Antimicrobial Resistance Pattern of the Staphylococcus Strains Isolated from Farm Animals, Exposed and Non-Exposed Personnel

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Abstract — Introduction: Staphylococcus aureus (S. aureus) resistant to Methicillin (MRSA) occupies a significant place in modern microbiology and infection control procedures. The frequency of MRSA infections continues to grow in hospital and community associated settings. The increase in the incidence of infections due to S. aureus is a consequence of the pathogen's ability to adapt to a changing environment. Methods: Analyzes were performed on 48 samples of Staphylococcus strains from farm animals, exposed and non-exposed personnel obtained from the Université des Frères Mentouri (Constantine) and Institute des Sciences Vétérinaires El Khroub (Algeria), collected during 2016. Each strain was tested against 30 antibiotics to detect antibiotic resistance. Results: Among 48 tested samples 41 (85,4%) were identified as S. aureus. Our results revealed that 14,6% of the S. aureus strains were resistant to Cefoxitin, which would presumably indicate MRSA strains. We also confirmed resistance to Methicillin, Penicillin, Oxacillin resistance and the majority of isolated strains were multi-resistant. Conclusion: These results confirmed that antibiotics resistance is present not only in nosocomial bacteria, but also in livestock and community environments microorganisms. The majority of isolated S. aureus strains were multi-resistant to more than three classes of antibiotics. MRSA strains were detected in 14,6% of our samples and was common among samples from farmers and non-exposed students.

Keywords **—** antibiotic resistance, Methicillin-resistant Staphylococcus aureus, occupational exposure, personnel.

I. INTRODUCTION

Staphylococcus aureus (S. aureus) belongs to the genus Staphylococcus and the family of Micrococcaceae. Among 41 recognized species of Staphylococci widely distributed in the living nature, 18 colonize humans. S. aureus is an important mammalian pathogen that has been recognized for its tendency to cause serious and invasive diseases. It is one of the most resistant and most adaptable non-sporogenic bacteria (1). Staphylococcus xylosus (S. xylosus) is a commensal of the skin of humans and animals and omnipresent bacteria naturally present in food, such as raw meat and milk and is one of the major starter cultures used for meat fermentation, but also is demonstrated that a few strains could potentially be hazardous and are related to animal opportunistic infections (2).

Staphylococcal food poisoning (intoxication) is one of the most common foodborne illnesses reported worldwide. When ingesting one or more preformed Staphylococcal enterotoxins (SEs) in contaminated food, intoxication will occur (3). The severity of symptoms may vary depending on the amount of SEs consumed in the food and the general health of individuals. The young and elderly population is more likely to develop more serious symptoms. Recently it has been reported that less than 1 μg of SE is sufficient to cause Staphylococcal food poisoning (4).

Before the discovery of antibiotics, invasive S. aureus disease was a significant cause of mortality. However, the introduction of Penicillin in the 1940s reduced numerous deaths from this microorganism. Early in the 1950s, isolated strains already showed resistance to Penicillin and then later, to semi-synthetic Penicillin derivatives such as Methicillin. Recently, Methicillin-resistant S. aureus (MRSA) strains manifesting intermediate and complete resistance to Vancomycin have been isolated in hospitals and some MRSA strains are now endemic in various community niches (5). The incidence of MRSA infections has dramatically increased in the last five years due to the worldwide emergence of community strains of MRSA among healthy people lacking traditional health care associated risk factors (6). MRSA is generally resistant to β-lactam antibiotics (Penicillins, Cephalosporins and Carbapenems). MRSA colonization in production animals detected in recent years has in several cases resulted in infections in humans and infections with livestock-associated strain of MRSA may today be considered as a zoonosis. MRSA has been detected in

cattle, chickens, horses, pigs, rabbits, seals, cats, dogs and birds. An assessment of the public health significance of MRSA in animals and food was issued by the European Food Safety Authority in 2009. Until now the primary route of transmission of livestock associated MRSA has been considered to be the occupational contact of livestock professionals with animals harboring this type of MRSA. Therefore, monitoring the occurrence and diversity of MRSA in primary production, including the slaughter, seems pivotal, while monitoring in food may also help with the assessment of consumer's exposure via this route, although to date this route of transmission has been deemed of minor importance. In addition, antibiotic susceptibility data on MRSA isolates are useful in directly informing on the emergence of strains of potential public health significance but can also provide important epidemiological information on the spread of particular strains between the animal and human populations, particularly when investigated in conjunction with molecular typing data (7).

