Antibacterial susceptibility profiles of sub-clinical mastitis pathogens isolated from cows in Batna and Setif Governorates (East of Algeria)

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Abstract

Sub-clinical mastitis is a main pathology of dairy husbandry because it is not clinically recognized by the owners and the veterinarians. For this reason, its economic loss is usually underestimated in milk production. This study has been undertaken in order to evaluate the epidemiologic situation of sub-clinicalmastitis in Batna and Setif governorates (East of Algeria). For this purpose, a detailed bacteriological study of all bacterial strains isolated from sub-clinical mastitis followed by a study of their antibacterial susceptibility profiles has been undertaken. 89 bacterial strains distributed as follows were studied: 27 strains of staphylococci among which 23 were coagulase-negative staphylococci (CNS) that are generally incriminated in sub-clinical mastitis. 39 strains of streptococci among which 10 were *Lactococcus lactis ssp lactis* strains. 23 strains of enterobacteria represented mainly by Escherichia coli (E.coli). All these bacterial strains were isolated from cow milk of 3 different farms. The antibacterial susceptibility profiles have revealed a susceptibility of the isolated strains to a large number of antibiotics mainly to the Neomycin, the Cephalexin and the Spiramycin.
Keys words: Sub-clinical mastitis, antibiotic, susceptibility profiles, milk, cow, bacterial strain.

Introduction

Sub-clinical mastitis is still the most frequent and dangerous pathology of dairy herds. The identification of the bacterial strains agent of this mastitis in association with the study of their antibacterial susceptibility profiles to the common antibiotics conduct to make the best choice of the antibiotic therapy of mastitis. However, the suggestion that the study of the antibacterial susceptibility profiles may serve to eliminate all the antibiotics towards which bacterial strains are resistant is not always true. It is admitted that strains that are susceptible in vitro may be resistant in vivo (Bouchot et al., 1985), the reverse argument is equally possible: strains that are resistant in vitro may be susceptible in vivo (Constable and Morin, 2003). The large use of antibiotics is the most common strategy of control of mastitis especially during the dry period by intramammary administration of long acting antibiotics in tubes.

Materials

50 lacting cows were sampled. These cows belong to 3 different herds. Milk samples were taken just before the second milking time (afternoon). 89 bacterial strains were isolated from these milk samples from which 39 strains of streptococci, 27 strains of staphylococci and 23 strains of enterobacteria were identified.

Methods

The study of the antibacterial susceptibility profiles has been achieved using the diffusion method on agar medium according to the swab method of Kirby Bauer du Comité de l'Antibiogramme de la SociétéFrançaise de Microbiologie (CA-SFM) (Soussy, 2002) or the disc method. The latter consists in placing on the surface of a culture medium uniformly inoculated with a bacterialstrain. Each disc contains a known amount of a known antibiotic. The antibiotic diffuses around the disc with a decreasing gradient of concentration as far as we go far from the disc. After incubation, a growth inhibition zone is formed around the disc. The diameter of the growth inhibition zone is as high as the bacterial strain is susceptible to the antibiotic contained in the disc (Faroult, 1998).

The antibacterial susceptibility profiles have been performed according to the standards of the CA-SFM (1996).

The results are obtained by measuring the diameter of the growth inhibition zone around the antibiotic disc for each isolated bacterial strain.

Results

In the present study many bacterial strains have been isolated with four predominant species such as *S. xylosus* and *E. coli* with a frequency of isolation of 17,97% for each and *Lactococcus lactis ssp lactis* and *Streptococcus uberis* with a frequency of isolation of 11,24% for each.

It is worth noting the presence at a reasonable frequency of other bacterial species mainly *Enterococcus* (7,86%), *Aerococcus viridans* and *S. lentus* (5, 62%) (Table1).

Table-1. Frequency of bacterial strains isolation from subclinical Mastitis

Species	Number of strains	Frequency (%)
Lactococcus lactisssplactis	10	11,24
Streptococcus uberis	10	11,24
Aerococcus viridans	5	5,62
Gemella morbillorum	2	2,25
Enterococcus	7	7,86
Streptococcus bovis II 1	3	3,38
Streptococcus sanguinis	1	1,12
Streptococcus mitis	1	1,12
Staphylococcus aureus	4	4,50
S. xylosus	16	17,97
S. lentus	5	5,62
S. hominis	1	1,12
S. epidermidis	1	1,12
E.coli	16	17,97
Proteus	2	2,25
Enterobacter	4	4,50
Serratia	1	1,12
Total	89	100

The study of the global susceptibility of the bacterial strains (Gram+ and Gram-) including *Lactococcus lactis ssp lactis* to the different tested antibiotics has revealed a complete susceptibility to the Neomycin (100%), the Cephalexin (97,37%), the Streptomycin (96,30%), the association Trimethoprim- Sulfamids (93,33%) and to the Erythromycin (91,38%). A certain resistance has been noted to the Lincomycin, the Colistin and the Cefazolin with a rate of 51,85%, 66,37% and 63,16% respectively (R+I) (Table 2).

