phenotypically and genotypically were evaluated, along with the molecular identification of the virulence genes of *slt1*, *slt2*, and *eaeA* in isolated *E. coli* strains.

MATERIALS AND METHODS

Sample Collection

Sixty samples (30 of each) of white soft cheese and Baladi yoghurt were randomly collected from small retailers and supermarkets in Giza governorate, Egypt. The samples were taken directly to the laboratory in an insulated ice box for rapid evaluation.

Microbiological Examination

Preparation of food homogenate and decimal dilutions of the examined samples were done according to the suggestions and recommendations of American Public Health Organization APHA (2015). Enumeration of total Staphylococci and Coliforms was done according to BAM (2016) and APHA (2015), respectively. Isolation of pseudomonas species was done according to Silva et al. (2018).

Biochemical Identification of the Isolated Strains

The isolated strains of Staphylococci, coliform, and Pseudomonas spp. were identified biochemically according to De Vos et al. (2009).

Profiling for Antibiogram Susceptibility of the Isolated Strains of *S. aureus, P. aeruginosa* and *E. coli*

Testing for antimicrobial susceptibility was employed using the Kirby-Bauer disc diffusion method in accordance with the direction directed by Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI 2020). The following antibiotic disks (Oxoid, Hampshire, England): Ampicillin-sulbactam (10/10µg), erythromycin (15µg), amoxicillin-clavulanic acid (20/10µg), tetracycline (30µg), cefoxitin (30µg), clindamycin (2µg), imipenem (10µg), and meropenem (10µg), were dispensed on Mueller-Hinton Agar (Oxoid, Hampshire, England) inoculated with 5-6 log cfu/mL of the isolated *E. coli* strains and incubated at 37°C for 16–18hr. While methicillin (5µg) and vancomycin (30µg) were used along with the preceding antibiotic discs for *S. aureus* and *P. aeruginosa*. The results were represented as resistant, intermediate, and sensitive strains depending on the diameter of inhibition zones recorded by the CLSI guidelines (2020).

Molecular Characterization

Nine strains of *S. aureus* out of 34, three strains of *E. coli* and 10 from 83 strains of *P. aeruginosa* were subjected to the molecular examination.

DNA Extraction

DNA of the isolated strains was obtained from the overnight incubated brain heart infusion broth cultures using the genomic DNA extraction kit (Fermentas Gene JET genomic DNA purification kit (Fermentas, Corporation, Germany), in agreement with the manufacturer's directions. DNA was preserved at -20°C for further analysis.

Molecular Identification

The PCR assays were carried out as a single assay utilizing *16S rRNA* in *S. aureus* and *23S rRNA* in *P. aeruginosa* isolates (Table 1). Furthermore, *mecA* and bla_{IMP} genes were identified in *S. aureus* and *P. aeruginosa*, respectively. Concerning *E. coli*, Multiplex PCR tests were used for the detection of *16srRNA* and *eaeA* genes, in addition to another reaction for the detection of *Slt1*, *Slt2*, as known virulence genes in *E. coli*. All used primers, with corresponding amplicon size, sequence, and references have been illustrated in Table 1.

From the extracted DNA, 50–100ng was added to 25pmol of each primer. Followed by adding 12.5µL of Master Mix reagent and completed to 25µL by distilled water. PCR amplifications were run in a thermo cycler 94°C/5min (Cycling conditions in a Bio RAD T100), 35 cycles of 94°C for 30s, 56°C for 30s, and 72°C for 30s; and extension at 72°C for 10min (Ombarak et al. 2018).

Statistical Analysis

Results were illustrated as mean±SEM using the program Statistical Package for Social Science (SPSS Inc., Chicago, IL, USA), version 26.

