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Clinical, Epidemiological, Bacteriological and Antimicrobial Resistance Studies on Bovine Mastitis in Menoufia Governorate

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ABSTRACT

Bovine mastitis globally is considered one of the most important diseases within dairy herds, mainly due to the associated economic losses. Mastitis can be classified as contagious and environmental mastitis. *Staphylococcus aureus, Escherichia coli, and Klebsiella pneumoniae are* the most common pathogens associated with mastitis cases. In this study, risk factors affecting the prevalence rate of clinical and subclinical mastitis were studied in 300 dairy cows. The clinical examination of 300 cows from El-Menoufia governorate revealed that 150 and 90 cows showed the clinical signs of acute and chronic mastitis, respectively. On the other hand, 60 cows showed no clinical signs and were positive in CMT and classified as subclinical mastitis. Bacteriological examination of 300 mastitis milk samples showed that 210 samples were positive in bacteriological culture (70 %). *Staphylococcus aureus* isolates are the most prevalent in clinical and subclinical mastitis cases. Sixty-two isolates were obtained from acute mastitis, 40 isolates from chronic mastitis, and 24 isolates from subclinical mastitis. On the other hand, 66 isolates of *E. coli* were obtained (52 isolates from acute mastitis, 11 isolates from chronic mastitis, and 3 from subclinical mastitis). The least causative agent isolates were *Klebsiella* species (a total of 18 isolates, from which 6 isolates were from acute mastitis, 9 isolates from chronic mastitis, and 3 from subclinical mastitis). Risk factor analysis showed that the El-Bagour region was the highest area affected by mastitis. The bacterial isolation percentages in relation to the stage of lactation showed the highest percentage at early lactation (31%), while the percentage of middle stage of lactation (22%), late stage of lactation (13%), and at the dry period (4%). From different breeds, the mixed breed was highly affected. The detection of antimicrobial resistance of the different isolates had been carried out in this study revealed that, among the randomly selected 126 *Staphylococcus aureus* isolates, showed high resistance to penicillin G, 66.6%, sulfamethoxazole/trimethoprim 57.1%, gentamicin 53.9%, chloramphenicol 51.5%. The highest rate of sensitivity was observed for spiramycin 80.1%, norfloxacin 59.5%, amoxicillin + clavulinic acid 57.1%, and ciprofloxacin 53.9%. Among the randomly selected 66 *E. coli* isolates, showed resistance to penicillin G by 86.3%, sulfamethoxazole/trimethoprim 57.5%, Tetracycline 56.06%. The highest rate of sensitivity observed was to Ciprofloxacin 84.8%, Norfloxacin 80.3%, Spiramycin 69.6%, chloramphenicol 62.1%, and amoxicillin+clavulinic acid 53%. On the other hand, among the randomly selected 18 *Klebsiella species*, these isolates showed resistance to amoxicillin + clavulinic acid 77.7%, Penicillin 72.2%, Spiramycin 72.2%, and Tetracycline 66.6%. The highest rate of sensitivity was observed for sulfamethoxazole/ trimethoprim, 72.2%, gentamycin, 61.1%, and Norfloxacin, 66.6%.

Keywords: Bovine mastitis, antimicrobial resistance, *S. aureus*, *Klebsiella*, *E. coli*, and Risk factors

INTRODUCTION

Bovine mastitis is defined as the inflammation of the mammary glands and is one of the most critical diseases affecting dairy herds worldwide. Its significant economic impact results not only from decreased milk production but also from increased culling rates (Sharun et al., 2021). Mastitis is primarily classified based on clinical presentation into clinical and subclinical forms, and etiologically into infectious and non-infectious causes. Infectious agents, particularly bacteria, are the most prevalent causative factors. These bacterial pathogens are further categorized as contagious, environmental, or opportunistic bacteria (Ndahetuye et al., 2019).

Multiple predisposing factors influence the incidence and prevalence of mastitis, including animal age, lactation stage, milking hygiene, environmental conditions, nutrition, and genetics. Around the calving period, high-producing dairy cows are particularly susceptible to environmental stressors related to housing, hygiene, and feeding, which may significantly increase disease occurrence early in lactation and adversely affect subsequent milk yield (Stanek et al., 2024). Additional risk factors include parity number, breed, and management practices. Notably, poor hygiene is significantly correlated with higher mastitis prevalence, highlighting the importance of improved hygiene and farm management to reduce infection rates (Djeddi et al., 2025).

