African Journal of Dairy Farming and Milk Production Vol. 1 (3), pp. 040-043, October, 2013. Available online at www.internationalscholarsjournals.org © International Scholars Journals

*Full Length Research Paper*

# Biodiversity of molecular profile of *Staphylococcus aureus* isolated from bovine mastitis cases in West Algeria

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Accepted 3 August, 2013

**Bovine mastitis is an inflammation of the mammary gland with local and or symptoms that occasionally result in a systemic infection. This disease has a profound impact on animal welfare and milk quality, and the most costly disease affecting dairy cows. The bacteria *Staphylococcus aureus* is one of the most frequently isolated pathogens from both subclinical and clinical infections. This study was conducted to investigate the phenotypic and genotypic characterization of *S. aureus* involved in dairy cow mastitis in West Algeria. A total of 141 isolates of *S. aureus* isolated from quarter milk samples were collected from dairy cows. All retained *S. aureus* species contained *gyr* gene and were identified by molecular typing. The presence of resistance was evaluated in *S. aureus*. Staphylococci antimicrobial resistance was performed by detection of *mecA* gene. Several virulence factors including toxin of the Pantin Valentine leukocidin coding gene (*pvl*) were also investigated by polymerase chain reaction (PCR). Only one strain of *S. aureus* was *mec*A - and** *pvl*+ gene.

**Key words***:* Mastitis, *S. aureus, Gyr A*, PCR, *mecA, pvl*.

## INTRODUCTION

Mastitis, the most expensive disease of dairy cows, continues to be a persistent problem in the dairy industry (Barkema et al., 2009; Le Marechal et al., 2011). Mastitis, inflammation of the mammary gland with local and or general symptoms that occasionally result in a systemic infection, can be caused by a wide range of microorganisms, including gram-negative and gram-positive bacteria (Le Marechal et al., 2011).

*Staphylococcus aureus* is one of the most frequently isolated pathogens from both sub-clinical and chronic infections (Watts 1988). *S. aureus* still remains one of the most significant organisms associated with clinical and subclinical bovine mastitis, not only in Algeria but worldwide. This disease is considered to be the most frequent and most costly production disease in dairy herds of developed countries (Fourichon et al., 2001). Mastitis is one of the dominant pathological dairy farming actually decreased milk production per cow due to the prevalence of clinical and subclinical mastitis (Le Marechal et al., 2011). The main etiology is infectious. It results in the majority of cases by cell type inflammatory response involving an increase in the cell concentration in milk, cell counts in the diagnosis of mastitis is essential and the results should be according to the results of the CMT (California Mastitis test).

Bovine mastitis produces a wide variety of problems in the dairy farm. The treatment of this disease is based on the use of antibiotics which are not always effective.

These drugs are also responsible for the presence of residues the milk the increase antibiotic-resistant strains.

Probiotic products were proposed as a valid alternative to antibiotic therapies and are also useful for the prevention of infectious syndromes (Espeche et al., 2012). *S. aureus* is the most predominant contagious pathogen responseble for clinical and subclinical infections in lactating cows (Kerro-Dego et al., 2002).

This study was conducted to investigate the phenotypic and genotypic characterization of *S. aureus* involved in dairy cow mastitis in West Algeria. The presence of resistance was evaluated in *S. aureus*. Staphylococci antimicrobial resistance was performed by detection of *mecA* gene and several virulence factors including toxin of the Pantin Valentine leukocidin coding gene (*pvl*) by polymerrase chain reaction (PCR).

## MATERIALS AND METHODS

### Bacteriological analysis

Milk samples were analyzed in Applied Microbiology Laboratory, Faculty of Sciences, Oran University. Milk samples were cultured on several media, on blood (5% sheep blood) agar plates, incubited at 37°C for 24 h. Growth on the plates was confirmed by additional laboratory tests in accordance. *S. aureus* was identified by means of typical colony and cells morphology, catalase reaction, by coagulase reaction using rabbit plasma (Quinn et al., 1994) (coagulase positive), or Pastorex (agglutination test) (Bio-rad, France) and biochemical characterization using the Api-staph system (Biomerieu, France). Strains expressed phenotypic resistance to cefoxitin con-firmed by polymerase chain reaction detection of the *mec*A gene, typing of the accessory gene regulator (agr) and detection of control specific gene Gyr of *S. aureus*. Coagulase-negative staphylococci were identified by typical colony and cell morphology and coagulase reaction.

