

Prevalence of Mastitis Pathogens in France: Antimicrobial Susceptibility of *Staphylococcus aureus*, *Streptococcus uberis* and *Escherichia coli*

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Abstract

The prevalence of bovine mastitis in France was established using 11 publications covering the period 1995-2012. The papers involved epidemiological surveys or treatment trials. Bacteriological analyses were performed on 777, 923 and 2341 aseptically collected quarter milk from acute, clinical and subclinical mastitis respectively. *Escherichia coli* and *Streptococcus uberis* were the most prevalent pathogens in acute and clinical mastitis whereas *Staphylococcus aureus* and coagulase negative staphylococci were the most frequently isolated pathogens in subclinical mastitis. The prevalence of *Corynebacterium bovis* and *Streptococcus agalactiae* was low whatever the severity of mastitis. The epidemiological data could be useful in the choice of treatment and provide indicators for future research with a view to developing new efficient vaccines. The susceptibility of 240 isolates (80 *Streptococcus uberis*, 80 *Staphylococcus aureus*, 80 *Escherichia coli*) isolated in 2013 from aseptically collected quarter milk from clinical and subclinical mastitis to 12 antimicrobial agents was determined by measuring their minimal inhibitory concentrations. Overall resistance levels were very low except for *S. aureus* towards penicillin G (21.3%) and for *Str. uberis* towards tylosin and cloxacillin (respectively 13.8% and 32.5%).

Keywords: Mastitis; Pathogens; Prevalence; Antimicrobial susceptibility; France

Introduction

Bovine mastitis is the most prevalent and most costly production disease in dairy herds. The economic impact includes cost for therapy, discarded milk, reduced milk production, culling and replacement. The most frequently isolated micro-organisms are Staphylococci, Streptococci and Coliforms, but other species may infect the udder. The panorama of udder pathogens varies between countries and also between subclinical and clinical mastitis. To our knowledge national surveys on microbial etiology of bovine mastitis have not been recently performed in France.

Antimicrobials are an important tool in mastitis control programs. Knowledge about antibiotic resistance trends is important to help the veterinarian in selecting the most appropriate antibiotic for treatment, because mastitis therapy is commonly initiated before bacterial susceptibility testing.

The aim of this study was to identify retrospectively the bacteria isolated from milk samples from French dairy herds and to determine the occurrence of antimicrobial resistance of major mastitis pathogens to commonly used antimicrobial agents.

Material and Methods

Prevalence of pathogens

The prevalence of mastitis pathogens was established using 11 publications covering the period 1995-2012 concerning epidemiological surveys [1-7] or treatment trials [8-11]. Quarter aseptic milk samples were collected according to the recommendations of the National Mastitis Council from cows in different farms, at different times if farms were repeatedly sampled, so that isolates could be considered epidemiologically independent [12]. Samples were submitted to different veterinary laboratories for identification of isolates. Bacteriological analyses were performed on 777, 923 and 2341 milk samples from cases of acute mastitis, clinical mastitis and subclinical mastitis respectively.

Acute mastitis involved both local (inflammation, modification of the milk....) and systemic (fever, loss of appetite, drop of the milk production...) clinical signs. Clinical mastitis was defined by the presence of local signs and subclinical mastitis by the absence of local and systemic signs and somatic cell count higher than 200.000/ml.

Antimicrobial susceptibility testing

The susceptibility to 12 antimicrobials agents of 240 isolates, 80 *Staphylococcus aureus*, (*S. aureus*), 80 *Streptococcus uberis* (*Str. uberis*), 80 *Escherichia coli* (*E. coli*) from aseptic quarter milk samples from cows with clinical or subclinical mastitis was determined. Milk samples were collected in 2013 from different regions in France, in different farms and on different cows and were considered epidemiologically independent. Isolates were identified by standardized methods [12]. Minimal inhibitory concentration (MIC) was determined using the broth microdilution method according to the recommendations of the Clinical and Laboratory Standards Institute [13]. Strains ATCC 29212 *Enterococcus faecalis*, 29213 (*Staphylococcus aureus*), and 27853 (*Pseudomonas aeruginosa*) were used as quality control on each day of testing. Breakpoints were defined according to Clinical and Laboratory Standards Institute [14] or European Committee on Antimicrobial Susceptibility Testing guidelines [15] when available.

