

METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS COLONIZATION IN NEWBORN AND OBSTETRICS DEPARTMENTS

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REZUMAT

Obiective: Evidențierea stării de colonizare cu Stafilococ aureus rezistent la metilicină (MRSA), precum și cu alți germeni microbieni cu potențial nosocomial în secții de terapie intensivă nou-născuți și obstetrică. **Material și metode:** Identificarea germeilor a fost realizată cu ajutorul sistemului mini API, iar testele de sensibilitate prin metoda difuzimetrică (NCCLS 2001) la 37 și 35°C cu citire automată prin sistemul Osiris Evolution. Tulpinile au fost apoi încadrate în fenotipuri de rezistență. **Rezultate:** Din cele 1065 de probe recoltate de la nou-născuți am izolat 127 tulpini de *S. aureus*, din care 30 (23.62%) au fost metilicilino-rezistente (MRSA). Din cele 1727 probe recoltate din Obstetrică, am izolat 45 tulpini de *S. aureus*, din care 8 (17.77%) au fost MRSA. Toate tulpinile MRSA au asociat și alte fenotipuri de rezistență. Procentul tulpinilor MRSA izolate la prematuri (33.33%) a fost net superior celui identificat în rândul nou-născuților la termen (8.82%). **Concluzii:** Prevalența ridicată a MRSA din studiul nostru, se încadrează în datele raportate de sistemul EARSS (European Antimicrobial Resistance Surveillance System) pentru România. Aceasta impune o supraveghere atentă a stării de portaj în rândul personalului medical, precum și o politică rațională a antibioterapiei în spitale.

Cuvinte cheie: Stafilococ aureus, rezistență la metilicină, nou născut, prevalență

ABSTRACT

Objective: To observe the colonization status with Methicillin resistant *Staphylococcus aureus* (MRSA) and other microbes with nosocomial potential in Newborn Intensive Care Units (NICU) and Obstetrics Departments (OD). **Material and methods:** Identifications were performed by mini API system and susceptibility tests by disk diffusion tests (NCCLS 2001), at 37 and 35°C and automatic reading on Osiris Evolution system. Then, we categorized the isolated germs according with their phenotypic resistance patterns. **Results:** From 1065 samples collected from newborns, we isolated 127 *S. aureus* strains, from which 30 strains (23.62%) were Methicillin resistant (MRSA). From 1727 samples collected from OD we isolated 45 *S. aureus* strains, with 8 MRSA strains (17.77%). All MRSA have been associated with other resistance patterns. In our study the percentage of MRSA isolated strains was higher in premature (33.33%) than in term newborns (8.82%). **Conclusions:** The high prevalence of MRSA in our study is similar to data reported by EARSS (European Antimicrobial Resistance Surveillance System) for Romania. This enforces a proper surveillance of medical staff and a rational policy in prescribing antibiotics in hospitals.

Key words: *Staphylococcus aureus*, Methicillin resistance, newborn, prevalence.

INTRODUCTION

Community-acquired and nosocomial infections caused by multidrug-resistant Gram-positive pathogens continue to increase in prevalence and have

become a serious problem in many parts of the world. *Staphylococcus aureus* or coagulase-negative staphylococci (CNS) resistant to methicillin (MRSA and MR-CNS) represent some of them. MRSA incidence in countries from Europe and US is estimated to be 10 to 50%.¹ According to the SENTRY program it is about 35%, with differences between hospitals, wards and geographic regions.

