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Subclinical mastitis on the raw milk as a risk factor for the transmission of Staphylococcus.aureus and coagulase-negative staphylococci, multidrug resistance in Sidi Bel Abbes, Algeria

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ABSTRACT

In Algeria, the milk is consumed directly, generally in the raw state thus escaping any quality control. The subclinical mastitis becomes a problem of food security. Our hypothesis is that consumption of the raw milk of the cows with subclinical mastitis is a tool for transmission of antibiotic resistant staphylococci, which can pass unobserved to man. A total of 981 raw milk samples were obtained from 250 cow's quarters. The California Mastitis test (CMT) has been performed to diagnose the presence of subclinical mastitis. Milk samples were subjected to a bacteriological study and presumptive colonies were confirmed by biochemical characterization using the API-Staph-20 system. The sensitivity tests were performed by disc diffusion method on Muller-Hinton agar, for 13 antibiotics commonly used in veterinary medicine in Algeria, and the VITEK 2 system is used for the determination of the minimum inhibitory concentrations (MIC) for the strains confirmed as S. aureus. The biochemical identification showed that 73.75% of the isolates are CoNS, and that 26.25% are S.aureus. Resistance to penicillin G touches 61.02% of the strains of CoNS that with the tetracycline is of 74.58%. For *S. aureus*, we found a high resistance to penicillin G 80.95% and with the tetracycline 71.43%. According to our results, 02 (9.52%) isolate is *S. aureus* resistant to the methicillin , with modified penicillin-binding proteins , identified by the test of the disk of the oxacillin with a diameter of: R≤10 mm, and confirmed by one MIC ≥ 4 µg / l. The MIC of the vancomycin has proved that one strain is a VRSA with one MIC ≥ 32 µg / ml, and a second strain is a hetero-VISA with one MIC > 1 µg / ml. We noted that for the 21 strains of *S. aureus*, 11 different phenotypes of resistances dominated by resistance to "penicillin G". Our results indicated that there's a multi-resistant staphylococci passage in raw milk consumed in the western region of Algeria, harmful for the health and the security of the consumer, with a higher prevalence of coagulase negative staphylococci compared with S.aureus. Despite unknown pathogenicity of the species, organisms may be the tanks of the resistant to antibiotics; which requires periodic monitoring of antimicrobial resistance of staphylococci in order to control their spread.

KEYWORDS: Antimicrobial, Minimum Inhibitory Concentration, Multi-resistance, Food Security.

INTRODUCTION

The raw milk has superior nutritional properties. Due to its neutral pH and the high activity of water, this is an excellent growth medium for the various microorganisms [7].

In most African countries, the milk is consumed directly, generally in the raw state thus escaping any quality control. The mastitis becomes a problem of food security [19].

The bovine Mastitis is the most common disease affecting the dairy herds worldwide [9]. Subclinical mastitis is the predominant form in the appearance and the staphylococci are the main causative agents [54]. Therefore, she is not easily recognized by the farmers and can lead to important production losses [16].

Staphylococcus aureus remains one of the most important organisms associated with the contagious bovine subclinical mastitis, not only in Algeria, but in the whole world. The investigation of antimicrobial resistance of staphylococci in dairy cows is important not only for the control of bovine mastitis, but also for public health [53].

This antimicrobial resistance developed by the pathogens is one of the main reasons for the low cure rates of mastitis [51].

The strategy adopted for the realization of our work, was based on the hypothesis that consumption of the raw milk of the cows with subclinical mastitis is a tool for transmission of antibiotic resistant staphylococci, which can pass unobserved to man and compromising food security.

MATERIALS AND METHODS

The present study was conducted in Sidi Bel-Abbes, situated in west of Algeria 433 km from the capital, in the laboratory of the Department of Agricultural Sciences of the Faculty of Natural Sciences and Life, University Djillali Liabés during the period from May 2013 to July 2015. The cows during lactation in the dairy farms of Sidi Bel-Abbes region were examined.

Sampling:

A total of 981 raw milk samples were obtained from 250 cow's quarters. Individual milk samples were taken from the four quarters of each cow milking, just before the second milking of the afternoon [28].

The California Mastitis Test (CMT):

The California Mastitis test (CMT) was performed to diagnose the presence of subclinical mastitis. This test was performed and interpreted by the methods described by [31] .

Inclusion criteria:

All the animals selected for this investigation was clinically healthy and did not show any mammary abnormality. In this study, a case of subclinical mastitis was defined as being a cow with at minimum a score of CMT $\geq$2 in one of the quarters.

