Physicochemical characteristics and antibiotic residue detection in raw cow’s milk delivered to three milk processing industries in Algeria

F. R. Meklati*, A. Meribai, Y. Titouche, T. Lyazidi, N. Yezli-Meklati, M. Abou-Mustapha and M. H. Ben-Mahdi

Abstract

The uncontrolled use of antimicrobials in veterinary medicine may result in the presence of their residues in food matrices of animal origin such as milk. The aim of this study was to examine the physicochemical quality and to detect antibiotic residues (β-lactams and tetracyclines) in milk, as their presence has resulted in the rejection of significant quantities of milk for marketing. The study was conducted on 274 milk samples delivered to three milk-processing units located in the suburbs of Algiers and surrounding cities (Blida and Boumerdes). Antibiotic residue detection was performed using a fast screening test (BetaStar® Combo), with a reading result within five minutes. The results indicated that the milk processing industry of Blida had the highest rejected milk volumes (43.4%), followed by Algiers (24%) and Boumerdes (19.1%). Non-compliance of milk acidity was the first cause for milk rejection (47.6 %), followed by the presence of antibiotic residues (26.8%), and incompliance with requirements for density (13.4%) and fat levels (12.2%). The study revealed 22 positive cases (8.03%) of antibiotic residues. Among these, 90.91% were positive for β-lactams, and only 9.09% for tetracyclines. All tetracyclines cases and half of the β-lactams cases were found in the Blida milk processing company, indicating that the good practice of milk production and collection must be strengthened in that company.

Key words: dairy industry; physicochemical properties; antibiotic residues; cow’s milk
Introduction

Milk consumption in Algeria has increased steadily in recent years, to an average of 110 litres per capita, due to the growing population. To respond to the growing demand and to enhance production, many high milk potential breeds were imported, mainly from Europe (Meklati et al., 2020). Successive development programmes were more focused on quantitative than on qualitative aspects. However, as in other North African countries, the improvement of milk quality has become a requirement to be considered (Srairi et al., 2013; Meklati et al., 2020). To protect consumer health, raw milk produced locally on farms and forwarded to the dairy industry should be controlled in situ upon delivery. Once its conformity is established, it will have to undergo pasteurisation and further processing. This process aims to assess its compliance with the national legislation in force, including antibiotic residues in food matrices of animal origin (JORA, 2016).

The occurrence of antibiotic residues in foodstuffs of animal origin could have adverse consequences for consumer health (such as allergic reactions, bacterial resistance, etc.). At the level of industrial production, that same presence in milk may lead to a decrease or loss of value of raw materials by delaying or completely preventing the fermentation processes (Lee et al., 2001; Grunwald and Petz, 2003). Maximum Residues Limits (MRLs) have been established in many countries worldwide. To protect consumers, public authorities have also implemented this concept with legislative texts (JORA, 2014), enhanced by the establishment of MRLs per compound in foodstuffs of animal origin (JORA, 2016).

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Constraints in financial resources and personnel training are among the main difficulties in implementing national food safety management systems for human health. Nevertheless, despite the absence of any official national data, regular controls of milk collectors’ deliveries were already being performed at the level of the dairy companies well before the promulgation of these texts. This action reduced the risk of any collected milk testing positive for antimicrobials from ending up in the bulk tank of the dairy (Meklati et al., 2022).

Owing to their widespread action spectra on Gram + and Gram - bacteria and their use in prevention and treatment, β-lactams and tetracyclines are the most frequently antibiotics used in veterinary treatments (Mimoune et al., 2021) and therefore are the main compounds tested for upon the delivery of milk to dairy processors.

The aim of this study was to assess the physicochemical quality of milk, and to detect the antibiotic residues in 274 milk samples delivered for processing in three milk dairy industries located in Algiers and two surrounding areas (Blida and Boumerdes).