According to the European Antimicrobial Surveillance System (EARSS), MRSA prevalence varied almost 100-fold, from < 1% in northern Europe to > 40% in southern and Western Europe. In Romania is considered to be more than 20%.²

Special attention should be given to newborn wards. Here, there are two kinds of residents:

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- permanent – provided by hospital staff, and
- transitory – provided by the newborns and visitors.¹

The large majority of newborns enter the ward without a stable micro flora on their skin and mucosae. Nosocomial infections are frequently due to microbes as *S. aureus*, which have a low colonization rate. For example, *S. aureus* causes 27% of the total number of nosocomial infections from Newborn Intensive Care Units (NICU).³ The onset of infection may be after a prolonged incubation period, when the child leaves hospital, or is still admitted, premature newborns being hospitalized for longer period of time. This may cause an omission or a delayed diagnosis and occurs especially with MRSA and MR-CNS.⁴

Hospital-acquired infections in obstetric patients have a long and dramatic history, but modern obstetric practices have produced low infection rates and extremely low maternal mortality.¹ Outbreaks of staphylococcal infections, in the era of MRSA, are uncommon in modern Obstetric Departments (OD). Even though infection rates are low, good surveillance data in obstetric infection are limited because of difficulties in specific diagnosis and short hospital stays of most obstetric patients.

METHODS

Our clinical material was gathered from NICU and OD from Timisoara. We compared the results obtained in the period 2001-2002 (P1) with those obtained in 2003-2004 (P2). EPI 6 software package was used for statistical analysis (chi square test). We collected a number of:

- 208 samples from newborns in 2001 – 2002;
 - 857 samples from newborns in 2003 – 2004;
- (Table 1)

Table 1. Samples collected from newborns

| Samples | 2001 - 2002 | | 2003 - 2004 | | TOTAL | |
|------------------------|-------------|------------|-------------|------------|-------------|------------|
| | Number | Percent % | Number | Percent % | Number | Percent % |
| Pharyngeal swabs | 77 | 37.01 | 253 | 29.52 | 330 | 30.98 |
| Nasal swabs | 69 | 33.17 | 276 | 32.21 | 345 | 32.39 |
| Blood | 19 | 9.13 | 135 | 15.76 | 154 | 14.46 |
| Bronchoalveolar liquid | 16 | 7.69 | 4 | 0.47 | 20 | 1.88 |
| Umbilical secretions | 3 | 1.44 | 20 | 2.33 | 23 | 2.16 |
| Uroculture | 8 | 3.84 | 36 | 4.21 | 44 | 4.13 |
| Conjunctival swabs | 9 | 4.32 | 19 | 2.21 | 28 | 2.63 |
| Vernix | 6 | 2.88 | 15 | 1.75 | 21 | 1.97 |
| Faeces | 1 | 0.48 | 7 | 0.81 | 8 | 0.75 |
| Gastric content | - | 0 | 81 | 9.45 | 81 | 7.61 |
| Skin swabs | - | 0 | 10 | 1.17 | 10 | 0.94 |
| Cerebrospinal fluid | - | 0 | 1 | 0.11 | 1 | 0.09 |
| Total | 208 | 100 | 857 | 100 | 1065 | 100 |

- 1727 samples from patients hospitalized in OD in 2003 – 2004. (Table 2)

Table 2. Samples collected from obstetrical patients

| Samples | 2003 - 2004 | |
|-------------------------|-------------|------------|
| | Number | Percent % |
| Pharyngeal swabs | 16 | 0.92 |
| Nasal swabs | 12 | 0.69 |
| Blood | 142 | 8.22 |
| Sputum | 1 | 0.05 |
| Col secretions | 614 | 35.55 |
| Uroculture | 842 | 48.75 |
| Othic secretions | 1 | 0.05 |
| Uterine content | 5 | 0.29 |
| Faeces | 3 | 0.17 |
| Wound secretions | 11 | 0.63 |
| Douglas puncture fluid | 2 | 0.11 |
| Amniotic fluid | 2 | 0.11 |
| Peritoneal fluid | 4 | 0.23 |
| Intrasurgical product | 2 | 0.11 |
| Drain secretions | 2 | 0.11 |
| Placenta | 1 | 0.05 |
| Post delivery discharge | 67 | 3.87 |
| Total | 1727 | 100 |

