Prevalence and Antimicrobial Resistance of Methicillin-Resistant *Staphylococcus aureus* Isolated from Raw Meat and Bovine Milk in Algeria

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**ABSTRACT**

Recently, reports of methicillin-resistant *Staphylococcus aureus* (MRSA) from several foods have become more frequent. The aim of this study was to estimate the prevalence of MRSA from 108 foodstuff samples of animal origin in Algeria. The strains were characterized by studying their resistance properties against several antibiotics; production of β-lactamase by Clover Leaf technique; their ecological origin was determined by biotyping. Of the 57 analyzed *S. aureus* strains, two (3.5%) were MRSA. Strain isolated from chicken meat showed the human biovar, β-lactamase production positive and resistance against 13 antibiotics while the other isolated from bovine milk showed the bovine biovar, β-lactamase production positive and resistance against 14 antibiotics. However none of the two strains were resistant to glycopeptides. This paper is first publication regarding MRSA isolate from foods of animal origin in Algeria.

**INTRODUCTION**

*Staphylococcus aureus* is an important food-borne pathogen. It is a versatile pathogen of humans and animals and causes a wide variety of diseases ranging in severity from mild skin infections to more severe diseases such as pneumonia and septicemia [18,16].

Severity of *S. aureus* infections relies on the production of virulence factors and the pathogenic attitude is frequently enhanced by acquisition of resistance to antimicrobials [3] such as the resistance to methicillin.

MRSA remain a major healthcare burden all over the world due to the emergence and spread of isolates with decreased susceptibilities to several antibiotics classes [19], which constitutes an important clinical problem due to the limited therapeutic options [9]. Resistance to methicillin was first described for *S. aureus* in 1960, shortly after the introduction of methicillin into clinical practice [1,11]. Since then, methicillin-resistant *S. aureus* (MRSA) has gradually disseminated and began causing serious nosocomial infections worldwide in the 1970s [20]. By the mid-1990s MRSA had increased dramatically worldwide, becoming a serious clinical problem in hospital environments. In recent years a major change in epidemiology of MRSA has been observed, with the appearance of cases in the community affecting people having no epidemiological connection with hospitals [8,30].

Methicillin-resistant *S. aureus* has become widespread in Algeria. Prospective, multicentre study was conducted between 2003 and 2004 with participation of nine university hospitals in the Mediterranean area, and the percentage of *S. aureus* strains demonstrating resistance to methicillin (MRSA) is 35.5% in Algeria [25].

With respect to MRSA infections in the community, colonization and infections of animals, including also the farmed ones, are of particular interest with regard to a mutual dissemination. Considering the increasing evidence of MRSA in food animals, it is logical that concerns would emerge about MRSA contamination of food and to assume that colonized animals were the source of contamination [33], although food handlers have been reported to serve as a potential source of the pathogenic bacteria [29]. During slaughtering of MRSA-positive animals, contamination of carcasses and the environment with MRSA may occur and consequently meat of these animals may get contaminated [5]. Food can be then considered an excellent way for introducing pathogenic microorganisms in general population [28]. MRSA strains have been detected in different foods, including bovine milk and cheese [21], meat products [31] and raw chicken meat [13,14]. Transmission of
MRSA by consumption of food products has not been investigated thoroughly [15], although S. aureus may be often detected in food and may be involved in food-borne diseases [21,4].

The increasing prevalence of MRSA multi-drug resistant strains which limits the therapeutic options available for the management of MRSA associated infections has become a worrisome issue worldwide [8]. Therefore, the determination of susceptibility or resistance of strains to antibiotics is very important from a clinical and economic point of view. Moreover, the public health of this issue is of great importance because antibiotic therapy of infectious diseases in animals poses the risk of selection of resistant strains and introduction of these strains into the food chain [20].

Sufficient and valid data are an indispensable component in the assessment of a possible health risk related to MRSA-contaminated food animals, especially meats and bovine milk. This paper reports the results on the occurrence of MRSA strains isolated from foods of animal origin produced in Algeria. This study was undertaken to: i) assess the MRSA exposure of consumers by testing a substantial quantity of meat samples from retail outlets and bovine milk in Algeria for the prevalence of MRSA; ii) characterize the isolated strains based on their antimicrobial-resistance pattern; iii) biotype the isolated strains to relate them with the characteristics mentioned above.

**MATERIALS AND METHODS**

**Sample collection:**

From November 2011 through June 2012, a total of 108 foods of animal origin samples were analysed microbiologically: 55 came from milk, 53 from meat included 24 chicken (breast and drumsticks) and 29 beef (ground beef and sirloin strips).

Meat samples were randomly collected in Tiaret city (North-West Algeria) from 14 butchers shops usually come from local or national breedings. Stores were visited only once and meat samples were obtained as offered to the consumer.

In addition, samples of cow’s milk are collected from five farms. All located at a distance of 10 Km to 30 Km from the city. Herd size varied from 4 cows to 20 cows.

All samples were immediately transported to the laboratory in a refrigerated box (4–8 °C), and processed usually at the same day of levy.

