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The prevalence and risk factors of *Staphylococcus aureus* carriage, analysis of antibiotic resistance and virulence factors

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Staphylococcus aureus nešiojimo paplitimas ir rizikos veiksniai, atsparumo antimikrobinėms medžiagoms ir virulentiškumo veiksnių analizė

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CONTENTS

ABBREVIATIONS

CA-MRSA	–	community associated methicillin–resistant *Staphylococcus aureus*
CA-SA	–	community associated *Staphylococcus aureus*
CDC	–	Centers for Disease Control
Cl	–	confidence interval
CLSI	–	Clinical Laboratory Standards Institute
C_V	–	coefficient of variation
DNA	–	deoxyribonucleic acid
dNTP	–	deoxyribonucleotide triphosphate
EARSS	–	European Antimicrobial Resistance Surveillance System
HA-MRSA	–	health care associated methicillin–resistant *Staphylococcus aureus*
HA-SA	–	health care associated *Staphylococcus aureus*
ICU	–	intensive care unit
IU	–	international unit
IDUs	–	injecting drug users
MIC	–	minimum inhibitory concentration
MLS	–	macrolide-lincosamide-streptogramin
MLST	–	multi locus sequence typing
MRSA	–	methicillin–resistant *Staphylococcus aureus*
MSSA	–	methicillin–sensitive *Staphylococcus aureus*
NHANE	–	National Health and Nutrition Examination Survey
OG	–	occupational group
OR	–	odds ratio
p	–	level of statistical significance
p_F	–	statistical significance, based on Fishers exact test, when number of cases in the cells of the contingency table is les than 5
PBP2a	–	penicillin-binding protein 2a
PCR	–	polymerase chain reaction
PFGE	–	pulsed field gel electrophoresis

PVL	–	Panton-Valentine leukocidin
rpm	–	revolutions per minute
S. aureus	–	*Staphylococcus aureus*
SCC*mec*	–	staphylococcal cassette chromosome *mec*
SD	–	standard deviation
spa	–	gene encoding Staphylococcal Protein A
SSI	–	surgical site infection
SSTI	–	skin and soft tissue infections
ST	–	sequence type
VISA	–	vancomycin-intermediate *S. aureus*
VRSA	–	vancomycin-resistant *S. aureus*
VCUH	–	Vilnius City University Hospital
VUHSK	–	Vilnius University Hospital Santariškių Klinikos

1. INTRODUCTION

Staphylococcus aureus (*S. aureus*) was discovered in Aberdeen, Scotland in 1883 by the surgeon Sir Alexander Ogston in pus from a surgical abscess [1]. He introduced the name "staphylococcus" (from the Greek ***σταφυλϖη***, "bunch of grapes") to describe "micrococci" responsible for inflammation and suppuration. Pasteur (1880), independently from Ogston, described small spherical bacteria isolated from pus obtained from furuncles and osteomyelitis and considered them to be pathogenic.

Members of the genus *Staphylococcus* form a coherent and well-defined group of related species that is widely divergent from those of the genus *Micrococcus*. The genus *Staphylococcus* consists of 36 species, 16 of which are found in humans. [2]

Only a few of the staphylococci are pathogenic in the absence of predisposing host conditions such as immunosuppresion or the presence of a foreign body. The most virulent ones include *S. aureus* and *S. lugdunensis* in humans. Although *S. epidermidis* and *S. saprophyticus* are commonly responsible for devise-related and urinary tract infections, they produce substantially less devastating disease syndromes than *S. aureus*. [2]

S. aureus is part of the usual bacterial flora of humans and can also be found in other mammals as well as birds. Nevertheless, throughout the recorded history this commensal has been a significant cause of infections in humans. Even Egyptian mummies are known to have pathological changes consistent with staphylococcal osteomyelitis. This microorganism has exceptional ability of transforming itself and being one step ahead of therapeutic novelties.

S. aureus is re-emerging as a major threat to human health and well-being the world over. With the evolution of human being and medicine, *S. aureus* also has evolved and adapted to a wide variety of human conditions and medical innovations. Historically, *S. aureus* was certainly a significant human pathogen prior to the development of antibiotics. For example, in the last

century, *S. aureus* was the major bacterial cause of death in the influenza pandemic of 1918, among those who developed secondary bacterial pneumonia. Following the introduction of antibiotics, *S. aureus* developed resistance to penicillin in the 1940s, and then emerged as an important cause of severe nosocomial infections in the 1950s [3]. With the development and widespread use of chloramphenicol and tetracycline in the 1960s, infections due to *S. aureus* occurred including staphylococcal enterocolitis. These were clearly related to two factors: eradication of the normal gut flora due to overuse of antibiotics and concomitant proliferation of *S. aureus* strains, which had developed antibiotic resistance during treatment. The timely discovery of beta-lactamase-resistant cephalosporins and later the semisynthetic beta-lactam antibiotics (methicillin, oxacillin, and nafcillin) saved the situation for the next 10 to 15 years.

Still, as early as the 1970s, sporadic reports of methicillin-resistant *S. aureus* (MRSA) began to appear. The first MRSA strain was found in a UK hospital in 1961 shortly after the introduction of methicillin in 1960 [4]. In the late 1970s epidemics of MRSA were reported in some unique facilities with extremely ill patients and with intensive antibiotic usage [5]. The widespread emergence of MRSA infections has been observed over the subsequent 20 to 30 years, in certain regions of Europe, throughout the United States, as well as in Japan and the Western Pacific [6, 7]. Until very recently, these MRSA strains have largely been associated with hospital-acquired infections [8, 9].

During the last 20 years, MRSA was reported in infections originating in the community. Multiple studies have documented cases of MRSA skin and soft tissue infections in persons in the community without traditional risk factors related to hospital associated methicillin-resistant *S. aureus* (HA-MRSA). In the 1980s, however, epidemic strains of MRSA emerged and attention shifted back to staphylococcal colonization and infection.

Carriage of *S. aureus* appears to play a key role in the epidemiology and pathogenesis of community and health acquired infections [10]. Patients with nasally colonized *S. aureus* have a significantly higher risk of developing

staphylococcal wound infection after a surgical procedure than those who are not colonized [11].

In Denmark, a national surveillance system of staphylococcal disease has been in operation at the Statens Serum Institute since the late 1950s. The implementation of strict infection control measures and low consumption of antibiotics helped successfully to reduce and keep at low levels hospital–acquired MRSA.

In Lithuania, so far, a national surveillance system of staphylococcal disease is not in place. Studies of the carriage rate of *S. aureus* in Lithuania are lacking. The prevalence of *S. aureus* among pre-school and school-aged children [12] and *S. aureus* carriage prevalence among hospitalized patients [13] were performed in a city of Kaunas. As far as we known there is no data about the prevalence of *S. aureus* in the healthy adult population in Lithuania.

There is limited data comparing community-acquired with health care-acquired *S. aureus* and MRSA cases in Lithuania. Furthermore, there has been no systematic comparison of the molecular characteristics (e.g., Panton-Valentine leukocidin gene profiles, *spa* types) of community and health care isolates. Such genetic information could improve understanding of the pathogenesis of different staphylococcal strains. The research on *S. aureus* carriage in Lithuania up to the present day was never aimed at evaluation of the risk factors which may influence the *S. aureus* colonization. This study will investigate the genetic relatedness and comparison between *S. aureus* in the community and hospital, and evaluate important risk factors for *S. aureus* colonization.

1.1. Aim of the study

To determine the extent of *S. aureus* carriage of selected adult population and hospitalized patients in Vilnius and evaluate potential risk factors of its carriage as well as antimicrobial resistance of isolated strains and, prevailing genetic elements of the resistance and virulence.

1.2. Objectives of the study

1. To assess *S. aureus* carriage in an adult community population and in hospitalized patients of some surgical departments in Vilnius.
2. To evaluate possible risk factors of *S. aureus* carriage in the community and in the hospitalized patient groups.
3. To determine antibiotic resistance differences and prevailing resistance genes of *S. aureus* strains isolated from selected community groups and hospitalized patients.
4. To define the prevalence of virulence gene and *spa* types of *S. aureus* strains isolated from community and hospitalized patients.

1.3. Scientific novelty of the study

The study of *S. aureus* carriage in adult community population outside hospital and in no connection to hospital risk factors was carried out for the first time. Hospitalized patients were also examined in order to determine *S. aureus* carriers on admission to and upon discharge from hospital.

In this study, for the first time in Lithuania, the analysis of risk factors was carried out to evaluate the prevailing factors of *S. aureus* carriage. According to the thorough survey of the participants of the study and their clinical record, the prevailing risk factors of *S. aureus* carriage in population and hospitalized patients were identified.

All the isolated *S. aureus* strains were tested according to the extended antibiogram for the resistance to antimicrobials. The results of resistance determination were compared among the community population group and the hospitalized patients group. The applied molecular testing enabled to determine and compare the prevailing genetic elements coding resistance to methicillin, erythromycin and tetracycline.

Although Panton-Valentine leukocidin is very important in the pathogenesis of *S. aureus* infections, the prevalence of this gene was never studied in Lithuania. The applied molecular testing, in this study, enabled us to determine its prevalence among the isolated *S. aureus* strains in the community population and hospitalized patients.

S. aureus typing helps to determine congenerical *S. aureus* strains, their origin and spread. According to the molecular testing methods, for the first time in Lithuania, *spa* types were identified in this study. The data obtained was compared between the studied groups.

1.4. Practical value of the study

During the study, a relatively high carrier rate was found for *S. aureus* in Lithuanian individuals. The carriage of this microorganism may cause wound and development of other infections. According to the results of the study, post-operative wound infection prevention measures may be implemented.

The detection of the risk factors influencing *S. aureus* carriage helps direct epidemiological surveillance and infection control measures towards the control of the factors and prevent *S. aureus* and MRSA from spreading.

The data obtained, on the resistance of *S. aureus* strains to antimicrobials, in this study may be used for implementing the Antimicrobial resistant microorganism spread prevention program (2008–2014). One of the aims of this program is to minimize the occurrence and spread of antimicrobial resistant microorganisms; to develop the antimicrobial resistant microorganism

monitoring system; to perform research in connection with antimicrobial-drug therapy and the growing resistance levels of microorganisms. [14]

1.5. Defensive statements of the dissertation

- More than half of the Vilnius adult population under the study is *S. aureus* carriers.
- Most of the isolated *S. aureus* strains are resistant to penicillin, and only a small part are resistant to methicillin.
- Panton-Valentine leucosidin toxin gene is identified in clinical CA-MRSA strains; methicillin susceptible *S. aureus* strains rarely have this toxin coding gene.
- When cases of *S. aureus* carriage have been identified, a thorough investigation usually revealed a history of recent hospitalization; close contact with a person who has been hospitalized; or other risk factors, such as previous antimicrobial-drug therapy.

2. LITERATURE REVIEW

2.1. Diversity of *S. aureus* phenotypic characteristics

Staphylococci are Gram-positive spherical bacteria that occur in microscopic clusters resembling grapes (Figure 1). Staphylococci are non-motile, non-spore forming, usually catalase positive, and have a thin capsule. *S. aureus* are perfectly spherical cells about 1 micrometer in diameter. The staphylococci grow in clusters because the cells divide successively in three perpendicular planes with the sister cells remaining attached to one another following each successive division. Since the exact point of attachment of sister cells may not be within the divisional plane and the cells may change position slightly while remaining attached, the result is formation of an irregular cluster of cells. [2]

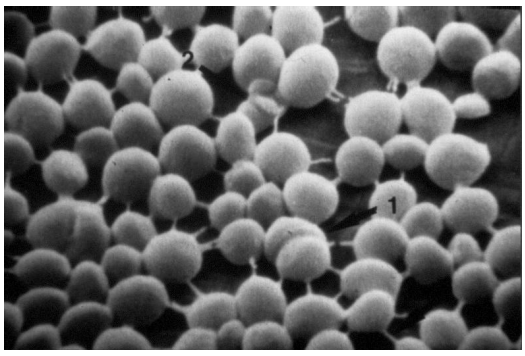


Figure 1. *Electron micrograph of Staphylococcus aureus.*
Adapted from: http://bioinfo.bact.wisc.edu/themicrobialworld/staph.html

S. aureus grow readily on most bacteriological media under facultative aerobic conditions. They grow most rapidly at 37^0C but best form pigment at room temperature (20–25^0C), they will survive and replicate at temperatures between 10^0 and 45^0C. Colonies on solid media are round, smooth, raised and glistening. *S. aureus* usually form gray to deep golden yellow colonies. Many

colonies develop pigment only upon prolonged incubation. No pigment is produced anaerobically or in broth. When grown on blood agar, staphylococci form small (1 to 2 mm), smooth, round colonies that are often pigmented and may be surrounded by a zone of β–hemolysis. [2]

The staphylococci produce catalase, which differentiates them from the streptococci. Staphylococci slowly ferment many carbohydrates, producing lactic acid but not gas. Nearly all strains of *S. aureus* produce the enzyme coagulase. The bacteria are oxidase-negative. [2]

2.1.1. Virulence factors and encoding genes of S. aureus

S. aureus can produce disease both through their ability to multiply and spread widely in tissues and through their production of many extracellular substances. Virulence factors expressed by *S. aureus* [15]:

- Surface proteins that promote colonization of host tissues;
- Invasins that promote bacterial spread in tissues (leukocidin, kinases, hyaluronidase);
- Surface factors that inhibit phagocytic engulfment (capsule, Protein A);
- Biochemical properties that enhance their survival in phagocytes (carotenoids, catalase production);
- Immunological disguises (Protein A, coagulase, clotting factor);
- Membrane-damaging toxins that lyse eukaryotic cell membranes (hemolysins, leukotoxin, leukocidin);
- Exotoxins that damage host tissues or otherwise provoke symptoms of disease (TSST, SEA-G, ET);
- Inherent and acquired resistance to antimicrobial agents.

Lately, the scientific literature turns greater attention to staphylococcal Panton-Valentine leukocidin. The description of this toxin is in chapter 2.1.1.1.

2.1.1.1. Panton–Valentine leukocidin

The Panton–Valentine leukocidin (PVL) toxin is one of the most clinically significant virulence factors in *S. aureus*. This cytotoxin was discovered in 1894 by van de Velde and distinguished from haemolysins in 1932 by Panton and Valentine. MRSA that encode PVL were first reported in Western Australia in the 1990s [16].

Staphylococcal leukotoxins, including PVL, are secreted as bicomponent toxins consisting of S and F proteins [17]. Depending on the combination of particular S and F proteins, a toxin is formed with varying leukocytolytic, erythrocytolytic, and dermonecrotic properties [18]. The PVL synthesis coding gene consists of *lukS-PV* and *lukF-PV* genes and 4 units of each form octameric β-barrel pores in leukocyte membranes in vitro, resulting in cell lysis [19]. This may cause cells such as neutrophils to release inflammatory enzymes and cytokines [20]. PVL also appears to induce apoptosis of neutrophils via a mitrochondrial pathway at lower concentrations, whereas, at higher concentrations, PVL induces necrosis [21]. In vivo, PVL causes necrosis of a skin when injected intradermally in rabbits [22].

PVL is a virulence factor rather specific to CA-MRSA. It is rarely identified in methicillin-sensitive *Staphylococcus aureus* (MSSA) and HA-MRSA. The bicomponent cytotoxin has previously been reported to be produced by less than 5% of *S. aureus* isolates [23, 24, 25]. Some studies, which investigated nasal carriage of *S. aureus,* demonstrate that from 0.5% [26] to 0.65% [27, 28] isolated MSSA strains carry PVL genes.

The frequency of PVL production among skin and soft tissue infections (SSTI) -associated MSSA isolates in the Chini et al. study was 12% [29]. Diep et al. [24] identified 6.7% MSSA isolates as PVL-positive, while Johnsson et al. [25] reported only 2.1% MSSA isolates carried PVL genes.

Some nasal carriage study have reported that 19% [30], 20% [26] and 22% [31] of CA-MRSA strains were PVL-positive, while the incidences of PVL in clinical isolates of CA-MRSA were from 25.3% to 72% [29, 32, 33]. In

an epidemiologic study from Minnesota, PVL was present in 77% of all CA-MRSA and in only 4% of HA-MRSA [34].

Above mentioned studies found that CA-MRSA carriage isolates have much lower rates of PVL carriage than do clinical CA-MRSA isolates, although strain types of CA-MRSA differed greatly between clinical and carriage isolates. At face value, these findings support the hypothesis that clinical strains of *S. aureus* are more virulent than carriage strains, by virtue of possessing virulence factors such as PVL.

Given this evidence and the strong epidemiological association between PVL-containing CA-MRSA strains and necrotizing pneumonia and skin and soft-tissue infections, it is plausible that PVL is partly responsible for the enhanced virulence of CA-MRSA (other leukocidins may also play a role).

Lina et al. [35] determined the presence of *lukS-pv* and *lukF-pv* in 172 *S. aureus* strains collected from patients with a variety of clinical syndromes. PVL was significantly associated with community-acquired pneumonia (85% of strains), compared with hospital-acquired pneumonia (0%). In patients with pneumonia, presence of the PVL gene results in a much higher mortality, i.e. 37% compared to 6% for patients with PVL-negative pneumonia [36]. A group of investigators found that *S. aureus* strains possessing PVL genes showed greater affinity for collagen and laminin than PVL-negative strains, and they hypothesized that this might permit binding of such strains to damaged respiratory epithelia [37].

PVL was also significantly associated with strains causing invasive skin infections such as furunculosis (93%) and cutaneous abscess (50%), compared with superficial folliculitis (0%). PVL was not observed in strains associated with infective endocarditis, urinary tract infections, toxic shock syndrome, or mediastinitis, although only few strains were tested. Diep et al. [24] reported a similar association of PVL and skin and soft-tissue infections caused by MRSA isolated from inpatients and outpatients from San Francisco General Hospital and inmates in county jails. By contrast, PVL-producing *S. aureus* are

rarely responsible for other infections such as osteomyelitis, septicemia and endocarditis [35].

PVL is usually present in CA-MRSA types USA300 and USA400 [38, 39] and is often harbored by other SCC*mec*IV-containing strains [39].

Saïd-Salim et al. [39] compared human polymorphonuclear cell lysis among PVL-positive and PVL-negative CA-MRSA strains with similar genetic backgrounds and found no difference in polymorphonuclear lysis. Voyich et al. [40] compared PVL-positive strains and PVL-negative strains with similar genetic backgrounds in mouse sepsis and abscess models, as well as PVL knockouts created for the USA300 and USA400 strains. There was no difference in survival in the mouse sepsis model. In the abscess model, PVL-negative strains unexpectedly caused slightly larger abscesses than did the PVL-positive strains. Isogenic PVL strains of USA300 and USA400 showed no difference in the ability to cause polymorphonuclear lysis in vitro. It is possible that the mouse models used in this study were not optimal to assess the in vivo effects of PVL, or, as the authors suggested, that PVL either is a marker for other virulence factors present in these strains or is one of many factors causing the enhanced virulence of particular CA-MRSA strains.

Due to conflicting results represented by these studies comparing PVL positive and PVL negative strains, the exact role of PVL as a virulence determinant in relation to infections remains controversial.

2.1.2. Molecular typing of S. aureus

Bacterial strain typing distinguishes epidemiologically related or clonal isolates from unrelated isolates and helps to understand the origin and spread of MRSA clones [41].

Numerous techniques are available to differentiate *S. aureus*, and specifically MRSA, isolates. Isolates can be distinguished by phenotypic methods, including antibiotic susceptibility testing and bacteriophage typing. Both methods have limitations, as genetically unrelated isolates commonly

have the same antibiogram, and many *S. aureus* isolates are nontypeable by phage typing [42].

During the 1990s, pulsed field gel electrophoresis (PFGE) was called the "gold standard" for outbreak investigation. However, PFGE had some disadvantages too. Using the PFGE typing method, it is difficult to prove that an outbreak results from the transmission of the same strain and due to its complexity and cost it has limitation as a tool for evaluation of large strain materials [43].

The new generation of genotyping methods includes multilocus sequence typing (MLST) and *spa* typing for unambiguous characterization of *S. aureus* [44]. Indeed, by sequencing an internal fragment of seven unlinked house-keeping genes, allelic profiles or sequence type (ST) can be determined. A clone is defined as a group of isolates having a strictly identical sequence of all seven genes [45]. Related STs can be grouped into clonal complex (CC) using the eBURST analysis (www.MLST.net) [46]. By the 20th of August, 2010 a total of 1702 STs were recorded at the shared database at www.MLST.net.

However, MLST is not suitable for routine surveillance of MRSA because of the high cost and the necessity of access to a high-throughput DNA sequencing facility.

Single locus DNA-sequencing of the repeat region of the *Staphylococcus* protein A (*spa*) can be used for reliable, accurate and discriminatory typing of MRSA [47, 48]. *spa* typing is especially interesting for rapid typing of MRSA in a hospital setting since it offers high resolution. The repeat region of the *spa* gene is subject to spontaneous mutations, as well as loss and gain of repeats. Repeats are assigned an alpha-numerical code, and the *spa* type is deduced from the order of specific repeats. There is a good correlation between clonal groupings determined by MLST and the respective *spa* types [49, 50]. Examples have been reported of isolates with the same *spa* type belonging to related MLST sequence types that arose by single-locus variation [51]. On the other hand, there seems to be a considerable degree of *spa* gene repeat number

variation within a given sequence type, suggesting that *spa* typing in some instances provides greater resolution than MLST [49, 50].

At present, 408 different *spa* repeats and 7072 *spa* types (20.08.2010) are recognized and can be clustered into *spa* CC groups in a similar way as for MLST CC groups (www.ridom.de, Ridom Staphyte software). Some important *S. aureus spa* types frequencies are listed in Table 1.

The collected data show a great variety of staphylococcus strains. Nevertheless, the most important in the pathogenesis of staphylococcal infections on humans is *S. aureus*. This microorganism has a variety of virulence factors, and is quite simple to culture and identify.

2.2. Antimicrobial resistance in staphylococci

S. aureus has developed resistance to basically all antibiotic classes available for clinical use. These include cell wall inhibitors such as β-lactams and glycopeptides, ribosomal inhibitors including macrolide-lincosamide-streptogramin B (MLS_B), aminoglycosides, tetracyclines, fusidic acid and oxazolidinones, the RNA polymerase inhibitor rifampin, the DNA gyrase blocking quinolones, and the antimetabolite trimethoprim-sulfamethoxazole [52].

The resistance falls into one of three classes [52]:

- Alteration of the molecular target of the antibiotic;
- Inactivation of the drug;
- Prevention of accumulation within the bacterial cells usually via efflux of the agent out of the bacterial cell by either dedicated or general efflux pumps.

In fact, there are some resistance genes that do not fall neatly into any of these three categories (e.g., resistance to the semisynthetic tetracycline minocycline via ribosome protection), and, in the case of some if not most classes of drugs, more than one resistance mechanism is at play [53, 54]. The main resistance mechanisms are summarized in Table 2.

Table 1. Global prevalence of some *spa* type's

spa type	Frequency	Countries of origin
t001	1.59 %	Austria, Belgium, Croatia, Czech Republic, France, Germany, Israel, Italy, Netherlands, Norway, Slovenia, South Africa, Spain, Sweden, Switzerland, United Kingdom, United States
t002	5.61 %	Austria, Belgium, Canada, China, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Iceland, Israel, Italy, Japan, Jordan, Lebanon, Martinique, Netherlands, New Zealand, Norway, Poland, Romania, SE, South Africa, Spain, Sweden, Switzerland, Taiwan, United Kingdom, United States
t003	12.83 %	Austria, Belgium, Canada, Croatia, Czech Republic, Denmark, France, Germany, Netherlands, Norway, Poland, Spain, Sweden, Switzerland, United States
t008	6.29 %	Austria, Belgium, Bulgaria, Canada, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Gabon, Germany, Hungary, Iceland, India, Israel, Italy, Japan, Jordan, Lebanon, Netherlands, New Zealand, Norway, Poland, Slovak Republic, South Africa, Spain, Sweden, Switzerland, United Kingdom, United States
t011	3.14 %	Austria, Belgium, Czech Republic, France, Germany, Italy, Netherlands, Norway, Poland, Spain, Sweden, Switzerland
t012	1.30 %	Austria, Belgium, Canada, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Iceland, Italy, Jordan, Latvia, Lebanon, Netherlands, New Zealand, Norway, Poland, South Africa, Spain, Sweden, Switzerland, United Kingdom, United States
t015	1.35 %	Austria, Belgium, Croatia, Czech Republic, Denmark, Finland, France, Germany, Hungary, Iceland, Indonesia, Italy, Latvia, Netherlands, New Zealand, Norway, Poland, Romania, South Africa, Spain, Sweden, Switzerland, Taiwan, United Kingdom, United States
t032	10.51 %	Austria, Belgium, Czech Republic, Denmark, France, Germany, Hungary, Iceland, Italy, Lebanon, Malaysia, Netherlands, New Zealand, Norway, South Africa, Spain, Sweden, Switzerland, United Kingdom, United States
t037	2.51 %	Austria, Belgium, Bulgaria, Canada, China, Croatia, Czech Republic, Denmark, France, Germany, Iceland, Italy, Jordan, Latvia, Lebanon, Malaysia, Netherlands, New Zealand, Norway, Poland, South Africa, Spain, Sweden, Switzerland, Taiwan, United Kingdom
t044	2.02 %	Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, France, Germany, Hungary, Iceland, Italy, Jordan, Lebanon, Netherlands, Norway, Spain, Sweden, Switzerland, United Kingdom
t050	0.24 %	Austria, Belgium, Denmark, France, Germany, Indonesia, Netherlands, Norway, Spain, Sweden, Switzerland, Taiwan
t056	0.28 %	Austria, Belgium, Czech Republic, Denmark, France, Germany, Netherlands, Norway, Romania, Spain, Sweden, Switzerland
t084	1.28 %	Belgium, Denmark, Finland, France, Gabon, Germany, Indonesia, Italy, Jordan, Lebanon, Netherlands, New Zealand, Norway, Poland, Spain, Sweden, Switzerland, Taiwan, United Kingdom, United States
t127	1.20 %	Austria, Belgium, China, Croatia, Cyprus, Denmark, Finland, France, Germany, Iceland, Indonesia, Latvia, Lebanon, Netherlands, New Zealand, Norway, Poland, Romania, South Africa, Spain, Sweden, Switzerland, Taiwan, United Kingdom, United States
t156	0.11 %	Austria, Belgium, France, Germany, Netherlands, Norway, Poland, Spain, Sweden, Switzerland
t346	0.27 %	Belgium, Denmark, France, Germany, Jordan, Netherlands, Norway, Poland, SE, South Africa, Sweden, Switzerland, United States
t359	0.10 %	Austria, Belgium, Denmark, France, Germany, Hungary, Israel, Netherlands, Norway, Spain, Sweden, Switzerland, Taiwan, United Kingdom
t589	0.02 %	Germany, Netherlands, New Zealand, Norway
t1255	0.02 %	Estonia, Germany, Netherlands, Spain
t1541	0.01 %	Germany, Netherlands, Norway, Sweden

Adapted from www.ridom.de

From the genetic point of view, resistance falls into one of two classes:

- Mutation of a bacterial gene;
- Acquisition of a dedicated resistance gene from some other organism by some form of genetic exchange (transduction, conjugation or transformation).

In general, resistance via mutation is an alteration of the target site of the antibiotics, although increased expression of either the target or of a nonspecific efflux pump also can be the result of a mutation. Acquired resistance determinants are, by and large, dedicated to narrow classes of compounds but can run the summation of mechanisms and are usually inducible. Dissemination of these acquired resistance determinants vary in frequency, depending on the type of genetic element carrying the resistance determinant. [54]

2.2.1. β-lactam antibiotics

The mortality of patients with *S. aureus* bacteremia in the pre-antibiotic era exceeded 80%, and over 70% developed metastatic infections [55]. The introduction of penicillin in the early 1940s dramatically improved the prognosis of patients with staphylococcal infection. However, as early as 1942, penicillin-resistant staphylococci were recognized, first in hospitals and subsequently in the community [3]. By the late 1960s, more than 80% of both community- and hospital-acquired staphylococcal isolates were resistant to penicillin and that number has increased since that to over 90% of *S. aureus* isolates.

Several studies on *S. aureus* carriage in the community demonstrated that 80.5% and 83.7% of the isolates showed resistance to penicillin [12, 56].

Recent reports demonstrate that resistance to penicillin in HA-MSSA is 68.4% in Italy, 76.4% - in Germany, 79.9% - in France, 86.8% - in UK, 95.4% - in Spain [57] and 89.0% - in Lithuania [58]. At the same time penicillin resistance in some HA-MRSA strains was found in 100% isolates [58].

Table 2. Mechanisms of *Staphylococcus aureus* resistance to major classes of antibiotics

Antimicrobials	Resistance mechanisms			Resistance gene		
	Target Modification	Drug Inactivation	Decreased Accumulation	Nature	Origin	Location
β-lactams						
Penicillinase-S	±	+	–	Penicillinase[(b)]	Acquired	Plasmid
				PBP2A[(a)]	Acquired	SCCmec (chromosome)
Penicillinase-R	+	–	–	PBP2A	Acquired	SCCmec (chromosome)
Glycopeptides						
Intermediate-R	+	–	–	Mutation in wall-building genes	Intrinsic	Chromosome
Fully-R	+	–	–	*vanA* and *vanH*	Acquired	Plasmid or transposon
Macrolide-Lincosamide-Streptogramin B						
Macrolides	+	–	+	*erm*[(a)]	Acquired	Plasmid or chromosome
				msrA[(c)]	Acquired	Plasmid
Lincosamide	+	+	–	*erm*[(a)]	Acquired	Plasmid or chromosome
				linA[(b)]	Acquired	Plasmid or chromosome
Streptogramin B	–	+	+	*erm*[(a)]	Acquired	Plasmid or chromosome
				vgb[(b)] (rare)	Acquired	Plasmid or chromosome
				msrA[(c)] (rare)	Acquired	Plasmid or chromosome
Streptogramin A	–	+	+	*vat, vatA*[(b)] (rare)	Acquired	Plasmid or chromosome
				vga, vgaB[(c)](rare)	Acquired	Plasmid or chromosome
Quinupristin-dalfopristin	+	+	+	Combinations of above (rare)		

R – reistant; S – susceptible. [(a)]target modification, [(b)]drug inactivation, [(c)]decreased drug accumulation. vanA, vanH, vancomycin resistance A and H genes; erm, erythromycin resistance methylase; msrA, macrolide-streptogramin resistance, ABC transporter; linA', lincosamide nucleotidyl transferase; vgb, virginiamycin hydrolysis; vat and vatA, acetyl transferase genes; vga and vgaB, streptogramin A efflux gene.

Continuation

Antimicrobials	Resistance mechanisms			Resistance gene		
	Target Modification	**Drug Inactivation**	**Decreased Accumulation**	**Nature**	**Origin**	**Location**
Other classes						
Linezolid	+	–	–	Mutation in 23S rRNA gene	Intrinsic	Chromosome
Tetracyclines	+	–	+	*tet*(K), *tet*(L) [c],*tet*(M),*tet*(O) [a]	Acquired	Plasmid or chromosome
Gentamicin	–	+	+	*aac(6')-aph(2'')* [b]	Acquired	Plasmid or chromosome
				Respiratory chain mutants [c]		Chromosome
Chloramphenicol	–	+	–	*cat*	Acquired	Plasmid or chromosome
Fusidic acid	+	–	+	*fusA* mutation [a]	Intrinsic	Chromosome
				pUB101 [c]	Acquired	Plasmid
Rifampin	+	–	–	*rpoβ* mutation	Intrinsic	Chromosome
Fluoroquinolones	+	–	+	*grlA, gyrA* [a]	Intrinsic	Chromosome
				norA [c]	Intrinsic	Chromosome
Trimethoprim	+	–	–	*dfrA* mutation	Intrinsic	Chromosome
				dfrA	Acquired	Plasmid or chromosome
Sulfamethoxazole	+	–	–	*dpsA*	Intrinsic	Chromosome
					Acquired	Plasmid (probable)

tet(K), tet(L), responsible for active efflux of tetracyclines; tet(M),tet(O), responsible for ribosomal modification and protection; aac(6')-aph(2''), bifunctional aminoglycoside acetyltransferase and phosphortransferase determinant; cat, chloramphenicol acetyltransferase; fusA, gene encoding elongation factor G; rpoβ, gene encoding the β-subunit of RNA polymerase; grlA, gyrA, genes encoding the DNA topoisomerase and gyrase, respectively; norA, gene encoding a staphylococcal efflux pump; dfrA, dihydrofolate reductase gene; dpsA, dihydropteroate synthase. Based on: Livermore DM, Winstanley TG, Shannon K. Interpretative reading: recognizing the unusual and inferring resistance mechanisms from resistance phenotypes. J Antimicrob Chemother. 2001;48:87-102 [53]. Jensen SO, Lyon BR. Genetics of antimicrobial resistance in Staphylococcus aureus. Future Microbiol. 2009;4:565-82. [54]

Results obtained from the studies indicate that staphylococcal resistance to penicillin is mediated by *blaZ*, the gene that encodes β-lactamase (Figure 2a) [59, 60].

