



Occurrence of Methicillin Resistant *Staphylococcus aureus* Carriage in Different Animal Species in Eastern Algeria

Belkadi Souhila, Meradi Manel¹, Oucheriah Yasmine, Yahiaoui Fatima Ezzahra, Bouakaz Ahlam, Nezzar Kebaili Wissam, Heleili Nouzha

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ABSTRACT

Background: Several animal species may act as a possible reservoir for transmission of MRSA to human. The aim of this study was to determine the antibiotic resistance profiles of *Staphylococci* from animals.

Methods: A total of 754 nasal and rectal swab samples collected from apparently healthy animals at Batna and Setif governorate, eastern Algeria. For this purpose, we studied the antimicrobial susceptibility of the isolates by conventional methods (Disk diffusion test and methicillin resistance by Cefoxitin disk diffusion test).

Result: The overall prevalence of *S. aureus* isolates from 754 samples was 43.61%, with a high rate of *S. aureus* isolation in rabbits (92%). Goats, bovine, dogs, cats, horses, poultry presented a medium prevalence with 31.91%, 25%, 23.75%, 21.25%, 15% and 15% respectively, while the lowest rate was observed in sheep with 10%. MRSA were isolated in all animal species (29.46%). All detected isolates were multiple drug resistant (MDR). A complete resistance (100%) was noted for ciprofloxacin and gentamicin in sheep and horses and to penicillin in dogs. MRSA is a serious problem for human and animal health; therefore, several experiments must be carried out to demonstrate possible transmission of MRSA between companion or food-chain animals and humans, as well as some MRSA clones of human origin that have adapted to new animal hosts eventually by losing useless virulence factors or acquiring new mobile genetic elements.

Key words: Companion animals, Livestock, Methicillin resistant, Nasal portage, Rectal portage, *Staphylococcus aureus*.

INTRODUCTION

Livestock is an important agricultural activity in the world that plays a fundamental role in the economic, ecological, cultural and environmental level. As a result, the animal health is an important factor. However, bacterial pathogen is consistently striking this sector (Srouf, 2006).

Staphylococcus aureus is a bacterium of significant importance because of its ability to cause a wide range of diseases and capacity to adapt to diverse environmental forms (Grema *et al.*, 2015). It is an important opportunistic pathogen that frequently colonizes the skin, the nose and mucous membranes of healthy humans and animals and can cause multiple infectious diseases of diverse severity ranging from minor skin infections, such as furunculosis to severe and highly debilitating conditions such as pneumonia and endocarditis (Jensen and Lyon, 2009; Lozano *et al.*, 2016). In addition, *S. aureus* causes inflammation of the mammary gland in bovine and the lower part of the foot in poultry (Quinn *et al.*, 2000).

Antibiotics are commonly used in farms for the treatment and prevention of various diseases, to improve food and to increase milk production (Sharma *et al.*, 2011). Nevertheless, zoonotic bacteria like *S. aureus* develop resistance to these antimicrobial agents. The emergence of this bacterial resistance in animals and their products has highlighted the role that plays the resistance transfer to the human population via the food chain and to the environment by a direct contact with animals (Mesfin, 2015).

Laboratory ESPA, Veterinary and Agricultural Sciences Institute, Department of Veterinary Sciences, Batna 1 University-05000, Algeria.

¹Faculty of Natural and Life Sciences, Department of Microbiology and Biochemistry, Batna 2 University-05000, Algeria.

Corresponding Author: S. Belkadi, Laboratory ESPA, Veterinary and Agricultural Sciences Institute, Department of Veterinary Sciences, Batna 1 University-05000, Algeria.
Email: souhilabelkadi@gmail.com

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Among *Staphylococcus aureus* isolates, methicillin-resistant *S. aureus* (MRSA) represents the major cause of antibiotic-resistance. For many years, MRSA was considered as a specific pathogen of human (Baptiste *et al.*, 2005; Leonard *et al.*, 2008). In recent years, a clone of MRSA has appeared in farm animals (MRSA-L) and isolated in veterinarians, farmers and in foods of animal origin (Chairat *et al.*, 2015). Nowadays, it has been detected in other pets (dogs, horses, cats, pet birds, cattle and pigs) associated with serious infections and become public health concern since it could not be treated easily with many antibiotics (Habibullah *et al.*, 2017).

In Algeria, the problem of antimicrobial resistance in veterinary medicine is poorly documented. This study highlights the antimicrobial resistance status resulting from *S. aureus* and methicillin-resistant *S. aureus* (MRSA) carriage in animals.