Results and Discussion

Although there is a scientific limitation to a country wide survey

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such as the variation in herd size, management skills, parity, milk yield and other parameters, the size of this data base (4041 quarter milk samples) helps to give a fair indication of general etiology of udder infections in France according to the severity of the mastitis.

Concerning acute mastitis, *E. coli* was the most frequently isolated bacteria in milk samples followed by *Str. uberis* (Table 1). *S. aureus* was rarely involved and *Streptococcus. agalactiae* (*Str. agalactiae*) not found. The order of prevalence was reversed for clinical mastitis, *Str. uberis* was in the first position followed by *E. coli* and coagulase negative staphylococci (CNS) (Table 2). The majority of the subclinical mastitis was caused by gram positive pathogens, *S. aureus* and CNS (Table 3). Unexpectedly a high prevalence of CNS (57.5%) and *Corynebacterium bovis* (*C. bovis*) (28%) was reported one article [10]. Whatever the severity of mastitis considered *C. bovis* and *Str. agalactiae* were uncommon in this study. These results are probably related to the efficacy of the control methods of mastitis and good susceptibility to antibiotic of these bacterial species.

The pattern of major pathogens causing organisms as determined from culture of milk samples has changed significantly over the last 40 years with a substantial increase in the percentage of CNS and *Str. uberis*, a virtual eradication of *Str. agalactiae* and the disappearance of *Mycoplasma*. All *Str. uberis* isolates were susceptible to the β lactams with the exception of cloxacillin for which 26 isolates (32.5%) were resistant (Table 4). Susceptibility to gentamicin and tylosin was relatively low.

Concerning *S. aureus* resistance was found for the penicillin G (17 isolates-21.3%) cephalixin (2 isolates-2.5%) and cefquinome (2 isolates-2.5%) (Table 5). The level of resistance for penicillin was lower than level previously reported in France (36.2%) between 1998-2000 [16]. For reason of natural resistance, 4 antibiotics tested for *Str. uberis* and *S. aureus* were not evaluated with *E. coli* (Table 6). Except for cephalixin for which MCI_{90} is high, *E. coli* isolates were relatively susceptible to all antibiotics tested. Only 2, 2 and 7 isolates were resistant to the enrofloxacin, association amoxicillin-clavulanic acid, and cefapirin respectively.

The choice of the antimicrobial treatment of mastitis cannot be only based on the MIC values because high values are not closely related with bacteriological cure *in vivo*. Other parameters such as the distribution and persistence of the antibiotic in the udder depend on of

Pathogens (N=777)	Range of prevalence (%)
Isolates Gram +	29.9-47
<i>Staphylococcus aureus</i>	4.3-7.4
Coagulase negative Staphylococci	0.9-5.8
<i>Corynebacterium bovis</i>	0-1.1
<i>Streptococcus uberis</i>	10.6-26
<i>Streptococcus dysgalactiae</i>	1.1-7.2
<i>Streptococcus agalactiae</i>	0
<i>Streptococcus sp.</i>	0
<i>Enterococcus sp.</i>	0-5.3
<i>Arcanobacterium pyogenes</i>	0-3.2
Other	0-1.1
Isolates Gram-	49.1-69.6
<i>Escherichia coli</i>	47.2-64.9
<i>Klebsiella sp.</i>	0-4.3
<i>Pseudomonas sp.</i>	0-2.1
<i>Pasteurella sp.</i>	0-2.6
Yeast	0.4-1.6

Table 1: Acute Mastitis: Prevalence of the different pathogens [6-9].

the pharmacological properties of the product and must be considered. However high MIC values are generally associated with treatment failure. Overall more than 50% of all clinical and subclinical mastitis were caused by gram positive pathogens and acute mastitis by gram negative pathogens. These data may help the veterinarian for the choice

Pathogens (N=923)	Range of prevalence (%)
Isolates Gram +	57-78
<i>Staphylococcus aureus</i>	7-18.9
Coagulase negative Staphylococci	6-15.8
<i>Corynebacterium bovis</i>	0-5
<i>Streptococcus uberis</i>	18-37
<i>Streptococcus dysgalactiae</i>	03-10
<i>Streptococcus agalactiae</i>	0-3.2
<i>Streptococcus sp.</i>	0-6
<i>Enterococcus sp.</i>	0-6.8
Other	0-7.7
Isolates Gram-	23-33
<i>Escherichia coli</i>	16.7-24
<i>Klebsiella sp.</i>	0-6
<i>Pseudomonas sp.</i>	0-3
Other	0-6.3
No identified	0-22
Yeast	1.8-6

Table 2: Clinical Mastitis: Prevalence of the different pathogens [2-4,11].