Methicillin resistance

The resistance of *S. aureus* to methicillin is caused by the presence of the *mecA* gene, which encodes the 78-kDa penicillin-binding protein (PBP) 2a (or PBP2‘) [61]. The 2.1-kb *mecA* gene is located on a mobile genetic element, designated the Staphylococcal Cassette Chromosome *mec* (SCC*mec*) [62]. Five types of SCC*mec* (types I–V) have been distinguished, ranging in size from 20.9 to 66.9 kb (Figure 3). SCC*mec* types I (34.3 kb), IV (20.9–24.3 kb) and V (28 kb) [63], VI encode exclusively for resistance to β-lactam antibiotics. In contrast, SCC*mec* types II (53.0 kb) and III (66.9 kb) determine multiresistance, as these cassettes contain additional drug resistance genes on integrated plasmids (pUB110, pI258 and pT181) and a transposon (Tn*554*) [63]. A new type (VII) has recently been identified in a CA-MRSA isolates from Sweden [64]. Zhang et al. [65] described a novel SCC*mec* type harboring a previously uncharacterized and unique combination of class A *mec* and type 4 *ccr* gene complex (4A), tentatively designated type VIII, identified in a Canadian CA-MRSA epidemic strain.

PBP2a substitutes for the other PBPs and, because of its low affinity for all β-lactam antibiotics, enable staphylococci to survive exposure to high concentrations of these agents. Thus, resistance to methicillin confers resistance to all β-lactam agents, including penicillins, cephalosporins and carbapenem. Recent studies determined the crystal structure of a soluble derivative of PBP2a. PBP2a differs from other PBPs in that its active site blocks binding of all β-lactams but allows the transpeptidation reaction to proceed [66].

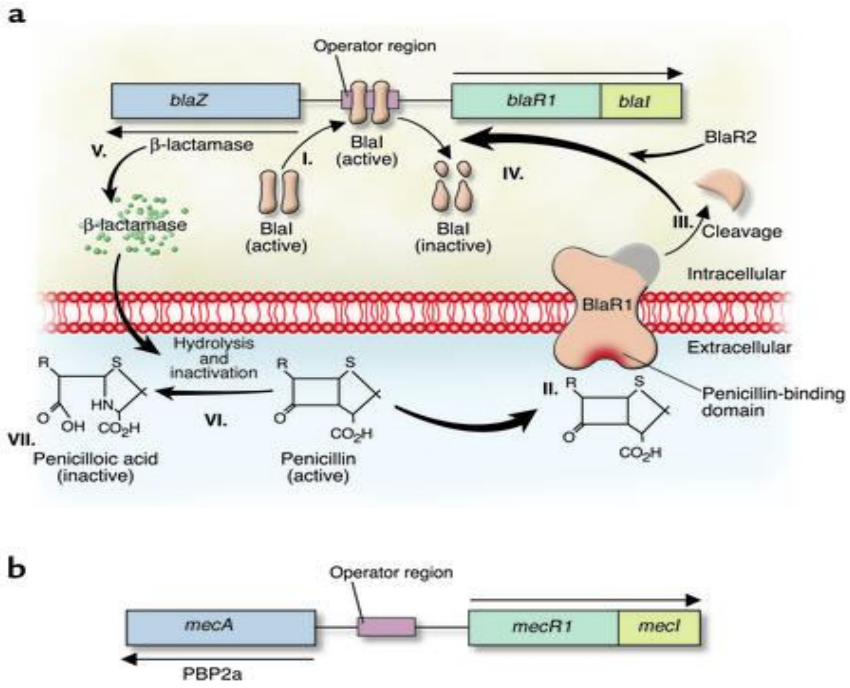


Figure 2. ***(a)*** *Induction of staphylococcal β-lactamase synthesis in the presence of the β-lactam antibiotic penicillin. I. The DNA-binding protein BlaI binds to the operator region, thus repressing RNA transcription from both blaZ and blaR1-blaI. In the absence of penicillin, β-lactamase is expressed at low levels. II. Binding of penicillin to the transmembrane sensor-transducer BlaR1 stimulates BlaR1 autocatalytic activation. III–IV. Active BlaR1 either directly or indirectly (via a second protein, BlaR2) cleaves BlaI into inactive fragments, allowing transcription of both blaZ and blaR1-blaI to commence. V–VII. β-Lactamase, the extracellular enzyme encoded by blaZ (V), hydrolyzes the β-lactam ring of penicillin (VI), thereby rendering it inactive (VII).* ***(b)*** *Mechanism of S. aureus resistance to methicillin. Synthesis of PBP2a proceeds in a fashion similar to that described for β-lactamase. Exposure of MecR1 to a β-lactam antibiotic induces MecR1 synthesis. MecR1 inactivates MecI, allowing synthesis of PBP2a. MecI and BlaI have coregulatory effects on the expression of PBP2a and β-lactamase.*

Adapted from Zhang HZ, Hackbarth CJ, Chansky KM, Chambers HF. A proteolytic transmembrane signaling pathway and resistance to beta-lactams in staphylococci. Science. *2001;291:1962–1965. [59]*

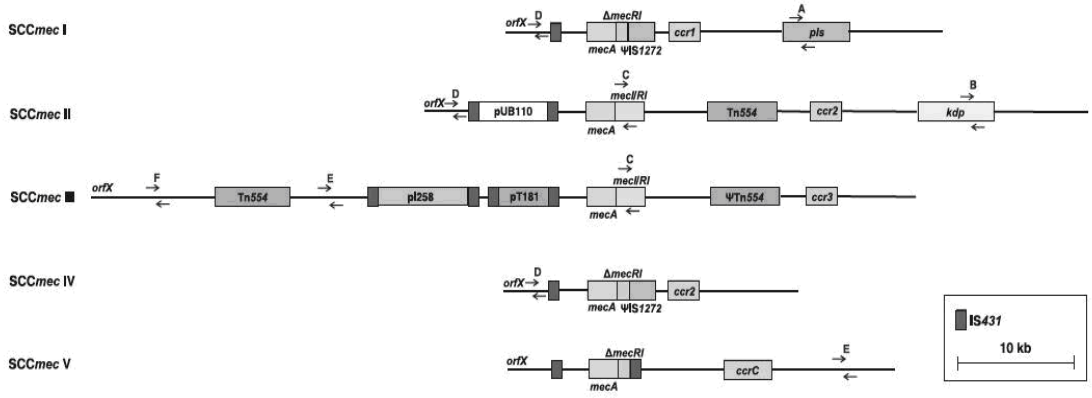


Figure 3. *Schematic arrangement of SCCmec types I–V. The major elements of the five SCCmec types (ccr genes, IS431, IS1272, mecA, mecI, mecRI, orfX, pI258, pT181, pUB101 and Tn554) are shown, as are the six loci (A–F) used for SCCmec typing according to the method of Oliviera et al. [67].*

Phenotypic expression of methicillin resistance is variable, and each MRSA strain has a characteristic profile of the proportion of bacterial cells that grow at specific concentrations of methicillin [68]. Expression of resistance in some MRSA strains is regulated by homologues of the regulatory genes for *blaZ*. These genes, *mecI* (repressor) and *mecR1* (inducer), regulate the *mecA* response to β-lactam antibiotics in a fashion similar to that of the regulation of *blaZ* by the genes *blaR1* and *blaI* upon exposure to penicillin (Figure 2).

In fact, the DNA sequences bound by the repressor genes to achieve inhibition of gene activation are identical. The sequence homology of *mecI-mecR1* with the *blaR1-blaI* regulatory genes results in the induction of *mecA* expression from this alternative system. Deletions or mutations in *mecI* or the promoter region of *mecA* result in constitutive expression rather than variable expression of *mec* [60]. Rosato et al. [69] have recently found that either *mecI* or *blaI* must be functional in all MRSA, and they suggest that this may be a protective mechanism preventing overproduction of a toxic protein. An

additional series of genes, the *fem* genes (**f**actor **e**ssential for resistance to **m**ethicillin resistance), play a role in cross-linking peptidoglycan strands and also contribute to the heterogeneity of expression of methicillin resistance [60].

Since no homologue of *mecA* exists in methicillin-susceptible staphylococci, it has been assumed that *mecA* was acquired from one of several coagulase-negative staphylococcal species [68]. Schnellmann et al. [70] identified a *mecA* gene in a methicillin-sensitive *Staphylococcus sciuri* with 88% homology on the amino acid level to MRSA. Transduction of the *S. sciuri mecA* into an MSSA resulted in increased resistance to methicillin coupled with the detection of PBP2a [70]. These studies therefore suggest one possible source of the *mecA* element in *S. aureus*. Hiramatsu et al. [71, 72] have speculated that the simultaneous detection of the new type IV *SCCmec* in different geographic regions of the world potentially reflects its enhanced mobility and multiple simultaneous transmissions from another coagulase-negative staphylococcus.

European antimicrobial resistance surveillance (EARSS) data indicate that MRSA proportions vary from less than 1% in the north to over 50% in southern Europe countries in 2008 [73]. In the northern part of Europe, MRSA rates were below 5%, except for Lithuania and Latvia (Figure 4).

Less than half of the countries (n=13) reported MRSA proportions equal or higher than 25%. Some Mediterranean and Balkan countries and the British Isles, except for France, Bosnia and Herzegovina, and Bulgaria could be included in this category.

Portugal and Malta had proportions of over 50%, these countries still showed a significant increase. MRSA proportions however increased in Portugal and Switzerland [73].

Ireland, Israel, Italy, UK and Romania showed a consistent decrease over the last four years (IE from 41.8% in 2005 to 33.1% in 2008, IL from 41.5% in 2005 to 35.5% in 2008, IT from 37.1% in 2005 to 33.5% in 2008, UK from 43.5% in 2005 to 30.7% in 2008 and RO from 59.8% in 2005 to 33.3% in 2008). [73]

Luxembourg showed a significant decrease, from 21% in 2007 to 9% in 2008. However, a significant increase was observed for Norway (from 0.0% to 0.6%), Denmark (from 0.28% to 2.3%) and Finland (from 0.95% to 2.9%) since 1999. For the first time since eight years, Iceland reported a single case of MRSA bloodstream infection in 2008. [73]

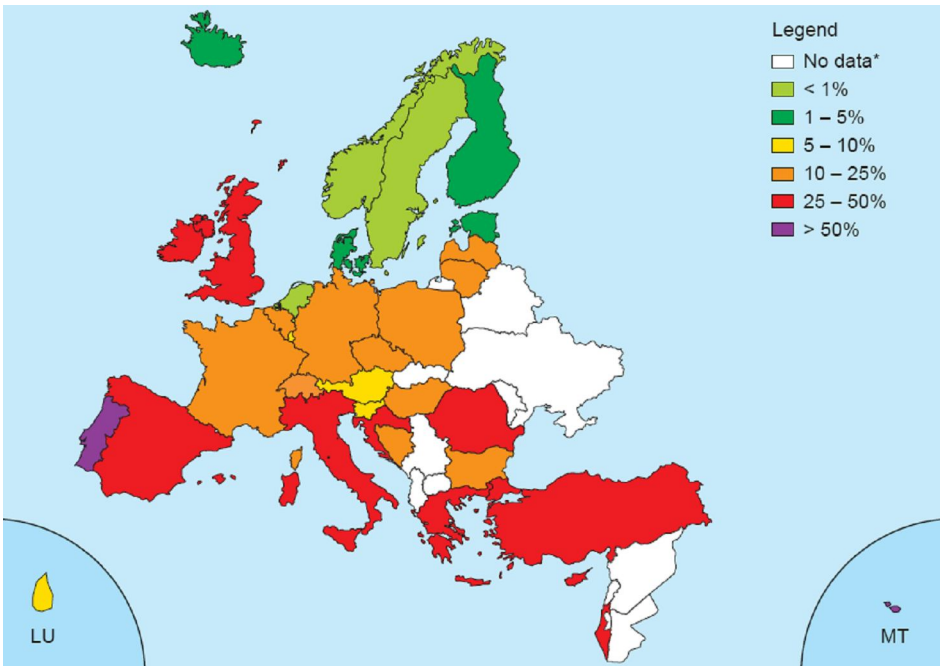


Figure 4. *Proportions of MRSA in invasive infections in 2008 in Europe. Obtained from the European Antimicrobial Surveillance System (EARSS) (www.rivm.nl/earss/).*

In Latvia, MRSA rates continue to decrease strongly, from 25% in 2004 to 13% in 2008, while decrease in Lithuania reached almost 2% (from 13.2% in 2006 to 11% in 2008). [73]

2.2.2. Glycopeptides

Vancomycin is currently the drug of choice for the treatment of MRSA, but its use is being compromised by the recent emergence of vancomycin-intermediate or –resistant *S. aureus* (VISA and VRSA) strains. In 1997, the first report of VISA came from Japan, and additional cases were subsequently

reported in other countries [74, 75, 76]. Finally, in 2002, MRSA with vancomycin resistance was isolated from two independent patients who were co-infected with vancomycin-resistant enterococci [77, 78]. In 2004, a third case was reported in New York, USA [79].

Overall, nine VISAs and one confirmed VRSA in Austria were reported to the EARSS database in 2006. Five countries reported vancomycin intermediate resistant *S. aureus*: Austria (n=1), Belgium (n=2), France (n=1), Ireland (n=1) and United Kingdom (n=4). Four confirmed VISAs and no VRSA were reported to the EARSS database in 2007. Vancomycin intermediate resistant *S. aureus* were reported by France (n=1), Ireland (n=1) and The Netherlands (n=2). Numbers of VISA and VRSA have increased in 2008. Two VRSA cases were found in Australia. VISA were reported by Australia (n=8), Hungary (n=2) and the UK (n=1) [73].

Several studies on *S. aureus* carriage in community demonstrate, that none of the isolates showed reduced susceptibility to this glycopeptide [12, 13, 56, 80, 81].

2.2.3. Rifampin

S. aureus resistance to this antibiotic is not high. The resistance of staphylococci isolated from clinical specimens range from 0 % to 6.7% [23, 58, 82, 83, 84, 85, 86]. Dailey et al. screened all patients who were admitted to the hospital for *S. aureus* and MRSA carriage [87]. It was determined that *S. aureus* resistance to rifampin ranged from 0% in 1999 to 1% in 2002. The similar findings were reported in other *S. aureus* carriage studies. Pavilonytė et al. [13] collected 61 patient admitted to the hospital. Resistance rate of *S. aureus* isolates of rifampin was 0%. Oguzkaya-Artan et al. [88] evaluated 200 children. All isolated *S. aureus* strains were susceptible to rifampin as well. Choi et al. [56] included 346 participants into *S. aureus* carriage study. 81 *S. aureus* strains were isolated. Only one strain (1.2%) was resistant to rifampin.

Despite the fact that rifampin is one of the most potent antistaphylococcal agents in vitro, with MICs of 0.03 μg/ml and lower, resistance readily arises by

mutation in the *rpoB* gene (with encodes the beta-subunit of RNA polymerase), resulting in a lower affinity for the antibiotic [89]. Therefore, when rifampin is used for the treatment, it is often used in combination with other antibiotics (e.g., vancomycin, fusidic acid, gentamicin) [83].

2.2.4. Fluoroquinolones

Fluoroquinolones originated in the 1960s as a by-product of the synthesis of antimalarial quinine. Fluoroquinolones were initially introduced for the treatment of Gram-negative bacterial infections in the 1980s. However, they have also been used to treat bacterial infections caused by pneumococci and staphylococci. Quinolone resistance among *S. aureus* emerged quickly, more prominently among the methicillin-resistant strains. The prevalence of quinolone (ciprofloxacin, norfloxacin) resistance in hospital MRSA is now close to 90% [90]. Some investigators from Spain found, that the prevalence of ciprofloxacin resistance in HA-MRSA was 88.8% in 2002 [85] and 96.7% in 2007 [86]. In a Canadian hospital, ciprofloxacin resistance was detected in 87% of MRSA isolates [91]. Several investigators from Lithuania have demonstrated ciprofloxacin resistance from clinical specimen in MSSA from 0.7% [82] to 8% [58] and from 75% [58] to 81.25% [82] in MRSA.

From 80% to more than 90% of CA-MRSA isolates are ciprofloxacin-susceptible [23, 92] and this relate also to the new fluoroquinolones, such as moxifloxacin, gatifloxacin and gemifloxacin. Naimi et al. [23] compared ciprofloxacin resistance in CA-MRSA and HA-MRSA strains and demonstrated 21% and 84% resistance respectively.

Resistance to ciprofloxacin differs in *S. aureus* carriage studies. Choi et al. [56] identified 13.6% of *S. aureus* intermediate resistant to this quinolone. But in similar studies performed in Lithuanian [12, 13] ciprofloxacin resistance was not found (0%).

Quinolone resistance results from chromosomal mutation. It proceeds by two types of mechanisms including over expression of the efflux pump NorA

and structural mutations in the quinolone targets topoisomerase IV and DNR gyrase genes [93].

2.2.5. Aminoglycosides

Aminoglycosides such as gentamicin, kanamycin, tobramycin, amikacin, and streptomycin are commonly used antimicrobial agents in the treatment of infections caused by both gram-negative and gram-positive organisms.

Several studies have evaluated the prevalence of nasal carriage of *S. aureus* and demonstrated low rates of gentamicin and kanamycin resistance. Pavilonytė el al. [12] found, that all MSSA and MRSA strains, isolated from children were susceptible to gentamicin. The same results were found in the study by Oguzkaya-Artan et al. [88]. Saxena et al. [80] studied 133 healthy residents. Resistance to gentamicin was found in 13 (76%) of 17 MRSA strains.

In 2008, Perez-Vazquez et al. [86] reported that MSSA and MRSA resistance to gentamicin in twenty-one Spanish hospital was 2.6% and 17.8% respectively. Cuevas et al. [85] studied 134 HA-MRSA isolates. 42.5% strains were resistant to gentamicin. Naimi et al. [23] compared 106 CA-MRSA and 211 HA-MRSA resistance patterns. Obtained data demonstrated that CA-MRSA and HA-MRSA resistance to gentamicin was 6% and 20% respectively. Grigaitė el al. isolated 221 *S. aureus* strains from burn wounds [58]. The data showed that 30% and 28% of isolated MSSA strains were resistant to gentamicin and kanamycin respectively. Higher rates of gentamicin and kanamycin resistance were demonstrated in MRSA strains, 96.5% and 98.0% respectively [58].

2.2.6. Tetracyclines

The tetracyclines are a class of structurally related compounds and are characterized by four interlocking six-carbon rings. These antibiotics inhibit protein synthesis by binding to the 30S subunit of the ribosome and block the entry of aminoacyl-tRNAs into the acceptor site [94, 95]. In general, the

tetracyclines are bacteriostatic for the staphylococci despite a mechanism of action similar to that of the aminoglycosides.

Schmitz et al. [96] investigated the tetracycline (TE) susceptibility of 3052 *S. aureus* isolates collected from April 1997 to February 1999 at 25 university hospitals participating in the European SENTRY program. Susceptibility to TE was 89.7% in the MSSA isolates and 42.9% in the MRSA isolates. Petrelli et al. [97] recently found that 6.3% of MRSA and 14.3% of MSSA strains were resistant to TE.

Limited data suggest that CA-MRSA isolates may be susceptible to TE (92%) [23]. In *S. aureus* carriage studies performed in Turkey [88] and Malaysia [56] resistance to TE was from 8.3% and 5.8% respectively.

It has been determined that two mechanisms play a role in resistance to tetracyclines in staphylococci [95]:

- active reflux that emerges with the acquisition of plasmid-based genes such as *tet*(K) and *tet*(L);
- ribosomal protection mediated by transposon- or chromosome-located *tet*(M) and *tet*(O) determinants.

It is well known that the majority of *tet*M-positive strains also contain *tet*K and that MRSA isolates possess the *tet*M or *tet*K genotype and that both drug efflux and ribosomal protection can be induced in vitro in *S. aureus* [98, 99].

The *tet*K gene, almost exclusively found on pT*181* and related plasmids, encodes a tetracycline efflux pump. Far less frequently found in staphylococci is *tet*L, which encodes an efflux pump similar in structure to that of *tet*K. A third tetracycline-resistance gene, *tet*M, occurring in *S. aureus,* is virtually identical to the *tet*M gene originally identified on the transposons Tn*916* and Tn*1545* in enterococci. However, in *S. aureus tet*M is apparently found at a constant chromosomal location and lacks the ability to transpose. While pT*181* plasmids are ubiquitous, the *tet*M determinant is probably more clinically significant in that it encodes resistance to all tetracyclines, including minocycline and doxycycline. [100]

Trzcinski et al. [99] investigated 66 MRSA isolates resistant to tetracycline, mainly from Eastern Europe. Their results showed that 36.4% of MRSA strains displayed *tetM* only, 31.8% had *tetK* only and 31.8% had both *tetK* and *tetM*.

In the Schmitz et al. [96] study, the *tetM* gene was detected in 304/400 (76%) and the *tetK* gene in 292/400 (73%) tetracycline-resistant MRSA isolates. Approximately half of the MRSA isolates (202/400, 50.5%) carried both the *tetM* and *tetK* genes. The *tetL* gene was detected in 6/400 (1.5%) MRSA isolates. The *tetM* gene was detected in 20/200 (10%) and the *tetK* gene in 192/200 (96%) tetracycline-resistant MSSA isolates. Twelve (6%) of the MSSA isolates carried both the *tetM* and *tetK* genes. The *tetL* gene was not detected in any of the MSSA isolates. The *tetO* gene was not found in any of the isolates tested. These results showed that the *tetM* and *tetK* genes were the most prevalent single tetracycline resistance determinants in MRSA and MSSA, respectively, and that the combination *tetM*/*tetK* was approximately 10 times more prevalent in tetracycline-resistant MRSA isolates than in tetracycline-resistant MSSA isolates.

Petrelli et al. [97] studied *tetK, tetM, tetL* and *tetO* to determine the TE resistance genotypic distribution. None of the tested isolates were positive for *tetL* or *tetO*. Two TE-resistant isolates carried *tetK*, while one MRSA strain was positive for *tetM*. One strain contained both *tetK* and *tetM*. The intermediate resistance phenotype did not harbor any Tet determinants.

2.2.7. Macrolides-Lincosamides-Streptogramin B

This group comprises separate classes of antibiotics: macrolide, lincosamide, and streptogramin (MLS) antibiotics are chemically distinct inhibitors of bacterial protein synthesis. Resistance proceeds by any of the three classical mechanisms: modification of the bacterial drug target, modification-inactivation of the drug itself, and decreasing intracellular accumulation of the drug. [34, 101]

Erythromycin was introduced in 1952 as the first macrolide antibiotic. Unfortunately, within a year, erythromycin-resistant staphylococci from the United States, Europe, and Japan were described [102]. Among CA-MRSA isolates, susceptibility to erythromycin ranges from 10% to 100%, with larger series generally reporting susceptibility rates of more than 50% [23, 80, 103].

Several studies have evaluated the prevalence of nasal carriage of *S. aureus* and demonstrate low rates of erythromycin and clindamycin resistance. Creech el al. [31] found 26% clindamycin resistance which was either constitutive or inducible. In Oguzkaya-Artan et al. [88] study this ratio was of 8.3%. A number of reports have documented lower (from 0% to 3.7%) resistance to erythromycin and clindamycin [12, 56].

In 2008, Perez-Vazquez et al. [86] reported that *S. aureus* resistance to erythromycin and clindamycin in twenty-one Spanish hospital was 81.1% and 31.1% respectively. Cuevas et al. [85] studied 134 HA-MRSA isolates. 59.7% strains were resistant to erythromycin, 42.5% – resistant to clindamycin. Among the isolates resistant to erythromycin and clindamycin, resistance was constitutive in 61 (67.0%) isolates, inducible in 11 (12.1%) isolates, and showed the M-phenotype (resistance to erythromycin, but susceptibility to clindamycin) in 19 (20.9%) isolates. Higher rates of erythromycin and clindamycin resistance were demonstrated by Naimi et al. [23] 91% and 79% respectively.

The first mechanism of macrolide resistance described was due to posttranscriptional modifications of the 23S rRNA by the adenine-*N*-*6*-methyltransferase. Target modification alters a site in 23S rRNA common to the binding of MLS_B antibiotics [104]. Modification of the ribosomal target confers cross-resistance to MLS_B antibiotics (MLS_B resistant phenotype) and remains the most frequent mechanism of resistance. In general, genes encoding these methylases have been designated *erm* (erythromycin ribosome methylation) and are located on mobile elements such as transposons (e.g., Tn*554* and *ermA*) [101] or plasmids (e.g., pE194 and *ermC*) [104]. *ermA* was first described in 1969 in a clinical strain, with inducible resistance [105]. The

second type of macrolide-lincosamide-streptogramin B resistance gene, *ermC*, was described by Iordanescu in 1976 [106].

Expression of MLS resistance in staphylococci may be constitutive or inducible. When expression is constitutive (MLS_Bc), the strains are resistant to all MLS_B type antibiotics. When expression is inducible (MLS_Bi), the strains are resistant to 14- and 15-membered macrolides only. A double disc diffusion test, called the D-test, has been devised in the routine laboratory [34, 107]. This test identifies inducible resistance that might highlight mutational clindamycin constitutive resistance. The 16-membered macrolides, the commercially available lincosamides, and the streptogramin antibiotics remain active against bacteria with this resistance mechanism.

In a Otsuka et al. study [108] of erythromycin and clindamycin resistance in *S. aureus* strains, found that MLS_B constitutive resistance (61.3% MRSA, 1.3% MSSA) and the *erm*A gene (95.0% MRSA, 53.3% MSSA) were predominant among MRSA strains, while MLS_B inducible resistance (38.7% MRSA, 94.0% MSSA) and the *erm*C gene (11.5% MRSA, 42.0% MSSA) were more prevalent among MSSA strains. Ardic et al. [109] also reported that the presence of the *erm*A gene was determined at a higher level (71.4%) in MRSA isolates. On the other hand, the *erm*C gene was determined in 64.3% of MRSA strains. Both erythromycin resistance genes existed in 14 (50%) of MRSA isolates. These data are consistent with some previous reports [110, 111].

Active macrolide efflux has been reported in Gram-positive cocci [104, 112, 113, 114, 115]. In *S. aureus* efflux is mediated by *msrA* and *msrB* genes [113, 116]. They belong to the complex ATP-binding cassette (ABC) transporter set of genes [113, 116], and confer resistance to both macrolides and streptogramins B (MS-resistance phenotype). In contrast to major facilitators, ABC transporters utilize ATP hydrolysis as a source of energy for active efflux. In addition to the *msrA* afflux pump, two efflux systems have been identified in staphylococci that confer resistance to streptogramin A antibiotics, *vga* and *vgaB* [104].

A variety of other mechanisms which usually confer resistance to only macrolides, lincosamides, or streptogramins (A or B) have been described [102, 104, 117], with diagnostic tests being available for only some of the genes encoding these mechanisms. The genes encoding enzymes which hydrolyze streptogramin B (*vgb* and *vgbB* genes) or modify the antibiotic by adding an acetyl group (acetyltransferases) to streptogramin A (*vat*, *vatB*, *vatC*, *satA*, and *satG* genes) have been described and detected in different assays.

Unlike target modification, which causes resistance to structurally distinct antibiotics, enzymatic inactivation confers resistance mostly only to structurally related drugs. The enzymes EreA and EreB (*ereA* and *ereB* genes), which hydrolyze the lactone ring of the macrocyclic nucleus, and the phosphotransferases (type I [*mphA*] and type II), which inactivate macrolides by introducing a phosphate on the 2'-hydroxyl-group of the amino sugar, have been found in *S. aureus* [108].

Lincomycin nucleotidyltransferases *linA*' have also been identified in *S. aureus* [102, 104, 117].

2.2.8. Fusidic acid

Fusidic acid (FA) is a narrow-spectrum bacteriostatic antibiotic, particularly active against staphylococci. Topical FA is commonly used in the management of atopic eczema and skin infections, such as impetigo. FA also systemically accumulates in the bones. Consequently, systemic FA is one of the treatments of choice for osteomyelitis [83].

Since the introduction of FA in the 1960s, there have been scattered reports of increased resistance [118, 119] but the majority of studies have reported a low prevalence of FA resistance among *S. aureus* and therefore resistance has not been regarded as a present or potential problem [120]. Recently, however, a number of groups have reported FA resistance to be on the increase [121, 122].

In the Scandinavian countries, FA has been used extensively for topical treatment of superficial skin infections for many years, irrespective of the

etiology. Until recently, <10% of *S. aureus* isolates from Norway were susceptible to FA [123]. However, Tveten et al. [124] recently demonstrated an increase of *S. aureus* resistance to FA, i.e. an increase from 3% in 1992 to 38% in 2001.

A review of 28 clinical centers shows that the incidence of resistance of *S. aureus* to FA between 1995 and 2001 has risen from 8.1% to 17.3% in the community in the UK [125]. A study by Shah et al. [126] found that 50% of dermatology patients had *S. aureus* isolates resistant to FA.

In *S. aureus* carriage studies performed in Lithuania [12, 13], Turkey [88], Australia [87] and Malaysia [56] resistance to FA ranged from 0% to 5% (0%, 0%, 2% and 5% respectively).

2.2.9. Mupirocin

Mupirocin has come into wide use as a topical agent for the treatment of Gram-positive infections and more recently has been employed successfully for elimination of staphylococci, particularly MRSA, from the nares and plays a crucial role in infection control protocols [127]. Mupirocin (pseudomonic acid A) is an analogue of isoleucine [128].

Several studies have proven mupirocin prophylaxis as being effective in preventing nosocomial *S. aureus* infections in randomized, placebo-controlled trials among dialysis and surgical patients and patients with recurrent skin infections [129, 130]. The resulting widespread use has lead to mupirocin resistance [131].

Resistance to mupirocin is nearly always related to MRSA. Kresken et al. [132] isolated 787 isolates of *S. aureus*. The majority of MRSA isolates exhibited low-level mupirocin resistance. It was detected in 22 of the 163 MRSA (13.5%) isolates. Five MRSA (3.1%) isolates exhibited high-level mupirocin resistance. Of the 624 MSSA isolates, mupirocin resistance was detected in 3 (0.5%), of which 1 (0.2%) was low-level resistant and 2 (0.3%) were high-level resistant.

Other investigators from Greek hospitals also found, that of 150 *S. aureus* isolated during 2000-2002, 1.3% were mupirocin resistant [133]. In New Zealand where mupirocin was available without prescription in the 1990s, the rate of high-level resistance in *S. aureus* was 14.2% in 1999 occurring mainly among community-acquired isolates [134]. A recent study from Canada showed that high-level mupirocin resistance in MRSA increased from 1.6% between 1995 and 1999 to 7% in the period 2000-2004 [91]. A rate of 8.6% was reported in 2007 from a study in surgical intensive care unit in the United States, where the isolates were predominantly healthcare-associated [135]. In a Spanish hospital, mupirocin resistance was detected in 11.3% of isolated MRSA strains [136].

Several studies on *S. aureus* carriage in the community demonstrated that mupirocin resistance in *S. aureus* range from 0% to 2.5% [56, 87, 88].

Thus HA-MRSA strains are typically multi-drug-resistant. Resistance of CA-MRSA strains are less expressed and they are susceptible to more classes of drugs. All CA-MRSA isolates are resistant to beta-lactams and many are resistant towards macrolides/azalides. Evidence suggests that CA-MRSA – the newest staphylococcal threat – will be increasingly prevalent in the near future. Moreover, although most strains are now susceptible to many non-β-lactam antibiotics, this may change due to exposure to multiple antimicrobials in various settings. Epidemiological studies are needed to specify if the patient populations are likely to harbor this pathogen. Alternative treatment options to β-lactams must be assessed under investigational trial conditions before specific treatment guidelines to be recommended.

2.3. Colonization and carriage of *S. aureus*

S. aureus is a commensal microorganism and a pathogen capable of causing a wide range of human diseases. It is spread in hospitals and in the community mainly by direct contact but also by aerial dissemination to the

environment from carriers and infected patients, especially from the skin. Transmission is therefore often seen among close contacts, e.g. among family members, who also tend to have similar carrier status [137]. *S. aureus* is easily transmitted and has the ability to survive under stress conditions (i.e. dryness, high salt concentration, and low pH), therefore making hygiene, especially hand hygiene pivotal in diminishing transmission [138].

