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Effect of using treated wastewater on the bacteriological quality of raw cow's milk: A case of a farm in Northeastern Algeria

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This study aims to assess the impact of the use of treated wastewater (without chlorination) in farming and dairy cattle breeding. Milk samples were collected from a farm in northeastern Algeria. The treated wastewater from the treatment plant is used on this farm for different activities. The results obtained show that the average contamination of milks with total flora is $3.7.10^5$ CFU /ml. Fecal coliforms are present at an average value of $1.5.10^3$ CFU/ml. All of the samples (100%) were positive for the count of fecal enterococci with an average value of 2.5.10 CFU/ml. Fungal flora was present with an average value of $1.36,10^3$ CFU/ml. *Escherichia coli* was isolated in 100% of the samples with high resistance rates for beta-lactam antibiotics. The results obtained for the search for pathogens belonging to the genus *Staphylococcus* show that 64% of the isolates were coagulase-negative *Staphylococcus* and 36% of the isolates were coagulase-positive. The study of *Staphylococcus* susceptibility/resistance to antibiotics revealed high frequencies of resistance, especially to beta-lactam antibiotics and macrolides. The bacteria tested show a majority resistance for Penicillin and Oxacillin (100%). These results reflect the microbiological risk that the consumption and marketing of this milk represents for the health of consumers and the need to implement preventive measures.

Key words: Irrigation, fecal coliforms, *Escherichia coli*, *Staphylococcus* sp, antibiotic resistance, microbiological risk.

INTRODUCTION

The emergence and spread of antibiotic resistance genes among pathogenic and non-pathogenic bacteria has been a growing threat in recent decades and there is a rapid lack of therapeutic options (Li and Webster, 2018;

Figure 1. Device for the use of treated wastewater at the outlet of the treatment plant.

Barancheshme and Munir, 2018). The emergence of this resistance in bacteria in animals and their products has attracted considerable interest due to the potential of transferring this resistance to the human population (Vásquez et al., 2017; McDermott et al., 2018). Suspected sites of resistance transmission include wastewater treatment plants where wastewater from various sources, including municipalities, sanitary wastewater, hospital effluents, storm water runoff and industries, is mixed and treated using a multi-stage purification process (Mohammadali and Davies, 2017; Hultman et al., 2018).

The use of wastewater for irrigation is observed as a way to address the imbalance between demand and supply of water. However, the literature shows that irrigation with treated wastewater is not without implications, some of which are negative (Gatto D'Andrea et al., 2015; Becerra-Castro et al., 2015).

Inadequately treated water from sewage systems represents both a risk to human and animal health if it is used to pasture or fodder crops grazed by livestock or otherwise consumed (Cass and Lowe, 2014). As a result, the water may contain bacteria, viruses, protozoa and helminth eggs that would be a risk to the livestock, or to humans who have contact with or consume livestock products (meat, milk, eggs, etc.) (Drechsel et al., 2010). The wastewater treatment plant on the wilaya of Khenchela (Northeastern Algeria) is a low load activated sludge with a capacity of 23,000 m^3/day for 192,000 equivalent/inhabitant. A mixture of urban, industrial, agricultural, storm water runoff and hospital wastewater from the city of Khenchela is discharged to this treatment plant, only to be finally discharged without tertiary treatment and disinfection into Baghai wadi.

The owner of traditional farm uses treated water at the outlet of a wastewater treatment plant to irrigate his pasture field and breed his cows (Figure 1). On the farm, this water is used for three main activities: pasture cultivation, dairy barn farming and cleaning, and for dairy cow consumption.

The main objective of this study is to assess the risks associated with the reuse of treated wastewater in agriculture. The dairy industry is particularly concerned about the potential effect on dairy cattle and milk quality following pasture irrigation with waste water. Therefore, monitoring bacterial pathogens, their survival and transfer is of the utmost importance to ensure that milk quality is not compromised.

MATERIALS AND METHODS

Raw milk samples were obtained after a manual milking of the four healthy cows, from the lactating udder, just before the first morning milking. Milk sample (100 ml) was collected in a sterile bottle after washing and disinfecting the teats and removing the first draft. The milk samples were immediately placed at a temperature of +4°C and then quickly sent to the laboratories for an analysis of its biochemical and microbiological composition.

