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Highlighting on the Incidence of Antimicrobial Resistance among Mastitic Buffaloes in Some Dairy Farms in Qalubiya governorate

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ABSTRACT

This study aimed to investigate somatic cell count (SCC) levels, and foodborne pathogenic bacteria in buffalo milk beside their antibiogram characteristics. Raw buffalo milk samples (n = 600) were randomly collected from Benha, Toukh, Kafr shokr and shebin elqanater provinces of Qalubiya Governorate, Egypt, during summer and winter seasons (75 of each/season). Results revealed the higher incidence of mastitis during summer season represented by higher SCC, with higher incidence of bacteria causing mastitis. Antibiotic resistant patterns and the presence of antibiotic resistance genes were evaluated in the isolates. Results revealed isolation of *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus, Klebsiella* spp. and *Enterococcus fecalis* from 59.3%, 44.0%, 35.8%, 10.8%, 6.7% and 4.3% of the examined samples, respectively. All isolates showed high rates of resistance to different antibiotics, and β-lactams particularly. The *bla*Z and *tet*K genes were detected in all of the examined *S. aureus* isolates, while *mecA* was detected in 66.6% only. In addition, *bla*TEM and *tet*A genes were detected in all of the examined *E. coli* and *Ps. aeruginosa* isolates; whereas, *aadB* gene was detected in 66.6% of the examined *Ps. aeruginosa* isolates. Moreover, *bla* gene was detected in all of the examined *B. cereus* isolates; while, *ermA* and *tetA* genes were detected in 33.3 and 66.6% of the examined isolates, respectively. On the other hand, higher level of somatic cell counts (SCC), and foodborne bacteria were determined in summer season than winter season indicating higher sus-

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ceptibility of buffalo herds to acquire different degrees of mastitis during humid and tropical weather (summer season) than cold weather (winter season). The high prevalence of bovine milk contamination with antimicrobial-resistant species in this study necessitates precise control on antibiotic prescription in veterinary medicine.

INTRODUCTION

Milk is a vital source of nutrients for humans, making any alteration in its composition a significant concern for the dairy industry. This sector must balance consumer demand, animal welfare, and product safety. Early detection of diseases is crucial for rapid treatment, especially in subclinical conditions like bovine mastitis, which often goes unnoticed (**Linehan et al. 2024**).

Bovine mastitis, an inflammation of the mammary gland, is economically impactful due to reduced milk production, increased culling rates, and substantial financial losses (**Zulfekar et al. 2025**). It is categorized into clinical, subclinical, and chronic forms based on symptoms and causes—infectious or noninfectious; where, infectious mastitis is further divided into contagious and environmental types, with bacterial pathogens such as *S. aureus*, *Streptococcus agalactiae*, *E. coli*, and *Pseudomonas spp.* being common culprits (**Morales-Ubaldo et al. 2023**).

Among these pathogens, *Staphylococcus aureus* is a prevalent Gram-positive bacterium linked to both clinical and subclinical mastitis. It produces toxins and enzymes that irreversibly damage mammary tissue, reducing milk yield **(Cheng and Han, 2020).** Similarly, *Bacillus cereus*, a Gram-positive spore-forming bacterium, can survive harsh conditions and cause mastitis in animals while posing food safety risks to humans **(Eid et al. 2023)**. On the other hand, coliform bacteria like *E. coli* are common Gram-negative environmental pathogens causing mastitis with symptoms ranging from mild udder inflammation to severe systemic effects that can lead to death **(Goulart and Mellata, 2022**). *Pseudomonas aeruginosa* is another Gram-negative bacterium associated with infections originating from contaminated environments such as bedding or water sources **(Diggle and Whiteley, 2021**).

Somatic cell count (SCC) serves as a key indicator of mastitis severity, particularly in subclinical cases. Elevated SCCs (>$2x10^5$ cells/ml) signal intramammary infections and lower milk quality. Diagnostic tools like the California Milk Test help detect these changes (**Laven, 2016**). Treatment strategies primarily involve hygienic practices and antibiotic administration through various methods such as intramammary infusion or injections. Common antibiotics include penicillin, cephalosporins, doxycycline, and quinolones (**Hossain et al. 2017**).

However, widespread antibiotic use raises concern about antimicrobial resistance (AMR), which threatens both animal and human health. Resistant bacterial strains can reduce drug efficacy and pose public health risks through foodborne transmission. Additionally, antibiotic residues in milk can cause allergic reactions or contribute to resistance development **(Kasimanickam et al. 2021; Sachi et al. 2019)**. Addressing AMR requires targeted research into bacterial isolation, resistance profiling, and gene detection in mastitic milk samples—a focus of this study on clinical and subclinically infected buffaloes (based on the regular milk yield, owner complain of abnormal milk properties) in Qalubiya governorate.

MAERIALS AND METHODS

2.1. Study area

This study was conducted on random milk production units located in four provinces (Benha, Toukh, Kafr-Shokr and Shebin-Elqanater) of Qalubiya governorate, Egypt.

2.2. Production system

Dairy animals in the study area were kept in rural traditional herd farms. They were kept under smallholder intensively managed dairy herds (≤20 cross-bred animals of all ages, Egyptian and Italian buffalo breeds). They were confined in an enclosure with dirt or concrete flooring. Hand milking is the only way for milk collection.

2.3. Collection of samples

A total of six hundred random samples of raw milk were collected aseptically from dairy buffaloes suffering from clinical, untreated, and subclinical mastitis that was positive for California Mastitis Test (CMT) accompanied by irregular milk yield with owner complain of abnormal milk properties**,** in the period of summer and winter seasons of 2024. Seventy-five samples were collected from each province / season. It is worth noted that, during summer season, 168 clinical mastitis samples (52, 43, 35, and 38 samples), and 132 subclinical mastitis samples (23, 32, 40, and 37 samples) were collected from Benha, Toukh, Kafr-Shokr and Shebin-Elqanater, respectively; while, during winter season, 118 clinical mastitis samples i.e. 28, 32, 29 and 29; and 182 subclinical samples (47, 43, 46, and 46 samples) were collected from the same provinces, respectively.

