Original article

PART OF METHICILLIN-RESISTANT STAPHYLOCOCCI ISOLATED FROM PATIENT MATERIAL

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Investigation comprised multivarious patient material like: swabs of wounds, eyes, ears, puncture fluid, aspirate, peritoneal liquid, blood and liquor samples.

Distribution of Staphylococcus aureus in nefrologic patients amounted to 21,71% and Coagulase Negative Staphylococci (CoNS) to 53,14% in 2003, of which methicillin-resistant Staphylococcus aureus (MRSA) was present in 63,27 and MRCoNS in 64,52% of cases. In surgical patients, the distribution of Staphylococcus aureus for the same period amounted to 22,75 and KNS to 18,11%, while MRSA was registered in 63,27 and MRCoNS 66,67% of cases. In 2004, the distribution of Staphylococcus aureus was 11,11 and KNS 24,07%. In nephrology patients, MRSA was present in 52,77 of cases and MRCoNS in 82,05%. In surgical patients, the distribution of Staphylococcus aureus was 43,38 and CoNS 35,29%, of which MRSA was present in 67,79 and MRCoNS in 81,25% of cases. In outpatients, the distribution of Staphylococcus aureus amounted to 26,02 and KNS to 12,07%, of which MRSA was present in 35,31 of cases and MRKNS in 53,65% of cases. The highest degree of resistance to other tested antibiotics was reported in nephrology patients. In these cases, in 2005, the resistance of MRSA to clindamycin was 81,82, erythromycin 90,91, ofloxacin 88,82%. Acta Medica Medianae 2008;47(2):10-14.

***Key words**: MRSA, MRCoNS, resistant, hospital isolates, outpatient isolates*

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Introduction

In the early 1970s, physicians were finally forced to abandon their belief that, given the vast array of effective antimicrobial agents, virtually all bacterial infections were treatable. Their optimism was shaken by the emergence of resistance to multiple antibiotics among such pathogens as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Mycobacterium tuberculosis*. The evolution of increasingly antimicrobial-resistant bacterial species stems from a multitude of factors that includes the widespread and sometimes inappropriate use of antimicrobials, the extensive use of these agents as growth enhancers in animal food, and, with the increase in regional and international travel, the relative ease with which antimicrobial-resistant bacteria cross geographic barriers (1-3).

Nowhere has this issue been of greater concern than with the Gram-positive bacteria pneumococci, enterococci, and staphylococci. Multidrug resistance is now the norm among these pathogens. *S. aureus* is perhaps the pathogen of greatest concern because of its intrinsic virulence, its ability to cause a diverse array of life-threatening infections, and its capacity to adapt to different environmental conditions (4,5). The mortality of *S. aureus* bacteremia remains approximately 20–40% despite the availability of effective antimicrobials (6).

S. aureus isolates from intensive care units across the country and from blood culture isolates worldwide are increasingly resistant to a larger number of antimicrobial agents (4,7). Inevitably this has left fewer effective bactericidal antibiotics to treat these often life-threatening infections.

Methicillin, introduced in 1961, was the first of the semisynthetic penicillinase-resistant penicillins. Its introduction was rapidly followed by reports of methicillin-resistant isolates (8).

An ideal method for MRSA detection should have a high sensitivity and a short time to the reporting of the results (9). However, some of the tests which are used in routine laboratorial work for detection of oxacillin resistance are not sensitive enough. Recommendations for improving the sensitivity include the increase of the inoculum size, incubation on lower temperature, adding NaCl in medium and increasing incubation time (10).

Recent reports of *S. aureus* isolates with intermediate or complete resistance to vancomycin portend a chemotherapeutic era in which effective bactericidal antibiotics against this organism may no longer be readily available (11,12).

Early recognition of patients colonized or infected with MRSA can have a direct impact on the selection of antibiotic therapy and the decision to initiate isolation procedures. Infections caused by MRSA result in lengthier hospital stays and rising health care costs and have a high attributable mortality rate (9).