The use of antibiotics in food animals is thought to contribute heavily to the emerging resistance patterns in human pathogens. Antibiotics are used in animal husbandry to improve growth and maintain the health of livestock. Antibiotic use falls neatly into three main categories: therapeutic, prophylactic and nutritive. Many of the broad and narrow spectrum antibiotics used for therapeutic treatment of infected animals are the same as those used in humans. Unfortunately, many of the antibiotics used for nutritive purposes to promote growth development are used at subtherapeutic levels (8).

The foodborne route of transfer of antimicrobial resistance is in addition to direct zoonotic spread resulting from contact with animals, e.g. livestock, pets and their excreta. Meanwhile, foods other than those originating from animals can also be vectors for the transmission of antibiotic resistant bacteria and bacteria-borne resistance genes to the consumer. In addition, food handlers can contaminate food during preparation (9). Most food processing technologies aim to reduce the numbers of foodborne pathogens present, including antibiotic resistant bacteria as well as the overall bacterial load. Hence, food deterioration and the possibility of foodborne infections are reduced. This important beneficial effect has to be considered when evaluating any potential hazards arising from food processing with respect to antibiotic resistance. Emerging non-thermal processing/preservation technologies (e.g. high-pressure processing, ionizing radiation, pulsed electric field and ultraviolet radiation) are technologies designed to produce safe food, while maintaining its nutritional and sensory qualities (10).

II. MATERIALS AND METHODS

The cross-sectional study was conducted during 2016. The aim of research was to study the phenotypic resistance pattern of Staphylococcus strains to wide spectrum of antibiotics and detection of number of asymptomatic carriers of MRSA strains among exposed and non-exposed farm personnel and farm animals. Testing of samples was performed in the laboratory of the Faculty of Pharmacy, Department of Preventive Medicine and Public Health of the University of Granada, Spain. The origin of isolated strains (N=48) was from exposed personnel (ranchers, veterinarians, students and academic staff), non-exposed personnel (1st year students of Veterinary faculty without praxis on farms) and farm animals (bovine and lamb). Samples of nasal swabs were collected by students from fifth year class of Veterinary faculty of the Université des Frères Mentouri (Constantine) and Institute des Sciences Vétérinaires El Khroub (Algeria), under supervision of academic staff.

All media for isolation and identification of Staphylococcus strains were prepared in the laboratory according to the manufacturer's instructions. For isolation of the strains we used Mannitol Salt Agar (MSA), Brain Heart infusion (BHI) broth and agar. The inoculum suspensions were prepared by selecting several morphologically similar colonies from overnight growth (16-24 h of incubation) on a BHI agar medium with a sterile loop and suspending the colonies in BHI broth to the density of a McFarland 0.5 standard. For agar plate inoculation a sterile cotton swab was dipped into the inoculum suspension and the excess fluid removed by turning the swab against the inside of the tube to avoid over-inoculation of plates. The inoculum was spread evenly over the entire surface of the MHA plate by swabbing in many directions. Plates were incubated for 24h at 37°C.

API Biochemical Test Strip based on enzymatic activity was used for identification of Staphylococcus species. To distinguish the difference between strains catalase, coagulase and oxidase test were performed.

Antimicrobial Susceptibility testing - The Disk diffusion test was performed by applying 30 commercially-prepared, fixed concentration paper antibiotic disks on the inoculated agar surface in Mueller Hinton agar plate. An inoculum turbidity of 0.5 McFarland was used. After streaking the colony on Mueller Hinton agar the antibiotic disks were added after 15 minutes on the plates by sterile forceps. Plates were incubated for 24h