2.4. Bacteriological examination

2.4.1. Isolation and identification of the targeted bacteria

All samples were subjected to bacteriological examination according to the procedures of **Radostits et al. (2007)**, where each milk sample was pre-incubated at 37°C for 18-24h pre-isolation, identification and AMR profile investigation. Each enriched milk sample was examined for isolation and identification of *S. aureus, B. cereus, E. coli* and *Ps. aeruginosa* according to **ISO 6888-1 (2023), ISO 7932 (2020), ISO 16649-2 (2001) and ISO 22717 (2006),** respectively for the bacteriological examinations. In addition, each sample was streaked on macconkey agar for detection of lactose fermenter and non-lactose fermenters, followed by biochemical identification.

2.4.2. Antibiotic Sensitivity Test

In-Vitro sensitivity test was done on each isolated *S. aureus, B. cereus, E. coli* and *Ps. aeruginosa* strains of each season to study their sensitivity for different antibiotics **(Tables 1 to 3)** using the disc method on Muller-Hinton agar and incubation for 24h in 37^{O}C **(CLSI, 2020) and EUCAST (2024)**.

2.5. Chemical analyses of the examined milk samples

Somatic cell count (SCC) was evaluated by an automated BacSomatic-SCC dye.60070030 according to **ISO 13366-2 (2006)**.

RESULTS

3.1. Prevalence of the most detected bacteria:

Referring to the recorded results in **Table (1)**, the prevalence of the food poisoning bacteria causing mastitis was higher in summer season's examined samples than those in winter season. While, **Table (2)** recorded that the collected samples from shebin-elqanater province had the highest prevalence of different food poisoning bacteria causing mastitis, followed by kafr-Shokr, Toukh and Benha, respectively.

Table 1. Prevalence of the detected foodborne bacteria in the examined milk samples (n=300/season)

	Winter		Summer		TOTAL	
	No.	%[1]	No.	%[1]	No.	%[2]
B. cereus	20	6.77	45	15.0	65	10.8
E. faecalis	10	3.33	16	5.3	26	4.3
E. coli	156	52.0	200	66.7	356	59.3
Klebsiella spp.	15	5.0	25	8.3	40	6.7
Ps. aeruginosa	85	28.3	130	43.3	215	35.8
S. aureus	126	42.0	138	46.0	264	44.0

%[1] Incidence in relation to the number of the examined samples of each season (300)
%[2] Incidence in relation to the total number of the examined samples (600).

Table 2. Number of the isolated pathogens from the examined milk samples in relation to the area of collection (n=150).

	Benha		Toukh		Kafr-Shokr		Shebin-Elqanater		Total	
	No.	%[1]	No.	%[1]	No.	%[1]	No.	%[1]	No.	%[2]
B. cereus	10	6.7	15	10	18	12.0	22	14.7	65	10.8
E. faecalis	4	2.7	7	4.7	5	3.3	10	6.7	26	8.7
E. coli	60	40.0	100	66.7	71	47.3	134	89.3	365	60.8
Klebsiella spp.	4	2.7	5	3.3	13	8.7	18	12.0	40	6.7
Ps. aeruginosa	25	16.7	62	41.3	53	35.3	75	50.0	215	35.8
S. aureus	48	32.0	57	38.0	74	49.3	85	56.7	264	44.0

Antibacterial resistance profile of the most detected bacteria

In addition, the following **figures (1-4)** showed wide range of variability in the resistance pattern of different most prevalent food poisoning bacteria causing mastitis to the different used antibiotics, where higher resistance was recorded in summer season than in winter season.

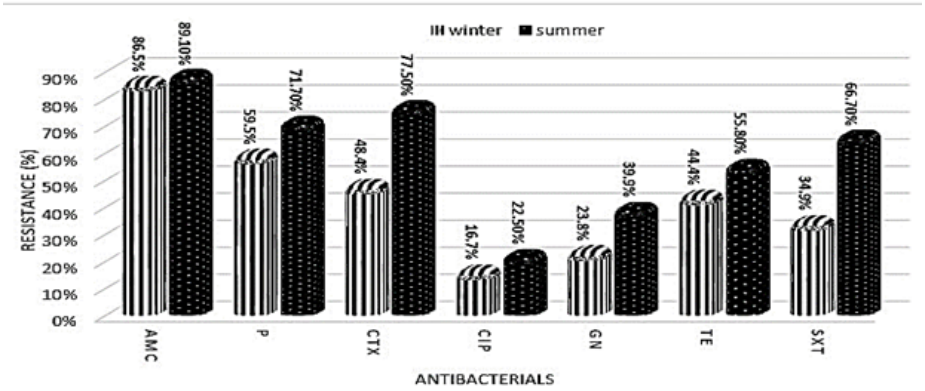

Fig. 1. In-Vitro anti-bacterial resistance ratios for isolated *S. aureus* strains of the examined milk samples in each season

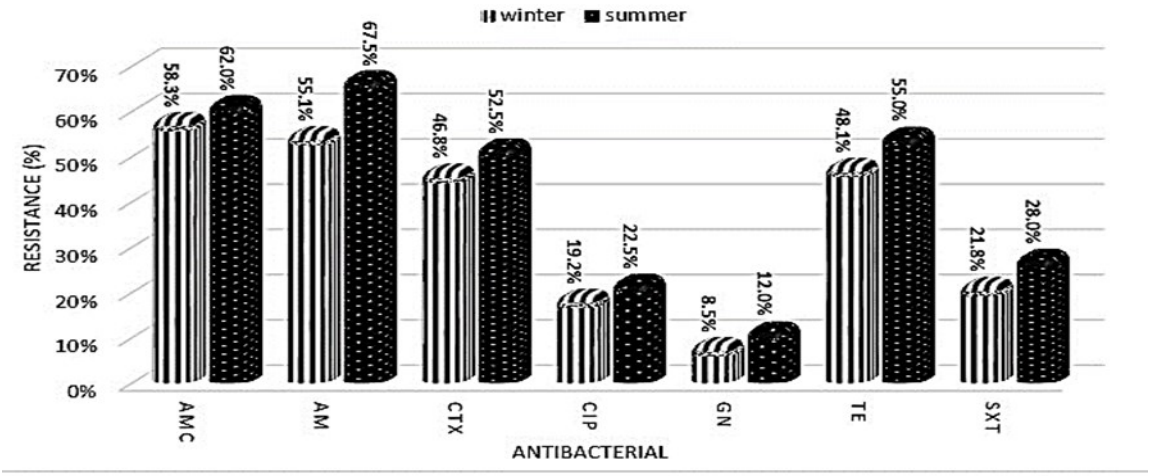

Fig. 2. In-Vitro anti-bacterial resistance ratios for isolated *E. coli* strains of the examined milk samples in each season

