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METHICILLIN - RESISTANT *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM CHILDREN IN THE COMMUNITY

Sofia Constantiniu[1], Angela Romaniuc[1], Aurora Ambăruş[2], Roxana Alexandrescu[1], Adriana Coşofreţ[3], Carmen Stanciu[4]

1. Institute of Public Health Iaşi, 2. "Sf. Spiridon" Hospital Iaşi,
3. "Nicolina" Medical Unit Iaşi, 4. Health District Authority Vrancea

Abstract. Aim. The aim of this study was to estimate the proportion of the MRSA strains in community by analysis of the pharyngeal swabs from children with acute and chronic pharyngitis assisted in medical ambulatory units. **Material and methods.** A number of 128 *S.aureus* strains isolated during the period of 2002-2004 from 1782 children were tested concerning their susceptibility to oxacillin. All *S.aureus* strains with oxacillin inhibition zones diameter <13 mm were tested concerning the Minimal Inhibitory Concentrations (MICs) by agar dilution method, recommended by National Committee for Clinical Laboratory Standards (NCCLS). The strains with MICs ≥ 4 μg/ml to oxacillin were considered methicillin -resistant *Staphylococcus aureus* (MRSA). **Results.** Among the 128 *S.aureus* strains isolated from community, a number of 14 strains had a zone of inhibition <13 mm diameter and 7 (5.4 %) strains had MICs ≥ 4 μg/ml, being MRSA. Only one strain (3.1%) from a number of 32 strains isolated in the year of 2002 was MRSA. From a number of 32 strains isolated in 2003, only 2 (6.2 %) were MRSA and from 62 strains isolated in the year of 2004, 4 (6.4 %) were MRSA. The MICs were ≥ 4 μg/ml for one strain, 8 μg/ml for 4 strains and 16 μg/ml for 2 strains. **Conclusions.** A number of 7 strains of 128 *S. aureus* strains were MRSA (5.4%). The community isolates which are generally susceptible to multiple antibiotics are in contrast to the typical, multiple-drug-resistant hospital MRSA ones. Although the percentage of the community - MRSA strains in our area is low, our data pointed out their presence in the community and underlined the importance of MRSA strains from community as possible sources for hospital infections.

Key-words: *S. aureus,* strains, methicillin-resistant, children, community

Rezumat. Scop. Estimarea proporţiei de tulpini *S. aureus* rezistent la meticilină (SARM) în comunitate, prin prelucrarea unor exsudate faringiene recoltate de la copii cu faringită acută şi cronică, din ambulator. **Material şi metodă.** Au fost izolate 128 tulpini de *S. aureus* de la 1782 copii, in intervalul 2002-2004 pentru care a fost testată sensibilitatea faţă de oxacilină. Toate tulpinile cu diametrul zonei de inhibiţie <13 mm au fost testate privind concentraţiile minime inhibitorii (CMI).Tulpinile cu CMI ≥ 4 μg/ml au fost considerate *S. aureus* SARM. **Rezultate.** Din 128 tulpini izolate de la copii din ambulator, 14 au prezentat zone de inhibiţie < 13 mm, 7 (5,4%) fiind SARM, având CMI ≥ 4 μg/ml. Ca evoluţie anuală a tulpinilor SAMR, s-a constatat: din 32 tulpini izolate în 2002 1 (3,1%) au fost SARM, în 2003 din 32 tulpini 2 (6,2%) au fost SARM şi 4 (6,4%) tulpini din 62 izolate în 2004. CMI: 4 μg/ml = 1 tulpină, 8 μg/ml = 4 tulpini şi 16 μg/ml = 2 tulpini. **Concluzii.** Din 128 de tulpini de *S. aureus*, 7 (5,4%) au fost SARM. Tulpinile izolate din comunitate, care sunt de regulă sensibile la multe antibiotice, se diferenţiază net de tulpinile SARM de spital, multiplu rezistente. Deşi prevalenţa SARM în zona noastră este redusă, rezultatele studiului nostru subliniază

importanța circulației SARM în comunitate, aceste tulpini reprezentând o potențială sursă de infecție pentru infecțiile nosocomiale.
Cuvinte-cheie: *S. aureus*, izolate, meticilin-rezistență, copii, comunitate

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of nosocomial infections worldwide. Traditionally, MRSA infections have been acquired almost exclusively in hospitals, long-term care facilities, or similar institutional settings (1-4). Risk factors for MRSA colonization or infection in the hospital include prior antibiotic exposure, admission to an intensive care unit, surgery, and exposure to a MRSA-colonized patient (1-5).