Materials and Methods

Study site and milk sampling conditions

A total of 274 raw milk samples were collected during the year 2016 from three milk-processing industries located in the suburbs of Algiers and surrounding cities (Blida and Boumerdes). Two of these enterprises belong to the public sector and are located in the Wilayas suburb of Algiers and the city of Boumerdes, while the third is privately owned and is situated in the Wilaya area of Blida. The climate of these regions is characterized by Mediterranean temperate weather with a long dry season extending from May to September, with irregular rainfall in the remaining months. Samples were taken from unpasteurised milk delivered by a tanker truck network collecting raw
cow’s milk from dairy farms affiliated with these enterprises. A volume of 0.5 L was collected from the milk tanker truck and then analysed immediately in the laboratory at each dairy enterprise.

**Physicochemical analysis and antibiotic residue screening**

The analyses involved the evaluation of the physicochemical quality of milk and screening for β-lactams and tetracyclines residues. pH was directly measured with a pH meter (Hanna Instruments, HI 2211). Milk acidity and density were measured in line with the AFNOR norms (AFNOR, 1969; AFNOR, 2004). Total dry extract (TDE) was determined using a Sartorius MA 35 moisture analyser (Sartorius, Germany), while fat level (FL) was determined by Gerber’s method (AFNOR, 2000). The antibiotic residues of β-lactams and tetracyclines families were investigated using a BetaStar<sup>®</sup> Combo test equipment (Neogen Corporation, Lansing, MI, USA), which is based on a fast immunochromatographic medium according to a “receptor assay” model for the analysis of both molecules. The antibiotic residue assessment was performed according to the manufacturer’s recommendations using a 200 µL volume for each test sample. This volume was placed in a vial containing the active agent and incubated at 47.5 ±1°C for 2 minutes, then the dipstick was inserted and kept for 3 minutes at that temperature. The result was interpreted by visually reading the staining intensity of two capture lines corresponding to β-lactams and tetracyclines respectively compared to the control line and classified as positive or negative. This method agrees with the European Committee recommendation 2002/657/EC relative to the validation criteria of analytical methods (European Commission, 2002; Reybroeck and Ooghe, 2012). Antibiotics of the β-lactam class (benzylpenicillin, ampicillin, amoxicillin, oxacillin, cloxacillin, dicloxacillin, nafcillin, ceftiofur, cephalaxin, cefazolin, cephalirin, cefaceftrile, cefoperazone, cefalonium) and those of the tetracycline group (tetracycline, oxytetracycline, chlorotetracycline, doxycycline) can be detected by the rapid screening test at thresholds or concentrations that may be lower than the Maximum Residue Limits (MRLs). However, according to Reybroeck and Ooghe (2012), the BetaStar<sup>®</sup> Combo revealed a limit of detection (LOD) exceeding the MRLs for cefalexin and desfuroylceftiofur. Furthermore, some metabolites of β-lactams may also escape detection when present at low concentration levels in cow’s milk (Meklati et al., 2022). In our experimental conditions, three replicates were performed for each sample. The average of these three values and the standard deviation was determined.

**Statistical Analysis**

For the three dairies, physicochemical analysis data were statistically analysed with ANOVA I, using Statistica<sup>®</sup> version 6.1 (Statsoft, France). When the ANOVA results were significant ($P < 0.05$), Duncan’s test was used to study in detail the significant variation recorded for the three regions.

**Results and Discussion**

**Physicochemical characteristics of milk**

For all milk samples delivered, the results revealed a highly significant difference ($P<0.001$) among the three dairies for titratable acidity, density, FL, and TDE, whereas no significant effect was recorded for pH (Table 1). Titratable acidity is an important parameter of milk quality delivered by collectors, as it provides an indication of bacterial acidification and the state of freshness or possible adulterations (Schmidt et al., 1996). pH and titratable acidity mean values were within the permitted values (Alais, 1984; JORA,
1993). Nevertheless, the minimum titratable acidity values (10.0–14.0°D), and the maximum titratable acidity scores (21°D), indicated large fluctuations and poor quality for some milk at delivery (Table 1). In addition, it is not uncommon to find farms producing milk with titratable acidity exceeding 18°D, particularly when hygiene and storage conditions do not comply with the required guidelines (Matallah et al., 2017).