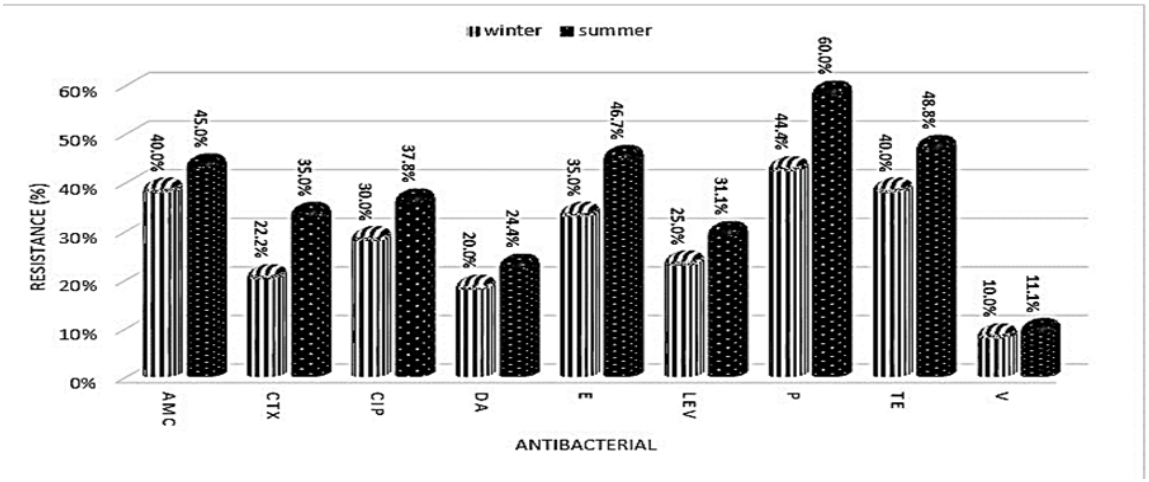

Fig. 3. In-Vitro anti-bacterial resistance ratios for isolated *Ps. aeruginosa* strains of the examined milk samples in each season

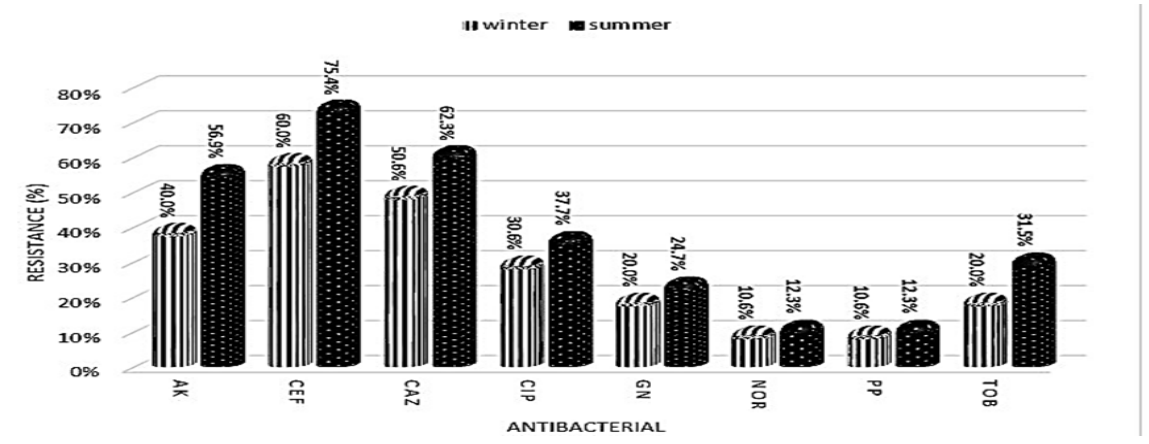

Fig. 4. In-Vitro anti-bacterial resistance ratios for isolated *B. cereus* strains of the examined milk samples in each season

Molecular detection of some antibiotic resistance genes

The molecular examination for three strains of *S. aureus* against three resistant genes "*bla*Z, *mec*A, and *tet*K" showed that all three strains amplified at 360bp. indicating positive for presence of *tet*K gene that encoding tetracycline efflux pump, and also all of them amplified at 833bp. indicating positive for *bla*Z gene "encoding β-lactamase resistance", while only two strains "2&3" able to amplified at 310bp. indicating presence of *mec*A gene on them as seen in **Fig (5).**