### Susceptibility testing

Susceptibility testing was performed by disk (Bio-rad, France) diffusion method on Muller-Hinton agar plates. Testing was performed according to recommendation of Ca-SFM-veterinarian 2012 (Committee on Antimicrobial Company Information French Microbiology). The antibiotics tested were penicillin G (PG-6 µ g), kanamyin (K-30 IU), gentamicin (Gm-15 µ g), tetracyclin (Te-30UI), erythromycin (E-15UI), lincomycin (L-15 µ g), pristinamycin (PT-15 µg), chloramphenicol (C-30 µ g), pefloxacin (Pef-5 µ g), fosfomycin (Fos-5 µ g), cefoxitin (Fox-30 µ g), fusidic acid (FA-10 µ g), vancomycin (VA-30 µ g), oxacillin (Ox 5 cmg), Amikacin (10 mcg), carbenicillin (cB 100 µ g) and ciprofloxacin (5 mcg). For testing susceptibility in staphylococci, 2% NaCl was added to the broth and to Muller-Hinton agar plates. Control strains, *S. aureus* ATCC 43300 and *S. aureus* ATCC 25923 were tested in parallel with each batch of isolates (Smyth et al., 2001). The susceptibility to the cefoxitin of *S. aureus* was confirmed by polymerase chain reaction of the *mecA* gene (Elazhari et al., 2010).

### Bacterial DNA extraction

DNA of each strain of *S. aureus* was extracted according to the standard protocol (Sambrook et al., 1989). The collected DNA was precipitated, described by electrophoresis on agarose gel and then stored at -20°C.

### Detection of *mecA* gene, *GyrA* gene and *pvl* gene by PCR

The confirmation *of S. aureus* species was performed on the basis of standard biochemical tests. The isolates were further characterized by molecular analysis amplifying the gene *gyr* typing of the accessory gene regulator (Brakstad et al., 1992). A duplex PCR for the simultaneous fragment 533 base pairs (bp) specific *mec*A gene and another 280 bp fragment of the gene *Gyr*A were used to prove the *S. aureus* species. The pathogenicity and virulence of *S. aureus* is associated with the capacity of this organism to produce several virulence factors including Panton-Valentine leukocidin (PVL) (Shittu et al., 2011). In addition, the Pantin-Valentine Leukocidin coding *pvl* gene was detected by simple PCR using specific fragment 433 pb (Sung et al., 2008); the primer used for mecA.

### Typing of *agr* gene (accessory gene regulator)

Using PCR multiplex for search simultaneous fragment *agr* type (*agr*1*; 440, agr2*; 550, *agr*3; 300, *agr*4; 650), the research has been carried out for strains of *S. aureus;* the fragment used to define the type of *agr S. aureus* isolates. All amplification products were separated by electrophoresis on agarose gel 1.5% stained with ethidium bromide (0.5 µg/ml) in Tris-borate-EDTA TBE (at a rate of one to two drops added). Photographs of gels were taken under ultraviolet (UV) device (Gel Doc) (Sambrook et al., 1989).

## RESULTS

Precise identification of *S. aureus* infected cows is important for successful implementation of a mastitis control program. Therefore, according to the phenotypic, biochemical properties as well as by amplification of the *gyr* gene, all of the isolates obtained in this study were identi-fied as *S. aureus*. Data descriptive of Staphylococci strains isolated from quarter milk samples from clinical or sub-clinical mastitis cases in the study is shown in Table 1.

Detection of *pvl-luk* toxin by amplification of the *pvl* gene from extracted DNA of the *S. aureus* strains reveled that positive amplification of the 533 pb fragment of *pvl* gene from the extracted DNA of only one strains from 11, this strain had the following molecular profile *agr*3, *mec*A-, *pvl*+, the rest of strains had a similar molecular profile (Figure 3).

### Antimicrobial susceptibility

Antimicrobial susceptibility testing reported a high susceptibility of *S. aureus* strains to antimicrobial agents which was confirmed by PCR by the absence of *mec*A gene. Our results shows absence of *mecA* gene for all *S. aureus* strains which were phenotypically susceptible to cefoxitin and oxacillin.

Results presented in Figure 2 shows the absence of the *mecA* gene from extracted DNA of *S. aureus* strains tested; this result confirmed the antibiogram results for susceptibility to methicillin.

## DISCUSSION

*S. aureus* is still considered one of the most common

**Table 1.** Data descriptive of Staphylococci strains isolated from quarter milk samples from clinical or subclinical mastitis cases in the study.

| Staphylococci strain | Data of strain | | | | | |
|---|---|---|---|---|---|---|
| | Clinical case % | subclinical case % | Winter % | Spring % | Summer % | Autumn % |
| *S. aureus* | 58.86 | 41.14 | 26 | 17.78 | 40 | 16.22 |
| *SCN* | 0 | 100 | 2.33 | 40 | 47.67 | 10 |

SCN, Staphylococcus coagulase negative.

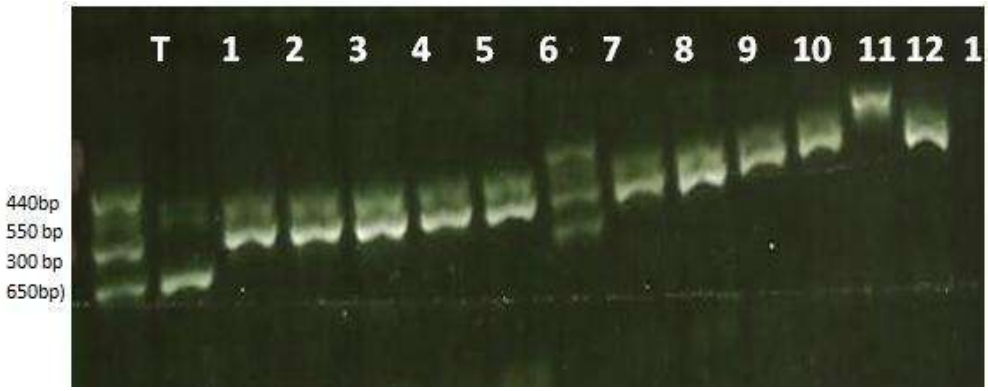


**Figure 1.** Agarose gel electrophoresis of polymerase chain reaction (PCR) amplification of agr1/2/3/4 gene.


**Figure 2.** Agarose gel electrophoresis of polymerase chain reaction (PCR) double amplification of *mecA* and *gyr A* gene.

