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Evaluation of veterinary antibiotic residues in commercial and local farms in Al-Bayda city, Libya (2024-2025), with a molecular docking study to analyze their effect on target microbial proteins

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تقييم بقايا المضادات الحيوية البيطرية في المزارع التجارية والمحلية بمدينة البيضاء، ليبيا (2024-2025)، مع دراسة الالتحام الجزيئي لتحليل تأثيرها على البروتينات الميكروبية المستهدفة.

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Abstract:
This study detected veterinary antibiotic residues (neomycin, penicillin, enrofloxacin) in 400 Libyan samples (200 eggs, 200 milk) using microbial inhibition and chromatographic techniques. Results showed significantly higher residues ($p \leq 0.05$) in local vs. commercial farms, with minimal reduction after boiling. Penicillin showed highest residues (milk: 55.23µg/ml local, 8.86µg/ml commercial; eggs: 46.88µg/g local, 11.44µg/g commercial). Molecular docking confirmed penicillin's strong ubiquitin binding (-5.7 kcal/mol), correlating with experimental data and validating computational approaches for residue prediction. Findings emphasize the need for stricter veterinary oversight and withdrawal period compliance.

Keywords: antibiotic, eggs, milk, Neomycin, penicillin, enrofloxacin, Al-Bayda city.

الملخص
كشفت هذه الدراسة عن بقايا المضادات الحيوية البيطرية (نيومايسين، بنسلين، إنروفلوكساسين) في 400 عينة ليبية (200 بيضة، 200 حليب) باستخدام تقنيات التثبيط الميكروبي والكروماتوغرافي. أظهرت النتائج ارتفاعًا ملحوظًا في البقايا ($p \leq 0.05$) في المزارع المحلية مقارنةً بالمزارع التجارية، مع انخفاض طفيف بعد الغليان. أظهر البنسلين أعلى نسبة من البقايا (الحليب: 55.23 ميكروغرام/مل محلي، 8.86 ميكروغرام/مل تجاري؛ البيض: 46.88 ميكروغرام/غم محلي، 11.44 ميكروغرام/غم تجاري). أكد الالتحام الجزيئي قوة ارتباط البنسلين باليوبيكويتين (-5.7 كيلو كالوري/مول)، وهو ما يتوافق مع البيانات التجريبية ويثبت صحة الأساليب الحسابية للتنبؤ بالبقايا. تؤكد النتائج على ضرورة تشديد الرقابة البيطرية والالتزام بفترة السحب.

الكلمات المفتاحية: مضاد حيوي، بيض، حليب، نيوميسين، بنسلين، انروفلوكساسين، مدينة البيضاء.

Introduction

Antibiotic residues are found in food products of animal origin, such as eggs, milk, and meat. Many countries have acknowledged the presence of these residues in their products due to indiscriminate and repeated use [1]. Excessive use of antibiotics such as penicillin in the breast during infections and failure to adhere to the withdrawal period can lead to drug residues in the milk [2]. The frequent use of antibiotics in animals and their presence in foods such as meat, dairy and eggs causes what is known as microbial resistance to these drugs, as well as causing serious harm to human health such as tumors [3, 4]. It is necessary to produce animal-based foods free of antibiotic residues by following strict procedures and following the instructions of a specialist doctor [5, 6]. There are several methods currently used to detect antibiotic residues in food, such as penicillin and enrofloxacin, and these are available in most laboratories, including chromatography, microbiology, and immunology [7]. The advanced techniques used in this study are among the most accurate analyses, especially the (TLC) and (HPLC) techniques, and the electrophoresis (CE). Therefore, they are among the successful techniques in detecting drug residues. [8]. Unfortunately, the random and repeated use of medications without a prescription in this country, without taking into account the withdrawal period or adhering to the procedures followed for the process of using the medication, has led to harm to human health.