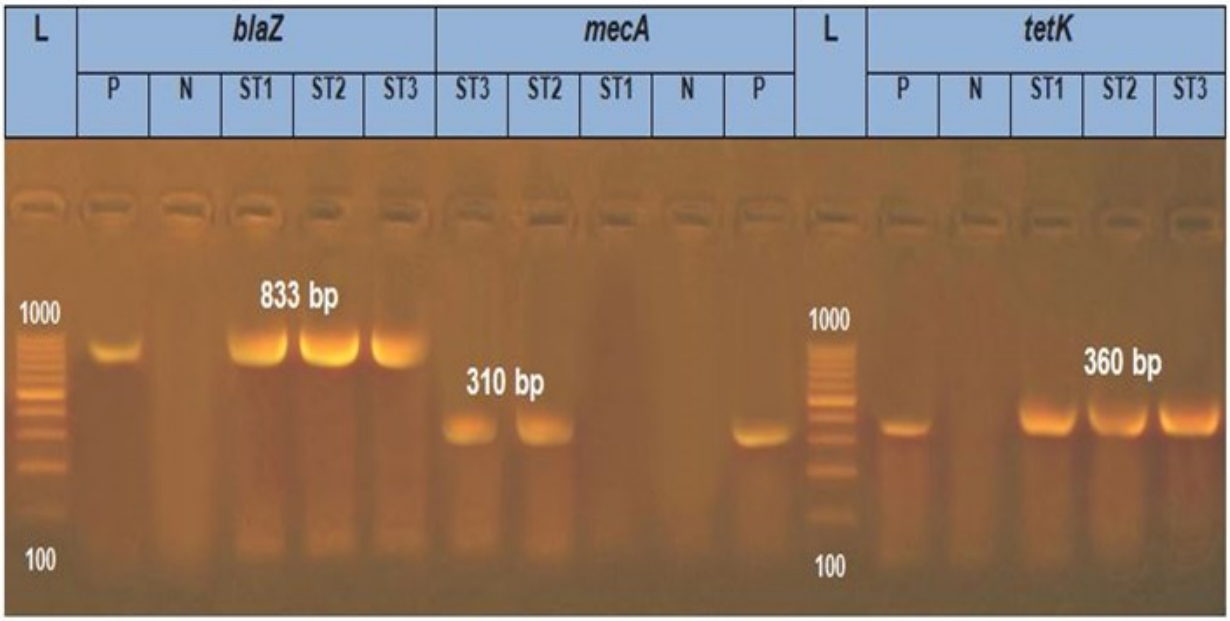

Fig. 5. Agarose gel electrophoresis of Uniplex PCR of *bla*Z (833 bp), *mecA* (310 bp) and *tetK* (360 bp) antibiotic resistant genes of *S. aureus*
Lane L: 100 bp ladder as molecular size DNA marker.
Lane P: Control positive *S. aureus* for the examined genes.
Lane N: Control negative (*E. coli*).
Lanes ST1, 2 and 3 showed positive bands at 833 and 360 bp for *blaZ* and *tetK* genes.
Lanes ST2 and 3 showed positive band at 310 bp for *mecA* gene, while ST1 was negative for the same gene.

The molecular examination for the resistant genes "*blaTEM* gene, *aadB gene, and tetA* gene" for three strains from each of *Escherichia coli* and *pseudomonas aeruginosa* showed that all examined strains able to amplify at 516 bp. which detected carrying *blaTEM* gene "coding for enzyme that confer resistance to beta-lactam antibiotics" (**Figure, 6)**. Moreover, the ability of the strains to amplify at 319 bp. indicating presence of *aadB gene* "coding for enzyme responsible for modifying aminoglycoside antibiotics" and as shown in **Figure (7)**, it presents in all examined *E. coli* while it presents in only two strains of *pseudomonas aeruginosa.* The *tetA* gene "coding for tetracycline efflux pump" was detected in all examined strains and amplified at 570 bp (**Figure, 8).**

The molecular examination for the occurrence of resistant genes "*bla* gene, *ermA* gene, and *tetA* gene" in three examined strains of *B. cereus* displayed presence of *bla* gene in all examined strains that amplify at 680bp. while *ermA* gene present in only one examined strain that amplify at 652 bp. and for *tetA* gene present in two isolates that amplify at 502 bp. **(Figure, 9)**

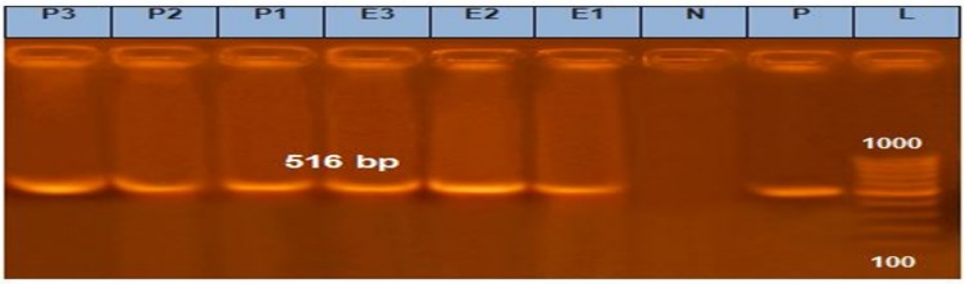

Fig. 6. Agarose gel electrophoresis of Uniplex PCR of *blaTEM* (516 bp) antibiotic resistant genes of *E. coli and Ps. aeruginosa*
Lane L: 100 bp ladder as molecular size DNA marker.
Lane P: Control positive *E. coli* for the *blaTEM* gene.
Lane N: Control negative (*S. aureus*).
Lanes E1, 2, 3, and P1, 2, 3 showed positive bands at 516 bp for *blaTEM* gene

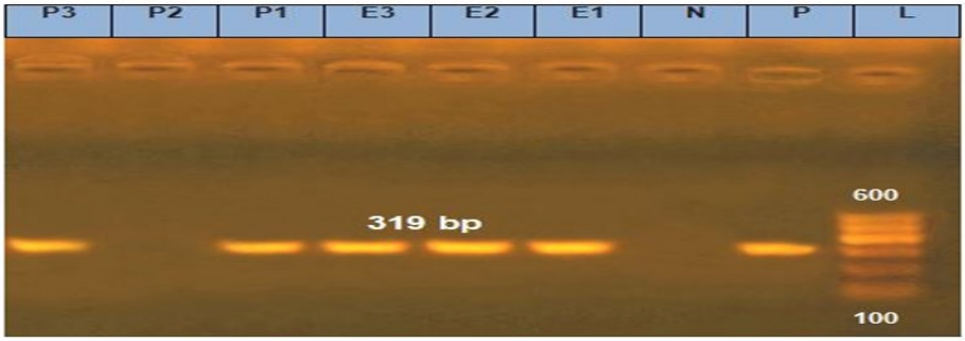

Fig. 7. Agarose gel electrophoresis of Uniplex PCR of *aadB* (319 bp) antibiotic resistant genes of *E. coli and Ps. aeruginosa*
Lane L: 100 bp ladder as molecular size DNA marker.
Lane P: Control positive *E. coli* for the *aadB* gene.
Lane N: Control negative (*S. aureus*).
Lanes E1, 2, 3 showed positive bands at 319 bp for *aadB* gene.
Lanes P2 and 3 showed positive bands at 319 bp for *aadB* gene, while P1 was negative for the same gene