Host-related risk factors encompass lactation stage, number of lactations, genetic makeup, peripartum-associated diseases, other health conditions such as lameness, breed differences, milk yield, spontaneous milk leakage between milkings, dry period management, and overall cleanliness of the dairy environment. Mastitis incidence is highest immediately after parturition and during early lactation. The prevalence also increases with successive lactations, associated with elevated somatic cell counts and higher culling rates. Genomic selection has emerged as a promising tool over the past decade to enhance herd health and performance by selecting for traits such as production, body conformation, somatic cell score, fertility, longevity, and health indices (Hisira et al., 2025).

Several studies identify *Staphylococcus aureus*, *Streptococcus* spp., *Escherichia coli*, and *Klebsiella pneumoniae* as the most common mastitis pathogens (Klaas & Zadoks, 2018; Ashraf & Imran, 2020; Cadona-Hernandez et al., 2021). Notably, some clinical *E. coli* mastitis cases observed during lactation result from infections acquired during the dry period, emphasizing this phase as critical for controlling *E. coli* mastitis (Berry & Hillerton, 2007).

Moreover, antimicrobial resistance has become an escalating global threat due to the widespread dissemination of resistance mechanisms. Conventional antibiotic treatments often lead to the emergence of resistant strains (Peng et al., 2022). Resistance to commonly used antimicrobials such as penicillin, amoxicillin, tetracycline, amikacin, gentamicin, and erythromycin has been extensively documented. Alarmingly, resistance to newer antibiotics—including piperacillin, ceftazidime, cefquinome, tigecycline, colistin, and vancomycin—is also on the rise. Additionally, concerns regarding antibiotic residues in milk are increasing (Bonardi et al., 2023).

Therefore, the aim of this study is to assess the prevalence of bovine mastitis in Menoufia Governorate, identify the causative bacterial agents, evaluate their antimicrobial susceptibility, and analyze the associated risk factors contributing to mastitis occurrence.

MATERIAL AND METHODS

Area of Study:

This study was conducted in nine centers of Menoufia Governorate: Shebeen El-Khoom, Menouf, Ashmoon, El-Bagoor, Berket El-Sabaa, El-Shohada, Quasna, Tala, and El-Sadat. Menoufia Governorate is located in the middle of the Nile Delta, bordered by El-Behera to the northwest, El-Kaliobia to the east, and Giza Province to the south. It covers an area of approximately 2,543.03 km².

Animal examination:

The study focused on dairy cows from various locations within Menoufia Governorate. Clinical examination followed the protocol described by Radostitis et al. (2007). The udder was visually inspected from behind, and the two hind quarters were examined for symmetry before and after milking. Abnormalities in udder size and consistency were assessed through palpation and inspection to detect signs of fibrosis, inflammatory swelling, or atrophy of mammary tissues. All quarters, including the milk cistern, supramammary lymph nodes, and teats, were palpated. Systemic signs such as fever, general depression, and anorexia were recorded. Housing conditions in the dairy farms varied; bedding materials included dust, sand, and rough concrete cement, with some farms having wet, muddy bedding and others dry bedding. Nutrition consisted mainly of concentrated feed, green fodder, and silage.

Sampling:

Milk samples were randomly collected from bovine farms in El-Menoufia Governorate presenting cases of clinical and subclinical mastitis. A total of 300 milk samples were obtained. Ten milliliters of milk were aseptically collected into sterile tubes, kept on ice, and transported immediately to the laboratory for bacteriological examination. Samples were collected from all four udder quarters.

California Mastitis Test (CMT):

Milk samples were screened for subclinical mastitis using the California Mastitis Test, following the method described by Schalm et al. (1971). The CMT reagent was procured from Kerbal® Company, Germany.

Bacteriological Isolation and Identification (Quinn et al., 2002):

Approximately 0.01 mL of each milk sample was cultured on different bacteriological media, including MacConkey agar, EMB agar, Paired Barker agar, and Mannitol salt agar. Plates were incubated at 37°C for 48 hours. Suspected colonies were examined for morphological characteristics, such as colony size, shape, color, pigment production, texture (smooth or rough), and lactose fermentation on MacConkey agar. Gram staining was performed on bacterial smears for microscopic examination. Biochemical identification followed the guidelines of Quinn et al. (2002). All culture media were supplied by Oxoid® Ltd., Basingstoke, UK.