Bacteriological analyzes:

Milk samples were analyzed by classical bacteriological isolation and identification techniques, as described by [37] and [31] .

Identification of staphylococci isolated by the API system:

The suspected colonies were confirmed by the biochemical characterization using the API-20-Staph system (BioMérieux, Marcy-France). The organism was cultivated on blood agar Columbia in 36 ° C ± 2 ° C for 18-24 hours. Inoculated a pure colony and well isolated in the API Staph medium to make a homogeneous bacterial suspension with a turbidity equivalent to 0.5 McFarland and this suspension was used immediately after the preparation. The identification was obtained with the 7-digit number for the profile 20 API strip test. Reading the digital profile is achieved by apiweb software version 4.1 [11].

Antibiotic susceptibility tests:

Disc diffusion test:

Sensitivity tests were performed by disc (Bio-Rad, France) diffusion method on Muller-Hinton agar. The disk diffusion test was conducted and judged according to the Clinical and Laboratory Standard Institute [8]. The *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) isolated from the mastitis milk samples were inspected for their behavior sensitivity against various antimicrobial agents used in the practice of veterinary and human medicine. The antibiotics tested were the following : Penicillin G (PG-10 UI), Amoxicillin + Clavunique acid (AMC- 20/10 µg) , Oxacillin (Ox- 1 µg), Cefoxitin (Fox-30 µg), Erythromycin (E-15 µg), Neomycin (N- 30 µg) , Gentamicin (Gm-10 µg), Enrofloxacin (ENR-5 µg) , Trimethoprim+sulfamethoxazole (SXT- 1,25/23,75 µg) , Tetracyclin (Te-30 µg), Vancomycin (VA-30 µg), Bacitracin (Ba-130 µg) , Clindamycin- Cm-2 µg) [8].

The quality control was carried before each antibiogram on reference strains: *Staphylococcus aureus* ATCC 25923; *Pseudomonas aeruginosa* ATCC 27853; *Escherichia coli* ATCC 25922.

Determination of minimum inhibitory concentrations (MIC) by the VITEK 2:

The VITEK 2 system (BioMérieux) is an integrated modular system which consists of a fill-seal unit, an incubator-reader, a computer control module, a data terminal and a multi copy printer. The system detects the bacterial growth and the metabolic changes in the micro wells of thin plastic cards by using a fluorescence-based technology. Different micro well cards contain the antibiotics or the biochemical substrates. The suspensions were prepared by emulsifying the bacterial isolates in 0.45% saline solution to the equivalent of a 0.5 McFarland turbidity standard according to the manufacturer's recommendations. The cards were automatically filled, sealed and charged into the instrument VITEK 2 for incubation and reading.

AST-P631 is plastic card of 64-well that contains the following antimicrobial agents used for staphylococci : Benzylpenicillin, Clindamycin, Erythromycin, Gentamicin, Neomycin, , Oxacillin, Test Cefoxitine screan , Tetracycline, Enrofloxacin , Trimethoprim-sulfamethoxazole, and Vancomycin. The instrument of VITEK 2 controls the growth in each well on this card within the specified period. At the end of the incubation cycle, the MIC values are determined for each antibiotic present in the card [8, 52].

Statistical analysis:

The risk factors were analyzed according to the CMT score results. The relationship between the occurrence of the subclinical mastitis and the bacteriological results was verified using chi square test or Fisher exact test, p value is considered as significant if $p<0.05$, highly significant if $p<0.01$ or strongly significant if $p<0.001$, by using the SPSS statistical software Version 23: (Statistical Package for the Social Sciences, IBM Corporation).

Results:

Nine hundred and eighty-one quarters examined of 250 lactating cows, clinically healthy, with normal appearance of udder examined for the presence of subclinical mastitis by using the California Mastitis test (CMT). On the total of the examined quarters, 19 (1.9%) quarters were inactive leaving 981 functional quarters.

According to the CMT the prevalence of the subclinical mastitis in the area is of (33.6%), as indicated in the (Table 1). The prevalence of the mastitis on the basis of the quarters is 336 with a higher infection rate in the hindquarters (173 quarters) compared to the forequarters (163 quarters) (Table 1).

Three hundred thirty six positive milk samples with a score of CMT≥2 were examined for bacteriology. The bacteriological examination revealed a percentage of positive samples of 14.2% (Table 1).