Table 1: Oligonucleotide primers for detection of Species specific and antimicrobial resistance genes in the examined isolates by conventional PCR

Microbial strain	Target gene	Primer Sequence (5`-3`)	Amplification size (bp)	Annealing Temp	Reference
S. aureus	*16S rRNA*	F:GCAAGCGTTATCCGGATTT R:CTTAATGATGGCAACTAAGC	597	55ºC	Al-Talib et al. (2009)
	mecA	F:ACGAGTAGATGCTCAATATAA R:CTTAGTTCTTTAGCGATTGC	293	60ºC	
E. coli	*16S rRNA*	F: AGA GTT TGA TCA TGG CTC A R: GGA CTA CCA GGG TAT CTA AT	798	56ºC	Holland et al. (2000)
	eaeA	F: AAG CGA CTG AGG TCA CT R: ACG CTG CTC ACT AGA TGT	450	56ºC	
	Slt1	F:AAATCGCCATTCGTTGACTACTTCT R:TGCCATTCTGGCAACTCGCGATGCA	370	72ºC	Brian et al. (1992)
	Slt2	F:CAGTCGTCACTCACTGGTTTCATCA R:GGATATTCTCCCCACTCTGACACC	283	72ºC	
P. aeruginosa	*23SrRNA*	F:CAAGCAATTCGGTTGGATAT R:GGCGTTGAGCTAACCAGTAC	192	65ºC	Lødeng et al. (2006)
	Bla$_{IMP}$	F:GGAATAGAGTGGCTTAAYTCTC R:GGTTTAAYAAAACAACCACC	232	52ºC	El-Mahdy and El-Kannishy (2019)

Table 2: Statistical analysis of the tested microbial parameters in the examined samples.

Parameter	White soft cheese			Baladi yoghurt		
	prevalence		Mean± SEM	prevalence%		Mean± SEM
	No.	%		No.	%	
Total Staphylococci count (log_{10} cfu/g)	30	100	7.8±0.2*	30	100	6.04±0.2*
Coliform count ((log_{10} MPN/g)	30	100	8.1±0.5*	30	100	2.7±0.1*
Detection of Pseudomonas species in the tested samples						
	No.	%		No.	%	
	30	100		20	67	

No.: number of positive samples: *: Means presence of significant difference (P<0.05).

RESULTS

Staphylococci species were present in all of the tested samples of white soft cheese and Baladi yoghurt with mean counts of 7.8±0.2 and 6.04±0.2 log_{10}cfu/g, respectively with presence of significant difference (*P*<0.05) (Table 2). Biochemical identification of the isolated strains revealed that *S. aureus* was present with percentages of 19 (16/84 isolates) and 15% (18/115 isolates) in white soft cheese and Baladi yoghurt, respectively (Fig. 1). Molecular identification of nine strains of *S. aureus* using *16S rRNA* gene confirmed its presence in all tested isolates (9/9 isolates) (Fig. 4).

Coliforms were found in all of the analyzed samples with mean counts of 8.1±0.5 and 2.7±0.1 log_{10} MPN/g in white soft cheese and Baladi yoghurt, respectively, with presence of significant (P<0.05) difference (Table 2). Biochemical identification revealed that *E. coli* was present in one (1/100 isolates) and three percentages (2/65 isolates) of the isolated coliform strains from white soft cheese and Baladi yoghurt samples, respectively, (Fig. 2). Molecular identification confirmed the existence of *16SrRNA* gene in all identified *E. coli* strains (3/3) (Fig. 5).

Pseudomonas spp. were present in the analyzed samples of cheese and yoghurt with percentages of 100 and 67%, respectively, (Table 2). Biochemical identification of the isolated strains displayed that *P. aeruginosa* were the most frequent ones in the examined cheese and yoghurt samples with percentages of 86% (56/109 isolates) and 56% (27/70 isolates), respectively (Fig. 3). Molecular identification confirmed their presence in 80% (8/10 isolates) using *23S rRNA* (Fig. 6).

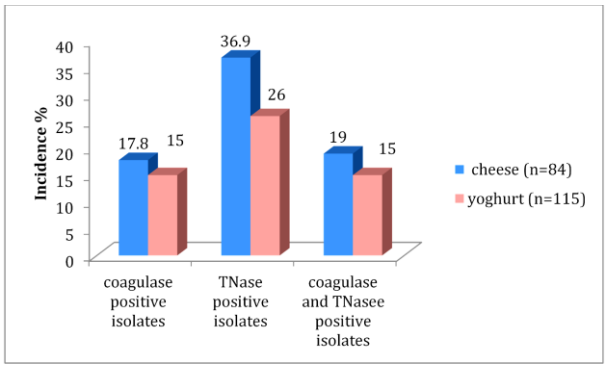

Fig. 1: Incidence of the biochemically identified *S. aureus* in the examined samples.

