Chapter

Distribution and Molecular Detection of Methicilin-Resistant *Staphylococcus aureus*

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Abstract

Isolation of *Staphylococcus aureus* is quite common in both the general population and hospital environment. The heterogeneity of the disease and the unique ability of *S. aureus* to develop resistance to the most recently discovered antibacterial drugs points to its ability to adapt and survive in different conditions. CA-MRSA is different from hospital strains of MRSA by its epidemiological, phenotypic and genotypic characteristics. The emergence of MRSA in the community suggests the need for a new approach to managing the indications and the certification of staphylococcal infections, with special emphasis on the selection of empiric antibiotic therapy. In the study, we analised of MRSA from 4341 samples taken from patients from the general population of Sarajevo Canton in the six-month period of follow-up processed at the Public Health Institute of Sarajevo Canton. We determined the epidemiological characteristics of the isolated strains. Methicillin resistance was determined by phenotypic methods. The following molecular methods were used for the confirmation of methicillin resistance: determination of the mecA gene, PFGE profile, genetic type of MRSA being determined by spa typing, the distribution of SCCmec types being examined, and the detected gene for PVL. The study stresses the need for national monitoring of spreading of the existing epidemic strains, as well as the monitoring of emergence of new strains which would enable the inclusion of our country in the international network of monitoring bacterial resistance.

Keywords: infection, CA-MRSA, phenotypic, antimicrobial susceptibility

1. Introduction

The first isolation of Staphylococci was carried out by Alexander Ogston during the investigation of the septicemia and wound infection bacteria in 1880, the microscopical examination of 88 pus specimens revealed the presence of Gram-positive cocci (*S. aureus*) [1]. In clinical observations, the most important species of Staphylococcus genus are *Staphylococcus aureus* and *Staphylococcus epidermids*, further falling into categories based on their coagulase activity. *S. aureus* is coagulase positive, expressing several virulence factors supporting host immune response

evasion. *S. epidermidis* being coagulase negative, usually less virulent, able to avoid the host immune system by forming and resulting in its hiding in a biofilm [2]. *Staphylococcus aureus* (SA) represents one of the most important microorganisms that are part of the normal micro flora in humans, with ability to cause very serious infections in certain conditions. Approximately 20–30% of the general human population is persistently colonized with SA. Primary and natural reservoir of *S. aureus* is the asymptomatic carriage by humans, using the anterior nasal mucosa as the main ecological niche. The risk of subsequent infection increases as the colonization provides a reservoir open to bacteria introduction as host defences are breached [3]. Adding to humans and domestic animals, livestock and fomites can also serve as joint reservoirs, providing this bacterial pathogen with dramatic relevance in human and veterinary medicine. *Staphylococcus aureus* can cause a wide variety of infections in range from common, mild skin and soft tissue infections to hematogenous infections with multi organ injuries. *Staphylococcus aureus* is infamous for its ability to become resistant to antibiotics, resulting in issues with treatment of these infections due to the resistance development, especially with methicillin-resistant SA [4]. The virulence of *S. aureus* is multifactorial due to the combined actions of a variety of virulence factors that facilitate tissue adhesion, immune evasion, and host cell injury [5]. These virulence factors involve both structural, such as surface adhesins which provide adherence to host tissues, and secreted factors, such as enzymes, that convert host tissue into nutrients. Anyway, a lot more significant is the secretion of a variety of pyrogenic toxins also known as superantigens; the Panton–Valentine leukocidin (PVL) and toxic shock syndrome toxin-1 (TSST-1) as most remarkable [6].

1.1 Methicillin-resistant *Staphylococcus aureus*

Methicillin-Resistant *Staphylococcus aureus* (MRSA) should be taken into accounted for great concern. It is the cause of bacterium infections in various parts of body. As it develops resistance to usually prescribed antibiotics, its treatments becomes more difficult than with most strains of *Staphylococcus aureus*. MRSA is sometimes called a super bug. Methicillin-resistant *Staphylococcus aureus* (MRSA), or multidrug-resistant *S. aureus*, first described in the United Kingdom in the early 1960s, are *S. aureus* strains which developed resistance through natural selection process to all available penicillins and other β-lactam antimicrobial drugs. Methicillin resistant *Staphylococcus aureus* (MRSA) being one of the most important hospital pathogens, becomes responsible for community infection in patients without previous health-care contact at the end of the last century. Worldwide, it is mainly responsible for a broad spectrum of nosocomial and community associated infections and cause of endemic and epidemic infections in many parts of the world. Methicillin resistance in S.aureus developed through the acquisition of the mecA gene located on mobile genomic island designated staphylococcal chromosome cassette mec (SCCmec) by methicillin-susceptible S.aureus [7]. The mecA gene is primary cause for the synthesis of a novel penicillin-binding protein known as penicillin-binding protein 2a, that decreased binding affinity for penicillin and cephalosporins. It follows that MRSA strains are resistant to all ß-lactam antibiotics. In the beginning, MRSA was susceptible to nonbeta lactam antibiotics. Later, namely, from the late 1979s to this day, new strains of MRSA resistant to multi-non- ß-lactam agents including aminoglycosides, with only vancomycin left as an antibiotic of last resort for treating MRSA infections, appeared. These strains, also described as epidemic MRSA (EMRSA or HA-MRSA), have had the capacity to spread extensively causing serious infections worldwide and mostly among in-hospital patients [8].

1.2 What is community-associated MRSA?

During 1990s, in Western Australia, a new MRSA type appeared and it was causing infections in the community of younger and healthy people without previously reported history of hospital admission or medical treatment [9]. These types of MRSA strains were described as community-acquired, community-originated, community-associated, or community-onset MRSA (CA-MRSA). HA-MRSA and CA-MRSA belong to different genetic lineages. While CA-MRSA strains are usually sensitive to antibiotics other than beta-lactams and contain staphylococcal cassette chromosome SCCmec type IV, V or VII, HAMRSA are generally multidrug-resistant and harbour larger SCCmec type I, II or III [10]. MRSA pathogenicity is related to extensive arsenal of virulence factors and toxins. The most common and probably important is the Panton-Valentine leukocidin (PVL) toxin being lethal to neutrophils and associated with skin and soft tissue infections as well as severe necrotizing pneumonia. Huge number of CA-MRSA clones have developed on every continent [11]. Notably, these CA-MRSA strains, initially, were associated with community-onset (CO) infections, have been entering hospitals and may be replacing the conventional HA-MRSA strains with significant clinical and public health implications [12]. However, CA-MRSA penetration is still mostly undefined due to lack of thorough exploration of in large number of hospitals as well as knowledge of the risk factors involved in nosocomial transmission of CA-MRSA compared with HA-MRSA [13]. The prevalence of in vitro resistance to non-β-lactam antimicrobial agents could be increasing among MRSA strains related to community transmission. MRSA typing is an essential component of an overall follow up system of describing epidemiological trends and control strategies for infections. Contemporary challenges for MRSA typing are focused on choosing the most appropriate technique in terms of efficiency, reliability, ease of performance and cost included. The phenotypic methods in general are prone easier performance, interpretation, cost efficiency and wide availability, and less discrimination. The genotypic methods are rather expensive and technically demanding, however more discriminatory. Latest technologies that involve sequencing of various genes are emerging as highly applicable with wide throughput typing systems. Still there is no consensus regarding the single best method for typing of MRSA strains [14]. Pulsed-field gel electrophoresis (PFGE) has become the 'gold standard' for genotyping method of MRSA for over a decade, and it has been used widely for local outbreak investigation, long-term surveillance of MRSA infections at regional and national levels and for international comparisons [7]. Recently, in Europe, harmonization efforts to standardize the PFGE typing protocol of MRSA as well as to enable multicentric comparison of PFGE data have been made. The macro restriction analysis of chromosomal DNA using PFGE is a reference method for *Staphylococcus aureus* typing and can be combined with other methods [15].