The aim of this paper was to perceive a part of staphylococcal kind and their sensitivity against antibiotics in hospital in relation to other bacterial kinds in our region for the period from 2003 to September 2005. For the period of 2005, the material from outpatients with established share of staphylococci in relation with other bacteria was processed.

Material and methods

The strains were isolated between January 2003 and September 2005 from the following specimens: swabs of wounds, eye, ear, puncture fluid, aspirate, peritoneal liquid, blood and liquor samples. Investigation was conducted in laboratories of the Sector for Microbiology of the Public Health Institute in Nis. Patients' material was processed using the standard microbiological procedure.

Investigation of sensitivity of the isolated strains was carried out by disk diffusion method (Kirby-Bauer) on Mueller-Hinton agar added with 2% NaCl and with disks of penicillin, oxacillin, erythromycin, clindamycin, genthamicin, ofloxacin, fusidin acid and vancomycin in the period of 2005, while in 2003 and 2004, instead of ofloxacin trimethoprim-sulphometoxazol was used (Bioanalyse, Turkey).

Disk diffusion method

Disk diffusion susceptibilitty test on antimicrobials was made on Mueller-Hinton agar with added 2% NaCl of 4 mm thicknes in Petri cup of 90 mm in diameter. After making suspension of isolates in glycosal broth of 0,5 Mc Farland, the cultivation was performed by the standard techni-que. On such culture medium, the disks (no more than 6) were plased on 25 mm distance. For the investigation of all isolates' sensitivity, the disks of penicilin, oxacillin, erythromycin, clindamycin, genthamicin, ofloxacin, fusidin acid and vanco-mycin were used (BioAnalise, Turska). After 30 minutes from plasing the disks, the plates were incubated in period of 24 hours at 35°C.

Blood agar and all other plates were made in laboratories of the Sector for Microbiology of the Public Health Institute in Nis. Control species used in investigation was *S. aureus* ATCC 25923.

Reading results

For reading results when used disk diffusion susceptibillity method, we used millimetar measurer and zone of inhibition was read by recommendation from CLSI (Institut for Standards in Clinical Laboratories) and marked as R, I or S. Border values of inhibition zone are given in Table 1.

Table 1. Border value for inhibition zone in S. aureus-a ATCC 25923 used during the investigation by disk diffusion susceptibility method

Antibiotics	Border values in mm		
	R	I	S
Penicillin 10 ij.	≤28	-	≥29
Oxacillin 1 µg	≤10	11-12	≥13
Erythromycin 15 µg	≤13	14-22	≥23
Clindamycin 2 µg	≤14	15-20	≥21
Genthamicin 10 µg	≤12	13-14	≥15
Ofloxacin 5 µg	≤14	15-17	≥18
Fusidin acid 100 µg	≤23	24-27	≥28
Vancomycin 30 µg	-	-	≥15
Doxiciclin 30 µg	≤12	13-15	≥16
Amycacin 30 µg	≤14	15-16	≥17
Chloramphenicol 30 µg	≤12	13-17	≥18
Rifampin 5 µg	≤16	17-19	≥20
Cefoxitin 30 µg	≤19	-	≥20

R-resistant; I- intermedier susceptible; S-susceptible

Multiple resistance presents an occurrence of resistance to at least three groups of antibiotics.

Results

Results of susceptibillity tests investigation against vancomycin are not presented in tabels, because all isolates showed susceptability.