It is known that humans are a natural reservoir for *S. aureus*, and asymptomatic colonization is far more common than infection. Colonization of the nasopharynx, perineum, or skin, particularly if the cutaneous barrier has been disrupted or damaged, may occur shortly after birth and may recur anytime thereafter (1). Family members of a colonized infant may also become colonized. Transmission occurs by direct contact to a colonized carrier. Carriage rates ranged are between 25% and 50%; higher rates than in the general population are observed in injection drug addicts, patients with insulin-dependent diabetes, dermatologic conditions, long-term indwelling intravascular catheters and health-care workers (1).

Recent studies suggest that the epidemiology of MRSA may be in change, as the isolation of MRSA is no longer limited to hospitalized patients or persons with predisposing risk factors (1,4-6). Although the MRSA strains can be isolated from healthy persons in community, the prevalence of MRSA colonization in healthy persons has been shown to be low, even when MRSA is highly endemic in hospital settings (1,7-12).

When cases of MRSA infection have been identified in the community, a thorough investigation usually reveals a history of recent hospitalization, a close contact with a person who has been hospitalized or other risk factors, such as previous antimicrobial-drug therapy (1,2-12).

In the last decade, there are frequently articles concerning the MRSA strains in community and the aspects of the evolution of *S. aureus* become very important.

The aim of this study was to estimate the proportion of the MRSA strains in community by analysis of the pharyngeal swabs from children with acute and chronic pharyngitis assisted in medical units.

MATERIAL AND METHODS

The presence of MRSA was estimated by analysis of the pharyngeal swabs from 1782 children with acute and chronic pharingitis assisted in medical units during the period of 2002 and 2004. In the context of the etiological agents of pharingitis, it was performed the isolation of *S. aureus* strains from pharyngeal swabs. Certainly, the isolation of *S. aureus* strains was not considered as etiological agents of pharingitis cases.

A number of 128 *S. aureus* strains were isolated from the children between the years 2002-2004 and tested concerning their susceptibility to oxacillin.

Antimicrobial drug susceptibility was tested by the disk diffusion method according to guidelines recommended by the National Committee for Clinical laboratory Standards (NCCLS), 1999 (13). All isolated *S. aureus* strains with oxacillin inhibition zones diameter <13 mm were tested concerning the MICs by agar dilution method recommended by NCCLS. All these strains were tested for their susceptibility to penicillin, kanamycin, erythromycin, cephalexin, cefuroxime, gentamicin, clindamycin, chloramphenicol, ciprofloxacin, rifampicin and vancomycin. The strains with MICs ≥ 4 μg/ml to oxacillin were considered MRSA and the strains with MICs < 4 μg/ml were considered methicillin - sensitive to *S. aureus* (MSSA).

RESULTS AND DISCUSSION

Of the 128 *S. aureus* strains isolated from community 14 strains had a zone of inhibition <13 mm diameter. From these only 7 (5.4 %) strains had MICs ≥ 4 μg/ml being MRSA.

From 733 children there were isolated 34 strains of *S. aureus* strains in the year of 2002: 33 (97,1%) MSSA strains and 1 MRSA strain (2,9 %). In the year of 2003 there were isolated 32 *S. aureus* strains from 563 children and from these 30 (93.8%) were MSSA and 2 (6.2 %) were MRSA. In the year of 2004 there were isolated 62 *S. aureus* strains from 486 children with 4 (6.5 %) MRSA strains and 58 (93.5%) MSSA strains, respectively (table 1).

Table 1. The structure of investigated children according to the presence of *S. aureus* and its sensitivity to methicillin

Year	Investigated children (No)	Positive *S.aureus* children No. (%)	MSSA No. (%)	MRSA No. (%)
2002	733	34 (4.6 %)	33 (97.1 %)	1 (2.9 %)
2003	563	32 (5.6 %)	30 (93.8 %)	2 (6.2 %)
2004	486	62 (12.7 %)	58 (93.5 %)	4 (6.5 %)
Total	1782	128 (7.1 %)	121 (94.5 %)	7 (5.5 %)

As figure 1 shows MRSA strains were 2.9% in 2002, 6.2% in 2003 and 6.5% in 2004. Figure 2 illustrates the MICs: 4 μg/ml for one strain, 8 μg/ml for 4 strains and 16 μg/ml for 2 strains.