Antibacterial Sensitivity Test (ClSI, 2024):

The antibiotic susceptibility of the predominant bacterial isolates (*E. coli*, *S. aureus*, and *Klebsiella* spp.) was assessed using the disk diffusion method, according to Clinical and Laboratory Standards Institute (CLSI, 2024) standards. Commercial antibiotic disks from Hi Media Laboratories Pvt. Ltd., India, and Oxoid® were used. Antibiotics tested included Amoxicillin-Clavulanic acid (20 µg), Penicillin (20 µg), Ciprofloxacin (20 µg), Gentamicin (10 µg), Streptomycin (10 µg), Erythromycin (20 µg), Norfloxacin (20 µg), Chloramphenicol (10 µg), and Tetracycline (20 µg).

Statistical Analysis

Prior to analysis, data were screened for outliers and missing values. No data were excluded due to unlikely values. Records with missing variables were excluded from analyses involving those variables. Statistical significance was set at $p = 0.05$. The distribution of intramammary infections (IMI) across herds was evaluated. Multivariate analysis was conducted using SPSS® software to examine interactions between the various factors studied.

RESULTS

Percentage of different types of mastitis among examined animals in El-Menoufia Governorate:

Among the 300 cows diagnosed with mastitis in El-Menoufia Governorate, clinical examination revealed that 150 cows (50%) exhibited signs consistent with acute mastitis, while 90 cows (30%) showed clinical signs indicative of chronic mastitis. The remaining 60 cows (20%) displayed no visible clinical signs but tested positive using the California Mastitis Test (CMT) and were therefore classified as cases of subclinical mastitis (Table 1).

Table (1): Percentage of different types of mastitis among examined cows in El-Menoufia Governorate:

Total examination of cattle	Types of mastitis	No	%
Clinical mastitis	**Acute clinical Mastitis**	150	50%
	Chronic clinical mastitis	90	30%
Subclinical mastitis		60	20%
Total		300	

The acute form of mastitis was characterized by systemic signs including fever, anorexia, and depression, along with marked local changes in the affected udder quarter such as heat, swelling, pain, and redness. Milk from affected quarters showed visible abnormalities, appearing reddish, yellowish, thick, caseated, or viscid. In contrast, chronic mastitis presented without systemic signs but exhibited local changes such as fibrosis, induration, hardness, and atrophy of the udder tissue. Milk from these cases was typically scant and watery, often containing flakes. Subclinical mastitis cases were identified using the California Mastitis Test (CMT), usually after the owner reported a noticeable decline in milk yield.

Percentage of S. aureus, E. coli and Klebsiella spp. causing clinical and subclinical bovine mastitis:

Out of 300 mastitic milk samples examined bacteriologically, 210 samples (70%) yielded positive cultures, while 90 samples (30%) showed no bacterial growth. *Staphylococcus aureus* was the most frequently isolated pathogen in both clinical and subclinical mastitis cases, with 62 isolates from acute mastitis, 40 from chronic mastitis, and 24 from subclinical mastitis. This was followed by *Escherichia coli*, which was identified in 66 cases—52 from acute mastitis, 11 from chronic, and 3 from subclinical cases. *Klebsiella* species were isolated in 18 samples, including 6 from acute mastitis, 9 from chronic mastitis, and 3 from subclinical mastitis (Table 2).

Table (2): Percentage of *S. aureus, E-coli* and *Klebsiella* spp. isolated from mastitic milk.

Isolates	Acute mastitis (No=150)		Chronic mastitis (No=90)		Subclinical mastitis (No=60)	
	No	**%**	**No**	**%**	**No**	**%**
S. aureus	62	**20.6%**	40	**13.3%**	24	**8%**
E.coli	52	**17.3 %**	11	**3.6%**	3	**1%**
Klebsiella species	6	**2%**	9	**3%**	3	**1%**
Total	120	**40%**	60	**20%**	30	**10%**
X^2*	***59.93***		***38.7***		***35.28***	
***P* value**	< 0.001		< 0.001.		< 0.001	

*X^2: The chi-square showed a significant correlation between the types of isolated bacteria and mastitis type.

<u>Effect of locality as a risk factor affecting the percentage of mastitis in El-Menoufia Governorate</u>.

A total of 300 mastitic milk samples were collected from cows across nine different centers in El-Menoufia Governorate to evaluate the effect of locality as a risk factor for mastitis. Bacteriological examination revealed varying rates of clinical and subclinical mastitis across locations. The distribution was as follows: El-Bagour (34 clinical, 16 subclinical), Ashmoun (32 clinical, 13 subclinical), Menouf (36 clinical, 14 subclinical), Shebin El-Kom (37 clinical, 8 subclinical), El-Sadat City (22 clinical, 8 subclinical), Berket El-Sabaa (9 clinical, 11 subclinical), Tala (12 clinical, 13 subclinical), El-Shohada (13 clinical, 2 subclinical), and Qwiesna (15 clinical, 5 subclinical). Statistical analysis using the chi-square test yielded a value of 11.65 with a p-value of 0.020, indicating a statistically significant association ($p \leq 0.05$) between locality and the type of mastitis diagnosed (Table 3).