Table 2. Part of *Staphylococcal species* in relation to entire number of isolated bacteria

Isolates	Outpatient	Hospital
Staphylococcus aureus	26,06%	62,05%
CoNS	12,07%	78,09%

Table 3. Part of MRCoNS and MRSA and their resistence against other antibiotics in outpatients for 2005

Tested antibiotics	MRCoNS N=88 or 53,65% Resistant		MRSA N=125 or 35,31% Resistant	
	Nu.	%	Nu.	%
Erythromycin	53	60,22	69	55,2
Clindamycin	39	44,31	53	42,4
Ofloxacin	64	72,72	41	32,2
Fusidin acid	67	76,13	22	17,6

Table 4. Part of MRCoNS and MRSA isolates at Nephrology and Surgery Clinics for every single year

Origin of isolates	MRCoNS (%)			MRSA(%)		
	2003	2004	2005	2003	2004	2005
Nephrology	64,52	82,05	77,77	52,46	52,77	47,82
Surgery	66,67	81,25	64,7	63,27	67,79	64,15

Table 5. Rates of MRCoNS and MRSA isolates originating from patients from the Nephrology Clinic, resistant against erythromycin, clindamycin, ofloxacin, and fusidin acid

Total number of isolates N=126	MRCoNS (%)			MRSA(%)		
	2003	2004	2005	2003	2004	2005
Erythro-mycin	71,66	65,62	73,47	55	68,42	90,91
Clinda-mycin	63,33	60,93	61,23	55	59,99	81,82
Ofloxacin	-	78,12	55,11	-	59,99	81,82
Fusidin acid	-	14,06	6,12	-	10,52	9,09

Table 6. Rates of MRCoNS and MRSA isolates originating from patients from the Surgery Clinic, resistant against erythromycin, clindamycin, ofloxacin, and fusidin acid

Total number of isolates N=121	MRCoNS (%)			MRSA (%)		
	2003	2004	2005	2003	2004	2005
Erythromycin	82,80	87,18	74,35	63,64	80	85,30
Clindamycin	81,72	78,21	69,23	54,55	82,50	79,42
Ofloxacin	-	-	46,15	54,55	45	79,42
Fusidin acid	-	-	10,12	4,54	7,50	0

Discussion

Part of infections caused by MRSA is constantly increasing, both in hospital patients and outpatients. Infection caused by MRSA cause lengthier hospital stay and increased hospital costs, and also have a high death rate (13).

In central Europe, the prevalence of MRSA and MRKNS in the period from 1990 to 1995 was reported. The rate of MRSA increased from 1,7 to 12,9%, while MRCoNS increased from 15,8 to 55,8%, with a tendency of futher increase of the rate (14).

Investigation of the part of MRSA in Europe has taken place in 25 hospitals around the continent in the period from April 1997 to February 1999. The highest rate of MRSA was in Portugal 54 and Italy 43-58% and the lowest rates were reported in Switzerland and Holland - 2%. In patient material from intensive care units, MRSA isolates were isolated most repeatedly - 38%, and the most rarerly MRSA isolates were isolated in patient material from internal clinic - 22%. MRSA caused nosocomial pneumonia in 34, urinary infections in 28,3, bacteriemias in 23,8 and infections of the skin and soft tissues in 22,4% (9). Similar investigations were conducted in the period from 2000 to 2002 in 26 countries around Europe, including the data from 495 hospitals. Parts of MRSA are different, prevailing in eastern parts of the continent. In Greece, the part of MRSA was 44,4, on Malta 43,8 in Great Britain 41,5, Ireland 41,2, Italy 40,09, Israel 38,4, Croatia 36,7, Portugal 34,7, Bulgaria 33,9, France 33,1, Spain 24,8, Belgium 23,6, Luxemburg 19,2, Slovenia 18,4, Poland 17,7, Germany 13,8, Slovakia 10,5, Austria 8,8, Hungary 7,1, Czech Republic 5,9, Finland 1,0%, Estonia 0,9, Sweden 0,8, Denmark 0,6, Holland 0,6 and Island 0,5% (15). Average of MRSA part in Europe for that period was 20,2% (15).

Part of MRSA in Italy from June 1, 2001 to July 1, 2002 was 41,2% (16).

In France, MRSA was 42 in 1992, and 37% in 1997. In 1995, MRKNS was 54 and MRSA 45,5% (17).

Unusual increase of the MRSA part which caused a nosocomial infections was in Taiwan. The part of MRSA increased from 26,7 in 1990 to 75% in 1998 (18).