etiological agents associated with clinical and subclinical infections in lactating cows by Esmat and Bader (1996) and El-Seedy et al., 2010.

Precise identification of *S. aureus*-infected cows is important for successful implementation of a mastitis control program. Therefore, according to the phenotypic, biochemical properties as well as by amplification of the *gyr* gene (Figure 1), all of the isolates obtained in this study were identified as *S. aureus* (Dastmalchi, 2012).

Results presented in Figure 2 show the absence of the *mecA* gene from extracted DNA of *S. aureus* strains tested, this result confirmed the antimicrobial susceptibi-lity testing results for susceptibility to methicillin. The strains of Staphylococcus aureus are (SASM) and showed high level of resistantance to eryhtromycine, ciprofloxacin, penicillin and a susceptibility to kanamycin. All strains showed also a high resistantance to to tetracycline, gentamicin and bacitracin (Figure 4).

Detection of *pvl-luk* toxin by amplification of the *pvl* gene from extracted DNA of the *S. aureus* strains reveled that positive amplification of the 533 pb fragment of *pvl* gene from the extracted DNA of only one strain from 11, this strain had the following profiles *agr*3, *mec*A-, *pvl*+ (Figure 3). This finding was comparable to the study of Sung et al. (2008). One isolate was positive for gene encoding the components of the Pantin-Valentine-Leukocidin (*pvl-luk)*; these results are similar to those obtained by Monecke et al. (2011) and Shittu et al.(2011).

## Conclusions

Our results indicate that several bacteria species of *S.*


**Figure 3.** Agarose gel electrophoresis of polymerase chain reaction (PCR) double amplification of *PVL* gene.

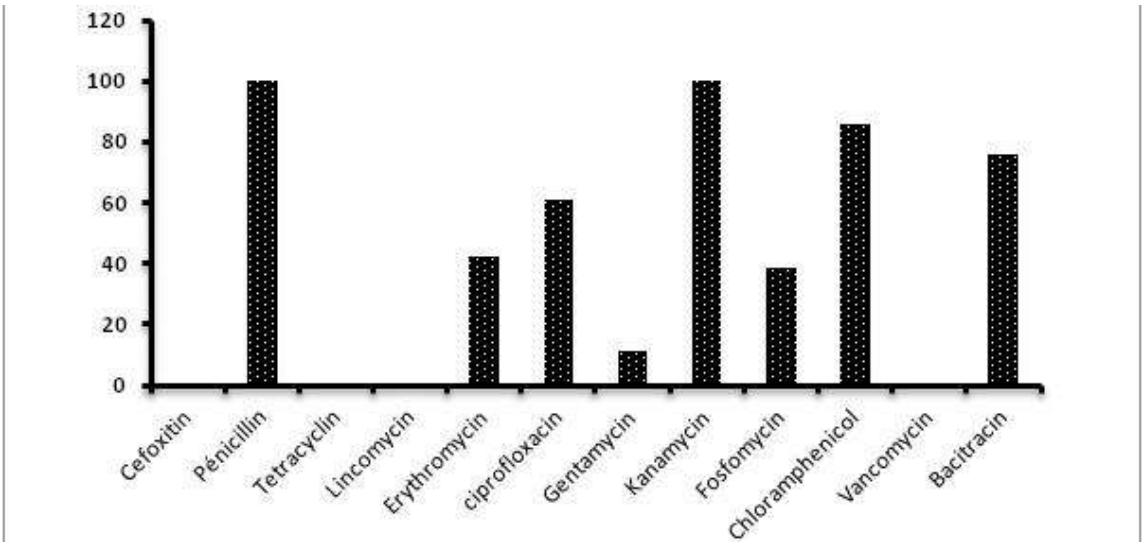


**Figure 4.** Antibiosusceptibility of strains of *Staphylococcus aureus* isolated.

*aureus* were found in mastitis cases. Genotypic and phenotypic identification of *Staphylococcus aureus* was confirmed by detection of *gene gyrA* and *agr* molecular typing. The antimicrobial susceptibility testing showed that *S. aureus* isolates from Oran Region West Algeria exhibited high susceptibility to all antimicrobial agents tested and due to the absence of *mec*A gene in all strains of *S. aureus* tested. The results of study show also that one strain of *Staphylococcus aureus* carried PVL-Luk toxin due to presence of *PVL* gene which showed a susceptibility to cefoxitin.

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