Pathogens (N=2341)	Range of prevalence (%)
Isolates Gram +	65-98.9
<i>Staphylococcus aureus</i>	6.2-41
Coagulase negative Staphylococci	9-57.5
<i>Corynebacterium bovis</i>	0-28
<i>Streptococcus uberis</i>	5.6-23.5
<i>Streptococcus dysgalactiae</i>	0-8
<i>Streptococcus agalactiae</i>	0-1
<i>Streptococcus sp.</i>	0-4
<i>Enterococcus sp.</i>	0-6.7
Isolates Gram-	0-15.7
<i>Escherichia coli</i>	0-15
<i>Klebsiella sp.</i>	0-6
<i>Pseudomonas sp.</i>	0-3
Other	0-11.2
No identified	1-22

Table 3: Subclinical Mastitis: Prevalence of the different pathogens [1-3,5,10].

Antibiotic	Resistance (%)	MIC (μ g/ml)		
		Breakpoints	MIC ₅₀	MIC ₉₀
Cephalonium	Not available	Not available	0.03	0.06
Cefapirin	0	≥ 16	0.03	0.25
Cefquinome	0	≥ 4	0.03	0.25
Amoxicillin	0	≥ 16	0.06	0.25
Clavulanic acid				
Cefalexin	0	≥ 16	0.25	0.25
Nafcillin	0	≥ 4	0.125	0.5
Cefazolin	0	≥ 4	0.125	0.5
Penicillin G	0	≥ 4	0.03	1
Enrofloxacin	0	>2	0.5	1
Cloxacillin	32.5	≥ 4	0.25	4
Gentamicin	67.5	≥ 8	8	32
Tylosin	13.75	≥ 4	1	>256

Table 4: Resistance and Minimal Inhibitory Concentrations for *Streptococcus uberis* isolates (N=80).

Antibiotic	Resistance (%)	MIC (µg/ml)		
		Break points	MIC ₅₀	MIC ₉₀
Cephalonium	Not available	Not available	≤ 0.06	0.125
Cefapirin	0	≥ 32	≤ 0.125	0.25
Cefquinome	2.5	≥ 4	0.25	0.5
Amoxicillin	0	≥ 8	0.125	0.5
Clavulanic acid				
Cefalexin	2.5	≥ 8	2	4
Nafcillin	0	4	0.25	0.5
Cefazolin	0	≥ 4	0.25	0.5
Penicillin G	21.25	≥ 0.25	≤ 0.06	0.5
Enrofloxacin	1.25	>2	0.125	0.25
Cloxacillin	0	≥ 4	0.25	0.25
Gentamicin	0	≥ 8	≤ 0.25	≤ 0.25
Tylosin	2.5	≥ 4	1	2

Table 5: Resistance and Minimal Inhibitory Concentrations for *Staphylococcus aureus* isolates (N=80).

Antibiotic	Resistance (%)	MIC (µg/ml)		
		Breakpoints	MIC ₅₀	MIC ₉₀
Cephalonium	Not available	Not available	2	4
Cefapirin	1.25	≥ 32	4	8
Cefquinome	0	≥ 8	0.06	0.06
Amoxicillin	2.5	>16	1	8
Clavulanic acid				
Cefalexin	0	>16	8	16
Cefazolin	0	>4	1	1
Enrofloxacin	2.5	>4	0.03	0.03
Gentamicin	1.25	>4	≤ 0.25	0.5

Table 6: Resistance and Minimal Inhibitory Concentrations for *Escherichia coli* isolates (N=80).

of the treatment when mastitis therapy is initiated before bacterial susceptibility testing. Presently recommendations exist for a limited use of antibiotics in order to prevent the increase of resistance both for animal and human isolates. This consideration underlines the need for efficient vaccines to prevent intramammary infections especially those caused by *S. aureus* and *Str. uberis*.

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