Numerous sites may be colonized with this microorganism, including skin, nasopharynx, perineum, vagina, rectum, gastrointestinal tract, and the axillae, but the anterior nares are the main ecological niche for *S. aureus*. Three patterns of carriage can be distinguished: persistent carriers, intermittent carriers, and non-carriers. [139]

Longitudinal studies show that approximately 20% (range 12–30%) of the population is constantly colonized with methicillin-susceptible *S. aureus*, and about 30% (range 16–75%) of the population may show transient colonization. Finally, between 5 and 69% almost never carry *S. aureus* and are called non-carriers [139, 140]. The very wide ranges found in the proportions of intermittent and non-carriers result from the use of different culture techniques, different populations being studied, and the use of different interpretation guidelines.

Genotyping data reveal that persistent carriers usually carry only one identical *S. aureus* strain over time and that intermittent carriers commonly carry different strains over time. [141]

Most infants become colonized shortly after birth, but carriage decreases with age (63.8% at the first month, 28.2% at six months) [142]. Young children tend to have higher colonization rates, probably because of their frequent contact with respiratory secretions [143]. The pattern of carriage in many people changes between the age of 10 and 20 years [140].

The persistent carriage rate is increasing to 50-55% in patients on hemodialysis, among insulin-dependent diabetes mellitus patients and in injecting drug users. In patients with skin lesions or atopic dermatitis, a much

higher nasal carriage rate is found (about 70%), and in skin lesions more than 90% of patients are colonized with *S. aureus*. [141]

Rates of MRSA colonization remain low among healthy persons. Rates of carriage among children with no risk factors for MRSA colonization have ranged from 0.8% to 3.0% [144, 145]. A MRSA carriage rate among children of 1.1% was found in a community-based study in Lithuania [12].

The prevalence of CA-MRSA in adults is currently low worldwide, but appears to be increasing. In a study of 225 healthy adults in New York City, none were colonized [146], while only 2 (0.24%) of 833 urban poor adults in San Francisco were found to carry MRSA in the nasopharynx [147]. A prevalence of 1% was found among 469 rural American Indians and was associated with recent antimicrobial use and large household [148]. A higher prevalence may be observed in settings that facilitate cross-transmission of bacteria, such as day-care centers and correctional facilities [143, 149]. In the United States, the prevalence of MRSA in the general population was also estimated from the 2001-2002 National Health and Nutrition Examination Survey (NHANES) data. The overall colonization rate of 5000 non-institutionalized persons with methicillin-sensitive *S. aureus* was 31.6% and 0.84% with MRSA. Analysis of 57 studies on CA-MRSA prevalence among hospitalized patients and persons in the community revealed that most individuals with CA-MRSA had at least one risk factor for MRSA [150]. This study suggested that the prevalence of CA-MRSA among persons without risk factors is 0.24%. Recent studies showed that the prevalence of CA-MRSA in Europe is 0.03-1.5% [151]: 0.7% - in Portugal, 0.1% - in Switzerland and 0.03% - in The Netherlands.

Asymptomatic colonization with *S. aureus* is far more common than infections. Nevertheless, colonization of the anterior nares with *S. aureus* has been shown to be a risk factor for invasive and surgical wounds infections [139]. This relationship was first established in the late 1950s. Casewell [152] reported that, approximately 30 – 40% of patients are *S. aureus* carriers on admission to hospital and this proportion increases during hospital stay.

Studies conducted in hospitals show a prevalence of MRSA carriage on admission ranging between 1% and 12% [153-155]. Carriage rate of *S. aureus* on the day of admission to Kaunas hospital in Lithuania was 67.3%, 4.9% of them were MRSA [13]. von Eiff et al. [156] found that in 12 of 14 patients (82%) the strain causing bacteremia was identical to the strain that had previously been recovered from the anterior nares. On the other hand, *S. aureus* – related death was four times more likely in non-carriers who developed an infection [157].

Lye et al. [158] compared MRSA carriers with MSSA carriers; they found a higher rate of peritonitis and exit site infection in MRSA carriers. Moreover, in the group of MRSA carriers, there were a significantly larger number of catheter losses and continuous ambulatory peritoneal dialysis (CAPD) patient dropouts. Muder et al. [159] studied the consequences of MRSA carriage in a long-term care facility and found that MRSA carriers were at increased risk for the development of *S. aureus* infections. MRSA colonization confers a three- to 16-fold increased risk for invasive infection compared with MSSA colonization [153, 160, 161]. The results of these studies are summarized in Table 3.

Table 3. Risk of infection following colonization with MRSA compared with MSSA

No. of participants:				OR	95% Cl	Reference
colonized with MRSA	infected with MRSA	colonized with MSSA	infected with MSSA			
26	5	137	2	16.07	2.37 – 173.81	153
20	3	141	6	3.97	0.58 – 20.50	160
63	24	84	8	5.85	2.25 – 16.31	161

Dall'Antonia et al. [162] determined that a protective factor against MRSA colonization is MSSA colonization. Furthermore it is clear that the risk of invasive infection following colonization with MRSA is greater than the risk

of infection following colonization with methicillin-susceptible *S. aureus*. This could be a result of the resistance itself, of an increased intrinsic virulence of MRSA compared with MSSA, or of a more vulnerable category of patients being colonized by MRSA.

Identification of patients likely to be colonized with MRSA on admission is a key to promptly deploying contact precautions. Jernigan et al. [163] estimated that the rate of HA-MRSA transmission was 0.14 transmissions per day; contact precautions lowered this rate sixteen-fold.

The spread of *S. aureus* in healthcare environment often depends on healthcare workers nasal colonization. Healthcare workers carry the organisms on their fingertips; this is believed to be a consequence of nasal colonization and of hand contamination from other patients and the inanimate environment. Passing staphylococci between patients on the hands of healthcare workers is the most frequent way in which these organisms are spread within the healthcare environment. Prevention of these infections requires careful hand washing or sanitization on the part of healthcare workers. [164]

Screening patients for MRSA carriage at hospital admission is widely accepted as an essential part of MRSA control programs and prevention of SSI. The rationale, evidence and strategies for MRSA screening and surveillance are reviewed by Tacconelli [165]. United Kingdom Department of Health recommends to screen all patients admitted to English hospitals for MRSA carriage [166]. However, because of a lack of evidence of its clinical effectiveness and cost-effectiveness, routine screening of all admissions to hospital is not advocated [165]. In some of the countries screening is performed only in the high risk groups of patients or foreign patients groups. The USA recommendations on MRSA suggest the screening of patients before admission to intensive care units, where the endemic level of MRSA cases is observed [167].

According to the research by Bagdonaitė et al. [168], it was found that screening for MRSA carriage on the admission day routinely is performed in 23.3% of hospitals in Lithuania. Only selective screening is applied. In most

cases, the screening is applied to patients who arrived from other hospitals, nursing homes, rehabilitation hospitals or those who had contact with MRSA patients.

The examining of samples taken on the admission day usually takes about 48-72 hours. Only rapid reporting of screening results would improve MRSA control, provided that a clear action plan for positive cases is in place and is being followed. However, in recent years, a number of different microbiological and molecular methods for the rapid detection of MRSA have been described. [165]

An effective culture screening method is direct inoculation of used swabs on a MRSA-selective chromogenic agar. Presumptive MRSA colonies can be confirmed rapidly by latex agglutination with antibodies direct against penicillin-binding protein 2a. This method will usually produce positive results after 24 h of incubation in >95% of true-positive cases. Full antimicrobial susceptibilities will be available on the next day. [169]

PCR methods are now available that can produce same-day results. High sensitivity and specificity have been reported in several studies, with results being available in 2 h [67, 110, 170]. The main barrier to the wide application of molecular screening for MRSA, in comparison with culture-based methods, that PCR tests are costly. [165]

The data on *S. aureus* carriage worldwide is diverse and shows vide variation. According to the data, the rate of *S. aureus* carriage is higher among children as compared to adults. Although the MRSA carriage rate is quite low, it continues increasing in hospitals and the community.

2.4. Risk factors for acquisition of *S. aureus*

A number of risk factors associated with *S. aureus* and CA-MRSA colonization have been identified. It is becoming clear that risk factors for acquiring healthcare-associated and community-acquired MRSA are markedly

different. With a reference to below cited articles the differences are summarized in Table 4.

An increase of CA-MRSA carriage and infections has been documented in several population groups and settings. CA-MRSA can spread by contact with infected skin or personal items, such as towels, linens, clothing, bandages, soap or razors that have been in contact with infected skin [164]. It is more likely to spread in places where there is close contact, such as locker rooms or in the correctional facilities. Investigations have shown transmission through the sharing of common objects, such as athletic equipment, towels, benches, personal items contaminated with MRSA, also through sport related abrasions, lacerations and physical contact [171, 172]. Outbreaks have been reported in football, wrestling, rugby, soccer, fencing, canoeing sports participants [173–175], and groups with close person-to-person contact, such as day care center, outdoor camps attendees [176], jail and prison inmates, and the military [177, 178]. CA-MRSA cases were revealed also in tattoo recipients [179].

Table 4. Summary of risk factors for acquisition of healthcare-associated and community-acquired MRSA

HA-MRSA	CA-MRSA
Resent hospitalization; Close contact with a person who has been hospitalized; Indwelling medical device; Recent surgery; Recent intubation; Renal dialysis; Previous use of antimicrobials; Chronic disease; Nursing home admission.	Inmates in correctional facilities; Native Americans, Native Alaskans, and Pacific Islanders; Children in day care centers; Military recruits; Men who have sex with men; Previous use of antimicrobials; Injecting drug use; Contact sports; Steam bath use; Direct contact with an individual who has a skin infection with CA-MRSA; Tattoo recipients; Homeless people.

Environmental surfaces with which people have bare-skin contact, such as sauna benches, have also been implicated as a possible route of MRSA

transmission. A high prevalence has been noted among non-white race, minority populations, including African Americans, American Indians, Australian aborigines and others [23, 180, 181, 182]. It is associated with lower socioeconomic status, crowded housing conditions and limited access to healthcare. Other population groups with high rates of CA-MRSA infections include men who have sex with men (MSM) and injecting drugs users (IDUs) [183, 184]. Sexually active gay men are up to 13 times more likely than the general population to acquire a highly drug resistant strain of CA-MRSA. Outbreaks of CA-MRSA have been reported from the USA, in MSM [183]. The first reports of CA-MRSA were in injecting drug users in Detroit [184]. Subsequently, many reports on CA-MRSA, especially from the USA, also from other countries, were related to the population of IDUs. There is evidence of the emergence of CA-MRSA in IDUs in Queensland and in New South Wales [185].

Though the relationship between the age and CA-MRSA colonization rate is still not strongly defined, it is thought that younger children may be at higher risk of CA-MRSA colonization than older children [176]. In 2001-2002, the incidence of all culture-confirmed CA-MRSA infections in Atlanta and Baltimore was highest among persons <2 years old [186]. This trend probably occurs because older children generally have a stronger immune system than do younger children.

A variety of risk factors have been linked to the acquisition and transmission of MRSA within the hospital environment; these include prolonged hospitalization or admission to a long-term care facility, peripheral vascular disease, recent antimicrobial therapy, intensive care unit stay, presence of an invasive indwelling device (intravascular or urinary catheter, endotracheal or tracheostomy tube, etc.), open wounds, severe underlying disease (diabetes, liver disease, renal failure) and close contact with colonized individuals [187, 188, 189]. Colonized and infected patients with *S. aureus* comprise the main reservoir of MRSA in healthcare facilities, and the primary

means of patients-to-patients transmission is via carriage on the hands of healthcare workers. [164]

In Lithuania, as well as in other countries, great attention is turned to epidemiological surveillance and control of hospital infections. Proper epidemiological surveillance enables to reduce the frequency of hospital infections, to evaluate the efficiency of control and screening (prophylactic), to adjust and implement them, to increase the safety of patients and staff. [190, 191]

Bagdonaitė et al. [168] analysed the control of MRSA status in Lithuanian hospitals and found that 46.2% of hospitals under study separate the patients with MRSA from others, 11.5% of hospitals appoint special nursing staff for such patients. For MRSA prevention screening of patients is performed only in 23.3% hospitals and screening of staff in 46.4% hospitals (mainly after contact with MRSA). Half of the hospitals have local MRSA control guidelines approved by hospital's manager.

The Institute of Hygiene coordinates the research of hospital infections and the spread of their risk factors in healthcare institutions according to the order (14 November, 2008) of the Health Minister of the Ministry of Health of the Republic of Lithuania No. V-1110 „On the epidemiological surveillance and control of hospital infections" [192, 193]. The program includes 42.6% service providers of health care institutions in 2010. The study analyzes the incidence of hospital-acquired infections, such risk factors as mechanical ventilation, vascular, bladder catheter use, operations, antimicrobials prescribed for treatment and prevention purposes and the extent of the prescription of their classes. According to the Institute of Hygiene in 2010 the overall incidence of nosocomial infections as compared to 2009 decreased by 3.9% [193].

In this field Ašembergienė et al. [194] has performed the study on the risk factors in Lithuanian hospitals. It was found that the highest risk to acquire a nosocomial infection had patients who spent more than 2 weeks in hospital, were treated in intensive care units, those who were given antimicrobial

therapy, or invasive measures were applied: mechanical ventilation, vascular, bladder catheter use, operations.

Previous hospital admission is another risk factor [150]. Furuno et al. [154] showed that hospitalized patients who had previous hospitalization within one year of the current admission had a high risk of developing MRSA.

Risk factor analysis shows that most healthcare-associated infections, whether caused by resistant or susceptible microorganisms, derive from invasive procedures or invasive devices. The vast majority of HA-MRSA bacteremias are intravascular device-related [195, 196]. These devices create openings in the natural protective barrier of the skin, allowing the bacteria to penetrate. Additionally, biofilm formation is likely to occur with indwelling devices, creating communities of *S. aureus* that are resistant to antimicrobials. Ventilator-associated pneumonia [197], surgical site infection [198], and catheter-associated urinary tract infection [199], are reported as well.

The importance of antimicrobial exposure has been highlighted as a risk factor for the acquisition and transmission of MRSA [200]. Administration of antibiotics to which bacteria are resistant predisposes the patient to colonization by that strain as competitive flora are destroyed and more resistant bacteria take their place [8, 201]. A number of different classes of antimicrobials have been implicated and both overall institutional use and individual patient use of antimicrobials increases the risk of MRSA (Table 5).

Muller et al. [200] attempted to define the relative contribution of institutional antimicrobial use and individual antimicrobial use and found that penicillin use at the hospital level and fluoroquinolone use at the individual level increased the risk of MRSA isolation from a clinical specimen. Other studies confirmed an increased risk with fluoroquinolone use [202, 203]. It is likely that the fluoroquinolone effect is mediated by eradication of MSSA and increased expression of adherence factors, both of which may promote colonization by MRSA [204].

Table 5. Risk factors for MRSA: class of antimicrobial therapy used before MRSA infection

Antimicrobial	MRSA (%) (n=121)
All beta-lactam antibiotics	67.8
Fluoroquinolones	41.3
First-generation cephalosporins	40.5
Beta-lactam/beta-lactamase inhibitor combinations	37.2
Vancomycin	24.8
Aminoglycosides	19.0
Macrolides	16.5
Metronidazole	11.6
Trimethoprim-sulfamethoxazole	10.7
Second-generation cephalosporins	8.3
Third-generation cephalosporins	6.6
Penicillins	6.6
Carbapenems	5.8

Adapted from Graffunder EM, Venezia RA. Risk factors associated with nosocomial methicillin-resistant Staphylococcus aureus (MRSA) infection including previous use of antimicrobials. J Antimicrob Chemother. 2002; 49:999-1005. [8]

According to the study by Stefanovič et al. [205] in Lithuania in 2004, it was found that antibiotics were prescribed for 58.3% of children and 33.35% of adults, who turned to outpatients clinics. Most frequent reasons for turning to the outpatients clinics were the respiratory tract infections (95.8% of children and 83.3% of adults). Antibiotics were prescribed without microbiological testing for upper respiratory tract infection etiology. Penicillins were prescribed most frequently, especially the wide spectrum aminopenicillins. Macrolides were also prescribed, as well as tetracycline group antibiotics. [205]

Besides the irrational antibiotic prescriptions in primary healthcare institutions, self-medication with antibiotics among population is also worth mentioning. Beržanskytė et al. [206] has found that 22.0% of the population in

West Lithuania were treating themselves with the remainders of antibiotics at home without medical consultation, or purchased them without prescription. This rate is the highest among other European countries [207].

A growing number of studies have found that MRSA may be recovered from the hospital equipment and from items, such as stethoscopes [208], blood pressure cuffs [208], tourniquets [209], and computer terminals [210]. Hardy et al. [211] performed serial environmental and patient screening cultures using selective media and found that MRSA could be recovered from the environment at every sampling; however, only 3 of 26 patients who acquired MRSA in the hospital acquired them from the environment.

The information presented shows that great attention is paid to the identification and evaluation of *S. aureus* carriage risk factors. However, researches analyzing the risk factors in Lithuania are scarce. Colonization pressure, use of invasive devices and antimicrobial exposure are important risk factors for health-associated acquisition of MRSA. Groups at a high risk of CA-MRSA include children, incarcerated persons or in the military service and participants in team sports.

3. MATERIALS AND METHODS

3.1. Study participants and selection

A *S. aureus* carriage study was performed in two different respondent groups: community group and hospitalized patients group.

Sample size calculations were made using software Winpepi. The method based on a normal approximation to the Poisson distribution, which uses a chi-square test, is described by Breslow and Day [212]; a continuity correction is applied. All sample sizes are rounded up to the next whole number.

For the calculations the following formula was used:

$$SS = \frac{Z^2 * (p) * (1 - p)}{c^2}$$

Where:

Z – Z value (e.g. 1.646 for 90% confidence level);

p – percentage picking a choice, expressed as decimal (0.5 used for sample size needed);

c – confidence interval, expressed as decimal (e.g. 0.04 = ± 4).

Community group

According to the calculations, recommended sample size was 539.

Criteria for inclusion were: currently Vilnius city residents, ≥18 years of age, not hospitalized within the previous 3 years, and not employed in health care service.

The community study group included two different groups: young adults [213], and older adults.

- *Young adults group* consisted of students from Vilnius University Faculty of Physics, Faculty of Natural Sciences, Vilnius College and Agricultural vocational school. The specialties of students included into the study were picked at random. Deans of Academic Affairs issued their permit to perform the study. Students from the Faculty of Medicine were not included

into the study as the lectures and work in hospitals are one of the risk factors for *S. aureus* colonization. The study was carried out in the period from March 2, 2006 to November30, 2007.

Auditoriums at university were picked at random during the lectures. Students were informed about the aim of the study, the criteria of suitability for the study and the procedure to be performed. Students, who met the criteria of the study, filled in the questionnaire and let the samples to be taken from their nose and throat.

- *Older adults group* includes healthy volunteers from National Blood Center, as well as individuals from non-medical state and private sector institutions meeting the age criterion.

The study in the National Blood Center was carried out in the period from March 27, 2008 to September 2, 2008. The respondents meeting the study criteria were examined by their consent. The participants filled in the questionnaires in the presence of a nurse, who afterwards took the samples from nose and throat for *S. aureus* carriage identification.

Private and state non-medical sector institutions were picked at random. The presentations on the aim of the study, the criteria of suitability for the study and the procedure to be performed were given in the institutions by their consent. The respondents, who agreed to participate in the study and met the study criteria, were examined. The study was carried out in the period from January 15, 2007 to March 20, 2008.

All the respondents according to the standard occupational classification (SOC 2000) [214] were grouped into occupational groups. Working participants were grouped as follows:

- Occupational group 1 (OG-1) – top management positions;
- Occupational group 2 (OG-2) – professional administrative or technical job, with great responsibility;
- Occupational group 3 (OG-3) – professional job in trade, service sectors, handling equipment, etc.;
- Occupational group 4 (OG-4) – unskilled labour.

Non-working participants of the study were grouped as follows:

- Occupational group 5 (OG-5) – students;
- Occupational group 6 (OG-6) – retired individuals (including the disabled respondents);
- Occupational group 7 (OG-7) – unemployed, housewives.

Hospitalized patient group

According to the calculations, the recommended sample size was 261. As methicillin resistant *S. aureus* carriage is a relatively rare phenomenon, the number of respondents was tripled to minimize sampling error.

A couple of the largest, multiprofile hospitals in Lithuania were invited to take part in the study: Vilnius University Hospital Santariškių klinikos and Vilnius University Emergency Hospital, which offer emergency and elective health care services. As the latter, for subjective reasons, refused to take part in the study, Vilnius City University Hospital was included. Surgery and Urology departments are of increased risk, therefore, the study was performed in the Abdominal surgery, Angiosurgery, Urology and Nephrology departments of these hospitals.

The respondents included into the study were 18 and older, hospitalized and put into the departments with higher risk of *S. aureus* colonization and those with planned hospitalization time 2 days the least.

On the admission day the department nurse explained the respondents the aim and procedure of the study. On the agreement to participate in the study, each participant filled in the given questionnaire. Afterwards, the nurse took the samples from nose and throat to examine *S. aureus* carriage on the admission day. Samples from nose and throat were also taken from the same respondents on the day of discharge from hospital.

The continuous study in Vilnius University Hospital Santariškių klinikos was carried out from October 22, 2007 to September 10, 2008. The same study in Vilnius City University Hospital was performed from January 15, 2008 to September 5, 2008.

3.2. The study of risk factors influencing *S. aureus* carriage

Community group and hospitalized patient group respondents were interviewed to evaluate *S. aureus* carriage risk factors. Each group respondents filled in the specially adapted anonymous questionnaire on the risk factors determining *S. aureus* carriage.

The risk factors evaluation questionnaires for community and hospitalized patients groups were designed according to the Danish Statens Serum Institute risk factors questionnaires with the permission of the Institute. The questionnaires were translated from English into Lithuanian and vice versa. The questionnaires were also adapted to our study, some questions were added. The applicability of the questionnaires was also tested.

A pilot study was performed (Figure 5):

- In Vilnius University Faculty of Natural Sciences in October, 2006. This study was performed with the Faculty Rector‘s consent. 30 students were questioned (12 first year students, 18 second year students).
- 30 hospitalized patients were questioned in Vilnius University Santariškių klinikos in April, 2007. This study was performed with the director and doctor epidemiologist‘s consent.

It was evaluated during this pilot study whether all the possible answers are included into the questionnaire, whether all of the answer categories are necessary and the respondents understand the questions, whether the size and scope of the questionnaire and the sequence of questions are applicable. Degree of internal consistency for the particular groups of variables in the questionnaire was evaluated using Cronbach alpha coefficient [215]. In the separate statement groups of the community group questionnaire Cronbach alpha varied from 0.667 to 0.700. According to the pilot study the questions were specified and the final versions of questionnaires were constructed. They were used for evaluation of *S. aureus* carriage risk factors in both respondent groups.

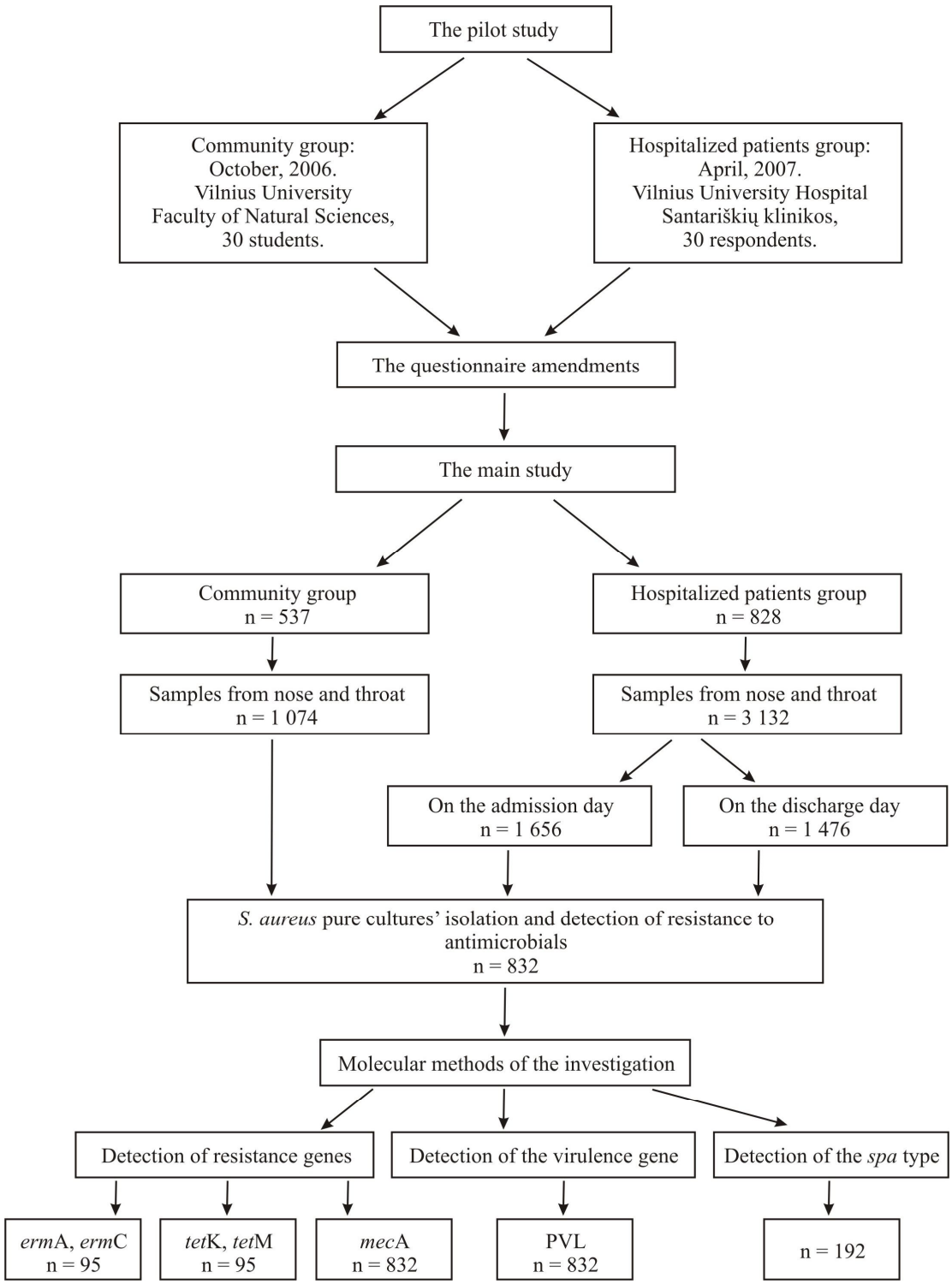


Figure 5. Data collection algorithm for investigating *S. aureus* carriage, resistance to antimicrobials and molecular methods.

Community group questionnaire (Questionnaire-1, APPENDIX 1) was filled in by the respondents themselves with the help of researcher, who explained the unclear questions before taking the samples.

The final version of the questionnaire consisted of two parts.

The first part of the questionnaire consisted of general demographic (gender, age, residence) questions and socio-economic (education, job, position, occupational group) questions.

The second part of the questionnaire consisted of questions regarding the risk factors that could be applied to the respondents and his/her family members. The questions were about skin, staphylococcal and other chronic diseases, the use of antibiotics and visiting outpatients' clinics in the last two years, contacts with the disabled, rest-home or prison residents, work in kindergarten, going in for sports, pets, trips abroad, etc.

The questionnaire consisted of 71 variables. 70 of them were discrete and one continuous. Most discrete variables (n=47) were nominal scale (dichotomous or multichotomous, 32 and 15 respectively), others – ordinal (n=23).

Hospitalized patients had to fill in two questionnaires. The first one was filled in on the admission day (Questionnaire-2, APPENDIX 2). The second questionnaire data was obtained from the clinical records on the discharge day (Questionnaire-3, APPENDIX 3).

The questionnaire for this group of patients was built of two parts as well. The first part consisted of the questions about age, gender, the use of antibiotics, hospitalization in the last year and chronic diseases. This questionnaire consisted of 6 continuous and 11 discrete variables. All variables were nominal, 4 dichotomous and 7 multichotomous.

The second part of the questionnaire was aimed at investigating the risk factors determining *S. aureus* colonization during hospitalization. The questions in this questionnaire were about the presence of surgical operation, antibiotic therapy before and after operation, post-surgical wound infections and their etiology, a catheter application, mechanical ventilation or application

of parenteral nutrition and the time of hospitalization. 3 variables were continuous and 13 – discrete. All categorical variables were nominal. Most of them were dichotomous (n=10), others – multichotomous (n=3).

Analysing the risk factors, the respondents were put into the case and control groups. The case group in the community group consisted of the respondents who had *S. aureus* isolated in their respiratory tract. The control group consisted of the respondents had no *S. aureus* isolated.

Hospitalized patient group respondents were put into the case group who had no *S. aureus* isolated on the admission, but had *S. aureus* isolated on the discharge day from the hospital as well as the respondents who had *S. aureus* isolated on the admission, but on the discharge day they had phenotypically different *S. aureus*. The respondents from the control group had no *S. aureus* isolated neither on the admission, nor on the discharge day from the hospital. Every case had the control matched according to the hospital, gender and age using frequency matching procedure (± 5 years).

3.3. *S. aureus* isolation and detection of resistance to antimicrobials by microbiological methods

To identify *S. aureus* carriers in the community group, on the agreement with the respondents to participate in the study, samples were taken from nose and throat. From hospitalized study respondents samples were taken on the admission and discharge days.

The respondents were classified as *S. aureus* carriers if they had this bacterium at least in one of the screened sites (nose or throat).

3.3.1. Samples and culturing

Bacterial culture samples for *S. aureus* carriage were taken from the anterior nares and throat with sterile cotton-wool swabs (Transwabs, Corsham, United Kingdom). The swab was inserted 1.5 – 2 cm into one naris, rotated

against the anterior nasal mucosa for 3 seconds, and this procedure repeated using the same swab in the second naris. Cultures from the throat were obtained with sterile cotton–wool swabs as well. Swabs were immediately placed into transtubes which contained 5 ml of Stuart's transport medium (Corsham, United Kingdom).

The swabs were labeled. Within 2 hours samples were cultured on mannitol-salt agar and Columbia 5% sheep blood agar. The mannitol salt agar and blood agar plates were incubated at 35^0C (24 h) and both of them for one day at room temperature.

3.3.2. Identification of *S. aureus*

Identification of *S. aureus* was based upon the growth and mannitol fermentation on phenol red-mannitol-salt agar, colony morphology on blood agar, the tube coagulase test results with rabbit plasma, DNase and latex agglutination tests, and microscopy. Morphological and physiological properties, enzyme reactions, and intrinsic resistance to certain antibiotics, have been included in practical identification schemes, which were used in our study.

Isolation and cultivation on mannitol salt agar

Procedure

- Sample or isolated colonies from specimens cultured on mannitol salt agar (Liofilchem, Italy);
- The plates were incubated at 35ºC for 24 hours in an aerobic atmosphere;
- The plates were incubated at room temperature for 24 hours, in the darkness.

Interpretation of results

Mannitol salt agar (MSA) was used for the isolation of presumptive pathogenic staphylococci from clinical and nonclinical materials according to

the recommendations of Chapman [216]. Most of the other bacteria were inhibited by the high concentration (7.5%) of sodium chloride. The fermentation of mannitol by bacteria produced acid products that changed pH and the color of the medium around the colony from pink to yellow.

Typical colonial morphology on MSA was as follows:

Staphylococcus aureus	Small to large surrounded by a yellow zone
Staphylococci other than *S. aureus*	Small to large with red or purple zones
Streptococci	No growth to trace growth
Micrococci	Large, white to orange
Gram-negative bacteria	No growth to trace growth

Investigation of hemolytic activity on blood agar

Procedure

- Sample or isolated colonies from specimens cultured on blood agar (Bio-Rad, France);
- The plates were incubated at 35ºC for 24 hours in an aerobic atmosphere;
- The plates were incubated at room temperature for 24 hours.

Interpretation of results

Abundant growth of most staphylococcal species occurred within 18 to 24 h on blood agar. Since most species cannot be distinguished from one another on the basis of colony morphology within a 24 h incubation period, colonies were allowed to grow for at least an additional two days before the primary isolation plate was confirmed for species and/or strain composition [217]. *S. aureus* produced hemolysin and colonies 1 to 3 mm in diameter, circular, smooth, butyrous consistency and with clear and colorless areas around were selected for further investigation.

Microscopy

All isolated cultures were Gram stained [218]. Cultures which were viewed through a microscope as Gram-positive, were about one micrometer in

diameter, and occurred in clusters resembling grapes were used for further identification.

Coagulase identification test [219]

Procedure

- 0.5 ml of rabbit plasma (Bio-Rad, France) were added to a clean test tube;
- A large loop full of a pure colony added from blood agar plate;
- Mixed thoroughly to suspend organism;
- The tube was incubated at 35^0C in incubator;
- The tube examined after 4 h incubation;

Test negative at 4 h was re-examined at 24 h because a small proportion of strains require longer than 4 h for clot formation.

Interpretation of results

- Positive test – clot formation;
- Negative test – no change, suspension remained homogenous.

