

## **STAPHYLOCOCCUS INFECTIONS: METHICILLIN HIGH-RESISTANCE AND INCIDENCE BASED ON ENVIRONMENTAL FACTORS AND INDIVIDUAL CHARACTERISTICS OF THE PATIENTS**

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### **INTRODUCTION**

*Staphylococcus* is the most common genus, from *Micrococcaceae* family, which is involved in human pathology. Staphylococci infection outbreak and evolution, like any other infectious process, depends on a few conditions like: the aggressivity of the microbes, the receptivity of the attacked organism and also its ability to defend. The decreasing of the infection rate in the healthcare setting remains a major challenge, especially for MRS infections, although, in many hospitals, for example in Singapore, policies concerning universal screening or isolation of MRS carriers have been implemented (Al-Anazi, 2009). However, this represents a significant and additional burden for the medical system around the world, with higher mortality and financial costs (Al-Anazi, 2009; Ang et al., 2018), and requires more space in terms of rooms with hospital beds.

The frequency of nosocomial infections with staphylococci has increased in the recent decades on account of using a high number of medical devices, such as catheters, which are development-friendly environments for this type of infection (Ciszewski et al., 2015). According to a study made in 2011, in South Korea, MRSH strains were found on 51.4% of the X-ray cassettes in a radiology department (Gomes et al., 2011).

Nevertheless, some people could be carriers of a certain bacteria and spread it into the community, without even knowing. For example, around 20-30% of people from the entire human population are carriers for *S. aureus* (Bruggeman et al., 2013), a type of *Staphylococcus* whose survival can lead to haematogenous infection and further on, to threatening the patients lives (Algeorge et al., 1985; Corrales et al., 2018; Kong et al., 2016; Lall and Sahni, 2014; Camacho-Ortiz et al., 2015).

Haematogenous *Staphylococcus* infections usually occur at children, adults who have suffered from burns or traumas and elderly people treated with corticosteroids or subjected to intensive care manners (Alizadeh et al., 2017).

The bacteriological examination of the blood is one of the most important investigations in clinical microbiology. It is done every time clinical signs, such as chills, increased body temperature or prostration indicate a septicemic or bacteremic condition. This method allows the isolation and subsequent identification of a bacteria. Hemoculture is at all times an emergency. It is necessary to apply the criteria for differentiation in order to distinguish the contamination bacteria from the bacteria with clinical significance.

In this study, the aim was describing the *Staphylococcus* hemoculture infection rate in 2016, the main way of spreading this infection being the nosocomial transmission. The colonization ratio depends on various factors such as environmental agents or patients characteristics. Also, studying the sensitivity/resistance to different antibiotics has contributed to the administration of more appropriate ones.

### **MATERIAL AND METHODS**

In order to collect the blood samples from Sibiu county emergency hospital patients, certain steps needed to be followed. First of all, using sterilized syringes, the blood was collected and distributed into two vials: aerobic and anaerobic. Afterwards, these vials were incubated in the Bactec 9050 System and tested for positivity for maximum 5 days.

The next step was either performing a Gram staining, or inoculating the blood samples. The Gram staining was done automatically by a system called Previ ColorGram, making possible the examination of a blood smear under the microscope.

The inoculation of blood samples on culture mediums was done under the laminar flow hood, using inoculating loops followed by incubating the culture mediums inside the thermostat, where they were incubated at 37°C, for 18-20 hours. The culture media used for *Staphylococcus* were: blood agar and blue agar.

Other tests that were made after the incubation include: the latex slide agglutination test and the catalase test. For the agglutination test, Staphytest Plus (Diagnostic Reagents) kit was used in order to differentiate the pathogenic species of *Staphylococcus* from the non-pathogenic ones. The coagulase-positive species, especially *S. aureus* but also *S. haemolyticus* and *S. xylosus*, are pathogenic species, while *S. epidermidis* and *S. saprophyticus* are non-pathogenic species. For the catalase test, the bacterial isolate was mixed in a drop of hydrogen peroxide solution. If the test was positive, a bubble formation appeared due to the release of oxygen, allowing *Staphylococcus*, which is catalase-positive, to be differentiated from other pathogens like *Streptococcus* or *Pneumococcus*, which are catalase-negative.