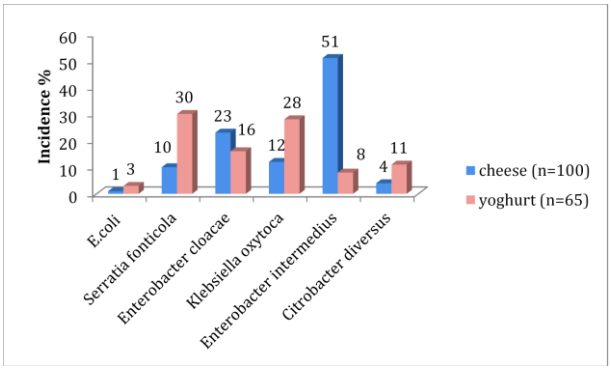

Fig. 2: Incidence of the biochemically identified coliform species in the examined samples: n= Total number of isolates.

The tested isolates of *S. aureus* (n=34) strains were resistant to cefoxitin (85.3%), ampicillin/sulbactam (76.4%), vancomycin (47.1%), methicillin (100%) and

Table 3: Phenotypic antimicrobial resistance patterns of *S. aureus*, *E. coli*, and *P. aeruginosa*

Antimicrobial class	Antimicrobial agent	*S. aureus* (n=34)			*E. coli* (n=3)			*P. aeruginosa* (n=83)		
		%								
		S	I	R	S	I	R	S	I	R
Beta lactam	Methicillin (5µg)	0		0	100	ND		0	0	100
	Ampicillin/sulbactam (10/10µg)	23.6	0	76.4	66.7	33.3	66.7	0	0	100
	Amoxy/clavulinic acid (20/10µg)	35.3	0	64.7	33.3	0	0	0	0	100
Glycopeptide	Vancomycin (30µg)	52.9		0	47.1	ND		0	0	100
Macrolides	Erythromycin 15µg)	17.6	14.7	67.6	0	0	100	0	0	100
Lincosamides	Clindamycin (2µg)	23.5	0	76.5	0	0	100	0	0	100
Tetracyclines	Tetracycline (30µg)	29.4	3	67.6	33.3	33.3	33.3	0	0	100
Cephalosporin	Cefoxitin (30µg)	14.7	0	85.3	33.3	0	66.7	0	0	100
Cabapenem	Imipenem (10µg)	91.2	0	8.8	100	0	0	0	0	100
	Meropenem (10µg)	76.5	0	23.5	100	0	0	100	0	0

ND: not detected: R: Resistant I: Intermediate S: Sensitive

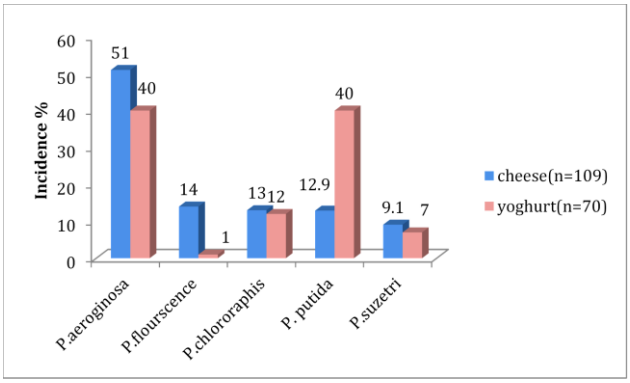

Fig. 3: Incidence of the biochemically identified Pseudomonas species in the examined samples.

erythromycin (67.6%). Concerning *E. coli*, all tested strains (n=3) were resistant to clindamycin and erythromycin, whereas, two strains (67%) were resistant to amoxy/clavulinic acid and cefoxitin. Nonetheless, the three strains were susceptible to carbapenem (meropenem and imipenem). On the other hand, all isolated *P. aeruginosa* (n=83) were resistant to all examined antibiotics except meropenem (Table 3).