Some other species of staphylococci, including *S. schleiferi* and *S. intermedius*, may also give positive results in tube coagulase tests, but are not common isolates from human infections. In addition, rare strains of *S. aureus* are negative in coagulase tests.

DNase identification test [220]

Procedure

- A heavy band streak (2 cm in length) of the test organism was inoculated on the surface of the plate with DNase test agar (bioMerieux sa, France);
- Plates were incubated for 24 hours at 35°C;
- On the next day a drop of 1N hydrochloric acid was added;

Results of this test were used to differentiate *S. aureus* from majority coagulase-negative staphylococci and morphologically similar species.

Whereas the reaction with DNA in the culture in the presence of diluted hydrochloric acid medium forms a hazy precipitate, growth producing deoxyribonuclease appears surrounded by a zone or a clear halo which contains fractions of soluble nucleotides from the degradation of DNA.

Interpretation of results

- DNase positive: when there was clear zone surrounding the inoculums' streak with the rest of the plate remaining opaque. The positive reaction took approximately 5 minutes to form.
- DNase negative: absence of clear halo around the inoculums' streak.

Detection of clumping factor, protein A, capsular polysaccharides antigens by latex agglutination test [169]

Procedure

- A drop of latex test reagent (Pastorex, Staph-Plus, Bio-Rad, France) was deposited into one of the circles of the agglutination card.
- A drop of negative control latex reagent was deposited in another circle.
- 1 to 3 Gram positive, pure and fresh colonies from blood agar were taken with a loop and were emulsified in a drop of latex for 10 seconds.
- The step 3 was repeated for the negative control latex.
- Homogenized by gently rotating the card. Results were red within 30 seconds of beginning the card rotation.

Interpretation of results

- A positive reaction was evidenced by the formation of aggregates with the reagent test only, visible to the naked eye under normal

lighting within 30 seconds of beginning the card rotation. The aggregates of latex particles may be of varying sizes with a more or less cloudy, pink background.

- In a negative reaction, the suspension does not produce any aggregates and retains its milky appearance.

S. lugdunensis and *S. schleiferi* have been reported to possess a fibrinogen affinity factor and may react with the detection test for clumping factor, giving false-positive results, depending on the strains and the isolation medium.

3.3.3. Antimicrobial susceptibility testing

A standard recognized disk diffusion method, published by the USA Clinical Laboratory Standards Institute (CLSI) was used for susceptibility testing of isolated *S. aureus* strains [221]. The disk diffusion method (also known as the Kirby-Bauer method), which we have chosen, is mostly applicable for testing of rapidly growing bacteria. To perform the test, filter paper disk impregnated with a specific amount of antimicrobial agenda were applied to the surface of an agar medium that had been inoculated with a known amount of the test organism. In areas where the concentration of drug was inhibitory, no growth occurred, forming a zone of inhibition around each disk. The diameter of the zones of complete inhibition was measured in millimeters. Results were reported categorically as Susceptible (S), Intermediate (I) or Resistant (R) [221].

All *S. aureus* isolates were tested against oxacillin (1µg), cefoxitin (30µg), rifampin (5µg), kanamycin (30µg), clindamycin (2µg), erythromycin (15µg), streptomycin (10µg), norfloxacin (10µg), fusidic acid (10µg), penicillin (10U), ciprofloxacin (5µg), tetracycline (30µg) and gentamicin (10µg) using commercial discs. Inducible clindamycin resistance was examined by using the double-disk method [222].

Several *S. aureus* strains isolated in one respondent and having different sensitivity spectrum in the extended antibiogram were ranked as phenotypically different.

Procedure of disk diffusion method with paper disks

Preparation of inoculum

Each isolated *S. aureus* strain was cultured onto blood agar to obtain isolated, pure and fresh colonies. After incubation at 35^0C overnight, 2 or more well-isolated colonies were selected with a loop, transferred the growth to a tube of sterile saline and vortexed thoroughly. The bacterial suspension was compared to the 0.5 McFarland standard.

Inoculation procedure

Within 15 minutes after the turbidity of the inoculum suspension was adjusted, sterile cotton swab was dipped into the suspension. Pressing firmly against the inside wall of the tube just above the fluid level, the swab rotated to remove excess liquid. The swab was streaked in three directions over the entire Mueller-Hinton agar (MH) (Bio-Rad, France) surface. Finally, was swabbed all around the edge of the agar surface.

Antimicrobial disks

Within 15 minutes after inoculation using a disk dispenser the appropriate antimicrobial disks (Oxoid Limited, Hampschire, UK) were applied onto the agar. The disks were placed with an equal distance apart from each other and no more than 6 disks were applied on a 90 mm diameter plate.

Recording and interpretation of results

Plates were incubated at 35^0C for 24 h. After incubation, the diameter of the zones of complete inhibition was measured and recorded in millimeters.

Quality control

To verify that susceptibility test results were accurate, each time a new batch of MH was prepared and once weekly a *S. aureus* control strain was included. Zone diameters obtained for *S. aureus* ATCC 25923 were compared with CLSI published limits.

D zone test for Erythromycin and Clindamycin

Procedure

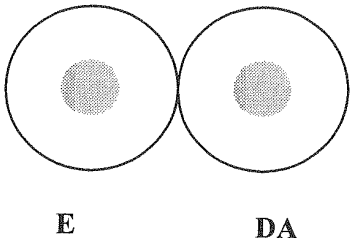
- A suspension of the test organism was prepared in sterile saline equivalent to a 0.5 McFarland standard using isolated colonies;
- Using sterile cotton swab, the standardized organism was inoculated onto an MH agar plate;
- Using a disk dispenser, clindamycin (2µg) and erythromycin (15µg) disks were applied onto the agar 12 mm apart from each other edge to edge;
- Plates were incubated at 35^0C for 24 h. After incubation, the diameter of the zones of complete inhibition was measured.

Interpretation of results

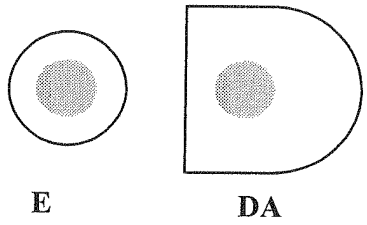
Organisms that showed flattening of the clindamycin (DA) zone adjacent to the erythromycin (E) disk in the shape of the letter D (referred to as a "D" zone) had inducible DA resistance [222].

Examples of zone of inhibition patterns and their interpretation:

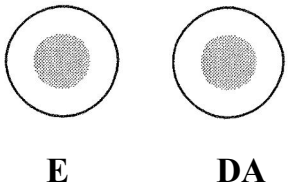
1. Both E and DA are Susceptible;



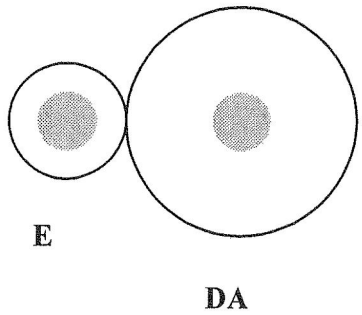
2. Both E and DA are Resistant; “D” zone is positive – inducible $MLS_{B;}$



3. Both E and DA are Resistant;



4. E is Resistant and DA is Susceptible; M phenotype.



Detection of resistance with NeoSensitabs

Antimicrobial tablets Neo-Sensitabs were used to confirm isolated *S. aureus* strains resistance to some antimicrobials. Neo-Sensitabs were standardized according to the MIC-breakpoints recommended by "Susceptibility Testing Standardization Groups" in several countries (e.g. Holland, Norway, Sweden, France, Germany, UK and Denmark) including the CLSI.

The above mentioned tablets were used for detection of resistance to cefoxitin (60µg), rifampin (30µg), kanamycin (100µg), clindamycin (25µg), erythromycin (78µg), streptomycin (100µg), norfloxacin (10µg), fucidic acid (100µg), penicillin (5µg), tetracycline (80µg) and mupirocin (10µg) of 263 randomly selected *S. aureus* strains.

Mecillinam (33µg) tablets were used for confirmation of β-lactamase production [223].

Procedure and interpretation of results

- Each isolated *S. aureus* strain was cultured onto 5 % horse blood agar (Statens Serum Institute) to obtain isolated, pure and fresh colonies. After incubation at 35^0C overnight.
- A suspension of the test organism was prepared in sterile saline equivalent to a 0.5 McFarland standard using isolated colonies;
- A sterile cotton swab was moved back and forth over the blood agar plate in at least two different directions by an inoculating device;
- Neo-Sensitabs (Rosco, Taastrup, Denmark) were applied onto 150 mm plate. Plates were incubated at 35^0C for 24 h. After incubation, semi-confluent growth was observed. The diameter of the zones of complete inhibition was measured.

Determination of minimum inhibitory concentration

The E test (also known as the Gradient Diffusion Method) was used to detect minimum inhibitory concentration. This method is based on the same principle as the disk diffusion method.

The E test was used to detect the resistance to vancomycin, ciprofloxacin and gentamicin of *S. aureus* strains, which by the disc diffusion method were identified to have the resistance to oxacillin/cefoxitin and intermediate resistance to ciprofloxacin and gentamicin.

Procedure

- The inoculum was prepared in Trypticase soy broth (TSB) and compared to the 0.5 McFarland standard;
- The tip of the swab was moved back and forth over the MH agar plate in at least two different directions by an inoculating device;
- Plates with E-test strips (AB Biodisk, Solna, Sweden) were incubated at 35-37^{0}C for 24 h;
- The MIC was read where inhibition growth merges with the strip at the sharp end of the ellipsoid inhibition zone. A magnifying glass was used to help identify the point where complete inhibition had been achieved which was where the MIC had to be read;
- *S. aureus* ATCC 29213 was used for quality control.

3.4. The identification of the resistance to some antimicrobials and virulence gene detection by molecular methods

S. aureus strains resistant to erythromycin and tetracycline had a multiplex PCR performed to detect *ermA, ermC, tetK, tetM* genes. All of the isolated strains during this study were tested for *mecA*, *spa* and *pvl* genes.

Sequence typing of 192 *S. aureus* isolates was performed to study the *spa* gene repeat region. 65 of typed strains were from community group, 64 – from VUHSK and 63 strains – from VCUH.

Staphylococci identified in the community group and included into the study were randomly selected. The following items were examined in hospitalized patients group: 1) all of the MRSA strains isolated on admission and discharge from hospital; 2) randomly selected *S. aureus* strains isolated from patients on the day of discharge, while no strains were isolated on the admission day; 3) randomly selected *S. aureus* strains isolated from the same patient on the admission and discharge days from hospital.

3.4.1. Preparation of chromosomal DNA

A single bacterial colony of the reference strains and isolates was picked in 300µl DNase free H_2O. The suspension was then heated to 95^0C for 10 min. After centrifugation for 6 min at 20000 rpm 4^0C to sediment the debris, the clear supernatant was ready to be used as template DNA in PCR.

3.4.2. Multiplex PCR for *ermA, ermC, tetK, tetM* genes detection

The DNA extract was amplified by PCR in a final volume of 50 µL, containing 0.1 mM of each dNTP, 2.5 U *Taq* DNA polymerase (Invitrogen), 0.2 µM each forward and reverse primers of *ermC, ermA, tetK and tetM gene* (Table 6), 1.5 mM $MgCl_2$, 1 x PCR buffer II and 5 µL of DNA template [109].

Amplification was performed in a DNA Engine DYAD (Bio-Rad, Hercules, CA, USA), with 3 min at 95^0C, followed by 30 cycles of 30 s at 95^0C, 59^0C and 72^0C, with a final 1 min at 72^0C. PCR products were visualized on E-Gels 2%w⁄v (Invitrogen, Carlsbad, CA, USA).

Table 6. Primers used for detection of genes encoding antibiotics resistance

Target gene	F/R	Primer sequence 5'-3'	Size	Reference
ermC	F	AAT CGT CAA TTC CTG CAT GT	299 bp	110
	R	TAA TCG TGG AAT ACG GGT TTG		
ermA	F	AAG CGG TAA ACC CCT CTG A	190 bp	
	R	TTC GCA AAT CCC TTC TCA AC		
tetK	F	GTA GCG ACA ATA GGT AATAGT	360 bp	
	R	GTA GTG ACA ATA AAC CTC CTA		
tetM	F	AGT GGA GCG ATT ACA GAA	158 bp	
	R	CAT ATG TCC TGG CGT GTC TA		

3.4.3. Multiplex PCR for *mecA*, *spa* and *pvl* detection

Each PCR contained 0.45 µM *mecA* primers, 0.18 µM spa primers, 1 µM *pvl* primers (Table 7), 1 × Multiplex PCR Master Mix (Qiagen, Valencia, CA, USA), 0.5 × Q-Solution (Qiagen) and 1 µL of DNA template preparation [224].

Amplification was performed in a DNA Engine DYAD (Bio-Rad, Hercules, CA, USA), with 15 min at 94^0C, followed by 30 cycles of 30 s at 94^0C, 1 min at 59^0C and 1 min at 72^0C, with a final 10 min at 72^0C. PCR products were visualized on E-Gels 2%w⁄v (Invitrogen, Carlsbad, CA, USA).

Table 7. Sequences of primers

Target gene	F/R	Primer sequence 5'-3'	Size	Reference
mecA	F	TCC AGA TTA CAA CTT CAC CAG G	162 bp	67
	R	CCA CTT CAT ATC TTG TAA CG		
spa-1113 *spa*-1514	F	TAA AGA CGA TCC TTC GGT GAG C	Variable 200-600bp	225
	R	CAG CAG TAG TGC CGT TTG CTT		
pvl	F	GCT GGA CAA AAC TTC TTG GAA TAT	80 bp	170
	R	GAT AGG ACA CCA ATA AAT TCT GGA TTG		

The PCR products were vacuum-purified using NucleoFast 96 PCR plates (Macherey-Nagel, Easton, PA, USA) according to the manufacturer's instructions. Sequencing of the amplicons with the PCR primers was performed as described previously using an ABI 3130 sequencer [59]. Ridom StaphType (Ridom, Munster, Germany) and BioNumerics v.4.6 (Applied Maths, Sint-Martens-Latem, Belgium) software, together with the multilocus sequence typing database (http://www.MLST.net), were used for analysis and annotation of the sequences generated from the isolates.

3.5. Study time and location

Samples were collected from March 2, 2006 to September 2, 2008, in Vilnius city, Lithuania. Bacteriological cultures for *S. aureus* and their susceptibility testing was performed at Vilnius University, Infectious diseases, Dermatovenereology and Microbiology department and the National Center for

Antimicrobials and Infection Control (NCAIC) at Statens Serum Institute, Denmark. Panton-Valentine leukocidin, *mecA, ermA, ermC, tetK, tetM* genes detection and *spa* typing was performed at the NCAIC at Statens Serum Institute, Denmark during 3-month period from November 2, 2008 to February 2, 2009.

3.6. The aspects of ethics

This study was approved by the Lithuanian Bioethics Committee. Permissions to conduct biomedical research were received on 2006-09-20 (protocol No. 41) and on 2007-12-27 (protocol No. 60). Written agreement was obtained from Vilnius University Hospital Santariškių klinikos and Vilnius City University Hospital directors. Each participant was informed about this research and its aim. After brief information, a sheet about *S. aureus*, MRSA and this study was given to each participant, and informed written consent was obtained. (APPENDIX 4)

3.7. Statistical analysis

Microsoft Excel program was used for data processing, and statistical packages for the Social Sciences (SPSS) for Windows (Version 13.0; SPSS, Chicago, III, USA) software and WinPepi were used for the statistical analysis of the data. [226, 227]

The extent of carriage was determined by calculating point estimate in percentage and its 95% confidence intervals.

For the continuous variables the standard descriptive analysis was applied using: mean, standard deviation, median, mode, minimal and maximal values. Correspondence to the normal distribution of the above mentioned variables was measured by Kolmogorov-Smirnov test.

When necessary, continuous variables converted to different discrete ordinal variables, using frequency analysis – i.e. grouping is based on the variability analysis of the same data.

For the categorical data (nominal and ordinal scale) p value was calculated using χ^2 statistics. If expected values were less than 5, Fishers exact iterative test was applied.

The significance level was taken at alpha = 0.05. The difference is statistically significant at $p \leq 0.05$. The real p value presented.

For the possible risk factors analysis univariate and multivariate methods were applied. The odds ratio and 95% confidence intervals were calculated to evaluate the risk factors. At the initial phase univariate analysis contingency tables 2 x 2 or 2 x k were generated using SPSS package. The information in the tables was later used for odds ratio calculation by WinPepi package. In case when the variable of interest was ordinal, in order to determine whether there is a time-dose-dependent relations between the exposure and outcome, a Mantel-Haenszel extension of the chi-square test for trend [228] was applied to test for an upward or downward trend in the odds ratios.

Frequencies were expressed as percentage for categorical data, crude odds ratio (OR), 95% confidence interval (CIs) were applied to determine significant associations between risk factor and *S. aureus* carriage. If the lowest 95% CI interval of the OR more than 1 exceeding unity, it was considered that OR is significantly increasing risk at the 95% level. If the OR is less than 1, and the upper 95% CI interval not reaching unity it was considered that the OR significantly decreasing risk at the 95% level. The level of significance was set at 0.05 using two-tailed method. This was calculated for all the potential risk factors with using WinPepi software.

Hierarchical backward elimination procedure was used for logistic regression model building. The variables were selected into the model when the obtained p value was < 0.25. However, epidemiologically important variables were included in to the model despite their statistical significance. The purpose for this was to ensure control of confounding factors. χ^2, Hosmer-

Lemshov test, classification table were used in the model for the assessment of the compatibility of the model. The significance of the coefficient β was assessed by Wald test. The statistical significance level was chosen $\alpha = 0.05$, the results were regarded as statistically significant when $p \leq 0.05$.

The Diversity index [229] was calculated by analyzing *spa* types. The Diversity index calculated by Simpsons formula:

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{s} n_j (n_j - 1)$$

Where:

N – the number of strains in the sample population;

s – the number of types described;

$\boldsymbol{n_j}$ – the number of strains belonging to the j-th type.

For the graphical presentation of the categorical data from the contingency tables, the special mosaic graph was used. A mosaic graph [230, 231] shows the frequencies in an n-way contingency table by nested rectangular regions whose area is proportional to the frequency in a cell or marginal sub table. The display, which is usually asymmetric, emphasizes the variations in the conditional probability of the categories of one variable, given the category of the second variable.

4. RESULTS OF THE STUDY

4.1. CHARACTERISTICS OF THE RESPONDENTS

4.1.1. Community group characteristics

A total of 537 individuals were recruited for this study. The study participants included 212 (39.5%) young adults and 325 (60.5%) older adults (Table 8).

Table 8. The study participants' sociodemographic characteristics

Variable	Study participants				Total (n=537)	
	Young adults (n=212)		Older adults (n=325)			
	n	%	n	%	n	%
Gender						
- Male	113	53.3	174	53.5	287	53.4
- Female	99	46.7	151	46.5	250	46.6
Age						
- Mean	19.8		40.4		32.2	
- Median	20.0		39.0		30.0	
- Mode	19		38		19	
- SD	1.2		11.1		13.3	
- C_v	6.0%		27.5%			
Education						
- uncompleted secondary	20	9.4	16	4.9	36	6.7
- secondary	192	90.6	103	31.7	295	54.9
- vocational school	0	0	17	5.2	17	3.2
- non-university higher ed.	0	0	56	17.2	56	10.4
- university	0	0	133	40.9	133	24.8
Occupational group						
- OG-1	0	0	38	11.7	38	7.1
- OG-2	0	0	88	27.1	88	16.4
- OG-3	0	0	84	25.8	84	15.6
- OG-4	0	0	37	11.4	37	6.9
- OG-5	212	100	0	0	212	39.5
- OG-6	0	0	5	1.5	5	0.9
- OG-7	0	0	73	22.5	73	13.6

The first group includes students from Vilnius University Faculty of Physics – 81 (38.2%), Vilnius University Faculty of Natural Sciences – 96 (45.3%), Vilnius College – 15 (7.1%) and agricultural vocational school – 20 (9.4%).

The second group includes volunteers from National Blood Center – 179 (55.1%), also individuals from non-medical institutions, 102 (31.4%) participants from state institutions and 44 (13.5%) participants from private sector.

The distribution of participants according to gender, age, education and occupation is presented in Table 1. There were 250 (46.6%) female and 287 (53.4%) male participants. The mean age of the participants was 32.2 years (median – 30). The youngest participant of the study was 18, the oldest – 72 years of age. The distribution of age in both groups of participants is presented in Figures 6 and 7.

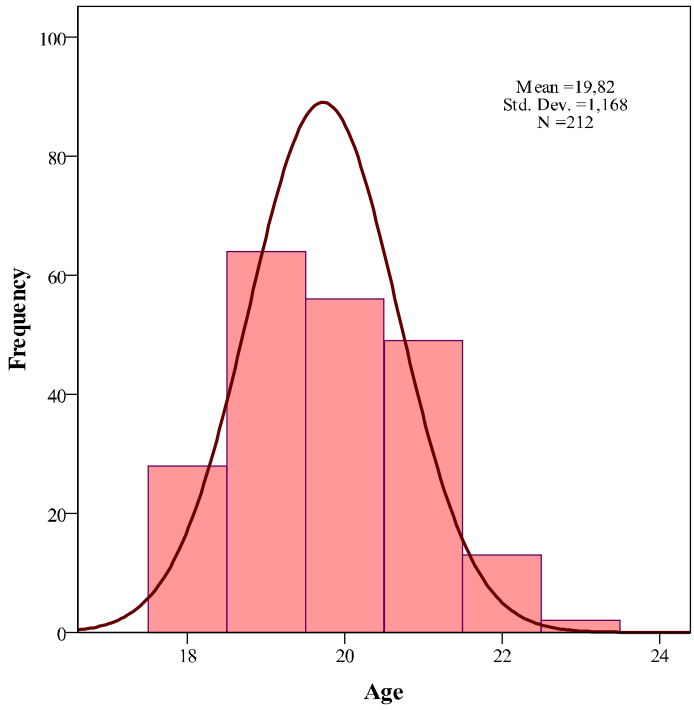


Figure 6. Histogram of the age distribution of younger adults' respondents.

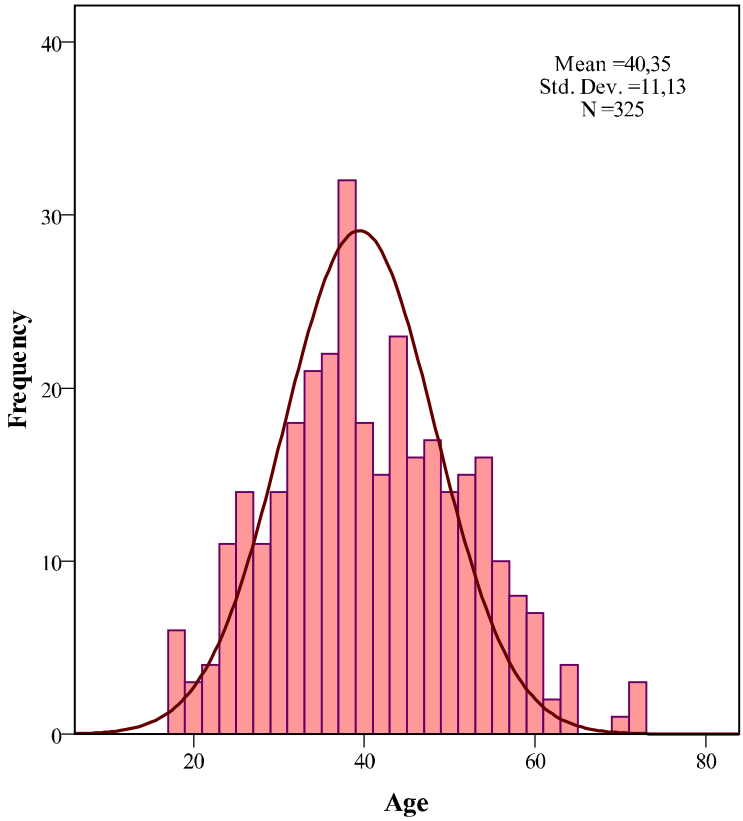


Figure 7. Histogram of the age distribution of older adults' respondents.

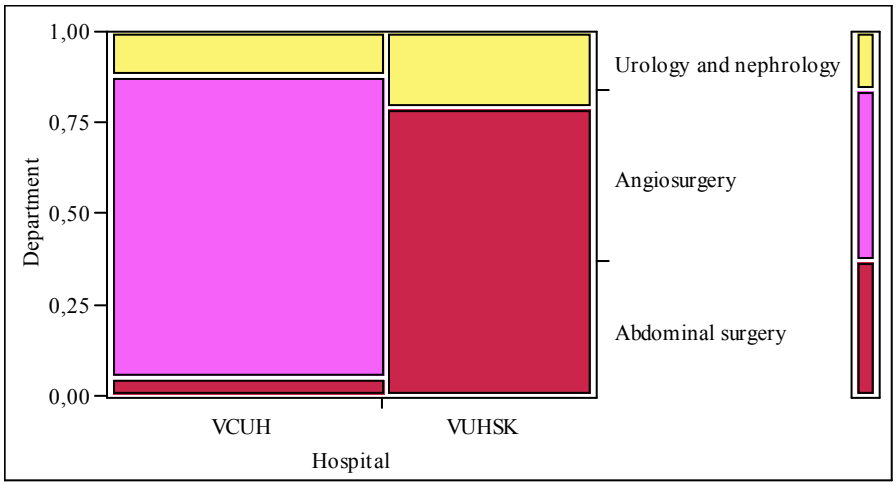
The largest of the occupational groups was OG-5 (students), one sixth of the respondents were from groups OG-2 (professional administrative or technical job, with great responsibility) and OG-3 (professional job in trade, service sectors, handling equipment, etc.).

4.1.2. Hospitalized patients' group characteristics

Vilnius City University Hospital (VCUH) and Vilnius University Hospital Santariškių Klinikos (VUHSK) took part in *S. aureus* nose and throat carriage identification study. All of the patients hospitalized during the study were examined, including a total of 828 patients. A little more than half of the

patients were from VCUH – 474 (57.2%) and the rest were from VUHSK – 354 (42.8%).

392 patients from VCUH Angiosurgery department were examined, representing 47.3% of all patients in the study. There were 307 (37.1%) respondents in Abdominal surgery department, 26 (8.5%) of them were from VCUH and 281 (91.5%) – from VUHSK. The smallest part of the patients' group 15.6% (n=129) were from VCUH and VUHSK Urology and Nephrology departments – (43.4%) and 73 (56.6%) respectively. The distribution of patients examined on the day of admission to each hospital and their departments is presented in the mosaic Figure 8.



χ^2=601.3; p=0.0001

Figure 8. Distribution of patients on the day of admission to VCUH and VUHSK hospitals.

A total of 47.2% (n=391) of hospitalized patients were females, 52.8% (n=437) – males. Demographic characteristics of hospitalized patients' group is presented in Table 9.

Table 9. Demographical characteristic of hospitalized patient respondents

	VCUH		VUHSK		Total	
	n	**(%)**	**n**	**(%)**	**n**	**(%)**
Gender						
- Female	173	44.2%	218	55.8%	391	47.2%
- Male	301	68.9%	136	31.1%	437	52.8%
Age (years)						
- Mean	65.7		56.8		61.9	
- Median	67.0		58.5		63.0	
- Mode	74		62		74	
- SD	12.9		15.3		14.6	
- C_v	19.6%		26.9%		22.4%	

The youngest patient and study participant was 19 years old, the eldest – 95. The mean age of the respondents was 61.9 years (median – 63). Figures 9 and 10 present the distribution of age among the hospitalized respondents in each hospital separately.

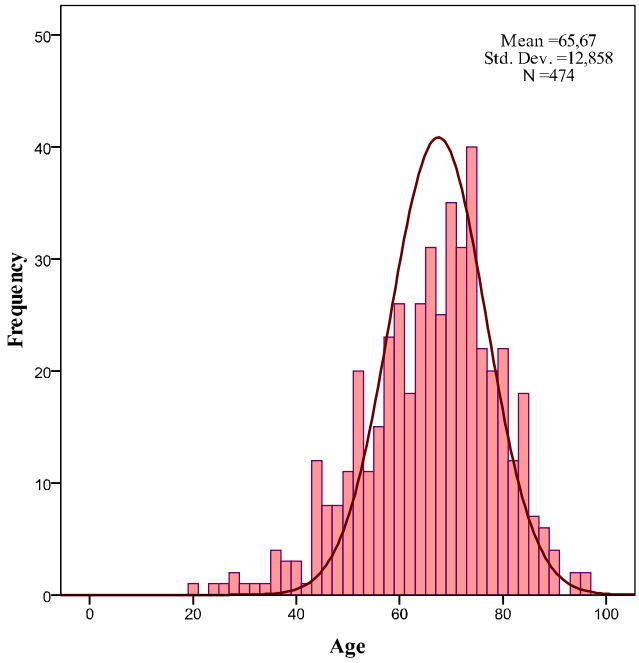


Figure 9. Histogram of the age distribution of the VCUH hospitalized respondents.

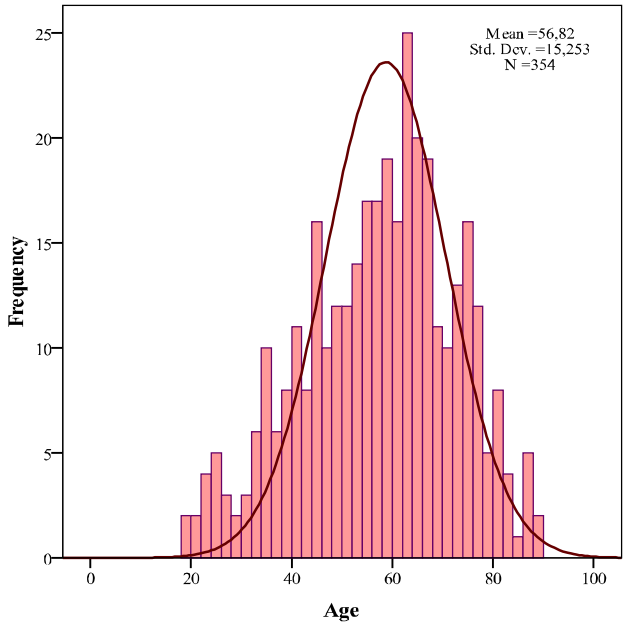


Figure 10. Histogram of the age distribution of the VUHSK hospitalized respondents.

On the discharge day from hospital, samples for *S. aureus* carriage identification were taken from 738 (89.1%) out of 828 already examined respondents (upon the day of admission to hospital). Patients examined in VCUH make up to 59.3% (n=438) of the respondents, patients from VUHSK – 40.7% (n=300) of all the examined patients on the discharge day from hospital. A total of 90 (10.9%) respondents already examined upon the day of admission to hospital were not included into the study on the discharge day from hospital. The loss rate of the patients in VCUH makes 7.6% (n=36), the loss rate in VUHSK – 15.3% (n=54). The loss rate of examined patients in each hospital is presented in Figure 11.

The highest number of examined patients upon admission to hospital included into the study upon discharge from hospital was from Angiosurgery department – 95.7% (375/392). The lower number of patients included were from Urology and Nephrology departments – 89.9% (116/129) and Abdominal surgery department – 80.5% (247/307). The distribution of the number of

patients upon discharge from hospital in every department of each hospital is presented in Figure 12.

