

Study of Toxic Profile of *Staphylococcus Aureus* Isolated From Raw Milk Samples of Mastitis SOR Consumption in Oran Area, Algeria

Nadjia BENHAMED^{*1,2}, Fatma BELHOUCINE¹, Mustapha MOHAMED BENKADA¹, Philippe GAUTIER³,
Amel ALIOUA¹, P-Y DONNIO³, Mebrouk KIHAL²

^{*1}Department of Biotechnology, University of USTO-MB, Oran, Algeria

²Laboratory of Applied Microbiology, University of Oran 1 Ahmed Benbela, Oran, Algeria

³EA 125Microbiologie-Risques Infectieux, University of Rennes 1, Rennes, France

ABSTRACT

Mastitis *S. aureus* is considered one of the major diseases in dairy cattle. The presence of *S. aureus* causes damage to the mammary gland tissue that will impact milk production in both volume and quality. The many virulence factors such as toxins and proteins or surface polysaccharides are involved in the pathology. This study determines the toxic profile of *S. aureus* strains isolated from raw milk samples from bovine mastitis diagnosed in dairy farms in Oran.

After identification by MALDI-TOF, spa typing and MLST have identified the phylogenetic position of the strains. Search for virulence factors is performed by real-time PCR TaqMan type, to detect manipulation in one of the four genes of virulence factors of *S. aureus*: tst for TSST-1, pvl leucocidin for ED, sea for enterotoxin A, seb for enterotoxin B, in this study sought.

Toxins research shows that only a few strains have proven carriers of different virulence genes including the gene encoding the pvl Panton-Valentine leukocidin. Other strains were positive for the presence of tst gene encoding the toxin of toxic shock syndrome (TSST-1), 100% of the *Staphylococcus aureus* isolates identified were sensitive to methicillin.

In conclusion, the results obtained in this work have determined the phylogenetic profile, toxic and sensitivity profile of the méticiline strains studied. Stem found produisantes as the Panton-Valentine leukocidin are sensitive to méticilline. Cette study is the first molecular characterization study of animal strains of *S. aureus* isolated in the region of Oran.

Keywords: milk, mastitis, *Staphylococcus aureus*, Toxic profile, pvl, TSST-1, sea, seb.

I. INTRODUCTION

S. aureus is an important human and animal pathogen responsible for skin and soft tissue infections, the bovine intramammary infections are de most infections caused by *S. aureus*. As an agent of intra-mammary infections, this pathogen can contaminate the bulk milk tank and thus may constitute a bacteriological hazard for raw milk dairy products consumed. In this context, molecular subtyping tools are of great interest for the comparison of genotypes in order to identify sources and

transmission routes for control improvement.

The pathogenicity of *S. aureus* is connected to the expression of virulence genes carried by the chromosome. Scientific studies have proven that in the case of *S. aureus* that has structural factors responsible for the pathogenic activity. The virulence factors such as the ability to secrete toxins, invasiveness and adhesiveness and hydrolytic enzyme production are the factors that determine pathogenicity. The Molecular biology studies on *S. aureus* have revealed the presence

of several genes involved in the virulent power or Table Some *S. aureus* strains are able to produce some virulence factors causing infections. 15 list the 14 genes that encode components of the cell wall, responsible for adhesion, attachment, invasion and the invasion of the host with super antigens.

Staphylococcus aureus is the most predominant contagious pathogen responsible for clinical and subclinical infections in lactating cows (KerroDego et al., 2002). The purpose of this study was to investigate a research staphylococcal virulence factors of *S. aureus* strains sensitive to the methicilin; Pantone Valentine leukocidin pvl, Enterotoxins sea, seb Enterotoxins and exotoxin, staphylococcal toxic shock syndrome: tst.

II. METHODS AND MATERIAL

We selected 52 *Staphylococcus aureus* Methicillin susceptible strains isolated from both subclinical and clinical cases. The strains were identified firstly by the spectrometry de mass (MALDI-TOF) and different spa types and MLST types.

Detection of virulence factors in *Staphylococcus aureus* by PCR time real. DNA extraction (the technique used InstaGene the kit).

Preparation of primers and probes : the reaction mixture contained 5µl of extract and 20 µl of the mix. It is performed in the extraction part. It is achieved, for sample (DNA extract of *S. aureus* strains) and virulence factors, positive control and negative control.

Table 1 : Taqman primer sequences and probes for real-time PCR

Amorce Forward (pvl)	5' -TTC ACT TGT ATC TCC TGA GCC TTT T -3'
Amorce Reverse (pvl)	5' -AGT ACA CAG TGG TTT CAA TCC TTC AT -3'
Sonde FAM-MGB (pvl)	5' -CAT GAG AAA CAG TTG CAA TA-3'
Amorce Forward (sea)	5' -CGA AAC GGT TAA AAC GAA TAA GAA AA-3'
Amorce Reverse (sea)	5' -CCT GTA AAT AAC GTC TTG CTT GAA GA-3'
Sonde FAM-MGB	5' -TGT AAC TGT TCA GGA GTT G-3'

