PREVALENCE OF CARRYNG MECA GENE *STAPHYLOCOCCUS AUREUS* STRAINS FROM PEDIATRIC INFECTIONS

MARIA CÎRLAN [1*], GABRIELA COMAN [2], OCTĂVIȚA AILIESEI [3]

Keywords: MRSA, gentamicin, *mec*A gene, „E-test", MIC, infection

Abstract: From the total *S. aureus* strains isolated in the Hospital for Children "Sf. Maria" Iasi during the period November 2000 - November 2003, 32,8% demonstrated to be methicillin-resistant (MRSA). From the two groups of MRSA: GR-MRSA and GS-MRSA, the last one had a slight prevalence during the years 2000, 2001 and 2002. Unexpectedly, during the first months of 2003 year, the number of these GS-MRSA strains triplicates. All the strains, including those with low resistance to O and those with high resistance to O are positive for the presence *mec*A gene.

INTRODUCTION

In many parts of the world, *S. aureus* resistant to methicillin (MRSA) has become a significant nosocomial pathogen, soon after the introduction of this antibiotic in the therapy (Aires et al. 1998).

The first MRSA isolates presented heterogeneous resistance, but progressively, the phenotype of heterogeneous resistance was replaced with that of the homogeneous resistance.

MRSA population disseminates rapidly from a region to another one. Some clones had become epidemic, being associated with a high spread within hospitals, between hospitals and between countries (Ayliffe et al. 1997; Aires et al. 1998; Lelievre et al. 1999;).

Thus, during '80 years, the gentamicin – resistant MRSA became epidemic in Australia, USA, and Europe. It was proved that these strains are generally resistant to a great number of antibiotics, including trimethoprim and / or ciprofloxacin.

During the last year, it was remarked in some countries like Germany that as the MRSA prevalence increases, the range of antibiotics to which the strains are resistant reduces (Witte et al. 2000; Pournaras et al. 2001). More than that, these strains are characterized by the surprising reappearance of the heterogeneous resistance to oxacillin, gentamicin susceptibility, variable resistance for macrolides, lincosamides, a great part of them remaining tobramycin resistant.

THE AIM OF INVESTIGATIONS

The objectives of this study are the determination of MRSA prevalence within the circulating strains, and evolution during a 3-year period in the Clinic Hospital of Emergencies for Children "Sf. Maria" Iasi, Romania.

MATERIAL AND METHODS

In the present study, 871 strains of *S. aureus* isolated from the patients hospitalized in the 5 pediatric clinics, 3 surgical clinics, an intensive care unit, and one of prematures within our hospital were included.

By the disk diffusion method, according to NCCLS criteria, the susceptibility to 12 antibiotics was tested. They were: penicillin (P), oxacillin (O), erythromycin (E), clindamycin (CL), kanamycin (K), tobramycin (TM), gentamicin (G), ciprofloxacin (CIP), tetracycline (T), rifampin (RIF), sulfamethoxazole-trimethoprim (SXT).

The agar plates were incubated at 35ºC for 24 h. The strains showing an inhibition zone of 13 mm diameter around the oxacillin disk (1μg) were considered to be resistant. For all these strains, the MIC (minimum inhibition concentration) was also determinated using the method of agar dilution (Mueller-Hinton agar supplemented with 2% NaCl.) Twenty strains randomly selected, having the MIC of 1 - ≥32 μg/ml have been evaluated by "E-test" (AB Biodisk Solna, Sweden) too.

We also performed the Oxacillin-Salt Agar Screening Test for *Staphylococcus aureus* using Mueller-Hinton agar supplemented with 4% NaCl and 6 μg O/ml in case of strains with the MIC <16 μg/ml.

To verify the work conditions, we used *S. aureus* ATCC 29213 control strain.

The mentioned tests were related to the results of *mec*A gene detection by PCR for 61 strains performed by collaboration with Tokyo Juntendo University, Department of Bacteriology headed by prof. Hiramatsu.K.

RESULTS AND DISCUSSION

Out of total number of tested strains (871), 585 (67,2%) were proved to be oxacillin-susceptible. These strains presented a quasiconstant resistance to P and variable resistance to E, K, and T. The number of resistance determinants was not greater than 4, and 88% of strains manifested mono- and biresistance.

286 strains (32,8%) have been confirmed by the quantitative method to be resistant to O, having MIC between 2 - ≥32μg (fig.1).

