Methicillin-resistance and prevalence of antibiotic resistance in bovine mastitis in france

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Introduction

Staphylococcus spp. are known to be responsible of about one third of bovine mastitis (Bradley *et al.*), infections that cause major economic loss worldwide.

In France, a recent survey of intramammary infections (Botrel *et al.*) showed that coagulase positive staphylococci were implicated in 30.2% of the subclinical mastitis and 15.8% of the clinical ones, versus 13.7% and 9.5% respectively for the coagulase negative staphylococci.

Since cows might be a source of contamination either by milk drinking or by direct contact, we performed a study on antibiotic resistance of bovine mastitis staphylococci isolated throughout the country in order to be representative of the French cattle population.
Moreover, virulence of a subset of strains was investigated by detecting staphylococcal enterotoxins (SE) and by testing their capacity to form biofilms.

Material and methods

In total, 199 *Staphylococcus* spp. were isolated from bovine mastitis between the end of 2007 and the end of 2008.

They were all characterised according to standard methods (catalase, coagulase, API20Staph).

Antibiotic resistance was determined by disk diffusion according to the recommendations of the Antibiogram Committee of the French Society for Microbiology.

PCR were performed to detect the *mecA* gene. The presence of 12 virulence-associated genes (SEs, *eta*, *etb*, *tst*; Akineden *et al.*) was assessed in a subset of 61 strains.

Biofilm formation was assessed in 96-well plates.

Results

1. Methicillin and associated resistances

* Overall, 137 isolates (70%) were characterized as *S. aureus* and 60 as coagulase negative staphylococci.
* Cefoxitin resistance was detected in 3 strains but only one MRSA and one resistant coagulase negative (MRS) were confirmed by PCR.
  - the MRSA strain was resistant to penicillin, kanamycin and tobramycin only
  - the MRS presented a multidrug resistance pattern (penicillin, kanamycin, tobramycin, gentamicin, lincomycin, erythromycin and marbofloxacin)
* The prevalence of resistance in non-MRS(A) was low (see below), with only penicilin (33.0%), erythromycin (11.7%) and tetracycline (10.6%) above the 10% of resistances.

Antibiotics	No. of isolates	Percentage (%)
penicillin	65	33.0
kanamycin	1	0.5
gentamicin	0	0
tobramycin	2	1
streptomycin	0	0
erythromycin	23	11.7
lincomycin	14	7.1
spiramycin	14	7.1
tetracycline	21	10.6
vancomycin	0	0
teicoplanin	0	0
florfenicol	0	0
bactrim	1	0.5
tylosin	13	6.5
marbofloxacin	0	0

* This reflects the prudent use of antibiotics by veterinarians and farmers, but attention must be maintain to avoid any emergence of resistance as seen for other animal species.

2. Virulence determinants

* A subset of 61 strains (31 *S. aureus* and 30 coagulase negative) were arbitrarily chosen to investigate the presence or absence of virulence determinants.
* PCR were carried out to detect thre presence of nine SE genes, the exfoliative toxins A and B, and the *tst* gene.
  - 18 strains (29.5%) carried at least one virulence gene and 16 contained a combination of 2 or more genes.
  - *see*, *eta* and *etb* were never detected
  - two associations of genes were predominantly found: *sed-sej* in 5 strains (8.2%) and *seg-sei* in 8 strains (13.1%). These associations were reported with a high incidence in cow mastitis or raw milk (Zschök *et al.*)
  - only the MRSA strain contained 7 SE genes (all the tested genes except *sea* and *see*)

Genes	No. of isolates	Percentage (%)
sea	2	3.3
seb	2	3.4
sec	2	3.5
sed	7	11.5
see	0	0
seg	13	21.3
seh	1	1.6
sei	8	13.1
sej	7	11.5
eta	0	0
etb	0	0
tst	1	1.6

* The capacity to form biofilm *in vitro* was harboured by only 4 strains (6.5%).

Conclusions and perspectives

The prevalence of MRS isolated from French bovine mastitis is very low (1%), as well as the resistance rates to other families of antibiotics.

Since bacterial antibioresistance does not explain the poor response of staphylococcal mastitis to antibiotic treatment, we investigated the virulence of a subset of strains. The results indicate that the capacity of forming biofilms was rare (4/61, 6.5%) and might not generate phenotypic resistance. But 29.5% (18/61) of the isolates presented at least one virulence-related gene, and 21.3% (13/61) displayed the already described *sed-sej* or *seg-sei* associations. Whether the presence of virulence-associated genes could explain the pathogenicity of such isolates remains to be determined.

Yet, our results suggests that cattle mastitis do not constitute a high risk of transmission of resistance to humans, considering the overall low prevalence of both antibiotic resistance and virulence determinants.

References

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