at 37°C prior to determination of results. Tested antibiotics and concentrations were: Amoxicillin (AX 25µg), Amoxicillin and clavulanate (AC 20/10µg), Ampicillin (AP 25µg), Carbenicillin (CB 100µg), Cloxacillin (CX 5µg), Oxacillin (OX 5µg), Penicillin G (PC 6µg), Cephalothin (CF 30µg), Cefoperazone (CZ 75µg), Cefoxitin (FX 30µg), Amikacin (AK 30µg), Apramycin (AM 15µg), Gentamicin (GM 10µg), Kanamycin (KM 30µg), Neomycin (NM 30µg), Netilmicin (NT 30µg), Paromomycin (PM 10µg), Spectinomycin (SP 100µg), Streptomycin (SM 10µg), Tobramycin (TM 10µg), Chloramphenicol (CM 30µg), Fosfomycin (FM 50µg), Tetracycline (TC 30 µg), Trimethoprim/ Sulfamethoxazole (TS 1,25 + 23,75µg), Nalidixic acid (NA 30µg), Mupirocin (MUP 5µg), Erythromycin (E 5µg), Ciprofloxacin (CIP 5µg), Vancomycin (VA 5µg) and Clindamycin (DA 2µg). Diameters of the zones of inhibition around the disks were measured to the nearest millimeter using a ruler. Results were interpreted according to criteria of the Société Française de Microbiologie (SFM 2017), British Society for Antimicrobial Chemotherapy (BSAC 2013), Clinical and Laboratory Standards Institute (CLSI 2007), EUCAST 2017, BD BBL New Jersey and CASFM 2003.

For the statistical analysis of the data the SPSS program for Windows (version 21) was used. Absolute and relative frequencies (%) have been used for the study of qualitative or categorical variables and means and standard deviations have been used for the study of the quantitative variables. The association between the different variables included in the study has been analyzed by mean comparison test.

III RESULTS

Among 48 strains, 41 (85,4%) were identified as *S. aureus* and 7 (14,6%) as *S. xylosus.* The origin of the strains was from exposed personnel (31,4%), non-exposed personnel (27,1%), and farm animals (41,5%). Table 1. represents antimicrobial resistance rate of isolated strains. Strains from non-exposed personnel were more resistant compared to the rest, but without statistically significant differences (p=0.394). *S. aureus* strains were more sensitive than *S. xylosus* strains, but not significantly (p=0.592).

Table 1. Antimicrobial resistance rate of isolated strains of *S. aureus* and *S. xylosus* (%)

Antibiotic	***Staphylococcus aureus***	***Staphylococcus xylosus***
Amoxicillin	78	14,3
Amoxicillin and Clavulanate	68,3	42,9
Ampicillin	85,4	57,1
Carbencillin	80,5	42,9
Cloxacillin	24,4	57,1
Oxacillin	70,7	85,7
Penicillin G	87,7	71,4
Cephalothin	17,1	0
Cefoperazone	56,1	28,6
Cefoxitin	14,6	42,9
Amikacin	19,5	14,3
Apramycin	61	0
Gentamicin	61	42,9
Kanamycin	29,3	28,6
Neomycin	53,7	0
Netilmicin	39	0
Paromomycin	26,8	14,3
Spectinomycin	2,4	0
Streptomycin	34,1	14,3
Tobramycin	56,1	0
Vancomycin	22	0
Clindamycin	17,1	42,9
Ciprofloxacin	4,9	0
Nalidixic acid	95,1	100
Erythromycin	22	14,3
Trimethoprim-Sulfamethoxazole	7,3	0
Tetracycline	68,3	42,9
Chloramphenicol	4,9	0
Fosfomycin	0	57,1
Mupirocin	4,9	0

In our study *S. aureus* isolates were highly resistant to Penicillin G and those isolates were widely distributed among all of samples: bovines (83,3%), non-exposed personnel (36%), exposed personnel (17%), lambs (16,7%), farmers (8%), exposed professors (6%) and veterinarian (3%).