MATERIALS AND METHODS

Samples collection

A total of 754 nasal and rectal swab samples were collected from randomly selected farms including cattle, sheep, goats, rabbits, cats, poultry and dogs from Batna governorate while horses were sampled from Setif governorate, eastern Algeria. The study included 377 animals from both genders. This investigation was performed between January 2018 to April 2019 in Microbiology laboratory, Batna 1 University, Algeria. All samples were aseptically collected and examined for detection of *S. aureus* (El Seedy *et al.*, 2017).

Isolation and identification of *S. aureus*

First, samples were incubated in a nutrient broth for enrichment (Schmidt *et al.*, 2014). After overnight incubation at 37°C, 1 µl of this broth was streaked into mannitol salt agar (MSA) which is a selective medium for *S. aureus*. The plates were then incubated for 24 hours. The incubation was extended to 48 hours if the result of the culture was negative or uncertain. Morphologically distinguishable staphylococcal colonies were cultured again on MSA plate to obtain pure cultures. The appearance of yellow-ish colonies on MSA was presumed to be *S. aureus*, subcultured and identified by conventional methods (gram's staining, catalase test, free coagulase production and biochemical tests (API Staph gallery) according to Giacitini *et al.* (2017).

Antimicrobial sensitivity testing

Disc diffusion testing was performed on all staphylococcal isolates in accordance with CLSI (2011) against the following panel of 10 antimicrobial agents; Pénicillin (10 µg), Oxacillin (5 µg), Cefoxitin (30 µg), Erythromycin (15 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Rifampicin (5 µg), Vancomycin (30 µg), Tetracyclin (30 µg), Amoxicillin and Clavulanic acid (20 µg /10 µg).

Using the commercially prepared disc, freshly sub-cultured isolates were emulsified in 3-4 ml of sterile distilled water. The turbidity of the suspension was adjusted to the turbidity of standard equivalent to 0.5 McFarland (Mustapha *et al.*, 2016). A sterile swab was dipped in the bacterial suspension and then smeared on Mueller-Hinton agar media surface. Five discs of antibiotics commercially available were dispensed into each inoculated plate and incubated at 37°C for 24 h. Strains were classified as resistant in accordance with EUCAST (2019) for all antibiotics tested.

Cefoxitin disk diffusion test

Resistance to methicillin was tested using a cefoxitin disc whose load is 30 µg and by an oxacillin disc of 5 µg load. The reading has been done in accordance with the

recommendations of the antibiogram committee of CA-SFM (Comité de l'Antibiogramme de la Société Française de Microbiologie).

RESULTS AND DISCUSSION

In veterinary medicine, the study of the carriage of *S. aureus* in animals remains limited to a few pets such as cats, horses and pigs (Labrecque, 2007). The interest about *S. aureus* and MRSA in livestock, domestic and wild animals has significantly increased (Lozano *et al.*, 2016); Methicillin resistant *Staphylococcus aureus* emerged 50 years ago, as a nosocomial pathogen but in the last decade it has become also a major cause of serious infections in both human and animals (Stefani *et al.*, 2012).

Our study was focused on the prevalence of *S. aureus* and methicillin resistant strains in eight categories of farm animals (Horse, sheep, cattle, goats, rabbits, poultry, dogs and cats) (Table 1).

Occurrence of *Staphylococcus aureus*

The results of *S. aureus* and MRSA strains prevalence on the monitored farms are summarized in Table 2.

S. aureus was recovered from all examined animal species. Of the 754 samples, 43.61% samples were positive for *S. aureus*. Wang *et al.* (2019) reported a lower isolation rate with 40.66%. The highest recovery rate was noted in rabbits (92%). It was followed by goats (31.91%), bovine (25%), dogs (23.75%), cats (21.25%), poultry and horses (15% both) and lowest with sheep (10%). Radwan *et al.* (2015) reported the same result for low isolation rate in sheep while, Giacitini *et al.* (2017) reported a higher prevalence with 53.5% in dairy sheep farms. Recovery of *S. aureus* from nasal and rectal swabs in goats was lower than those found in the investigation of Daaloul-Jedidi *et al.* (2016) (86.6%). The isolation rates from bovine and poultry was lower than those of Akkou *et al.* (2016) in Algeria who reported 29.81% and 55%, respectively.

