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FREQUENCY OF ISOLATION AND INVOLVEMENT OF *S. AUREUS* AND COAGULASE-NEGATIVE STAPHYLOCOCCI (CNS) IN INFECTIONS IN HOSPITAL PATIENTS

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Summary

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Four thousand two hundred and seventy specimens from materials obtained from patients of the Clinic of Intensive and Critical Care (CICC), the Clinic of Vascular Surgery (CVS) and the Clinic of Thoracal Surgery (CTS) at the University Hospital – Stara Zagora for the period 1998 − first half of 2001 have been studied. The analysis of data showed that the incidence of isolates was as followed: *S. aureus* – 14.43%, CNS – 21.60%, *Enterobacteriaceae* – 24.51%, Gram-negative non–fermenting bacteria – 28.97%, *Enterococcus spp.* – 7.58% etc. The sensitivity of isolated staphylococci, evaluated as clinically important, to β-lactams was determined. The methicillin/oxacillin resistance of all isolates was tested. The involvement of MRSA and MRCNS for the period of the study was followed up. Among the methicillin-resistant strains, there were no isolates with reduced (intermediate) sensitivity to glycopeptides.

Key words: β-lactams, CNS, hospital infections, methicillin/oxacillin, MRSA, MRCNS, *S. aureus*

INTRODUCTION

Compared to the 70−80-ies of past century, when the primary causative agents of multiple infections were Gram−negative bacteria, the last decade was also characterized by an increase in the frequency rate and the incidence of *S. aureus* (Adcock *et al.*, 1998; De Sousa *et al.*, 1998; Kerodle *et al.*, 1998). At the same time, coagulase−negative staphylococci (CNS) also play a pronounced role (Galbard *et al.*, 1999; Cetinkaya *et al.*, 2001). The role of *S. aureus* among the primary agents of infections is beyond any doubt, taking into account the wide spread of staphylococcal carriership among people, including the hospital personnel (Hackbarth *et al.*, 1995; Pavlova and Toshkova, 2002).

The coagulase−negative staphylococci (CNS), comprising more than 30 species and a part of the normal human microflora, were considered as saprophytes and clinical material contaminants for a long time. In the antibiotic era and the age of medical high technologies, however, they became especially importnant in human pathology. Their ability to adhere to various medical equipment units, the appearance of new genetic and phenotypic features, particularly those related to resis-

tance to antibiotics are improtant traits, helping their epidemic distribution and their involvement as nosocomial agents. The most commonly isolated species are *S. epidermidis* and *S. haemolyticus*, although by now, about 16 CNS species are considered as relatively pathogenic (Lang *et al.*, 1999; Mack, 1999; Mlinarczyk *et al.*, 2000; Cetinkaya *et al.*, 2001). As causative agents of nosocomial infections, CNS are primarily encountered in risk patients such as immunosuppressed patients, transplantation patients, in intravascular catheters, prostheses, infections after cardiosurgery etc. (Wang *et al.*, 1993; Rupp and Archer, 1994; Lang *et al.*, 1999; Crump and Colligon, 2000). Commonly encountered CNS infections are sepcicaemiae, endocardites, intravascular catheter-related infections, urological infections, surgical wound infections, joint prostheses, peritoneal dialysis catheters, various infections following transplantations, infections in neonates, in intensive care units, in neutropeniae, cardiosurgical infections etc. (Raad and Bodey, 1992; Yip and Rotstein, 1998; Crump and Colligon, 2000).

The principal problem today are methicillin–resistant staphylococci – MRSA (methicillin–resistant *S. aureus*) and methicillin–resistant coagulase-negative staphylococci (MRCNS) that possess an entirely new mechanisms of resistance – production of an extra penicillin-binding protein (PBP-2a), with a low affinity to methicillin and all other β−lactam antibiotics, as well as the presence of "normal" proteins (PBP 1, 2, 3), that are also with a low affinity to β−lactams. Apart their resistance to methicillin and β−lactams, those isolates are most commonly resistant to aminoglycosides, macrolides etc. (Hackbarth *et al.*, 1995; De Sousa *et al.*, 1998; Kerodle *et al.*, 1998, Tsakaris *et al.*, 2002). They are responsible for severe, predominantly hospital infections, have an epidemic distribution and generally present a multiresistance to almost all used antibacterial drugs (Adcock *et al.*, 1998, De Sousa *et al.*, 1998, Lang *et al.*, 1999, Tammelin *et al.*, 2000).