However, these cases of non-compliance for acidity (Figure 1) were the main reason for milk rejection, with a proportion evaluated at 47.6% for the three regions studied. The region of Algiers had the highest number of rejected samples (51.5%) due to the large number of samples with low titratable acidity (min. 10°D), closely followed by Blida (50%) with high values (max. 21°D), then by Boumerdes (37%). As a consequence, this type of milk delivered by the tanker trucks network is systematically refused and thus rejected without seizure, penalty, or destruction. The informal circuit, which eludes any control and whose products are usually of dubious quality, occupies a prominent position in the dairy sector (Meklati et al., 2020). Moreover, it plays a significant part in the incorporation of products discarded by the regular network.

This rejected milk is also directed towards the artisanal manufacture of fermented milk (Lben, Raib), which is highly appreciated by the local population as an accompaniment to some traditional foods. Furthermore, in recent few years, more and more farmers are opting to transform their production into fermented milk and sell it themselves rather than the official channel which is less lucrative. Collectors are also involved in the direct sale of Lben and Raib products to consumers through the

### Table 1. Physicochemical analysis results of milk samples collected across the three units

<table>
<thead>
<tr>
<th>Physicochemical Parameters</th>
<th>Algiers</th>
<th>Blida</th>
<th>Boumerdes</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Mean ± SD 6.7±0.11</td>
<td>6.6±0.09</td>
<td>6.5±0.13</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Min. 6.4</td>
<td>6.4</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Max. 7.2</td>
<td>6.8</td>
<td>6.9</td>
<td></td>
</tr>
<tr>
<td>Titratable acidity(°D)</td>
<td>Mean ± SD 15.9±1.52c</td>
<td>18.1±0.92a</td>
<td>16.7±1.38b</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>Min. 10.0</td>
<td>16.5</td>
<td>14.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Max. 21.0</td>
<td>21.0</td>
<td>21.0</td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>Mean ± SD 1029±1.09a</td>
<td>1031±0.91a</td>
<td>1029±0.92b</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>Min. 1027</td>
<td>1028</td>
<td>1026</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Max. 1032</td>
<td>1034</td>
<td>1031</td>
<td></td>
</tr>
<tr>
<td>FL [g/L]</td>
<td>Mean ± SD 33±3.11b</td>
<td>35.3±4.10a</td>
<td>31.9±2.62c</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>Min. 23.0</td>
<td>27.0</td>
<td>22.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Max. 42.0</td>
<td>41.5</td>
<td>41.0</td>
<td></td>
</tr>
<tr>
<td>TDE [g/L]</td>
<td>Mean ± SD 117±5.27b</td>
<td>124±5.58a</td>
<td>115±4.11c</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>Min. 106</td>
<td>108</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Max. 130</td>
<td>134</td>
<td>127</td>
<td></td>
</tr>
</tbody>
</table>

P: probability; *: P<0.05; **: P<0.01; ***: P<0.001; NS: non-significant (P>0.05). In each line and for each physicochemical parameter, the values (mean± standard deviation) marked with different letters are significantly different (P<0.05), Duncan’s Test. The letter “a” corresponds to the highest adjusted mean.
informal network (Belhadia et al., 2014). Except for Blida, the mean density value recorded was lower than the range (1,030–1,034) established by JORA (1993). The minimal values observed at Boumerdes and Algiers (1,026 and 1,027 respectively) led to systematically rejection of milk delivered at these values. Non-compliance for density was the reason for rejection in 21% and 21.2% of samples, due to the addition of water to the milk (Figure 1). Indeed, some breeders are inclined to add high volumes of water to milk to increase the benefit margin at the expense of quality. Adulteration of milk by the addition of water may introduce chemical or microbial health hazards, and reduce the nutritional and processing quality and marketing value of milk (Swai and Schooman, 2011). In our previous study (Meklati et al., 2017), the density of raw milk for processing, recorded on dairy farms located in the Mitidja region, including Algiers and Blida, was evaluated in the range of 1,030–1,031.