As a whole there was a strong significant association between the bacteriological examination and the CMT in the milk obtained by the $\chi 2$ test ($P <0.001$) . There is a good sensitivity to detecting subclinical mastitis by using CMT with a score ≥2. The subclinical mastitis and bacteriological examination are related even if the quarters will be changed.

The bacterial isolates were identified biochemically by using the API 20 STAPH system and the species of Staphylococci were summarized in (Table 2). A strong difference is marked between species distribution ($p<0,001$) indicating a large variation in resistance witch is highest in *S.aureus.*

The sensitivity of the CoNS strains vis-a-vis 13 antimicrobial agents tested in this study are presented in (Figure 1). Resistance to penicillin G touches 61.02% of the strains of CoNS, that with the oxacillin is of 35.59%. The frequencies of resistance to the clindamycin, erythromycin and tetracycline are respectively 27.12%, 38.98% and 74.58%. The percentage of the resistance of the CoNS to the enrofloxacin was of 10.17%, 06.78% of the resistance to the vancomycin and 03.39%, 05.08% to the amoxicillin + Clavunique acid and the trimethoprim-sulfamethoxazole respectively. No resistance was observed for the bacitracin, the neomycin and the gentamicin for CoNS strains, we also noted a low resistance rate of 01.69% to the céfoxitin.

Intermediate resistance to the clindamycin was the most frequent result 08.47%, followed by the enrofloxacin 06.78%, the trimethoprim-sulfamethoxazole 03.39% and 01.69% for the tetracycline, the gentamicin and the neomycin, respectively .

On the whole, we noted us for 59 CoNS strains 25 different resistance phenotypes, the most isolates (72.88%; n = 43) were resistant to more than one antimicrobial agent; they are largely dominated by the phenotype "Tetracycline", that is represent in (Figure 2).

For *S. aureus*, we found a high resistance to penicillin G 80.95% and with the tetracycline 71.43%. For the vancomycin, the gentamicin, the bacitracin, the trimethoprim-sulfamethoxazole and with the cefoxitin, resistance is null and thus a very good sensitivity. The resistance to the erythromycin is the most frequent result with 28.57%, followed by the clindamycin 14.29%, 14.28% for the Amoxicillin + Clavunique acid and the neomycin, the oxacillin 09.52% and the enrofloxacin 04.76%.

In general, one observed a good association between the antibiogram and measuring of the MIC for all strains of *S. aureus*; what is represented in (Table 3). There is a significant association ($p<0,001$) between antibiotics and the resistance level obtained by the two methods. This means that antibiotics do not have the same effects on S.aureus, since the most effective antibiotics are SXT and GM using both methods: standard method and the CMI test.

The phenotypic method based on the agar disc diffusion failed to detect the positive results of the test cefoxitin screen, on the other hand 02 *S. aureus* strains were positive to this test confirmed by the MIC.

The MIC of VITEK 02 detects 02 *S.aureus* strains with modified penicillin-binding proteins (PBP), a strain with Hetero-VISA (Vancomycin-intermediate *S. aureus*) and the other with VRSA (Vancomycin-resistant *S. aureus*).

We noted that for the 21 strains of *S. aureus*, 11 different phenotypes of resistances dominated by resistance to penicillin G, which is represented in (Figure 3).

Discussion:

The present study was conducted to study the antimicrobial susceptibility of the staphylococci isolated from the raw milk of the lactating cows with the subclinical mastitis cases in the western Algeria. The CMT is a safety assurance tool for raw milk intended for human consumption. According to [4] the CMT with a score ≥ 2 or more was associated at an increasing risk of infection by *S. aureus*. In our study the results show good sensitivity of the CMT for a bacteriological identification of the subclinical mastitis in the cows; of the similar results were found by [38]. The association between the bacteriological examination and the CMT in the milk obtained by the Chi 2 test ($P < 0.001$) is strongly significant.

In this study the prevalence of quarters with a subclinical mastitis is of 33.6% which is higher than the results found in other studies conducted in Algeria. In the region of the Algerian east [28] found 29.44%, same in another study in the central of Algeria [40] reported 29.2%. In India [33] one reported 30.73% and in Egypt [10] 23.47%.

According to the bacteriological examination , among the 336 positive samples with the CMT 142 samples are positive bacteriological cultures and the staphylococci represents (56.34%), confirming that the staphylococci are the main causative agent of the bovine mastitis in the different countries [51] . The Staphylococcal mastitis reported like more common by many studies; 41.04% in India by [33] , and 63.6% in Egypt by [10].