Table (3): Effect of locality as a risk factor affecting the percentage of mastitis in El-Menoufia Governorate.

Region*	**No. of + samples**	**%**	**Clinical mastitis**	**%**	**Subclinical mastitis**	**%**
1- El-Bagour	50	16.6%	34	11.3%	16	5.3%
2- Ashmoun	45	15 %	32	10.6%	13	4.3%
3- Menouf	50	16.6%	36	12%	14	4.6%
4- Shebin El-kom	45	15%	37	12.3%	8	2.6%
5- El-Sadat city	30	10%	22	7.3%	8	2.6%
6- Brekit El-Sabaa	20	6.6%	9	3%	11	3.6%
7- Tala	25	8.3%	12	4%	13	4.3%
8- El-Shohada	15	5%	13	4.3%	2	0.6%
9- Qwiesna	20	6.6%	15	5%	5	1.6%
Total	300		210		90	

*The chi-square statistic is 11.651235. The *p*-value is $p = .020142$. Result significant at $p \leq .05$.

<u>The percentage of bacterial strains in relation to different centers in El-Menoufia Governorate.</u>

The distribution of bacterial mastitis cases across various centers in El-Menoufia Governorate was analyzed based on the identified pathogens. In El-Bagour, 29 cases were positive for *S. aureus* and 11 for *E. coli*. In Ashmoun, there were 21 cases of *S. aureus*, 8 of *E. coli*, and 3 of *Klebsiella* species. Menouf reported 25 *S. aureus*, 16 *E. coli*, and 5 *Klebsiella* isolates. In Shebin El-Kom, 24 cases were due to *S. aureus*, 11 to *E. coli*, and 2 to *Klebsiella*. El-Sadat City showed 12 *S. aureus*, 8 *E. coli*, and 2 *Klebsiella* cases. Berket El-Sabaa had 5 *S. aureus*, 3 *E. coli*, and 1 *Klebsiella* case. In Tala, there was 1 case of *S. aureus* and 5 of *E. coli*. El-Shohada reported 6 *S. aureus* and 2 *Klebsiella* cases, while Qwiesna had 3 *S. aureus*, 4 *E. coli*, and 3 *Klebsiella* isolates (Table 4).

Table (4): Percentage of each bacterial strain in each center of El-Menoufia governorate.

Region	Total Sample No	No .of *S. aureus*	%	No. of *E.coli*	%	No of *Klebsiella species*	%
1- El - Bagour	50	29	9.6%	11	3.6%	0	0%
2- Ashmoun	45	21	7%	8	2.6%	3	1%
3- Menouf	50	25	8.3%	16	5.3%	5	1.6%
4- Shebin El-kom	45	24	8%	11	3.6%	2	0.6%
5- El-Sadat	30	12	4%	8	2.6%	2	0.6%
6- Brekit El –Sabaa	20	5	1.6%	3	1%	1	0.3%
7- Tala	25	1	0.3%	5	1.6%	0	0%
8- El-Shohada	15	6	2%	0	0%	2	0.6%
9- Quewisna	20	3	1%	4	1.3%	3	1%
Total	300	126	42%	66	22%	18	6%

Effect of age of mastitic cows as a risk factor affecting the percentage of bacterial mastitis in El-Menoufia Governorate:

The cows in the study were classified into three age groups. The first group consisted of cows older than 7 years, among which mastitis cases were distributed as 36% acute, 34.4% chronic, and 25% subclinical. The second group included cows aged 5 to 7 years, with 37.3% of cases classified as acute, 17.7% as chronic, and 55% as subclinical mastitis. The third group, comprising cows aged 2 to 5 years, showed 26.6% acute, 24.4% chronic, and 20% subclinical mastitis cases. These findings indicate that older cows are at a higher risk of developing mastitis. Statistical analysis using the chi-square test yielded a value of 26.48 with a p-value of 0.000025, indicating a highly significant association between age and mastitis incidence ($p \leq 0.05$) (Table 5).