1.3 Detection and diagnosis of MRSA strains

Identifying the causative organism can be challenging in treatment *Staphylococcus aureus* infection, especially for resistant strains. Traditional culture and susceptibility testing for MRSA lasts between 48 and 72 h, taking a 16- to 24-h incubation and 16 to 24 h more in completing the susceptibility tests. Latest progress in molecular and nonmolecular testing methods greatly reduced the time needed to detect MRSA [16]. These rapid and sensitive screening assays could contribute to infection control and reducing overall costs. With a rapid test,

Bauer et al. [17] observed bacteremia patients diagnosed with MRSA had a shorter length of stay and lower hospital costs, and for patients with MSSA, the switch from empiric to targeted therapy was 1.6 days shorter. Use of rapid molecular diagnostic tests rather than conventional methods is also related to a significantly lower mortality risk for patients with bloodstream infections (odds ratio (OR) [95% CI] 0.66 [0.54–0.80]), including those caused by Gram-positive organisms (OR [95% CI] 0.73 [0.55–0.97]). Combining rapid molecular testing with an antibiotic stewardship program will be able to lower the mortality risk [18]. Individual hospitals in decision making about the tests used should consider the specificity, sensitivity, price, turnaround time, and expertise, necessary for each test [16, 19]. Modification to the traditional culture method is the use of chromogenic agar, producing a colour reaction in the bacterial cultures. These media also contain antibiotics where only resistant bacteria is able to grow. According to this, MRSA can be detected in 20 to 26 h [16]. In clinical practice, the use of chromogenic media has been seen to shorten the time to aimed MRSA treatment by 12 h [20]. Another innovation in MRSA detection is the development of real-time polymerase chain reaction (PCR) tests with the ability to detect genes specific to *S. aureus*. In making difference in MRSA strains from MSSA or methicillin resistant coagulase-negative staphylococci, PCR methods are aimed at a part of DNA where the MRSA-specific SCCmec gene meets the *S. aureus* orfX gene. The PCR tests are usually performed directly on samples taken from blood or a nasal or wound swab, and results can be available within 1 to 3 h [16]. In clinical practice, generally, the turnaround times from taking samples to complete results are generally longer due to the length of time needed to transport samples, conduct the test, and send the results. As a rule, the overall time is usually much shorter with PCR-based assays than with chromogenic media culture [21]. Moreover, PCR tests showed pooled estimates for sensitivity and specificity of 92.5 and 97.0%, respectively, in the meta-analysis described earlier. In addition, the sensitivity of PCR has been notably higher than the one on chromogenic media, and the specificity was significantly higher than the one on traditional culture [19]. In relation to MRSA detection by chromogenic agar, PCR shortened the overall time of patient isolation as well as number of days patients were inadequately isolated during their hospital stay [21].

2. Aims

- To determine the prevalence and distribution of methicillin-resistant *Staphylococcus aureus* from different patient samples in general population of Canton Sarajevo, Bosnia and Herzegovina
- To determine epidemiological characteristics of isolated strains
- To evaluate isolated MRSA with phenotypic methods: disc diffusion test (DD), E-test, latex agglutination test with antibodies to penicillin-binding protein 2a (PBP2a), and a selective chromogenic medium
- To determine the presence of mecA gene in isolated outpatient strains
- To determine the genetic profile of MRSA strains by DNA Fingerprints by Pulsed-field Gel Electrophoresis (PFGE) and spa-typing test methods
- To evaluate the distribution of SCCmec types

- To determine the prevalence of Panton-Valentine leukocidin (PVL) -positive *Staphylococcus aureus* strains

- To estimate infection and colonization risk of outpatient MRSA strains

3. Materials and methods

Samples taken from January to August 2015 were collected from 4,341 patient samples admitted to the Laboratory of Institute of Public Health of Sarajevo Canton, Bosnia and Herzegovina (B&H) and they showed a total number of 653 methicillin-resistant *Staphylococcus aureus*, that is, out of 2279 found *Staphylococcus aureus* strains, 653 were methicillin-resistant *Staphylococcus aureus.* Those samples included nose swabs, throat, ear, eye, umbilicus swabs, wound and skin swabs. All MRSA isolates collected were identified by standard microbiological methods based on the demonstration of deoxyribonuclease, bound coagulase (rabbit plasma, bioMerieux, France) and free coagulase (Slidex Staph Plus, bioMerieux, France) [22]. Antibiotic susceptibility determination used the agar disk diffusion method in compliance with the guidelines of the Clinical Laboratory Standards Institute [23, 24]. The following twelve antibiotics were tested to the susceptibility of the *S. aureus* isolates: sulfamethoxazole-trimethoprim, oxacillin, cefoxitin, erythromycin, clindamycin, linezolid, ciprofloxacin, tetracycline, fusidic acid, rifampicin, vancomycin and gentamicin. Multiplex PCR was used for testing those MRSA strains for the presence of the mecA gene. Molecular analysis of the SCCmec cassette was conducted using a method earlier depicted by Oliveira et al. [25], with certain modification indicated by Budimir et al. [26]. Presence of the gene for PVL was found by use of PCR primers previously accounted for by Lina et al. [27]. In addition, all of these isolates passed analysis for epidemiological relatedness by pulsed-field gel electrophoresis (PFGE). Macrorestriction of chromosomal DNA used the restriction enzyme SmaI analysis, complying with previously described procedure. DNA fragments were separated using a CHEF-DR III electrophoresis system (Bio-Rad laboratories, Hercules, California, USA) at 6.0 V/cm for 2O h, with pulse times ramped from 5 s to 40 s. Gels used with ethidium bromide staining and they were photographed under UV illumination. PFGE patterns analysis was done by using GelCompare software (Applied Maths, Ghent, Belgium) according to the Tenover et al. scheme [28]. Isolates with indistinguishable band patterns were assigned to the same PFGE pattern type. Isolates that differed by ≤ three fragments were considered to be subtypes closely related i.e. of a given clonal group. The dendrogram was produced by use of 0, 5% optimization, a 3% band tolerance and the unweighted pair group method with arithmetic averages based on Dice coefficients.