The biochemical identification showed that 73.75% of the isolates are CoNS, and that 26.25% are S.aureus. This result is in agreement with [32] in Ethiopia and by [22] in Zimbabwe where CoNS, and *S. aureus* were reported as the organisms most frequently isolated by descending order of importance. This stresses the importance of CoNS in the subclinical mastitis and that some of them are more pathogenic than what is previously admitted.

Several species of CoNS are isolated in this study; the data suggest that the distribution of the main species of CoNS in the milk of mastitis was different between the dairy farms or the herds [53]. The CoNS species isolated in the present study in agreement with the results are isolated by [46] and [13] . In this study, the isolates from CoNS are 73.75% what higher of 29.12% found by [28] in east of Algeria and lower than [17] in Iran with 95.6%.

In the present study, the antibiotics are selected for the antibiogram by taking account of drugs most frequently used for the treatment of the bovine mastitis in Algeria. For this the activity of 13 antibacterial agents is tested in vitro against strains of staphylococci isolated from the subclinical mastitis in the lactating cows.

Our results indicate an increase in resistance to antibiotics of Staphylococci isolated from the mastitis, what is agreement with the results of [36].

In the present study the isolates of *S. aureus* showed a strong resistance to penicillin G and the tetracycline 80.95% and 71.43% respectively. The high prevalence of this resistance is agreement with the results of [20] in Malaysia (86% and 76.6%) and the results of [51] in China of (90.4% and 74.4%). Our results diverge with that from [5] in France which found a very low prevalence (17% and 1.4%), and with the study carried out in the European Union by [45] in which resistance to penicillin is of (50.0%) in Italy , (15.5%) in the Netherlands and (37.5%) in France.

The high percentage of resistant isolates of *S. aureus* to penicillin and tetracycline can be due to the wide administration of these antimicrobials in the dairy farms [20]. In addition it is postulated that resistance is exacerbated by the frequent use of intra-mammary preparations by the farmers [21]. In Algeria the beta-lactams including penicillin G are largely used in the treatment of the mastitis without any test of the sensitivity of the bacterial strains with respect to these antimicrobials.

The Gentamicin is very active against *S. aureus*, no resistance was found. This result is similar to the results got by [34] in India, [1] in Ethiopia and of [41] in Algeria.

It is interesting to note that the present study revealed a complete susceptibility (100%) of *S. aureus* strains to many antibiotics, including the trimethoprim-sulfamethoxazole, the gentamicin, the cefoxitin and the bacitracin; the same result was reported in east Algerian with [2].

The high percentage of sensitivity to these families of antibiotics could be the cause of their less frequent uses in veterinary practice in Algeria, for the reason of the high cost of these drugs.

Resistance to the erythromycin is of 28.57%, the same result was reported by [48] in India of 27.94%, and distinct to [51] in China, which found a high prevalence for this drug 79.9%. A low resistance to the clindamycin of 14.29% noted in the present study which is lower than the resistance found by [20] in Malaysia with 34.9% and largely lower than [51] in China with 77.2%.

In this study a low resistance is detected for the amoxicillin-clavulanic acid with 14.28%, but remains frankly lower than the result of [48] in India which shows a resistance of 63.23%. The high synergistic activity of the amoxicillin and clavulanic acid depending on their resistance to the enzyme of ß-lactamase. The clavulanic acid is an inhibitor of ß-lactamase [25].

In the present study a low resistance was detected for the enrofloxacin 4.76% which is similar with the results of [35] in Egypt with 4.8%. For the neomycin, one reported a low resistance of 14, 28%, diverging the results from [41] , which found a sensitivity of 100% for this antibiotic.

In spite of the frequent use of the neomycin in veterinary practice in Algeria in the intra mammary pomades especially, the high sensitivity noticed in the present study is justified by the use of this molecule associated with other antibiotics to ensure an broad activity spectrum covering all the bacteria commonly met in the mastitis.

Of 21 isolates of *S. aureus*, 08 (30.1%) isolates with a multidrug resistance phenotype. According to [27] , the definition most frequently used for the multi-resistant bacteria, is resistance to three classes or more of antimicrobials. The various phenotypes of resistance observed in the present study can be due to emergence of new strains of organisms in our area.