Table (5): Percentage of different types of mastitis among examined cows in relation to animal age:

Total examination of cattle	No	Age*	Number	%
Clinical mastitis	150	(2-5) years	40	26.6%
		(5-7) years	56	37.3%
		More than 8 years	54	36%
	90	(2-5) years	22	24.4%
		(5-7) years	16	17.7%
		More than 8 years	52	34.4%
Subclinical mastitis	60	(2-5) years	12	20%
		(5-7) years	33	55%
		More than 8 years	15	25%
Total	300		300	

*The chi-square statistic is 26.478461. The p-value is $p = .000025$. The result is significant at $p \leq .05$.

Effect of quarter as a risk factor affecting the percentage of bacterial mastitis in El-Menoufia Governorate:

The animals were classified according to the udder quarter affected by mastitis. In the right fore quarter, there were 20 cases of acute mastitis, 11 cases of chronic mastitis, and 13 cases of subclinical mastitis. The left fore quarter showed 24 cases of acute mastitis, 8 chronic, and 7 subclinical. In the right hind quarter, 41 cases of acute mastitis, 23 chronic, and 8 subclinical were recorded. The left hind quarter had 35 cases of acute mastitis, 18 chronic, and 2 subclinical mastitis cases (Table 6)**.**

Table (6): Effect of quarter as a risk factor affecting the percentage of bacterial mastitis in El-Menoufia Governorate.

Udder Quarter	**Acute Mastitis**	**Chronic Mastitis**	**Subclinical Mastitis**
Right Fore Quarter	20	11	13
Left Fore Quarter	24	8	7
Right Hind Quarter	41	23	8
Left Hind Quarter	35	18	2
X^2*	15.70		
***P* value**	.015		

*The chi-square statistic is 15.7048. The p-value is .01543. The result is significant at $p < .05$.

Effect of stage of lactation as a risk factor affecting on percentage of mastitic milk samples collected from El-Menoufia Governorate

The sampled animals were categorized into four groups based on their stage of lactation: Group 1 (early lactation), Group 2 (mid-lactation), Group 3 (late lactation), and Group 4 (dry period). The prevalence of bacterial mastitis in these groups was 31%, 22%, 13%, and 4%, respectively (Table 7).

Table (7): Effect of stage of lactation as a risk factor affecting on percentage of mastitic milk samples collected from El-Menoufia Governorate

Stage of lactation	No. of examined cows	No. Of positive samples	%
Early stage of the lactation (0- 2 months after parturition)	115	93	31
Middle stage of lactation (2-5 months after parturition)	92	66	22
Late stage of lactation (5 months until dryness)	55	39	13
Dry period (from 40- 50 days before parturition)	38	12	4
X^2*	33.33		
P value	< 0.001		

*The chi-square statistic is 33.3359. The *p*-value is < 0.00001. The result is significant at $p < .05$.\

Effect of cattle breed as a risk factor affecting the percentage of bacterial mastitis in El-Menoufia Governorate

The effect of cattle breed as a risk factor for bacterial mastitis was evaluated by classifying sampled animals into three groups: Balady breed (45 mastitic cows), mixed breed (160 mastitic cows), and pure breed (36 mastitic cows). Among these, 90 Balady, 160 mixed, and 50 pure-breed cows were examined for bacterial mastitis (Table 8).

Table (8): Effect of cattle breed as a risk factor affecting the percentage of bacterial mastitis in El-Menoufia Governorate.

Cattle breed	No. of examined cows	No. Of positive samples	%
Balady breed	90	45	15
Mixed breed	160	129	43
Pure breed	50	36	12
Total	300	210	70
X^2*	25.83		
P value	< 0.001		

*The chi-square statistic is 25.8393. The *p*-value is < 0.00001. The result is significant at $p < .05$.

Cumulative Impact of Risk Factors on Mastitis Incidence and Bacterial Isolation in Dairy Cows of El-Menoufia Governorate

The combined effects of various risk factors on mastitis incidence were evaluated (Tables 9 and 10). Statistical analysis using ordinary logistic regression revealed significant associations between bacterial isolation and the stage of lactation. Specifically, bacterial isolation was three times more likely in acute mastitis cases compared to chronic or subclinical cases. The stage of lactation also significantly influenced mastitis incidence: the late lactation stage was strongly associated with acute mastitis, whereas the dry period and early lactation stages were predominantly linked to chronic and subclinical mastitis.