Isolation (on conventional culture media) and identification of germs with nosocomial potential were performed at the hospital laboratory. Confirmation of identification tests, as well as extensive antimicrobial tests, were performed at the laboratory of the Department of Microbiology. Identifications were performed using the API system (BioMerieux France) and susceptibility tests, by disk diffusion tests (NCCLS 2001 standard) at 37 and 35°C, with interpretation on Osiris Evolution (Bio Rad Laboratories). This interpretation not only supplied us with data concerning the sensitivity of the studied strains, but it also helped identify various resistance phenotypes.^{5,6}

Inhibition zones for Oxacillin (1µg Oxacillin/disk) in *S. aureus* strains (NCCLS 2001) are presented below.

- Resistance: corresponding to an inhibition zone less or equal with 10 mm;
- Intermediate resistance: corresponding to 11-12 mm inhibition zone;
- Sensitive: corresponding to an inhibition zone more than 13 mm.

The following resistance patterns (according to international standards) were used for describing MRSA isolates:

- High level resistance (homogenous), corresponding to an inhibition zone less than 10 mm for Oxacillin.
- Low level resistance (heterogeneous) characterized by the growth inside of inhibition area for Oxacillin (valid only for resistance test to oxacillin at 30°C).

RESULTS

The objective of our study was to observe the colonization and infectious status with Methicillin resistant *S. aureus* (MRSA) and other microbes with nosocomial potential in NICU and OD.

We isolated 832 microbial strains with nosocomial potential (489 from newborns and 343 from Obstetrics) and our attention focused on 172 *S. aureus* strains (20.67%). In most samples, a single microbial specimen was isolated. Figure 1 illustrates the percents calculated from the number of microbial strains with nosocomial potential isolated in each department and each period of time (NICU 2001-2002, NICU 2003-2004 and OD 2003-2004).

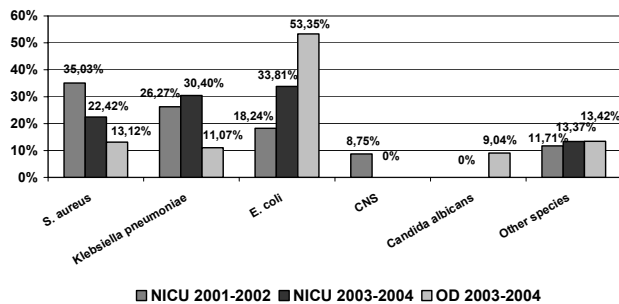


Figure 1. Germs with nosocomial potential isolated from NICU and Obstetric Department

Respectively:

- from 1065 samples collected from newborns we isolated 127 *S. aureus* strains, from which, 30 strains were methicillin resistant (MRSA) (23.62%). (Table 3, 4)
- from 1727 samples collected from OD we isolated 45 *S. aureus* strains, with 8 MRSA strains (17.77%). (Table 5)

The rest of *S. aureus* isolates (97 strains from NICU, 37 strains from OD) belonged to Penicillin-resistant Methicillin-sensitive (Peni-R Meti-S) phenotype. (Table 6)

Table 3. Distribution of MRSA strains according to the sample type (2001-2002) in NICU

| No. | Strain | Sample | Phenotype |
|-----|---------|-------------------------------|----------------|
| 1. | 4/5257 | Umbilical swab | MRSA+KTG+MLSBc |
| 2. | 5/5314 | Nasal swab | MRSA+KTG+MLSBc |
| 3. | 6/5310 | Nasal swab, pharyngeal swab | MRSA+MLSBi |
| 4. | 51/5645 | Gastric aspirate | MRSA+MLSBi |
| 5. | 55/5680 | Nasal swab | MRSA+MLSBi |
| 6. | 82/153 | Nasal swab, conjunctival swab | MRSA+KTG+MLSBc |
| 7. | 84/72 | Conjunctival swab | MRSA+KTG+MLSBc |
| 8. | 90/208 | Pharyngeal swab | MRSA+MLSBc+Fq |
| 9. | 97/298 | Nasal swab | MRSA+MLSBi |
| 10. | 98/291 | Pharyngeal swab, nasal swab | MRSA+KTG+MLSBc |
| 11. | 102/316 | Pharyngeal swab | MRSA+KTG |
| 12. | 110/436 | Blood | MRSA+MLSBi |

