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Antimicrobial Resistance of Staphylococcal Strains Isolated from Various Pathological Products

Laura-Mihaela SIMON[1*], Lia-Monica JUNIE[1], Daniela HOMORODEAN[2]

[1] "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca, 13 Emil Isac, 400023 Cluj-Napoca, Romania.
[2] Pneumology Clinic Leon Daniello, 6 B.P. Haşdeu, 400371 Cluj-Napoca, Romania.
E-mails: lauramihaelasimon@yahoo.com; mjunie@yahoo.com;

* Author to whom correspondence should be addressed; Tel.: +4-0740-230141.

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Abstract

Background: The optimal choice of antimicrobial therapy is an important problem in hospital environment in which the selection of resistant and virulent strains easy occurs. S. aureus and especially MRSA(methicillin-resistant S. aureus) creates difficulties in both treatment and prevention of nosocomial infections. *Aim:* The purpose of this study is to determine the sensitivity and the resistance to chemotherapy of staphylococci strains isolated from various pathological products. *Material and Method*: We identified Staphylococccus species after morphological appearance, culture properties, the production of coagulase, hemolisines and the enzyme activity. The susceptibility tests were performed on Mueller-Hinton medium according to CLSI (Clinical and Laboratory Standards Institute). *Results:* The strains were: MSSA (methicillin-susceptible *S. aureus*) (74%), MRSA (8%), MLS B (macrolides, lincosamides and type B streptogramines resistance) (12%) and MRSA and MLS B (6%). MRSA strains were more frequently isolated from sputum. MRSA associated with the MLS B strains were more frequently isolated from pus. MLS B strains were more frequently isolated from sputum and throat secretions. All *S. aureus* strains were susceptible to vancomycin and teicoplanin. *Conclusions*: All staphylococcal infections require resistance testing before treatment. MLS B shows a high prevalence among strains of *S. aureus*. The association between MLS B and MRSA remains a major problem in Romania.

Keywords: Resistance; Antibacterial agents; MRSA (methicillin-resistant *S. aureus*); *Staphylococcus*.

Introduction

The motivation of this study was the increased frequency of *S. aureus* strains involved in nosocomial infections. Rates of MRSA (methicillin-resistant *S. aureus*) resistance was variable, high rates of resistance have been reported particularly in southern Europe.

90% of staphylococcus strains were resistant to penicillin and penicillin-derived antibiotics. Next line of attack, methicillin, is becoming less efficient, between 1975 and 1991 the prevalence of methicillin-resistant *S. aureus* strains was 26%.

Also, antiseptic *S. aureus* resistance makes possible the survival of these bacteria in the hospital environment. S. aureus is currently considered as one of the most common cause of nosocomial infections.

Thus, while S. aureus community infections can still be treated with penicillin derivatives, nosocomial infections are increasingly resistant to penicillin and require more aggressive antibacterial agents.

VISA strains (S. aureus with intermediate susceptibility to vancomycin) were identified in Japan in 1996, then in USA and France. S. aureus with reduced susceptibility to vancomycin (SARV) are associated with the clinical failure of treatment with vancomycin [1]. The VISA and SARV strains were susceptible to rifampicin, co-trimoxazole and tetracycline. Hetero-VISA strains are susceptible to

vancomycin but contain subpopulations, at a frequency of 10−6 CFU/mL or higher that grow at a vancomycin concentration of 4 mg/L and have MICs of >4 mg/L [2].

After VISA and SARV strains, vancomycin-resistant MRSA (VRSA) has now emerged.

There are estimated to be over 53 million healthy carriers of MRSA (2.7%) of the 2 billion of S. aureus carriers. The annual incidence of illness with S. aureus in the U.S. (2001-2002) was 18 - 25.7/100.000 inhabitants, of which 23% required hospitalization. The prevalence of MRSA infections is increasing: from 29% in 1991 to 43% in 2002. Septicemia with S. aureus had a mortality rate between 11-48% [3].

SENTRY surveillance programme 1997–1999 reported that the infections with MRSA in USA and Europe have similar prevalence [4].