Our results revealed that 14,6% of the S. aureus and 42,9% of the S. xylosus were resistant to Cefoxitin. The comparisons of the means of the size for Cefoxitin zones between non-exposed personnel and bovines were statistically significant (p=0.038). Our data revealed Fosfomycin to be the most effective drug for S. aureus without registered resistance. Moreover, almost the opposite of Fosfomycin is the data of the efficacy of Penicillin G (87,8 %) and Nalidixic acid (95,1%) which proves to be ineffective in treating S. aureus. The resistance to Oxacillin was 70,7%. The strains from non-exposed students were the most resistant compared to the rest, although the differences are not statistically significant (p=0.874). Strains of S. xylosus (100%) and almost all strains of S aureus (95,1%) revealed resistance to Nalidixic acid. Vancomycin testing was performed as part of a routine procedure in this work and S. aureus represents a number of 22% of resistance,

while S. xylosus revealed 0% of resistance. Strains that came from farmers were the most resistant compared to the rest and also are statistically significant (p=0.023). Strains of S. aureus presented greater resistance in the grouped sample (university + faculty) than the other group (all the farms) and also the differences are statistically significant (p=0.010). The comparison of the means of the halos between farmer and lamb strains showed almost statistically significant value (p=0.070) and according to the installations, a statistically significant value was found (p=0.006). All of the isolates from farmers in the present study were resistant to Tetracycline. Our study also revealed worrisome data of a total of 76,9% resistant isolates from non-exposed personnel. Strains from farmers were the most resistant compared to the rest but with no statistically significant differences (p=0.207) and according to the installations there was no statistically significant value (p=0.344). Strains of S. aureus presented greater resistance in the grouped sample (university + faculty) than the other group (all the farms) but the differences are not statistically significant (p=0.763). No resistance to Apramycin, Netilmicin, Mupirocin, Trimethoprim-sulfamethoxazole Spectinomycin, Ciprofloxacin, Vancomycin, Tobramycin and Cephalothin was detected for S. xylosus strains, while we can note that a group of antibiotics tested on S. aureus showed rather low resistance, such as group of antibiotics consisting of Spectinomycin (2,4%), Mupirocin (4,9%), Ciprofloxacin (4,9%), Chloramphenicol (4,9%), Trimethoprim-sulfamethoxazole (7,3%), Cephalothin (17,1%), Amikacin (19,5%), Cloxacillin, (24,4%), Paromomycin (26,8%) and Kanamycin (29,3%). Two group samples of animal origin (lamb and bovine) revealed the same resistance to Nalidixic acid where a total of 100% resistances was confirmed.

No resistance to Fosfomycin, Cloxacillin, Cephalothin, Paromomycin, Streptomycin, Chloramphenicol, Trimethoprim-Sulfamethoxazole, Clindamycin, Cefoxitin, Spectinomycin, Mupirocin, Vancomycin and Ciprofloxacin was recorded among samples from bovine group. Resistances in lamb group of samples didn't show any resistance to Cefoxitin, Spectinomycin, Chloramphenicol, Fosfomycin, Vancomycin, Ciprofloxacin nor Mupirocin. The percentage of strains resistant to Mupirocin is encouraging since for S. aureus the recorded value was 4.9%, while the S. xylosus strains showed no resistance. When we compared the mean value of Mupirocin halos we found they were statistically significant (p=0.027) between lamb and farmer samples but according to the installations there was no statistically significant values between them (p=0.941 and p=0.732 respectively). Among all tested samples neither was resistant to Mupirocin (0%) except the non-exposed students group where 15,4% of resistance was recorded. The study of this sample group showed high level of resistance among all tested antibiotics with a 100% resistance to Penicillin and Ampicillin. Surprising results were confirmed among veterinarian samples where we expected high resistance as they were in daily contact with farm animals and the possibility of transmission of resistant strains was much higher. The results revealed total resistance to four of thirty antibiotics as follows: Ampicillin, Cefoperazone, Apramycin and Nalidixic acid. Isolates from farmer group of samples revealed a total of 100% resistance to Carbenicillin, Amoxicillin, Gentamicin, Tetracycline, Tobramycin, Penicillin, Ampicillin, Cefoperazone, Apramycin, Netilmicin, Nalidixic acid and Oxacillin, while no resistance to Fosfomycin, Chloramphenicol, Clindamycin, Ciprofloxacin and Mupirocin was detected. Resistance to Streptomycin was also common among farmers. The strains of S. aureus presented resistance to Streptomycin with statistically significant differences (p=0.033) between the university + faculty group than in total of farms. Samples from exposed professors showed a slightest level of resistance among all tested antibiotics. S. aureus samples from this group didn't show any resistance to Fosfomycin, Kanamycin, Cephalothin, Cloxacillin, Amikacin, Trimethoprim-Sulfamethoxazole, Streptomycin, Chloramphenicol, Cefoxitin, Cefopreazone, Spectinomycin, Netilmicin, Paromomycin, Mupirocin, Vancomycin, Erythromycin and Ciprofloxacin.