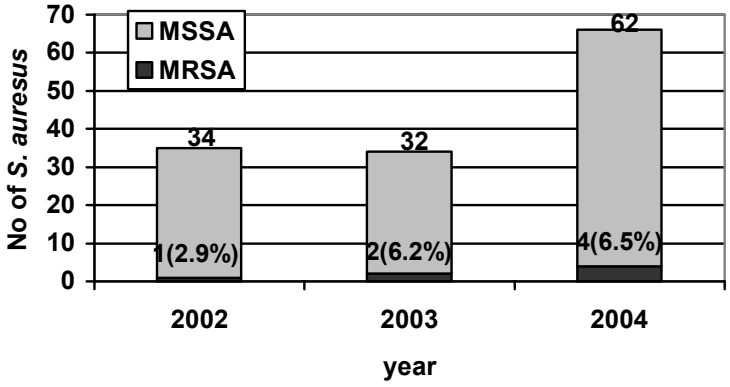

Fig. 1 Community isolated *S. aureus* strains (MRSA and MSSA)

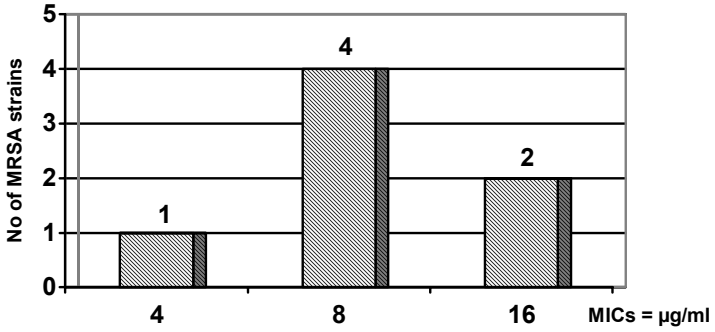

Fig. 2 The minimal inhibitory concentrations (MIC) of the MRSA strains (µg/ml)

The patterns of resistance of MRSA strains are presented in the table 2. All MRSA strains were resistant to penicillin, kanamycin and gentamicin. A number of 3 strains (42.8%) were resistant to erythromycin and to ciprofloxacin was resistant only one strain (14.2%). The community MRSA strains had a limited resistance, in concordance with the findings of many other authors (1-7).

The community isolates generally susceptible to multiple antibiotics are in contrast to the typical, multiple-drug-resistant hospital MRSA isolates.

Table 2. Patterns of resistance of the S. aureus strains

Patterns of resistance	Strains No. %
penicilin + oxacilin +kanamicyn+ gentamicin	4 (57.1 %)
penicilin +oxacilin+erythromicyn+kanamicyn+gentamicin	2 (28.5 %)
penicilin+oxacilin+erythromicyn+kanamicyn+gentamicin+ciprofloxacin	1 (14.2 %)

The presence of MRSA in community is a very important aspect of the epidemiology of the staphylococcal infections. Though the percentage of the community MRSA strains in our area is low, our data point out the aspect of their presence in the community. The presence of acquired-community MRSA infections in children and adults hospitalized with staphylococcal infections indirectly proves the presence in a high number of MRSA in community. If we consider that the frequency of acquired-community *S. aureus* infections is increasing in general population, it is understandable the importance of the presence of MRSA strains in community.

In Romania, the percentages of MRSA in different categories of hospitals are varied. According to some findings it is proved the behaviour of many *S. aureus* strains isolates in hospitals from different human infections according to antimicrobial agent. On the other hand, there are studies that present the hospitalized cases of severe infections caused by MRSA or others medical circumstances all reported from Romanian studies (table 3).

Table 3. MRSA prevalence reported by Romanian studies

Author reference	Year	Medical conditions	MRSA prevalence
Coman G (14)	2002	Hospitalized children	13.2%
Dorobăţ C (15)	2002	Systemic severe infections	3.3%
Corcaci C (16)	2002	Meningitis	13.7%
Mihalache D (17)	1999	Nosocomial meningitis	16.0%
Iancu LS (18)	2001-2004	Hemodialised patients	32.3%
Iancu LS (19)	2001	Medical staff	25.0%
Codiţă I (20)	2002-2004	Surveillance study	36-46%

Community-associated methicillin-resistant *S. aureus* (CA-MRSA) is an emerging phenomenon that has been reported from almost every continent in the world. MRSA infection first emerged in the early 1961 and its prevalence has increased steadily in hospitals as well as in communities (1-4, 11).

There are many retrospective studies concerning the increase of MRSA in the last years (1-11). Chambers elaborated a very interesting synthesis

concerning the evolution of community-acquired MRSA in the United States (1). According to his findings, data collected by the Centre for Disease Control (CDC) in the early to mid - 1980s showed that MRSA were limited mainly to relatively large urban medical centres, of about 5% to 10%. Smaller, unreferral centres were relatively free of MRSA, with prevalence rates below 5%. By the 1990s, rates among these smaller community hospitals (<200-bed) had increased to 20%, and twice that rate were found in the larger urban centres. More recent surveillance data have indicated that rates have continued to rise, with a prevalence of 50% of MRSA isolates from intensive care units by the end of 1998. At these high rates, the emergence of correspondingly high rates of MRSA strains in the community can be anticipated. The true prevalence of MRSA cannot be determined because no systematic, population-based surveillance of community isolates of *S. aureus* exists (1-3). One hospital - based study has found that up to 40% of MRSA infections in adults were acquired before admission to the hospital (1).