The antibiogram was done either manually, using the disc diffusion method, or automatically, using the Vitek 2 Compact system. The disc diffusion method used Kirby-Bauer technique and the antibiotic disks were placed on a Muller-Hinton agar dish using a dispenser with an eye to position them on a proper distance from each other. After incubating the dishes at 35-37°C for 18-20 hours, the inhibition zone formed around each disk made it possible to appreciate the sensitivity or resistance to different antibiotics. The automatic method was done with the help of Vitek 2 Compact system that uses specialized *Staphylococcus* identification cards named AST-P 592.

## RESULTS AND DISCUSSION

### The incidence of *Staphylococcus* infections in hemocultures

In order to establish the incidence of *Staphylococcus* in hemocultures (Figure 1), the influence of certain factors need to be analyzed.

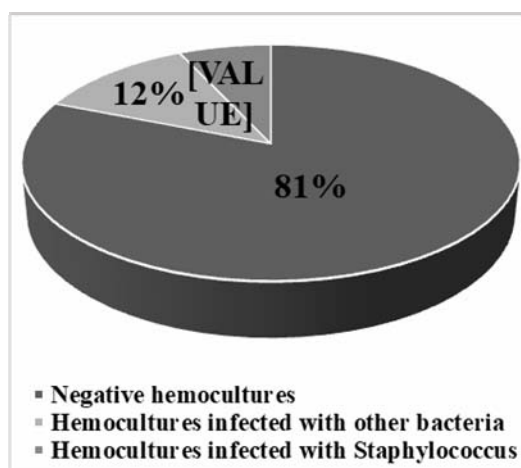


Figure 1. The division of hemocultures depending on negativity/positivity of the hemoculture and the type of infectious bacteria

The blood samples were collected from a total of 1207 patients in Sibiu county emergency hospital during the year 2016. After being tested in the Bactec system, the blood samples could have been differentiated into negative or positive and then the positive ones were tested to identify the type of bacterial colonisation.

### The incidence of *Staphylococcus* infections based on the month of the year

The fewest number of cases was recorded in the months with lower temperatures such as: February, September, November and December, when there were identified only 4-5 hemocultures infected with *Staphylococcus* each month. In contrast, an increase in infection is observed in spring and summer months, with August being the month when the highest number of *Staphylococcus* infections in hemocultures were recorded (Figure 2).

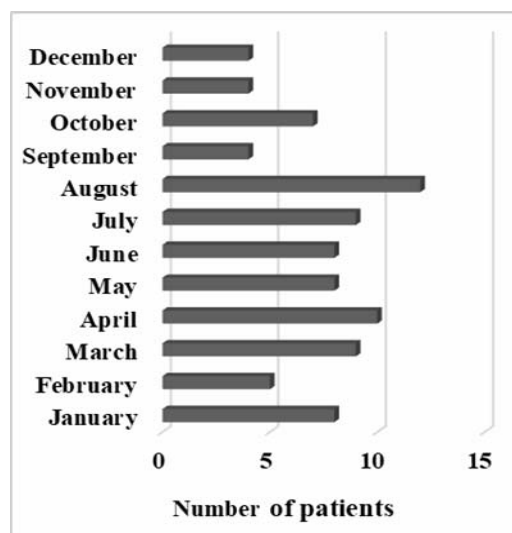


Figure 2. *Staphylococcus* infections classified on the months of 2016 with the highest number of infected patients in summer and only a few cases in the colder months

The increased incidence of this months' infection can be attributed to the heat which causes not only the secretion but also the resistance of enterotoxins that are thermally stable (Pai et al., 2010).