Fat levels (FL) is a criterion for milk payment according to its quality upon delivery in Algeria. The FL showed values in compliance with the requirements of JORA (1993) for milk at Blida (35.3±4.10 g/L) and under the threshold (34 g/L) with important fluctuations for the dairies at Boumerdes and Algiers (31.9±2.62 g/L and 33±3.11 g/L, respectively). The lipid fraction can vary according to feed and season (Månsson, 2008), but also with lactation stage (Meklati et al., 2017). The lower FL content in the Algiers and Boumerdes areas could be explained by a highly unbalanced diet, due to the expensive price of feed on the local market, exacerbated for many years by marginal national fodder production (Meklati et al., 2020). Minimal values were identified for all regions (Table 1) with suspicious cases of fat removal, a rejection reason estimated at 12.2% for the three dairies. However, Boumerdes had the largest number of rejections (21%) for this cause (Figure 1), followed by Blida (10 %) and Algiers (9.1 %). Matallah et al. (2017) reported that one-third of dairy farms studied in northeastern Algeria

![Figure 1](image-url). Distribution of the causes for milk rejection for each dairy enterprise
displayed a FL content of less than 30 g/L.

The highest mean content of Total Dry Extract (TDE) was recorded in Blida (124±5.58 g/L). This value remains, however, lower than the content (128 g/L) reported by Alais (1984) but higher than the average content (120±8.04 g/L) reported by Matallah et al. (2017). According to Croguennec et al. (2008), an increase or decrease of TDE is closely related to protein and FL changes.

Antibiotic residue detection

The results indicated that 22 (8.03%) of the 274 milk samples collected from the three dairies were positive for antibiotic residues. Among these positive samples, 20 (90.91%) were positive for the β-lactams family, while only two (9.09%) were positive for tetracyclines (Table 2), denoting the important use of penicillins in Algerian veterinary medicine. Many antibiotic classes used in humans (medically important) are currently utilized in the beef, dairy, pork, and poultry industries. Antibiotics commonly used for the treatment, prophylaxis and growth promotion of food animals include doxycycline, colistin sulphate, neomycin, tetracycline, enrofloxacin, ciprofloxacin and amikacin (Bungau et al. 2021; Hassan et al. 2021). Our findings were higher than those obtained by Debeche et al. (2018), who revealed that of the 8.9 million litres of raw milk collected from a dairy (Mila district, North-eastern Algeria), 290,720 litres (3.25%) were contaminated with antibiotic residues. However, the results obtained were slightly lower than those reported (9.17%) by Zeghilet et al. (2022) on raw milk from the Constantine region (northeastern Algeria). Titouche et al. (2016) reported a high rate of contamination in raw milk produced in the Tizi Ouzou area. Greater proportions (21.05%) were recorded in processed milk producers from Romania as detected using the BetaStar® Combo analysis (Pogurschi et al., 2015).

All cases of tetracycline detection were in the Blida area, suggesting that industry-affiliated farms use more tetracyclines compared to the other regions under study, confirming the existence of a geographic effect in the use of antibiotics (Debeche et al., 2018). Our data indicated that β-lactams were more widely used than tetracyclines, as previously reported by Baazize-Ammi et al. (2019). However, Mimoune et al. (2021), who investigated the presence of antibiotic residues in the Algiers and Boumerdes areas, found the opposite tendency, as the latter were predominant in these regions. The same pattern also occurred in Romanian processed milk producers, with the exclusive occurrence of tetracyclines (Pogurschi et al., 2015). As reported by Patel et al. (2020), in 2018, tetracyclines accounted for 66%, penicillins for 12%, macrolides for 8%, sulphonamides for 5%, aminoglycosides for 5%, lincosamides for 2%, cephalosporins for 1%, and fluoroquinolones for <1% of antibiotic sales for livestock.