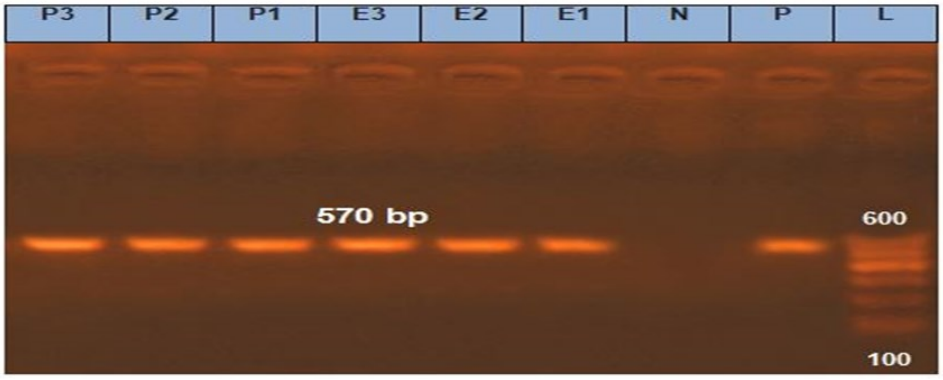

Fig. 8. Agarose gel electrophoresis of Uniplex PCR of *tetA*(A) (570 bp) antibiotic resistant genes of *E. coli and Ps. aeruginosa*
Lane L: 100 bp ladder as molecular size DNA marker.
Lane P: Control positive *E. coli* for the *tetA*(A) gene.
Lane N: Control negative (*S. aureus*).
Lanes E1, 2, 3, and P1, 2, 3 showed positive bands at 570 bp for *tetA*(A) gene.

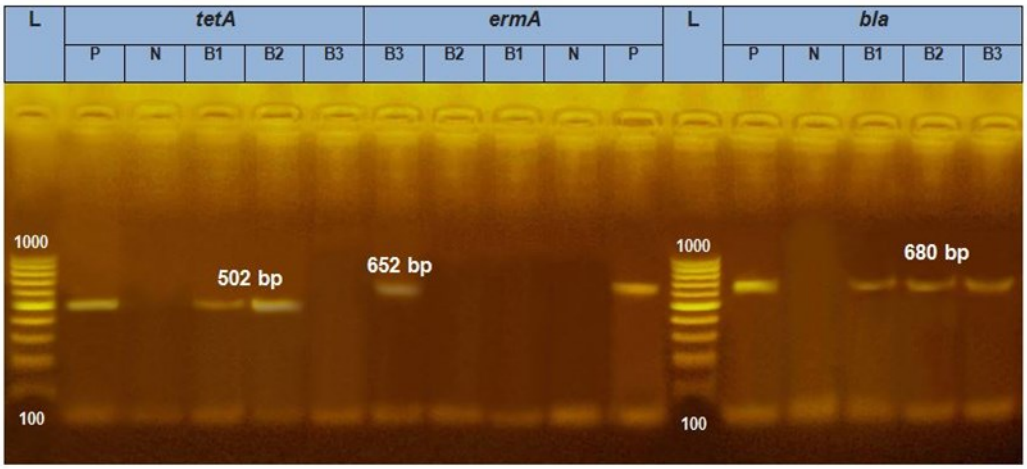

Fig. 9. Agarose gel electrophoresis of Uniplex PCR of *tetA* (502 bp), *ermA* (652 bp), and *bla* (680 bp) antibiotic resistant genes of *B. cereus*
Lane L: 100 bp ladder as molecular size DNA marker.
Lane P: Control positive *B. cereus* for the examined genes.
Lane N: Control negative (*E. coli*).
Lanes B1, 2 and 3 showed positive bands at 680 bp for *bla* gene.
Lanes B3 showed positive band at 652 bp for *ermA* gene, while B1, 2 was negative for the same gene.
Lanes B1, 2 showed positive band at 502 bp for *tetA* gene, while B3 was negative for the same gene

Moreover, as significant indicators, **Table (3)** showed higher SCC in the examined milk samples during summer season, either for clinical or subclinical mastitic examined samples, than in the winter collected samples indicating higher prevalence of mastitis in summer season than in winter season

Table 3. Statistical analytical results of chemical examination of raw buffalo milk samples (n=75/season/location).

Location	Season	SCC ($x10^3$/ml)	
		Clinical	Subclinical
Benha	Winter	350.30±40.2	276.70±25.2
	Summer	540.60±60.1*	342.33±23.6*
Toukh	Winter	444.0±45.77	263.33±37.9
	Summer	620.±65.3*	355.0±15.0*
Kafr-Shokr	Winter	420.7±40.3	282.70±1.2
	Summer	517.1±45.9*	361.70±17.5*
Shebin-Elqanater	Winter	398.0±31.6	281.33±13.3
	Summer	580.2±53.26*	365.70±15.0*

* Superscript star within the same column means significant difference when P<0.05.

DISCUSSION

One of the most serious diseases facing the milking livestock sector is bovine mastitis, that not only causes production of low quality of milk, but also increases the economic pressure on the breeders and the dairy industry as all. Such cases represent as an inflammatory response of the udder tissue to different causative agents "physical or bacterial cause", and based on the degree of inflammation, bovine mastitis may divide into clinical mastitis, subclinical, and chronic mastitis (**El-Shenawy, 2024**). The abrupt change in the climate, also, plays a significant role in potentiation of the bacterial growth, and so increases incidence of the bovine mastitis (**Jingar et al. 2014**).

This research highlights the incidence of the most detected bacterial causes that may be isolated from infected mastitic buffalos across different seasons (**Table, 1)**, and within different regional areas at Qalubiya governorate, Egypt **(Table, 2).** Based on the result of **Table (1),** the most prevalent bacterial agents across all seasons were *E. coli*, *S. aureus* and *Ps. aeruginosa* with prevalence of 59.3%, 44.0%, and 35.8%, respectively across all seasons. Their prevalence was notably higher in summer (66.7% for *E. coli,* 46.0% for *S. aureus,* and 43.3% for *Ps. aeruginosa*) compared with winter season (52.0%, 42.0%, and 28.3%, respectively). Their incidence with the same manner was previously recorded by **Zeinhom et al. (2013)** and **El-Demerdash et al. (2023).** Previous records attributed such findings to the hygienic conditions of milking workers and equipment; while the health status of animal and environmental conditions play a crucial role in udder infection and inflammation. *E. coli* and *Ps. aeruginosa* are environmental pathogens entering the udder through fecal contamination or contaminated water and equipment, leading to acute inflammation, while *S. aureus* is primarily a contagious pathogen transmitted during milking from infected udders, causing chronic infections due to its ability to evade strong immune responses and survive intracellularly; furthermore, *E. coli* triggers a robust innate immune response with high levels of inflammatory cytokines, whereas S. aureus induces a weaker, often chronic infection with limited cytokine activation, complicating treatment and eradication efforts **(Bannerman et al. 2004; Cheng and Han, 2020 and El-Demerdash et al. 2023).**