As a result of the increased resistance of staphylococcal strains to antibacterial drugs, the glycopeptide antibiotics vancomycin and teicoplanin are the alternative for treatment of severe infections. That is why, the reports for isolation of *S. aureus* strains with reduced sensitivity to vancomycin are particularly worrying. Since 1996 there are data for isolation of staphylococcal strains with reduced (intermediate) sensitivity towards glycopeptides – glycopeptide–intermediate *S. aureus* (GISA) and vancomycin–intermediate *S. aureus* (VISA) (Biavasko *et al.*, 2000; Mlinarczyk *et al.*, 2000; Viedma *et al.*, 2000; Tsakaris *et al.*, 2002).

The aim of the present study was to analyse the data from current investigations of various clinical materials, to determine the frequency rate and the incidence of *S. aureus* and CNS and to evaluate their resistance to antibacterial drugs. Those data are useful both for therapeutical purposes and for the epidemiological control. Their follow-up aimed to monitor the appearance of strains with reduced (intermediate) sensitivity to glycopeptides.

MATERIALS AND METHODS

A total of 4 270 materials obtained from patients of the Clinic of Intensive and Critical Care (CICC), the Clinic of Vascular Surgery (CVS) and the Clinic of Thoracal Surgery (CTS) at the University Hospital – Stara Zagora for the period 1998 − first half of 2001 have been studied.

The analysed strains were isolated only from hospitalized patients.

The number of the different types of specimens and their distrubution according to the clinics of origin are presented in Table 1. Blood cultures were the most numerous (39.86 %), followed by wound discharges (14.89%). The respiratory discharges were 21.33%, surgical infection materials − 14.89 %, whereas the bronchial discharges, assayed primarily in intubated patients − 6.42%. This distribution was corresponding to the most commonly encountered clinical manifestations of infections: suspected bacteriaemiae, surgical and wound infections, as stated also by others (Raad and Bodey,1992; Kerodle *et al.*, 1998; Crump and Colligon, 2000).

Routine microscopic and culture methods were used, selecting the optimal culture medium for each type of material. The major part of blood cultures were investigated by means of a Bactec 9050 automated system (Becton Dickinson).

Isolated staphylococcal strains were identified using routine methods and by a Sceptor automated system (Becton Dickinson). The antibacterial resistance was determined by the disc diffusion method of Kyrbi-Bauer and according to the criteria of NCCLS−2000. The resistance to methicillin was determined by a screening test with oxacillin agar (6 μg/ml), confirmed by test with oxacillin discs (1 μg) − BBL (NCCLS 2001) and a Crystal system for MRSA (Becton Dickinson). The clinical significance of CNS was established by routine criteria: reproducibility of isolates, an identical phenotype and resitotype, type of studied material, presence of risk factors in patients etc. (Galbard *et al.*, 1999; Terpstra *et al.*, 1999; Crump and Colligon, 2000; Cetinkaya *et al.*, 2001).

RESULTS AND DISCUSSION

The incidence of isolates was as followed: *S. aureus* – 14.43 %, CNS – 21.60 %, *Enterobacteriaceae* – 24.51 %, Gram-ne-

Table 1. Distribution of the overall number of analysed samples by type of the pathological material and clinic of origin

Type of the material	CTS	CVS	CICC	Percentage
Blood cultures	219	861	622	39.86 %
Wound discharges	86	418	132	14.89 %
Urine	119	223	150	11.52 %
Bronchial discharges	18	0	256	6.42 %
Punctates	46	10	99	3.63 %
Venous catethers	5	20	75	2.34 %
Expectorates and materials from the upper respiratory tract	533	217	161	21.33 %
Total	1026	1749	1495	100.00 %

CICC = Clinic of Intensive and Critical Care; CVS = Clinic of Vascular Surgery; CTS = Clinic of Thoracal Surgery.

Table 2. Incidence of *S. aureus* strains and coagulase-negative staphylococci (CNS) in clinical materials and clinics of origin

Type of the material	*S. aureus* (n=105)					CNS (n=187)				
	CTS	CVS	CICC	Total		CTS	CVS	CICC	Total	
				n	%				n	%
Blood cultures	1	8	10	19	18.10	2	18	43	63	33.69
Wound discharges	4	54	11	69	65.70	6	76	10	92	49.20
Bronchial discharges	6	3	8	17	16.20	14	8	10	32	17.11
Total	11	65	29	105	100.00	22	102	63	187	100.00

CICC = Clinic of Intensive and Critical Care; CVS = Clinic of Vascular Surgery; CTS = Clinic of Thoracal Surgery.