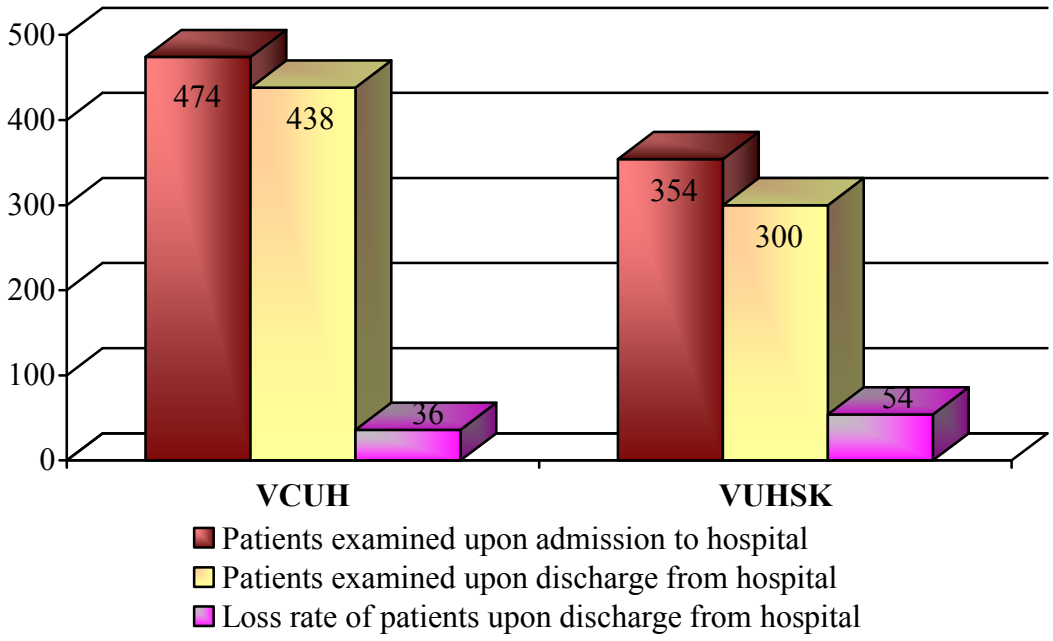
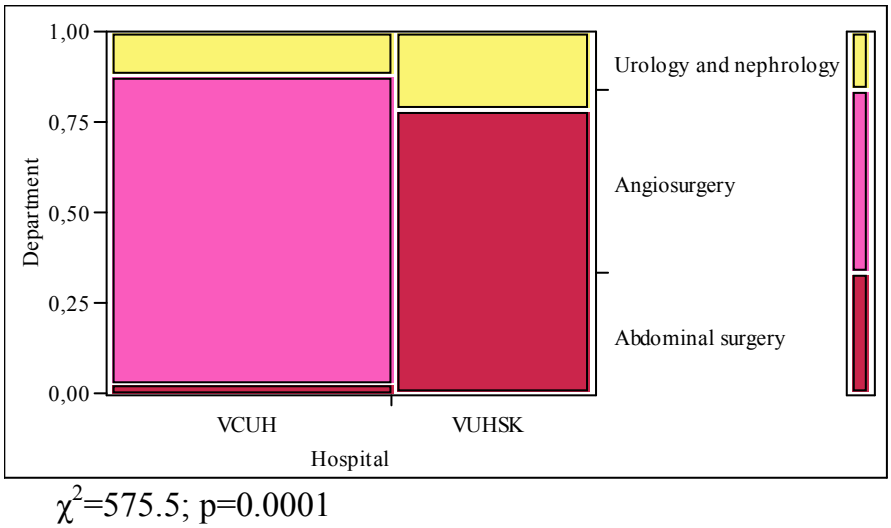


Figure 11. Number of patients examined and not included into the study in each hospital.



χ^2=575.5; p=0.0001

Figure 12. The distribution of examined patients upon discharge from VCUH and VUHSK hospitals.

4.2. ISOLATION OF *S. AUREUS* FROM PARTICIPANTS OF THE STUDY

4.2.1. *S. aureus* carriage in the community groups

The extent of *S. aureus* carriage in the Vilnius selected adult population was 50.8% [95% Cl 46.52 - 55.14] or 273 persons from the 537 participants.

The percentage of persons who had *S. aureus* detected in any of two samples was 35.9% (193 of 537), whereas 14.9% (80 of 537) had *S. aureus* detected in both samples.

Out of 273 carriers, 107 participants had *S. aureus* detected in nose, 86 – in throat, and 80 participants in both – nose and throat (Figure 13). If nose had been the only screening site, 34.8% (187/537) of *S. aureus* carriers would have been identified, while taking samples from only the throat would have identified 30.9% (166/537), instead of 50.8% (273/537).

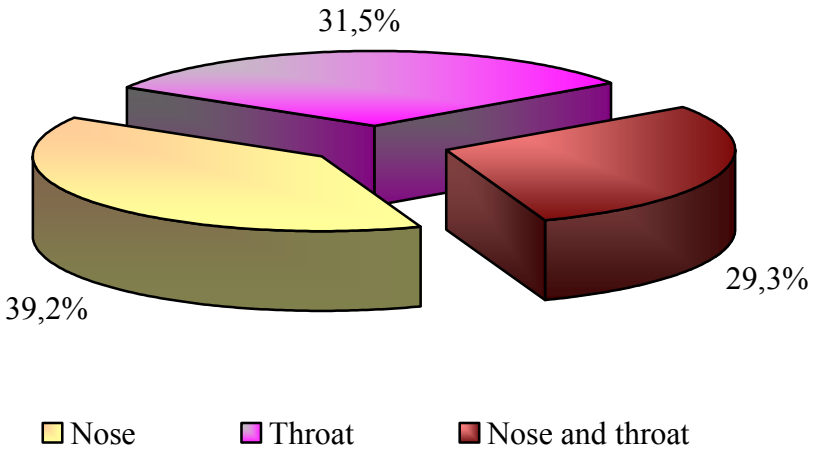
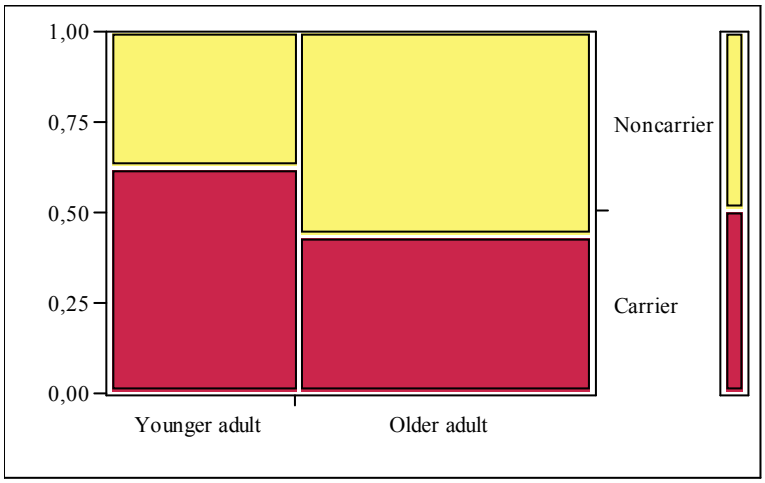


Figure 13. Culture results from different swabbed sites in community groups.

There were more *S. aureus* carriers in younger adult group (n=132, 62.3%) than in older adult group (n=141, 43.4%). *S. aureus* carriage differs significantly between the two age categories (p=0.0001) (Figure 14).

The analysis of *S. aureus* carriage peculiarities according to gender showed that the fraction of male carriers (54.0%) was greater than the fraction

of female carriers (47.2%), which is statistically insignificant (χ^2=2.447, df=1, p=0.068).



χ^2=18.3; p=0.0001

Figure 14. Distribution of *S. aureus* carriers in two age categories.

S. aureus carriage among occupational groups was statistically significant (p_F=0.0001). This was determined by a greater fraction of OG-5 and considerably smaller fraction among OG-4, OG-6 and OG-7. *S. aureus* carriers and non-carriers among OG-1, OG-2 and OG-3 distributed equally (Figure 15).

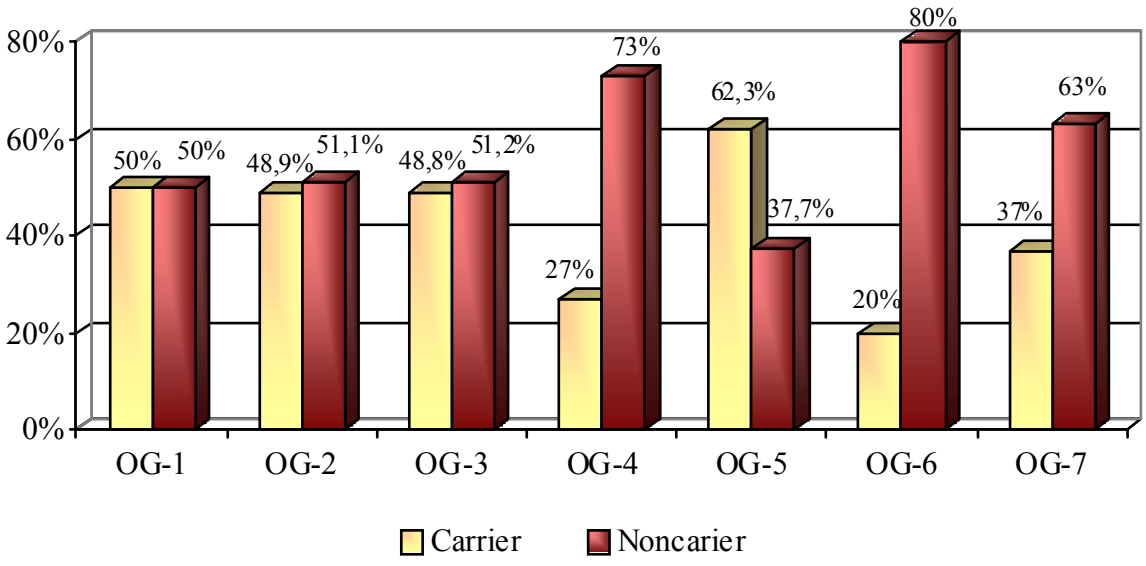


Figure 15. *S. aureus* carriage according to occupational groups (page 53).

4.2.2. *S. aureus* carriage in the hospitalized patients‘ group

S. aureus carriage determination on the day of admission to hospital

A total of 828 respondents were examined upon admission to hospital; it was found that 29.7% (n=246) of the respondents were *S. aureus* carriers. A slightly higher number of *S. aureus* carriers were found among the respondents admitted to VUHSK – 30.8% (109/354) than to VCUH – 28.9% (137/474) (Figure 16). Patients‘ *S. aureus* carriage evaluation showed, that there was no statistically significant difference between two hospitals (χ^2=0.346, df=1, p=0.556).

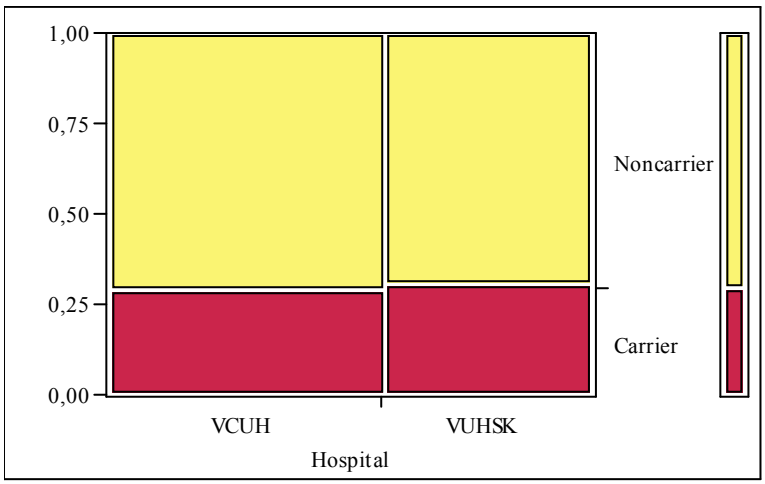


Figure 16. Distribution of *S. aureus* carriers in two hospitals on the day of admission.

Analysis of *S. aureus* carriage frequency according to gender showed, that contrary to community group results, women slightly more often were found to be *S. aureus* carriers than men (31.5% and 28.1% respectively), which was statistically insignificant (χ^2=1.084, df=1, p=0.298).

Analysis of *S. aureus* site location showed, that only the nares were the most prevalent site of *S. aureus* colonization (59.8% compared to 15.9% in the

throat). In both swabbed sites colonization was found in 24.4% of colonized respondents. (Figure 17)

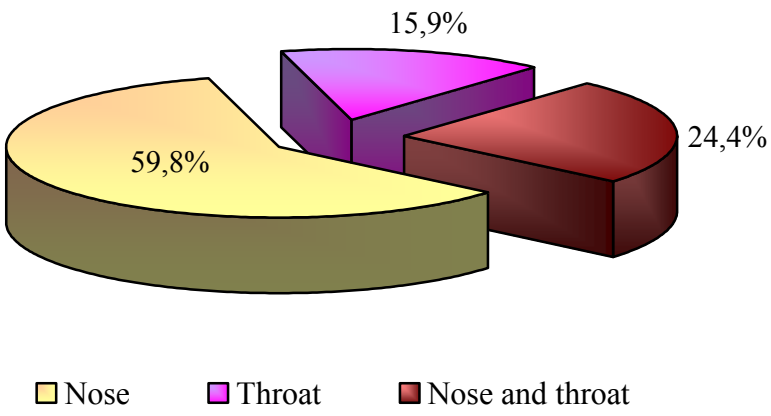


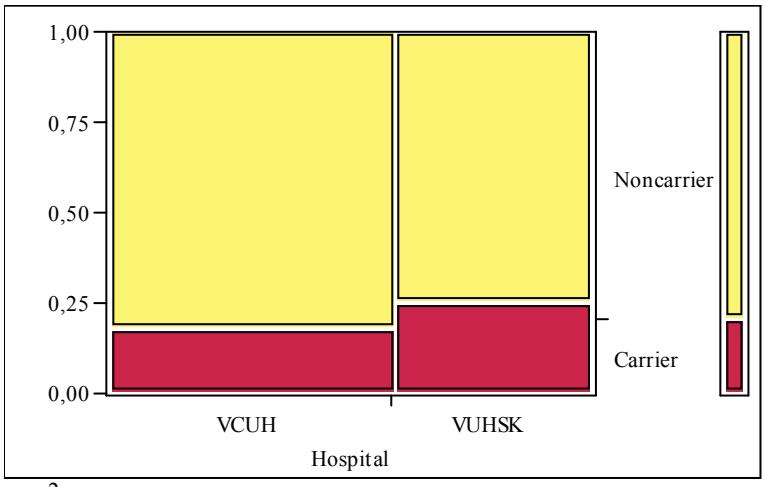
Figure 17. Frequency of *S. aureus* isolates, according to the swabbed sites in hospital group on the day of admission.

S. aureus carriage determination on the day of discharge from hospital

On the day of discharge from hospital 738 respondents were examined. It was found that 20.7% of them were *S. aureus* carriers. The number of *S. aureus* carriers identified upon admission to and discharge from hospital was compared, so the results of 438 respondents from VCUH and 300 respondents from VUHSK were analysed. The respondents, from which the samples were not taken, because of the reduced hospitalization time, were not included into the analysis (n=90).

The comparison of the respondents from each hospital on the day of discharge showed that significant higher number of *S. aureus* carriers was found in VUHSK. (Figure 18).

Our study revealed, that women were more frequently colonized with *S. aureus* than men, (24.4%, 85/263 and 17.4%, 68/322, respectively), that on the day of discharge higher number of carriers were determined than upon admission to hospital. This difference is statistically significant (χ^2=5.467; df=1; p=0.019).



χ^2=5.604; p=0.018

Figure 18. Distribution of *S. aureus* carriers in two hospitals on the day of discharge.

During respondent testing on the day of discharge, the same trends of *S. aureus* isolates in nose prevailed - 79.0%, the other swabbed sites were less common. (Figure 19)

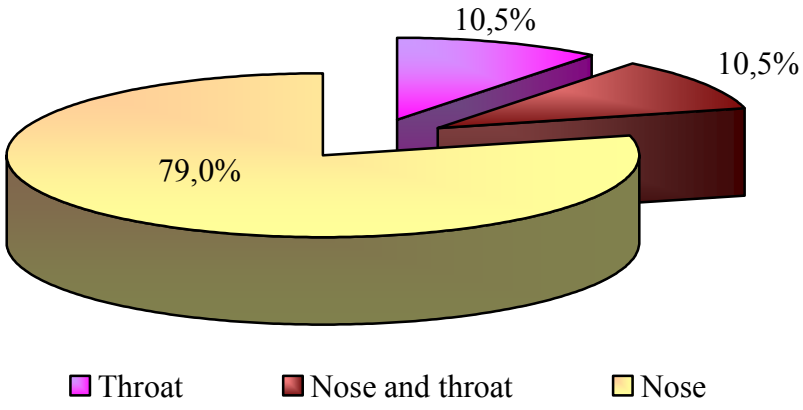


Figure 19. Frequency of *S. aureus* isolates, according to the swabbed sites in hospital group on the day of discharge.

A total of 53.9% (n=69) of the respondents in VCUH, who were colonized on the day of admission to hospital, had no *S. aureus* isolates on the day of discharge. Whereas, 6.1% (n=19) of all the respondents, who had no *S. aureus* isolates on the day of admission, were colonized with new *S. aureus* on the day of discharge. For more than half (68.4%, n=13) of newly colonized respondents *S. aureus* was isolated from nose. (Figure 20)

After hospitalization this microorganism was not isolated for a smaller number of *S. aureus* carriers in VUHSK (37.6%, n=35) as compared to VCUH. The greater number of respondents, who were not *S. aureus* carriers on the day of admission to VUHSK (8.2%, n=17), after hospitalization were colonized with the new *S. aureus* strains (Figure 20). The nose was a more frequent site of colonization in this group of respondents as well (76.5%, n=13).

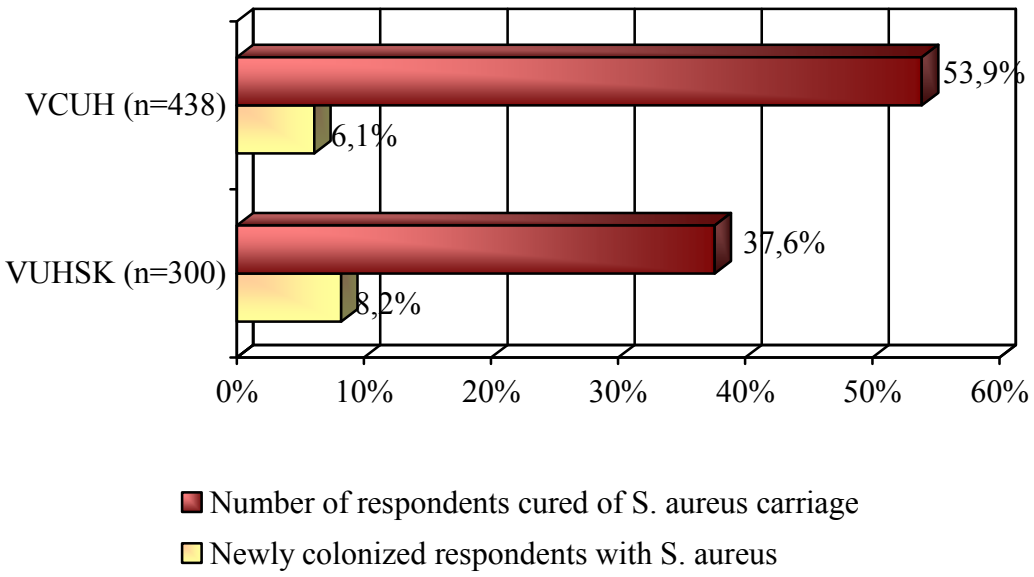


Figure 20. Number of respondents cured and newly colonized with *S. aureus* upon discharge in VCUH and VUHSK.

Seven respondents (4 from VUHSK and 3 from VCUH) on admission were colonized with *S. aureus*, but on discharge day phenotypically different *S. aureus* were isolated.

Comparing all the respondents colonized with the new *S. aureus* strains upon discharge from hospitals, it was found, that the rate in separate hospital departments was significantly different (χ^2=6.459; df=2; p=0.040). A greater number of respondents discharged from Abdominal surgery departments had new *S. aureus* isolates (48.8%, n=21). 14 (32.6%) of all newly colonized patients on the day of discharge were registered in Angiosurgery department; Urology and Nephrology departments had the lowest number of such respondents (18.6%, n=8).

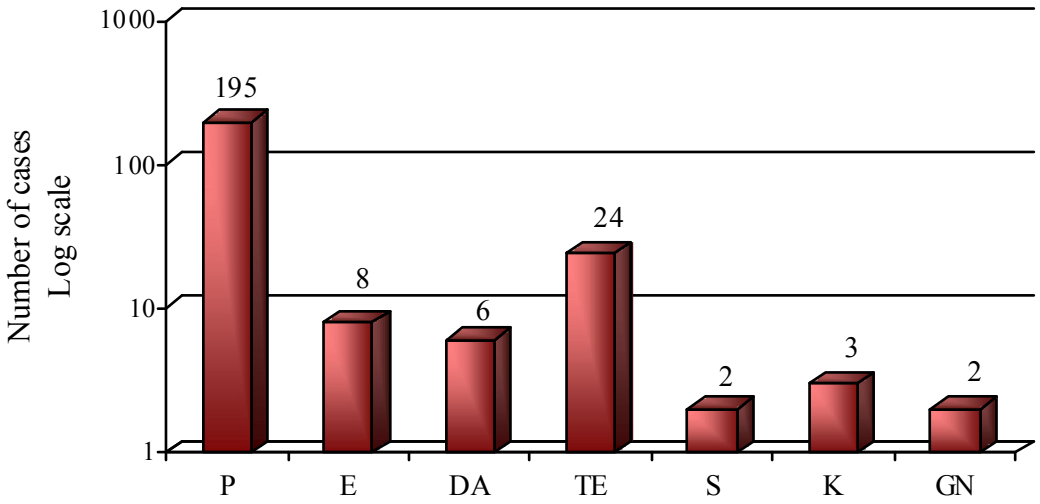
4.3. THE RESISTANCE OF *S. AUREUS* ISOLATES TO ANTIBIOTICS ANALYSED

4.3.1. The resistance of *S. aureus* isolates to antibiotics analysed in the community group

Based on the resistance pattern, 298 different *S. aureus* strains of 357 were isolated. Different *S. aureus* strains in the anterior nares and throat were found after examination of 21 participant; four volunteers had two different *S. aureus* strains at the same site.

Sixty-five percent of the isolates (n=195) were resistant to penicillin. Low resistance rates to other antibiotics were detected: 8.1% (n=24) were resistant to tetracycline and <3% of the isolates were resistant to kanamycin (1.0%), gentamicin (0.7%), erythromycin (2.7%), clindamycin (2.0%) and streptomycin (0.7%). (Figure 21)

All isolates were sensitive to oxacillin/cefoxitin, rifampin, norfloxacin, ciprofloxacin and fusidic acid. Ninety-nine (33.2%) of the isolated *S. aureus* strains were sensitive to all antibiotics used in this study. All 122 randomly selected *S. aureus* strains were sensitive to mupirocin.



Antibiotic abbreviations: P-penicillin, E-erythromycin, DA-clindamycin, TE-tetracycline, S-streptomycin, K-kanamycin, GN-gentamicin.

Figure 21. Resistance rates of isolated *S. aureus* strains from the community group.

Among the isolates resistant to erythromycin and clindamycin, resistance was inducible in 6 (75%) isolates (Figure 22), and the M-phenotype (resistance to erythromycin but sensitivity to clindamycin) was present in 2 (25%) isolates.

Resistance to two or more antibiotics was noted in 30 (10.1%) isolates. Twenty isolates were resistant to two antibiotics (tetracycline-penicillin [18 strains], erythromycin-clindamycin [one strain], and penicillin-streptomycin [one strain]). Nine strains were resistant to three antibiotics (erythromycin-clindamycin-penicillin [four strains], kanamycin-gentamicin-penicillin [two strains], kanamycin-tetracycline-penicillin [one strain], erythromycin-tetracycline-penicillin [one strain], tetracycline-penicillin-streptomycin [one strain] and one strain was resistant to four antibiotics (erythromycin, clindamycin, tetracycline and penicillin).

All 103 (34.6%) penicillin-susceptible *S. aureus* strains were found to have mecillinam zones ranging from 22 to 30mm [223]. A total of 195 (65.4%)

penicillin-resistant isolates were positive in the β-lactamase test, and all these strains were resistant to mecillinam (e.g. zones were from 9 to 13mm).

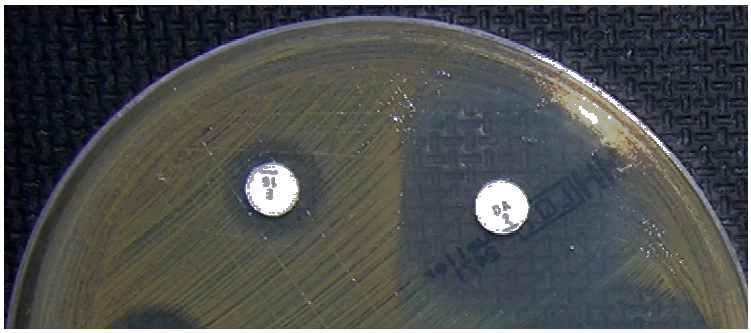


Figure 22. Positive disk induction D-test indicating inducible lincosamide resistance.

4.3.2. The resistance of *S. aureus* isolates to antibiotics analyzed in the hospitalized patients‘ group

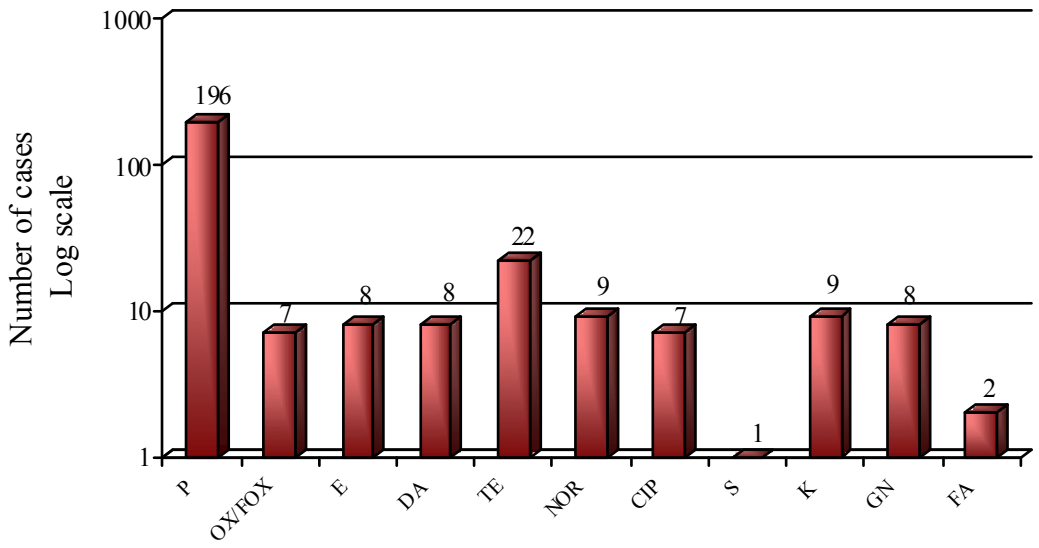
The resistance to antibiotics of S. aureus isolated on the day of admission to hospital

After patients were examined on the day of their admission to hospital, 306 *S. aureus* strains were isolated. According to the extended antibiogram, 254 phenotypically different *S. aureus* were isolated; 111 of them were isolated in VUHSK and 143 - in VCUH.

Antimicrobial resistance study of *S. aureus* isolates from hospitalized patients showed that 77.2% (n=196) of isolates were resistant to penicillin. A total of 8.7% (n=22) of the isolates were resistant to tetracycline. Resistance testing also revealed low resistance rates to oxacillin/cefoxitin and ciprofloxacin (2.8%), to erythromycin, clindamycin and gentamicin (3.2%), to kanamycin and norfloxacin (3.5%). Less than 1% of the *S. aureus* isolates were resistant to fusidic acid and streptomycin, 2/254 and 1/254 respectively. (Figure 23)

Among the isolates resistant to erythromycin and clindamycin, resistance was inducible in all 8 (100%) isolates (Figure 22).

In this study only seven (2.8%) of the *S. aureus* isolates were methicillin resistant. All MRSA were also resistant to kanamycin, norfloxacin and ciprofloxacin, while 85.7% (n=6) were resistant to gentamicin. Lower resistance rates were observed for tetracycline (28.6%, n=2), and erythromycin, clindamycin (14.3%). None of the MRSA isolates showed reduced sensitivity to vancomycin.



Antibiotic abbreviations: P-penicillin, OX-oxacillin, FOX-cefoxitin, E-erythromycin, DA-clindamycin, TE-tetracycline, NOR-norfloxacin, CIP-ciprofloxacin, S-streptomycin, K-kanamycin, GN-gentamicin, FA-fusidic acid.

Figure 23. Antimicrobial resistance profile of *S. aureus* strains isolated from patients on the day of admission.

Resistance to two or more antibiotics was noted in 37 (14.6%) *S. aureus* isolates. 20 isolates were resistant to two antibiotics (tetracycline-penicillin [16 strains], erythromycin-clindamycin [one strain], penicillin-norfloxacin [one strain], tetracycline-fusidic acid [one strain] and penicillin-streptomycin [one strain]). Eight strains were resistant to three antibiotics (erythromycin-

clindamycin-penicillin [five strains], kanamycin-gentamicin-penicillin [one strain], kanamycin-tetracycline-penicillin [one strain], tetracycline-penicillin-norfloxacin [one strain]), and two strains were resistant to four antibiotics (erythromycin-clindamycin-tetracycline-penicillin [one strain], penicillin-kanamycin-fusidic acid-gentamicin [one strain]).

Isolated MRSA strains were resistant to five or six antibiotics. Four strains were resistant to oxacillin/cefoxitin-kanamycin-norfloxacin-ciprofloxacin-gentamicin; two strains were resistant to oxacillin/cefoxitin-kanamycin-norfloxacin-ciprofloxacin-tetracycline-gentamicin and one strain was resistant to oxacillin/cefoxitin-kanamycin-clindamycin-erythromycin-norfloxacin-ciprofloxacin.

All 254 isolates were sensitive to rifampin. Fifty-six (22.1%) of the isolated *S. aureus* strains were sensitive to all used antibiotics in this investigation.

Out of 196 penicilin resistant *S. aureus* isolates, 193 (98.5%) of them were positive in the β-lactamase test.

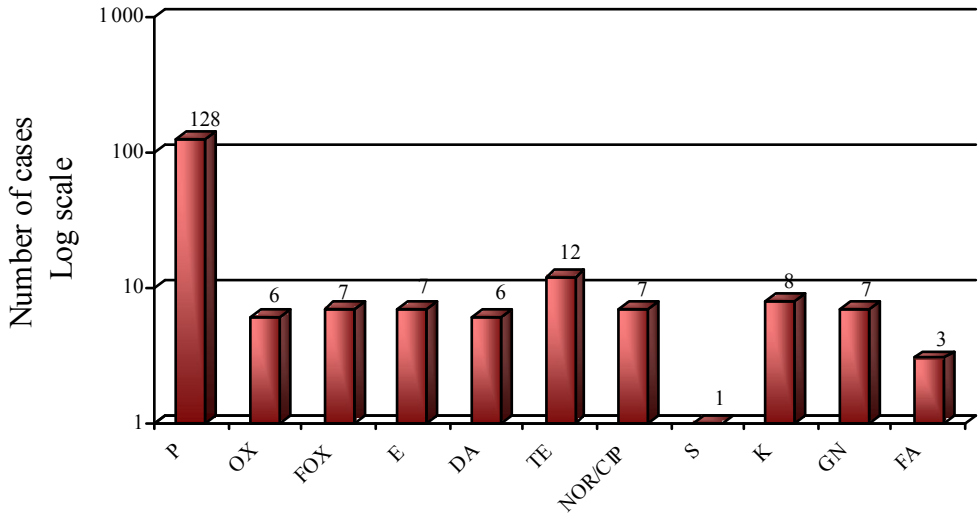
The resistance to antibiotics of S. aureus isolated on the day of discharge from hospital

After patients were examined on the day of their discharge from hospital, 169 *S. aureus* strains were isolated. According to the extended antibiogram, 155 phenotypically different *S. aureus* were isolated; 75 of them were isolated in VUHSK and 80 - in VCUH.

82.6% (n=128) of *S. aureus* isolates were resistant to penicillin. A total of 7.7% (n=12) and 5.2% (n=8) of the isolates were resistant to tetracycline and kanamycin, respectively. Antimicrobial resistance study of *S. aureus* isolates revealed that 4.5% (n=7) of strains were resistant to cefoxitin, erythromycin, norfloxacin, ciprofloxacin and gentamicin. Resistance to oxacillin and clindamycin was 3.9%. (Figure 24)

Among the isolates resistant to erythromycin and clindamycin, resistance was inducible in six (85.7%) isolates (Figure 22), and the M-phenotype (resistant to erythromycin but sensitive to clindamycin) was present in one (14.3%) isolate.

Antimicrobial resistance studies of seven MRSA isolates by disc diffusion methods showed that all strains were resistant to kanamycin, norfloxacin and ciprofloxacin, while 85.7% (n=6) were resistant to gentamicin. Low resistance rates were observed to tetracycline, erythromycin and clindamycin (14.3%), whereas none of the isolates showed reduced sensitivity to vancomycin.



Antibiotic abbreviations: P-penicillin, OX-oxacillin, FOX-cefoxitin, E-erythromycin, DA-clindamycin, TE-tetracycline, NOR-norfloxacin, CIP-ciprofloxacin, S-streptomycin, K-kanamycin, GN-gentamicin, FA-fusidic acid.

Figure 24. Resistance rates of isolated *S. aureus* strains on the day of discharge.