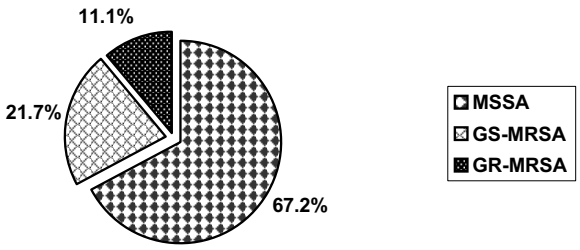

Fig.1. The frequency of *S. aureus* methicillin-susceptible strains (MSSA), and methicillin resistant strains (MRSA) isolated from pediatric infections.

These MRSA strains were also differentiated in two distinct groups.

The first group which comprised 189 strains with MIC between 2-8μg/ml represented the group of MRSA strains susceptible to G (GS-MRSA).

They had 7 resistance phenotypes, 3 being predominant (table 1).

It is remarked from this table that the number of resistance determinants does not exceed 5, only 4 strains being resistant to TM and all are susceptible to CIP.

The second group comprised 97 strains with a different behavior, presenting a constant resistance to P, O, K, TM, G, T and / or CIP and SXT. This is the group of GR-MRSA strains. The number of the resistance determinants was between 6-10, being evidenced 12 phenotypes from which 5 were predominant. A sole strain with 4 resistance determinants (P, O, K, C), and susceptible to G and TM has been, however, classified in this group having a MIC=16μg/ml (table 2).

Table 1. Resistance phenotypes of GS-MRSA strains

PHENOTYPES	No. OF STRAINS	%	MIC (μg/ml)
P.O.	4	2	2-8
P.O.K.	51	26	2-8
P.O.E.	4	2	2-8
P.O.E.K.	2	1	8
P.O.K.TM.	6	3	2-8

PHENOTYPES	No. OF STRAINS	%	MIC (µg/ml)
P.O.K.T.	81	40	2-8
P.O.E.K.T.	41	20	8

Table 2. Resistance phenotypes of GR-MRSA with high resistance to oxacillin

PHENOTYPES	No. OF STRAINS	%	MIC (µg/ml)
P.O.K.C.	1	1	16
P.O.K.TM.G.T.	1	1	16
P.O.E.K.TM.G.T.	2	2	16
P.O.E.K.TM.G.SXT.T.	2	2	16
P.O.E.K.TM.G.RIF.T.	1	1	16 - ≥32
P.O.K.TM.G.CIP.RIF.T.	23	24	≥32
P.O.E.K.TM.G.RIF.C.T.	6	6	≥32
P.O.K.TM.G.CIP.RIF.C.T.	14	15	≥32
P.O.K.TM.G.CIP.SXT.RIF.T.	3	3	≥32
P.O.E.K.TM.G.CIP.RIF.T.	13	13	≥32
P.O.E.K.TM.G.CIP.RIF.T.	1	1	≥32
P.O.E.K.TM.G.CIP.RIF.C.T.	19	20	16 - ≥32
P.O.E.K.TM.G.SXT.RIF.C.T.	11	11	≥32

After 1975 year the MRSA strains have received with variable frequency the resistance mechanisms to the quasitotality of the antibiotic families. The reservoir of resistance genes was considered to be *S. epidermidis* and in 2001 it has been demonstrated the in vivo transfer of *mec*A gene between different species of *Staphylococcus* (Wilders et al. 2001). The results recorded by the method of agar dilutions have been concordant with "E-test" results, the obtained value by these methods allowing the identical classification of the strains belonging to the both described groups (fig. 2)

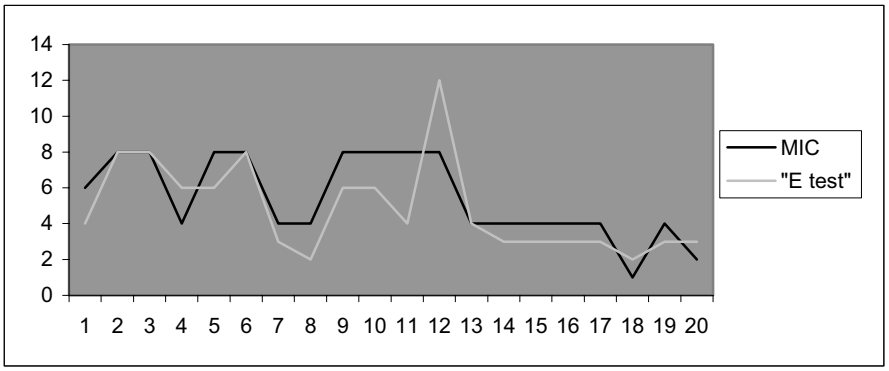

Fig.2. The concordance of the values obtained by the method of dilutions in agar and "E-test"

The results of *mec*A gene detection by PCR have been positive for all the tested strains, including those with low resistance to O (2µg/ml).