According to our results, 02 (9.52%) isolate of *S. aureus* is *S. aureus* resistant to the methicillin (MRSA), identified by the test of the disk of the oxacillin with a diameter of: R≤10 mm, and confirmed by one MIC ≥ 4 µg / liter. The Céfoxitin disc diffusion test (30 mcg) to detect the methicillin resistance of *S. aureus* gave a result with a diameter of: S ≥22 mm thus negative, what is contradictory with which indicate the MIC ≥ 8 µg / l which indicates a positive test for the test of céfoxitin screen by the automated system VITEK2 .

A similar conclusion, found by a study out in Switzerland, demonstrated that on 142 *S.aureus* strains obtained from 2,662 samples taken on bovine mastitis, only 2 (1.42%) isolates were positive for MRSA [49].

In the present study the Vitek 2 system (BioMérieux) was used for the first time in Algeria to testing the MIC in the studies on the sensitivity of the germs isolated from the subclinical mastitis from the cows, the test was carried out according to the manufacturer's instructions. Readings were taken automatically every 15 minutes. Several studies have developed the reliability and the capacity of the automated systems like the VITEK2 system to detect resistance to deferent antibiotics in the staphylococci [52].

The break point values of MIC for *S. aureus* are used according to the recommendation of CLSI (Clinical and Laboratory Standards Institute). For the test of sensitivity to oxacillin : one MIC ≤ 2 µg / liter indicates susceptibility, and one MIC ≥ 4 µg / liter indicates the resistance. Thus for the test of cefoxitin screen: one MIC ≤ 4 µg / L indicates a negative test, and one MIC ≥8 µg / L indicates a positive test.

In general, one observed a good agreement between the antibiogram and the MIC for all the strains of *S. aureus*, except for the phonotypical method of céfoxitin disc (30 mcg) which found a contradictory result with the MIC.

According to [12] the study realized in France indicate that the test of céfoxitin disk (30 mcg) showed a specificity and sensitivity of 100 % for the detection of the MRSA with the criteria of interpretation of diameter of <27 mm. For the study of [43] in Denmark, the method of the céfoxitin was excellent with a sensitivity of 100% and a specificity of 99% using a diameter of the interpretation area of S ≥ 29 mm and of R <29 mm. These diameters are higher than those corresponding to the prospects of CSLI for the values of disk diffusion, can be the justification of our results.

The automated system VITEK 2 is proven able in reproducible detection and interpretation with a high level of precision and standardization of the resistance mechanisms of the *S. aureus* strains [24, 26, 39].

Few studies announced the resistance of the vancomycin of *S. aureus* in the bovine mastitis in Algeria. The vancomycin is the most reliable choice for the control of *S. aureus* strains resistant to methicillin (MRSA). Recently, the MRSA become resistant to the vancomycin, which generates a crisis to control them [29].

The CLSI lowered the critical values of the MIC of the vancomycin from 4 µg / mL to 2 µg / mL for the sensitive isolates and of 32 µg / mL at 16 µg / mL for resistant strains in 2006 [18].

For the vancomycin resistance by the disk diffusion method in agar, our results are in agreement with [5] in France in which no resistance was reported. In the present study, we found an intermediate resistance strain for the vancomycin.

After the confirmation by the MIC of the vancomycin, it has proved that this strain is a (VRSA) which means a strain of *S. aureus* resistant to the vancomycin with one MIC ≥ 32 µg / ml, and a second strain hetero-VISA with one MIC > 1 µg / ml.

The hetero-Visa is defined like of *S. aureus* strains sensitive to the vancomycin but presence of under intermediate population to the vancomycin [6, 15] . For *S. aureus*, a heterogeneous intermediate resistance to the vancomycin seems to be the stage which precedes the development of intermediate resistance to this molecule [30].

The mechanism of genetic and biochemical resistance to the vancomycin in *S. aureus* is always poorly known [14].

The MIC of the hetero-VISA isolates is in the range of susceptible strains, but the subpopulations of the VISA are present at a frequency of $<10^5$-10^6 [30] .

The interpretation criteria (in millimeters) of the CLSI define only the character of the sensitivity for the disk of vancomycin which are however ≥ 15 mm, these last are not reliable to detect the VISA strains.

In the present study, the bacteriological results indicate a high prevalence of CoNS with 73.75%; several studies noted that majority of bacteria coming from cows with subclinical mastitis are CoNS, in Algeria [2] and in Uganda [21]. For a long time the CoNS are considered as minor pathogens, and they are generally associated with a light increase amongst the number of somatic cells in milk during intra mammary infections [3].