Molecular identification of the *mecA* gene in the isolated *S. aureus* strains (n=9) revealed that 77% (7/9) of isolates carried this gene (Fig. 4). While, the bla_{IMP} gene was present in all molecularly identified *P. aeruginosa* isolates (n=8) (Fig. 6). Additionally, *slt1* and *slt2* were present in the three examined *E. coli* isolates, while, *eaeA* gene was present in two of them (Fig. 5).

DISCUSSION

S. aureus is an urgent threat to public health. Therefore, estimating the frequency of Staphylococci and *S. aureus* is always necessary to develop preventative policies to stop the transmission of this pathogen through food chain. The prevalence of Staphylococci and *S. aureus* in this study were relatively high (Table 3). For delicate products like yoghurt and fresh cheeses, thermal treatment is not suitable for post-manufacturing treatment, where *S. aureus* could be introduced to such products through post-manufacturing steps (i.e., handling, transportation, and storage), which might explain this high prevalence, that was aligned with Halim et al. (2022), while Mohamed et al. (2020) revealed a higher count in cheese samples.

E. coli contamination renders dairy products of bad keeping quality or even unsafe for human consumption, resulting in financial losses (Laslo and György 2018). In the current investigation, high coliform contamination levels were found in the examined samples. A comparable coliform count was described by Fathi et al. (2019) who linked this high finding to manual milking and the low-quality water. However, Mohamed et al. (2020) reported a lower coliform count in white soft cheese and a 4.4% *E. coli* isolation rate. Rahimi et al. (2011) on the other hand, were unable to isolate *E. coli* from yoghurt samples.

Pseudomonas is known to be the primary determinant of the shelf-life of most dairy products, as their growth is correlated with the production of protease, lipase, and lecithinase heat-resistant enzymes that cause putrefaction, fermentation, rancid- and bitter odors (Ahmed et al. 2021). *P. aeruginosa* was present in nearly half of the examined cheese and yoghurt samples. Comparable prevalence percentage was stated by Atia et al. (2020), in contrast, Ibrahim et al. (2022) isolated *P. aeruginosa* with a lower percentage of 16 and 18% from yoghurt and soft cheese, respectively. The demonstrated relatively high incidence of *P. aeruginosa* might be attributed to the fecal contamination, unhygienic measures applied during processing, transportation, storage, and the contaminated water used (Atia et al. 2020).

The isolated *S. aureus* strains had high levels of cefoxitin and erythromycin resistance, which was comparable to Kasem et al. (2021) who found that the isolated *S. aureus* showed high resistance to cefotaxime (82.4%) and erythromycin (50%). Additionally, Abd El Halem (2019) and Taher et al. (2022) reported that 65.4, 42, and 12.3% of the isolated *S. aureus* from milk and certain dairy products were resistant to tetracycline, cefoxitin, and erythromycin, respectively. On the contrary, Kassem et al. (2021) conveyed that the isolated *S. aureus* showed intermediate resistance to erythromycin (50%) and tetracycline (40%).

Recently, the development of multi-drug resistant *E. coli* has become a global concern as it causes high sickness and death (Xiao et al. 2019). The beta-lactam resistance in the isolated *E. coli* (67%) was considered relatively high when compared to the prevalence of beta-lactam-resistant *E. coli* (55%) isolated by Wu et al. (2021). Similarly, Gudogan and Avci (2014) and El Bagoury et al. (2019) stated that isolated *E. coli* was mainly resistant to erythromycin (100%), with intermediate susceptibility to oxytetracycline (44.4%).