### The incidence of *Staphylococcus* infections based on the species of *Staphylococcus*

*Staphylococcus hominis* is considered to be one of the most common isolates found in the blood of the newborns and immunosuppressed patients. This is mostly because of its difficulty in treatment due to resistance to antibiotics (Cloud et al., 2006), making it the top species that infects hemocultures, in the current study.

*Staphylococcus aureus* is naturally plentiful in the whole human body and its survival can lead to blood infections which might even threaten the life of patients (Algeorge et al., 1985; Kong et al., 2016), fact confirmed also by the incidence in hemocultures in Sibiu county emergency hospital.

*Staphylococcus epidermidis* is ranked as the third most commonly found species in this study (Table 1) and it can become pathogenic when is found in blood or when it colonizes different medical devices. The most common way of entering the body is through intravascular catheters (Beswick et al., 2010; Akoda et al., 2013).

Table 1. The incidence of *Staphylococcus* infected hemocultures categorized by species

Species	The number of infected hemocultures
<i>S. hominis</i>	29
<i>S. aureus</i>	24
<i>S. epidermidis</i>	23
<i>S. haemolyticus</i>	5
<i>S. auricularis</i>	2
<i>S. xylosus</i>	1
<i>S. simulans</i>	1
<i>S. warneri</i>	1
<i>S. capitis</i>	1
<i>S. saprophyticus</i>	1

#### The incidence of *Staphylococcus* infections based on the age category of the patients

The cases of *Staphylococcus* infections covered patients of all ages starting with newborns and ending with 90-year-old people. The lowest number of infected hemocultures came from patients aged between 11 and 40 years old, identifying a total of only 6 cases in the three categories (Figure 3).

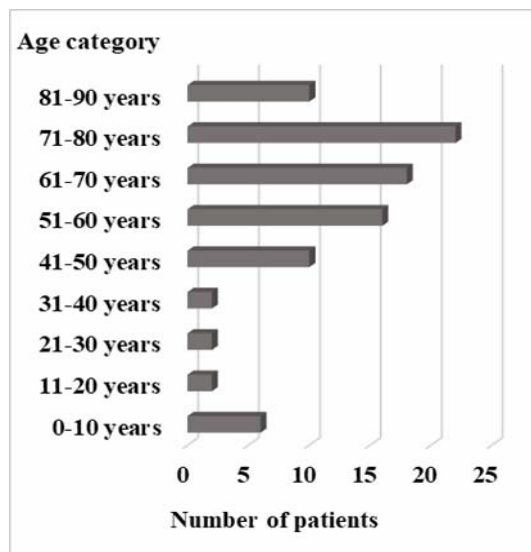


Figure 3. The number of infected hemocultures differed based on the patients age category, with the most cases of the 22 recorded in elderly patients

The highest number of *Staphylococcus* infected patients was in the 71-80 age category, where it was recorded a quarter of the total number of cases. Elderly patients are mostly affected by this kind of infections due to the low cellular and humoral immunity as well as the decrease of physiological functions.

#### The incidence of *Staphylococcus* infections based on the gender of the patients

Considering the gender of the patients, there are no significant differences in terms of proportion, the number of males and females being almost equal (Figure 4). According to this statistics, *Staphylococcus* species did not prefer any gender to colonize the blood.

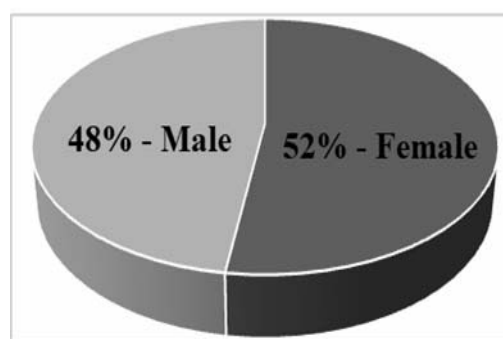


Figure 4. The gender balance between male and female patients infected with *Staphylococcus*

#### The incidence of *Staphylococcus* infections based on the blood samples department of origin

The positive hemocultures came from one of the 2 departments of the hospital: surgical or medical. The fact that the blood samples coming from the surgical department were much more numerous (Figure 5) led to believing that most of the infections were contacted in a nosocomial way.