Other foodborne microbes also were isolated from the mastitic buffalos such as *B. cereus, Klebsiella spp.,* and *E. faecalis* with higher incidence in summer season 15%, 8.3%, and 5.3%, respectively**.** Their higher prevalence during summer (humid and tropical weather) months mainly contributed to heat stress that causes immunosuppression to the mammary gland and so facilitate its inflammation (**Olde Riekerink et al. 2007).** In addition, the higher temperature potentiates the microbial growth and their wide distribution by different vectors like flies which are most abundant during summer season (**Hoffmann et al. 2020; Heinicke et al. 2021**).

The bacterial prevalence differs within geographical areas **(Table, 2)**, where areas like Shebin-Elqanater, that in most of the examined cases suffered from low hygienic conditions and workers knowledge about personal hygiene and good hygienic practices during milking and storage of collected milk, showed the highest incidence of bacterial growth across all other studied areas, which may be contributed to the presence of certain issues in the hygienic practice within this area, or with the surrounding environments like poor housing or bedding equipment, bad hygienic circumstances, preceding history of mastitis, poor milking systems and\or contaminated milking equipment, lacking supervision, control, and prevention measures of mastitis all represent most common causes of mastitis in certain area as was described by **Rahularaj et al. (2019).** So, further studies must be conducted in such areas plus taking in consideration the targeted interventions, such as training on hygienic milking practices and proper equipment sanitation, that may significantly reduce contamination, and potentiality of mastitis occurring.

Antibiotics remain one of the primary approaches for treating mastitis, but their efficacy is increasingly weakened by the emergence of multidrug-resistant strains (**Liu et al. 2022**). The recent study revealed seasonal variations in antibiotic sensitivity patterns among *S. aureus* isolates from mastitic cases **(Figure 1)**. Resistance rates were consistently higher during summer compared to winter seasons, particularly for amoxicillin-clavulanic acid (89.1% vs. 86.5%), cefotaxime (77.5% vs. 48.4%), penicillin (71.7% vs. 59.5%), trimethoprim-sulfamethoxazole (66.7% vs. 34.9%), tetracycline (55.8% vs. 44.4%), gentamicin (39.9% vs. 23.8%), and ciprofloxacin (22.5% vs. 16.7%), respectively. This seasonal amplification of resistance may stem from elevated horizontal gene transfer rates among bacterial strains in warmer temperatures **(Li et al. 2022**). Moreover, Higher bacterial antibiotic resistance in summer compared to winter is mainly caused by environmental and management factors that promote increased infection rates and antibiotic use during warmer months.

In summer, conditions such as higher temperatures, humidity, muddy paddocks, and wet bedding favor the proliferation and transmission of mastitis pathogens like *S. aureus* and *E. coli*, leading to more frequent infections and consequently more antibiotic treatments. This increased antibiotic use exerts selective pressure on bacteria, encouraging the emergence and persistence of resistant strains. Additionally, insect vectors that transmit pathogens are more active in summer, further spreading resistant bacteria. Conversely, colder and drier winter conditions reduce mastitis incidence and antibiotic usage, resulting in lower resistance levels. Studies have shown significantly higher resistance rates to antibiotics such as penicillin and ampicillin in summer, linked to these seasonal environmental factors and treatment patterns **(Karzis et al. 2019 and Naranjo-Lucena et al. 2025).**

Despite their historical dominance in staphylococcal mastitis treatment, β-lactam antibiotics now face escalating resistance (**Ahmed et al. 2020 and Talaat et al. 2023**). The intermammary use of β-lactam antibiotics has been linked to the development of methicillin-resistant *S. aureus* (MRSA), complicating herd-level mastitis management (**Javed et al. 2022**). Resistance to cefotaxime also reported by **Elias et al. (2020),** and tetracycline aligns with earlier findings by **Abdi et al. (2018); Shrestha et al. (2021) and Talaat et al. (2023).** In addition, gentamicin resistance was reported by **Kotb et al. (2018)** and **Munive Nuñez et al. (2023)** who found that the mastitis-borne bacterial isolates exhibited resistance toward gentamicin by about 43.5% and 10.5%, respectively; which reflect broader antimicrobial resistance trends in mastitis pathogens.

Furthermore, the resistant patterns for the isolated *E. coli* were demonstrated in summer and winter seasons **(Figure, 2)**, in which the isolated strains, also, showed higher drug resistance during summer seasons than winter season as follow: the resistance against ampicillin (67.5% vs. 55.1%), amoxicillin-clavulanic acid (62% vs. 58.3%), tetracycline (55% vs. 48.1%), cefotaxime (52.5% vs. 46.8%), trimethoprim-sulfamethoxazole (28% vs. 21.8%), ciprofloxacin (23% vs. 19.2%), and gentamicin (12% vs. 9%), respectively. Based on the recorded results, the isolated *E. coli* exhibited resistance to β-lactam antibiotics such as ampicillin and amoxicillin which came in agree with the recorded findings by **Brown (2015); Tekiner and Özpınar (2016);** and **Aliyo and Teklemariam (2022).** The resistance of *E. coli* toward β-lactam antibiotics render the microbe to be also resistant to cephalosporin (**Ombarak et al. 2019**), as seen recently the resistance toward cefotaxime mainly came in the same line with **Yakovlieva and Bahlai (2019), Ahmed et al. (2021) and Campos et al. (2022).** Resistance of E. coli toward tetracycline was noticed earlier in the mastitis cases as mentioned by **Supré et al. (2014),** and reached to 48% by **Das et al. (2017**) and to (15.93 %) by **Majumder et al. (2021).** However, the isolated E. coli showed high susceptibility to ciprofloxacin in both seasons, and this agree with findings reported by **Mahdavi et al. (2022)** who detected the low resistance of *E. coli* to ciprofloxacin by 3.33%. Additionally, the low resistance of *E. coli* to gentamicin disagreed with findings of **Abed and Menshawy (2021).**