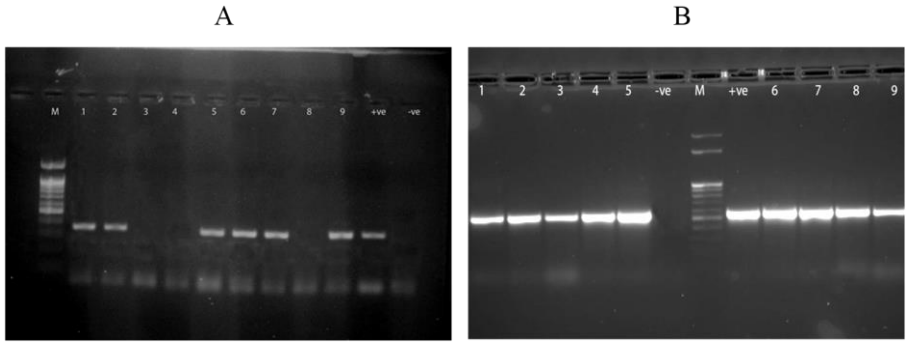

Fig. 4: PCR assay for *16srRNA gene* (293bp) (B) and *mecA* gene (597bp) (A) to identify *S. aureus &MRSA* A: Lane-M: 100 bp DNA ladder-step; Lane-+ve: (*S. aureus* ATCC25923); Lane--ve : negative control; A: Lanes 1, 2,5,6,7, 9: positive *S. aureus f*or *mecA* gene. B: Lanes 1, 2,3,4,5,6,7,8,9: positive *S. aureus.*

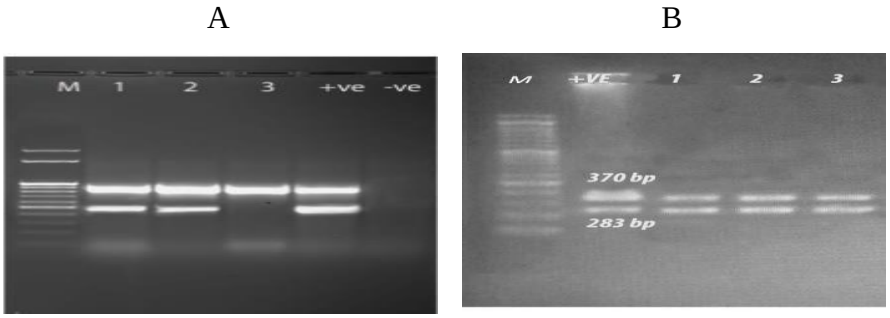

Fig. 5: Multiplex PCR for detection of *16srRNA* gene (798 bp), *eaeA* (450bp) (A), *slt1* (370bp) and *slt2* (283bp) genes (B) in *E. coli*. A: Lane-M: 100 bp DNA ladder-step; Lane +ve: (*E. coli* ATCC25922); Lane−ve: negative control A: Lanes 1, 2, 3: positive *E. coli* for *16srRNA* and lanes 1, 2: positive *E. coli for eaeA* gene. B: Lanes 1,2,3: positive *slt1* and *slt2*

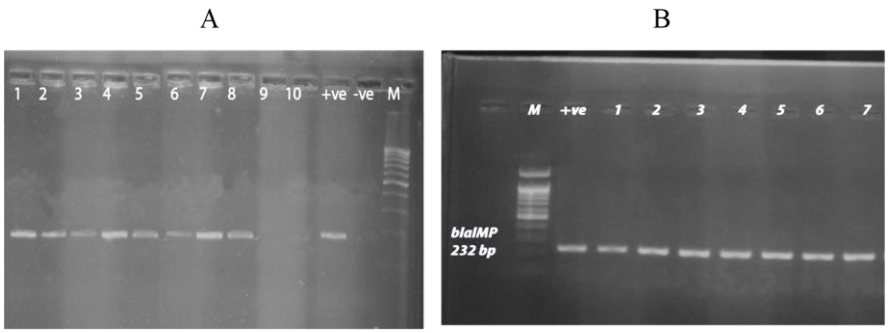

Fig. 6: CR detection of *23srRNA* gene (192 bp) (A), and *bla$_{IMP}$* (232 bp) (B) in *P. aeruginosa:* A: Lane-M: 100 bp DNA ladder-step; Lane-+ve: (*P. aeruginosa* ATCC27853); Lanes 1, 2,3,4,5,6,7,8: positive *P. aeruginosa* B: Lane 1,2,3,4,5,6,7: positive *P. aeruginosa* for *bla$_{IMP}$* gene

P. aeruginosa multidrug resistance may be attributed to the enzymes that can change or degrade drug targets, as well as reduced cell wall permeability, which restricts its capacity to absorb medicines (Ammar et al. 2016). *P. aeruginosa* identified strains were phenotypically resistant to all analyzed antibiotics with the exception of meropenem. Similarly, Nasirmoghadas et al. (2018) found that *P. aeruginosa* isolated strains were resistant to the studied antibiotics, with the exception of ceftazidime and polymixin B. Moreover, Ammar et al. (2016) observed that most of *P. aeruginosa* isolated strains were resistant to erythromycin and clindamycin.