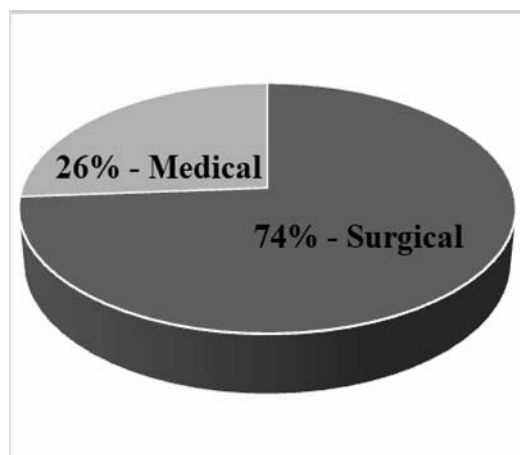


Figure 5. The proportion of infected hemocultures according to the department they came from

### The incidence of *Staphylococcus* infections based on the presence/absence of a previous treatment

When the blood sample is taken, the patient could be already under treatment with antibiotics, like in this study where almost half of the patients were treated before the blood collection (Figure 6).

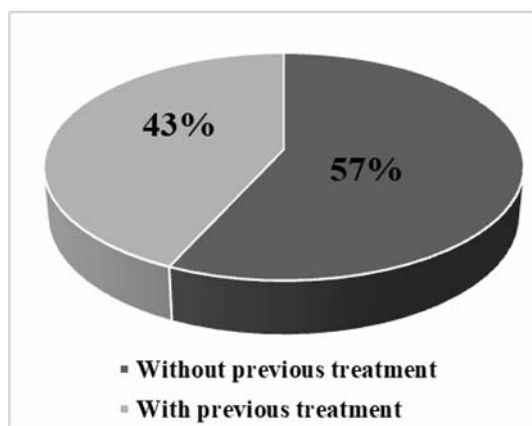


Figure 6. The statistics of staphylococcal infections according to the presence or absence of a treatment before collecting the blood sample

### The incidence of *Staphylococcus* infections based on MRS and MLSBi infections

Some species of *Staphylococcus* may gain 2 types of major resistance because of the clinical environment: methicillin resistance (MRS) or inducible clindamycin resistance (MLSBi) (Figure 7).

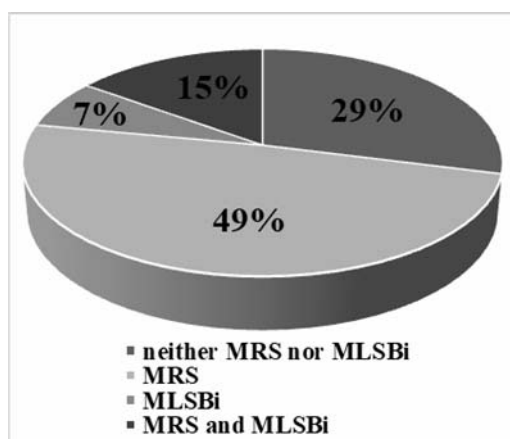


Figure 7. The methicillin resistance is increased, being identified alone in almost half of the probes and also combined with inducible clindamycin resistance; the clindamycin resistance alone was identified only in a few cases

MRS infections are a worrying factor not only because of the resistance to methicillin but also because of the resistance to many chemotherapeutic

agents (Ruff and Shinefield, 2009). Also, accepting the patients infected with MRS in hospitals is a major challenge considering the patients health safety (Al-Anazi, 2009).

To test the MLSBi resistance, the D phenomenon must appear, or at least a small growth on the clindamycin inhibition zone (Mathur et al., 2001; Otto and Vuong, 2002).

### The incidence of *Staphylococcus* infections based on the antibiotics resistance

Staphylococci acquire increased resistance to **beta-lactamines**. This is done by producing enzymes that inactivate them and changing the target of action. These enzymes are called beta-lactamases and, in staphylococcal infections, they preferentially hydrolyze penicillins. This increased resistance to penicillins is due to their use as a common antibiotic in the hospital environment.