Table 4. Distribution of MRSA strains according to the sample type (2003-2004) in NICU

| No. | Strain | Sample | Phenotype |
|-----|---------|------------------------------------|--------------------------|
| 1. | 60/5263 | Nasal swab (premature) | MRSA+KTG+MLSBi+Fq |
| 2. | 77/5990 | Nasal swab (premature) | MRSA+KTG+Fq+ M phenotype |
| 3. | 74/5965 | Bronchoalveolar liquid (premature) | MRSA+KTG+Fq+ M phenotype |
| 4. | 8/127 | Nasal swab (premature) | MRSA+KTG+Fq+ M phenotype |
| 5. | 10/134 | Nasal swab (premature) | MRSA+KTG+Fq+ M phenotype |
| 6. | 11/135 | Pharyngeal swab (premature) | MRSA+KTG+Fq+ M phenotype |
| 7. | 32/1085 | Nasal swab (premature) | MRSA+KTG+Fq+ M phenotype |
| 8. | 16/216 | Nasal swab (premature) | MRSA+KTG+MLSBc |
| 9. | 34/1123 | Nasal swab (premature) | MRSA+KTG+MLSBc |
| 10. | 37/1402 | Nasal swab (premature) | MRSA+KTG+MLSB+Sa |
| 11. | 39/1639 | Nasal swab (premature) | MRSA+KTG+MLSB+Sa |
| 12. | 40/1653 | Pharyngeal swab (premature) | MRSA+KTG+MLSBc+Fq |
| 13. | 44/1702 | Nasal swab (premature) | MRSA+KTG+MLSBc+Fq |
| 14. | 48/1745 | Nasal swab (premature) | MRSA+KTG+MLSBc+Fq |
| 15. | 52/1843 | Pharyngeal swab (premature) | MRSA+KTG+MLSBc+Fq |
| 16. | 24/529 | Nasal swab (newborn at term) | MRSA+KTG+MLSBc+Fq |
| 17. | 26/540 | Pharyngeal swab (newborn at term) | MRSA+KTG+MLSBc+Fq |
| 18. | 29/673 | Nasal swab (newborn at term) | MRSA+KTG+MLSBc+Fq |

Table 5. Distribution of MRSA strains according to the sample type (2003-2004) in OD

| No. | Strain | Sample | Phenotype |
|-----|-----------|-------------------------|-------------------------|
| 1. | 1035/5040 | Col secretions | MRSA+KTG+MLSBi+Fq |
| 2. | 1040/5169 | Col secretions | MRSA+KTG+MLSBi+Fq |
| 3. | 1052/5204 | Col secretions | MRSA+KTG+MLSBc |
| 4. | 8/249 | Wound secretions | MRSA+K(Nm)+MLSB+Sa |
| 5. | 15/757 | Wound secretions | MRSA+KTG+Fq+M phenotype |
| 6. | 25/999 | Post delivery discharge | MRSA+KTG+MLSB+Sa+Fq |
| 7. | 26/1001 | Col secretions | MRSA+KTG+MLSBc+Fq |
| 8. | 29/1108 | Col secretions | MRSA+KTG+MLSBc+Fq |

All MRSA have been associated with other resistance patterns like: aminoglycosides (KTG

Table 6. Resistance phenotypes in *S. aureus* strains, isolates from NICU and OD.