4. Results

Out of the total number of isolates, the largest number of isolates included in our research was isolated from the nasal or nasopharyngeal swab (41%), while 35% isolates were found in a skin swab samples. There was 1% isolates isolated from the umbilical swab and sputum sample, and 2% from an abscess puncture sample and conjuctival swab.

Based on the analysis of data from the epidemiological questionnaire, we estimated the distribution of examinees by gender. Out of a total of 100 subjects

included in the study, 49 (49%) were male and 51 (51%) female and there was no statistically significant difference in the percentage of the particular sex. The average age of the respondents was 10.9 ± 18.7 years.

Prior to the start of the study, 59% of subjects had hospital treatment according to the anamnestic data, but there was no statistically significant difference in the groups with and without hospital treatment in the year before the start of this study. Out of the 59 examinees who were hospitalized, 86.4% were hospitalized in the maternity hospital, while a significantly smaller number was hospitalized in orthopedics (5.1%), pediatrics (3.4%), dermatology, gynecology and surgery (1.7%).

Antibiotic usage within 12 months before the follow-up period was recorded in 32% of examinees, while 68% of them did not recieve therapy in that period, which is statistically significant. Out of the group of examinees who were taking antibiotics, 34.4% recieved amoxicillin and clavulanic acid, 18.8% recieved fourth-generation cephalosporins and sulfethoxazole-trimethoprim, while only 6.3% recieved macrolides.

MRSA was present in only 3% examinees out of those who had previosly documented MRSA strains (one year before this study). 6.0% of examinees included in the study had surgical procedure at the same time, while 94% of examinees didn't have surgery.

Epidemiological data are a significant variable for differentiating CA and HA MRSA. Therefore, variables that may have influenced the transmission and development of staphylococcal infections have been included in the epidemiological questionnaire. None of the subjects had been on dialysis therapy, nor had they been placed in a nursing home for 12 months prior to our study. 10% of examinees stated that they own a pet (80% own a dog as a pet and 20% a cat as a pet).

Out of total number of examinees, 8.0% of examinees had previous contact with a MRSA infected patients, 13% were involved in sports and 2.0% of examinees used an invasive /orthopedic device.

4.1 Microbiological analysis of the strains

During the follow-up period, out of a total of 4341 examined patient samples, 2279 *Staphylococcus aureus* were isolated, out of which 653 were methicillin-resistant SA. The prevalence of *Staphylococcus aureus* was 52.5% and the prevalence of MRSA was 28.7%.

We selected a representative sample of 100 strains, eliminating the "copy" strains (strains from the same patient at different places and those that are repeated), by random selection, trying to monitor the dynamics and even representation throughout the months of the study period).

4.2 Results of antibiotic susceptibility testing

In our study, 100 strains were tested for susceptibility to 12 antibiotics. Antibiotics that were tested include antibiotics that are therapeutically important for staphylococci, as well as other antibiotics that are known to be resistant to them as a marker for a virulent clone, such as fusidic acid.

All MRSA isolates were resistant to the β-lactam antibiotics tested, i.e. penicillin, oxacillin, and cefoxitin, 68% of MRSA strains were resistant to erythromycin, 5% to clindamycin, 5% to gentamicin and 4% to ciprofloxacin. All isolates were susceptible to sulphamethoxazole-trimethoprim, rifampicin, fusidic acid, linezolid and vancomycin (**Table 1**).

Based on the antibiogram, all examined strains were divided into 5 profile groups. Group A was represented in 32%, group B in 62%, group C 4%, group D 1% and 1% group E. (**Table 2**).

	Resistant	Sensitive
Penicillin	100 (100%)	0
Oxacillin	100 (100%)	0
Cefoxitin	100 (100%)	0
Erythromycin	68 (68%)	32 (32%)
Clindamycin	5 (5%)	95 (95%)
Sulphamethoxazole- trimethoprim	0	100 (100%)
Rifampicin	0	100 (100%)
Fusidic acid	0	100 (100%)
Gentamicin	5 (5%)	95 (95%)
Ciprofloxacin	4 (4%)	96 (96%)
Linezolid	0	100 (100%)
Vankomycin	0	100 (100%)

Table 1.
Antimicrobial susceptibility patterns (N = 100).

Groups	Profile groups
A	resistant to penicillin, oxacillin and cefoxitin; sensitive to erythromycin, clindamycin, sulphamethoxazole-trimethoprim, rifampicin, fusidic acid, garamycin, ciprofloxacin, linezolid and vancomycin
B	resistant to penicillin, oxacillin, cefoxitin and erythromycin; sensitive to clindamycin, sulphamethoxazole-trimethoprim, rifampicin, fusidic acid, garamycin, ciprofloxacin, linezolid and vancomycin
C	resistant to penicillin, oxacillin, cefoxitin, erythromycin, clindamycin, garamycin, ciprofloxacin; sensitive to sulphamethoxazole-trimethoprim, rifampicin, fusidic acid, linezolid and vancomycin
D	resistant to penicillin, oxacillin, cefoxitin, erythromycin, clindamycin; sensitive to sulphamethoxazole-trimethoprim, rifampicin, fusidic acid, garamycin, ciprofloxacin, linezolid and vancomycin
E	resistant to penicillin, oxacillin, cefoxitin, and garamycin; sensitive to erythromycin, clindamycin, sulphamethoxazole-trimethoprim, rifampicin, fusidic acid,ciprofoxacin, linezolid and vancomycin

Table 2.
Profile groups based on the antibiogram.

4.3 Results of phenotypic methods in MRSA detection

In our study, conventional phenotypic methods were used to detect MRSA isolates: disk diffusion oxacilin test and disk diffusion cefoxitin test. To confirm the MRSA isolates, a latex agglutination test with antibodies to PBP2a, a selective chromogenic medium, ChromID MRSA, and an E test were used to determine the value of the minimum inhibitory concentration for oxacillin.

Oxacillin disk diffusion and cefoxitin disk diffusion tests, due to their accuracy and economic acceptability, have proven to be good options for the detection methicillin resistance of S.aureus. The results of this study indicate a previously proven fact that the latex agglutination test, ChromID MRSA, and E test, due to their high sensitivity and specificity, play a significant role as confirmatory tests for the detection of methicillin resistance.

All phenotypic methods that were used had 100% sensitivity as well as specificity, except for the DD cefoxitin and DD oxacillin tests, 98.9 and 96.8.

4.4 Molecular analysis of tested strains

4.4.1 MecA gene detection

Reduced sensitivity or resistance to oxacillin or cefoxitin was found in all isolates, including the study by phenotypic methods. After detection of MRSA isolates by phenotypic methods, all isolates were subjected to molecular methods that tested the presence of the mecA gene.

All tested isolates were positive for the mecA gene. The control mecA positive strains used in our study are MRSA isolates described in the Methods section. One of the isolates used is COL and the other is WIS.

COL is an MRSA strain isolated for the first time in 1965 in Great Britain, known as a representative of the Archaic clone, whose characteristics are: SCCmec type I, ccr AB 1, sequential type (ST) 4.

WIS is an MRSA isolate native to Australia, SCCmec type V, possesses ccrC.