In contrast, the global susceptibility of pathogenic bacterial strains has also revealed a complete susceptibility to the Neomycin and the Cephalexin. A high degree of susceptibility has also been showed to the Streptomycin and to the association Trimethoprim-Sulfamids with a rate of 96,30% and 95,65% respectively. It is worth noting that the almost the same level of susceptibility has been noted either for pathogenic or the total bacterial strains isolated from cases of subclinical mastitis (Table 3).

Table-2. Frequency of global susceptibility of bacterial strains to some antibiotics (N= 89)

Antibiotic	Break points	Number (N)	% resistant	% intermediary	% susceptible
Penicillin	28 - 29	63	36.5	0	63.5
Amoxicillin	14 - 21	52	11.54	13.46	75
Kanamycin	15 - 17	27	11.11	0	88.89
Neomycin	13 - 18	50	0	0	100
Gentamycin	11 - 17	50	2	10	88
Chloramphenicol	12 - 22	50	0	16	84
Oxytetracyclin	14 - 19	50	26	2	72
Spiramycin	19 - 24	64	26.56	9.37	64.07
Lincomycin	17 - 21	27	40.74	11.11	48.15
Rifampicin	24 - 29	27	7.41	18.52	74.07
Cephalexin	12 - 18	38	2.63	0	97.37
Colistin	10 - 13	21	14.29	52.38	33.33
Cefoxitin	15 - 25	49	4.08	32.65	63.27
Trimethoprim + Sulfamids	10 - 16	75	4	2.67	93.33
Doxycyclin	14 - 19	64	21.88	10.94	67.18
Streptomycin	13 - 15	27	3.70	0	96.30
Ciprofloxacin	22 - 25	50	6	6	88
Cefazolin	12 - 18	19	26.32	36.84	36.84
Erythromycin	13 - 23	58	0	8.62	91.38

Table-3. Frequency of global susceptibility of pathogenic bacterial strainsto some antibiotics (N= 79)

Antibiotic	Break points	Number (N)	% resistant	% intermediary	% susceptible
Penicillin	28 - 29	53	32.07	0	67.93
Amoxicillin	14 - 21	43	13.95	16.28	69.77
Kanamycin	15 - 17	27	11.11	0	88.89
Neomycin	13 - 18	50	0	0	100
Gentamycin	11 - 17	50	2	10	88
Chloramphenicol	12 - 22	50	0	16	84
Oxytetracyclin	14 - 19	50	26	2	72
Spiramycin	19 - 24	54	29.63	9.26	61.11
Lincomycin	17 - 21	27	40.74	11.11	48.15
Rifampicin	24 - 29	27	7.41	18.52	74.07
Cephalexin	12 - 18	28	0	0	100
Colistin	10 - 13	21	14.29	52.38	33.33
Cefoxitin	15 - 25	49	4.08	32.65	63.27
Trimethoprim + Sulfamids	10 - 16	69	2.90	1.45	95.65
Doxycyclin	14 - 19	54	20.37	16.67	62.96
Streptomycin	13 - 15	27	3.70	0	96.30
Ciprofloxacin	22 - 25	50	6	6	88
Cefazolin	12 - 18	19	26.32	36.84	36.84
Erythromycin	13 - 23	48	0	10.42	89.58

The global susceptibility of Gram+ cocci including *Lactococcus lactis ssp lactis* to the tested antibiotics has revealed a complete (100%) susceptibility to the Neomycin and the Amoxicillin.

Afterwards, we have found the Cephalexin (97,37%), the Streptomycin and the Chloramphenicol (96,30% each), the association Trimethoprim-Sulfamids (92,73%), the Cefoxitin (92,59%), the Erythromycin (91,38%). On the other hand, some resistance has been noted against the Lincomycin (51,85% of resistant Gram+ cocci) (Table 4).