| | Newborns | | | | Obstetric Patients | |
|-------------|-----------|-------------|-----------|-------------|--------------------|-------------|
| | 2001-2002 | | 2003-2004 | | 2003-2004 | |
| | MRSA | PeniR-MetiS | MRSA | PeniR-MetiS | MRSA | PeniR-MetiS |
| No. strains | 12 | 36 | 18 | 61 | 8 | 37 |
| %* | 25.0 | 75.0 | 22.78 | 77.22 | 17.78 | 82.22 |

* Percents calculated from the number of *S. aureus* isolate from each department, in each period

and K (Nm) phenotypes), fluoroquinolones (Fq phenotype), macrolides (MLSBc, MLSBi, phenotypes), or Cotrimoxazole. (Fig. 2) Many strains were multiresistant, being sensitive only to vancomycin (NCCLS 2001).

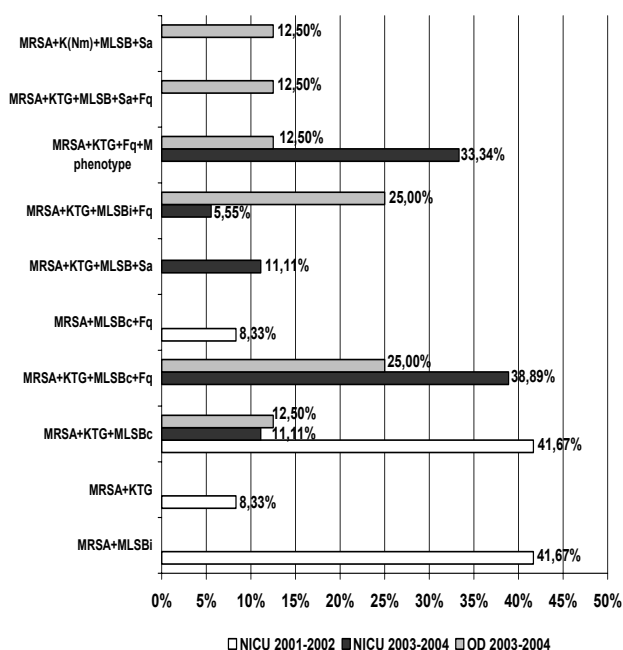


Figure 2. Resistance phenotypes in MRSA strains, isolates from NICU and Obstetric Department.

DISCUSSIONS

Acquired resistance to Methicillin develops by changes in antibiotic target. Alterations in Penicillin binding proteins (PBPs) will offer resistance to all beta-lactam antibiotics. This type of resistance may be homogenous (high level resistance) or heterogeneous (low level resistance). The last one is encountered only in a small part of the microbial population.⁷⁻¹⁰

The percentage of 23.62% MRSA in NICU and 17.77% in OD in our study is similar to data reported by EARSS (European Antimicrobial resistance Surveillance System) for Romania (they consider more than 20% MRSA strains for our country). According to the SENTRY program this percentage is about 35%, with differentiation between hospitals, wards and geographic regions. Comparing the percentage of MRSA from NICU versus OD in 2003-2004 reveals insignificant statistical differences ($p=0.51$).

Methicillin-resistant staphylococci were isolated especially from premature newborns with prolonged hospitalization. Figure 3 illustrates the superior percent of MRSA (33.33%) in premature children versus term newborns (8.82%), with significant value ($p=0.01$).

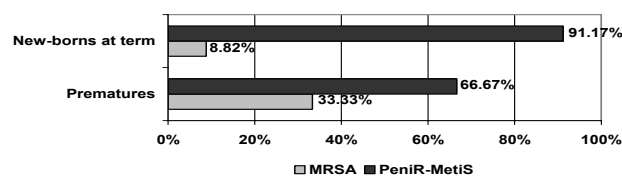


Figure 3. Distribution of MRSA strains isolated from newborns at term and premature in 2003-2004.

In fact, contributing factors in neonatal infection are considered to be:

- the mother's immune status;
- placenta status;
- gestational age;
- the immune status of the newborn.