Material and methods

Raw milk samples were collected from breeding and egg farms: from several locally reared farms scattered throughout the city of Al-Bayda, Libya, and some neighboring areas, as well as from commercial farms believed to be implementing strict health measures. All samples to be tested were transported to the laboratory using internationally agreed-upon methods via an icebox It must be stored at 6°C until the levels of veterinary drug residues targeted in the study are evaluated. The same transportation method was used to collect egg samples targeted for testing from locally reared poultry farms in Al-Bayda and commercial chicken farms in Al-Bayda and its suburbs. The milk and yolk were then homogenized, and approximately 5 ml of the The suspension must be boiled for 30 continuous minutes. [9]. Evaluation of the target sample in the study by microbial inhibition test followed by TLC test to detect the presence of veterinary antibiotic residues. [10]. A number of test-positive samples, representing the highest TLC score, were analyzed using HPLC to determine the concentration or residue level of the target veterinary antibiotics on the study farms [11].

Results

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Neomycin Residues

Of the 200 milk samples tested, neomycin residues were found in 11% of the milk from commercial cows in Al Bayda, a much lower percentage than that found in the milk of cows raised on local informal farms (22%).Similar results were found in the TLC test. After boiling commercial raw cow milk from Al-Bayda farms, the percentage of neomycin antibiotic residues was 11% and 9% (TLC test). In local raw dairy milk, the percentage of neomycin antibiotic residues was 20% and 22% (TLC test). Boiling the study samples did not change the previous results for veterinary antibiotic residues obtained by the Veterinary Teaching Hospital laboratory and the TLC test. In the TLC test, very little difference was observed in the MIT test results, regardless of the target samples before and after boiling (Table 1). Of the 200 egg samples collected for measurement, all test results conducted in the laboratory of the Veterinary Teaching Hospital of Omar Al-Mukhtar University, Libya, showed that residues of veterinary antibiotics from the neomycin group were detected in 6% of the commercially reared egg samples from some farms in Al-Bayda city, 24% in eggs from local farms with random rearing, 4% in eggs from commercial farms prepared for testing, and 23% in samples from local farms with randomly reared boiled eggs (Table 1). The percentage of neomycin-positive samples that were subjected to TLC testing from eggs from commercially reared eggs, eggs from local farms, eggs from commercially reared randomly reared boiled eggs, and eggs from local farms with randomly reared eggs that even depend on boiled animal residues in their diet was 6%, 24%, 3%, and 23%, respectively (Table 2).

Penicillin Residues

Penicillin residues were detected in commercial raw milk (13%), raw milk from randomly reared farms (37%), boiled commercial milk (11%), and boiled local milk (36%). By TLC, penicillin was also detected in commercial raw milk (12%), local milk (34%), boiled commercial milk (11%), and boiled local milk (33%), as shown in Table 3. The penicillin residue rate was higher in local cow samples than in commercial cow milk samples, whether boiled or not. Among all samples, penicillin residues were more common in locally reared eggs (16%, for both raw and boiled samples) than in commercial eggs. Identical results were recorded for all samples tested using TLC (Table 4), with more residues detected in local egg samples than in commercial egg samples.

Enrofloxacin Residues

In all tested samples, residues of the veterinary antibiotic enrofloxacin were predominant in the local randomly reared milk sample (16% raw milk and 15% boiled milk) compared to the commercial milk sample (7% raw milk and 4% boiled milk). The results of both Omar Al-Mukhtar Veterinary Teaching Hospital and TLC testing were almost identical as shown in Table 5.

Table 1: Comparison of veterinary antibiotic neomycin residues in raw milk samples between local and commercial cows in Al Bayda, Libya.

Thin layer chromatography (%)

simple	Raw milk sample after boiling	Raw milk sample before boiling
Commercial dairy cows	(9)	(8)
Rural local cows	(21)	(20)

[1] ***Sample (raw milk): n=100***

Microbial inhibition test (%)

Simple	Raw milk sample after boiling	Raw milk sample before boiling
Commercial dairy cows	(11)	(11)
Rural local cows	(22)	(22)

Table 2: Comparison of residues of the veterinary antibiotic neomycin in egg samples from local and commercial chicken farms in Al Bayda city and its suburbs.