Resistance to 2 or more antibiotics was observed in 25 (16.1%) isolates. 11 strains were resistant to two antibiotics (tetracycline-penicillin [10 strains], and tetracycline-fusidic acid [one strain]). Five strains were resistant to three antibiotics (erythromycin-clindamycin-penicillin [four strains], and

erythromycin-fusidic acid-penicillin [one strain]), and two strains were resistant to four antibiotics (erythromycin-clindamycin-streptomycin-penicillin [one strain], penicillin-kanamycin-fusidic acid-gentamicin [one strain]).

Isolated MRSA strains were resistant to five or six antibiotics. One strain was resistant to cefoxitin-kanamycin-norfloxacin-ciprofloxacin-gentamicin. Four strains were resistant to oxacillin/cefoxitin-kanamycin-norfloxacin-ciprofloxacin-gentamicin; one strain was resistant to oxacillin/cefoxitin-kanamycin-norfloxacin-ciprofloxacin-tetracycline-gentamicin and one strain was resistant to oxacillin/cefoxitin-kanamycin-clindamycin-erythromycin-norfloxacin-ciprofloxacin.

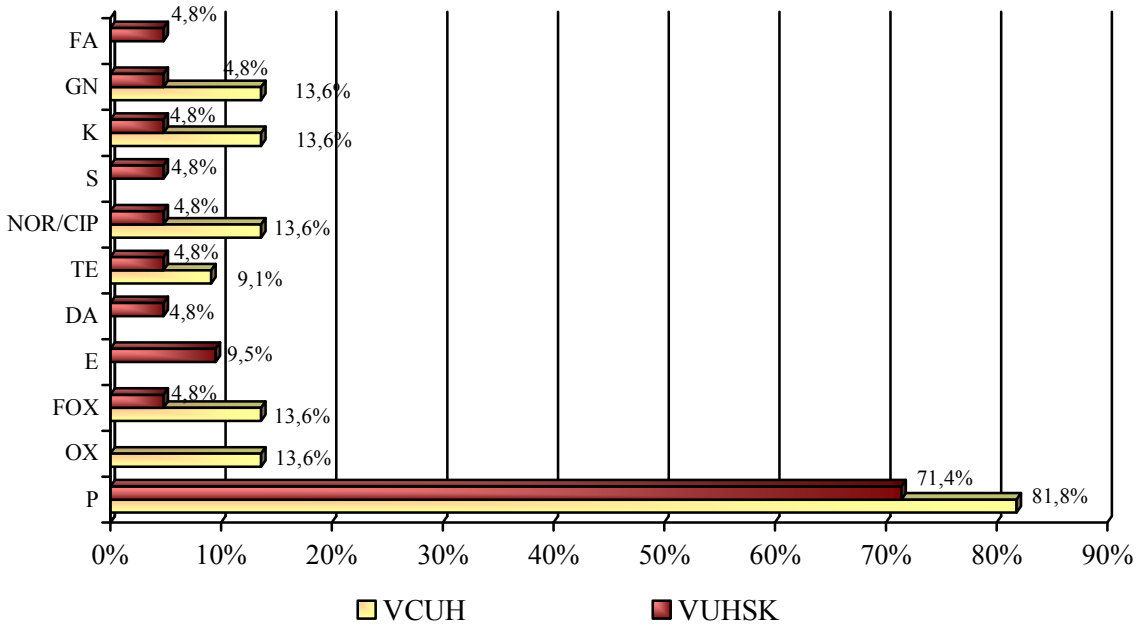
All 155 isolates were sensitive to rifampin. Twenty-six (16.8%) of the isolated *S. aureus* strains were sensitive to all antibiotics used in our study. 141 *S. aureus* strains randomly selected in hospitalized patients group on admission and discharge days were sensitive to mupirocin.

Out of 128 penicillin resistant *S. aureus* strains, 126 (98.4%) isolates were positive in the β-lactamase test.

It was found, during the study, that 43 respondents on the day of discharge from hospital were colonized with the new *S. aureus* strains. 9.3% (4/43) of the respondents were colonized with MRSA on the day of discharge from hospital.

Comparing the newly isolated strains‘ resistance to antibiotics in each hospital, more respondents with the methicillin resistant *S. aureus* strains were identified upon discharge from hospital in VCUH than in VUHSK (13.6% and 4.8% respectively) (Figure 25). This difference is statistically insignificant (p=0.607), which was due to the small numbers of methicillin resistant *S. aureus* strains.

The new *S. aureus* strains isolated from the respondents upon discharge from VCUH were also more resistant to penicillin (81.8%) and tetracycline (9.1%). Whereas the new *S. aureus* strains isolated from the respondents upon discharge from VUHSK were more resistant to erythromycin (9.5%), clindamycin (4.8%), fusidic acid (4.8%) and streptomycin (4.8%). (Figure 25)



Antibiotic abbreviations: FA-fusidic acid, GN-gentamicin, K-kanamycin, S-streptomycin, RD-rifampin, NOR-norfloxacin, CIP-ciprofloxacin, VA-vancomycin, TE-tetracycline, DA-clindamycin, E-erythromycin, OX-oxacillin, FOX-cefoxitin, P-penicillin

Figure 25. The comparison of newly isolated *S. aureus* strains‘ resistance to antibiotics in VCUH and VUHSK.

4.4. DISTRIBUTION OF SELECTED *erm* AND *tet* GENES AMONG ISOLATED *S. AUREUS*

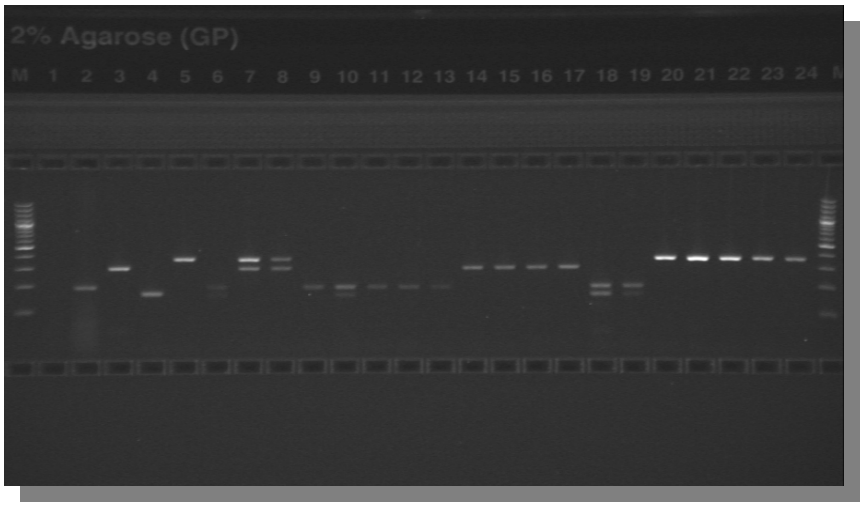
4.4.1. The presence of resistance genes of *S. aureus* isolated in the community group

Distribution of methicillin resistance genes

A total of 357 *S. aureus* strains isolated in the community group were tested for *mecA*. Resistance to methicillin was tested by disc diffusion method. All strains were methicillin sensitive and PCR testing confirmed that they did not harbor this resistance gene.

Distribution of erythromycin and tetracycline resistance genes

A total of 31 *S. aureus* strains, 30 of which were phenotypically different, were tested for *ermA*, *ermC*, *tetK* and *tetM* by multiplex PCR. (Figure 26)



Lines: M–molecular marker; 1 – *ermA*, *ermC*, *tetK* and *tetM* negative standard strain; 2 – control isolate *ermA*, 3 – control isolate *ermC*, 4 – control isolate *tetM*, 5 – control isolate *tetK*. 6–24 tested different *S. aureus* isolates: 6, 9, 11-13 – positive for *ermA*, 7, 8 – *ermC, tetK*, 10, 18, 19 – *ermA, tetM*, 14-17 – *ermC*, 20-24 – *tetK*.

Figure 26. Image of the *ermA*, *ermC*, *tetK* and *tetM* genes in 2% agarose gel as a result of the multiplex PCR test.

Eight of 30 *S. aureus* strains were resistant to erythromycin and/or clindamycin. A total of two (25.0%) strains contained *ermA*, and five (62.5%) contained *ermC*. No *erm* gene was found in one strain (12.5%). Among erythromycin-resistant isolates with MLS_B inducible resistance (n=6) in community group the *ermC* gene was predominant (66.7%), while *ermA* gene was present in 33.3% of tested isolates. Positive strains for *ermA* and *ermC* genes produced a band of 190 and 299 bp, respectively, in 2% agarose gel (Figure 26).

tetK and *tetM* were evaluated to determine the tetracycline resistance genotypic distribution of 30 *S. aureus* strains, 24 of which were resistant to tetracycline. In the community group the *tetK* gene was detected in 22/24 (91.7%) and in two (8.3%) of tetracycline resistant *S. aureus* strains the *tet* gene was not detected.

One *S. aureus* strain had inducible MLS resistance and *ermA* gene, and it was sensitive to tetracycline, but positive for *tetM*.

Positive strains showed a band with 360bp (*tetK*) and 158bp (*tetM*) in agarose gel.

4.4.2. The presence of resistance genes of *S. aureus* isolated in the hospital group

Distribution of methicillin resistance genes

475 *S. aureus* strains isolated from the hospitalized respondents group were tested for the *mecA* gene. Analysis of 297 phenotypically different *S. aureus* strains showed, that 3.7% (n=11) of them had *mecA* gene. All eleven phenotypically different strains were oxacillin/cefoxitin resistant, which was determined by disc diffusion method. Furthermore, among the 286 oxacillin/cefoxitin sensitive strains, the *mecA* gene was not detected. Positive strains showed a band with 162bp in 2% agarose gel (Figure 27).

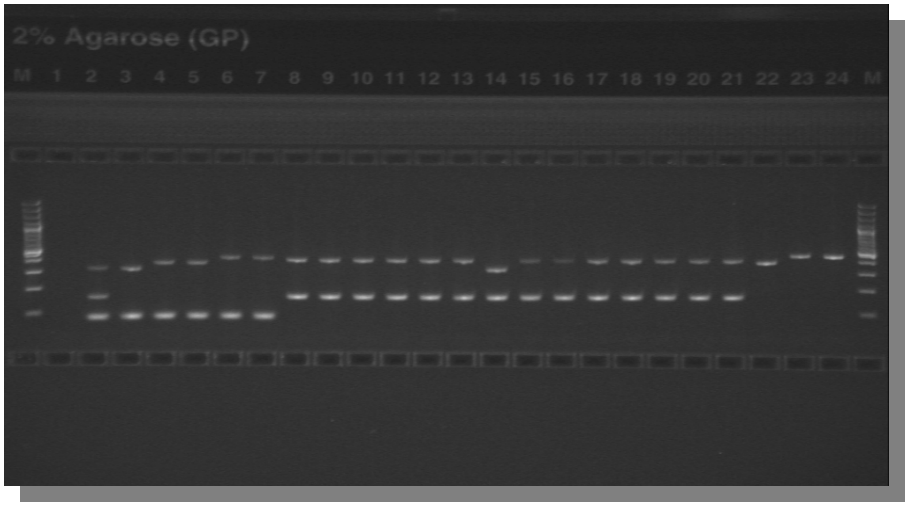
Distribution of erythromycin and tetracycline resistance genes

The prevalence of the macrolide resistance genes *ermA*, *ermC* and tetracycline resistance genes *tetK*, *tetM* were analyzed using PCR in 64 erythromycin or/and tetracycline resistant *S. aureus* isolates, comprising 31 phenotypically different MSSA and 4 MRSA strains.

A total of 10 phenotypically different MSSA and one MRSA strains were resistant to erythromycin and clindamycin. Among erythromycin resistance methylase genes, more prevalent was *ermA*, which was detected in 54.5%

(n=6) of the *erm*-positive subpopulation, including erythromycin-resistant MRSA strain, whilst *ermC* occurred in four isolates (36.4%). In one (9.1%) erythromycin resistant *S. aureus* strains the *erm* gene was not detected.

All *ermA* and *ermC* genotypes were associated with inducible macrolide, lincosamide and streptogramin B phenotype.



Lines: M – molecular marker; 1 – *mecA*, PVL, and *spa* negative standard strain; 2 – control isolate *mecA*, PVL, *spa*; 3–24 tested different *S. aureus* isolates. 3-7 – positive for PVL, *spa*, 8-21 – positive for *mecA*, *spa*, 3-24 – *spa*.

Figure 27. Agarose gel electrophoresis of PCR amplicon of *mecA* (162bp), PVL (80bp) and *spa* (variable) genes.

In this study the distribution of two tetracycline resistance genes in 23 tetracycline-resistant phenotypically different MSSA, three MRSA isolates and nine tetracycline-susceptible strains were determined.

All three tetracycline-resistant MRSA isolates displayed *tetK* gene. One MRSA strain had inducible lincosamide resistance and *ermA* gene, also it was sensitive to tetracycline, but positive for *tetM*.

The *tetK* gene was detected in 22/23 (95.7%) MSSA isolates. The *tetM* gene was not found in tetracycline-resistant MSSA isolates. One MSSA strain

contained *ermA* and *tetM* although it was sensitive to tetracycline. No tetracycline resistance gene was encountered in one (4.4%), of the 23 MSSA isolates investigated.

Our results show that the *tetK* gene was the most prevalent tetracycline resistance determinant in MSSA and MRSA.

4.5. THE PRESENCE OF PANTON-VALENTINE LEUKOCIDIN GENE

The prevalence of the PVL gene was assessed in 832 *S. aureus* isolates from both the community and hospital groups. In total, 595 phenotypically different *S. aureus* strains were analyzed. Isolates were tested for the PVL genes by multiplex PCR (Figure 27) as described in the chapter "Materials and methods".

PVL genes were detected in 15 (2.5%) phenotypically different isolates, all of them were MSSA. All tested MRSA strains were PVL-negative.

A higher proportion of PVL-positive isolates was found among community group – 3.0% (9/298). Among *S. aureus* strains recovered from hospital respondents, the proportion of PVL-positive isolates was 2.0% (6/297). No statistically significant differences (p=0.310) were found between the rates of carriage of PVL among community and hospital groups.

Eight participants with PVL-positive MSSA were male (53.3%) and seven were female (46.7%). No statistically significant differences (p=0.585) were found between the rates of carriage of PVL between genders. The mean age of participants with determined PVL-positive *S. aureus* was 38.3 years (range, 19 to 73). Analysis of *S. aureus* with the virulence coding gene, considering the swabbed site, showed that 14 PVL-positive strains were isolated from nose, throat site contained 3 times less (n=4) of *S. aureus* isolates with the same gene. (Table 10)

The antimicrobial resistance patterns of the 15 PVL-positive MSSA isolates are shown in Table 10. Most of the isolates 93.5% (14/15) were resistant just to penicillin. Only one *S. aureus* isolate (6.7%) was resistant to penicillin and tetracycline.

Table 10. Association of 15 PVL-positive *S. aureus* isolates with some characteristics of recipients, swabbed site and a phenotypic characteristic of MSSA isolates

Sample No.	Group	Swab site	Gender	Age (years)	Resistance pattern
31	Community	Nose and throat	M	19	P
34		Nose	M	19	P
43		Nose	F	19	P
58		Nose and throat	M	19	P
130		Nose	M	19	P
172		Nose	F	19	P
215		Throat	M	33	P
441		Nose	M	34	P
499		Nose	F	38	P
1107	Hospital	Nose	F	48	P
1141		Nose	F	63	P, TE
1167		Nose	F	45	P
2072		Nose	M	55	P
2360		Nose	M	73	P
2408		Nose and throat	F	72	P

Antibiotic abbreviations: P – penicillin, TE – tetracycline.

4.6. *SPA* TYPING OF ISOLATED *S. AUREUS* STRAINS

A total of 192 *S. aureus* isolates were typed by *spa* typing. Sixty five (33.9%) of *S. aureus* typed strains were obtained from community group, 64 (33.3%) – from patients in VUHSK and 63 strains (32.8%) – from patients in VCUH.

All 152 strains, out of 192 typed *S. aureus,* were phenotypically different. Sixty five (42.8%) *S. aureus* strains were obtained from community group, 44 (28.9%) – from VCUH and 43 (28.3%) isolates from VUHSK.

In this study, 80 different *spa* types were identified among the 152 *S. aureus* isolates. The diversity index of 152 *S. aureus* strains was 0.978. This index shows that, if any two strains were sampled randomly from the population, on 97.8% of occasions they would fall into different types. This percentage varies from 95.8 to 99.8 with 95% probability (Table 11). Two phenotypically different *S. aureus* strains were not typeable: one *S. aureus* strain was from VUHSK and one – from VCUH. Both strains were isolated from each patient on the days of admission and discharge. One *S. aureus* strain isolated from the patient in VCUH, on the days of admission and discharge, was of unknown *spa* type, although the repeated succession was identified.

Table 11. Typeability and diversity of *spa* types within isolated *S. aureus* strains

	Typeability	**No. of *spa* types**	**No. (%) of singular *spa* types**	**Diversity index (95% Cl)**
Different *S. aureus* strains (n=152)	98.7%	80	57 (71.3%)	0.978 (0.958-0.998)
CA (n=65)	100%	41	31 (75.6%)	0.970 (0.929-1.011)
VUHSK (n=43)	97.7%	29	22 (75.9%)	0.957 (0.896-1.018)
VCUH (n=44)	97.7%	30	24 (80.0%)	0.972 (0.923-1.021)

In total, *spa* typing resolved 41 distinct *spa* types for the 65 different *S. aureus* strains from community (diversity index 0.970). However, more different *spa* types within community population isolates were found, compared to the number of types detected in the VUHSK and VCUH (29 and 30 respectively) (Table 11). Among the MSSA strains isolated in the community group, most frequently identified were t084, t056, t015, t002 and t359 *spa* types (13.8%, 7.7%, 6.1%, 4.6%, and 4.6% respectively).

Additionally, a much higher percentage of *spa* types occurred only once in VCUH population (80.0%). Only 5 *spa* types occurred in all three studied groups (t002, t015, t056, t127, t346). The one predominant *spa* type among the MRSA isolates (t008) represented more than 80% of all MRSA isolates (Table 12). Diversity index of isolated MRSA strains was 0.345 (95% CI 0.0-0.795). It means that if two MRSA strains were sampled randomly from the studied population, then on 65.5% of occasions they would fall into the same types.

Table 12. The most frequent *spa* types within MRSA and MSSA isolates

Study group	Organism group	Most frequent *spa* types*	No. (%) of isolates
Community	MSSA	t084	9 (13.8%)
		t056	5 (7.7%)
		t015	4 (6.1%)
		t002	3 (4.6%)
		t359	3 (4.6%)
		t050	2 (3.1%)
		t127	2 (3.1%)
		t156	2 (3.1%)
		t159	2 (3.1%)
		t803	2 (3.1%)
VUHSK	MRSA	**t008**	1 (2.3%)
	MSSA	**t002**	2 (4.7%)
		t012	3 (7.0%)
		t015	4 (9.3%)
		t056	6 (14.0%)
		t065	2 (4.7%)
		t127	2 (4.7%)
		t589	2 (4.7%)
VCUH	MRSA	**t008**	8 (18.2%)
		t190	1 (2.3%)
		t002	1 (2.3%)
	MSSA	t056	2 (4.5%)

	t127	2 (4.5%)
	t148	2 (4.5%)
	t330	2 (4.5%)
	t647	1 (2.3%)
	t002	1 (2.3%)
	t008	1 (2.3%)

* – types in boldface indicate their occurrence in both MRSA and MSSA strains.

In summary, this suggests that due to the high number of isolated MSSA strains in the study, they tend to have greater diversity of singular *spa* types than MRSA strains. This fact contributed to the calculation of a distinctly higher diversity index for the MSSA population.

After *S. aureus spa* types isolated in VUHSK and VCUH were analysed, it was identified that t056, t008 and t002 circulated in both hospitals under study. Figure 28 shows some *S. aureus spa* types, which were different in both hospitals.

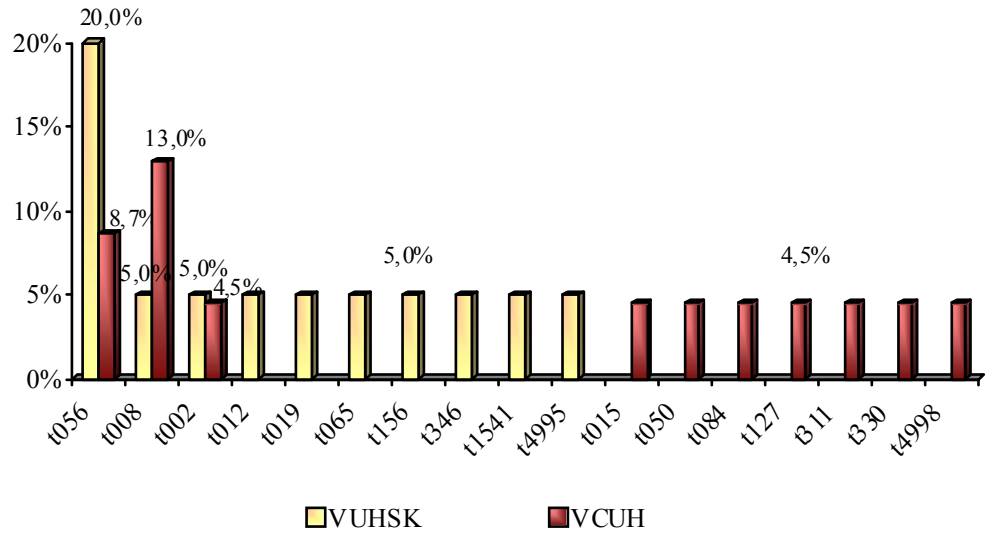


Figure 28. Frequencies of *spa* types collected in both hospitals from colonized respondents during hospitalization.

4.7. THE RISK FACTORS FOR COLONIZATION OF *S. AUREUS* IN UPPER RESPIRATORY TRACT

4.7.1. The risk factors influencing the colonization of *S. aureus* in upper respiratory tract in the community group

The analysis was conducted on 273 *S. aureus* carriers and 264 respondents without *S. aureus* at the same time period. Two examined adult groups were compared to evaluate the age influence on *S. aureus* carriage. The young adult group had a higher rate of *S. aureus* carriage (Figure 14) (OR=2.15; Cl 1.49-3.12). As the examined adult groups were divided into 5 subgroups, it was found that with age the risk of becoming a *S. aureus* carrier gradually decreases, except with the last age subgroup, which is statistically insignificant. (Tables 13 and 14).

Table 13. The age influence to become *S. aureus* carrier in the young adult group

Young adults	*S. aureus* carriers		OR
	Yes	No	
	abs. no. (%)	abs. no. (%)	
18	18 (64.3%)	10 (35.7%)	1.000
19	40 (62.5%)	24 (37.5%)	0.926
20	34 (60.7%)	22 (39.3%)	0.859
21	29 (59.2%)	20 (40.8%)	0.806
22	11 (73.3%)	4 (26.7%)	1.528

χ^2_{MH}=0.004; df=1; p=0.948

Table 14. The age influence to become *S. aureus* carrier in the older adult group

Older adults	*S. aureus* carriers		OR
	Yes	No	
	abs. no. (%)	abs. no. (%)	
< 31	35 (49.3%)	36 (50.7%)	1.000
32-37	30 (44.1%)	38 (55.9%)	0.812
38-43	30 (46.9%)	34 (53.1%)	0.908
44-51	22 (35.5%)	40 (64.5%)	0.566
52 >	24 (40.0%)	36 (60.0%)	0.686

χ^2_{MH}=1.985; df=1; p=0.159

The number of *S. aureus* carriers in the young adult group was higher among men (69.0%) than women (54.4%). This risk factor was determined as having a statistically significant influence on *S. aureus* colonization (OR=1.86; CI 1.02-3.39, p=0.034).

It was analysed whether different skin diseases, skin injuries or other infections caused by *S. aureus* (such as osteomyelitis, pneumonia, scalded skin syndrome, toxic shock syndrome and etc.) may have the influence on the respiratory tract colonization with this microorganism. It was determined that the factors mentioned above increase the risk of *S. aureus* colonization, especially after having staphylococcal skin infection (OR=2.63). Nevertheless, the data was statistically insignificant. This was mostly due to the low number of observations in groups. (Table 15)

Table 15. Potential risk factors for *S. aureus* carriage

Analysed risk factors		*S. aureus* carriers		OR (95% CI)	*p*
		Yes	No		
		abs. no. (%)	abs. no. (%)		
Skin diseases	Yes	41 (15.0%)	30 (11.4%)	1.38 (0.81-2.37)	0.252
	No	232 (85.0%)	234 (88.6%)		
Skin staphylococcal infection	Yes	8 (2.9%)	3 (1.1%)	2.63 (0.62-15.51)	0.222
	No	265 (97.1%)	261 (98.9%)		
Other staphylococcal infection	Yes	2 (0.7%)	1 (0.4%)	1.94 (0.10-114.94)	1.000
	No	271 (99.3%)	263 (99.6%)		
Use of antibiotics	Yes	125 (45.8%)	96 (36.4%)	1.48 (1.03-2.12)	0.029
	No	148 (54.2%)	168 (63.6%)		

A total of 41.2% (221/537) of all community group respondents indicated that in the last two years they used antimicrobial medications. It was determined that antimicrobial medication usage significantly increases the risk of becoming *S. aureus* carrier (p=0.029). (Table 15)

The relation of *S. aureus* carriage with chronic diseases was also analysed. 67 respondents (12.5%) indicated that they had one or several diseases mentioned in the questionnaire. After the connection of respondents‘

chronic diseases and *S. aureus* carriage was analysed, it was found that there was no statistically significant influence on *S. aureus* carriage (OR=0.81; Cl 0.47-1.40). Table 16 shows the influence of some chronic diseases on *S. aureus* colonization. According to the risk factors presented, the OR varied widely and none of the factors had statistically significant influence on both studied groups. This was determined by the low factor rate in the studied groups; 4 table grids had case number below 5.

Table 16. Potential risk factors for *S. aureus* carriage related to current chronic diseases

Analysed risk factors		***S. aureus* carriers**		**OR (95% Cl)**	***p***
		Yes	**No**		
		abs. no. (%)	**abs. no. (%)**		
Chronic diseases	Yes	31 (11.4%)	36 (13.6%)	0.81 (0.47-1.40)	0.436
	No	242 (88.6%)	228 (86.4%)		
Diabetes	Yes	2 (0.7%)	2 (0.8%)	0.97 (0.07-13.43)	1.000
	No	271 (99.3%)	262 (99.2%)		
Bronchi and lung chronic diseases	Yes	13 (4.8%)	16 (6.1%)	0.78 (0.34-1.76)	0.569
	No	260 (95.2%)	248 (93.9%)		
Digestive system diseases	Yes	11 (4.0%)	15 (5.7%)	0.70 (0.28-1.66)	0.425
	No	262 (96.0%)	249 (94.3%)		
Oncological diseases	Yes	2 (0.7%)	1 (0.4%)	1.94 (0.10-114.94)	1.000
	No	271 (99.3%)	263 (99.6%)		

It was found that visits to outpatient clinics (in the last 2 years) statistically significantly increase *S. aureus* colonization risk (OR=1.56; Cl 1.09-2.24) (Table 17). Such risk factors as the blood sampling from a vein, hormone consumption, and intravenous drug use were not statistically significant for *S. aureus* colonization in the community group. By analyzing young adult group and older adult group separately, it was found that in the latter group hormone consumption is an important risk factor for colonization of the microorganism under study (OR=5.47, CI 1.06-53.47, p=0.023).

Table 17. Potential risk factors for *S. aureus* carriage related to medical impact

Analysed risk factors		*S. aureus* carriers		OR (95% Cl)	*p*
		Yes	No		
		abs. no. (%)	abs. no. (%)		
Visits to outpatients clinics	Yes	131 (48.0%)	98 (37.1%)	1.56 (1.09-2.24)	0.012
	No	142 (52.0%)	166 (62.9%)		
Blood taking from a vein	Yes	18 (6.6%)	17 (6.4%)	1.03 (0.49-2.17)	1.000
	No	255 (93.4%)	247 (93.6%)		
Hormone consumption	Yes	16 (5.9%)	10 (3.8%)	1.58 (0.66-3.97)	0.317
	No	257 (94.1%)	254 (96.2%)		

The information on contacts with people having a higher risk of being potential *S. aureus* carriers due to their work or activities was analyzed. It was found during the study that only three respondents had a close contact with a person infected with MRSA. Due to these low numbers a relation of this factor and upper respiratory tract colonization with *S. aureus* was not found (OR=0.48). (Table 18)

According to the study, the contact of the respondents in the older adult group with the people having dermal problems increased the risk of *S. aureus* colonization (OR=2.62, CI 1.00-7.34, p=0.047). Meanwhile, this possible risk factor in the young adult group did not have any influence on becoming *S. aureus* carrier (OR=0.50, CI 0.24-1.06, p=0.051). Regarding the period of time of contacts with owners of dermal problems it was found that the older the contact the less risk it takes (up to 6 months OR=1.000, 7-12 months OR=0.5, up to 24 months OR=0.38); this tendency is statistically insignificant (χ^2_{MH}=1.046; df=1; p=0.306).

37% of carriers and 29.5% of non-carriers pointed out that they had contacts with people working in the health care sector. This risk factor is quite important for *S. aureus* colonization (OR=1.40), nevertheless the data is not statistically significant. 5.9% of carriers and 10.2% of the respondents with no *S. aureus* isolated claimed that they had children visiting kindergarten OR=0.55. 2.6% of carriers and 6.1% of the respondents who had no *S. aureus*

isolated worked in a kindergarten OR=0.41. However, no statistically significant result was found. (Table 18)

Table 18. Selected risk factors for *S. aureus* carriage related to the contact with persons from to the risk group

Analysed risk factors		*S. aureus* carriers		OR (95% Cl)	*p*
		Yes	No		
		abs. no. (%)	abs. no. (%)		
Contacts with MRSA infected person	Yes	1 (0.4%)	2 (0.8%)	0.48 (0.01-9.31)	0.618
	No	272 (99.6%)	262 (99.2%)		
Contacts with person having dermal problems	Yes	35 (12.8%)	29 (11.0%)	1.19 (0.68-2.09)	0.594
	No	238 (87.2%)	235 (89.0%)		
Contacts with health care sector employees	Yes	101 (37.0%)	78 (29.5%)	1.40 (0.96-2.04)	0.068
	No	172 (63.0%)	186 (70.5%)		
Contacts with rest-home residents	Yes	8 (2.9%)	12 (4.5%)	0.63 (0.22-1.72)	0.368
	No	265 (97.1%)	252 (95.5%)		
Having children, who go to kindergarten	Yes	16 (5.9%)	27 (10.2%)	0.55 (0.27-1.08)	0.080
	No	257 (94.1%)	237 (89.8%)		
Kindergarten employee	Yes	7 (2.6%)	16 (6.1%)	0.41 (0.14-1.07)	0.055
	No	266 (97.4%)	248 (93.9%)		
Contacts with prison inmates	Yes	13 (4.8%)	17 (6.4%)	0.73 (0.32-1.63)	0.455
	No	260 (95.2%)	247 (93.6%)		
Contacts with disabled people	Yes	21 (7.7%)	21 (8.0%)	0.96 (0.49-1.91)	1.000
	No	252 (92.3%)	243 (92.0%)		

37% of carriers and 29.5% of non-carriers pointed out that they had contacts with people working in the health care sector. This risk factor is quite important for *S. aureus* colonization (OR=1.40), nevertheless the data is not statistically significant. 5.9% of carriers and 10.2% of the respondents with no *S. aureus* isolated claimed that they had children visiting kindergarten OR=0.55. 2.6% of carriers and 6.1% of the respondents who had no *S. aureus* isolated worked in a kindergarten OR=0.41. However, no statistically significant result was found. (Table 18)

The respiratory tract colonization with *S. aureus* odds ratio between carriers of this microorganism and non-carriers of those who take exercise was 1.24 (0.81-1.91). The use of public sports equipment during exercise was not identified as a statistically significant factor. (Table 19)

Table 19. Various risk factors for *S. aureus* carriage

Analysed risk factors		***S. aureus* carriers**		**OR (95% Cl)**	***p***
		Yes	**No**		
		abs. no. (%)	**abs. no. (%)**		
Going in for some sports	Yes	65 (23.8%)	53 (20.1%)	1.24 (0.81-1.91)	0.348
	No	209 (76.2%)	211 (79.9%)		
The use of public sports equipment	Yes	51 (78.5%)	43 (81.1%)	0.85 (0.30-2.30)	0.820
	No	14 (21.5%)	10 (18.9%)		
Pets at home	Yes	148 (54.2%)	130 (49.2%)	1.22 (0.86-1.74)	0.262
	No	125 (45.8%)	134 (50.8%)		
Trips away from Lithuania	Yes	111 (40.7%)	99 (37.5%)	1.14 (0.80-1.64)	0.480
	No	162 (59.3%)	165 (62.5%)		
Were parents born in Lithuania?	Yes	239 (87.5%)	228 (86.4%)	1.11 (0.65-1.90)	0.702
	No	34 (12.5%)	36 (13.6%)		

Such factors as pets (OR=1.22), trips away from Lithuania in two-year period (OR=1.14) are presented in Table 19 and were not qualified as the factors with statistically calculable influence on the upper respiratory tract colonization with *S. aureus*. Some of the risk factors mentioned are not widely spread among the respondents as well as control groups.