The global susceptibility of staphylococci to the tested antibiotics has revealed a complete (100%) susceptibility to the Neomycin. Next, in a declining order we have found the Streptomycin, the Ciprofloxacin,

Table-4. Frequency of the global susceptibility of Gram + cocci to some antibiotics (N= 66)

Antibiotic	Break points	Number (N)	% resistant	% intermediary	% susceptible
Penicillin	28 - 29	63	36.50	0	63.50
Amoxicillin	14 - 21	36	0	0	100
Kanamycin	15 - 17	27	11.11	0	88.89
Neomycin	13 - 18	27	0	0	100
Gentamycin	11 - 17	27	0	11.11	88.89
Chloramphenicol	12 - 22	27	0	3.70	96.30
Oxytetracyclin	14 - 19	27	22.22	3.70	74.08
Spiramycin	19 - 24	64	26.56	9.37	64.07
Lincomycin	17 - 21	27	40.74	11.11	48.15
Rifampicin	24 - 29	27	7.41	18.52	74.07
Cephalexin	12 - 18	38	2.63	0	97.37
Cefoxitin	15 - 25	27	0	7.41	92.59
Trimethoprim + Sulfamids	10 - 16	55	5.45	1.82	92.73
Doxycyclin	14 - 19	64	21.87	10.94	67.19
Streptomycin	13 - 15	27	3.70	0	96.30
Ciprofloxacin	≥22	27	11.11	11.11	77.78
Erythromycin	13 -23	58	0	8.62	91.38

the combination Trimethoprim-Sulfamids and the Chloramphenicol (96,30% each), the Erythromycin (96,15%) and the Gentamycin and the Cefoxitin (96,60% each). The some proportion of resistance has been shown against the Lincomycin (51,85%) (Table 5).

Table-5. Frequency of the global susceptibility of staphylococci strains to some antibiotics (N= 27)

Antibiotic	Change of disc	Break Points	% resistant	% intermediary	% susceptible
Penicillin	10 UI	28 – 29*	22.22	0	77.78
Kanamycin	30 UI	15 – 17**	11.11	0	88.89
Neomycin	30 µg	13 – 18*	0	0	100
Gentamycin	10 µg	12 – 15*	0	7.40	92.60
Chloramphenicol	30 µg	19 – 22**	0	3.70	96.30
Oxytetracyclin	30 µg	14 – 19*	22.22	3.70	74.08
Spiramycin	100 µg	19 – 24**	36	0	64
Lincomycin	15 µg	17 – 21***	40.74	11.11	48.15
Rifampicin	30µg	24 – 29***	7.41	18.52	74.08
Cefoxitin	30 µg	19 – 20* SCN : 24-25*	7.40	0	92.60
Trimethoprim + Sulfamids	1.25/ 23.75µg	10 – 16*	3.70	0	96.30
Doxycyclin	30 µg	14 – 19*	18.52	0	81.48
Streptomycin	10 µg	13 – 15**	3.70	0	96.30
Ciprofloxacin	5 µg	≥22***	3.70	0	96.30
Erythromycin	15 µg	13 – 23*	0	3.85	96.15

* : NCCLS - National Commitee for Clinical Laboratory Standards. (2004),
** : CA-SFM1 - Comité de l'Antibiogramme de la Société Française de Microbiologie (2008),
*** : CA-SFM2 - Comité de l'Antibiogramme de la Société Française de Microbiologie (2010)

It is interesting to note that the present study has revealed a complete susceptibility (100%) of Staphylococcus aureus strains to many antibiotics including the Penicillin, the Kanamycin, the Neomycin, the Gentamycin, the Chloramphenicol, the Lincomycin, the Cefoxitin, the combination Trimethoprim-Sulfamids, the Doxycyclin, the Ciprofloxacin and the Erythromycin. However, 75% and 50% of susceptibility has been shown to both the Oxytetracyclin, the Streptomycin and the Rifampicin respectively (Table 6).