IV. DISCUSSION

As previously indicated, it is extremely important to know all the aspects related to the transmissibility of S. aureus, the role of healthy carriers and the antibiotic resistance characteristics of isolated strains. It is necessary to remember the role of the general community and not only infected patients in the spread of S. aureus strains. The complete control of MRSA presence in the community is impossible. Anyway, monitoring the presence of MRSA in determined populations may be the first step in the struggle against the high prevalence. Among healthy carriers, as explained in the Introduction chapter, nasal carriers play a very important role in the epidemiological chain of infections and food poisoning. This work was based on the aim of detecting the number of asymptomatic carriers of MRSA strains in a small population of the students of the Université des Frères Mentouri (Constantine) and Institut des Sciences Vétérinaires El Khroub (Algeria). Given the diversity of analyzed samples from these groups, our expectation was to obtain results that would point to the prevalence and

phenotypic characteristics of particular strains. Since the antibiotic resistance testing was the primary goal of our study we mostly focused on reviewing and comparing resistances levels of tested antibiotics knowing that among analyzed samples some of them belonged to university students who would represent a population of healthy and young people, therefore with a good level of resistance to infections.

The problem of antibiotic resistance has been proven in our work through testing of samples up to 30 commercial antibiotics among which were antibiotics of first line of therapy for Staphylococcal infections. The results of analyzed samples clearly pointed to the problem of increasing resistance to antibiotics among the general population.

The results of comparative analysis of two different Staphylococcus strains pointed to uncontrollable antibiotic use which as a result has a growing appearance of resistant strains, no matter which microorganism is involved. Inadequate application of antibiotics, as shown in this work, surprisingly had the greatest influence among the population from one of the tested groups that actually did not have any risk factor for contact and infection (contact with farms and animals).

The above indicates that the general part of the population is constantly exposed to the risk of contact with resistant strains, or in the worst case of getting the infection. Frequent and uncritical prescribing of systemic antibiotics is a prerequisite for the emergence of increasingly antibiotic resistance, so the appearance and multiplication of multi-resistant strains is a major challenge in the control of infection.

The results in this work are worrisome primarily because of multi-resistance, as well as the fact that S. aureus is highly resistant to most available antibiotics which are often used in the choice of empirical therapy.

Statistically, MRSA was detected in 6 (14,6%) of our samples which in percentages almost coincides with the results obtained in a study made at the Faculty of Veterinary Medicine in Belgrade using the disc diffusion method, where resistance to Cefoxitin was detected in 17 (11,64%) isolates (146 out of 200 swabs) (11).

Our result of MRSA carriers was detected in farmers and non-exposed students. Although S. aureus was isolated in all group samples, the recorded rate of strains that supposedly are MRSA seems to be higher compared to those reported among Malagasy veterinary students (Madagascar) (12). Results obtained in their work showed a 9,04% prevalence of MRSA colonization among 30 S. aureus isolates (30/155).

The investigation study carried out by researchers in Serbia (13) represented a low prevalence of MRSA strains: 0.37% in 533 samples from second, third and fourth year students of the School of Medicine in Belgrade. However, our investigation was performed on fewer people but the prevalence of MRSA was 14,6 %. Due to direct contacts with hospital patients, the students of the School of Medicine in Serbia were at significantly higher risk of colonization by MRSA strains. On the other hand, students from our sample group, in particular non-exposed students, had no risky contacts, thus it remains unclear what is the reason for the higher prevalence of MRSA strains in this population compared to medical students.

The results of our study were quite clear for farmers and exposed students; they harbored much more resistant strains of S. aureus than people who do not have contact with farm and animals. This contact is a risk factor not only for MRSA carriage but also for carriage of S. aureus strains sensitive to Methicillin, but resistant to other significant antibiotics. Besides, our results showed that a total of 31,7% (13/48) of farm animals carried S. aureus. Colonized lambs represented 19,5% and bovines an 12,2% of total isolates. According to the location of these samples, farms BOU, KD and BENL presented the origin of major percentage of isolates.