Staphylococci are naturally resistant to **quinolones** of the first generation but are sensitive to fluoroquinolones, the ratio of resistant and susceptible species being approximately 1:1.

The **aminoglycoside**-acquired resistance mechanisms relate to the acquisition of the modifying enzymes that trigger phosphorylation, adenylation of a hydroxyl group, or acetylation of an amino group.

The resistance to **cyclins** is relatively high, as it is shown in Figure 8, and there are two tetracycline-acquired resistance mechanisms: diminishing intracellular accumulation of antibiotics and altering the affinity of the target for these antibiotics.

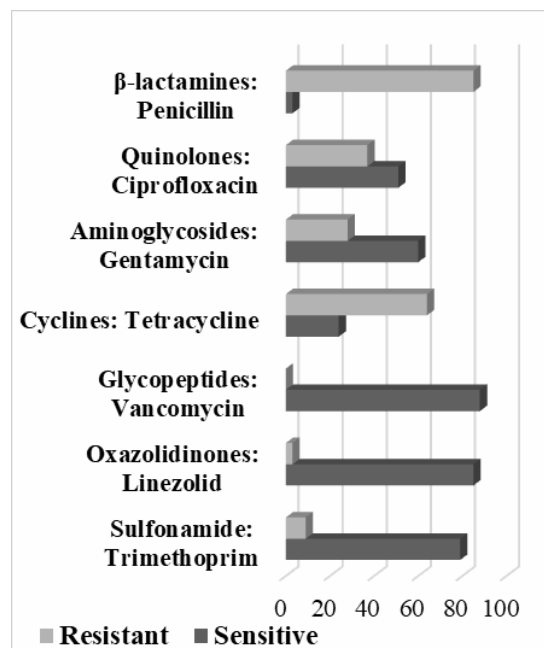


Figure 8. The sensitivity and resistance of *Staphylococcus* to different classes of antibiotics

Increased sensitivity can also be noticed among **glycopeptides**, especially vancomycin, which is the alternative treatment for methicillin-resistant *Staphylococcus* infections.

There is an increased sensitivity of the staphylococci to **oxazolidinones**, but the mechanism of resistance remains unknown.

Staphylococci have a low **sulfonamide** resistance, which is achieved by altering or overproducing the target enzymes.

## CONCLUSIONS

*Staphylococcus* species can enter and infect the human body in various ways and even cause serious conditions. This study provided further insight into the influence of environmental factors and characteristics of the patients on the incidence of *Staphylococcus* infections in hemocultures. High temperatures have favored this type of infections due to the increased secretion of enterotoxins, which are thermally stable. *Staphylococcus* infections were more common among the elderly people because of their decreased immunity and the body functions that are slowed down.

Unfortunately, a significant number of blood infections were nosocomial, fact confirmed by the multitude of probes that came from the surgical department. Also, staphylococci can gain resistance to different antibiotics like methicillin or clindamycin, which can imply resistance to other classes of antibiotics, making the patients with MRS or MLSBi infections hard to be treated and a real medical challenge if hospitalized.

## ABSTRACT

In 2016, out of 1207 hemocultures from patients at Sibiu county emergency hospital, 7% were infected with *Staphylococcus*, identifying more cases in the months with higher temperatures. Elderly people were mostly affected, regardless of gender. Due to nosocomial infections, 64% of staphylococci were MRS and they came from the surgical section of the hospital. In the present study, the *Staphylococcus* hemoculture infection rate was described, aiming the identification of the infections, the assessment of the colonization ratio based on the total number of cases recorded in 2016 at Sibiu county emergency hospital and pointing out of possible correlations between colonization and age, gender, hospital section origin of infections and previous treatment. The main characteristics of the species were identified using Gram staining, microscope examination, catalase test, coagulation test and antibiograms. *Staphylococcus* species cause a lot of conditions, especially if they are not treated properly.

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