The data collected on the family members‘ diseases, hospitalization, work and other activities showed that the contact with family members who had some staphylococcal infection was a highly significant factor (OR=2.33) for the respondents‘ colonization with *S. aureus*, nevertheless, it was not statistically significant. This may be due to relatively low frequency of this risk factor in the respondent groups.

It was revealed, that such risk factors as family members‘chronic diseases (OR=1.65) and hospitalization in the two-year period (OR=1.49) increase the risk for the people in contact to become *S. aureus* carriers. The respondents

who share the same living space with sick or hospitalized family members more often had *S. aureus* isolated from their upper respiratory tract than those who did not share the same living space (Table 20). After examining the importance of family members‘ admittance to the hospital as the risk of colonization, it was found that the earlier a family member was hospitalized, the less risk for the family member living together to become *S. aureus* carrier (up to 6 months OR=1.00, 7-12 months OR=0.80, up to 24 months OR=0.68). Nevertheless, this tendency was not statistically significant (χ^2_{MH}=0.972; df=1; p=0.324).

Table 20. Potential risk factors for *S. aureus* carriage related to family members

Analysed risk factors		***S. aureus* carriers**		**OR (95% Cl)**	***p***
		Yes	**No**		
		abs. no. (%)	**abs. no. (%)**		
If family members had staphylococcal infections	Yes	14 (5.2%)	6 (2.3%)	2.33 (0.83-7.52)	0.109
	No	253 (94.8%)	253 (97.7%)		
Family members having chronic diseases	Yes	59 (22.1%)	38 (14.7%)	1.65 (1.03-2.66)	0.033
	No	208 (77.9%)	221 (85.3%)		
The use of hormonal medications by family members	Yes	10 (3.7%)	15 (5.8%)	0.63 (0.25-1.54)	0.309
	No	257 (96.3%)	244 (94.2%)		
Family members‘ admittance to the hospital	Yes	95 (35.6%)	70 (27.0%)	1.49 (1.01-2.20)	0.039
	No	172 (64.4%)	189 (73.0%)		
Family members‘ visits to outpatient clinics	Yes	110 (41.2%)	90 (34.7%)	1.32 (0.91-1.90)	0.151
	No	157 (58.8%)	169 (65.3%)		
Family members working in kindergarten	Yes	10 (3.7%)	11 (4.2%)	0.88 (0.33-2.32)	0.826
	No	257 (96.3%)	248 (95.8%)		
Family members‘ contacts with prisoners	Yes	11 (4.1%)	10 (3.9%)	1.07 (0.40-2.86)	1.000
	No	256 (95.9%)	249 (96.1%)		
Family members‘ contacts with disabled	Yes	17 (6.4%)	16 (6.2%)	1.03 (0.48-2.24)	1.000
	No	250 (93.6%)	243 (93.8%)		

Family members‘ staphylococcal infections, visits to outpatient clinics, contacts with disabled and prisoners all increased the risk for the respondents to become *S. aureus* carriers, however, this data was not statistically significant (Table 20).

After summarizing the results, risk factors were grouped into separate groups to identify the most significant *S. aureus* carriage related risk factors.

A statistically significant association was found between the *S. aureus* colonization in the upper respiratory tract and the group of risk factors, which included male gender, visits to outpatients' clinics and living with family members who have chronic diseases. (Table 21)

Table 21. Association between risk factors and colonization with *S. aureus* of upper respiratory tract

Risk factor	General OR	95% CI	OR_p	95% CI
Gender (male)	1.31	0.92–1.87	1.59	1.11–2.28
Visits to outpatients clinics	1.56	1.09–2.24	1.71	1.19–2.47
Family members having chronic diseases	1.65	1.03–2.66	1.60	1.01–2.55

OR_p – corrected OR

4.7.2. The risk factors possibly influencing respiratory tract colonization with *S. aureus* in the hospitalized patients group

A total of 20.7% (153/738) of the respondents were *S. aureus* carriers on the day of discharge from hospital. 28.1% (n=43) of the respondents were colonized with a new *S. aureus* strain.

The influence of chronic diseases on the respiratory tract colonization with *S. aureus* was evaluated. It was found that a hospitalized person suffering chronic diseases has a higher risk of becoming *S. aureus* carrier (OR=1.60). Such diseases as diabetes (OR=2.59), oncological cases (OR=4.31) were found as significant risk factors for upper respiratory tract colonization; nevertheless,

due to the small extent of the risk factors in the case groups, the results obtained were statistically insignificant (Table 22).

Table 22. The influence of chronic diseases on hospitalized patients to become colonized with a new *S. aureus* strain

Analysed risk factors		Case group	Control group	OR (95% Cl)	p
		abs. no. (%)	abs. no. (%)		
Chronic diseases	Yes	22 (51.2%)	17 (39.5%)	1.60 (0.63-4.11)	0.386
	No	21 (48.8%)	26 (60.5%)		
Diabetes	Yes	7 (16.3%)	3 (7.0%)	2.59 (0.54-16.52)	0.313
	No	36 (83.7%)	40 (93.0%)		
Oncological cases	Yes	4 (9.3%)	1 (2.38%)	4.31 (0.40-217.09)	0.360
	No	39 (90.7%)	42 (97.7%)		
Whether the surgical operation was performed	Yes	37 (86.0%)	35 (81.4%)	1.41 (0.38-5.45)	0.771
	No	6 (14.0%)	8 (18.6%)		
Antibiotics prescribed before surgical operation	Yes	26 (60.5%)	24 (55.8%)	1.21 (0.47-3.12)	0.827
	No	17 (39.5%)	19 (44.2%)		
Antibiotics prescribed after surgical operation	Yes	16 (40.3%)	12 (51.7%)	1.53 (0.56-4.22)	0.490
	No	27 (59.7%)	31 (48.3%)		

The questionnaire data analysis showed that 86.0% (n=37) of the case group respondents and 81.4% (n=35) of the control group respondents had surgery performed. Surgical intervention may influence colonization with *S. aureus* (OR=1.41), yet the results obtained were not statistically significant.

It was analyzed whether antimicrobials' prescription before and after the surgical operation had any influence on the upper respiratory tract colonization with *S. aureus* strain; it was found that the factors mentioned increased the risk of colonization with this microorganism, but statistical significance was not observed. (Table 22)

It was also analysed which antimicrobials used had an influence on colonization with *S. aureus*. The results showed that 84.0% (n=42) of the respondents had a single doze of cephalosporins prescribed before the surgical

operation, 10% (n=5) – penicillins. The prescription of cephalosporins may influence the colonization with a new *S. aureus* strain, however not statistically significant (OR=1.65, CI 0.17-21.43, p=0.666) due to the low number of cases.

After surgical operation 60.7% (n=17) of the respondents were prescribed cephalosporins and 32.1% (n=9) – penicillins. The analysis of prescription of antimicrobials after surgical operation showed that penicillins increased the likelihood of carriage, yet the results were not statistically significant (OR=1.11, CI 0.17-7.76, p=1.000).

It was also determined the influence of the time span of after-surgery prescribed antimicriobials' use on colonization with a new *S. aureus* strain. When the time span was grouped into 3 time periods, there was a significant tendency towards that the longer the time span of antimicrobials' use, the risk of becoming new *S. aureus* carrier significantly decreased (Table 23).

Table 23. The influence of the time span of antimicrobials' use after surgery on colonization with a new *S. aureus* strain

Time span of antimicrobials' use after surgery (days)	**Case group**	**Control group**	**OR**
	abs. no. (%)	**abs. no. (%)**	
2-3	6 (75.0%)	2 (25.0%)	1.000
4-5	9 (64.3%)	5 (35.7%)	0.600
6-9	1 (16.7%)	5 (83.3%)	0.067

χ^2_{MH}=4.227; df=1; p=0.040

Regarding the relation between some frequently applied medical procedures, such as vein or urinary catheter introduction, mechanical ventilation and parenteral nutrition, and upper respiratory tract colonization with a new *S. aureus* strain it was found that 55.8% (48/86) of the respondents had vein catheter introduced, 17.4% (n=15) - had urinary catheter introduced. Mechanical ventilation was applied to 1.2% (1/86) of the respondents, parenteral nutrition to – 3.5% (n=3) of the respondents. The analysis of these possible risk factors showed that urinary catheter introduction increased the

risk of colonization, but not statistically significantly (OR=1.63, CI 0.46-6.17, p=0.571). The results of our study showed that the colonization with a new *S. aureus* strain does not statistically significantly depend on the factors mentioned above.

It was found that the longer the duration of hospitalization, the greater the risk to become colonized with a new *S. aureus* strain. If we take the risk of hospitalization up to 4 days as 1, the risk of hospitalization up to 5-9 days was 1.412; 10 days and longer period OR= 1.524. Yet χ^2_{MH}=0.494; df=1; p=0.482, which means that the tendency was insignificant. (Table 24)

Table 24. Hospitalization period influence on colonization with a new *S. aureus* strain

Hospitalization period (days)	Case group	Control group	OR
	abs. no. (%)	abs. no. (%)	
2-4	9 (42.9%)	12 (57.1%)	1.000
5-9	18 (51.4%)	17 (48.6%)	1.412
10->	16 (53.3%)	14 (46.7%)	1.524

χ^2_{MH}=0.494; df=1; p=0.482

The hospitalized patients group comprised of a low number of observations. This may be why there were no statistically significant results obtained through analysis of 95% and 90% confidence intervals. It is possible that statistically significant results could have been obtained by increasing the case and control groups substantially.

Due to relatively small number of respondents in the case and control groups, and because of the coefficient of determination (R^2) of the models tested were not significantly exceeding zero, logistic regression was not performed.

5. DISCUSSION

S. aureus (MSSA as well as MRSA) is ranked as the second most common cause of hospital-acquired and community-acquired infections. Staphylococcus caused nosocomial infection increase morbidity, mortality, hospital stay, and costs. [232, 233]

Due to increasing number of infections caused by MRSA strains, which are often multiresistant, therapy has become problematic. Therefore, prevention of staphylococcal infections has become more important than ever before.

S. aureus carriage has been identified as a risk factor for the development of community-acquired and nosocomial infections in general hospital populations [164, 195, 211], surgical patients [139, 198], patients on haemodialysis or continuous peritoneal dialysis [158], patients with liver cirrhosis and after liver transplantation [234], HIV-infected patients [235], and patients admitted to intensive care units [236].

S. aureus carriage in community

We found in our study that the carriage rate of *S. aureus* in community group is as high as 50.8%. This was similar to the carriage rate found in other community-based studies in Lithuania [12, 13]. Although, higher rates (59.8%) have been found in various studies [237, 238], most of the studies report a carriage rate of 20–30% [139].

The obtained results suggested that the presence of *S. aureus* was more frequent in the young adults' group (62.3%) than in the older adults' group (43.4%). The carriage rate reported in young adults' group in our study was similar to those found in other studies [237, 239]. The higher carriage rate among young adults group may be due to the more active way of life and close communication among individuals.

The rates of MRSA colonization remain low among healthy people worldwide. The rates of carriage among children with no risk factors for

MRSA colonization have ranged from 0.8% to 3.0% [144, 145]. The prevalence of CA-MRSA in adults is currently low, but appears to be increasing. In the United States, the prevalence of MSSA and MRSA in the general population reached 31.6% and 0.84% respectively [240]. The analysis of 57 studies on CA-MRSA prevalence among hospitalized patients and community members revealed that the prevalence of CA-MRSA among people without risk factors is 0.24% [150]. Tiemersma et al. [151] state that the prevalence of CA-MRSA in Europe is from 0.03 to 1.5%.

MSSA and MRSA isolation rates are higher among children than among adults [12, 238]. Hamdan-Partida et al. [237] found that 33.6% of all MRSA isolates in the study were detected in the 1 to 10 year-old group, followed by the 11 to 20 year-old group (25.2%), and for the other groups, the percentage decreased as the age increased. Other studies [239, 241] also showed that colonization with *S. aureus* decreases as people grow older.

In our opinion, the carriage rate in above mentioned studies varies depending on the different investigated population groups, the use of culture methods and sample sites. The high prevalence of *S. aureus* colonization in our study could at least partly be related to the use of both nasal and throat swabs for screening of *S. aureus* carriage.

Our results indicate that the prevalence of MRSA in the healthy adult population of Vilnius is low. This finding was contrary to an earlier report of MRSA carriage in Lithuania [12, 13]. In earlier studies in Lithuania *S. aureus* carriage was analyzed in children group [12] and hospitalized patients group [13]. As mentioned before, the spread of this microorganism in the children group is higher and may be partly due to their close contact at school allowing ready interchange of bacterial flora. It is also not clear whether the scope of the study was formed applying the selected criteria (e.g.: within a year hospitalized children were not included into the study). It is possible to make an assumption that part of the hospitalized patients during the study arrived from the nursing homes, rehabilitation clinics or other hospitals i.e. had a contact with hospital environment [13]. Therefore, it is possible that the difference among the results

obtained was due to the different study groups and the applied criteria for group formation.

Further studies have to be carried out to determine the carriage rate of MRSA in the Lithuania on the population level, covering large samples and different groups of subjects including children in day care centers, sport teams, prison inmates, and etc.

S. aureus carriage in hospitalized patient group

Rates of MRSA carriage among patients on admission varies with geographical location, the rate can vary from 2% to 17% of general medical and surgical patients [13, 153, 158, 242, 243, 244]. Casewell [152] reported that, approximately 30 – 40% of patients are *S. aureus* carriers on admission to hospital and this proportion increases during hospital stay. After admission, colonized patients may become endogenously infected with their own organism or act as the source of further cross-colonization of others [139, 156, 159]. Carriage rate of *S. aureus* on the day of admission to Kaunas hospital in Lithuania was 67.3%, 4.9% of them were MRSA [13].

Our findings in surgical departments of two hospitals were similar to the mentioned studies (30.8% in VUHSK and 28.9% in VCUH). We found that generally 29.7% of respondents from both hospitals were *S. aureus* carriers, 2.8% of them were colonized with MRSA. 28.6% of these MRSA carriers developed SSI MRSA infection.

Foreign researchers such as Mest et al. [242] reported a prospective study on patients who were treated in the surgical intensive care unit from all surgical wards. They screened all patients preoperatively on the day of admission for nasal carriage of MRSA and found that 4% of patients had MRSA-positive nasal cultures. 26% of these MRSA carriers developed MRSA infections. Davis et al. [153] reported a prospective study on subsequent MRSA infections in patients admitted to 5 units in their hospital. 3% of the patients were MRSA carriers. The rate of subsequent MRSA infections in nasal carriers of MRSA was 10 times as much as that in non-carriers. Lye et al. [158]

reported a prospective study on MRSA infection in patients entering into the CAPD program. 17% of these patients were nasal carriers of MRSA. Yano et al. [243] in prospective observational cohort study screened 2423 patients who were admitted to orthopedic surgery department and found that 2.6% of patients were colonized with MRSA. 6.3% patients with preoperative nasal cultures that were positive for MRSA had SSI occurrence. The results of our study slightly differ from some mentioned studies and differences may have been due to discrepancies in the condition of patients, and differences in sampling procedures.

The admission of MRSA-positive patients has become a major mode of introduction and dissemination of MRSA within health care institutions. In addition, it has recently been shown that hospital discharge of MRSA carriers can result in the spread of MRSA within the community [245] or a large proportion of MRSA colonized patients can be admitted to other wards, other hospitals, rehabilitation centers or long-term care facilities where active surveillance methods are not used and these patients would not have been identified in the absence of clinical cultures. These patients may have extended hospital stay, which would enable them to potentially transmit MRSA to healthcare workers and other patients while their colonization status remained unrecognized.

Foreign countries‘ studies show, that 1.9% - 12.7% of MRSA carriers were identified upon discharge from hospital [244, 246-249]. Fewer MRSA carriers were identified during our study. It was found that 20.7% of the respondents from both hospitals were *S. aureus* carriers on the discharge day. 5.3% of the discharged respondents were colonized with a new MSSA strain. The percentage of new MRSA carriers found in our study was only 0.5%.

Several factors may explain the differences in results across our and other studies. The high rate of MRSA carriage in the following studies [244, 247, 249] may be related to the involved adult patients admitted to the ICU. By contrast, the patients studied by Gener et al. [246] were from dermatology department. However, we did not include ICU or dermatology departments in

our study. There also were differences in the length of patient stay in the hospital, and in the number of examined respondents. These differences are probably an underestimation of the true prevalence of new MRSA carriers' cases on the discharge day.

Our study also showed that patients discharged from VUHSK were more frequently (p=0.018) colonised with *S. aureus*. Such results might be due to the higher number of examined respondents (78.7%), as compared to VCUH (2.5%), from abdominal surgery department. Furthermore, the higher number of *S. aureus* carriers on admission day were also detected in VUHSK.

Overview of S. aureus screening sites

It was found during our study that the nares were the most prevalent site of *S. aureus* colonization (sensitivity of 39.2% in community group, 59.8% on the day of admission and 79.0% on the day of discharge in hospitalized patients groups). Without using throat sampling, 31.5% in community group, 15.9% on the day of admission and 10.5% on the day of discharge in hospitalized patients' groups' respondents would not have been identified. The sensitivity of nares and throat sampling were 72.1% and 27.9% respectively in newly colonized patients on hospitalization period.

Current guidelines differ in their recommendations for sampling anatomical sites in search of *S. aureus* colonization. Recommended sampling includes the anterior nares and secretions or visible wounds [250], and various combinations of the nares, throat, skin and perineum, axilla, and rectum [251, 252]. Girou et al. [251] claim that the sensitivity of combined sampling of the nares, throat, and perineum may be as high as 98%. Coello et al. [253] performed the largest reported study on multiple-site screening of 403 MRSA-colonized patients: nasal swabs identified 79% of patients colonized at any site; combined swabs from nose and throat, nose and perineum, and nose, throat and perineum identified 86%, 93% and 98%, respectively. A recent study from an orthopedic ward in a Swedish hospital claims that, when a single-site sampling is employed, *S. aureus* colonization is more prevalent in

the throat and rare at the nares [254]. This study and similar reports [237, 255], demonstrated the importance of the throat as a very common site of colonization and it was suggested that *S. aureus* sampling should include throat sampling. Interestingly, Armstrong-Esther et al. [238] after examining children and adult groups came to the conclusion that transient carriers quite often yielded the organism from throat or hand alone, a finding rare among the persistent carriers.

We agree with Struelens et al. [256] that the most important *S. aureus* screening site is the nose and not the throat as was claimed by Bignardi et al. [255]. However, in contrast to Struelens et al., and consistent with Girou et al. [251], Hamdan-Partida et al. [237] and Eveillard et al. [252] the nose is not the only relevant screening site but should be considered alongside the throat, and, if necessary, other (clinical) sites for efficient detection of *S. aureus* and MRSA carriers.

The resistance of isolated S. aureus strains to some antimicrobials

Previous community studies in Lithuania and other investigators have reported penicillin resistance rates of *S. aureus* strains much higher reaching 80.5–83.7% [12, 13, 56]. Our data showed that only 65.4% of the isolated *S. aureus* strains were resistant to penicillin. Similarly, the resistance to erythromycin which was only 2.7% in this study compared to the 16.8% to 34.6% reported by Perez-Vazquez et al. [86] and Otsuka et al. [108] respectively. The results of our study show that the investigated *S. aureus* strains were less resistant to clindamycin (2%) as compared to some data in literature sources (8.3–26%) [31, 86, 88]. Lower clindamycin resistance levels in previous studies from Lithuania [12, 13] may be explained by the fact that to determine *S. aureus* resistance dilution method was used and the inducible clindamycin D zone test was not performed.

Our data showed that more than 8% of the *S. aureus* isolated from community group were resistant to tetracycline and that corresponds to data of the previous studies [56, 88, 96, 97].

According to the data published, 0–2.6% of *S. aureus* strains are resistant to aminoglycosides [12, 13, 56, 86]. In comparison, our data is very similar. 0.7% of *S. aureus* strains were resistant to streptomycin, gentamicin and 1% to kanamycin.

In contrast, oxacillin, cefoxitin, fusidic acid, norfloxacin, ciprofloxacin and rifampin were active against all the isolated *S. aureus* strains. Our results are similar to those in some other investigations [12, 13, 56, 80, 88].

Some differences in resistance data could be related to the number of participants, differences in the investigated groups or diverse techniques in the investigation of resistance to antimicrobials [56]. We believe that the main explanation for the low antibiotic resistance rates detected stems from the fact that the antibiotic consumption in primary health care in Lithuania is comparatively lower in comparison with other European countries [257].

Review of antimicrobial susceptibility patterns on the admission day in our study demonstrated that resistance to penicillin and tetracycline were predominant. Decreased activity of ciprofloxacin, erythromycin, clindamycin, gentamicin, kanamycin and norfloxacin was noted. Oxacillin and cefoxitin resistance was found in 2.8% of *S. aureus* isolates. All the isolates isolated on the discharge day were susceptible to rifampin. The higher MRSA carriers' rate on discharge day compared with community group respondents may be due to selection criteria applied to community group. Among the patients examined on the day of admission, part of the respondents (37.8%) was previously admitted to hospital within a year, whereas community group respondents were not admitted to hospital for 3 years time. Presumably, one of the well known MRSA carriage risk factors - hospitalization, has influenced the detection of MRSA carriers as well as the differences of the resistance to antibiotics of *S. aureus* isolates detected on the admission day and among community group respondents.

The antimicrobial susceptibility patterns of *S. aureus* isolates on the discharge day also differ. In most of the studies conducted on clinical *S. aureus* strains high resistance to penicillin ranging from 68.4% to 95.4% was reported

[57, 58], our results are in keeping with the data (82.6%). Penicillins quite often (for 31% respondents) were used after operation in hospitals included in the study. An increase in resistance to oxacillin (3.9%) and cefoxitin (4.5%) was noted.

High resistance development against tetracycline (89.7% in the MSSA isolates and 42.9% in the MRSA isolates) was reported in Schmitz et al. [96] investigation. Petrelli et al. [97] has recently found that 6.3% of MRSA and 14.3% of MSSA strains were resistant to tetracycline, while in this study tetracycline resistance was 7.7%.

In 2008, Perez-Vazquez et al. [86] and Cuevas et al. [85] reported that clinical *S. aureus* strains resistance to erythromycin and clindamycin was 59.7% – 81.1% and 31.1% – 42.5% respectively. In this study, on the contrary, 4.5% of *S. aureus* strains were resistant to erythromycin and 3.9% - to clindamycin.

The resistance rate to aminoglycosides and fluoroquinolones was lower than noted in recent studies among adult patients in Lithuania, Spain and Canada [58, 82, 85, 86, 91].

After examining the resistance to mupirocin of 263 randomly selected *S. aureus* isolates from community as well as from hospitalized patients groups, it was found that all of the *S. aureus* isolates were susceptible to this antimicrobial. Nevertheless, Kresken et al. [132] detected mupirocin resistance in 13.5% MRSA isolates, and in 0.5% of MSSA strains. Other investigators from Greek hospitals also found, that 1.3% of *S. aureus* were mupirocin resistant [133]. Several studies on *S. aureus* carriage in the community demonstrated that mupirocin resistance of isolated *S. aureus* isolates range from 0% to 2.5% [56, 87, 88]. In Lithuania, nasal mupirocin ointment is not available in the market, because it is not registered by State Medicines Control Agency of Lithuania. Hence, use of mupirocin nasal ointment is rare in a Lithuanian community setting. The finding of negligible baseline mupirocin resistance was gratifying, as mupirocin is recommended as part of the

eradication therapy for recurrent staphylococcal skin infection or before thyroidectomia.

The differences of the resistance of isolated *S. aureus* strains to antimicrobials in our study as compared to the data presented by other scientists may be linked not only to the consumption of antibiotics in the country, but also to the internal policy of antibiotics consumption in the hospitals under study.

Erythromycin and tetracycline resistance genes in S. aureus

The use of multiplex PCR for the *S. aureus* identification and for detection of antibiotic resistance genes has been described previously [110]. The multiplex PCR assay performed in our study included the detection of four different resistance genes in one reaction. The advantage is that it is easy to perform and is much more cost-effective.

In general, we have found that isolated erythromycin resistant MRSA strain was positive for *ermA*. Some researches [108, 109] reported that *ermA* gene is predominant in MRSA strains. Most (50.0%) of the 18 MSSA isolates from both groups in this study contained the *ermC* gene, 38.9% had *ermA*. These findings were consistent with previous reports [109, 110, 111].

In terms of the incidence of tetracycline resistance genes in isolated *S. aureus* strains, *tet*K genes were dominant in MSSA and MRSA in both community and hospital groups. These findings were similar to Petrelli et al. [97] who found that *tet*K genes were dominant. However, our results contradict the data of some investigations. Schmitz et al. [96] reported that the *tet*M gene was found more prevalent in MRSA isolates (76%) while *tet*K was detected in 96% of tetracycline-resistant MSSA strains. Trzcinski et al. [99] found the *tet*K gene in 21 (31.8%) of 66 MRSA isolates, the *tet*M gene in 24 (36.4%) and both genes together in 21 isolates (31.8%).

The other tetracycline resistance coding gene, *tet*M, was detected in one MSSA and one MRSA strains which were phenotypically sensitive to tetracycline, but had inducible MLS resistance and *ermA* genes. Phenotypically

tetracycline sensitive, *tet*-positive isolates let us suppose that presence of the gene but lack of the phenotype is possible.

We also noted that two strains were found to be resistant to erythromycin and two strains resistant to tetracycline in both groups but did not carry any *erm* or *tet* genes. Similarly, Sekiguchi et al. [258] also found discordance among resistance genes and phenotyping susceptibility. They stated that this discordance might be due to mutations in the coding or promoter region of the resistance genes. On the other hand, this result can be explained by the location of these genes in small plasmids, which were occasionally lost. Also erythromycin resistance can be caused by presence of *msrA* or *ermB* [109]. However, we did not determine them.

Since *S. aureus,* especially MRSA caused hospital infections are the growing problem, rapid, sensitive and reliable molecular identification techniques should be developed and introduced for the genotypic determination of resistance profiles. It could be very helpful to much more rapidly determine treatment options.

The prevalence of PVL in isolated S. aureus strains

Some reports have suggested that certain strains of CA-MRSA may be more virulent than HA-MRSA [259, 260]. The expression of PVL has been more strongly associated with CA-MRSA than with MSSA and HA-MRSA [259].

This bicomponent cytotoxin previously reported to be produced by less than 5% of *S. aureus* isolates [23, 24, 25]. Some studies, which investigated nasal carriage of *S. aureus* in the community, demonstrated that from 0.5% [26] to 0.65% [27, 28] of isolated MSSA strains carry PVL gene. The genotyping data in our study provides a unique picture of prevalent PVL-positive *S. aureus* in some groups of Vilnius adult population. A remarkable finding from our study was that 2.5% of the *S. aureus* isolates studied had PVL genes. Obtained results were in contrast to those reported in other studies. Munckhof et al. [26] did *S. aureus* carriage study in Queensland adults'

population, and found that 0.5% MSSA and 20% CA-MRSA strains were PVL positive. Lo et al. [261] examined 300 colonizing *S. aureus* strains isolated from children and found that 6% of them had the PVL locus. This higher prevalence is probably related to the greater proportion of MRSA colonized pediatric respondents (7.4%).

It can be assumed that PVL positive MSSA strains are rather common, but they are usually overlooked, as no routine test for PVL is available. This reinforces the need to study the prevalence of PVL-encoding genes among MSSA isolates from carriage isolates.

Regarding the study by Munckhof et al. [33] in the clinical cohort, the prevalence of PVL was found in 16% of MSSA and 55% of CA-MRSA strains. Other studies found that CA-MRSA and MSSA carriage isolates have much lower rates of PVL presence than do clinical *S. aureus* isolates [29, 30, 31, 32]. Daskalaki et al. [262] found that children with PVL-positive CA-MRSA and MSSA SSTIs were more likely to have severe local diseases and require incision and drainage. The clinical manifestations of PVL-positive *S. aureus* may initially correspond to skin infections and then progress to severe necrotizing haemorrhagic pneumonia with a high death rate. However, as stated, the pneumonia resulting from PVL-positive CA-MRSA is far more aggressive than that associated with either HA-MRSA or other, more typical, community acquired organisms. Therefore, in clinical practice PVL detection in patients with severe community acquired pneumonia is essential for patients' survival and as it may lead to earlier started empirical, aggressive treatment. The Health Protection Agency and the UK government's Department of Health recently published revised guidance on the diagnosis and management of PVL-associated *S. aureus* infections in England [263].

Our results demonstrate that PVL-positive *S. aureus* strains were more commonly isolated from nose than from throat. The cause of this occurrence is unclear, but may relate to strain-specific characteristics such as adherence factors.

PVL-positive MSSA and MRSA strains display variable antimicrobial susceptibility profiles, which can be geographically distinct. In the present study, the majority of PVL-positive MSSA isolates were resistant just to penicillin. In contrast, other studies demonstrated, that PVL-positive *S. aureus* were more frequently methicillin-resistant and had higher rates of resistance to ciprofloxacin, erythromycin, fusidic acid [39, 40].

spa types in the analysed S. aureus strains

The diversity of some isolated *S. aureus* strains from community and hospitalized patients was determined by *spa* typing. We found that *spa*-type t056 was one of the dominant in MSSA strains in both groups. It should be emphasized that *spa*-type t008 and t002 were associated in both MRSA and MSSA isolates in our study. The observation that 15% *spa* types were found in both community and in hospital groups, suggest that transmission of *S. aureus* from the clinical settings to the community and vice versa is of frequent appearance.

We have detected in this study a high prevalence of single *spa* types (e.g. t119, t1023, t2120, t2771, t3850, t4135 and etc.) in *S. aureus* isolates. However, according to the Ridom StaphType^{TM} database, the geographical distribution of these *spa* types is very limited and mainly reported in Germany, Norway, the Netherlands, Poland and France. [264]

The *spa* types t4995 – t4999, t5001 – t5005 detected in this study were added into the Ridom StaphType^{TM} database. Lithuania currently is listed as the only country where these *spa* types were detected [264].

Most of the MSSA isolates from community group belonged to *spa*-type t084. This *spa*-type has previously been found in Denmark, Poland, Spain, United Kingdom, the United States, Italy and etc. The second most prevalent *S. aureus spa*-type in community group was t056. It is circulating in northern European countries and Germany, the Netherlands, Romania, Spain. [264]

This study also showed that most of *S. aureus* strains isolated in the hospital group were associated with t008 spa-type, previously described in

European countries such as Austria, Belgium, Bulgaria, Estonia, United Kingdom, Poland, Denmark, Finland, Norway, Sweden also India, Iceland, South Africa, the United States, etc. This *spa*-type was dominant among MRSA isolates as well. The second most prevalent *S. aureus spa*-type in hospital group as well as in community group was t056. [264]

Although *spa* typing is still a quite expensive method of testing, its implementation would enable us to examine the outbreaks of hospital infections in greater detail, as well as their origin and reasons. Additionally, Ridom StaphType^{TM} database enables us to specify the data via the Internet. Moreover, this SpaServer can be used to collate and harmonize data from various geographic regions.

We believe that after *spa* typing for the greater number of isolated *S. aureus* strains in our study more plausible results would be obtained and it would also be possible to compare them among the groups examined and the data obtained by the authors of other countries in greater detail.

Risk factors in community and hospitalized patients' groups

In the analysis of various risk factors influencing community members' colonization with *S. aureus,* different authors analyze such risk factors as age, gender, socioeconomic status, obesity, having a household member who was an MRSA carrier, close contact with a person with risk factor(s) for MRSA acquisition, recent hospitalization, recent outpatient visit, recent nursing home admission, recent antibiotic exposure, chronic diseases (e.g., renal disease, diabetes, malignancy and ect.), injection drug use, contact sports or other athletic activities, living conditions, contact with specific close population, having children attending day care centers and others [15, 20, 26, 173, 175, 177, 178, 265, 266, 267].

We have joined in our study some of the undergoing analysis and already detected risk factors reported by other authors'; we also included some new ones with the aim to evaluate their influence. As our target was to detect the scope of *S. aureus* carriers in the healthy community group, taking into

consideration the CDC offered CA-MRSA definition [268], such risk factors as hospitalization, surgery, dialysis, or residence in a long-term-care facility <1 year (in our study 3 years) before the study, permanent indwelling catheters or percutaneous medical devices or a previous positive MRSA culture were exclusion criteria for study participants.