S. xylosus also reflected a different capacity to antibiotic resistance. Isolated strains were, among others, also tested to Methicillin and Penicillin and showed that lamb samples expressed more resistance to both antibiotics (60%, 66,7%).

The biggest confusion in this issue is the question who is the primary carrier of S. aureus, animals or humans? Do MRSA strains found in animal farms come from humans (farm workers, owners etc) or are transferred to farm workers by animals? Therefore, all aspects of the presence of S. aureus strains in animals have not yet been clarified. Especially difficult is the organization and implementation of epidemiological research on farms where pigs, bovines and poultry are kept together. MRSA isolates in some of the listed animals only adds to the lack of clear answers to this issue: what is the primary animal carrier: cattle, pigs or chickens, whether strains of MRSA can be delivered from cows to cows and cattle or vice versa.

The "golden standard" reference method for determining Methicillin resistance is the presence of mecA genes. Nonetheless, most routine microbiological laboratories use phenotypic methods; disk diffusion and Oxacillin

probing plate, also there are commercial and automated detection systems for antibiotic resistance. There are a number of factors influencing the outcome of phenotypic methods such as: type of antibiotics, temperature, incubation time and NaCl concentration. Methicillin, which is no longer commercially available, has been replaced by Oxacillin and more recently by Cefoxitin as a representative of β-lactam antibiotics. Cefoxitin used in the disc diffusion method for detecting Methicillin resistance showed a certain advantage in a series of studies relative to Oxacillin. Cefoxitin is a better inducer of the mecA gene and Cefoxitin used in disk diffusion tests gives sharper end points and is easier to read than those with Oxacillin. Staphylococcus aureus strains become resistant to Penicillin by producing enzymes (penicillinase) that destroys the antibiotic. This is a form of β-lactamase which breaks down the β-lactam ring of the penicillin molecule. The first line therapy is penicillinase-resistant penicillins like Oxacillin or Flucloxacillin. Oxacillin was tested in this study and S. aureus revelead resistance of 70,7% in tested samples.

S. aureus resistant to Cefoxitin was found in 6 samples; farmers and non-exposed students groups. In comparison with above mentioned study of MRSA isolates from exposed veterinary students (9) in Malagasy (Madagascar) (12), we obtained similar rates of resistance but for non-exposed students , particularly for Trimethoprim-Sulfamethoxazole (71,2 % versus our result of 76,9 %), Erythromycin (64,28 % versus 30,8 %), Tetracycline (78,57 % versus 76,9 %) Surprisingly, compared resistances to Gentamicin (20% versus 69,2%) and Vancomycin (7,14% versus 30,8) are unusual and not similar. This should also be elaborate in order to confirm and clarify the reason for such high levels of resistance and taking in consideration that these above mentioned antibiotics are accessible without any special medical prescription. In available literature we found recommendations for the use and recovery of older antibiotics: Trimethoprim-Sulfamethoxazole, Fusidic acid and Tetracycline in the therapy of community associated MRSA infection (14).

Our results for S. aureus revealed low resistance to Trimethoprim-Sulfamethoxazole (7,3%) while no recorded resistances were found for S. xylosus. Opposite of that, we found a high level of resistance for Tetracycline (S. aureus 68,3%, S. xylosus 42,9%), specifically referring to isolates from farmers whose samples were all resistant to Tetracycline. This was particularly striking in comparison with a study case of 48 S. aureus isolates from pig farmers and veterinarians collected in Western Switzerland (15) where 50% of farmer's isolates were resistant, whereas people without exposure to animals harbored none (0/123).

The re-evaluation of older antibiotic agents may prove valuable for expanding therapeutic options against contemporary pathogens with advanced drug resistance patterns. Fosfomycin, which has been known for approximately 40 years, has shown good antimicrobial activity against a broad spectrum of pathogens, including Gram-positive cocci. It has a unique mechanism of action, inhibiting an early step in cell wall synthesis (16). Our data revealed Fosfomycin to be the most effective drug for S. aureus with a 0% resistance.