A study at Veterans Affairs (VA) hospitals made between August 2007and October 2009, revealed that the prevalence of MRSA was 10.4% (61 of 585) (15.0% in patients with electronic medical record (EMR) and 5.6% in patients without EMR-documented antibiotic use during the past year [5].

A study made in Taiwan revealed an rapid increased incidence (from 39% in 1991 to 75% in 2003) of MRSA infections correlated with the use of glycopeptides, beta-lactam-beta-lactamase inhibitor combinations, extended-spectrum cephalosporins, carbapenems and fluoroquinolones [6]. Consequently, the clinical management of serious infections is increasingly difficult.

Most MRSA infections are severe infections, 23% of patients requiring hospitalization. In Great Britain the number of deaths due to MRSA is 3.000/year. In the same country, the MRSA bacteriemia (between 2004 and 2005) produced 5.53 deaths/100000 population [7]. Therefore, we studied the prevalence of *S. aureus* strains and coagulase-negative staphylococci (CNS) isolated from 'Leon Daniello' Hospital Cluj-Napoca between January 2006 - September 2008.

Material and Method

Pathological Samples

The sampling and transport of pathological products intended for bacteriological examinations directly influences the efficiency of subsequent stages and ultimately achieve of a correct diagnostic.

Sampling of patients was performed strictly respecting the general rules for the collection of pathological products for bacteriological examination.

The samples were transported to the laboratory as soon as possible using Stuart and Amies media embedded in sterile tubes.

Isolation and identification of microorganisms in samples

Microscopic examination

We examined Gram stain smears using immersion objective of optical microscope.

Bacterial Isolation

The solid media used for isolation were Columbia agar (BioRad, Oxoid or BioMerieux). Samples were cultivated on solid media type: Columbia agar added 5% sheep blood and chocolate blood agar and after they were incubated at 37°C for 24 hours in an atmosphere of 5-10% CO_2.

Bacterial Identification

The identification of microorganisms was based on morphological characters, culture characters, biochemical tests and antigen tests for pathogenicity.

Staphylococccus species have been identified on morphological appearance, culture properties, the production of coagulase, hemolisines, presence of the enzyme activity. Since morphology and culture is not an absolute criterion for S. aureus identification, we used free coagulase and coagulase tests related to each strain of staphylococcus isolated. We used the following techniques:

- The kit PASTOREX coagulase-Staph-Plus (Bio-Rad)
- Coagulase tubes with human plasma oxalate

The first test show related coagulase "clumping factor" and the second, free coagulase. For definite identification of bacterial species were used API20E galleries.

Chemotherapy sensitivity testing of isolated germ was carried out using disc diffusion technique on standardized Kirby-Bauer method with manual reading.

In some cases, for classification of resistance phenotypes were performed additional tests based on the same disc diffusion technique.

Culture Media

The susceptibility tests were performed on Mueller-Hinton medium recommended by CLSI.

The antibacterial kit (Oxoid) consisted of: penicillin, oxacillin, clindamycin, erythromycin, kanamycin, tobramycin, gentamicin, pristinamycin, vancomycin, teicoplanin, ciprofloxacin, trimethoprim / sulfamethoxazole, tetracycline, nitrofurantoin, rifampicin, chloramphenicol, linezolid.

The disk diffusion method provided important data relating to resistance phenotypes.

Statistical Analysis

Quantitative variables were summarized as percentage. Microsoft Excel was used for graphical representation of the obtained results.

Results

359 *S. aureus* strains were isolated from: sputum (42%), bronchial lavage (17%), pharyngeal swabs (31%), pus (8%), pleural fluid, conjunctival discharge, ear, nose (3%) (Figure 1).

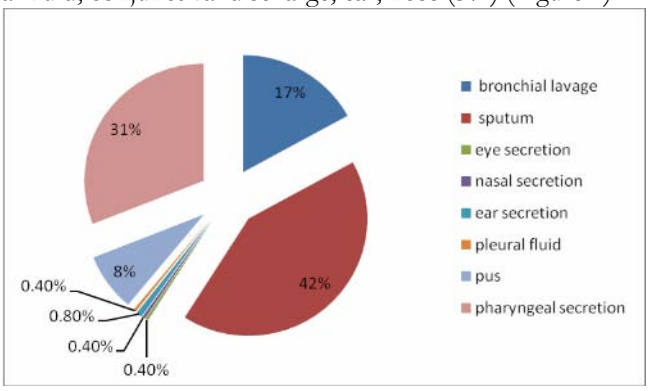

Figure 1. Sample distribution

We isolated 39 CNS strains from: 55% urinary tract infections and 45% from breakthrough wounds. The average age of patients from whom CNS strains were isolated was 36 years.