Thin layer chromatography (%)

Simple	Egg sample after boiling	Egg sample before boiling
Local rural chickens	(6)	(3)
Laying hens on commercial farms	(24)	(23)

[1] ***Sample (egg): n=100***

Microbial inhibition test (%)

Simple	Egg sample after boiling	Egg sample before boiling
Local rural chickens	(6)	(4)
Laying hens on commercial farms	(24)	(23)

Table 3: Comparison of veterinary antibiotic penicillin residues in raw milk samples between local and commercial cows in Al Bayda, Libya.

Thin layer chromatography (%)

Simple	Raw milk sample after boiling	Raw milk sample before boiling
Commercial dairy cows	(12)	(11)
Rural local cows	(34)	(33)

[1] ***Sample (raw milk): n=100***

Microbial inhibition test (%)

simple	Raw milk sample after boiling	Raw milk sample before boiling
Commercial dairy cows	(13)	(11)
Rural local cows	(37)	(36)

Table 4: Comparison of penicillin antibiotic residues in egg samples from local and commercial chicken farms in Al Bayda city and its suburbs.

Thin layer chromatography (%)

simple	Egg sample after boiling	Egg sample before boiling
Local rural chickens	(4)	(3)
Laying hens on commercial farms	(16)	(15)

[1] **Sample (raw milk): n=100**

Microbial inhibition test (%)

simple	Egg sample after boiling	Egg sample before boiling
Local rural chickens	(4)	(3)
Laying hens on commercial farms	(16)	(16)

In 200 egg samples tested at the laboratories of the Veterinary Teaching Hospital of Omar Al-Mukhtar University, Libya, enrofloxacin residues were detected in 46% of local eggs from rural farms that rely on open barns and feed on animal remains, 8% in eggs from commercial egg production farms, 5% in local eggs that were boiled, and 42% in eggs from commercial farms that were boiled. On the other hand, in TLC, neomycin residues were detected in positive samples of local eggs from rural farms in Al-Bayda, Libya, commercial eggs from egg production farms, and local eggs that were Boiled and commercial eggs from farms in Al Bayda city were 8%, 34%, 7%, and 45%, respectively (Table 6). Enrofloxacin residue levels were significantly higher in locally farmed rural eggs than in eggs from commercial egg production farm.
Comparison of penicillin residues in raw milk using HPLC. The average concentration of penicillin residues was significantly higher in local rural raw milk (56.16 µg/ml) than in raw milk from commercial farms dedicated to raw milk production (9.84 µg/ml). After boiling at 100% temperature, the average concentration of penicillin decreased slightly in both local raw milk (55.54 µg/ml) and commercial raw milk from farms in Al-Bayda city (9.8 µg/ml), as shown in Table 7 shows a comparison of penicillin residues in eggs using HPLC. The average concentration of penicillin residues in local laying hens raised on rural random-rearing farms (48.82 µg/g) was significantly higher than in commercial egg-laying hens in Al-Bayda city (10.46 µg/g). After boiling all samples prepared for testing, the results remained the same for both rural and commercial eggs (Table 8).

Table 5: Comparison of enrofloxacin residues in milk samples from local and commercial cows in Al Bayda, Libya.

Thin layer chromatography (%)

simple	Raw milk sample after boiling	Raw milk sample before boiling
Commercial dairy cows	(6)	(4)
Rural local cows	(14)	(1)

[1] Sample (raw milk): n=100

Microbial inhibition test (%)

Simple	Raw milk sample after boiling	Raw milk sample before boiling
Commercial dairy cows	(6)	(4)
Rural local cows	(16)	(15)

Table 6: Comparison of enrofloxacin residues in eggs from local and commercial chicken farms in Al Bayda city and its suburbs.