4.4.2 Spa-typing test results

As previously described in the Introduction to Spa Typing, the polymorphic region X consists of a variable number of 24 base pairs of repeating fragments. From a total of 100 strains examined, we selected a representative sample of 29 strains that underwent spa-typing. Seven different spa types were discovered: t008, t919, t041, t1179, t2187, t2674 and t10807. The most common type was t008 (55.2%).

4.4.3 SCCmec typing

We analysed and subjected 100 isolates to SCCmec typing. The obtained amplification products differ in molecular weight, and the typing result is obtained by visual comparison with the control strains. SCCmec loci of control strains were also amplified in each reaction cycle. A marker was applied to each individual gel, with a range of DNA fragments of 100-1500pb. The PCR products of the control strains were electrophoretically parallel separated. Each amplification cycle in which no corresponding PCR product was obtained in the control strains was repeated, as well as PCR reactions and electrophoresis of isolates that could not be typed with this method.

The distribution of SCCmec types is shown in **Figure 1**. The most prevalent SCCmec element was type IV (86%), followed by SCCmec I (4%) and SCCmec type

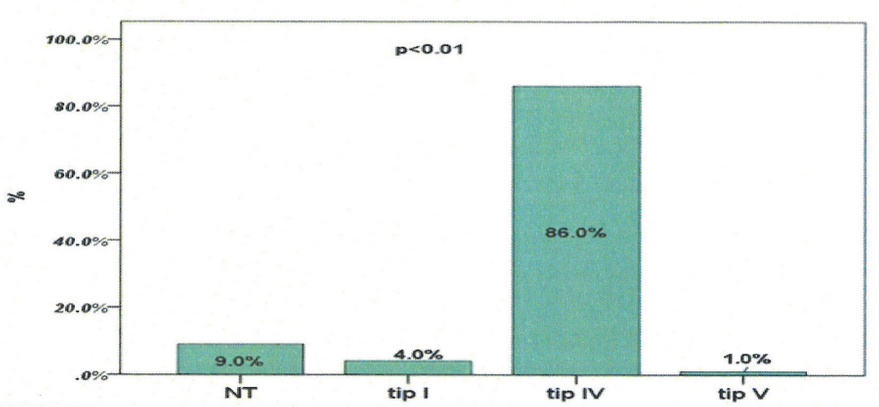

Figure 1.
SCCmec types distribution among MRSA isolates. Note: NT- non-typeable.

V (1%). Non-typical strains were found in 9.0% of cases. No isolates with SCCmec III were found during this analysis. Chi-square test was used for the analysis of categorical variables.

4.4.4 Determination of the presence of the PVL gene

Using the primers and amplification cycle conditions described in the Method section, 100 isolates were tested for the presence of the PVL toxin gene. MW2 strain, MRSA strain isolated in 1998 in the USA, SCCmec type IV, ST131 was used as a positive control strain.

Positive expression of PVL gene was found in 24.0% of isolates, while 76% showed a statistically significant higher negative expression of PVL gene.

The current prevalence of CA MRSA PVL positive isolates was 0.05% and the periodic prevalence was 0.037%.

Common to all positive PVL MRSA isolates is SCCmec type IV and the fact that they are outpatient isolates.

4.4.5 Results of PFGE analysis

Results PFGE - electrophoretic profiles of isolates.

Pulsed field electrophoresis after the splitting of chromosomal DNA by the restriction enzyme SmaI yielded clearly separated DNA fragments that were visualized by UV light illumination after staining with ethidium bromide and photographed with a Polaroid camera.

Figure 2 shows a photograph of electrophoretic gels that are part of the results obtained by this analysis. The number of fragments greater than 10 for each isolate is visible, the minimum of the bands to which the comparison standards described in the Methods section can be applied. **Figure 2** is a representation of a PFGE electrophoretic gel after cleavage with SmaI enzyme and separation in an electric field and staining with ethidium bromide.

M means a lambda marker, and genotyped strains are numbered 1–20. Blank fields 7 and 12 mean that the isolation probably failed in the first attempt and we repeated these isolates.

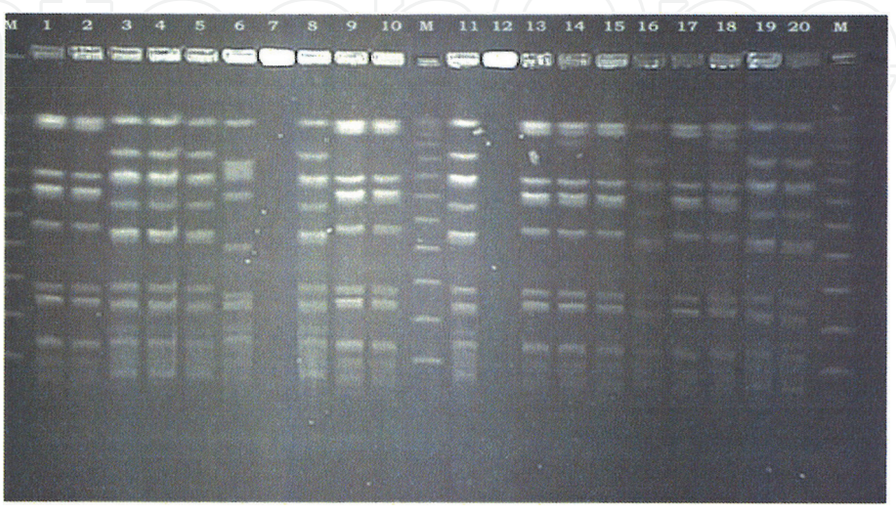

Figure 2.
Representative PFGE patterns of MRSA strains isolated from population included in the study, after splitting enzyme SmaI and separation in an electric field and staining with ethidium bromide.

Determination of PFGE groups using dendrogram percentage similarity:

The photos were scanned and stored in the database of the computer software system GelCompar. Each individual strip was subjected to a normalization procedure, in which, for each individual photograph, the size of the fragments was compared with the same molecular standard found on each gel in the electrophoretic reaction.

After normalization and entering basic data into the database, a dendrogram of similarity percentage was made, using Dice coefficient from PFGE data, using a limit value of ≥80%, with a tape tolerance of 3% and an optimization parameter of 0.5%.

In the PFGE profile analysis of the isolates with the computer system GelCompar, the isolates fell into five similarity groups: A-E. The largest number of isolates (87%) belonged to one of two groups: C (60%) and D (27%).

Group A includes 3 isolates, described as SCCmec type IV. There were no PVL positive isolates in this group.

Group B included four isolates, with three isolates being SCCmec type I, while one was SCCmec type IV. There were no PVL positive isolates in this group.

Group C represents the largest group in the PFGE analysis, including 60 isolates, and all isolates of this group belonging to SCCmec type IV. In this group, 28.33% (17) of isolates were PVL positive.

Group D includes 27 isolates, three of which were nontypeable, and the rest of the isolates belong to SCCmec type IV. In this group, only two isolates were PVL positive.

Group E included 3 isolates, SCCmec types I, IV and V. These three isolates were not PVL positive.

Three PFGE types were singletons, that is, not similar to any of the other strains, two of which are SCCmec IV, while one is nontypeable.