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FT-IR instrumental analysis

The biochemical composition of raw milk samples was performed by Fourier Transform Infrared Spectroscopy (FTIR). It is a rapid biochemical fingerprinting technique (Nicolaou et al*.,* 2010). It can potentially be applied to produce results with the same accuracy and sensitivity as reference methods in a short period of time (Nicolaou and Goodacre, 2008). Measurements were made using a Fourier Transform Infrared Spectrometer (FTIR) (DYNASCAN Border Spectrum, Perkin-Elmer Ltd, England), equipped with a deuterated triglycerin sulfate (DTGS) detector stabilized at an optimized temperature in the far and mid infrared. Infrared spectra were recorded at 64 scans in the range of 8 300 to 50 cm^{-1} with a resolution of 4 cm^{-1}. KBr separator was used to record milk spectra. The milk sample was ground with KBr powder to be pressed into a tablet. Then, the IR spectrum was collected.

Microbiological analyses

The microbiological analyses were carried out at the Microbiology Laboratory of the University of Khenchela (Algeria). From milk previously homogenized, serial decimal dilutions were prepared in peptone saline diluent using standard methods. (ISO 6887 - 5: 2010). All raw milk samples were analyzed for the presence of total aerobic mesophilic bacteria (TAMB), fecal coliforms (CF), fecal enterococci (FE), yeasts and viable molds and for the detection of *Escherichia coli* and positive and negative coagulase *Staphylococcus* using the standard methods described below.

Total aerobic mesophilic bacteria enumeration

Total aerobic mesophilic bacteria (TAMB) were measured according to the standard method (ISO 4833-1: 2013). The Petri dishes were inoculated separately with 1 ml of each dilution to which Plate Count Agar (PCA) was added (Pasteur Institute, Algeria). After 72 h of incubation, all colonies were counted and the results were expressed in units forming colony per ml of milk (CFU/ml).

Fecal coliforms and *E. coli* counts

Fecal coliforms (FC) and *E. coli* were measured using the standard method (ISO 4831:2006 and ISO 7251:2005). Fecal coliforms were counted using the most probable number (MPN) technique in brilliant green bile (2%) broth (Pasteur Institute of Algeria). After the incubation period of 24 to 48 h at 44°C, the pattern of positive results was compared with a table of most probable numbers. The counts were expressed in units forming colony per ml of milk (CFU/ml). For the isolation and identification of *E. coli*, positive tubes showing turbidity and gas production were cultured on selective medium Hektoen agar (Pasteur Institute, Algeria) and incubated at 37°C for 24 h. Large yellow salmon colonies on Hektoen agar were suspected as *E. coli* strains and further confirmation was made by following standard microbiological techniques which include colony morphology studies, Gram staining. Biochemical analysis of *E. coli* isolates was performed using API 20E strips (BioMérieux).

Fecal *Enterococci* enumeration

Intestinal enterococci were counted using the most probable number method in Rothe broth (Pasteur Institute, Algeria). After incubation from 48 h at 37°C, the contents of the positive tubes, showing turbidity, were inoculated on BEA (*Bile Esculine Azide*) medium at 37°C for 24 and 48 h, for confirmation. Enterococcal colonies were small, translucent and surrounded by a black halo (positive esculin) (Maury, 1987).

***Staphylococcus* detection**

Staphylococcus detection was performed according to the standard method (ISO 6888-1:2003) on Baird Parker agar supplemented by egg yolk and potassium tellurite (Pasteur Institute, Algeria) by a spread plate technique; after enrichment on Giolitti Cantoni Base broth (Pasteur Institute, Algeria) (ISO, 2003). The agar plate was aerobically incubated for 24 - 48 h at 37°C. The positive result of the test is the appearance of colonies surrounded by a light halo with a black or grey center. Suspected colonies were sub-cultivated on the same selective medium plates and incubated at 37 °C for 24 h to obtain a pure culture. Pure cultures were further examined for morphological staining and cultural characteristics as well as biochemical characteristics (fermentation of mannitol, catalase and coagulase). For the identification of *Staphylococcus* species, API 20 Staph strips (BioMérieux) were used (Zangerl and Asperger, 2003).