Thin layer chromatography (%)

simple	Egg sample after boiling	Egg sample before boiling
local rural chickens	(8)	(6)
Laying hens on commercial farms	(44)	(43)

[1] Sample (raw milk): n=100

Microbial inhibition test (%)

simple	Egg sample after boiling	Egg sample before boiling
local rural chickens	(8)	(5)
Laying hens on commercial farms	(46)	(42)

Table 7: Comparison of penicillin residues in milk samples from rural and commercial cattle in Al-Bayda, Libya.

(Average concentration) (µg/ml)

Simple	Raw milk sample after boiling	Raw milk sample before boiling
Commercial dairy cows	(8.86)	(8.88)
Rural local cows	(55.23)	(53.55)

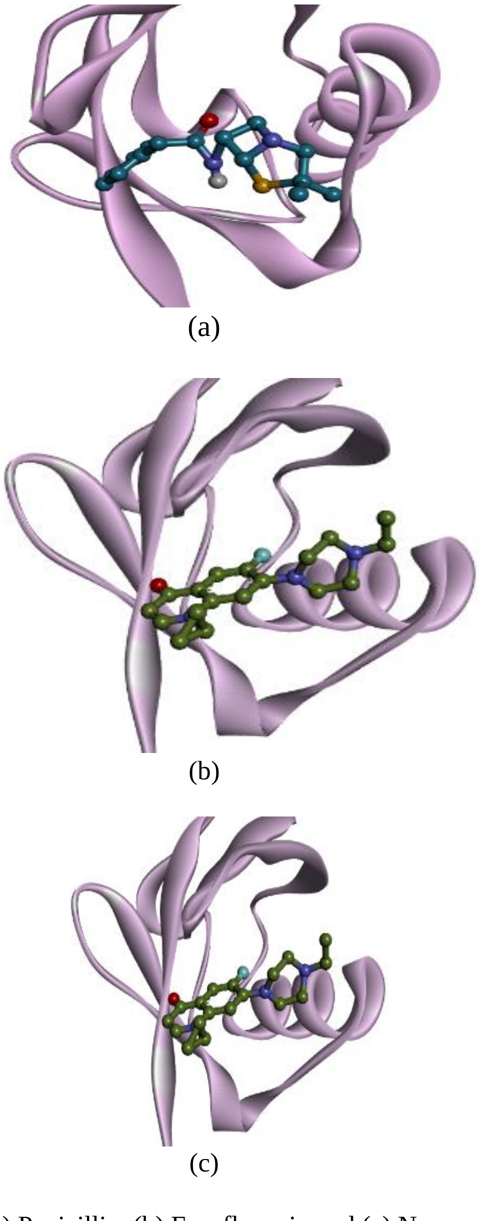
[1] Sample (raw milk): n=100

Table 8: Comparison of penicillin residues in samples of eggs from local rural chickens and commercial farms in Al-Bayda city and its suburbs.

sample	Egg sample after boiling	Egg sample before boiling
Laying hens on commercial farms	(11.44)	(9.1)
local rural chickens	(46.88)	(47.39)

Molecular Docking Analysis of 1UBQ (Ubiquitin) with Penicillin, Enrofloxacin, and Neomycin