The isolates were: MSSA - 184 strains (74%), MRSA - 21 strains (8%), MLS B – 29 strains (12%) and MRSA + MLS B -14 strains (6%)(Figure 2).

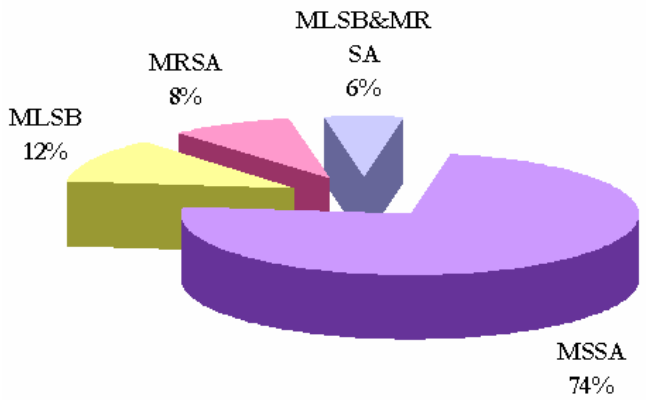

Figure 2. Distribution of *S. aureus* resistance to chemotherapeutic agents

From the strains involved in nosocomial infections 42% were resistant to methicillin. *S. aureus* was isolated from single bacteria infections in 226 cases of which 171 were MSSA, 25 MLSB, 18 MRSA, 12 MRSA and MLS B (Figure 3).

S. aureus was isolated from multiple-bacteria infections in 22 cases of which 13 were MSSA, 4 MLS B, 3 MRSA and 2 strains were MRSA associated with MLS B (Figure 4).

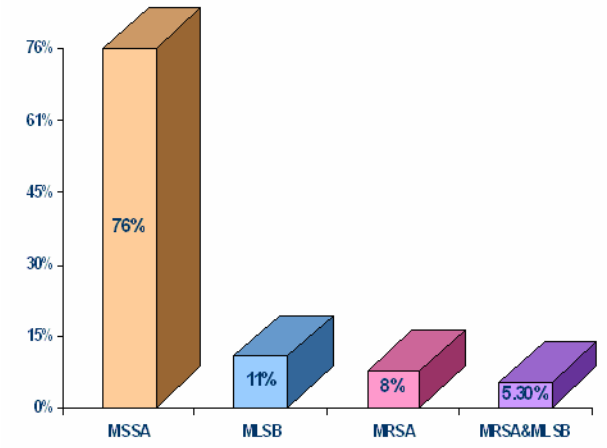

Figure 3. Resistance profile distribution (strains isolated single bacteria infections)

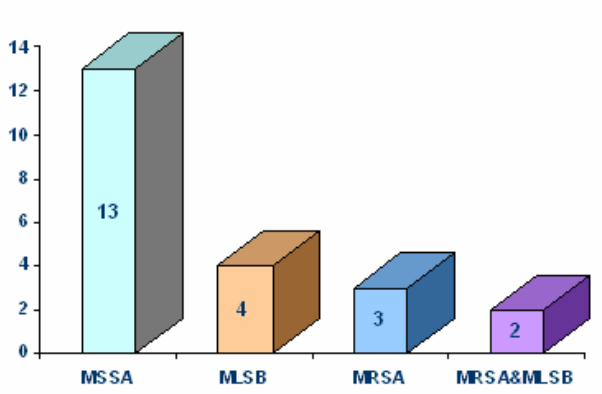

Figure 4. Resistance profile distribution of *S. aureus* strains (isolated from multiple-bacteria infections)

The distribution of macrolide susceptibility was: 6% were susceptible to erythromycin and lincomycin, 56% were resistant to erythromycin and 38% were resistant to MLS B (14% presented inducible MLS B and 24% presented constitutive MLS B) (Figure 5).