Viable yeasts and molds enumeration

ISO 21527-1:2008 specifies a horizontal method for the enumeration of viable yeasts and molds in products intended for human consumption or animal feeding with a water activity greater than 0.95 (eggs, meat, dairy products (except milk powder), fruit, vegetables, fresh pasta, etc.), using the colony counting technique at 22 -25°C (ISO, 2008).

The spread-plate technique is strongly preferred to the pour-plate technique for enumeration of yeasts and molds in foods using dilution plating. Spread plating avoids any risk of thermal inactivation of fungal propagules which may be associated with the pour-plate technique and facilitates maximum exposure of cells to atmospheric oxygen (Beuchat, 2003). A sample of 0.1 ml of appropriately diluted sample is deposited in duplicate on the surface of the oxytetracycline glucose yeast extract agar (OGYE) (Pasteur Institute, Algeria). Then it was uniformly spread on the surface using a curved sterile glass rod. The rods must not exceed 2 mm in diameter in order to minimize the adhesion of the sample at the end of the spreading procedure. The agar plates were aerobically incubated for 5 days at 22°C (Beuchat, 2003). All colonies were counted and the results were expressed in units forming colony per ml of milk (CFU/ml).

Antimicrobial susceptibility/resistance test

An antimicrobial susceptibility/resistance test by disc diffusion on Mueller-Hinton agar (Pasteur Institute, Algeria) (Bauer et al*.,* 1966) was performed for all *E. coli* and *Staphylococcus* isolates according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2017). The antimicrobial agents and disc charges used in this study on *E. coli* isolates were ampicillin (AMP 10 µg), amoxicillin + clavulanic acid (AMC 30 µg), ceftazidime (CAZ 30 µg), Imipenem (IMP 10 µg), Ofloxacin (OFX 05 µg), Nitrofurantoin (F 300 µg), Gentamicin (CN 10 µg), Amikacin (AK 30 µg), Colistine (CT 50 µg) and Fosfomycin (FF 200 µg) (Thermo Scientific oxoid, France).

The antimicrobial agents and disc charges used in this study on *Staphylococcus* isolates were Penicillin (P 10 µg), Oxacillin (OX 1 µg), Amikacin (AK 30 µg), Gentamicin (CN 10 µg) and Kanamycin (K 30 µg), Erytromycin (E 15 µg), Clindamycin (DA 2 µg), Pristinamycin (PT 15 µg), Ofloxacin (OFX 5 µg), Levofloxacin (LEV 5µg), Vancomycin (VD 5µg), Rifampicin (RD 5µg) and Cotrimoxazol