5. Discussion

Staphylococcus aureus is one of the most important and adaptable human pathogens, causing most often skin and soft tissue infections, although it can cause any organ and organic system infection as well as infections related to toxin production [29]. Colonisation is important step in pathogenesis of infections caused by *S. aureus*. Approximately 20–30% of overall population is persistently colonised by *S. aureus*, with verified colonisation at nasal mucosa [30]. This study microbiologically analyses 4341 different biological specimens from out of hospital respondents. *Staphylococcus aureus* is isolated in 2279 specimens, with prevalence of 52,5%. Out of 2279 identified *Staphylococcus aureus*, methicillin resistance was shown in 653 isolates proved by use of phenotype methods, thus MRSA prevalence was 28,7%. Considering the use of MRSA prevalence as an indicator of success in conducting infection control programme, there are series of data from various geographical areas that point to high variability of MRSA prevalence in the whole world. European research show high variability in MRSA prevalence results. Thus, percentage of MRSA in Scandinavian countries and Netherlands is less than 1%, while prevalence in Spain is more than 50% [31]. At latest, growth of bacterial isolates number resistant to methicillin becomes serious clinical and epidemiological problem. Introducing methicillin into clinical practice in 1961, MRSA soon becomes one of the main intra-hospital problems around the world. Nowadays, MRSA still holds primate in hospital environment, and in the last few years in out of hospital environments as well. Namely, in the nineties of the last century, the problem of out of hospital MRSA (Community-acquired methicillin-resistant *Staphylococcus*

aureus or CA-MRSA) arises, in many features different from hospital MRSA strains. *Staphylococcus aureus* resistant to methicillin is placed highly in modern microbiology and infection control procedures. Recent studies show change in microbiology of MRSA as well as its limitation to hospital environment so the infection can appear in general population [29]. CA-MRSA strains become important health issue connected with high morbidity and mortality in general population [32].

In our study, we selected representative sample of 100 MRSA strains, eliminating "copy" strains (strains of the same patient from different places and those repeated ones), by random choice method, trying to follow the dynamics and equal representation through individual months of research period. In our study, 41% cases confirmed presence of the pathogen in the nasal, and 35% samples isolated *S.aureus* in skin smear. Creech et al. confirmed colonisation of nasal mucosa in paediatric cases with *S.aureus* in 35% cases, and in 9% cases they bore nasal MRSA [33]. Male sex was positive to MRSA in 49%, while females were positive in 51% of analysed MRSA specimens. In Farr et al. study [34] has shown that females were highly represented in verified CA-MRSA infections. The same study shows the greatest number of out of hospital infections in females aged 18–44. In our study average age was 10,9 (±18,7). Those results could be explained by high number of new-borns analysed in our research. Anamnestic data in epidemiology questionnaire about eventual hospitalisation within a year prior to beginning of the study has shown that 59% of respondents had been hospitalised at some clinical department, while the greatest percentage had been hospitalised at maternity department of the clinic (86.4%), adding to the data of high new-borns representation in the research. However, the German study done at in-hospital and out-of-hospital patients showed high prevalence of CA-MRSA in patients with hospitalisation data or prolonged stay at special purpose facilities (nursing homes) [35]. According to the definition of CDC, as well as numerous modifications of the definition, there are epidemiological circumstances among risk factors in appearance of out-of-hospital MRSA infection. Having that in mind, we analysed series of general prior data and the analysis followed the criteria for differentiating in-hospital from out-of-hospital MRSA. The data showed that only 6% of respondents had undergone surgeries in the prior year, while 2% of positive respondents have used some invasive apparatus. Pets had earlier been identified as the source and transmitters of MRSA infections to humans in contact [36].

Analysing data of epidemiological questionnaire it shows that 10% of the respondents keep a pet, 3% have had documented MRSA infection before, 8% have had contact with MRSA carrier, while 13% practiced collective sports. Resistance development becomes issue of priority, and the follow up system for resistance measurement in relevant data, according to which antibiotics would be given by recommendations to lower the resistance, e.g. try to stop or slow down the development of new resistance, has been established.

Data on resistance in the close by environment have to be basis for empirical therapy development, so it can be more successful in every single patient treatment, as well as efficient in spreading resistant types in the community.

For those reasons it is necessary to have local epidemiological data on sensitivity of out-of-hospital strains and specific hospital close at hand, due to variation of bacterial resistance level between hospital centres in different countries, between centres of the same country, as well as between hospital wards and departments of the same centre.

Staphylococcus aureus is a microorganism that developed resistance mechanisms to all antibiotics available for treating infections caused by staphylococci. The most important among those antibiotics that *S. aureus* developed resistance to, are the ones indispensable in therapy schemata for infection caused by staphylococci treatment and resistance mechanisms that mark antibiotics era. Penicillin as the

medicine of first choice for staphylococci infection treatment is almost abandoned, while the remaining number of isolates sensitive to penicillin activity is 20% by some studies [37].

According to the data extracted by isolate testing in disc – diffusion method, resistance to penicillin, methicillin (oxacilin) and cefoxitin showed all MRSA strains. Among isolates of the collection, resistance to vancomycin, linezolid, rifampicin, sulfamectasole – trimethoprim and fusidine acid was not shown. For vancomycin the sensitivity out of inhibition zone less than 14 mm in disc – diffusion method according to CLSI standards was not confirmed [38].

Resistance to macrolides is 68%, while to clindamycin it is significantly less and in amount of only 5%.

Resistance to gentamicin is 5% while to ciproflaxcin is 4%.

It is well-known that great percentage of hospital MRSA strains is resistant to quinolone, in total 90%. Similar results are gathered in in the research in the area of Republic of Croatia (91%), where the progressive test to quinolone has been followed in the last 20 years [39].

In the world resistance phenomenon among *S.aureus* strains context, exact and early assessment of resistance is of key importance in infection caused by *S.aureus* prognosis.

Although many phenotype methods have been developed to achieve fast methicillin resistance detection, lack of these methods is lessened sensitivity, which in the end cannot ensure appropriate and timely treatment of all patients infected by MRSA.

Several studies have shown that revelation of *mec*A gene is the "golden standard" method for diagnosing MRSA in microbiological laboratories [40].

However, not all laboratories, especially in transition countries such as ours is, have the necessary equipment and educated stuff for establishing molecular techniques. Thus, the need for fast, exact and economically profitable identifying MRSA strains by phenotype methods appeared [41].

As for disc diffusion tests for MRSA detection, sensitivity of oxacilin disc diffusion test and cefoxitin disc diffusion test was 100%, while specificity was 96% and 98%.

Rao Venkatakrishna et al. also found high sensitivity and specificity of oxacilin DD and DD Cefoxitin test in MRSA detection [42].

Chromogenic surface use (ChromID) has shown 100% specificity and sensitivity. Morris et al. in their study compared chromogenic surfaces and showed sensitivity for ChromID 93% [43].