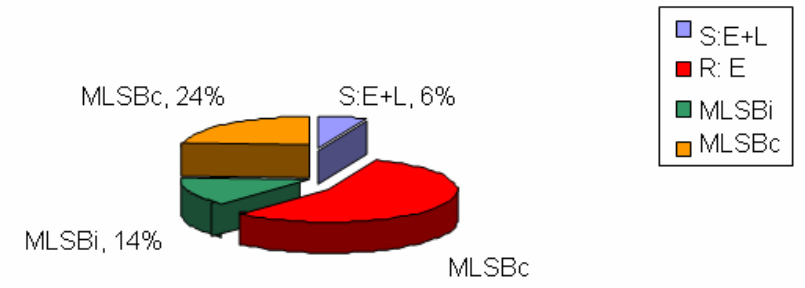

Figure 5. Distribution of macrolide-resistant *S. aureus* strains

MRSA strains were more frequently isolated from sputum. MRSA associated with the MLS B strains were more frequently isolated from pus. MLS B strains were more frequently isolated from sputum and throat secretions (Figure 6).

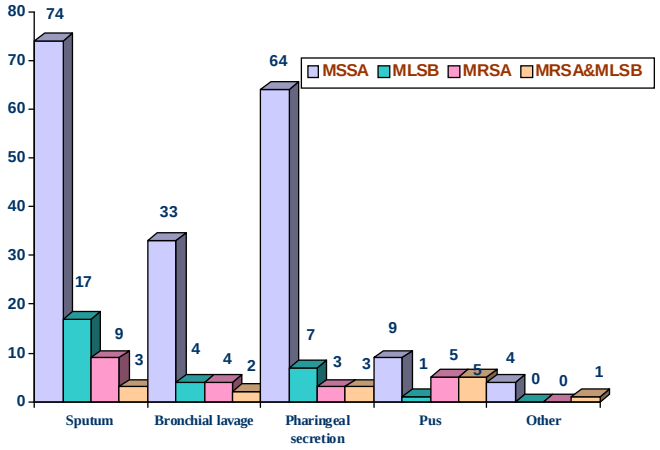

Figure 6. Strains distribution according to the pathological product

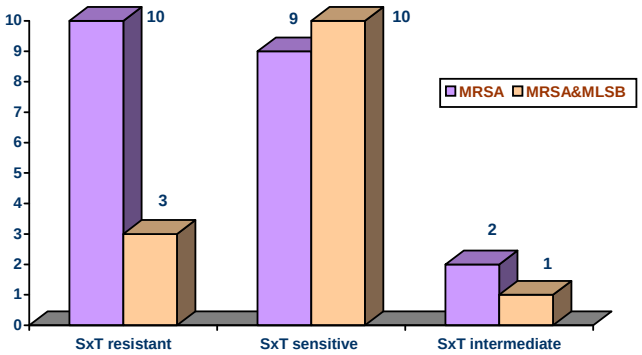

Figure 7. Distribution according to resistance to trimethoprim-sulfamethoxazole and MRSA/MLS B

All *S. aureus* strains were susceptible to vancomycin and teicoplanin. From CNS strains, 12% were resistant to vancomycin and 4% to teicoplanin.

Discussion

In the European Union MRSA infections are estimated to affect more than 150.000 patients/year. There is a great incidence variability of MRSA among the States of EU. The MRSA incidence variates from less than 1% to more than 50% [8].

A study of MRSA prevalence made in 39 German hospitals (in the period 1 November to 30 November 2006) and to one Dutch hospital (in the period 1 July to 30 September 2007) revealed 1.6 MRSA/100 patients (6.5% of all S. aureus) in the German and 0.5 MRSA/100 patients (1.4% of all *S. aureus*) in the Dutch part of the border region [9].

The rate of colonization with S. aureus in Georgia was 24.7% of the tested pacients. MRSA strains were isolated in 21.7% of the patients and they were resistant to multiple non-beta-lactam antimicrobial agents [10].