Additionally, geographic location significantly affected mastitis incidence, with Tala center exhibiting the highest rate among all surveyed centers. The analysis further showed that bacterial isolation in chronic mastitis was 1.2 times higher than in subclinical mastitis, while in acute mastitis it was 3.68 times greater compared to subclinical cases. Regarding cattle breed, mixed breeds showed a 2.4-fold increase in the likelihood of chronic mastitis compared to subclinical mastitis. The influence of lactation stage on mastitis incidence was also evident: early and mid-lactation stages recorded the highest incidence of subclinical mastitis, whereas both dry and late lactation stages were associated with increased chronic mastitis. Acute mastitis incidence was 1.7 times higher than subclinical mastitis during these stages.

Table (9): Analysis of some risk factors associated with bovine mastitis

Parameter		B	Std. Error	95% Wald Confidence Interval		Hypothesis Test			Exp(B)	95% Wald Confidence Interval for Exp(B)	
				Lower	Upper	Wald Chi-Square	df	Sig.		Lower	Upper
Threshold	[mastitis=1]	.145	.7825	-1.389	1.678	.034	1	.853	1.156	.249	5.357
	[mastitis=2]	1.620	.7886	.074	3.165	4.218	1	.040	5.051	1.077	23.696
Bacteria		.991	.2593	.483	1.499	14.612	1	.000	2.694	1.621	4.478
Locality		.081	.0460	-.009	.171	3.115	1	.078	1.085	.991	1.187
Age		.048	.1840	-.313	.409	.067	1	.795	1.049	.731	1.505
Quarter		-.083	.0986	-.276	.110	.709	1	.400	.920	.759	1.117
Stage		-.311	.1143	-.535	-.087	7.423	1	.006	.732	.585	.916
Breed		.132	.1553	-.172	.437	.723	1	.395	1.141	.842	1.547
(Scale)		1[a]									

Dependent Variable: mastitis
Model: (Threshold), bacteria, locality, age, quarter, stage, breed

a. Fixed at the displayed value.

Table (10): The effect of some factors in relation to the incidence of mastitis

mastitis[a]		B	Std. Error	Wald	df	Sig.	Exp(B)	95% Confidence Interval for Exp(B) Lower Bound	Upper Bound
2	Intercept	-2.020	1.217	2.755	1	.097			
	bacteria	.239	.397	.361	1	.548	1.270	.583	2.766
	Locality	.072	.074	.962	1	.327	1.075	.930	1.242
	Age	.384	.281	1.860	1	.173	1.468	.845	2.549
	Quarter	.146	.172	.722	1	.395	1.158	.826	1.622
	Stage	-.703	.182	14.941	1	.000	.495	.347	.707
	Breed	.902	.258	12.225	1	.000	2.465	1.487	4.088
3	Intercept	-1.770	1.094	2.615	1	.106			
	bacteria	1.304	.366	12.687	1	.000	3.683	1.798	7.548
	Locality	.105	.068	2.390	1	.122	1.111	.972	1.269
	Age	.163	.248	.430	1	.512	1.177	.723	1.915
	Quarter	-.063	.161	.150	1	.698	.939	.685	1.289
	Stage	-.566	.159	12.710	1	.000	.568	.416	.775
	Breed	.561	.229	5.969	1	.015	1.752	1.117	2.746

a. The reference category is: 1.

Antimicrobial sensitivity of each bacterial strain isolated from mastitis milk:

Among 126 randomly selected *Staphylococcus aureus* isolates, high resistance rates were observed to penicillin G (66.6%), sulfamethoxazole/trimethoprim (57.1%), gentamicin (53.9%), and chloramphenicol (51.5%). Conversely, the highest sensitivity rates were recorded for spiramycin (80.1%), norfloxacin (59.5%), amoxicillin-clavulanic acid combination (57.1%), and ciprofloxacin (53.9%) (Table 11).

Of the 66 *Escherichia coli* isolates tested, resistance was most notable against penicillin G (86.3%), sulfamethoxazole/trimethoprim (57.5%), and tetracycline (56.1%). The greatest sensitivity was observed to ciprofloxacin (84.8%), norfloxacin (80.3%), spiramycin (69.6%), chloramphenicol (62.1%), and amoxicillin-clavulanic acid (53%) (Table 12).

Among 18 *Klebsiella* species isolates, resistance was highest against amoxicillin-clavulanic acid (77.7%), penicillin G (72.2%), spiramycin (72.2%), and tetracycline (66.6%). The highest sensitivity rates were found for sulfamethoxazole/trimethoprim (72.2%), gentamicin (61.1%), and norfloxacin (66.6%) (Table 13).

Table (11): Antimicrobial sensitivity test of *S. aureus* isolates recovered from milk samples of cows.