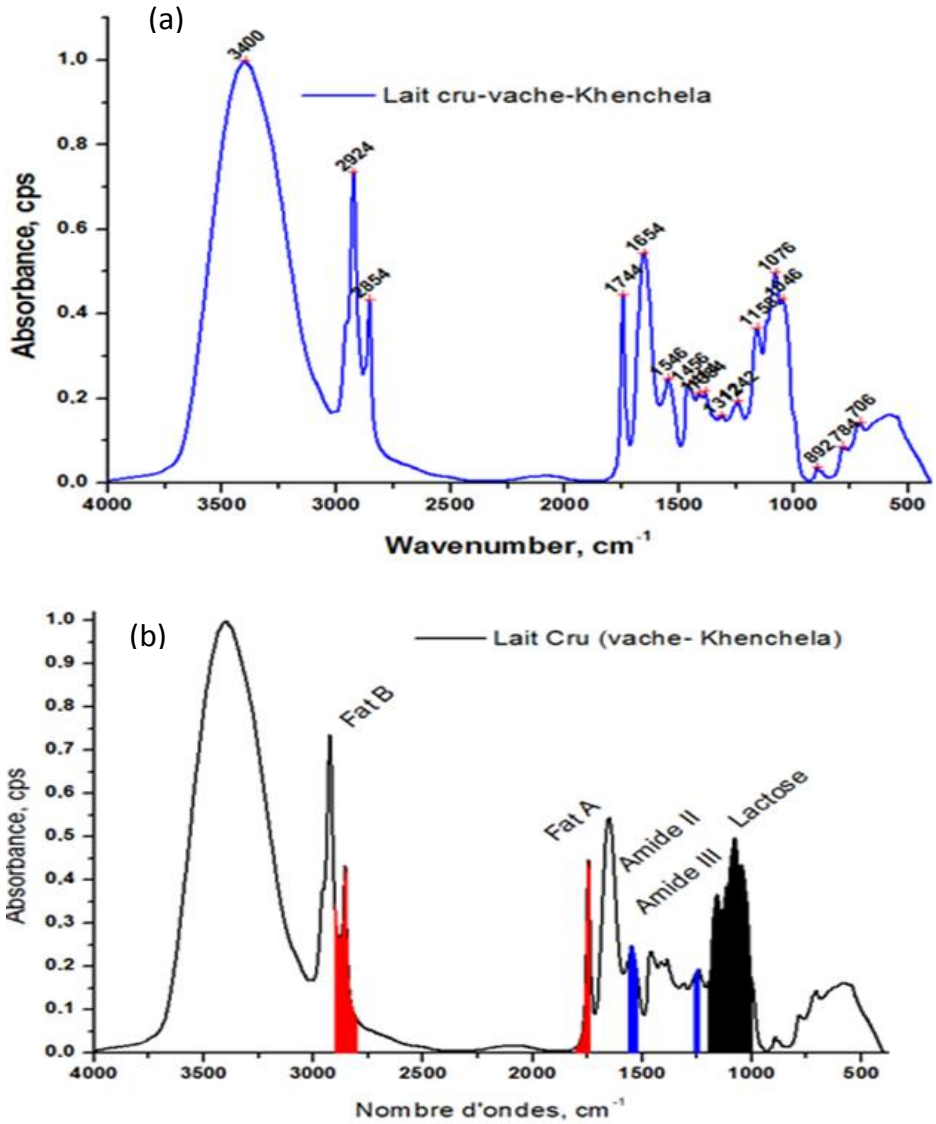

Figure 2. (a) Typical spectra raw cow milk samples obtained by Fourier transform infrared spectroscopy in selected spectral range 4 000-400 cm^{-1}. (b) Principal component analysis showing score plot of Fourier transform infrared measurements.

(SXT 1.25 µg) (Thermo Scientific oxoid, France). The area diameter for each antimicrobial agent was then transformed into sensitive, intermediate and resistant categories according to the performance standards interpretation table for antimicrobial susceptibility testing (MHPHR, 2014). For quality control, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 were used as reference strains.

RESULTS

Fourier transforms infrared (FTIR) spectroscopy

Figure 2a and b. show the Fourier transform infrared absorption spectra of raw milk samples in the spectral range of 400 - 4 000 cm^{-1}. There are numerous peaks that correspond to the different molecular bonds of milk components interacting with infrared radiation. Three main components, fat, protein and lactose, all have strong and characteristic peaks.

Determination of the fat content of milk samples from Fourier Transform Infrared (FTIR) spectra is mainly based on 2 specific regions. The spectral region between 1 725 and 1 850 cm^{-1} showed bands of low absorption due to the carbonyl group (C=O) of milk lipids (commonly called fat A). Another spectral region of medium intensity between 2 800 and 2 924 cm^{-1} absorbed infrared light due to the alkyl chain of fatty acids (commonly called fat B) (Lefier et al., 1996; Eskildsen et al., 2016). The

Table 1. Microbiological criteria for raw milk (JORA, 2017).

Raw milk	n	m	M
Total aerobic mesophilic flora	1	10^5 µo ml^{-1}	3.10^6 µo ml^{-1}
Fecal coliforms	1	10^3 µo ml^{-1}	3.10^4 µo ml^{-1}
Fecal enterococci	1	Absence / 0.1 ml	-
Staphylococcus aureus	1	Absence/ 1 ml	-
Clostridium sulfite-reducing agents at 46 °C	1	5×10^1 µo ml^{-1}	$1,5.10^2$ µo ml^{-1}
Antibiotics	1	Absence	-

m: Threshold below which the product is considered to be of satisfactory quality. M: Acceptability threshold beyond which the results are no longer considered satisfactory. M = 10 m, when counting in solid media. M = 30 m, when counting in liquid medium. n: Number of units in the sample (equal to 1 for raw milk); µo ml^{-1}: microorganism per milliliter.

infrared peaks at 2 854 and 1 744 cm^{-1} observed in Fig. 2.a. could be related to the milk fat contents B and A respectively.