As reported in few hospitals, there was a decrease in resistance of MRSA isolates to individual antibiotics. In a couple of French hospitals after a five-year, controlled, reduced prescription of aminoglycosides, there was an increased number of Gentamicin-sensitive MRSA strains ranging from 46,8% to 94,4% though this phenomenon can not be explained by the reduced use of aminoglycoside antibiotics. However, the growth of Gentamicin-sensitive MRSA strains and the reduction of gentamicin-resistant strains provides an opportunity for reintroduction of aminoglycosides in MRSA infection therapy and less reliance on the use of glycopeptide antibiotics (17).

Vancomycin still remains the reference standard in the treatment of infections caused by MRSA. However, disk diffusion is unreliable and cannot distinguish between wild type isolates and those with non-vanA-mediated glycopeptide resistance, according to EUCAST Clinical Breakpoint Tables v. 7.1, valid from 10.03.2017. The current Clinical and Laboratory Standards Institute (CLSI) recommendation is that MIC tests should be performed to determine the susceptibility of Staphylococci to Vancomycin. The disk test does not differentiate Vancomycin-susceptible isolates of S. aureus from Vancomycin-intermediate strains. CLSI lists only susceptible disk diffusion interpretive criteria (in mm) for Vancomycin and Staphylococcus spp. There has not been a sufficient number of non-susceptible isolates to develop resistant and intermediate breakpoints. Organisms for which the Vancomycin zone diameters are ≥15mm are considered susceptible, although several studies show that this breakpoint is unreliable for detecting VISA strains (18). Vancomycin testing was performed as part of a routine procedure in disk diffusion method for this work and S. aureus represents a number of 22% of resistance, while S. xylosus revealed 0% of resistance.

Resistance to Erythromycin and Streptomycin was also common in farmers where 33% of the isolates showed

resistance, and it was similar among isolates from farmers in above mentioned study where recorded resistance was 35% for Erythromycin and 34% for Streptomycin (15).

Clindamycin is frequently used to treat skin infections because of its patient's tolerability and excellent tissue penetration. This antibiotic accumulates in wound abscess and is a good alternative treatment for MRSA and MSSA infections (19). Also, it was successfully used in the treatment of soft tissue and bone infections and pneumonia. Due to the efficacy and the possibility of oral and parenteral administration, it was a good choice for initial empirical therapy. However, concerns about the possible occurrence of clindamycin resistance during therapy have discouraged clinicians to prescribe this drug. Using this antibiotic when Erythromycin resistance has already been shown may lead to induction of cross resistance among macrolide and lincosamide antibiotics. When standard susceptibility testing, erythromycin-resistant MRSA may have an inhibition zone around Clindamycin on the basis of which we could conclude that it is falsely susceptible to this drug (20). Findings in this study revealed 17,1% resistance of S. aureus strains and 42,9% resistance of S. xylosus strains to Clindamycin. Fosfomycin is the only antibiotic in this study that is proven to be effective for treating S. aureus since there was no recorded resistance among the tested samples (0%).

V. CONCLUSION

Among 48 analyzed samples, strains of S. aureus were isolated and confirmed in 41 of them. S. aureus carriers were confirmed among all groups, and the majority of isolated S. aureus strains were multi-resistant to more than three classes of antibiotics. Our data revealed Fosfomycin to be the most effective drug since S. aureus strains were 100% sensitive to this antibiotic. MRSA strains were detected in 6 (14,6%) of our samples and was common among samples from farmers and non-exposed students. Although we couldn't establish a molecular characterization (mecA) of isolated MRSA strains to confirm MRSA identification, we establish baseline information of nasal carriage of MRSA in exposed and non-exposed personnel. However, our samples are represented by a restricted population groups and selected groups have contact with farms and animals. Also, we were not able to distinguish neither locality origin nor their activities and social conditions that may have an influence in the carriage rate.

REFERENCES

[1] Ogsten, A., (1881): Report upon microorganisms in surgical diseases. Br. Med.J.1:369-375.