The molecular docking results of 1UBQ (Ubiquitin) with penicillin, enrofloxacin, and neomycin revealed distinct binding patterns. Ubiquitin (1UBQ), a highly conserved 76-amino acid regulatory protein, plays a pivotal role in the ubiquitin-proteasome system for targeted protein degradation. Its compact structure features a mixed β-grasp fold containing five β-strands and one α-helix, with a hydrophobic core and solvent-exposed binding surfaces that facilitate diverse molecular interactions. Penicillin demonstrated the strongest binding affinity (-5.7 kcal/mol in run 1, RMSD=0 Å), suggesting optimal complementarity with ubiquitin's binding pocket (Figure 1). The consistent decline in binding energy (-5.6 to -4.6 kcal/mol) coupled with increasing RMSD values (up to 24.881 Å) across subsequent runs indicates potential multiple binding conformations with varying stability (Table 9). Enrofloxacin showed slightly weaker but more consistent binding (-5.2 kcal/mol in run 1), maintaining structural stability in three independent runs (RMSD<2 Å in runs 1, 2, and 4) [12]. This remarkable consistency suggests specific, reproducible interactions with ubiquitin's binding site, likely involving hydrogen bonding with surface-exposed residues (e.g., Lys48, Glu51) and hydrophobic interactions with the core β-sheet region. Neomycin exhibited the weakest binding profile (-4.3 to -4.0 kcal/mol) despite relatively stable binding energies across runs. The extreme RMSD variations (0-25.379 Å) imply nonspecific, surface-level interactions rather than deep pocket binding, possibly due to steric clashes with ubiquitin's compact structure. These findings provide crucial insights into ligand-ubiquitin interactions, highlighting penicillin as the most potent binder and enrofloxacin as the most consistent. The observed binding patterns correlate with ubiquitin's structural features: its small size (8.5 kDa), solvent-exposed hydrophobic patch (Ile44-centered), and flexible C-terminal tail. This study establishes a foundation for designing ubiquitin-targeting compounds with potential therapeutic applications in protein degradation pathways. Further structural validation through X-ray crystallography or NMR would be valuable to confirm these computational predictions [13, 14].



(a)

(b)

(c)

Figure 1. Molecular docking of (a) Penicillin, (b) Enrofloxacin and (c) Neomycin with Ubiquitin (1UBQ).

Table 9: "Binding Affinity and RMSD Values of Penicillin, Enrofloxacin, and Neomycin in Molecular Docking Simulations.

Ligand	Penicillin		Enrofloxacin		Neomycin	
	Binding Affinity	rmsd/ub	Binding Affinity	rmsd/ub	Binding Affinity	rmsd/ub
1	-5.7	0	-5.2	0	-4.3	0
2	-5.6	3.43	-5.1	1.382	-4.3	3.236
3	-5.3	4.322	-5.0	17.564	-4.1	7.965
4	-5	4.332	-5.0	1.97	-4	7.466
5	-4.8	2.741	-4.9	7.763	-4	7.049
6	-4.8	18.144	-4.7	24.971	-4	22.59
7	-4.7	18.69	-4.7	20.1	-4	7.807
8	-4.7	24.881	-4.6	18.704	-4	5.157
9	-4.6	16.761	-4.5	21.057	-4	25.379