In some authors' studies [44] E-test in MRSA detection has been used as the golden standard and its advantages in the sense of conducting, as well as precision that approaches those of molecular methods PCR, *mec*A detection, have been proven. In our study we have also shown 100% sensitivity and specificity for E-test. Also, for latex agglutination test we have confirmed identical results. Ahmad has proven superiority of the test as the alternative method MRSA detection in his study [45].

After conducting phenotype methods, we have examined MRSA strains by molecular typing methods, and by analysis of data gathered in that way, we have come to interesting clinical and epidemiological findings.

With all isolates included in the research after detecting methicillin resistance by phenotype methods, they were subjected to molecular methods by which the presence *mec*Agene was tested. All examined isolates were positive to *mec*Agene, according to the claim that for *mec*Agene identification, polymerase chain reaction (PCR) is considered the golden standard [40].

By method *spa*-typing, developed by Freney et al. [46], based on sequencing polymorph region X gene protein A *Staphylococcus aureus* (*spa*), we typed 29 out of our 100 types. We had seven various *spa*-types, from which the most represented t008 16/29 (52,2%), t1179 5/29 (17,2%), t10807 3/29 (10,3), t2674 2/29 (69%), t041 1/29 (3,4%), t919 1/29 (3,4%), t2187 1/29 (3,4%). If we compare it with relative global frequency of appearance for single *spa*-types by Ridom Spa Basa, we can notice that t008 is globally represented in 6,32%. Spa t008 is present in the countries such as Australia, Austria, Bulgaria, Canada, Croatia, Check Republic, Denmark, Estonia, Finland, Germany, Hungary, Israel, Norway, Poland, Portugal, Spain, Great Britain, Switzerland and Sweden. The *spa* type is found in sequence types ST-8, ST-247, ST-250 and ST-254, and placed in CC8, Northern German MRSA, USA300 ORSA IV and Archaic/Iberian clonal complex.

In this study t041 was represented 3,4%, while globally it is less represented with only 0,31% (Austria, Belgium, B&H, Croatia, Check Republic, Denmark, France, Germany, Hungary, Island, Ireland, Italy, Netherlands, Norway, Slovenia, Sweden and Switzerland), in sequence types ST-111 i ST-228, placed in CC5 and Southern German MRSA clonal complexes.

Representation of t919 in our study is the same as t041 (3,4%), and it appears in Austria, Germany, Norway and Sweden, with globally significantly less representation of 0,01%.

In our study we had four, so far unknown, *spa* types, and it can be explained by polymorph region X consisting of 24 base pairs of repeating fragments.

In our analysis the SCC*mec* results of typification have shown dominance of SCC*mec* type IV (86%), typical for out-of-hospital population. It is necessary to emphasize there is appearance trend for SCC*mec* type IV inside hospital population, with the tendency of shifting SCC*mec* type I and dominant Iberian clone so far characteristic for in-hospital environment. SCC*mec* typing of strains included in our research we have proven that da SCC*mec* type I is seen in total of 4% strains, while SCC*mec* type V is seen in very small percentage (1%) in the examined strains. Valsesia et al. [47] have recently confirmed that SCC*mec* type IV and V was registered in examined population in the amount of 87% cases, while SCC*mec* type I and II appeared only sporadically, and SCC*mec* type III was completely absent in out-of-hospital population. In our analysis we have not confirmed any case of SCC*mec* type II or III. Those results are in accordance with our results, except that SCC typing of strains included in the study has not shown presence of SCC*mec* type V types.

Previous studies have indicated that HA-MRSA infections are mainly caused by multiresistant strains carrying SCC*mec* type I, II or III, but rarely SCC mec type IV. On the other hand, CA-MRSA strains carrying SCC*mec* type IV, V or VII, are usually sensitive to majority of un-β-lactam antibiotics, although series of studies indicate spreading CA-MRSA strains in hospital centres and consequently taking place of traditional HA-MRSA strains [48, 49].

Our study indicates possible existence of new combinations of SCC*mec* fragments and ccr genes evolving by recombining already described segments, and by completely new SCC*mec* types, due to genome *S.aureus* susceptibility to dynamic changes, changes of genetic parts inside the species as well as species typical for *S.aureus.* This conclusion is based on presence of 9% atypical strains.

In our collection of strains subjected to molecular analysis, all MRSA isolates have been tested to presence Panton-Velentine leukocidin (PVL) toxin. Number of PVL-positive MRSA was 23 isolates, while one isolate was atypical.

Some studies in which virulence factors with PVL of in-hospital and out-of-hospital MRSA were examined at the same time and it is discovered that less than 5% MRSA isolates are SCCmec I, II and III PVL positive, while 40–90% MRSA

SCCmec type IV contains PVL gene. [50]. CA-MRSA can represent serious problem for public health due to distribution of strains with potential to produce PVL toxin. Presence of genes coding Panton-Valentine leukocidin is important marker of virulence as well as determinant of clinical consequences of infections caused by PVL positive types which are far heavier than PVL negative *S. aureus* infections. Cocchi et al. [51] in their study showed the transmission of PVL -positive CA-MRSA, member of Southwest Pacific clone (SWP) with epidemic capacity. The same study shows transmission from father with recurrent skin and subcutaneous tissue infections, over mother with nasal colonisation, to their child with symptoms of necrotising pneumonia. These data indicated that recurrent skin infections, usually not given great clinical importance, can represent serious threat in development of severe clinical picture of the carrier with possibility of further transmission PVL positive causer.

However, the role of positive MRSA strains in predicting possible severe clinical manifestations for the carrier still remains undefined.

CA-MRSA is linked to the production of Panton-Valentin leukocidin. Probably the PVL is direct virulence factor in staphylococci necrotising pneumonia [52], while its role in skin and soft tissues infection remains controversy.

There is epidemiological connection between MRSA and PVL, especially in the USA where USA300 clone dominates which is PVL positive. But counterargument to the attitude, at the same time confirming still undefined role of PVL as virulence marker in prediction of clinical infection manifestations, is the fact that there are several types PVL negative with the same clinical outcome that this toxin cannot be taken as universal marker of CA-MRSA. In the conducted analysis on 100 MRSA isolates, it is confirmed that 23 isolates SCCmec type IV containing gene z PVL, one atypical isolate while there were 76 PVL negative isolates of various SCCmec types, but with dominance of type IV. By examining isolates origin we established that 11 PVL positive PVL isolates were from skin area, 8 from nasal mucosa, 2 from pustule smear, while others were represented by 1 PVL positive isolates from different corporal regions (sound conductor, wound smear).

In Great Britain, according to the National Reference Laboratory data, genes coding PVL are present in less than 2% of clinical isolates *S. aureusa*, whether MSSA or MRSA [52]. While PVL is currently accepted as important factor for *S. aureus* virulence, the latest research give preference to alternative virulence factors such as arginine catabolic mobile element (ACME), α toxin, regulatory genes coding expression, and newly described peptides.

PVL role is still very important, primarily due to fact it represents important marker in screening virulent *S.aureus* strains.