Milk protein is expected to have absorption bands around 1 650, 1 550 and 1 250 cm^{-1} due to amide I, amide II and amide III groups, respectively (Dagnachew et al*.,* 2013). It also has an absorbance peak in the region between 1 060 and 1 100 cm^{-1}, associated with a phosphate group bound to casein protein (Etzion et al*.,* 2004). Figure 2b shows two spectral bands of medium intensity, the peak of 1 242 cm^{-1} in the wavelength range of 1 225-1 280 cm^{-1} corresponds to the N-H bending and C-N stretching vibrations of amide III (Lei et al*.,* 2010). The other peak of 1 546 cm^{-1} in the spectral range of 1 525-1 580 cm^{-1} was obtained by bending the n-plane N-H with the C-N stretching vibrations of amide II (Moros et al*.,* 2006).

Lactose is expected to have an absorption peak in the infrared region between 1 030 and 1 150 cm^{-1} due to the presence of various C-O stretching vibrations in carbohydrates (Grappin et al*.,* 2006; Zhou et al*.,* 2006). Figure 2a shows a high intensity peak of 1 076 cm^{-1} in the spectral range between 1 000 and 1 150 cm^{-1} and this peak could be related to the lactose content of the milk. A typical water transmittance spectrum between 3 650 and 3 000 cm^{-1} was represented in the hydroxyl group (O-H) (Coitinho et al*.,* 2017) (Figure 2a).

Microbiological analyses

The average contamination of milk samples with total flora is $3.7.10^5$ CFU/ml. fecal coliforms were present at an average value of $1.5,10^3$ CFU/ml. All of the samples (100%) were positive for the count of fecal enterococci with an average value of 2.5.10 CFU / ml. For fungal flora, it was present with an average value of $1.36,10^3$ CFU/ml.

According to the microbiological criteria of the inter-ministerial decree of 04-10-2016 of the OJ No.: 39/17 of the Algerian Republic (Table 1) (JORA, 2017), the overall quality of the study sample is compromised. Fecal enterococci were the first causes of non-compliance: A hundred percent of the samples was unsatisfactory, the total aerobic mesophilic flora and fecal coliforms do not exceed the acceptability limit according to the Algerian standard.

E. coli isolation and identification

For *E. coli* testing, 25 isolates were isolated, purified and identified. Antibiotic resistance was tested for each of the bacteria identified against 10 different antibiotics. The values of inhibition diameters were compared with the values in the reading table (MHPHR*,* 2014). Resistance rates for each antibiotic were calculated; the results obtained are grouped in Table 2.

Seventy two percent of *E. coli* isolates were resistant to Ampicillin (AMP). Forty per cent (40%) of isolates were to Amoxicillin/Clavulanic acid (AMC). The resistance frequencies for Ceftazidime (CAZ) (third generation cephalosporin) and Imipenem (IMP) were 28 and 00% respectively. The resistance rate obtained for antibiotics belonging to the aminoglycosides class is variable, with a resistance rate of 08% for Gentamicin and 32% for Amikacin. This variability would be due to the low consumption of Gentamicin giving the existence of less toxic and more effective molecules. Eight percent of *E. coli* isolates was resistant to Colistin, 12% to fosfomycin, ofloxacin and Nitrofurantoin.

Staphylococcus isolation and identification

The second cause of non-compliance of the milk sample is the presence of *Staphylococcus*. All samples were positive for *Staphylococcus*; colonies that developed on Baird Parker agar after incubation were stained with Gram stain and staphylocoagulase tested to distinguish strains with pathogenic potential (*S. aureus)* from non-pathogenic strains. Twenty five strains were isolated and

Table 2. Antimicrobial resistance profiles of *E. coli* isolates.