Antimicrobial agent	Conc	Sensitive		Intermediate		Resistant	
		No.	%	No.	%	No.	%
amoxicillin+clavulinic acid	Amc	72	57.1%	22	17.4%	32	25.3%
penicillin G	P	14	11.1%	28	22.2%	84	66.6%
Ciprofloxacin	Cip	68	53.9%	32	25.3%	26	20.6%
Gentamicin	CN	24	19.0%	34	26.9%	68	53.9%
sulfamethoxazole/trimethopr	SXT	33	26.1%	21	16.6%	72	57.1%
Spiramycin	S	101	80.1%	15	11.9%	10	7.9%
Norfloxacin	Nor	75	59.5%	21	16.6%	30	23.8%
Chloramphenicol	C	34	26.9%	27	21.4%	65	51.5%
Tetracycline	TE	60	47.6%	25	19.8%	41	32.5%

Table (12): Antimicrobial sensitivity test of *E. coli* isolates.

Antimicrobial agent	Con	Sensitive		Intermediate		Resistant	
		No.	%	No.	%	No.	%
amoxicillin+clavulinic acid	Amc	35	53%	5	7.57%	26	39.3%
penicillin G	P	7	10.6%	2	3.03%	57	86.3%
Ciprofloxacin	Cip	56	84.8%	5	7.57%	5	7.57%
Gentamicin	CN	50	75.7%	5	7.57%	11	16.6%
sulfamethoxazole/trimethoprir	SXT	20	30.3%	8	12.1%	38	57.5%
Spiramycin	S	46	69.6%	5	7.5%	15	22.7%
Norfloxacin	NOR	53	80.3%	4	6.06%	9	13.6%
chloramphenicol	C	41	62.1%	14	21.2%	11	16.6%
Tetracycline	TE	25	37.8%	4	6.06%	37	56.06%

Table (13): Antimicrobial sensitivity test of *Klebsiella species* isolates

Antimicrobial agent	Conc	Sensitive		Intermediate		Resistant	
		No.	%	No.	%	No.	%
amoxicillin+clavulinic acid	AMc	3	16.6%	1	5.5%	14	77.7%
Penicillin G	P	2	11.1%	3	16.6%	13	72.2%
Ciprofloxacin	Cip	4	22.2%	2	11.1%	12	66.6%
Gentamicin	CN	11	61.1%	2	11.1%	5	27.7%
sulfamethoxazole/trimethoprim	SXT	13	72.2%	1	5.5%	4	22.2%
Spiramycin	S	2	11.1%	3	16.6	13	72.2%
Norfloxacin	NOR	12	66.6%	1	5.5%	5	27.7%
Chloramphenicol	C	6	33.3%	2	11.1%	10	55.5%
Tetracycline	TE	4	22.2%	2	11.1%	12	66.6%

DISCUSSION

Effective control of mastitis relies heavily on the early detection of inflammation, particularly for subclinical mastitis (SCM), which often goes unnoticed without sensitive diagnostic methods. Timely identification and management of SCM can significantly reduce the disease burden in dairy herds. In this study, 300 mastitic cows from El-Menoufia Governorate were clinically examined. Among them, 150 cows (50%) exhibited acute mastitis signs, 90 cows (30%) showed chronic mastitis, while 60 cows (20%) were asymptomatic but tested positive by the California Mastitis Test (CMT), classifying them as subclinical cases.

The clinical presentation of acute mastitis included systemic signs such as fever, anorexia, and depression, alongside pronounced local udder inflammation characterized by heat, swelling, pain, and redness. Milk abnormalities such as reddish or yellowish discoloration, increased viscosity, and presence of clots were also observed. These findings align with the observations of Sayed and Rady (2008), who described similar acute clinical manifestations including swelling, firmness, and altered milk consistency. Chronic mastitis was characterized by udder fibrosis, induration, hardness, and atrophy, with milk secretion reduced to watery drops containing flakes, but without systemic signs. These observations corroborate Ibrahim’s (2017) report on chronic mastitis pathology. Subclinical cases were detected mainly through CMT following owners' complaints of reduced milk yield, consistent with the findings of Abed et al. (2021), who highlighted the economic importance of subclinical mastitis globally.