In Europe the CoNS of bovine origin are most of the time reported to be sensitive to the antimicrobial agents [44].

According to [50] resistance to other antimicrobials one that the penicillin of CoNS was rare .What it is not the case of our study.

In the present study the CoNS are more sensitive than *S.aureus* with respect to penicillin with 61.02% compared with 80.95%. For the CoNS penicillin is the least effective antibiotic, what agree with the study realized in Turkey by [47].

A lower activity was found for the tetracycline with 74.58% of resistance, the same decreased sensitivity is observed in a study in Korea between 2003 and 2008 by [23] with 80.1% of resistance.

In the present study, the study of antibiotic susceptibility of CoNS emphasizes frequencies of the resistance of 5.08% for SXT, and for the sensitivity to 100% for the gentamicin and the neomycin, the same results were found by [2] in the east of Algeria.

In this study a resistance of 38.98% and 27.12% is for the erythromycin and the clindamycin, respectively. Our result is significantly higher than that found to erythromycin in Algeria by [2] which found 100% of sensitivity, and higher than that of [5] in France with 7.3%. For the clindamycin 2.9% and 15.4% were founded by [5] in France and [46] in Turkey.

Concerning the enrofloxacin and the vancomycin, one noted a good activity of these antimicrobials with low resistance of 10.17% and 6.75%, which diverge with the results from [5] in France which found a sensitivity of 100%.

These CoNS isolates are resistant to the oxacillin with 35.59%, but one strain (1.69%) was resistant to the céfoxitin by the disk diffusion method , what implies that it is methicillin resistant (MRS), this observation agree with it of [3] in Tunisia with 1.7%.

In the present study a difference in sensitivity to antimicrobial among CoNS species was observed, what is also described by [49] . These CoNS can thus be used like reservoir of resistant genes to antimicrobial in the environment [41].

Table 1: The prevalence of the mastitis and bacteriology compared to quarters

Quarters	Number of examined quarters	Subclinical Mastitis (SM)		Bacteriological results (BR)		Inactive teats		SM vs BR		
								χ^2	DF	p
AD	250	80	32.0%	35	14.0%	6	2.4%	126.015	1	<0.001
PD	250	83	33.2%	32	12.8%	3	1.2%			
AG	250	83	33.2%	33	13.2%	7	2.8%			
PG	250	90	36.0%	42	16.8%	3	1.2%			
Total	1000	336	33.6%	142	14.2%	19	1.9%			

Table 2: The frequency of the staphylococcal species isolated from quarters with a subclinical mastitis (CMT positive).

Species	Frequency	Percentage (%)	χ^2	DF	p
Xylosus	11	13.75	67.375	14	<0.001
Hominis	6	7.50			
Caprae	6	7.50			
Intermidius	6	7.50			
Auricularis	2	2.50			
Aureus	21	26.25			
Chromogenes	5	6.25			
Scuiri	3	3.75			
Simulans	1	1.25			
Epidermis	6	7.50			
Warneri	2	2.50			

Capitis	2	2.50			
Hemolyticus	5	6.25			
Lentus	2	2.50			
Hyicus	2	2.50			
Total	80	100.00			

Table 3: The test of sensitivity of S. aureus for different antibiotics and the MIC realized by automat VITEK 2

Antibiotics [a]	Resistance degree [b]						χ2	DF	p	The CMI test (VTTEK2) [c]				χ2	DF	p
	R	%	I	%	S	%				S	%	R	%			
PENI	17	80.95	0	0.00	4	19.04				5	23.80	16	76.19			
AMC	3	14.28	1	4.76	17	80.95										
ENR	1	4.76	1	4.76	19	90.47				20	95.23	1	4.76			
VAN	0	0.00	1	4.76	20	95.23				20	95.23	1	4.76			
TE	15	71.43	0	0.00	6	28.57				5	23.80	16	76.19			
SXT	0	0.00	0	0.00	21	100				21	100	0	0.00			
CM	3	14.29	0	0.00	18	85.71	124.9	24	<0.001	18	85.71	3	14.29	92.0	10	<0.001
E	6	28.57	0	0.00	15	71.43				15	71.43	5	23.80			
N	3	14.28	1	4.76	17	80.95				17	80.95	4	19.04			
OX	2	9.52	1	4.76	18	85.71				19	90.47	2	9.52			
GM	0	0.00	0	0.00	21	100				21	100	0	0.00			
FOX	0	0.00	0	0.00	21	100				19	90.47	2	9.52			
BA	0	0.00	0	0.00	21	100										

a, b, c : CLSI 2008.