The region of Blida also recorded half the whole cases of β-lactams (10 cases), which represents 23.53% of all positive
samples (Table 2). These results were almost twice those from the same district detected using the Delvotest SP®, in which 11.97% of raw cow milk samples were positive (Baazize-Ammi et al., 2019). However, the value was lower than recorded in Iran, in which 32.9% of the bulk tank milk was exclusively positive for β-lactams (Mokhtari et al., 2013). In addition, the remaining β-lactams cases were attributed to Algiers with 6 cases (4.76%) of 126 milk samples and to Boumerdes with 4 cases (4.12%) of 97 samples (Table 2). These displayed values remained lower than those reported by Mimoune et al. (2021) for the same regions (8.33% and 6.25%, respectively). The displayed values were close to those obtained by Bilandžić et al. (2011) for 1259 raw milk collections evaluated in Croatia over a three-year period with the Delvotest SP-NT microbiological method (3% positive cases). For all the regions studied, milk adulteration cases due to the presence of antibiotic residues was the second widest reason for rejection (26.8%) after acidity. The Blida region was most affected, with twice the number of cases than in Algiers or Boumerdes (Figure 1).

According to Lievaart et al. (2005) and Fabre et al. (2010), contamination that is intentional (non-abidance of the withdrawal period) or due to neglect (not marking animals undergoing antibiotic treatment) of only a single cow under antibiotic treatment is enough to make an entire truck of several thousand litres of milk unfit for receipt by the dairy. Not separating animals in the drying-off period that might still be under antibiotic treatment from other lactating cows is another point of particular attention to be taken into account, which could explain the presence of antibiotic residues in milk. Indeed, a study conducted in northern Algeria (Hamlaoui et al., 2021) revealed that only 2.7% of farmers always separated dry cows from lactating ones. There is also a tendency for some farmers to direct the milk of cows undergoing antibiotic treatment to calf feed, which is another critical point (Hamlaoui et al., 2021).

Moreover, due to the limited screening resources and the lack of restrictive legislation, only a few collectors regularly perform antibiotic inspection before collecting the milk from each farm in Algeria. Indeed, there are currently no disciplinary actions (financial sanctions or milk destructions) in the country, except the prohibition of delivery to a dairy for milk with a positive antibiotic test. Nevertheless, the Algerian framework legislation states that milk reserved for human consumption must not contain antibiotic substances (JORA, 1993). This regulation has since evolved, introducing the concept of MRLs (JORA, 2014), with more subsequent details on the denomination and the threshold of each substance that may be present in milk and other foodstuffs of animal origin (JORA, 2016).

The BetaStar® Combo as a screening test is used by many quality control laboratories in the national dairy industry as it quickly (5 min) detects β-lactams and tetracyclines, as the most frequently used compounds in veterinary medicine at levels close to the MRLs (European Commission, 2010; Reybroeck and Ooghe, 2012). Previous studies (Reybroeck and Ooghe, 2012; Meklati et al., 2022) highlighted the potential occurrence of false-positive results with the rapid test. These were more marked for tetracyclines when the milk tested had titratable acidity values exceeding 19°D, and fat content at the low (27 g/L), or high (56–62 g/L) extremes. Nevertheless, under routine laboratory conditions in dairy industries, this type of milk is usually systematically rejected from the outset since titratable acidity is typically performed first for compliance purposes, followed by fat content assessment,
and then finally antibiotic residue investigations. Except for β-lactams and tetracyclines, the BetaStar® Combo (Neogen Corporation, Lansing, MI, USA) is unable to detect other groups such as quinolones and sulfonamides. The dairies in this study had equipment that could provide a rapid screening test only for penicillins and tetracyclines since neither checked for other antibiotic classes at the time of milk delivery. There is therefore a major health risk to the consumer if compounds or their metabolites of other families of antibiotics are present in milk at concentrations exceeding the thresholds (Meklati et al., 2022).

Milk quantities accepted and rejected at each dairy

The delivered milk volumes by collectors, either accepted or rejected on sampling day, were recorded (Table 3). The dairy in Algiers displayed the most important delivered quantities, whereas the milk processing industry in Blida recorded the highest quantity rejected (43.4%), followed by Algiers (24%) and Boumerdes (19.1%).