Bacteriological culture remains the gold standard for pathogen identification in mastitis. Of the 300 milk samples cultured, 210 (70%) yielded positive bacterial growth, with 90 samples testing negative. *Staphylococcus aureus* was the predominant isolate, recovered from 62 acute, 40 chronic, and 24 subclinical mastitis cases, followed by *Escherichia coli* (66 isolates) and *Klebsiella* species (18 isolates). These results concur with Paulin-Curlee et al. (2007) and Bertolami et al. (2015), who emphasized the role of *S. aureus* as a major mastitis pathogen. Similarly, the presence of coliform bacteria such as *E. coli* and *Klebsiella* spp. aligns with Goulart et al. (2022), highlighting their environmental ubiquity and pathogenic potential.

Age was a significant risk factor in this study. Cows older than seven years demonstrated the highest prevalence of mastitis, with 36% acute, 34.4% chronic, and 25% subclinical infections. Middle-aged cows (5–7 years) had a lower chronic mastitis prevalence but a higher subclinical rate (55%). Younger cows (2–5 years) showed comparatively lower incidence rates. These findings are supported by Dego and Tareke (2003) and Abera et al. (2013), who reported increased mastitis risk with advancing age due to factors like teat canal dilation and immune senescence.

The lactation stage also influenced mastitis occurrence. Early lactation (0–2 months postpartum) recorded the highest incidence (31%), followed by mid-lactation (22%), late lactation (13%), and the dry period (4%). The elevated susceptibility during early lactation may be attributed to physiological stress and immunosuppression around parturition, as noted by Santos et al. (2004) and Mungube et al. (2004).

Breed differences were observed with mixed breeds showing a 43% mastitis incidence, Balady breeds 15%, and pure breeds 12%. This contrasts with findings by Curone et al. (2018), who reported lower clinical mastitis in some native breeds, likely due to genetic resistance. The higher susceptibility of high-yielding breeds, such as Holstein-Friesians, compared to low-producing breeds like Jersey cattle has been documented by Shaheen et al. (2015) and Washburn et al. (2002).

Antimicrobial susceptibility testing revealed significant resistance among isolates. Among 126 *S. aureus* strains, high resistance was observed to penicillin G (66.6%), sulfamethoxazole/trimethoprim (57.1%), gentamicin (53.9%), and chloramphenicol (51.5%). Highest sensitivity was noted against spiramycin (80.1%), norfloxacin (59.5%), amoxicillin-clavulanic acid (57.1%), and ciprofloxacin (53.9%). These findings are consistent with Gundogan et al. (2014).

Among 66 *E. coli* isolates, resistance rates were highest for penicillin G (86.3%), sulfamethoxazole/trimethoprim (57.5%), and tetracycline (56.1%), while ciprofloxacin (84.8%), norfloxacin (80.3%), and spiramycin (69.6%) showed the highest sensitivity. Similar patterns have been reported by Gundogan et al. (2014) and Bag et al. (2021), with variable resistance rates due to antimicrobial usage patterns.

For 18 *Klebsiella* isolates, resistance was high against amoxicillin-clavulanic acid (77.7%), penicillin G (72.2%), spiramycin (72.2%), and tetracycline (66.6%), while sensitivity was notable for sulfamethoxazole/trimethoprim

(72.2%), gentamicin (61.1%), and norfloxacin (66.6%). These results align with Osman et al. (2014) and Wu et al. (2022), though discrepancies with other studies (Enferad & Mahdavi, 2020) suggest methodological differences in susceptibility testing may influence resistance profiles (Schwarz et al., 2010).

This study's findings emphasize the need for integrated mastitis control programs encompassing improved hygiene, regular screening (including CMT), strategic antimicrobial use guided by susceptibility testing, and genetic improvement strategies. Future research should focus on molecular characterization of pathogens to understand virulence and resistance mechanisms, alongside intervention trials assessing management practices' effectiveness. Additionally, educating farmers on mastitis prevention and rational antibiotic use is critical to reduce disease incidence and AMR risks.

CONCLUSION

Mastitis remains one of the most significant diseases causing substantial economic losses in Egypt's dairy industry. This study revealed a high prevalence of multidrug-resistant strains of *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella* species isolated from mastitic cows in El-Menoufia Governorate, particularly against commonly used antibiotics. These findings underscore the urgent need to regulate antibiotic use and enhance awareness among veterinarians and farmers regarding antimicrobial resistance risks. To improve treatment outcomes and mitigate resistance development, antibiotic therapy for mastitis should be guided by antimicrobial susceptibility testing prior to administration.

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DISCLOSURE OF POTENTIAL CONFLICT OF INTEREST

The authors state that they have no conflicts of interest.

ETHICAL APPROVAL

This study adheres to the ethical guidelines established by the Faculty of Veterinary Medicine, El-Sadat city University, Egypt.

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