Classes	Antimicrobial agents and disc charges	Resistance rate, % (n)
β - lactam	Ampicillin (AMP) (10 µg),	72 (18)
	Amoxicillin + Clavulanic Acid (AMC) (30 µg)	40 (10)
	Ceftazidime (CAZ) (30 µg)	28 (7)
	Imipenèm (IMP) (10 µg)	00 (-)
Fluoroquinolones	Ofloxacin (OFX) (05 µg)	12 3
Nitrofurans	Nitrofurantoïn (F) (300 µg)	12 3
Aminoglycosides	Gentamicin (CN) (10 µg)	08 (2)
	Amikacin (AK) (30 µg)	32 (8)
Polymyxins	Colistin (CT) (50 µg)	08 (2)
Phosphonic Acids	Fosfomycin (FF) (200 µg)	12 (3)

purified; their identification by the API 20 Staph system revealed the predominance of coagulase-negative *Staphylococcus* (64%) compared to the coagulase-positive S*. aureus* species (36%). The main species of coagulase negative *Staphylococcus* isolated and their respective frequencies were: *S. hominis* (36%), *S. xylosus* (08%), *S. warneri* (08%), *S. epidermidis* (04%), *S. chromogenes* (04%) and *S. lugdunensis* (04%).

Antibiotic resistance was tested for each of the bacteria identified against 13 antibiotics. Resistance rates for each antibiotic were calculated and the results obtained are presented in Table 3. It can be observed that resistance rates vary significantly from one antibiotic to another. The *Staphylococcus* species studied showed a 100% resistance rate to Penicillin, and Oxacillin. The resistance rate obtained for antibiotics belonging to the aminoglycosides class varies, a zero resistance rate for Gentamicin, 04% for Amikacin and 08% for Kanamycin. For macrolides; the *Staphylococcus* species studied showed 100% resistance to Erytromycin, 48% to Clindamycin and 64% to Pristinamycin. Eighty per cent (80%) of the isolates were resistant to Rifampicin and 12% to Cotrimoxazole. A resistance rate of 2% for Ofloxacin and a zero resistance rate for Levofloxacin and vancomycin. Multiple antibiotic resistance phenotypes were generated from 25 *S. aureus* isolates showing resistance to three or more antibiotics. Data indicating the predominant multiple antibiotic resistance phenotypes are shown in Table 4.

DISCUSSION

Fourier transforms infrared (FTIR) spectroscopy

The possibility of FTIR analysis for milk and dairy products has been mentioned by Lanher (1991), Van de Voort (1992) and Lefier et al*.* (1996). Milk FTIR spectra could possibly give more useful information on how the quality of milk is influenced by environmental factors. This could be used to define new traits and also used as a herd management monitoring tool to detect aberrations due to feeding and other environmental changes (Dagnachew et al*.,* 2013). The results obtained showed that the weight composition of water, carbohydrates, lipids and proteins in the milk samples were always balanced in descending order: The vast majority of water, carbohydrates mainly represented by lactose, lipids and finally proteins. The use of treated wastewater in the study site obviously did not influence the biochemical and nutritional composition of the milk samples.

Bacteriological qualities of raw milk

The water used on the study farm is the wastewater treated and discharged by the treatment plant; this is mixture of urban, industrial, agricultural and hospital wastewater from the city of Khenchela. On the farm, this water is used for three main activities: pasture cultivation, dairy barn farming and cleaning, and for dairy cow consumption. The main objective of this study is to assess the risks associated with the reuse of treated wastewater in agriculture. The dairy industry is particularly concerned about the potential effect on dairy cattle and milk quality following pasture irrigation with waste water. Therefore, monitoring bacterial pathogens, their survival and transfer is of the utmost importance to ensure that milk quality is not compromised.

Contamination of raw cow's milk with microorganisms is influenced by the health status and hygiene of dairy cows (Chambers, 2002; Cempírková, 2007). Due to the high nutritional value, water content and almost neutral pH of milk, many pathogenic and spoilage microorganisms can develop (Ray, 2004). The value of the total mesophilic aerobic flora of raw milk indicates a very poor quality of

Table 3. Antimicrobial resistance profile of *Staphylococcus* isolates.

Classes	Antimicrobial agents and disc charges	Resistance rate, % (n)
β - lactam	Penicillin (P) (10 µg)	100 (25)
	Oxacillin (OX) (1 µg)	100 (25)
Aminoglycosides	Amikacin (AK) (30 µg)	08 (2)
	Gentamicin (CN) (10 µg)	0
	Kanamycin (K) (30 µg)	04 (1)
Macrolides	Erytromycin (E) (15 µg)	100 (25)
	Clindamycin (DA) (2 µg)	48 (12)
	Pristinamycin (PT) (15 µg)	64 (16)
Fluoroquinolones	Ofloxacine (OFX) (5 µg)	4 (1)
	Levofloxacin (LEV) (5 µg)	0
Glycopeptides	Vancomycin (VD) (5 µg)	0
Rifamycin	Rifampicin (RD) (5 µg)	80 (20)
Sulfonamides	Cotrimoxazole (SXT) (1.25 µg)	12 (3)

Table 4. Multiple antibiotic resistant phenotypes for *Staphylococcus* isolates.