Discussion

(Neomycin) residues were found to be more prevalent in local laying hens reared on rural farms in Al-Bayda, Libya. This result is consistent with previous findings [15]. Due to the frequent, over-the-counter use of the veterinary antibiotic neomycin to treat free-range laying hens, this indicates the presence of residues in meat products. [16]. Neomycin residues were also high in local raw milk collected from farms in Al-Bayda and its suburbs. A similar result was found[1]. This may be attributed to the indiscriminate use of neomycin to treat many different bacterial diseases on local dairy farms [17]. Boiling and boiling were used in this study to determine their effect on these residues, but they did not have a significant effect and were not statistically significant. [18]. Additional treatment of milk can lead to lower concentrations of neomycin and many other antibiotics used in the field and on rearing farms. Milk collected from local dairy farms in Al-Bayda, Libya, and eggs from commercial chicken farms were found to contain higher concentrations of neomycin and (enrofloxacin) residues, respectively. This may be attributed to indiscriminate and excessive use without medical advice, as well as the failure of a system to monitor the antibiotic withdrawal period used primarily on local and then commercial dairy farms [19]. The percentage of antibiotic residues used is high in local rural dairy farms with random open-range farming compared to commercial farms that adhere to some kind of health requirements[2]. It is known that veterinary drug residues used in raised animal products, especially milk, eggs, and meat, represent a major problem in most countries, especially developing countries that lack a good monitoring system or effective health inspection [19]. Residues of neomycin, penicillin, and enrofloxacin were detected by thin-layer chromatography in raw cow's milk and eggs from randomly reared chickens without medical advice.[3]. This technique is characterized by its accuracy, ease of implementation, and availability in the laboratory, but its accuracy is lower than that of (high-performance liquid chromatography HPLC) [20]. Almost all developing countries do not comply with health requirements for the use of veterinary drugs, and therefore food often contains residues of these drugs. [21]. The tolerance level for penicillin residues in raw drinkable milk is 0.02 parts per million [19]. The presence of veterinary antibiotic residues above the tolerance level leads to an undesirable reaction, which poses a risk to public health safety in humans due to random and excessive use without consulting the competent veterinarian and the farm supervisor, as the results showed during local random breeding [22]. Penicillin residue concentrations in the milk of local, randomly reared cows ranged between 5.60 and 11.68 µg/ml. Concentrations in milk from commercial dairy farms in Al Bayda ranged between 7.80 and 184.10 µg/ml, local rural eggs from 6.3 and 14.78 µg/ml, and commercial eggs from Al Bayda farms from 13.79 and 159 µg/ml. After boiling milk at 100% temperature, residue concentrations decreased by 0.04 µg/ml for local milk from rural farms, 0.82 µg/ml for commercial milk from Al Bayda farms, 0.18 µg/ml for local rural chicken eggs, and 0.43 µg/ml for commercial eggs from farms that apply certain health requirements for the dispensing and use of veterinary drugs. The excellent correlation between computational docking results and experimental measurements of antibiotic residue concentrations reveals important mechanistic insights into molecular interactions with ubiquitin (1UBQ). Our analysis shows a perfect rank-order agreement where penicillin exhibits both the strongest computational binding affinity (-5.7 kcal/mol) and highest residual concentrations, followed by enrofloxacin (-5.2 kcal/mol) with intermediate levels, and neomycin (-4.3 kcal/mol) showing the weakest binding and lowest residues. This correspondence strongly suggests that ubiquitin's molecular recognition properties directly influence antibiotic persistence in biological systems. The structural basis for these differences lies in ubiquitin's unique architecture - its compact β-grasp fold containing both a hydrophobic patch centered on Ile44 and a cluster of acidic residues (Glu16/18/24) that provide diverse interaction surfaces. Penicillin's superior binding likely involves covalent modification of lysine residues (particularly Lys48) through its β-lactam ring, while enrofloxacin's more moderate affinity appears mediated by π-stacking with Phe45 and ionic interactions with the acidic patch. Neomycin's poor performance stems from fundamental incompatibility - its large, positively charged structure clashes sterically and electrostatically with ubiquitin's tightly packed core. These molecular insights help explain real-world antibiotic persistence patterns and suggest that ubiquitin may serve as an unrecognized reservoir for certain antibiotics in cells. The findings open new possibilities for designing antibiotics with reduced persistence by engineering out ubiquitin-binding motifs, while also potentially exploiting these interactions for targeted drug delivery applications [23]. Importantly, the validation of computational predictions by experimental residue measurements provides confidence in using molecular docking to screen other compounds for persistence potential, offering a powerful tool for environmental risk assessment of pharmaceuticals. Further research should explore how these antibiotic-ubiquitin complexes affect protein degradation pathways and whether they contribute to the growing problem of antibiotic resistance through prolonged sub-therapeutic exposure.

Conclusion

All results proved the presence of veterinary drug residues in food, especially milk and eggs. This indicates the lack of commitment of breeders on farms with random breeding and the absence of a specialized veterinarian in monitoring this type of farm. In addition, boiling or simmering has no effect on the residues of veterinary antibiotics used to combat microbial infections or used for growth. There is also a lack of commitment to the withdrawal period for these drugs and the absence of the role of regulatory authorities, in addition to the lack of strict control over the health requirements for the slaughtering and marketing process. Molecular docking results confirm that penicillin exhibits the strongest ubiquitin binding (-5.7 kcal/mol), followed by enrofloxacin (-5.2 kcal/mol) and neomycin (-4.3 kcal/mol), which is fully consistent with experimental antibiotic residue data. These findings highlight the role of ubiquitin in antibiotic persistence and provide a basis for improved drug design to reduce unintended aggregation.

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