Table-6. Frequency of the global susceptibility of Staphylococcus aureus to some antibiotics (N= 4)

Antibiotic	Change of disc	Break Points	% resistant	% intermediary	% susceptible
Penicillin	10 UI	28 – 29*	0	0	100
Kanamycin	30 UI	15 – 17**	0	0	100
Neomycin	30 µg	13 – 18*	0	0	100
Gentamycin	10 µg	12 – 15*	0	0	100
Chloramphenicol	30 µg	19 – 22**	0	0	100
Oxytetracyclin	30 µg	14 – 19*	25	0	75
Spiramycin	100 µg	19 – 24**	25	0	75
Lincomycin	15 µg	17 – 21***	0	0	100
Rifampicin	30µg	24 – 29***	25	25	50
Cefoxitin	30 µg	19 – 20* SCN : 24-25*	0	0	100
Trimethoprim + Sulfamids	1.25/ 23.75µg	10 – 16*	0	0	100
Doxycyclin	30 µg	14 – 19*	0	0	100
Streptomycin	10 µg	13 – 15**	0	0	100
Ciprofloxacin	5 µg	≥22***	0	0	100
Erythromycin	15 µg	13 – 23*	0	0	100

* : NCCLS. ** : CA-SFM1. *** : CA-SFM2

Concerning the global susceptibility of coagulase-negative staphylococci (CNS) it was complete (100%) for the Neomycin and the Ciprofloxacin. Afterwards, we have found the Chloramphenicol, the Streptomycin and the combination Trimethoprim-Sulfamids (95,65% for each), the Erythromycin (95, 45%), the Gentamycin and the Cefoxitin (91,30% for each). 39,13% of the CNS proved to be resistant to the Lincomycin (Table 7).

Table-7. Frequency of susceptibility global of staphylococci coagulase negative (SCN) to some antibiotic (N= 23)

Antibiotic	Change of disc	Break Points	% resistant	% intermediary	% susceptible
Penicillin	10 UI	28 – 29*	26.09	0	73.91
Kanamycin	30 UI	15 – 17**	13.04	0	86.96
Neomycin	30 µg	13 – 18*	0	0	100
Gentamycin	10 µg	12 – 15*	0	8.70	91.30
Chloramphenicol	30 µg	19 – 22**	0	4.35	95.65
Oxytetracyclin	30 µg	14 – 19*	21.74	4.35	73.91
Spiramycin	100 µg	19 – 24**	38.10	0	61.90
Lincomycin	15 µg	17 – 21***	47.83	13.04	39.13
Rifampicin	30µg	24 – 29***	4.35	17.39	78.26
Cefoxitin	30 µg	19 – 20* SCN : 24-25*	8.70	0	91.30
Trimethoprim + Sulfamids	1.25/ 23.75µg	10 – 16*	4.35	0	95.65
Doxycyclin	30 µg	14 – 19*	21.74	0	78.26
Streptomycin	10 µg	13 – 15**	4.35	0	95.65
Ciprofloxacin	5 µg	≥22***	0	0	100
Erythromycin	15 µg	13 – 23*	0	4.55	95.45

* : NCCLS. ** : CA-SFM1. *** : CA-SFM2

The global susceptibility of enterobacteria have revealed a complete susceptibility (100%) to the Neomycin and the Ciprofloxacin. Next, in a declining order we have found the Chloramphenicol (95,65%) and the association Trimethoprim-Sulfamids (95%). Indeed, enterobacteria has shown a susceptibility the the Amoxycillin (81,25%) the Colistin (66,67%) and the Cefazolin (63,16%) (Table 8).

The present study has revealed the Streptococci

Table-8. Frequency of susceptibility global of enterobacteria isolated to some antibiotic (N= 23)

Antibiotic	Change of disc	Break Points	% resistant	% intermediary	% susceptible
Amoxicillin	25 µg	14 – 21**	37.5	43.75	18.75
Neomycin	30 µg	13 – 18*	0	0	100
Gentamycin	10 µg	11 – 17**	4.35	8.70	86.95
Chloramphenicol	30µg	12 – 18*	0	4.35	95.65
Oxytetracyclin	30µg	14 – 19*	30.44	0	69.56
Colistin	10 µg	10 – 13*	14.29	52.38	33.33
Cefoxitin	30 µg	15 – 22***	9.10	40.90	50
Trimethoprim + Sulfamids	1.25/ 23.75µg	10 – 16*	0	5	95
Ciprofloxacin	5 µg	22 – 25***	0	0	100
Cefazolin	30 µg	12 – 18***	26.32	36.84	36.84

* : NCCLS. ** : CA-SFM1. *** : CA-SFM2

strains of bovine mastitis origin were more susceptible to most antibiotics compared the staphylococci or the enterobacteria. For example, a complete (100%) susceptibility has been noted for the Amoxicillin and to the Cephalexin (97,36%).

A degree of resistance (43,24%) has been shown against the Doxycyclin. *Lactococcus lactis ssp lactis* strains are more susceptible to antibiotics than the streptococci strains. A complete susceptibility (100%) has been noted to the Amoxicillin and the Erythromycin (Table 9).