Analysing the age influence on *S. aureus* carriage, it was found that the respondents from young adults group statistically significantly more often were colonized with *S. aureus* than older adults (OR=2.15, CI 1.49-3.12). As the young adults group consisted of students, some of them came from other towns and have studied for a year or longer now, so the result obtained may be linked to the living conditions in students' dormitory, sharing of personal items, more frequent re-infection, etc. The study data has shown that the respondents which belong to OG-5 (students) more frequently are *S. aureus* carriers as compared to other OG respondents (p_F=0.0001) as well.

The literature reports that a higher *S. aureus* carriage rate is characteristic of males [269, 270]. Our study has also found that males were more frequent *S. aureus* carriers. Nevertheless, it was detected that the variable of gender had statistically significant influence on colonization only in the group of young adults (OR=1.86, CI 1.02-3.39). This might have been due to the lower level of attention to hygiene among young males (especially hand washing) and more frequent sharing of personal belongings. However, why males are more frequent *S. aureus* carriers has not been clearly investigated yet and this association remains unclear. The reasons for this male preponderance need to be further studied, including the possible role of personal hygiene, smoking and may be hormone impact.

The finding that antibiotic consumption in two year period was the important factor (OR=1.48, CI 1.03-2.12) associated with *S. aureus* colonization in community subjects is consistent with the findings of previous reports [200, 202, 271, 272, 273]. These findings are indicative of the presence of strong selective pressure from antimicrobial use in the community. Further

measures to control antibiotic usage to reduce selective pressure for antibiotic resistance are needed in the community as well as in hospitals.

The present analysis also found that visiting outpatients' clinics was significant risk factor for *S. aureus* colonization (OR=1.56, CI 1.09-2.24). The outpatients' clinics provide not only general practice services, but also other doctors' consultations (surgeons', LOR, cardiologists') as well as different other services (surgical wound treatment, throat and nose examination, blood tests, etc.). Moreover, the clinics are visited by sick people, patients recently discharged from hospital, people with injuries and patients after operation. It should also be noted that some of the specialists consulting in outpatients' clinics also work in hospitals. All these factors may be related to *S. aureus* strains spread by direct and close contact [274] and also could indicate a connection between respondents and the health care environment [165].

Our data also indicate that hormone users in older adult group are at increased risk of harboring *S. aureus* carriage (OR=5.47, CI 1.06-53.47). In young adult group 7.5% of the respondents admitted using hormone medicines. The proportion of young adults' group respondents with hormone (contraceptive) consumption might have been underestimated because many subjects might not have recognized they had been using hormones. Choi's et al. [56] study on nasal carriage of *S. aureus* among healthy adults revealed that those who used oral contraceptives were nearly 5 times more likely to be colonized with *S. aureus* as compared to non-users. As there is a scarcity of data on this specific interaction in our study, further studies are required to determine the actual relationship of hormone consumption to *S. aureus* carriage.

Another risk factor markedly associated with higher *S. aureus* carriage in older adults group was the contact with people with skin disorders (OR=2.62, CI 1.00-7.34). Other researchers reported that different skin infections are quite important risk factor for *S. aureus* colonization [81, 246, 275]. So, tending of such patients, care and treatment increase *S. aureus* spread by contact. Dermatological factors, like skin diseases or other skin infections of our

respondents, which have been reported to be associated with *S. aureus* carriage [81, 275, 276] were not determined in this study.

In this study, subjects who shared living space and had close contact with family members suffering from chronic diseases (OR=1.65, CI 1.03-2.66) and family members recently admitted to the hospital (OR=1.49, CI 1.01-2.20) had a significantly higher rate of *S. aureus* colonization. Family members suffering from chronic diseases significantly more often have visits to outpatients' clinics, receive medical service, are admitted to hospital, consume medications (including antibiotics). Therefore, they have contact with hospital environment more freequently and this may be the source of *S. aureus* [81]. Living together with *S. aureus* colonized person, sharing of things increase the probability of *S. aureus* spread by contact and therefore, predetermine the colonization or re-infection of other subjects [274, 277, 278, 279]. A study among an elderly population demonstrated that not only persistent but also non-carriage or intermittent *S. aureus* nasal carrier states are shared among household members [280]. Up to 65% of people with positive cultures living within one household shared genotypically identical strains. [280]

Multivariate logistic regression analysis of risk factors revealed that three factors together – male gender, attending outpatients' clinics and having family members suffering from chronic diseases were significant risk factors for *S. aureus* carriage in upper respiratory tract.

Our ability to identify other important risk factors previously identified by others (suffering from chronic diseases, having family members, who work in a health care institution, or having children, who go to kindergarten, kindergarten employee, having pets and ect.) for *S. aureus* carriage of the studied groups was limited and all mentioned risk factors were not significantly associated with carrier status. Large community-based studies are needed to improve the understanding of the epidemiology and to confirm risk factors of *S. aureus* carriage in the community.

S. aureus, especially MRSA, is a serious threat to hospitalized patients globally. Nasal *S. aureus* colonization has been shown to be a risk factor for

nosocomial infections [156, 158, 159]. Subjects, whose activities involved contact with a health care facility, had a significantly higher rate of *S. aureus* or MRSA colonization than community subjects [165]. Some investigations demonstrated, that recent admission to a hospital, longer length of stay, presence of chronic diseases, catheterization, endotracheal intubation, enteral catheterization, consumption of antibiotics, contact with MRSA carriers were the most important factors associated with MRSA colonization among hospitalized patients. [150, 154, 189, 281, 282].

Finally, specific characteristics of hospitals, such as the size of hospital, a low ratio of nurses to beds, services provided and hospital infections' prevention and control policy may influence the level of patients' colonization with *S. aureus* or MRSA. [190, 283, 284]

Greater attention in the studies in Lithuania as well as other countries is turned to the detection and control of hospital infections' risk factors. At the planning stage of this study we were mainly interested in the risk factors influencing hospitalized patients' colonization with *S. auerus* in hospital environment. It was detected in this study that 5.8% of patients' acquired a new *S. aureus* strain during their hospitalization. After examining the duration of antimicrobial prescription in post-operative period, the tendency was found in this study that the risk of becoming *S. aureus* carrier decreases when the time span of antimicrobial consumption increases (Table 23). This leads us to an assumption that consuming antimicrobials for a longer post-operative period (in the antimicrobial background) prevents the patients from colonization with a new *S. aureus* strain, presumably circulating in hospital environment. The data obtained contradict most of the previously mentioned studies. Nevertheless, this phenomenon was described in the studies by Bischoff el al. [271] and Winkler et al. [285].

However, other risk factors, which may influence the upper respiratory tract colonization during hospitalization, were not detected as statistically significant due to the relatively low number of cases (n=43). It is plausible that in the future valuable data can be obtained by expanding this study and

studying other risk factors in greater detail, as well as evaluating their influence on colonization with *S. aureus* in hospital environment; the data could also be used to improve epidemiological surveillance and control in hospitals.

6. CONCLUSIONS

1. It has been found that individuals in the community in Vilnius city have a high carriage rate of *S. aureus* (50.8%) in the upper respiratory tract. Total of 29.7% of patients were colonized with *S. aureus* on the admission day to the hospital and 20.7% – on the discharge day. Total of 5.8% of the respondents were colonized with a new *S. aureus* strain.

2. The most significant risk factors influencing the colonization of the upper respiratory tract with *S. aureus* in the community group were young age, consumption of antimicrobials in a two-year period, attending outpatient clinic, having family members with chronic diseases or recently hospitalized, male gender, hormone consumption and contact with those who have skin disorders. A statistically significant association was found between the *S. aureus* colonization and the group of several risk factors, which included male gender, attending outpatients' clinics and having family members suffering from chronic diseases. Inverse relationship between antibiotics prescribed and *S. aureus* colonization was found in the hospitalized patient group.

3. *S. aureus* resistance to penicillin in community group was lower than of the strains isolated from hospitalized patients on the admission and discharge days. *S. aureus* strains resistant to methicillin were detected only in the hospitalized patient group. There were no *S. aureus* strains resistant to rifampin and mupirocin. *ermC* gene coding the resistance to erythromycin was predominant (62.5%) in the community group; ermA (54.5%) – in the hospitalized patient group. Among *S. aureus* strains resistant to tetracycline *tetK* gene was predominant in community and hospitalized patients' groups (91.7% and 96.2%, respectively).

4. The Panton–Valentine leukocidin gene was found in 2.5% of the phenotypically different *S. aureus* strains. A higher proportion of PVL-positive

isolates were found among the community group, i.e. 3.0%. The proportion of PVL-positive isolates among *S. aureus* strains isolated from hospital respondents was 2.0%. *spa* types t084 and t056 were mostly prevalent in the community group, t008 and t056 *spa* types in the hospitalized patient group. The highest proportion of the examined MRSA strains belonged to t008 and t002 *spa* types. A total of 15% of all the determined *spa* types circulated in community and hospitalized patient groups.

7. RECOMMENDATIONS

I. Information and education

- To inform and educate the society in order to explain the importance of personal hygiene for the spread of microorganisms; as well as to influence the proper attitude to the consumption of antimicrobials.
- To inform and educate the representatives of the media on how to present the information to the society on the consumption of antimicrobials, their influence on the selection of microorganisms and the spread of resistant strains.
- To deepen the knowledge of health care specialists and doctors on the antimicrobial medicine, prescription indications and relevance.

II. Implementation of molecular research

- To suggest the implementation of methods determining resistance genes in the microbiological clinical research laboratories.
- To implement the detection of virulence determining genes (PVL) by molecular tests and do *spa* typing in microbiological laboratories.

III. Continuous studies' implementation

- To regularly perform the detection of *S. aureus* carriers in hospitals on the admission and discharge days, monitor the variation.
- During hospitalization systematically analyze the risk factors influencing *S. aureus* carriage in the upper respiratory tract.

8. LIST OF PUBLICATIONS

Articles in peer-reviewed journals:

- **Agnė Kirkliauskienė**, Niels Frimodt-Moller, Arvydas Ambrozaitis. Epidemiological aspects of community associated methicillin-resistant *Staphylococcus aureus*. *Medicinos teorija ir praktika (Theory and Practice in Medicine)* 2009; 15(4): 349-355.
- **Agnė Kirkliauskienė**, Arvydas Ambrozaitis, Robert L. Skov, Niels Frimodt-Moller. The prevalence of *Staphylococcus aureus* nose and throat carriage by healthy adults. *Visuomenės sveikata (Public Health)* 2010; 2(49): 124-131.

Oral presentations:

- **A. Kirkliauskienė**. *Staphylococcus aureus* strains isolated from clinical specimen resistance to antibiotics. The report in the conference of VU Medicine and Natural Sciences Faculties on the topic: *Molecular epidemiology of the resistant to antibiotics bacteria spread in Lithuania and pathogenic to humans and pets*. 28 October, 2006, Vilnius
- **A. Kirkliauskienė**, A. Ambrozaitis. Hospital infections and *Staphylococcus aureus*: history and the present. The report in the seminar of Hygiene Institute and Hospital Infections Control Association on the topic: *The control of methicillin resistant Staphylococcus aureus infection.* 8 February, 2008. Vilnius.

Poster presentation:

- **Agnė Kirkliauskienė**, Arvydas Ambrozaitis, Robert L. Skov, Niels Frimodt-Møller. Prevalence of *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* in the community in Lithuania. Poster presentation in the 8th Nordic-Baltic Congress on Infectious Diseases "Well-known infections – the hottest features of diagnostics and treatment". Saint Petersburg, Russia, September 23-26, 2009.

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APPENDIX 1

Questionnaire (No. 1) to assess the risk factors of *S. aureus* carriage in the community group

ANKETOS NR. _____

LYTIS ☐ V – vyras, M – moteris **AMŽIUS** ☐ (metai)

GYVENAMASIS MIESTAS _____

IŠSILAVINIMAS ☐ **1**–nebaigtas vidurinis,
2–vidurinis,
3–profesinė ar amatų m-kla,
4–neuniversitetinis aukštasis,
5–universitetinis aukštasis.

UŽIMTUMO GRUPĖ ☐ **1**–užimantys vadovaujančias pareigas,
2–kvalifikuotas atsakingas, administracinis ar techninis darbas,
3–kvalifikuotas darbas prekyboje, paslaugų srityje, darbas su įranga,
4–nekvalifikuotas darbas,
5–studentas, moksleivis,
6–pensininkas,
7–bedarbis, namų šeimininkė.

DARBOVIETĖ _____

PAREIGOS _____

RIZIKOS VEIKSNIŲ ĮVERTINIMAS VISUOMENĖS GRUPĖJE

1. **Ar turėjote odos ligų?**

   ☐ (**1**–Taip, **2**–Ne)

2. Jei taip, prieš kiek mėnesių?

   ☐ <6 mėn, ☐ 6-12 mėn, ☐ 3-24 mėn, ☐ >24 mėn.

3. **Ar turėjote odos pažeidimų (infekcijų), kurias sukeltų stafilokokai?**

   ☐ (**1**–Taip, **2**–Ne)

4. Jei taip, prieš kiek mėnesių?

   ☐ <6 mėn, ☐ 6-12 mėn, ☐ 13-24 mėn, ☐ >24 mėn.

5. **Kurioje kūno vietoje buvo infekcija?**

_____

6. **Ar turėjote kitų, stafilokokų sukeltų, infekcijų?**

☐ (**1**–Taip, **2**–Ne)

7. Jei taip, prieš kiek mėnesių?

☐ <6 mėn, ☐ 6-12 mėn, ☐ 13-24 mėn, ☐ >24 mėn.

8. Ar tai buvo meticilinui atsparus stafilokokas?

☐ (**1**–Taip, **2**–Ne)

9. Jei taip, ar buvote gydomas/-a nuo stafilokokinės infekcijos?

☐ (**1**–Taip, **2**–Ne)

10. **Ar esate vartojusi/-ęs antibiotikus?**

☐ (**1**–Taip, **2**–Ne)

11. Jei taip, prieš kiek mėnesių?

☐ <6 mėn, ☐ 6-12 mėn, ☐ 13-24 mėn, ☐ >24 mėn.

12. Jei taip, tai kiek kartų per pastaruosius 2 metus?

☐ 1 kartą, ☐ 2–3 kartus, ☐ >3 kartus.

13. Gal atsimenate vartotų antibiotikų pavadinimus?

_____

14. **Ar sergate kuria nors iš šių ligų?**

1–cukrinis diabetas,
2–lėtinė inkstų liga,
3–bronchų ir plaučių lėtinės ligos,
4–imuninės sistemos ligos,
5–virškinimo sistemos ligos,
6–onkologinės ligos,
7–kita lėtinė liga _____.

15. **Ar lankėtės poliklinikoje?**

☐ (**1**–Taip, **2**–Ne)

16. Jei taip, prieš kiek mėnesių?

☐ <6 mėn, ☐ 6-12 mėn, ☐ 13-24 mėn, ☐ >24 mėn.

17. Jei taip, prieš kiek mėnesių?

☐ <6 mėn, ☐ 6-12 mėn, ☐ 13-24 mėn, ☐ >24 mėn.

18. **Ar jums buvo imtas kraujas iš venos?**

☐ (**1**–Taip, **2**–Ne)

19. Jei taip, prieš kiek mėnesių?

☐ <6 mėn, ☐ 6-12 mėn, ☐ 13-24 mėn, ☐ >24 mėn.

20. **Ar reguliariai vartojate (kortiko) steroidus (pvz.:prednizoną), kitus hormoninius vaistus?**

☐ (**1**–Taip, **2**–Ne)

21. **Ar turėjote kontaktą su MRSA užsikrėtusiu asmeniu?**

☐ (**1**–Taip, **2**–Ne)

22. Jei taip, prieš kiek mėnesių?

☐ <6 mėn, ☐ 6-12 mėn, ☐ 13-24 mėn, ☐ >24 mėn.

23. **Ar turėjote kontaktą su žmogumi, turinčiu odos pažeidimų?**

☐ (**1**–Taip, **2**–Ne)

24. Jei taip, prieš kiek mėnesių?

☐ <6 mėn, ☐ 6-12 mėn, ☐ 13-24 mėn, ☐ >24 mėn.

25. **Ar kontaktavote su žmonėmis, dirbančiais sveikatos priežiūros sektoriuje?**

☐ (**1**–Taip, **2**–Ne)

26. Jei taip, prieš kiek mėnesių?

☐ <6 mėn, ☐ 6-12 mėn, ☐ 13-24 mėn, ☐ >24 mėn.

27. **Ar turėjote kontaktą su žmonėmis, gyvenančiais senelių namuose?**

☐ (**1**–Taip, **2**–Ne)

28. Jei taip, prieš kiek mėnesių?

☐ <6 mėn, ☐ 6-12 mėn, ☐ 13-24 mėn, ☐ >24 mėn.

29. **Jei turite vaikų, ar jie lanko vaikų darželį?**

☐ (**1**–Taip, **2**–Ne)

30. **Ar kada dirbote vaikų darželyje?**

☐ (**1**–Taip, **2**–Ne)

31. Jei taip, prieš kiek mėnesių?

☐ <6 mėn, ☐ 6-12 mėn, ☐ 13-24 mėn, ☐ >24 mėn.

32. **Ar kontaktavote su žmonėmis, esančiais įkalinimo įstaigoje?**

☐ (**1**–Taip, **2**–Ne)

33. **Ar turėjote kontaktą su neįgaliais žmonėmis, esančiais asmens sveikatos įstaigoje?**

☐ (**1**–Taip, **2**–Ne)

34. **Ar kultivuojate kokią nors sporto šaką?**

☐ (**1**–Taip, **2**–Ne)

35. Jei taip, kokią?

_____

36. Jei taip, ar naudojate inventorių, kuris tiesiogiai liestųsi prie jūsų ir kitų asmenų odos?

☐ (**1**–Taip, **2**–Ne)

37. **Ar vartojate intraveninius narkotikus?**

☐ (**1**–Taip, **2**–Ne)

38. **Ar auginate naminius gyvūnėlius?**

☐ (**1**–Taip, **2**–Ne)

39. Jei taip, kokius? _____

40. **Kur gyvenate?**

1–nuosavas namas,
2–butas,
3–bendrabutis,
4–kitos sąlygos, nurodyti _____.

41. **Tualetas?**

1–bute,
2–kieme,
3–bendras

42. **Ar buvote išvykęs už Lietuvos ribų?**

☐ (**1**–Taip, **2**–Ne)

43. Jei taip, prieš kiek mėnesių?

☐ <6 mėn, ☐ 6-12 mėn, ☐ 13-24 mėn, ☐ >24 mėn.

44. Jei taip, kokiose šalyse lankėtės?

_____

45. Jei taip, ar išvykos metu lankėtės pas gydytoją ar gulėjote ligoninėje?

☐ (**1**–Taip, **2**–Ne)

46. Jei taip, kreipėtės į gydytoją, polikliniką ar ligoninę?

_____

47. Jei taip, prieš kiek mėnesių?

☐ <6 mėn, ☐ 6-12 mėn, ☐ 13-24 mėn, ☐ >24 mėn.

48. **Ar jūsų tėvai gimę Lietuvoje?**

☐ (**1**–Taip, **2**–Ne)

49. Jei ne, tuomet kur?

_____

50. **Kiek jūsų šeimoje yra jaunesnių nei 18 metų narių?**

☐ **0**, ☐ **1–2**, ☐ **3–4**, ☐ **5–6**, ☐ **>6**.

51. **Kiek jūsų šeimoje yra 18 metų ir vyresnių narių?**

☐ **0**, ☐ **1–2,** ☐ **3–4**, ☐ **5–6**, ☐ **>6**.

52. Ar kas nors iš šeimos narių sirgo stafilokokų sukeltomis infekcijomis?

☐ (**1**–Taip, **2**–Ne)

53. Jei taip, prieš kiek mėnesių?

☐ <6 mėn, ☐ 6-12 mėn, ☐ 13-24 mėn, ☐ >24 mėn.

54. Ar kas nors iš šeimos narių serga kuria iš išvardintų ligų?

1–cukrinis diabetas,
2–lėtinė inkstų liga,
3–bronchų ir plaučių lėtinės ligos,
4–imuninės sistemos ligos,
5–virškinimo sistemos ligos,
6–onkologinės ligos,
7–odos ligos,
8–kita lėtinė liga_____.

55. **Ar kas iš šeimos narių reguliariai vartoja (kortiko)steroidus (pvz.:prednizoną), kitus hormoninius vaistus?**

☐ (**1**–Taip, **2**–Ne)

56. **Ar kuris šeimos narys yra gulėjęs ligoninėje?**

☐ (**1**–Taip, **2**–Ne)

57. Jei taip, prieš kiek mėnesių?

☐ <6 mėn, ☐ 6-12 mėn, ☐ 13-24 mėn, ☐ >24 mėn.

58. Jei taip, kiek dienų praleido ligoninėje?

☐ ≤3 dienas, ☐ 4–7 dienas, ☐ >7 dienas.

59. **Ar jiems teko lankytis poliklinikoje?**

☐ (**1**–Taip, **2**–Ne)

60. Jei taip, prieš kiek mėnesių?

☐ <6 mėn, ☐ 6-12 mėn, ☐ 13-24 mėn, ☐ >24 mėn.

61. **Gal kas nors iš jūsų šeimos narių dirba/-o vaikų darželyje?**

☐ (**1**–Taip, **2**–Ne)

62. Jei taip, prieš kiek mėnesių?

☐ <6 mėn, ☐ 6-12 mėn, ☐ 13-24 mėn, ☐ >24 mėn.

63. **Ar kas nors iš jūsų šeimos narių kontaktavo su žmonėmis, esančiais įkalinimo įstaigose?**

☐ (**1**–Taip, **2**–Ne)

64. **Ar kas iš šeimos narių turėjo kontaktą su neįgaliais žmonėmis, esančiais asmens sveikatos įstaigoje?**

☐ (**1**–Taip, **2**–Ne)

APPENDIX 2

Questionnaire (No. 2) to assess the risk factors of *S. aureus* carriage in the hosptalized patients group

Ligoninėje įgyto *Staphylococcus aureus* nešiojimo tyrimas

ANKETA (1)

1.	Paciento numeris	
2.	Paguldimo data (diena, mėnuo, metai)	____ – ____ – _____
3.	Skyriaus kodas	
4.	Klinikinė diagnozė (kodas)	
5.	Amžius (metai)	____
6.	Lytis	□ – Vyras □ – Moteris
7.	Ar 12 mėnesių bėgyje vartojo antibiotikus?	□ – Taip □ – Ne
8.	Vartotų antibiotikų pavadinimai	1. _____ 2. _____ 3. _____
9.	Antibiotiko vartojimo trukmė (dienų skaičius)	1. _____ 2. _____ 3. _____
10.	Antibiotikai vartoti	1. □ – paskyrus gydytojui □ – savo nuožiūra 2. □ – paskyrus gydytojui □ – savo nuožiūra 3. □ – paskyrus gydytojui □ – savo nuožiūra
11.	Ar 12 mėnesių bėgyje gydėsi ligoninės stacionare?	□ – Taip □ – Ne
12.	Ligoninės pavadinimas	1. _____ 2. _____ 3. _____
13.	Prieš kiek laiko gydėsi ligoninės stacionare? (d.; sav.; mėn.)	1. _____ 2. _____ 3. _____
14.	Kuriame (-iuose) skyriuje (-iuose) buvo gydomas kiekvienu buvimo ligoninėje atveju?	1. _____ 2. _____ 3. _____
15.	Kiek dienų buvo gydomas kiekvienoje ligoninėje? (atskirai žymėti dienų skaičių)	1. _____ 2. _____ 3. _____
16.	Kuriame (-iuose) skyriuje (-iuose) buvo gydomas ligonis tiriamoje ligonineje? (skyriaus kodas)	1. _____ 2. _____ 3. _____
17.	Lydintys susirgimai, būklės	□ – cukrinis diabetas; □ – inkstų lėtinės ligos; □ – virškinimo trakto ligos; □ – bronchų ir plaučių lėtinės ligos; □ – imuninės sistemos ligos; □ – onkologinės ligos; □ – lėtinės odos ligos, pragula, nudegimas (pabraukti); □ – kiti _____
18.	Išrašymo data (diena, mėnuo, metai)	____ – ____ – _____

APPENDIX 3

Questionnaire (No. 3) to assess the risk factors of *S. aureus* carriage in the hosptalized patients group

Ligoninėje įgyto *Staphylococcus aureus* nešiojimo tyrimas

ANKETA (2)

1.	Paciento numeris	
2.	Ar buvo atlikta chirurginė operacija?	□ – Taip □ – Ne
3.	Kokia buvo atlikta operacija?	
4.	Ar buvo vykdoma antibiotikų terapija prieš operaciją ar jos metu?	□ – Taip □ – Ne
5.	Skirtų antibiotikų pavadinimai	1. _____ 2. _____ 3. _____
6.	Antibiotiko vartojimo trukmė (dienų skaičius)	1. _____ 2. _____ 3. _____
7.	Ar buvo vykdoma antibiotikų terapija po operacijos?	□ – Taip □ – Ne
8.	Skirtų antibiotikų pavadinimai	1. _____ 2. _____ 3. _____
9.	Antibiotiko vartojimo trukmė (dienų skaičius)	1. _____ 2. _____ 3. _____
10.	Ar išsivystė pooperacinės žaizdos infekcija?	□ – Taip □ – Ne
11.	Ar iš žaizdos išskirtas *Staphylococcus aureus*?	□ – Taip □ – Ne
12.	Ar išskirtas *S. aureus* buvo atsparus meticilinui?	□ – Taip □ – Ne
13.	Ar buvo įvesti venų kateteriai?	□ – Taip □ – Ne
14.	Ar buvo įvesti šlapimo takų kateteriai?	□ – Taip □ – Ne
15.	Ar buvo atliekama mechaninė ventiliacija?	□ – Taip □ – Ne
16.	Ar buvo taikomas parenterinis maitinimas?	□ – Taip □ – Ne
17.	Hospotalizacijos laikotarpis (dienų skaičius)	

APPENDIX 4

Informed consent form for study respondents

Infekcinių ligų, dermatovenerologijos ir mikrobiologijos klinika
Rengimo data: 2006 gegužės 25 d.

ASMENS INFORMAVIMO FORMA

Tyrimo pavadinimas

***Staphylococcus aureus* nešiojimo paplitimas ir rizikos veiksniai, atsparumo antimikrobinėms medžiagoms ir virulentiškumo veiksnių analizė**

Tyrėjai

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Įžanga.

Šiuo dokumentu siekiame potencialiam tyrimo dalyviui suteikti informacijos, kuri padėtų apsispręsti dėl dalyvavimo šiame klinikiniame tyrime. Pateikiame informaciją apie tyrimo svarbą, sutikusio dalyvauti tyrime teises ir naudą.

Jei sutikimo formoje yra Jums nežinomų ar nesuprantamų žodžių ar terminų, tyrėjas ar medicinos personalas būtinai Jums paaiškins.

Šio tyrimo svarba.

Staphylococcus aureus yra patogeninis mikroorganizmas, sukeliantis žmonėms labai įvairiai pasireiškiančias infekcijas. Šis sukėlėjas gali būti paviršinių odos pažeidimų, bakteremijos (būklė, kai bakterijos patenka į kraują ir juo išplinta organizme), endokardito (širdies vidinės sienelės uždegimas), absceso (pūlinys), centrinės nervų sistemos infekcijų, osteomielito (pūlingas procesas, susidarantis tarp kaulo ir jį dengiančio antkaulio), pneumonijos (plaučių uždegimas), šlapimo takų infekcijų ir net mirties priežastimi. Stafilokokų toksinai sąlygoja įvairių sindromų išsivystymą (Pvz.: impetigo – pūslinė-šašinė odos liga, apsinuodijimai maistu, nudegusios odos, toksinio šoko sindromas).

Pasauliniais tyrimų duomenimis beveik 20 proc. populiacijos yra pastovūs S. aureus nešiotojai, 30–50 proc. populiacijos yra laikini auksinio stafilokoko nešiotojai. Šis mikroorganizmas lokalizuojasi žmogaus nosies šnervėse, nosiaryklėje, pažastų srityje, tarpvietėje, makštyje.

Literatūros duomenimis šiuo metu 0,8–3 proc. sveikos visuomenės gali būti infekuoti meticilinui atspariais *S. aureus* (MRSA), šis procentas visuomenėje vis didėja. Šio mikroorganizmo nešiojimas prailgina hospitalizacijos laiką, apsunkina šio sukėlėjo sukeltų infekcijų gydymą, kadangi jis atsparus daugeliui antibiotinių vaistų, to pasėkoje didėja ir mirties rizika.

Patvirtinimas.

Šio tyrimo protokolą peržiūrėjo ir tyrimo vykdymui pritarė Lietuvos Bioetikos komitetas.

Dalyvavimas.

Kviečiame Jus dalyvauti tyrime, jei:

- pageidaujate būti ištirtas ar esate meticilinui atsparaus auksinio stafilokoko nešiotojas;
- atitinkate šiam tyrimui reikalingų respondentų apibūdinimą;
- duosite raštišką sutikimą;

Tyrimo metu bus:

- gautas raštiškas Jūsų sutikimas dalyvauti tyrime;
- pateikta užpildyti anketa apie MRSA rizikos veiksnius;
- paimtas mėginys iš nosies ir gerklės.

Mėginių paėmimas ir S. aureus identifikacija.

Tiriamoji medžiaga steriliais tamponais bus imama iš nosies ir gerklės. Steriliu tamponu 1,5-2 cm gylyje palei nosies vidinę sienelę apsukame 4 kartus tiek dešinėje, tiek kairėje šnervėse.

Tiriamoji medžiaga iš gerklės bus imama tiriamajam plačiai išsižiojus ir kitu steriliu tamponu prisilietus prie tonzilių.

Tiriamieji tamponai talpinami į transportinę terpę ir laikomi 4^0C temperatūroje. Vilniaus universitete, Medicinos fakultete, Mikrobiologijos skyriuje bus atliekami mikrobiologiniai mėginių tyrimai: išskiriama gryna kultūra, įvairiais identifikaciniais metodais nustatomas *S. aureus*, diskų difuzijos metodu nustatomas išskirto mikroorganizmo jautrumas antibiotikams. Genetiniais tyrimais: pulsuojančio gelio lauko elektroforezės metodu bei polimerazės grandinine reakcija bus nustatomi genai atsakingi už išskirto *S. aureus* atsparumą kai kuriems antibiotikams Danijos Valstybiniame Serumų Institute.

Be to, kiekvienas dalyvis turės užpildyti tokią pačią, anoniminę anketą apie galimus rizikos veiksnius, susijusius su *S. aureus* nešiojimu.

Su tyrimu susijusi rizika.

Mėginių iš nosies ir gerklės paėmimas gali sukelti trumpalaikį diskomfortą, tačiau jokios kitos su tyrimu susijusios rizikos nėra.

Tyrimo teikiama nauda.

Dalyvavimas šiame tyrime naudingas, kadangi mikrobiologiniais ir genetiniais tyrimais bus nustatyta ar esate meticilinui atsparaus auksinio stafilokoko nešiotojas. Efektyvūs ir teisingi Jūsų šeimos gydytojo paskirti šio sukėlėjo likvidavimo būdai sumažins odos, pooperacinių žaizdų infekcijų bei kitų MRSA sukeliamų ligų

galimybę, o ligos atveju sutrumpės hospitalizacijos laikas, bus racionaliau parenkami antibiotikai.

Dalyvavimo savanoriškumas.

Jūsų dalyvavimas šiame tyrime yra savanoriškas. Atsisakymas dalyvauti tyrime nesukels jokių pasekmių, neturės jokio poveikio medicininei priežiūrai, kurią turite teisę gauti.

Jūs gausite pasirašytą šios formos kopiją.

Konfidencialumas.

Jūsų dalyvavimas šiame tyrime bus konfidencialus. Jokios Jūsų asmenybę identifikuojančios informacijos nebus renkama ir skelbiama. Jums bus suteiktas numeris, kuris neleis identifikuoti tyrime dalyvavusio asmens. Nurodę tyrėjams identifikacinį numerį (paskambinę tel.: 8 610 31050 arba atsiuntę jį elektroniniu paštu (adresu agne.kirkliauskiene@mf.vu.lt), sužinosite savo tyrimo rezultatus.

Teisė užduoti klausimus.

Jei Jums kiltų klausimų dėl savo, kaip mokslinio tyrimo dalyvio teisių, galite kreiptis į Lietuvos bioetikos komitetą, tel. (8~5) 2124565.

Iškilus klausimams, susijusiems su šiuo tyrimu, Jūs taip pat turite teisę juos užduoti Pagrindiniam tyrėjui:

Tyrėjo vardas	prof. Arvydas Ambrozaitis;
Tyrėjo adresas	Birutės al. 1/20, Vilnius, LT-08117, Lietuva;
Tyrėjo telefono numeris	(5) 2 724 657
Tyrėjo fakso numeris	(5) 2 758 885