SHORT COMMUNICATION

Antibiotic susceptibility of *Staphylococci* isolated from bovine mastitis in Algeria

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ABSTRACT

Objective: This work aimed to determine the resistance and/or the susceptibility to antibiotics of staphylococci isolated from cattle with mastitis in the North of Algeria.
Materials and Methods: The disk diffusion method was carried out to reveal the antibiotic resistance in accordance to the National Committee for Clinical Laboratory Standards guidelines in the Mueller-Hinton agar.
Results: Coagulase-negative *Staphylococci* (CNS) isolates showed more resistance to Cefoxitin, Amoxicillin + Clavulanic Acid, Vancomycin, Trimethoprime Sulfamethoxazole, Clindamycine, Neomycin, and Erythromycin than Coagulase-positive *Staphylococci* (CPS). CPS were more resistant to Penicillin and Tetracycline as compared to CNS strains; however, all these strains presented sensitivity to Gentamicin and neomycin.
Conclusion: The *Staphylococci* showed high resistance to the beta-lactam antibiotics. As far as the authors know, these molecules are used with or without control in different protocols to prevent and cure the mastitis in Algeria.

Introduction

Farmers and dairy producers are exposed to serious economic losses due to a variety of diseases, and one of the foremost diseases is the inflammation of the mammary gland "Mastitis." Mastitis by its nature is a very complex and costly disease and is very common in dairy herds [1].

Mastitis has two forms clinical and subclinical, and etiologically speaking the major agent of both forms is considered to be *Staphylococcus aureus* [2]. Coagulase-negative *Staphylococcus* (CNS) being one of the major pathogens isolated from the subclinical mastitis in many countries, it has captured more attention in the last years, despite being previously considered to be an inconsequential pathogen [3]. CNS is known to be a possible cause of the subclinical form of mastitis; however, other reported many cases where it caused the clinical form [4].

In dairy herds, mastitis is the main cause for the use of antibiotics by dairy producers. Despite that, antibiotic treatments are mostly inefficient against staphylococci infections. Many of the staphylococci agents isolated from this disease have had multi-antimicrobial resistance [5]. And this resistance is in addition to others the main reason for the low efficiency of the antibiotic treatment of staphylococcal mastitis, moreover, in the last decade, resistance to the most commonly used antibiotics have been acquired by various human diseases causing bacteria species in several countries raising some serious red flags [6,7]. In Algeria, the antibiotic resistance is not well investigated. We aimed through this study to investigate, identify, and determine the *in vitro* activity of 11 antimicrobial drugs against several mastitis causing staphylococci pathogens.

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Materials and Methods

Milk collection and microbiological tests

In this study, 45 strains of staphylococci (10 *S. aureus* and 35 CNS) were isolated from milk samples of mastitis infected cows from herds located in Ain Defla province of Algeria from 2011–2013.

First, alcohol swabs were used to clean the teat end, they were left to dry up, and then 5 ml of the milk secretion was collected in sterile tubes after the first few drops were eliminated.

The samples were transferred directly after cooling down to the laboratory and then the antibiotic responsiveness test was carried out on the isolated staphylococcal strains according to the commonly accepted principles [8].

Briefly, 0.1 ml from every sample was plated on Columbia blood agar medium, containing 5% of sheep blood, and incubated at 37°C for 48 h. Then conventional methods (Gram staining, colony morphology, hemolysis, tests for catalase, coagulase, and anaerobic fermentation of mannitol) were used to identify the isolates. All the tests were performed as described by Koneman et al. [9]. Trypticase soy broth containing 20% of glycerol was used to store the isolates at –20°C.

Antibiotic resistance analysis

The antibiotics used in the antimicrobial sensitivity test were: Penicillin G (P; group Penicillins; 6 µg), Céfoxitin (FOX; group Cephalosporins; 30 µg), Amoxicillin + Clavulanic Acid (AMC; group Penicillins; 10 µg), Enrofloxacin (ENR; group Fluoroquinolones; 5 µg), Vancomycin (VA; group Aminoglycosides; 30 µg), Trimethoprim-Sulfamethoxazole (SXT; group Sulfonamides; 1.25 µg), Clindamycin (CM; group Macrolides 2 µg), Gentamicin (GM; group Aminoglycosides; 10 µg), Tetracycline (TE; group Tetracyclines; 30 µg), Neomycin (N; group Aminoglycosides 30 µg), and Erythromycine (E; group Macrolides; 15 µg). The method used in the test was the disk diffusion method on Mueller Hinton Agar (Oxoid), and the test was performed according to National Committee for Clinical Laboratory Standards (NCCLS) [10] guidelines.