**Isolation and identification of S. aureus:**

For staphylococci isolation, 10 g/ml of each sample were transferred to flasks with 90 mL of Peptone Water and then plated onto Baird Parker Agar with Egg Yolk Tellurite Emulsion (BP, Merck, USA ; Tellurite, Pasteur Institute, Algeria) according to ISO 6888-1 [10]. The plates were incubated under aerobic conditions at 37°C for 24-48 h.

From each positive sample, 5 typical S. aureus colonies (black colonies surrounded by 2–5mm clear zones) were transferred to on Mannitol Salt agar (Fluka, Spain) for further purification. Typical colonies-yellow colonies showing Mannitol fermentation were cultured in Brain Heart Infusion broth (Fluka, India) (BHI) for 24 h at 37 °C and tested using standard microbiological procedures such as Gram staining, catalase and oxidase reactions, coagulase by test tube technique and TDNase. Strains were also streaked on blood agar plates to test hemolytic activity. Identification of S. aureus was confirmed with biochemical test API STAPH (bioMérieux, Marcy l’Etoile, France). After identification, strains were stored at 20 °C in Brain Heart Infusion Broth with glycerol (50% v/v).

**Biotyping:**

Biotyping was carried out according to the simplified scheme [6,7,13], which uses four discriminative tests: the production of staphylokinase and β-haemolysin, the coagulation of bovine plasma within 6 h and the type of growth on crystal violet agar.

**Antimicrobial susceptibility:**

All S. aureus isolates were screened for methicillin-resistance using disc diffusion. This was performed on Mueller-Hinton agar plates (Fluka, India) as per the Clinical and Laboratory Standards Institute [2] guidelines using 1 μg oxacillin and 30 μg cefoxitin discs (Bioanalyse, UK).

A zone diameter ≤ 10 mm for oxacillin and ≤ 21 mm for cefoxitin were classified as resistant. The MIC of oxacillin was determined by an agar dilution method in accordance with NCCLS recommendations [23] on Mueller-Hinton agar containing 4% NaCl (Fluka, Spain) and oxacillin at concentrations ranging from 0.016 to 16 μg/ml for S. aureus.

Furthermore, the antibiotic susceptibility pattern of methicillin-resistant S. aureus strains was determined by disc diffusion method for pénicillin G (10 UI), gentamicine (10 μg), tobramycin (10 μg), kanamycine (30 μg), amikacin (30μg ) erythromycin (15 μg), spiramycin (10 μg), lincomycin (10μg), la pristinamycin (15μg), la vancomycin (30 μg), l’ofloxacin (5μg), tetracyclin (30 μg), chloramphenicol (30 μg), fosfomycin (50 μg),
fusidic acid (10 μg), la bacitracine (8 μg), nalidixic acid (30 μg), novobiocine (30 μg) (Bioanalyse, UK) and three antimicrobials such erythromycin, tetracycline, chloramphenicol were selected and confirmed by MIC (E-test) (Biomérieux, Marcy, l’Etoile, France) on Muller Hinton agar. The diameter of the zone of inhibition produced by each antibiotic disc was measured, recorded and the isolates were classified as “resistant”, “intermediate” and “sensitive” based on the standard interpretative chart updated according to the current NCCLS standard [24]. A methicillin-susceptible S. aureus strain (ATCC 25923), a MRSA strain (ATCC 43300) were used as control organisms for the disk diffusion.

Isolates were considered as multiresistant MRSA when they were resistant to three or more of the antibiotic listed above.

Detection of β- lactamase production:

All isolates showed resistance to penicillin, were tested for β-lactamase activity by Clover Leaf Technique according to the method described by Parvathi and Appalaraju [27].

Result:

Prevalence of MRSA in Foods of animal origin:

A total of 57 (52, 7%) S. aureus strains were isolated from foods of animal origin, including 34 (64,1 %) of the 53 meat samples and 23 (41,8%) of the 55 milk samples. Of the 57 S. aureus isolates, two (3,5%) were found to be methicillin-resistant (MIC ≥ 16 μg/ml for oxacillin) (Table1), therefore the overall MRSA prevalence was 2/108 (1.8%). Among the MRSA strains, profile attributed to human biotype were observed for strain originated from poultry, whereas profile attributed to bovine biotype were observed for the other one belonged to bovine milk.

Antimicrobial susceptibility testing:

The antimicrobial resistance profile of the tested MRSA strains to different antibiotics was analysed; both isolates were thus found to be resistant, in addition to beta-lactams, where they show the capacity to produce β-lactamase. to erythromycin where isolates had MIC of > 256 μg/ml (Table1), spiramycin, lincomycin (constitutive type) also to bacitracine, oflaxacine, nalidixic acid and tetracycline where strains X3 and V7 have MIC values 24 and 64 μg/m, respectively to teracyclin (Table 1). However, differences were found with regard to the resistance to fusadique acid and fosfomycine which were recorded in a single strain (Table 1).

Our MRSA expressing two phenotypes of resistance to aminoglycosides involving two inactivating enzymes aph (3')-III, which confers resistance to kanamycin and amikacin (phenotype K) and ant (4') (4'”) which confers resistance kanamycin, amikacin and tobramycin (KTphenotype).