The antibiotic resistance patterns of *Ps. aeruginosa* isolated from mastitic cases demonstrate seasonal variations, with higher resistance rates observed during the summer compared to winter seasons **(Figure, 3)**. Resistance rates increased across all antibiotics during the summer, with the most significant rise observed for cefepime (75.4% in summer vs. 60% in winter), and Ceftazidime (62.3% in summer vs. 50.6% in winter), which contrast the results of **Huang et al. (2024)** who demonstrate the complete sensitivity of *Ps. aeruginosa* to ceftazidime, and higher than **Hancock (1998)** who reported resistance to ceftazidime by about 15.2%. Besides that, amikacin showed resistance at a rate of 56.9% in summer seasons vs. 40% in winter seasons, which agreed with the findings of **Ibrahim et al. (2017).** The other antibiotics like ciprofloxacin exhibited resistance rate of 37.7% in summer vs. 30.6% in winter that contrasted the results of **Huang et al. (2024)** who found the susceptibility of *Ps. aeruginosa* isolates to ciprofloxacin (31.5% vs. 20%), and gentamicin showed

(24.7% vs. 20%)**.** Both piperacillin and norfloxacin exhibited relatively low resistance rates, with 12.3% during summer and 10.6% during winter, which were higher than those reported by **Huang et al. (2024).** These antibiotics may still hold promise for treating mastitis caused by *Ps. aeruginosa* despite the seasonal increase in resistance levels.

However, all the preceding microbes exhibited higher resistance in warmer season as a result of their adaptation and proliferation during such season with their ability to transfer the resistant genes between them, the resistance pattern of *B. cereus* isolated from mastitic cases presents a unique trend compared to other pathogens **(Figure, 4)**. Notably, certain antibiotics such as amoxicillin/clavulanic acid, cefotaxime, and penicillin G exhibited higher resistance rates during winter than summer seasons. Specifically, the resistance percentages were as follows: 45% vs. 40% for amoxicillin/clavulanic acid, 35% vs. 22.2% for cefotaxime, and 60% vs. 44.4% for penicillin G. This unique trend may contribute to the increased incidence of clinical mastitis during colder weather because of the prolonged lactation period in winter acts as a cofactor that facilitates the proliferation of *B. cereus* (**Moosavi et al. 2014**). Additionally, the ability of *B. cereus* to form spores allows it to resist harsh environments, potentially enhancing its growth and survival during winter conditions. This adaptability could play a significant role in the higher resistance rates observed during this season.

While for other antibiotics like tetracycline, erythromycin, ciprofloxacin, levofloxacin, clindamycin, and vancomycin, the resistance rates were higher in summer season than that recorded in winter season. *B. cereus* showed resistance to tetracycline by about 48.8% in summer season vs. 40% in winter season, and this came nearly similar to **Eid et al. (2023)** who recorded resistance of *B. cereus* to tetracycline by 45.5%, and higher than that recorded by **Osama et al. (2020)** who showed 22.6% resistance, while counteract the result of **Mohammadin et al. (2023)** who demonstrate the susceptibility of the isolated strains to tetracyclin, ciprofloxacin, levofloxacin. Whilst erythromycin showed a resistance rate 46.7% in summer and 35% in winter, this is higher than that recorded by **Osama et al. (2020)** showed only resist to erythromycin by about 5.6%, and **Kowalska et al. (2022)** reported resistant in only 1.87%, but contrast findings of **Rosovitz et al. (1998)** who detect the susceptibility of *B. cereus* to erythromycin. For ciprofloxacin it recorded resistance rates of 37.8% in summer vs 30% in winter, which disagreed with the results of **Eid et al. (2023) and Mohammadin et al. (2023)** who demonstrated highly sensitivity of *B. cereus* to ciprofloxacin. Levofloxacin showed a resistance level of 31.1% in summer and 25% in winter, which opposes the results of **Eid et al. (2023) and Mohammadin et al. (2023).** In addition, clindamycin exhibits 24.4% resistant in summer and 20% in winter, which agreed with **Murray et al. (2007).** Vancomycin displayed fewer resistant rates in both seasons, where it showed resistance at 11.1% in summer vs. 10% in winter, which came nearly similar to **Mohammadin et al. (2023).**

The molecular exploration for the resistance genes in the examined *S. aureus* **(Figure, 5)** showed the ability of *blaZ* gene to intensify in all examined strains which detect the ability of such isolates to produce β-lactamase enzyme that resist penicillin, and this came in agree with **Bolte et al. (2020)** who demonstrated presence of such gene in *Staphylococcus* strains isolated from mastitis cases. Also, all examined strains carrying *tetK* gene, this came in agreement with **Ahmed et al. (2020); Abo-Shama et al. (2022) and Talaat et al. (2023).** While, *mecA* gene was detected in 66.6% of the examined isolates which came in harmony with the findings of **Algammal et al. (2020a).**

Testing other resistant genes carried by *E. coli* and *Ps. aeruginosa* **(Figure, 6)**, the *blaTEM* gene "coding for beta-lactamase enzyme that confer resistance to beta-lactam antibiotics" able to intensify in all the examined *E. coli* strains, this approves the data detected by **Yu et al. (2015), and Majumder et al. (2021)** who determined the presence of *blaTEM* gene in *E. coli* isolated from mastitis cases. Existence of such gene in all examined *Ps. aeruginosa* aligns with other studies reporting *blaTEM* in 86.36% of clinical *Ps. aerugino-*

sa isolates (including mastitis-related cases) (**Islam et al. 2024),** and 26.7% of ESBL-producing isolates in Iran (**Peymani et al. 2017**).