Table 3. Raw milk daily volumes (L) checked, accepted, or rejected during the sampling

<table>
<thead>
<tr>
<th></th>
<th>Milk volume checked</th>
<th>Milk volume accepted</th>
<th>Milk volume rejected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Min.</td>
<td>Max.</td>
</tr>
<tr>
<td>Algiers</td>
<td>21,855 ± 4,670</td>
<td>12,717</td>
<td>26,954</td>
</tr>
<tr>
<td>Blida</td>
<td>8,300 ± 756</td>
<td>6,328</td>
<td>9,379</td>
</tr>
<tr>
<td>Boumerdes</td>
<td>14,205 ± 1,832</td>
<td>10,748</td>
<td>16,214</td>
</tr>
</tbody>
</table>

Conclusion

This study revealed significant physicochemical differences and the presence of antibiotic residues in milk collected from all three studied dairies, particularly in Blida, which confirms the existence of deficiencies in good breeding practices, hygiene in the production at the farm and in the transport of collected milk. In addition to the loss of considerable milk quantities from the official circuit, these shortcomings lead to consumer health risks and increase the country’s dependence on imported milk powder. These findings highlight the need to reconsider not only the basic rules from livestock breeding to milk collecting, but also the importance of reinforcing the current regulations by enhancing the assessment of antibiotic residues. It is also essential to regulate the fate of milk that tests positive to prevent it from ending up in the informal circuit, and to encourage its destruction to guarantee the consumer a healthy, quality product, but also to avoid significant losses for the dairy industry caused by the inhibition of the fermentation process. It is also important to strengthen the ability of dairies to test for groups of antibiotics other than β-lactams and tetracyclines.

References

Physicochemical characteristics and antibiotic residue detection in raw cow's milk delivered to three milk processing industries in Algeria

Fizikalno-kemijske karakteristike i detekcija rezidua antibiotika u sirovom mlijeku isporučenom u tri industrije za preradu mlijeKA u Alžiru

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Fizikalno-kemijske karakteristike i detekcija rezidua antibiotika u sirovom kravljem mlijeku isporučenom u tri industrije za preradu mlijeka u Alžiru

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Nekontrolirana uporaba antimikrobnih sredstava u veterinarskoj medicini može rezultirati prisustvom njihovih rezidua u matricama hrane životinjskog podrijetla kao što je i mlijeko. Ovaj rad ima za cilj proučavanje fizikalno-kemijske kakvoće i detekcije ostataka antibiotika (β-laktama i tetraciklina), čije je određivanje u mlijeku prouzročilo odbijanje znatnih količina za plasman na tržištu. Istraživanje je provedeno na 274 uzorka mlijeka dostavljena u tri industrije za preradu mlijeka u predgrađima Alžira i drugih gradova (Blida i Boumerdes). Detekcija ostataka antibiotika provedena je brzim testom (BetaStar® Combo), s rezultatom očitanja unutar pet minuta. Rezultati su pokazali da je najveća količina odbijenog mlijeka u industriji prerade mlijeka iz jedinice Blida (43,4 %), a slijede Alžir (24 %) i Boumerdes (19,1 %). Nesukladnost kiselosti mlijeka bila je prvi uzrok odbacivanje mlijeka (47,6 %), a zatim slijede: prisutnost antibiotskih ostataka (26,8 %), gustoća (13,4 %) i nesukladnost razine masti (FL) (12,2 %). Od svih procijenjenih uzoraka, studija je otkrila 22 pozitivna slučaja (8,03 %) na ostatke antibiotika. Među njima je 90,91 % bilo pozitivno na β-laktame, dok je samo 9,09 % pozitivno na tetracikline. Svi slučajevi tetraciklina i polovica β-laktama pronađeni su u mljekoprerađivačkoj tvrtki Blida, što ukazuje da se u toj kompaniji mora ojačati dobra praksa uzgoja i prikupljanja mlijeka.

Ključne riječi: mliječna industrija, fizikalno-kemijska svojstva, mlijeko