This investigation revealed that nasal swabs were found more infected by *S. aureus* than rectal swabs in goats, rabbits, cats, dogs and poultry except in sheep in which equal rate (50%) was observed in both nasal and rectal swabs. In bovine and horses, *S. aureus* isolates were more from rectal than nasal swabs. This result does not corroborate with those of Linhares *et al.* (2015) and Daaloul-Jedidi *et al.* (2016) who reported a predominance of this pathogens in nasal samples than in rectal ones in pigs and goats respectively. This portage can constitute a potential risk of transmission to people who are in contact with these animals (Carlet and Shlemmer, 2015). The frequency of *S. aureus* in nasal swabs was higher than that reported by Mai-siyama *et al.* (2014) as 10% for sheep, 20% for cattle and 08% for goats. For rectal samples, Al-Thani *et al.* (2012) reported a total absence of *S. aureus* in these samples, especially for sheep and goats. This can be explained by the difference in breeding conditions such as the presence of several animals on the same farm (birds, pets) which facilitates the transmission of this germ.

Table 1: Prevalence of *S. aureus* by age and sex of different animal's species (in %).

| Species | Age | Examined samples | Global prevalence (%) | Male (%) | Female (%) |
|---------|-------------|------------------|-----------------------|----------|------------|
| Bovine | ≤ 1year | 100 | 25 | 48 | 52 |
| Sheep | < 1 year | 100 | 10 | 10 | 90 |
| | > 1 year | | 90 | | |
| Goats | < 1 year | 94 | 30 | 23 | 77 |
| | > 1 year | | 70 | | |
| Rabbits | < 3 months | 100 | 10.2 | 47 | 53 |
| | 5 months | | 4.08 | | |
| | 8 months | | 8.16 | | |
| | 12 months | | 73.5 | | |
| | 15 months | | 2.04 | | |
| | 24 months | | 2.04 | | |
| Horses | 0-2 years | 100 | 19 | 25 | 75 |
| | 2-4 years | | 31 | | |
| | 4-6 years | | 19 | | |
| | 6-8 years | | 6 | | |
| | 8-10 years | | 12.5 | | |
| | 10-12 years | | 12.5 | | |
| Dogs | 0-4 months | 80 | 6 | 65 | 35 |
| | 5-10 months | | 4 | | |
| | >10 months | | 11.25 | | |
| Cats | 0-12 years | 80 | 12 | 53 | 47 |
| | 3-5 years | | 6 | | |
| | > 5 years | | 10 | | |

Table 2: *S. aureus* recovery rates from animal's samples (In %).

| Source of the isolates | Number of animals | Examined samples | <i>S. aureus</i> | Nasal swab | Rectal swab | MRSA (+) |
|------------------------|-------------------|------------------|------------------|------------|-------------|----------|
| Bovine | 50 | 100 | 25 | 48 | 52 | 24 |
| Sheep | 50 | 100 | 10 | 50 | 50 | 40 |
| Goats | 47 | 94 | 31.91 | 24.46 | 75.5 | 23.33 |
| Rabbits | 50 | 100 | 92 | 57 | 43 | 7 |
| Horses | 50 | 100 | 15 | 40 | 60 | 46.66 |
| Dogs | 40 | 80 | 23.75 | 74 | 26 | 12.5 |
| Cats | 40 | 80 | 21.25 | 52.94 | 47.05 | 55.55 |
| Poultry | 50 | 100 | 15 | 80 | 20 | 26.66 |
| Rate | 377 | 754 | 29.23 | 53.3 | 46.69 | 29.46 |

The present study showed the influence of age in the carriage of *S. aureus*. It was more isolated in animals aged more than one year old, which is explained by the skin modifications which are considered as the first barrier against pathogens with decrease in elasticity and thickness of subcutaneous cell tissue and dry skin (Branchet *et al.*, 2012). In addition, some anomalies of the respiratory system, more often observed in elderly animals, will cause the aggression of the bronchial epithelium, expressed by a decrease in bronchial secretions which are the second barrier against the entry of many airborne infectious agents (Vareille *et al.*, 2011). Our findings are in consistent with data of Branchet *et al.* (2012) who also described age as a factor associated with the carriage of *S. aureus*. The results reported that females are most infected by *S. aureus* in all animal species. Weese (2005) confirmed that sex is a risk factor, the female

seems to be more susceptible to colonization by *S. aureus* than males.

Evaluation of antibacterial activity

Results of antibiotic sensitivity test on *S. aureus* iso-lates recovered from nasal and rectal swabs from different animal species revealed that all the isolates were completely resistant (100%) to ciprofloxacin and gentamicin in sheep and horses and to penicillin in dogs (Table 3).

The overall results of resistance to penicillin, gentamicin, ciprofloxacin and erythromycin was highly prevalent with 64.81%, 50.53%, 48.16 and 47.79 respectively. Moreno-Grúa *et al.* (2018) gave the same resistance rate to penicillin.