Oxacillin-Salt Agar Screening test evidenced the growth of the strains with MIC<16µg/ml. These results demonstrate that this test is useful for the detection of *mec*A gene positive strains.

Regarding the evolution of antibiotic resistance, table 3 reveals the following aspects:

- The average frequency of the MRSA strains for the years 2000, 2001, and 2002 is 24% with a slight predominance of the GS-MRSA group (53%) from the total of MRSA strains.

- During the first 10 month of 2003 year, we can observe a different situation. The incidence of the MRSA strains is doubled (44.7%) with a net distribution in favour of GS-MRSA strains (74.2%).

Table 3 – Evolution of resistance to antibiotics of MRSA strains during a period of 3 years

YEAR	**TOTAL**	**MRSA**	**%**	**GS-MRSA**	**%**	GR-MRSA	**%**
2000	38	9	23.6	5	55.5	4	44.4
2001	194	46	23.7	23	50.0	23	50.0
2002	248	56	22.5	31	55.3	25	44.6
2003	391	175	44.7	130	74.2	45	25.7

This surprising increasing could be correlated with the transfer of 89 newborns from the two maternities from Iasi by which probably there were introduced at least two distinct clones of GS-MRSA.

The emergence of some GS-MRSA clones in disadvantage of GR-MRSA clones has also been reported in other countries as Germany (Witte et al. 2000) and France (Thouvarez et al. 2003).

CONCLUSIONS

The majority of *S. aureus* strains isolated in our hospital are susceptible to oxacillin (67.2%).

During the period November 2000 - December 2002 there are remarked the approximately equal percentages of the two MRSA groups (GR-MRSA 47%, and GS-MRSA 53%). During the 10 months of the 2003-year, the number of GS-MRSA strains triplicated (74.2%) while GR-MRSA strains reduced to 25% from the total of MRSA strains.

The increasing of the number of MRSA strains multisusceptible to non-β-lactam antibiotics creates a new problem: the opportunity of some clinic studies regarding the therapeutic effect of the other antibiotic families to restrict the use of vancomycin.

REFERENCES

Aires de Sousa M., Sanches I.S., Ferro M.I., Vaz M.J., Saraiva Z., Tandeiro T, Serra J., and H. de Lencastre. 1998. Intercontinental spread of a multidrug-resistant methicillin-resistant *Staphylococcus aureus.* J. Clin. Microbiol. 36: 2590-2596

Ayllife G.A. 1997. The progressive intercontinental spread of meticillin-resistant *Staphylococcus aureus*. Clin. Infect. Dis. 25: S74-S79

Lelièvre H., Lina G., Jones M.E., Olive C., Forey F., Roussel-Delvallez M., Nicolas-Chanoine M.H., Bébéar C.M., Jarlier V., Andremont A., Vandenesch F., and Etienne J. 1999. Emergence and Spread in French Hospitals of Methicillin-Resistant *Staphylococcus aureus* with Increasing Susceptibility to Gentamicin and Other Antibiotics J.Clin. Microbiol. 37: 3452-3457

National Committee for Clinical Laboratory Standards. 1997. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically- Fourth Edition: Approved Standard M7-A4. NCCLS, Wayne, PA

Pournaras S., Slavakis A., Polyzou A., Sofianou D., Maniatis A.N., and Tsakris A. 2001. Nosocomial spread of an unusual methicillin-resistant *Staphylococcus aureus* clone that is sensitive to all non-β-lactam antibiotics, including tobramycin. J. Clin. Microbiol. 39: 779-781

Thouvarez M., Muller A., Hocquet D., Talon D., and Bertrand X. 2003 Relationship between molecular epidemiology and antibiotic susceptibility of methicillin-resistant *Staphylococcus aureus* in a French teaching hospital J. Med Microbiol. 52: 801-806

Witte W., Braulke D., Heuck C., and Cuny C. 2000. Methicillin resistant *Staphylococcus aureus* toward coordinated response to a continuing challenge European Communicable Disease Bulletin 5: 31-34

[1] Clinic Hospital of Emergencies for Children "Sf. Maria", Str. Vasile Lupu, 62, 700309 Iaşi – România
[2] University of Medicine and Pharmacy "Gr. T. Popa", Microbiology Department, Str. Universităţii, 16, 700115 Iaşi – România
[3] University "Al. I. Cuza", Faculty of Biology, B-dul Carol I, 20A, 700506 Iaşi – România
[*] corresponding author: Cîrlan Maria, Clinic Hospital of Emergencies for Children "Sf. Maria", Str. Vasile Lupu, 62, 700309 Iaşi - România