The latency of isolated strains was evaluated through unusual colonization with methicillin-resistant *S. aureus*. We observed from nasal swabs isolates that 18 newborns were carriers of *S. aureus*. At the same time we noticed multiresistant *Escherichia coli*, *Klebsiella*, *Pseudomonas* strains colonization. In NICU, we observed a significant reduction in *S. aureus* percentage ($p=0.004$) (in 2003-2004 versus 2001-2002), and also a significant difference between percentages of *S. aureus* in NICU and OD, in 2003-2004 ($p=0.001$). (Fig. 1) The hospital source of these strains is obvious, because the newborn do not possess a stable skin and mucosal flora. The medical staff's hands and nasal carriers represent the common way of staphylococci transmission. In our study, all *S. aureus* strains isolated from medical staff were Peni-R Meti-S, none of them were MRSA.

There is no concordance between resistance phenotypes in strains isolated in mothers, with those isolated in their own newborns, 4 resistance phenotypes fit in mother's group with the newborn's group, reflecting a hospital cross-contamination (Fig. 2):

- MRSA+KTG+MLSBc (Methicillin resistant *S. aureus* - associated with: resistance to Kanamicine, Tobramicine, Gentamicine + resistance to macrolides,

lincosamides, streptogramins, constitutive phenotype);
- MRSA+KTG+MLSBc+Fq (Methicillin resistant *S. aureus* - associated with: resistance to Kanamicine, Tobramicine, Gentamicine + resistance to macrolides, lincosamides, streptogramins, constitutive phenotype + resistance to fluoroquinolones);

- MRSA+KTG+MLSBi+Fq (Methicillin resistant *S. aureus* - associated with: resistance to Kanamicine, Tobramicine, Gentamicine + resistance to macrolides, lincosamides, streptogramins, inducible phenotype + resistance to fluoroquinolones);

- MRSA+KTG+Fq+ phenotype M (Methicillin resistant *S. aureus* - associated with: resistance to Kanamicine, Tobramicine, Gentamicine + resistance to macrolides + resistance to fluoroquinolones).

The continuous increasing resistance in germs with nosocomial potential is a reality mentioned in the literature, as well as in our study. We isolated germs with increasing associated resistance patterns (in 2003-2004 versus 2001-2002). (Fig. 2)

Unfortunately, we don't know the real number of MRSA nosocomial infections in the period when our study was performed, because of miss-cooperation with some clinicians on this issue. Anyhow, their presence in newborns, as well as in OD, demands a proper surveillance in prescribing antibiotics in hospitals, and a better team work involving clinicians-bacteriologists-epidemiologists.

CONCLUSIONS

Nosocomial infections caused by *S. aureus* represent an indicator of the quality of medical attendance, still raising problems in neonatology departments. The occurrence of multidrug resistant strains, like MRSA, especially in neonates, with an immature immune system, increases the gravity of infection, regarding its clinical evolution.

The MRSA percentage in NICU is higher than recorded in OD. All our MRSA strains were sensitive to vancomycin.

We recorded a slight decrease of MRSA percentage in neonates hospitalized in NICU in 2003-2004 versus 2001-2002, statistically insignificant ($p=0.775$) but also increasing associated resistance patterns (in 2003-2004 versus 2001-2002).

The percentage of MRSA isolated strains is higher in premature than in term newborns, reflecting their immune depression and, on the other hand, the multiple inoculation possibilities during their complex medical assistance.

There is no concordance between resistance

phenotypes in strains isolated in mothers, with those isolated in their own newborns, but 4 resistance phenotypes were found both in mother's group and in the newborn's group, reflecting hospital cross-contamination.

The high percentage of MRSA strains with clinical manifest or latent nosocomial potential in newborns, as well as in OD, require proper surveillance in prescribing antibiotics in hospitals, and better team work between clinicians-bacteriologists-epidemiologists.

Because in Romania the initiation of a unitary Surveillance System of Nosocomial Infections is in its early stages, the entire significance of the whole phenomenon is most probably understated.

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