[2] Dordet-Frisoni E., Dorchies G., De Araujo C., Talon R., Leroy S., (2007): Genomic Diversity in Staphylococcus xylosus. Applied and Environmental Microbiology. 2007;73 (22):7199-7209. doi:10.1128/AEM.01629-07.

[3] Bhunia A.K., (2008): Foodborne Microbial Pathogens: Mechanisms and Pathogenesis, 6:125-129.

[4] FDA (2012): Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed. US Food and Drug Administration, Silver Spring, p.87– 92.

[5] Alalem M.A., (2008): Antibiotic resistant Staphylococcus aureus infection studies in hospitals. PhD Thesis. Graduate School of Natural and Applied Sciences. Middle East Technical University, Ankara, Turkey

[6] Chambers FH., (2001): The changing Epidemiology of Staphylococcus aureus? Emerg. Infect. Dis. 7:178-82.

[7] The EFSA Journal, (2012): European Food Safety Authority; Technical specifications on the harmonised monitoring and reporting of antimicrobial resistance in methicillin-resistant Staphylococcus aureus in food-producing animals and food. 10(10):2897. 56 pp. doi:10.2903/j.efsa.2012.2897.

[8] McCormick J.K., Yarwood J.M., Schlievert P.M., (2001): Toxic shock syndrome and bacterial superantigens: an update. Annu. Rev. Microbiol. 55:77–104.

[9] The EFSA Journal (2008): Foodborne Antimicrobial Resistance, 765, 7-87.

[10] Lado B., and Yousef A., (2002): Alternative food preservation technologies: efficacy and mechanisms. Microbes and Infection 4, 433-440.

[11] Rađenović M., et al. (2016): Investigation of presence of methicillin resistant Staphylococci in students of the Faculty of Veterinary Medicine at The University Of Belgrade, Arhiv veterinarske medicine, Vol. 9, No. 2, 17 – 28.

[12] Rasamiravaka T., (2016) : Evaluation of methicillin resistant Staphylococcus aureus nasal carriage in Malagasy veterinary students, African Journal of Clinical and Experimental Microbiology, 17 (4): 250-255.

[13] Ćirković I., Đukić S., Vuković D., Stevanović G., Švabić-Vlahović M., Stepanović S. (2013): Nazalno kliconoštvo meticilin-rezistentnog Staphylococcus aureus kod studenata Medicinskog fakulteta Univerziteta u Beogradu. Srpski arhiv za celokupno lekarstvo, 141, 5-6, 349-353.

[14] Sabl K., (2006): Community associated methicillin resistant S.aureus: new bug, old drugs. Ann Pharmacother. 40(6): 1125-33.

[15] Oppliger A., et al. (2017): Antimicrobial Resistance of Staphylococcus aureus strains acquired by pig farmers from pigs. Applied and Environmental Microbiology 78.22 (2012): 8010–8014.

[16] Falagas M.E., Kanellopoulou M.D., Karageorgopoulos D.E., et al. (2008): Antimicrobial susceptibility of multidrug-resistant Gram negative bacteria to fosfomycin. Eur. J. Clin. Microbiol. Infect. Dis. 27:439-43.

[17] Leliévre H., Lina G., Jones M., Olive C., Forey F., Roussel-Delvalez M., et al, (1999): Emergence and spread in French hospitals of methicillin-resistant Staphylococcus aureus with increasing susceptibility to gentamicin and other antibiotics. J. Clin. Microbiol. 37(11): 3452-7.

[18] Clinical and Laboratory Standards Institute/NCCLS (2006): Performance Standards for Antimicrobial Susceptibility Testing. Sixteenth informational supplement. M100-S16. Wayne, PA: CLSI.

[19] Zorgani A., Shawerf O.,. Tawil K, El-Turki E., and Ghenghesh K.S., (2009): Inducible Clindamycin resistance among staphylococci isolated from burn patients. Libyan Journal of Medicine. vol. 4, no. 3, pp. 104–106.

[20] Chavez-Bueno S., Bozdogan B,. Katz K., Bowlware K., Cushion N., Cavuoti D., et al. (2005): Inducibile Clindamycin resistance and molecular epidemiologic trends of pediatric CA-MRSA in Dallas, Texas. Antimicrob Agents Cheother. 49(6):2283-8.