Phenotypes	Number of isolates	(%) Observed
P - OX - E	25	100
P - OX - E - RD	20	80
P - OX - E - RD - PT	16	64
P - OX - E - RD - PT - DA	12	48
P - OX - E - DA - PT - RD - SXT	3	12
P - OX - E - DA - PT - RD – SXT - AK	2	8
P - OX - E - DA- PT - RD - SXT – AK - K	1	4

raw milk compared to the required standards of 10^5 CFU/ml (JORA, 2017). In addition, the overall bacterial load was very high; 90% of the samples had a value greater than 10^5 CFU/ml of flora. This total flora load and the large number of samples exceeding the recommended limits can be attributed mainly to infected udders, unsanitary milking equipment or procedures and/or poor microbiological quality of water used for cleaning utensils and animals, as well as milk storage conditions (Chye et al*.,* 2004; Ghazi and Niar, 2011; Singh and Gupta, 2015; Wanjala et al*.,* 2018). The result of the fecal coliforms showed significant contamination and indicated very poor quality of raw milk compared to the required standards of 10^3 CFU/ml (JORA, 2017). In general, coliforms indicate fecal contamination and their number is proportional to the degree of pollution produced by the stool (Aggad et al*.,* 2009). However, the presence of coliforms indicates poor hygienic and sanitary conditions during milking and subsequent handling or water supply (Yucel and Ulusoy, 2006).

Some studies have shown that cattle excreta is not a significant source of coliform contamination of raw milk, but that water used for sanitation and milking environments is considered as one of the critical sources (Kagkli et al*.,* 2007; Martin et al*.,* 2016). Therefore, the use of poor quality and unsanitary water during sanitation procedures can indirectly contaminate milk (Robinson, 2005).

E. coli was isolated in 100% of the samples; the presence of this bacterium in milk indicates possible contamination by contaminated manure, soil and water (Chye et al*.,* 2004). The development of antibiotic resistance in bacteria such as *E. coli* is a serious public health problem. The results show that only one antibiotic, Imipenem, showed 100% efficacy against *E. coli* strains; of the 25 isolates tested, 72% showed resistance to at least one of the 11 antibiotics.

The highest resistance of *E. coli* isolates in this study was observed in antibiotics β-lactam. β-lactam antibiotics have low toxicity, a factor that has led to overuse of these

drugs in medical therapy (Moyane et al*.,* 2013). Few studies have noted resistance of enterobacteriaceae to the antibiotic β-lactam in milk samples (Ntuli et al*.,* 2016); a study by Geser et al*.* (2012) reported resistance to antibiotics CTX-M β-lactam in *E. coli* from milk samples.

The development of bacterial resistance to antimicrobial agents is a serious threat to human health (Zastempowska et al*.,* 2016). Although antibiotic-resistant bacteria and genes encoding antibiotic resistance have been commonly detected in wastewater and treatment system by-products, the role of wastewater treatment processes in the dissemination of antimicrobial resistance is not clear (Mohammadali and Davies, 2017). In recent years, a number of studies have focused on variables that influence the profiles of antibiotic-resistant bacteria and antibiotic resistance genes during treatment (Xia et al*.,* 2012; Yuan et al*.*, 2014).

Hospital wastewater is likely to contribute significantly to the spread of multidrug-resistant pathogenic bacteria in wastewater treatment plants (Lien et al*.,* 2016). Due to the presence of constant sub-inhibitor levels of broad spectrum antimicrobials, hospital wastewater creates an ideal situation for the exchange of antibiotic resistance genes and their combinations between clinical pathogens and environmental bacteria (Basode et al*.,* 2018; Amador et al*.,* 2015). *Staphylococcus* was detected in all samples. The high number of isolated coagulase-negative *Staphylococcus* is believed to be due to poor milking hygiene conditions and poor quality washing water (Kouamé et al*.,* 2010; Hamiroune et al*.,* 2016).