Methicillin-resistant *S. aureus* (MRSA) has emerged as a major threat to global health. The *mecA* gene is mainly accountable for *S. aureus's* resistance to methicillin and penicillin (Hussain et al. 2013; Schnitt and Tenhagen 2020). The high incidence of MRSA (100%) in the identified *S. aureus* strains in this study could be linked to the inappropriate and overuse of methicillin in dairy farms, and the presence of insufficient food safety regulations (Hassani et al. 2022). Comparable results were described by Al-Ashmawy et al. (2016) and Fadel and Ismail (2015). Contrary to our findings, Papadopoulos et al. (2018) were unable to identify any MRSA strains in the examined dairy products.

The development of carbapenemases acquired genes, mostly those coding metallo-lactamases (MBLs) in *P. aeruginosa*, makes them a therapeutic issue because they can breakdown β- lactams (Fritsche et al. 2005). The high frequency of carbapenem resistant *P. aeruginosa* is caused by the existence of *bla$_{IMP}$* genes (Livermore 2002).

The isolated *P. aeruginosa* were sensitive to meropenem but resistant to imipenem. This variable carbapenem resistance phenotype may be explained by a

chromosomally controlled mechanism that differs with every carbapenem, like the loss of porin (oprD), which contributes to imipenem resistance, and the excessive expression of efflux pumps, which contributes to meropenem resistance (Pragasam et al. 2016). Comparable results were reported by Saedii et al. (2022) and Bandyopadhyay et al. (2019). However, Bahar et al. (2010) found that the 23 strains of *P. aeruginosa* were phenotypically imipenem resistant, but none of them harbored bla_{IMP} gene.

Enterotoxigenic *E. coli* (ETEC) can produce an adhesion factor chromosomally encoded by the *eaeA* gene, which plays a role in producing diseases linked to the gastrointestinal tract, including diarrhea, bloody diarrhea, and hemorrhagic colitis. These conditions can be complicated by neurological disorders and renal failure (Pradel et al. 2008). *E. coli* that produces the *eaeA* gene can also produce shiga toxins *slt1* and *slt2* (Nweze 2009). Shiga toxins (*slt1* and *slt2*) were present in all isolated *E. coli* strains. In the same manner, Elhadidy and Mohammed (2013) and Ahmed and Samar (2017) found that *sxt1*, *sxt2*, and *eaeA* genes were present in 85.7, 42.9 and 28.6% of the *E. coli* isolated strains.

Conclusion

This paper investigated the prevalence of *S. aureus*, *E. coli*, and *P. aeruginosa* in white soft cheese and Baladi yoghurt as popular Egyptian products. The antimicrobial susceptibility pattern was estimated phenotypically and genotypically together with the presence of *slt1*, *slt2* and *eaeA* virulence genes in *E. coli*. The identified *S. aureus* strains were phenotypically multidrug resistant and carried the *mecA* gene. Whereas *E. coli* strains harbored the shiga toxin genes and were resistant to clindamycin (E) (100%) and erythromycin (100%). Moreover, *P. aeruginosa* isolated strains were insensitive to the studied antibiotics phenotypically and harbored the bla_{IMP} gene. The extensive use of antibiotics in dairy farms can be responsible for this high pattern of antibiotic resistance. Therefore, periodical evaluation of antimicrobial resistant strains in dairy products should be practiced to prevent their spread, in addition to applying the systems of food safety and Good Manufacturing Practices (GMP) to ensure the safety of dairy food by protecting the consumer's health.

Declarations

The authors declare that they have no competing interests.

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Authors' Contributions

Walaa G. Nadi: Methodology, Investigation, and Writing-Original draft preparation. Lamiaa Ibrahim Ahmed: Conceptualization, Visualization, Supervision Methodology, Investigation, Review, and Editing. Abeer Abdel Nasser Awad: Conceptualization, Methodology, Investigation, Supervision, Review and, Editing. Eman M. Taher: Conceptualization, Methodology, Investigation, Supervision, Review, and Editing.

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