To test the resistance, the antibiotic disks, containing the antibiotics were dispensed on the surface of the medium and incubated aerobically at 37°C for 18 h. After that, data revealed three cases (resistant, intermediate or susceptible) by identifying the inhibition zone diameter [11]. The control strain was *S. aureus* ATCC 25 923.

Results

Tables 1 and 2 represent the sensitivity of the 10 strains of Coagulase-positive *Staphylococci* (CPS) and the 35 CNS strains (isolated during 2012 from cow presenting the subclinical and clinical form of the inflammation) to the antibiotics.

All isolated strains, despite the difference in origin (clinical and subclinical mastitis), presented the same sensitivity toward the antibiotic agents. The order of *in vitro* effective antibiotic agents against *S. aureus* is Neomycin and Gentamycin (100%), Trimethoprim-Sulfamethoxazole (90%), Enrofloxacin (90%), Amoxicillin + Clavulanic acid (80%), Céfoxitin (80%), and Vancomycin (80%).

Both CPS strains and CNS strains presented a resistance toward the following agents Penicillin, Clindamycin, and Erythromycin; however, strains of *S. aureus* in addition presented a resistance to Tetracycline.

Table 2 presents the results of the antibiotic susceptibility tests carried out on the 35 CNS isolates. The order of *in vitro* antibiotic agents to which CNS were susceptible is Neomycin (97.14%), Trimethoprim + Sulfamethoxazole (91.42%), Enrofloxacin (85.71%), and Tetracycline (65.71%), followed by Céfoxitin (54.28%), Amoxicillin + Clavulanic Acid (51.42%), Vancomycin (48.57%), Clindamycin (45.71%), Erythromycin, and Penicillin (28.57%), whereas CNS isolates were totally sensible to Gentamicin (100%).

All staphylococcal strains presented the same sensitivity against Gentamycin and Neomycin; however, *S. aureus* strains presented higher resistance toward Penicillin and Enrofloxacin. Multi-antimicrobial resistance incidence is shown in Table 3, and strains with this phenomenon represent 57.78% (26/45) with none having a resistance toward only one or two antibiotics.

Discussion

In this work, data on the activity of different antibacterial substances facing staphylococcal strains were described. In the current study, various CNS from California Mastitis Test (CMT) positive samples have been determined. Data also confirmed that CNS was resistant to the beta-lactam antibiotics which are intensively used in the prevention and cure of mastitis. Our results were consistent with other works [12].

The high incidence of Penicillin resistance (100%) exceeded those reported in different regions including Tunisia (22.6%) [13], Estonia (61.4%) [14], Korea (52.9%), Switzerland (31%), Finland (32%), and the USA (22.1%) [15]. Coagulase-positive strains isolated from animals with mastitis presented more resistance to antibiotherapy than coagulase-negative strains, in accordance with reported data, especially on resistance to Penicillin [16].

Penicillin G resistance is very interesting because this antibiotic is the major treatment recommended against staphylococcal mastitis. These bacteria were more resistant than those isolated before [17]. This might be due

Table 1. Antibiotic susceptibility of CPS strains isolated from bovine mastitis to various antibiotics.

Antibiotics	Total	CPS (*n* = 10)					
	Profile	Sensitive		Resistance		Intermediate	
	Break points	Number	%	Number	%	Number	%
P	≤28–29≥	0	0	10	100	0	0
FOX	≤19–20≥	8	80	2	20	0	0
AMC	≤19–20≥	8	80	2	20	0	0
ENR	≤16–23≥	9	90	1	10	0	0
VA	≥15	8	80	2	20	0	0
SXT	≤10–16≥	9	90	0	0	1	10
CM	≤14–17≥	7	70	3	30	0	0
GM	≤12–15≥	10	100	0	0	0	0
TE	≤14–19≥	6	60	4	40	0	0
N	≤13–18≥	10	100	0	0	0	0
E	≤13–23≥	7	70	3	30	0	0

P = Penicillin, FOX = Cephalosporins, AMC = Amoxicillin + Clavulanic Acid, ENR = Enrofloxacin, VA = Vancomycin, SXT = Trimethoprim-Sulfamethoxazole, CM = Clindamycin, GM = Gentamicin, TE = Tetracycline, N = Neomycin, E = Erythromycine.