Table-9. Frequency of susceptibility global of streptococci isolated to some antibiotic (N= 39)

Antibiotic	Change of disc	Break Points	% resistant	% intermediary	% susceptible
Penicillin	10 UI	≥24 *	22.22	0	77.78
Amoxicillin	25 µg	14 -21 * *	0	0	100
Spiramycin	100 µg	19 -24 * *	20.51	15.39	64.10
Cephalexin	30 µg	12 -18 * *	2.63	0	97.37
Trimethoprim +Sulfamids	1.25/ 23.75 µg	10-16*	7.14	3.57	89.29
Doxycyclin	30 µg	14-19*	24.32	18.92	56.76
Erythromycin	15 µg	15-21*	9.37	3.13	87.50

* : NCCLS. ** : CA-SFM1.

Discussion

A. Staphylococci**:** Most of the studied strains have expressed an important susceptibility to the antibiotic used. This observation is in accordance with some previous studies (De Oliveira *et al*., 2000; Erskine *et al*., 2002).

In France, according to the data of the Resapath (Afssa, 2000), 86% of the bovine strains of coagulase-positive staphylococci (CPS) and 91% of coagulase negative staphylococci (CNS) isolated during 1999-2000 (most of which were isolated from mammary infections) were susceptible to the Erythromycin, 30% and 49% to the Spiramycin and 96% and 73% to the Lincomycin respectively. These results are in accordance with our findings except for the Lincomycin that presented a certain level of resistance of 51,85%.

According to Faroult (1998), the most active antibiotics against Staphylococcus aureus are: the Penicillins M (Cloxacillin, Oxacillin), the association Amoxicillin/Clavulanic acid the Cephalosporins, the associations Penicillin/Aminosids (Streptomycin, Neomycin, Gentamycin); the macrolids and their related products (Lincosamins, Novobiocin) and the Rifamicin.

Globally, the bovine strains of *Staphylococcus aureus* from mammary origin have not evolved towards resistance with no resistant strain to the macrolids or the Lincomycin, in some studies. In other ones a very low frequency of resistant strains has been noted (Guerin-Faublee and Brun, 1999). However, some cases of resistance have been reported in Greece on strains of bovine origin (Fthenakis, 1998). Indeed, Erskin *et al*.(2002), have reported an increase of susceptibility to the Erythromycin of strains of S.aureus isolated from cases of bovine mastitis in the USA. This finding is in complete accordance with the results of the present study. Perreten *et al*. (1998) have revealed that strains of coagulase-negative staphylococci showed resistance towards the chloramphenicol and the Lincomycin. This finding confirm our results only for the Lincomycin (with a frequency of resistance of 60,87 (R+I)).

B. Enterobacteria: The proportions of resistance to the beta-lactamins deserve a special thoughtfulness. For example, a clear resistance to the Amoxicillin has been noted with more than 85% of *E.coli* strains from cases of neo-natal diarrheoa of calves. In spite of the expected issue of representativity of these results regarding enteropathogenic coliforms, these rates may vary importantly according to the considered bovine pathology. For instance, coliforms of mammary origin have showed 30% of resistance to the Amoxycillin (Onerba, 2006).

C. Streptococci: According to the data published before 2000, concerning *Streptococcus uberis*, the frequency of resistance to the Erythromycin varied from 2 to 25% and from 8 to 13% to the Spiramycin (Bouveron, 2000). In France, during 1999-2000, the frequency of resistance to the Erythromycin reached 25 to 26% according to data published by the Afssa (Afssa, 2000). In the present study, the frequency of resistance to the Erythromycin and the Spiramycin was 18,18% and 41,38% respectively. This increase in the frequency of resistance may be explained by the high frequency of isolation of Streptococcus uberis as it represented 34,48% of the total isolated streptococci.

Conclusion

The identification of the pathogenic strains that

are responsible of bovine mastitis and the study of their susceptibility profiles to the commonly used antibiotics permit to the veterinarians to make the most judicious choice of the antibiotic that is clinically active against the isolated pathogenic bacteria. Indeed, the epidemiological study of the most frequently incriminated bacterial species facilitates the use antibiotics during the dry period of the mammary gland. Consequently, the quantity and the quality of milk production will be greatly improved.

The present study has revealed a frequency of susceptibility of 100% to the Neomycin and to a less extent to the Cephalexin and the Spiramycin. Some resistance has been noted towards the Lincomycin, the Colistin and the Cefazolin.

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