Although an ideal surveillance program does not exist, the establishment of international monitoring program allowed to obtain some good results in antimicrobial therapy and minimized the consequences of microbial resistance to antibiotics. These international surveillance programs are: EARSS (European Antimicrobial Resistance Surveillance System The), ICARE (CDC-NNIS) (Intensive Care Antimicrobial Resistance Surveillance), NNIS (Nosocomial Infection Surveillance System-National), SENTRY Antimicrobial Surveillance Program, MYSTIC (The Meropenem Yearly Susceptibility Test Information Collection Program). MYSTIC Program is a longitudinal antimicrobial surveillance study that has been

in existence since 1997 in centers that are actively prescribing meropenem. The purpose of these programs is to determine trends in antibiotic resistance of key pathogens involved in nosocomial infections and community.

SENTRY Program (1997) took into account 100 sentinel centers in over 30 countries in North America, Latin America and Europe, spanning a period of 5 years which followed the prevalence of germs isolated in different infections. In systemic infections, the study made 1997-2002 in North America revealed that the most frequent etiology was *S.aureus* (22.9% - 28.7%). In Latin America from 2502 cases of pneumonia, 23.3% of cases were caused by *S. aureus*. In skin and soft tissue infections, the study in 2000 in North America, Latin America, Europe, showed that the most frequent bacteria was S. aureus (45.9%). 100% of MRSA strain were susceptible to vancomycin and teicoplanin. 100% of CNS strains were susceptible to vancomycin and only 87.8% to teicoplanin.

The EARSS report for 2006, which covered 31 countries has reported that from 29552 S.aureus strains, about a quarter were MRSA. Oxacillin resistance was the following: 25% about half of the countries, between 1-5% in Northern Europe, between 7-22% in Central Europe, over 40% in 6 countries and over 50% in Romania. Except Romania, Belgium, Lithuania, most strains were isolated from intensive care units.

A study made in the Brazilian hospitals participating in the SENTRY Antimicrobial Surveillance Program between January 2005 and September 2008 revealed that the most frequently isolated from bloodstream infections were *S. aureus* (20.2% of total) and CNS coagulase-negative staphylococci (14.7%). S. aureus was the most frequent (28.1%) bacteria isolated from skin and soft tissue infections. The lower respiratory tract infections were produced by S. aureus (24.9% of cases). The percent of MRSA strains was 31.0%. These strains were also resistant to clindamycin, ciprofloxacin and levofloxacin. Vancomycin, linezolid and daptomycin were all very active against *S. aureus* strains tested (>99.9-100.0% susceptible) [11].

Another study made in Latin American hospitals (between January 2003 and December 2008) evaluated the resistance patterns of 12.324 Gram-positive strains. The frequence of MRSA strains was between 32.7% in Brazil and 49.7% in Chile. These strains were also resistant to erythromycin (90.1%), clindamycin (84.4%), and levofloxacin (86.8%) [12].

A study made in 2006 in 40 European centers revealed that MSSA were susceptible to amikacin (99.2%), meropenem and imipenem (98.1%), piperacillin + tazobactam (96.6%), gentamicin (91.8%), tobramycin (91.1%), ciprofloxacin (83.1%) and ceftazidime (59.7%) [13]. The MRSA strains isolated in Europe in 2007 were susceptible to imipenem (97.7%), meropenem (97.3%), piperacillin/tazobactam (96.2%), tobramycin (94.2%), gentamicin (92.0%), ciprofloxacin (84.0%) and ceftazidime (39.8%) [14].

A french multicentre study concluded that all staphylococci strains were susceptible to meropenem, imipenem and piperacillin/tazobactam [15].

There is a continuous need of surveillance programs, the results of these types of surveillance are very important because they can, hopefully, help in the correct choice of therapy. The knowledge of phenotypes circulation in our geographical area as the medium of choice of appropriate anti-infective therapies, and ultimately not a proper monitoring of staphylococcal infections.

Conclusions

1. MLS B shows a high prevalence among strains of *S. aureus*.
2. The association between MLS B and MRSA remains a major problem in Romania.
3. No VISA strains were isolated.
4. CNS strains were more resistant to antibiotic than those of *S. aureus*.

Conflict of Interest

The authors declare that they have no conflict of interest.

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