All isolates were susceptible to vancomycin, novobiocin, pristinamycine and gentamycine. chloramphenicol was also active often with weak MIC = 0,064μg/ml for V7 strain and 0,016 for X3 strain (Table 1).

Table 1: Characteristics of MRSA isolates from Foods of animal origin.

<table>
<thead>
<tr>
<th>Strains name</th>
<th>Sample origin</th>
<th>Biotype</th>
<th>Resistance profil</th>
<th>β-lactamas production</th>
<th>MIC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X3</td>
<td>Milk</td>
<td>Bovine</td>
<td>P/ Ox / Fox / K / An / Tm /E / B Sp / L / TE / NA / Ofx / Fos</td>
<td>+</td>
<td>16 &gt;265 24 0,016</td>
</tr>
<tr>
<td>V7</td>
<td>Chicken meat</td>
<td>Human</td>
<td>P/ Ox / Fox / K / An / B / E + 16 &gt;256 64 0,064 Sp / L / NA / Ofx / TE / FA</td>
<td>+</td>
<td>16 &gt;265 64 0,064</td>
</tr>
</tbody>
</table>

P, penicillin; OX, oxacillin; Fox, cefoxitin; K, kanamycin; An, amikacin E, erythromycin; TE, tetracycline; Tm, tobramycin; B, bacitracin; Sp, spiramycin; L, lincomycin; NA, nalidixic acid; Ox, ofloxacin; Fos, fosfomycin; FA, fusidic acid; C, chloramphenicol.

Discussion:

Staphylococcus aureus resistant to mithicillin (MRSA) is a major human pathogen in the world. Additionally MRSA is widely spread in different animals [12]. Its prevalence in most African countries has not been reported [25] particularly in the food sector.

The present study aimed to estimate the prevalence of MRSA strains isolated from foods of animal origin and characterized thanks to their antibiotic resistance profile. Biotype strains were also evaluated in order to determine if contamination is of human or animal origin.

In this survey of 57 S. aureus strains, 2 (3, 5%) were mithicillin resistant (MRSA) percentage which is according to that stated by López in [17], who found that 3% of 149 S. aureus strains isolated from food such as meat and milk [17]. Corrente also found in 2007 out of the 200 isolates of S. aureus, 6 (3%) were MRSA [3]. A study in Korea, including 930 slaughterhouse and retail meat samples, showed the presence of MRSA in two chicken meat samples (0.2%) but not in any pork or beef sample [14].

In another study, of 1913 specimens from food-producing animals, including milk and meat of beef, pig and chicken origin, Lee [15] found 15 strains harbouring the mecA gene. Most of the MRSA isolates were from...
milk and three from chicken. Lee concluded that contaminated foods of animal origin may represent a source of MRSA infection for humans [15]. This indicates that food-producing animals are important spreaders of MRSA.

However, in the present research, the ecological origins of the MRSA isolates were traced by using the simplified biotyping scheme of Devriese et al. [6,7,13]. One of the two strains V7 isolated from raw chicken meat belonged to the human biovar, Similar to Kitai et al. [13], out of 444 samples of raw chicken meat examined, two (0.45%) harboured MRSA strains. Interestingly, these two MRSA strains belonged to the human biovar, suggesting that food handlers had been the source of contamination [21,29]. This finding emphasized the role of humans for the introduction of these strains onto raw meats during processing. Thereby sanitary education of food handlers in hygienic practices is necessary to prevent the survival of MRSA in raw foods. While the other MRSA strain X3 isolated from cow's milk cattle belonged to the bovine biovar. In an Italian survey of 1634 foodstuff samples 6 (0.4%) MRSA strains were isolated from bovine milk and cheese where, three MRSA strains were found to belong to the non-hostspecific (NHS) biovar and three to the ovine biovar. This suggests that ruminants may act as reservoirs of MRSA strains [21].

The emergence of multidrug resistant pathogens is recognized as an environmental hazard to the food supply and human health, as it makes eradication more difficult and incidence to increase [32]. Investigation on antibiotic resistance showed that both MRSA strains isolated in this study exhibits properties of resistance against several families of antibiotics (β-lactam, macrolides, aminoglycoside and quinolones) due to the frequent use of these antibiotics in animals, in particular to treat staphylococcal infections, it is important to monitor the evolution of MRSA resistance to these groups of drugs, while no strain was resistant to vancomycin. These findings go in agreement with previously reported studies [13,21,33].

In conclusion, the present study highlighted that raw foods of animal origin contaminated with MRSA in Algeria may constitute a health hazard to consumers, though the frequency of isolation is very low. This requires the implementation of an active strategy where microbiological food safety must be ensured in order to prevent the emergence of MRSA or to avoid its spread by contamination of raw foods, emphasising the need for improved hygiene practices during food processing and also during the distribution and consumption of the final food products.

ACKNOWLEDGEMENTS

We are grateful to all the staff in the laboratory of hygiene and animal diseases for their contribution to this document.

REFERENCES