Table 3. Percentage of the total number of *S. aureus* isolates (n=131) in the clinics of origin

Clinic	MSSA		MRSA		Total	
	n	%	n	%	n	%
CVS	55	41.98	11	8.40	66	50.38
CTS	10	7.63	4	3.05	14	10.68
CICC	35	26.72	16	12.21	51	38.93
Total	100	76.34	31	23.66	131	100.00

CICC = Clinic of Intensive and Critical Care; CVS = Clinic of Vascular Surgery; CTS = Clinic of Thoracal Surgery; MSSA = methicillin-sensitive *S. aureus*; MRSA = methicillin-resistant *S. aureus.*

Table 4. Percentage of the total number of coagulase-negative staphylococci (CNS) isolates (n=188) in the clinics of origin

Clinic	MSCNS		MRCNS		Total	
	n	%	n	%	n	%
CVS	45	23.94	58	30.85	103	54.79
CTS	16	8.51	6	3.19	22	11.70
CICC	33	17.55	30	15.96	63	33.51
Total	94	50.00	94	50.00	188	100.00

CICC = Clinic of Intensive and Critical Care; CVS = Clinic of Vascular Surgery; CTS = Clinic of Thoracal Surgery; MSCNS = methicillin-sensitive coagulase-negative staphylococci; MRCNS = methicillin-resistant coagulase-negative staphylococci.

gative non–fermenting bacteria – 28.97%, *Enterococcus spp.* – 7.58% etc. The total number of staphylococci was 319−188 CNS (58.93 %) and and 131 *S. aureus* (41,07 %).

The number and percentages of isolated staphylococcal strains in the various materials and clinics are presented in Table 2. *S. aureus* was isolated in 18.1 % of blood cultures, whereas clinically significant CNS were 33.69 %. Similar data are reported by almost all authors, although the percentages vary along with the profile of the hospital, presence of risk factors, promoting CNS infections, the methods of investigations and evaluation etc. The important role of skin colonization with CNS, most commonly methicillin–resistant or multiresistant, as well as their frequent involvement as intrahospital infection agents, are well recognized (Raad and Bodey, 1992; Tammelin *et al.*, 2000). In our study, the highest percentage was that of staphylococcal isolates from wound

and surgical infections – 65.7 % for *S. aureus* and 49.2 % for CNS. In general, those isolates originated from the Clinic of Vascular Surgery. In other specimens, their incidence was lower.

The incidence of methicillin–sensitive (MSSA) and methicillin–resistant (MRSA) *S. aureus* in the different clinics is shown in Table 3. The MSSA isolates were 131 (76.34 %) and the MRSA – 23.66 %. Our findings correpond to data reported by numerous authors and the MRSA prevalence varies within a wide range in the various countries and hospitals (De Sousa *et al.*, 1998, Kerodle *et al.*, 1998, Mir *et al.*, 1998).

In our clinics, the highest MRSA percentage was observed in the Clinic of Intensive and Critical Care, followed by the Clinic of Vascular Surgery.

The findings about CNS were somewhat different. Both methicillin-sensitive and methicillin-resistant CNS isolates were 50% (Table 4). The percentage is rather high, but similar data are reported by Rupp and Archer (1994), Tammelin *et al.*, (2000). It must be stated that despite the use of criteria for the analysis only of clinically significant strains, the materials were most probably contaminated by CNS, originating mainly from the skin. It is known that the percentage of methicillin-resistant CNS, circulating in intensive care and surgical clinics is considerable, due to the strong selective pressure due to the extensive use of antibiotics (Terpstra *et al.*, 1999).

The highest incidence of MRCNS was again in the Clinic of Vascular Surgery – 30.85 % whereas in the Clinic of Intensive and Critical Care it was 15.96 %.

The study of the incidence of methicillin–sensitive and methicillin–resistant staphylococci is an important global problem with regard to the determination of their epidemiological impact as most important agents of severe infections, including hospital infections and for an adequate therapy. The informing of clinicians about their role is particularly important for the success of antiepidemical procedures and the limitation of infections.

In conclusion, our data evidenced that staphylococci were leading agents causing infections in surgical and intensive care clinics although the most important involved bacterial species are intestinal and Gram-negative non-fermentative bacteria. The incidence of isolation of *S. aureus* was 14.43 % and for CNS – 21.60 %. They were most commonly isolated from the Clinic of Vascular Surgery and the Clinic of Intensive and Critical Care. The percentage of methicillin-resistant *S. aureus* strains was 23.66%, being the highest in the Clinic of Intensive and Critical Care (26.72 %). Methicillin-resistant CNS (50 %) were the most frequently isolated in the Clinic of Vascular Surgery – 30.85 %.

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