In our region, a part of MRSA isolates isolated in patient material from the Clinics of Nephrology and Surgery was 60,95 and 72,05% for MRCoNS, which is similar to the rates of MRSA and MRCoNS in Portugal, Italy and Spain. In outpatients, the parts of MRSA were 35,31 and 53,65% for MRCoNS. A part of MRSA isolates isolated from hospital patient material from Surgery Clinic was significantly higher compared to the part of MRSA isolates isolated from hospital patient material from Nephrology Clinic, and their distinction increases for more than 2% per year. Part of MRCoNS isolates was very high in both clinics, and in 2004 it was 82,05%. Part of *S. aureus* isolates isolated in the material from surgical patients increased from 22,75 in 2003 to 43,38% in 2004. Part of CoNS isolates isolated from surgical patients, also increased from 18,11 to 35,29%. Isolates of MRCoNS isolated in the material from outpatients showed a high rate of resistance against tested antibiotics, while this rate is lower in MRSA isolates.

Rate of MRSA isolates resistance against erythromycin, clindamycin, ofloxacin, fusidin acid isolated in the material from nephrology patients increased during the investigated period. Rate of resistance of MRCoNS isolates is similar as in 2003. Rate of resistance of MRSA isolates isolated in the material of surgical patients against erythromycin, clindamycin, ofloxacin, fusidin acid was 85,3% in 2005, which was a moderate increase in relation to 2003. Rates of resistance MSCoNS and MSSA isolates isolated in patient material from both clinics against erythromycin, clindamycin, ofloxacin, fusidin acid were considerably lower.

Conclusion

The obtained results suggest that, in our region, there is a high rate of MRSA, just like isolates from coagulase negative staphylococci. In addition, it can be concluded that the parts of methicillin-resistant *Staphylococcus aureus* and coagulase negative staphylococci are constantly increasing. Therefore, we should focus our efforts on applying the measures which will decrease such high part.

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ZASTUPLJENOST METICILIN REZISTENTNIH STAFILOKOKA IZOLOVANIH IZ BOLESNIČKOG MATERIJALA

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Ispitivanje je obuhvatilo raznovrsni bolesnički materijal: briseve rana, oka, uha, punktata, aspirata, peritonealnih tečnosti, krvi i likvora.

Zastupljenost *Staphylococcus aureus*-a kod nefroloških bolesnika iznosi 21,71%, a koagulaza negativnih stafilokoka (KNS) 53,14% u 2003. godini, od čega je meticilin rezistentni *Staphylococcus aureus* (MRSA) u 63,27%, a meticilin rezistentni koagulaza negativni stafilokok (MRKNS) iznosio 64,52% slučajeva. Kod hirurških bolesnika, zastupljenost *Staphylococcus aureus* za isti period iznosila je 22,75%, a KNS 18,11%, dok je MRSA 63,27% a MRKNS 66,67%. Za 2004. godinu zastupljenost *Staphylococcus aureus*-a iznosi 11,11%, a KNS 24,07% kod nefroloških bolesnika od čega je MRSA 53,77%, a MRKNS 82,05%. Kod hirurških bolesnika zastupljenost je 43,38% za *Staphylococcus aureus*, a 35,29% za KNS, od čega je MRSA 67,79%, a MRKNS 81,25%. Kod ambulantnih bolesnika zastupljenost *Staphylococcus aureus*-a iznosi 26,06%, a KNS 12,07%, od čega je MRSA 35,31%, a MRKNS 53,65%. Najveći stepen rezistencije na ostale testirane antibiotike imali su sojevi sa Klinike za nefrologiju. Kod njih je MRSA rezistentan na klindamicin 81,82%, na eritromicin 90,91%, a na ofloksacin 88,82% u 2005. godini. *Acta Medica Medianae 2008;47(2):10-14.*

***Ključne reči**: MRSA, MRKNS, rezistencija, bolnički izolati, ambulantni izolati*