S. aureus of environmental origin can easily colonize cow udders (Piessens et al*.,* 2011). In addition, unhygienic cow milking methods, particularly manual milking and the use of contaminated utensils, could lead to contamination of milk with *S. aureus* from foreign sources (Hamiroune et al*.,* 2016). The presence of *S. aur*eus tends to reduce the quality of milk and milk products traditionally prepared by their metabolic activities and could precipitate food poisoning due to the development of toxins that could cause disease when consumed by humans (Omoshaba et al*.,* 2018). The study of the susceptibility/resistance of *S. aureus* to antibiotics revealed high resistance frequencies, particularly for penicillin and oxacillin. The mechanism of penicillin resistance is based on the bacterium's synthesis of an enzyme called β-lactamase or penicillinase (Guérin-Faublée and Brun, 1999). This inducible plasmid enzyme hydrolyzes the β-lactam cycle of penicillins A and G and renders them inactive (Kotra and Mobashery, 1998).

Staphylococcus in hospitals, and more recently in communities (present outside the hospital environment) have developed cross-resistance between penicillins M (methicillin, oxacillin) and other β-lactamins through the production of a protein, PLP2a, which binds penicillin (PLP) and has a low affinity for these compounds (Chambers, 2001). The gene encoding PLP2a, *mec*A, is carried by a chromosomal element that also contains other genes for resistance to heavy metals and other antibiotics, which explains the multi-resistance profile of MRSA (methicillin-resistant *S. aureus*) (Dumitrescu et al*.,* 2010).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important opportunistic pathogen in humans and cattle (Omoshaba et al*.,* 2018). In this study, methicillin-resistant *Staphylococcus aureus* could be transferred from livestock to humans through milk and dairy products. Multiple antibiotic-resistant strains of *Staphylococcus*, defined as isolates resistant to three or more antibiotics, were obtained in a large proportion of the milk samples analyzed. The development of multiple antibiotic resistances in most of these isolates can be attributed to the acquisition of plasmid-mediated resistance (factor R) (Yamamoto et al*.,* 2013; Akindolire et al*.,* 2015). Usually, *S. aureus* is known to contain a number of multiple antibiotic-resistant plasmids that may explain the observed phenotypes (Yamamoto et al*.,* 2013).

These results reveal that multiple antibiotic-resistant *Staphylococcus* isolates were isolated from milk samples. It is therefore suggested that these multiple antibiotic resistant isolates can have serious health implications for people who consume such dairy products. The high number of yeasts and molds in this study may be due to poor equipment hygiene during milk handling and processing, and indicating unsanitary conditions of handling and environmental contamination (Bonfoh et al., 2003; Prejit and Latha*,* 2007). Many foodborne molds, and possibly even yeasts, can also be dangerous to human or animal health because of their ability to produce toxic metabolites called mycotoxins. Human exposure to mycotoxins can result either from the consumption of contaminated food of plant origin or from the ingestion of mycotoxins transported from animal feed into animal tissues, meat, eggs or milk (Zastempowska et al*.,* 2016). Some foodborne molds and yeasts can also cause allergic reactions or infections.

Conclusion

This study aims to assess the impact of the use of chlorine-free treated wastewater in farming and dairy cattle breeding. Milk samples were collected from a farm in Northeastern Algeria. The treated wastewater from the treatment plant is used on this farm for different activities. The results of this study indicate that the overall microbiological quality of milk samples is well below current Algerian standards; they are heavily contaminated with fecal contamination germs and pathogenic bacteria with worrying antibiotic multi-resistance profiles. The source of contamination in milk samples can be water used for three main activities: pasture farming, dairy barn operations and cleaning, and for consumption by dairy cows. The presence of

multidrug-resistant bacteria in milk can pose a serious threat to public health and has a negative effect on the treatment of infections in humans. Newborns and children appear to be more exposed to milk contaminants than adults because they consume larger amounts of milk and are more sensitive. Urgent and effective measures must be taken to ensure proper wastewater management by the services concerned and farmers. Therefore, it is recommended that training and advice be given to farm owners and workers responsible for milking, emphasizing the need for hygiene practices on farms.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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