Table 2. Susceptibility of bovine CNS isolates to various antibiotics.

Antibiotics	Total	CNS (*n* = 35 isolates)					
	Profile	Sensitive		Resistance		Intermediate	
	Break points	Number	%	Number	%	Number	%
P	≤28–29≥	10	28.57	25	71.42	0	0
FOX	≤24–25≥	19	54.28	11	31.42	7	20
AMC	≤19–20≥	18	51.42	17	48.57	0	0
ENR	≤16–23≥	30	85.71	1	02.85	4	11.42
VA	≥15	17	48.57	18	51.42	0	0
SXT	≤10–16≥	32	91.42	1	02.85	2	05.71
CM	≤14–17≥	16	45.71	19	54.28	0	0
GM	≤12–15≥	35	100	0	0	0	0
TE	≤14–19≥	23	65.71	12	34.28	0	0
N	≤13–18≥	34	97.14	1	02.85	0	0
E	≤13–23≥	10	28.57	19	54.28	6	17.14

P = Penicillin, FOX = Cephalosporins, AMC = Amoxicillin + Clavulanic Acid, ENR = Enrofloxacin, VA = Vancomycin, SXT = Trimethoprim-Sulfamethoxazole, CM = Clindamycin, GM = Gentamicin, TE = Tetracycline, N = Neomycin, E = Erythromycine.

to the uncontrolled use of antibacterial agents in Algeria. Besides, regular use of these molecules may develop the resistant strains. The data are in agreement with reported studies [6].

The Tetracycline resistance noted in the present work can be the consequence of misuse in mastitis cure and prevention in our farms. No resistance to Neomycin and Gentamicin was determined in this work. This is not consistent with the data of Turutoglu et al. [12] in Turkey but it is similar to those reported by Gentilini et al. [18] and Kaszanyitzky et al. [19].

In this paper, results of antibiogram revealed the problem of multiple resistances to three or more antibiotics. The widespread use of antibiotics results in the emergence of multiresistant bacteria. In the present study, it was observed multi-resistance from two to eight tested

Table 3. Antimicrobial resistance patterns of 45 *Staphylococci* isolated from bovine mastitis.

Pattern	Resistance profile	Resistant isolates	
		Number	%
1	TE	3	6.67
2	P	5	11.11
3	P TE	4	8.89
4	P CM TE	1	2.22
5	P CM E	1	2.22
6	P AMC E	2	4.44
7	P AMC E VA CM	2	4.44
8	P AMC E VA CM TE	3	6.67
9	P FOX AMC E	1	2.22
10	P FOX AMC VA CM	2	4.44
11	P FOX AMC VA CM E	6	13.33
12	P FOX AMC VA CM E TE	1	2.22
13	P FOX AMC VA CM E TE ENR	1	2.22
14	P FOX AMC VA CM TE	1	2.22
15	P FOX VA CM E	1	2.22
16	P VA CM E	1	2.22
17	P VA CM E TE N	1	2.22
18	P VA SXT CM E	1	2.22
19	ENR TE E	1	2.22
20	All susceptible	7	15.56

P = Penicillin, FOX = Cephalosporins, AMC = Amoxicillin + Clavulanic Acid, ENR = Enrofloxacin, VA = Vancomycin, SXT = Trimethoprim-Sulfamethoxazole, CM = Clindamycin, GM = Gentamicin, TE = Tetracycline, N = Neomycin, E = Erythromycine.

antimicrobial classes. In the same context, Machado et al. [20] detected that all CNS isolates from bovine mastitis showed resistance to two or more anti-microbial agents.

Conclusion

In the end, all most of staphylococcal strains isolated had no resistance to Neomycin and Gentamicin and all of them were resistant to Penicillin. Antibioresistance in animals can present an important danger for human health. This serious problem was extremely revealed with Penicillin against *S. aureus* and CNS.

Acknowledgments

Noting to disclose.

Conflict of Interest

The authors declare no conflict of interest.

Authors' contribution

S.R. and K.R. performed the design of the study; M.N. and K.D. realized statistical analysis. All the authors interpreted the results and supervised the manuscript.

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