Early reports pointed out that the community isolates of MRSA had affected persons with known risk factors as contact with health-care facilities, previous antimicrobial therapy, whereas more recent reports describe colonization and transmission in populations free of risk factors (1). Herold concluded also that the prevalence of community-acquired MRSA without identified risk is increasing (2). A CDC report pointed out the importance of this aspect, presenting findings concerning deaths of four children from rural Minnesota and North Dakota which were caused by infection with community-acquired MRSA strains. These children were free of risk factors for MRSA infection. In these cases infections were caused by strains susceptible to several antibiotics, except beta-lactams and different from typical nosocomial isolates circulating in local hospitals (1).

The community isolates are generally susceptible to multiple antibiotics, an aspect which is in contrast to the typical, multiple-drug-resistant hospital MRSA isolates. The community MRSA strains were susceptible to other classes of antibiotics. Often they are resistant only to beta-lactam antibiotics. The presence of *S. aureus* strains with the lack or loss of resistance to multiple antibiotics suggests a community origin due to antibiotic selective pressure which is much lower within the community than in hospitals (1-12).

The problem of the origin of MRSA strains in community is not completely understood. The methicillin resistance determinant, *mec*, is chromosomally encoded (21). Ribotyping (a genotyping scheme that uses Southern blot analysis to identify DNA restriction enzyme polymorphisms of the five to six ribosomal RNA genes distributed throughout the *S. aureus* chromosome) and cluster analysis indicate that *mec* has integrated into at least three distinct methicillin-susceptible chromosomal

backgrounds, A, B, and C (1). The determinant *mec* itself is polymorphic; three types have been identified: I, II, and III. Okuma et al, recently identified a novel staphylococcal cassette chromosome *mec* (SCC *mec*) as the methicillin resistance determinant on their chromosome designated type IV SCC*mec* (9).

Whether strains of MRSA in the community also arise *de novo*, as a consequence of horizontal acquisition of the *mecA* gene is unclear. The transfer of *mecA* DNA to a susceptible *S. aureus* strain has occurred *in vitro* and recently in a hospitalized patient during antibiotic treatment (6). The mechanism of transfer of *mec* DNA from a donor to a recipient also is not completely understood. However, the excision and integration of the *mec* DNA from and to the chromosome are apparently catalyzed by cassette chromosome recombinants A and B (Ccr A and B) coded by *mec*-associated genes (*ccrA* and *B*), with homology to the invertase-resolvase family of DNA recombinants (20).

Concerning the origins, which are likely to become obscured as clones move back and forth between hospital and community over time, emergence of MRSA within the community is a major threat with several important clinical implications: treatment failure with accompanying complications or death may result if an anti-staphylococcal beta-lactam antibiotic is used and the infecting strain proves to be resistant; infections caused by methicillin-resistant strains may be more difficult to manage or more expensive to treat (1). Recently it was pointed the nosocomial infections with community-associated MRSA in neonatal intensive care unit. These CA-MRSA strains can become nosocomial pathogens (22). The increasing prevalence of MRSA will inevitably increase the use of vancomycin, adding further to the problem of antibiotic-resistant gram-positive bacteria (1).

Antimicrobial resistance to penicillin, methicillin, or vancomycin is an unavoidable consequence of the selective pressure of antibiotic exposure. Although the details of the epidemiology of staphylococcal drug resistance may change, the fundamental forces driving it are similar. The question is not whether resistance will occur, but how prevalent resistance will become. Minimizing the antibiotic pressure that favours the selection of resistant strains is essential to controlling the emergence of these strains in the hospital and the community, regardless of their origins (1-12).

CONCLUSIONS

- A number of 128 *S. aureus* strains were isolated from 1782 children in the period of 2002-2004.
- A number of 7 (5.4 %) strains were MRSA (MICs $\geq$ 4 μg/ml).
- The MICs were of 4 μg/ml for one strain, 8 μg/ml for 4 strains and 16 μg/ml for 2 strains
- All MRSA strains were resistant to penicillin, kanamycin and gentamicin. 3 (42.8%) strains were resistant to gentamicin and one (14.2%) was resistant to ciprofloxacin.

- The community isolates which are generally susceptible to multiple antibiotics are in contrast to the typical, multiple-drug-resistant hospital MRSA ones.
- Although the percentage of the community - MRSA strains in our area is low, our data pointed out their presence in the community. Taking into consideration that the acquired-community *S. aureus* infections have an increased frequency, this study has underlined the importance of this aspect of MRSA in community.

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