(sea)	
Amorce Forward (seb)	5' -CTG TTA GGG TAT TTG AAG ATG GTA AAA AT-3'
Amorce Reverse (seb)	5' -TCT AAT TCT TGA GCA GTC ACT TTT TTC T -3'
Sonde FAM-MGB (seb)	5' -TCT TTT GAC GTA CAA ACT A-3'
Amorce Forward (tsst)	5' -GCT TGC GAC AAT CGC TAC AG -3'
Amorce Reverse (tsst)	5' -GAT GCT TTT GCA GTT TTG ATT ATT TG -3'
Sonde VIC-MGB (tsst)	5' -TTT TAC CCC TGT TCC C -3'

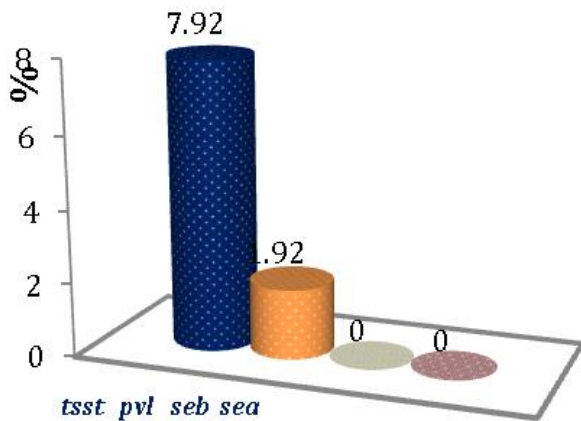
III. RESULT AND DISCUSSION

Production of toxins: Two strains cards are producing test, two producing strains and the pvl. No strains produced the other two toxins (sea and seb). TST + (t007) (4 strains) (2 strains in winter, in summer one isolated strain 1 strain isolated in spring). pvl + (t267) (1 strains) (isolated in winter), presence of the gene encoding the toxin (TSST) by the detection of toxins through the real-time PCR StepOne. The results obtained are comparable to those shown in the study of Velusamy et al., (2006). The distribution of the types spa of *Staphylococcus aureus* strains revealed a diversity of strains of three to two types in each studied herd, as in farms F2, F3 presence of three types of strains against farms F4, F5 two types of strains of *Staphylococcus aureus* and one type of strain was isolated in the F6 farm.

Staphylococcus aureus is considered common agent of clinical and subclinical mastitis in dairy cows. The main reservoir of *S. aureus* is the mammary gland and transmission from one cow to another usually occurs during milking. *Staphylococcus aureus* produces a spectrum of extracellular protein toxins and virulence factors, which are thought to contribute to the pathogenicity of the microorganism. The enterotoxin (SE) and staphylococcal toxic shock syndrome (TSST-1) are recognized as food poisoning syndrome and officers may be involved in other types of infections in humans and animals (Akineden et al., 2001). The results obtained in this first study in the region of Oran, we determined four strains of *S. aureus* carriers of the gene

encoding the toxic shock syndrome (TSST-1) and two other strains carrying gene (*pvl-luk*) encoding the toxin 'Pantin-Valentine-leucocidin.

Search SE enterotoxins show an absence in all strains tested. We know that about 95% of outbreaks of staphylococcal food poisoning are caused by type SE SEA-SEE, and the remaining 5% epidemics can be combined with other new identified SE (Kokan and Bergdoll, 1987).



Staphylococcal Toxin

tsst+ (t007) (4 souches) (2 souches en hiver, 1 souche isolée en été, 1 souche isolée en printemps).
pvl+ (t267) (1 souches) (isolée en hiver).

Figure 1: Detection of Staphylococcal Toxin Genes

IV. CONCLUSION

This first study on bovine mastitis in the region of Oran allowed us to determine the molecular profile of toxic bacterial species *S. aureus* that is dominant among bacterial isolates in the study.

S. aureus strains isolated in these study antibiotic susceptible strains and some strains of *Staphylococcus aureus* have proved sensitive carrier and gene encoding toxins (*Luk-pvl*, *TSST-1*) for the toxin-Pantin Valentine - leucocidine and toxic shock syndrome. These results revealed for the first time an animal strain identified with this toxic molecular profile in the region of Oran.

V. REFERENCES

- [1] Akineden, O., Annemuller, C. and Hassan, A.A, Toxin genes and other characteristics of *Staphylococcus aureus* isolates from milk of cows with mastitis. 2001.Clin. Diagn. Lab. Immunol. 8:959–964.
- [2] Velusamy, S, Ashish, A, Sawant, A, Barbara, E, Gillespie, S, Headrick, J, Lorenza, C. and Stephen, P., Prevalence of Enterotoxin and Toxic Shoc Sydrome Toxin Genes in *S. aureus* isolated from milk of cow with Mastitis,food. 2006. Path. And disease 3:274-283.
- [3] Kerro-Dego, O., J.E. van Dijk, H. Nederbragt, Factors involved in the early pathogenesis of bovine *S. aureus* mastitis with emphasis on bacterial adhesion and invasion. 2002. Areview . Vet.
- [4] Kokan, N.P. et Bergdoll, M.S, Detection of low enterotoxin producing *Staphylococcus aureus* strains. 1987. Appl. Environ. Microbiol. 53:2675–2676.