Moreover, the *aadB gene* "coding for enzyme responsible for modifying aminoglycoside antibiotics rendering them ineffective" was detected in all examined *E. coli* strains **(Figure, 7)**, which aligns with the results of **Mostafa et al. (2025).** While it was detected in 66.6% of the examined *Ps. aeruginosa* isolates, this is nearly agreed with **Ahmadian et al. (2020)** who detected its presence in 54.76% of isolated *Ps. aeruginosa.* In addition, the tetracycline resistant gene (*tetA gene)* was demonstrated in all examined strains of *E. coli* **(Figure, 8)** that confirmed the reported data by **Bag et al. (2021)** who found that all the tetracycline resistant *E. coli* isolated from mastitis in dairy farms carrying *tetA* gene. Also, it was detected in all examined isolates of *Ps. aeruginosa.* Up to our knowledge, there is no previous study demonstrated presence of *tetA* gene in *Ps. aeruginosa* isolated from mastitis case, but **Algammal et al. (2020b)** demonstrate presence of such gene in 75.6% of *Ps. aeruginosa* isolated from fish.

The molecular demonstration of the resistant genes "*bla* gene, *tetA* gene, and *ermA* gene" in the isolated *B. cereus* **(Figure, 9)** displayed presence of *bla* gene in all examined strains, this consistent with the findings of **Abd El-Tawab et al. (2020)** who noticed presence of the *bla* gene in all tested isolates (100%) and **Eid et al. (2023)** who found *bla* gene in 98.18% of examined *B. cereus* isolated from subclinical mastitis. The *tetA* gene is also presented in two out of three tested isolates and this nearly disagrees with the result of **Eid et al. (2023)** who lack detect of the *tetA* gene in any of examined strains. The *ermA* gene that modifies macrolide antibiotic present in 33.3% of the examined *B. cereus,* this slightly resembles **Algammal et al. (2022)** and **Algammal et al. (2024)** who mentioned that *B. cereus* strains often exhibit multidrug resistance (MDR), carrying genes like *bla1*, *bla2*, *tetA*, and *ermA* for resistance to β-lactams, tetracyclines, and macrolides, respectively.

Milk production and udder health are among the most critical concerns for breeders, making early inspection of udder health essential. Somatic cell count (SCC) stands as the major indicator for mammary gland health which directly affects milk quality and serves as a fundamental requirement for milk acceptance at purchase points **(Król et al. 2010**). It represents a judging indicator for the inflammatory status of the udder tissue that help in recognizing the degree of infection "subclinical or clinical mastitis" (**Ebrahimie et al. 2018**).

The measurement of the SCC across different seasons "summer and winter" within four provinces (Benha, Toukh, Kafr-Shokr and Shebin-Elqanater) of Qalubiya governorate, Egypt **(Table, 3)** showed significantly higher SCC values during the summer compared to winter seasons across all locations. This increase is consistent with findings from other studies that reported elevated SCC during warmer months due to heat stress and its impact on animal health **(Sarubbi et al. 2013; Amin et al. 2017; Kabelitz et al. 2024; and Viana et al. 2025).**

Heat stress during summer season reduce feed intake and weaken udder defense mechanisms, and so increasing susceptibility to mastitis and other infections, which consequently lead to elevating SCC levels (**Zhang et al. 2022**). Also, summer season often coincides with higher humidity, promoting bacterial growth such as *S. aureus* and *E. coli*, which directly elevates SCC (**Sharma et al. 2011**). In addition, heat stress may be an important factor for buffalo milk, whose heat dissipation by sweating is less efficient than cattle (**Viana et al. 2025).**

In contrast, cooler weather was better for the health status of buffalo and so, the winter season across all locations showed lower SCC levels, that appear due to reduced environmental stress and better physiological conditions for buffaloes during cooler months.

The somatic cell count (SCC) is a vital indicator widely used to evaluate mastitis in dairy animals, serving as a reliable marker for

both clinical and subclinical infections. An elevated SCC, typically above 200,000 cells/mL, signals an inflammatory response in the mammary gland due to bacterial invasion, even when clinical symptoms may not yet be apparent. This makes SCC an essential tool for early detection of subclinical mastitis, allowing timely intervention before the disease progresses. Monitoring SCC helps differentiate between healthy and infected quarters or cows, guiding treatment decisions such as the need for antibiotics or other management practices **(Sharma et al. 2011 and Ramuada et al. 2024)**.

Among the locations **(Table, 3)**, significant variations were noted between winter and summer collected samples either in clinical or subclinical affected milk samples, suggesting potential differences in management practices, environmental conditions, or herd health. The elevation in SCC value in certain areas may contribute to inadequate milking equipment sanitation and increasing mastitis risk in such areas as mentioned by **El-Bramony et al. (2004**). Also, insufficient nutrition in certain regions weakens immune function so increasing susceptibility to infections **(Alhussien and Dang, 2018).**

As heat stress factor**,** temperature and seasonal variations have a profound impact on the health and function of the mammary gland in dairy cows. High temperatures, particularly during the summer season, cause heat stress that adversely affects mammary epithelial cell proliferation and function, leading to reduced milk production and altered gland physiology. Heat stress during critical periods such as late gestation and the dry period impairs mammary gland development and remodeling, resulting in lower milk yield in subsequent lactations. Additionally, heat stress can disrupt immune cell activity within the gland, increasing susceptibility to infections and raising somatic cell counts, which indicate inflammation or mastitis **(Lengi et al. 2022)**.

Because of using antibiotics as a primary treatment in mastitis cases, the presence of antibiotic residues poses a significant food safety and dairy industry concerns, as it can lead to antimicrobial resistance (AMR) and health risks for consumers, beside that it can inhibit the effectiveness of starter culture in dairy production. So, effective monitoring programs and adherence to withdrawal periods are essential to minimize residue levels (**Forouzan et al. 2014)**.

CINCLUSION

In conclusion, this study underscores the seasonal variation in the prevalence and antimicrobial resistance of mastitis-causing bacteria in Qalubiya Governorate, Egypt. The higher incidence and resistance observed during the summer months highlight the role of heat stress and environmental factors in promoting bacterial growth and resistance. The identification of specific resistance genes in prevalent bacteria provides insights into the mechanisms driving antimicrobial resistance in the region. Furthermore, the study emphasizes the importance of geographical location and associated management practices in influencing the incidence of mastitis and SCC levels. These findings emphasize the necessity for targeted interventions, including improved hygiene practices, proper equipment sanitation, and strategic antibiotic use, to mitigate the impact of mastitis on buffalo milk production and quality, especially during the summer season.

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