PFGE is genetic typing method used as means of molecular – epidemiological study of genetic variants of *S. aureus* and other numerous bacterial pathogens. For its highly discriminating capacity PFGE is considered golden standard for local epidemics of bacterial infections [53]. Combination of molecular typing methods (PFGE, mecA, SCC*mec* and MLST) with epidemiological and clinical data enable revelation of MRSA groups and their appearance, thus ensuring application of rational, appropriate, infection control measures [54]. Also, this study has enabled detection of MRSA groups in limited geographical area by applying methods of molecular typing so it can represent basis for similar studies on broad area and enable application of this region into European MRSA infection control network.

Comparing PFGE analysis results, by criteria of Tenover et al.(28), applying cut-off similarities 80%, our study has shown that most of the isolates is classified into two larger groups, indicating clonal connection and genetic similarity of isolates. Dendogram contains 5 groups, marked alphabetically from A to E.

PFGE analysis of profile isolates by computer system GelCompar isolates are grouped in similarity groups, inside which similarity between isolates is 80% and more. Thus we had results in five groups, marked alphabetically from A to E. Most of the isolates fell into two most numerous groups, C and D.

Group A contains 3 isolates, falling into SCC*mec* type IV. This group had no PVL positive isolates.

Group B contains four isolates, with 3 isolates belonging to SCCmec type I, while one belongs SCC*mec* type IV. This group does not have PVL positive isolates.

Group C contains 60 isolates, and represents the most numerous group in our PFGE analysis. All isolates of the group belong to SCC*mec* type IV. This group had 28,33% (17) PVL positive isolates.

Group D has had 27 isolates, three of them atypical and the rest of them also fall into SCC*mec* type IV. In this group only two isolates were PVL positive.

Group E has had 3 isolates, belonging to SCC*mec* type I, IV and V. These three isolates were not PVL positive.

Three PFGE types are single genotypes, with two of them SCC*mec* IV, while one is atypical.

Among mostly used definitions CA-MRSA that take into account epidemiological data, as well as genetic origin of isolates are modified definitions of the CDC. In cases of suspicion to CA-MRSA the first step is to eliminate any connection to hospitals and hospital system, because the connection for such isolates places them into HA-MRSA species [55].

Due to the fact it is necessary to have molecular analysis for the types aiming to avoid classifying MRSA strains into CA or HA MRSA strains based on epidemiological data, for it might lead to possibility of crosswise mistakes.

Concerning the received results indicating presence of isolates SCC*mec* type IV and with respondents of paediatric age with positive epidemiological data of hospital environment contact, traditional division to in-hospital and out-of-hospital MRSA is questionable and demands further revision.

Prevalence of "real" CA MRSA strains in general population broadly varies in different geographical areas. In meta analysis, Salgado et al. [56], showed prevalence in amount of 1,3% for MRSA colonisation in community. However, it must be pointed out that most of the people colonised by MRSA strains, had risk factors connected to hospitalisation.

After excluding these patients prevalence of "real" CA-MRSA colonised was 0,2%, responding to the prevalence of the study (0,13%).

About presence of CA MRSA in Bosnia and Herzegovina there is not enough data. There are several genotyping isolate methods *S.aureus* for epidemiological research. However, Harbarth et al. [57] still give advantage to molecular methods of MRSA identification comparing to standard cultivation methods. Anyway, length of the procedure and the need for specialised laboratories still represents limiting factor for broad use of molecular analysis.

Earlier reports on CA-MRSA strains describes appearance of new strains in patients with the lack of traditional epidemiological risk factors for MRSA infection and/or colonisation. Those patients with CA-MRSA infection are very often of younger age, with minimum comorbidities and negative epidemiological data of hospital environment contact, comparing to patients with infections caused by HA-MRSA strains. Furthermore, different socio-economic characteristics related to CA-MRSA infections, including ethnical background and socio-economic status.

Recently, [58] however, with the growth of CA-MRSA prevalence, epidemiological difference between these and HA-MRSA types became less defined, concerning numerous reports of hospital epidemics caused by CA-MRSA types.

CA-MRSA is more often defined as cause of infections arising at hospital environment and infections connected to hospital environment. On the other hand, hospital clones are described as cause of infections in general population, indicating the fact that some clones are capable of successful barrier crossing between hospitals and general population [59].

In spite of its clinical importance, multicentric research of the entire area of Bosnia and Herzegovina for prevalence and epidemiology of MRSA as the cause of infections in general population, has not been conducted. This imposes the necessity for national monitoring of spreading and presence of these types.

6. Proposal for algorithm of treatment CA-MRSA infection

The first choice for empirical antimicrobial medication depends on MRSA prevalence in the community, type and severity of infection. Vancomycin should be prescribed in cases of severe infections [60], while microbiological data is available, in areas where similar infections of outpatient MRSA strains were documented. Also, it is necessary to prescribe vancomycin in cases when it is known that patient had been colonised by MRSA strain, or is intravenous addict or when MRSA infection risk factors are included. In the last ten years skin and soft tissue infections caused by CA-MRSA have reached epidemic level. The key in their treatment is surgical care, incision and drainage, in avoiding further tissue destruction; with empirical application of antibiotics modified according to microbiological findings. In the areas of low prevalence CA-MRSA and in treatment of mild infections therapy should be started with some penicillin antibiotics resistant to penicillinase or the first generation of cephalosporins [61]. The carrier selection, if undertaken, is important in process of hospitalisation, in the open community it is impossible to be carried out. However, the follow up for discharged patients and control of their laboratory diagnostics with MRSA sensitivity examination, is more important than ever before. Screening can reduce MRSA incidence during hospitalisation admission process. MRSA eradication or decolonisation by topical application of mupirocin or cotrimaxazole are used with various success, and some studies show that sulfamethoxazole-trimethoprim in available oral antibiotics has the fastest bacteria impact [62]. Due to fast resistance development, decolonisation of all known carriers is not recommended [63].

Due to high morbidity and mortality connected to staphylococci isolates coding PVL, the selection of carriers and decolonisation by muciprocin is recommended with people having recurring abscesses despite antimicrobial therapy and their contacts if they have MRSA isolated in nasal vestibule.

PVL toxin and better understanding of toxin role in pathogenesis of CA-MRSA infections can have therapy implications. Some studies have shown that intravenous immunoglobulin use has benefits in positive treatment outcomes in shock therapy [64]. PVL neutralisation as well as other toxins by intravenous immunoglobulin is shown in vitro [65]. Based on the prevalence CA-MRSA of 0,13%, where none of the isolates caused severe infection, we estimate that empirical therapy for outpatient pneumonia treatment does not need any modification by adding antibiotics with impact on MRSA isolate, such as vancomycin.

6.1 Detection algorithm/treatment of outpatient MRSA

Having in mind the fact that in the world and Europe number of outpatient MRSA increases, in diagnostic and therapeutic approach it must be taken into account that it is MRSA strain.

Good sampling and choice for microbiological testing, (smear, wound aspirate in the case of localised infection and blood in the case of system infection), fast detection of MRSA in microbiological laboratory, as well as the result of sensitivity to adequate nonbetalactam antibiotics may contribute to adequate infection treatment and timely measure taking in infection control, aiming at reducing spreading of very adaptable outpatient MRSA strains in ambulances and hospital environment.