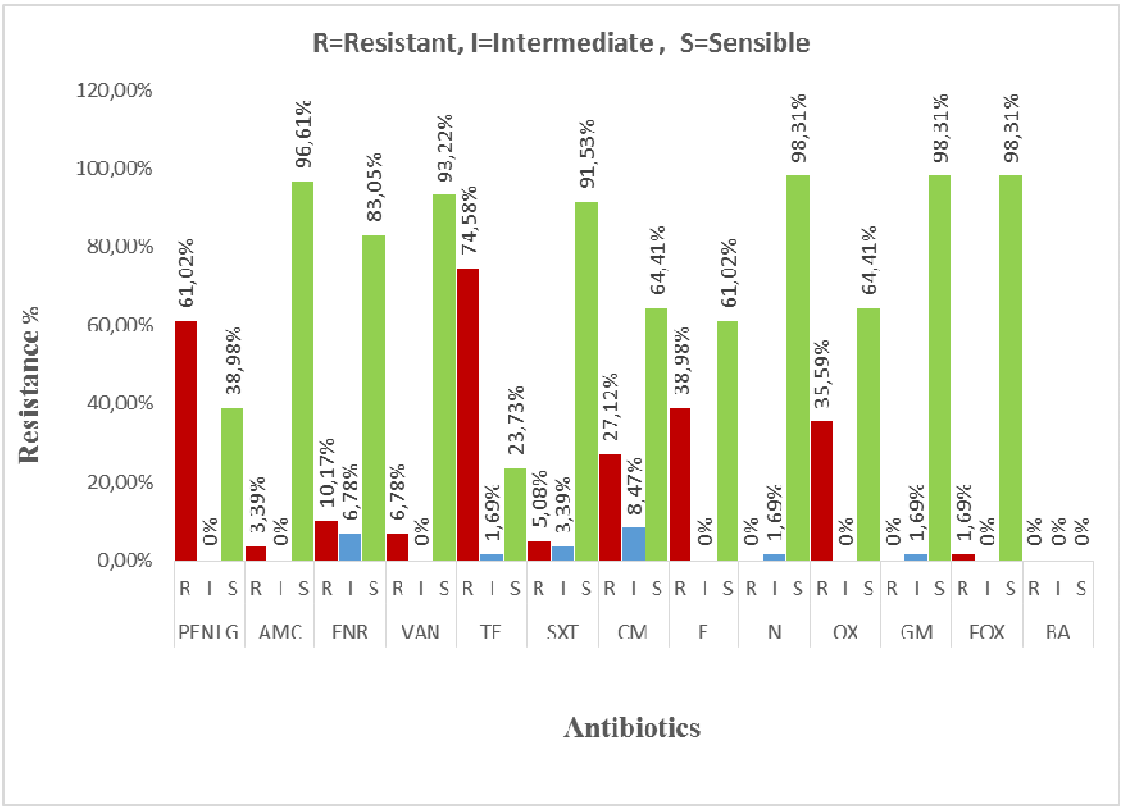

Fig. 1: Antibiogram of coagulase negative staphylococci isolated

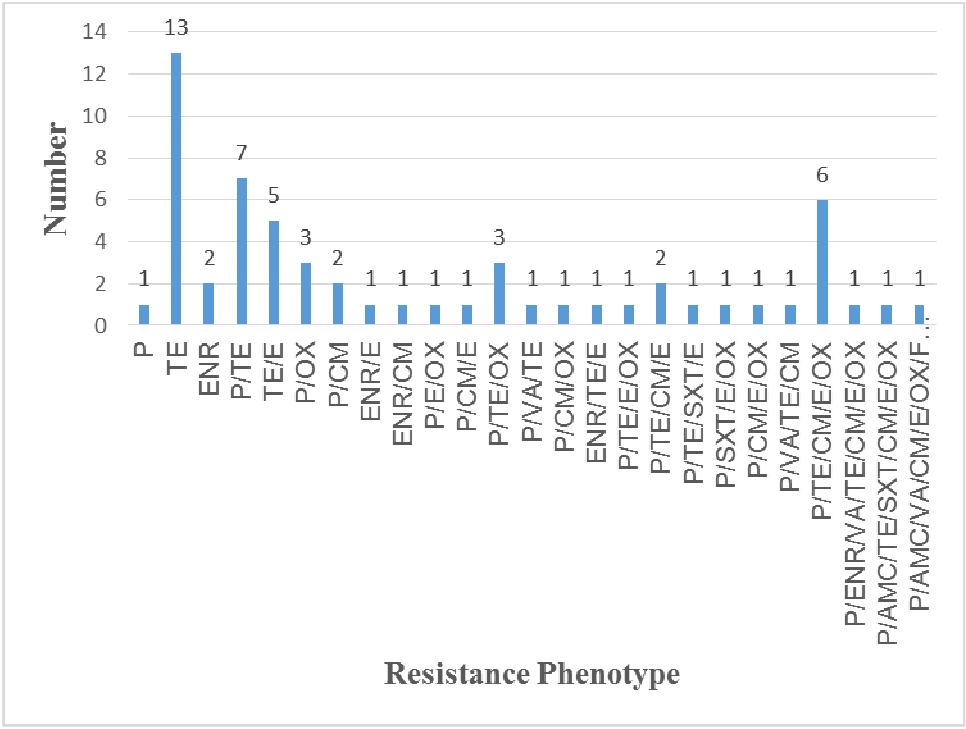

Fig. 2: Profiles of resistance to antibiotic of coagulase negative staphylococci isolates.

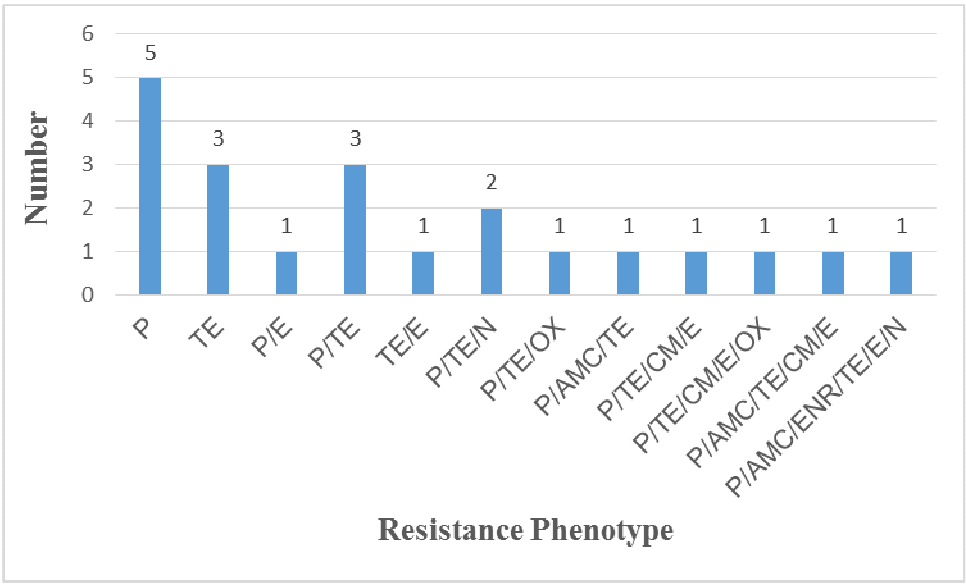

Fig. 3: Profiles of resistance to antibiotic of *S.aureus* isolates.

Conclusion:

Our results indicated that there's a passage of *S. aureus* and coagulase-negative staphylococci, multi-resistant in the raw milk consumed in the western area of Algeria that indicates the antibiotic resistance invaded the dairy cattle in our region.

The Staphylococci in particular *S. aureus*, are always considered as a major cause of the subclinical mastitis; this mastitis has paramount importance for public health, because it passes unperceived and it is the main reason for the antimicrobial treatment. This rate of resistance raised to certain antimicrobial agents can be explained by their frequent uses in veterinary practice in Algeria.

The results of this study showed a higher prevalence of coagulase negative staphylococci compared to *S.aureus*. In spite of the pathogenic power ignored for certain species, these organisms can be reserves of resistant to antibiotics ; what requires a periodic monitoring of antimicrobial resistance of staphylococci in order to control their spread. This monitoring could be useful to treat the infections more effectively, and to

prevent in the future the passage in raw milk and dairy products of the harmful microorganisms for the health and the security of the consumer.

Our results present a paradigm for future studies of the evolution of multidrug résistance of pathogenic bacteria in the western area of Algeria.

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