Table 3 shows a high resistance to vancomycin. Effectively, this may be due to the fact that the clinical isolates of *S. aureus* with intermediate and complete resistance to vancomycin have emerged within the past two decades and

Table 3: Overall antibiotic resistance of *S. aureus* isolated from animals (In %).

| | OX | AMX | FOX | VAN | CIP | RIF | E | GEN | TE | P |
|---------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Bovine | 60 | 40 | 24 | ≥ 4 | ≥ 4 | 80 | 5 | 5 | ≥ 4 | 50 |
| Sheep | 40 | 40 | 40 | ≥ 4 | 100 | ≥ 4 | ≥ 4 | 100 | ≥ 4 | 50 |
| Goats | ≥ 4 | ≥ 4 | 23.33 | ≥ 4 | 14 | 90 | 43 | 28 | ≥ 4 | ≥ 4 |
| Rabbits | 22.22 | 11.11 | 7.41 | ≥ 4 | ≥ 4 | 14.82 | 11.11 | ≥ 4 | ≥ 4 | 53 |
| Horses | 55 | 16.7 | 46.66 | 50 | 100 | 25 | 75 | 100 | 20 | 65 |
| Dogs | 77.78 | 33.33 | 12.5 | 44.44 | 33 | 44.44 | 55 | ≥ 4 | 22.22 | 100 |
| Cats | 70.58 | ≥ 4 | 55.55 | 14.64 | 23.52 | 23.52 | 58.82 | 23.52 | 17.64 | 82.35 |
| Poultry | 46.66 | ≥ 4 | 26.66 | ≥ 4 | 66.66 | 13.33 | 86.66 | 46.66 | ≥ 4 | 53.33 |
| Total | 35.17 | 28.22 | 29.46 | 36.36 | 48.16 | 41.58 | 47.79 | 50.53 | 19.95 | 64.81 |

have become a serious public health problem (Cong *et al.*, 2020). Noble *et al.* (1992) demonstrated that there is Vancomycin resistance transfer, mediated by transposons mainly found on plasmids, which raised considerable worry about the risk of dissemination of vancomycin-resistant determinants to universally susceptible microorganisms of medical importance, especially *S. aureus*. This concern was subsequently confirmed by the successful transfer of the van element from *Enterococcus faecalis* to a MRSA strain in mix-infected mice (Cong *et al.*, 2020). Haaber *et al.* (2015) discovered that susceptibility of *S. aureus* to vancomycin is reduced by concurrent exposure to colistin, a cationic peptide antimicrobial employed to treat infections by gram-negative pathogens.

Giacinti *et al.* (2017) revealed in their investigation that *S. aureus* isolates were phenotypically resistant to all the β -lactams tested and to erythromycin, streptomycin, kanamycin and tetracycline. Same results were revealed in our study for erythromycin (47.79%).

S. aureus strains (29.46%) showed a resistance to cefoxitin and were thus identified as methicillin-resistant *S. aureus* (MRSA) strains as described by Hachemi *et al.* (2019). Radwan *et al.* (2015) reported high rate of methicillin resistance with 58.3%.

According to Table 1, cats were more affected by SARM strains followed by horses and sheep (55.55%, 46.66% and 40% respectively). Faires *et al.* (2009) gave a little lower rate for cats (10%). For Sarhan and Mohammed (2019), MRSA isolates prevalence in sheep (92.30%) were significantly higher than our results.

Weese (2005) reported same MRSA isolation rate in horses with 42.5% while most published reports indicated that MRSA colonization in horses ranged from 0 to 11% (Grema *et al.*, 2015). The previously prevalence might under-represent true carriage as this study samples were only from the nasopharynx.

Bounar-Kechih *et al.* (2018) isolated also MRSA from poultry at 53.5% which was higher than our results with 26.66%.

Dogs and rabbits were the least affected by *S. aureus* carriage (12.5% and 7.41% respectively). Loeffler *et al.* (2005) and Moreno-Grúa *et al.* (2018) gave also a low rate with 9% for dogs and 12.5% for rabbits, respectively.

All the isolated MRSA strains showed cross-resistance to antibiotics used in the study. They were more resistant towards two or multiple antibiotic combinations; therefore, they were cross-resistant to many other antibiotic families other than β -lactam antibiotics. In Algeria, selling antibiotics is totally uncontrolled what means abusive and indiscriminate use in human but also in the animal.

CONCLUSION

The main finding of this study indicates clearly that *S. aureus* and MRSA were highly isolated from all animal species joining the results of other studies. They were also resistant to β -lactam antibiotics (Penicillin and Oxacillin). This result also suggests that it may be a potential risk for human health because of a possible transmission from animals. Because of the few studies done in this area in Algeria, it will be interesting to realize sampling from multiple anatomic sites when screening animals for MRSA and to undertake molecular studies are needed in order to understand the cross transmission from animal to human or from human to animals.

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