Diagnosis CA-MRSA infection should be considered with seriously ill young people who previously had had symptoms similar to influenza, pneumonia disease with hemoptysis symptoms, high febrility, leucopenic and and hypotensive. Those symptoms are signal to life threatening infection, necrotising pneumonia, and septic shock and, in consequence, even death outcome.

Important ways of presenting CA-MRSA infections are skin infection, with developing abscess, furuncle, carbuncle, without drainage infections progress to fasciitis, deep infections of soft tissues, after which, in case of recovery, tissue deformities are left.

Epidemiological definition and connection to hospital stay lose in importance because it is not unusual for typical CA-MRSA to cause epidemics in hospital wards [66].

As the first step in successful treatment the selection of suitable sample for microbiological testing is recommended. In cases of severe skin and subcutaneous tissue infections taking abscess aspirates or deep subcutaneous change will also have therapeutic effect.

In case of suspicion to necrotic pneumonia, blood sample usually contains cause, while sample from respiratory system is desirable.

For cause diagnostics of mild skin change, it is enough to take skin smear. Depending on sat down of infection, for sampling, standard rules of microbiological testing apply.

For treatment outcome it is important to apply suitable antimicrobial medication and in the case of suspicion to MRSA infection the confirmation is of extreme value.

With application of antistaphylococci antibiotics, penicillin or cephalosporin, skin and subcutaneous tissue infection and fascia it is necessary to surgically treat, incise and debride abscess.

By standard processes of isolation and detection of *S.aureus*, after which the testing is undertaken for isolates sensitivity to oxacilin/cephoxitin and the result due is in 48 hours later.

A lot better time frame is given by molecular MRSA detection based on PCR method. The latest PCR methods do not use amplification mecA as proof for MRSA, because there is a possibility of getting falsely positive results by amplification *mec*A gene coagulaze-negative staphylococci contaminating the sample. IDI-MRSA (Infecto Diagnostic, Canada), GenoType MRSA Direct (Hain Lifescience, Germany), detect part of orfX gene, specific For*s. aureus* and adjacent part of SCC*mec* chromosome region. In that way one PCR reaction detects MRSA in unsterile samples. Results are available in 24 hours.

Small number of routine laboratories in Bosnia and Herzegovina have molecular methods available, and they are limited to great and reference centres.

Also, the method by which MRSA diagnostics time can be significantly shorter, is method of latex agglutination by which PBP2a is detected (BioMerieux, France). Method does not require special equipment and stuff training, antigen extraction is simple, results relevant [67]. The method's purpose is not for detection MRSA coding *mec*A gene, which is not prominent, because such test is falsely negative.

Detection of virulent CA-MRSA strains is also important for application of measures for spreading prevention during admission to hospital [68] of the patient

with CA MRSA infection and stay with seriously ill patients to which infection can have a fatal outcome. Some authors suggest selection of patients in intensive care units aiming at improving treatment [69]. During patient treatment with infection CA-MRSA, it is necessary to undertake prevention measures for spreading isolates, and avoid using common objects with other patients. It is considered that relatively great number of epidemic infections CA-MRSA is caused just by transmitting pathogens over objects of common use (soaps, towels...).

Transmission of out-of-hospital MRSA to laboratory employees is registered. Wagenvoort et al. [70] as well as to the doctor who resuscitated child with necrotic pneumonia caused by CA-MRSA [71] strain so it takes utmost care in treating this transmissible, more often described pathogen.

At this moment, MRSA strains as causes of epidemic should be typed in single hospitals or reference laboratories to establish whether they are CA-MRSA or HA-MRSA strains, for larger group of patients and stuff can represent risk that could demand new control strategies for CA-MRSA strains. New strategies can include screening the hospital stuff, enhanced follow up for cases with the infection beginning in hospital and out-of-hospital environment and vice versa. They would, also, include improved prevention and infection control measures in general population, focusing on MRSA in contaminated area [72].

Periodical examination of antimicrobial sensitivity profile of MRSA strains (cause of infection), in combination with representative isolate set typing of specific area, is useful to ensure necessary empirical therapy, having in mind that appearance CA-MRSA in certain parts of the world brought changes in empirical therapy of staphylococci infections.

Reference laboratories should periodically continue representative isolate sets typing to ensure adequate follow up of MRSA trends, as well as appearance of new types.

For complete estimation of their epidemiology, MRSA infections should be characterised as:

1. Caused by HA-MRSA or CA-MRSA strains.

2. Infections acquired in hospital or out of hospital environment.

3. Infections beginning in general population or in health care facilities [73].

7. Conclusions

- Prevalence *Staphylococcus aureus* in Sarajevo Canton is 52.5%, and methicilin resistance of *Staphylococcus aureus* is 28,7%.

- Phenotype methods in MRSA detection have high specificity and sensitivity, available commercially and can provide broad span application in laboratories as routine methods. However, they lack possibility of differentiating hospital from out of hospital MRSA.

- Differentiating HA and CA MRSA with epidemiological and phenotype isolate characteristics is possible only with molecular method application.

- MRSA strains in typing by spa-typing as follows: the most common type is t008 type (55.2%), than t1179 (17.2%) and t10807 (10.3%). Comparing relative

global frequency of single type appearance of spa-types on Ridem Spa Server, t008 is globally the most registered type. New spa – types discovered in this study are t1179, t2187, t2674 and t10807.

- Prevalence of CA MRSA in six months period of follow up in geographical area of Sarajevo Canton in the amount 0,13%, while prevalence of CA MRSA PVL positive types is 0,037%.

- From total number of typed MRSA strains, 24% of them was PVL positive. Most of PVL positive isolates was resistant to beta lactam and macrolide antibiotics.

- Comparing PFGE genetic profile MRSA isolates were classified into 5 similarity groups, in which two of them are dominant, groups C and D, containing 60% and 27% isolates. Group C had 28,33% PVL positive types, while group D had only 2 PVL positive isolates.

- The greatest number of MRSA isolates, 84% has SCCmec type IV speaking on behalf of very high representation of out of hospital MRSA. SCCmec type I is present in 4%, while type V was present in only 1% isolates. Atypical isolates were 9%, while 95,8% PVL positive isolates fell into SCCmec type IV.

- The goal of every centre for microbiological diagnostics is to improve and speed up diagnostics of MRSA positive patients for treatment personalisation, and efficient spreading prevention of CA MRSA in hospital and out of hospital population.

- Results of the study, conducted in the most densely populated area of Bosnia and Herzegovina, indicate high level of MRSA prevalence.

- Standardization of national laboratory work is necessary, clinical as well as public health in testing antimicrobial bacteria sensitivity, due to transfer of patients between institutions, communication between institutions and follow up resistance development. For reduction of resistance level it is necessary to control spending and supervising, and prescribing antibiotic medications, in medicine and in veterinary and food industry.

- This study emphasizes the need for national monitoring of spreading existing types, as well as emerging of new strains thus enabling inclusion of our country into international network of follow up for resistant bacteria.

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