

Medical Microbiology Department, Department of Vascular Surgery and Angiology  
Medical University of Lublin

ELŻBIETA MAZUR, JUSTYNA NIEDŹWIADEK, ANNA NIWIŃSKA,  
PIOTR TERLECKI, JACEK WRÓŃSKI, MARIA KOZIOŁ-MONTEWKA

*Colonization by resistant Staphylococcus epidermidis strains  
in patients qualified for routine vascular reconstructions*

Coagulase-negative staphylococci (CNS) create a great problem in surgical procedures connected with biomaterials implantation. Forming biofilm on plastic surfaces CNS become the cause of polymer associated staphylococcal infections (PASI) (10). During implantation polymers are easily colonized by the patient's own flora or by bacteria present on the personnel's hands (3). *Staph. epidermidis* is the most important species responsible for these infections (10). Multicenter studies proved that about 40% of implanted biomaterials were primarily contaminated by bacteria colonizing patients' skin, in most cases by *Staph. epidermidis* (4). It is estimated that about 50–70% of infections associated with implantation or catheterization is caused by this species (3, 10). It is particularly important in vascular surgery where reconstruction procedures are so frequently performed. What is more, vascular graft infections are associated with mortality rate estimated to be as high as 75% (13). Anterior nares are the main reservoir of staphylococci present on the skin and mucose surfaces thus their microbiological examination is important in assessment of strains colonizing a given individual (1, 2, 10, 16).

The aim of the study was: 1) to examine *Staph. epidermidis* colonization of the anterior nares in patients qualified for routine vascular reconstructions on the day of admission to the hospital and after a week of staying there, 2) to evaluate antimicrobial susceptibility of isolated strains, including detection of resistance mechanisms.

#### MATERIAL AND METHODS

The study included 35 patients with peripheral arterial occlusive disease (PAOD) qualified for routine vascular reconstruction in the Department of Vascular Surgery and Angiology from February through June 2005 (32 men and 3 women; mean age of 60 years). All subjects showed chronic leg ischemia IIb according to Fontaine classification. There were no necrotic changes on the skin or any symptoms of bacterial infection in the examined group. As an antibiotic prophylaxis cephalosporins (cephazoline 2x1.0) were used till the wound drainage was removed. Staphylococcal carriage or infection risk factors investigated included: diabetes mellitus, neoplasm tumors, skin diseases, respiratory tract infections, hospital stay or any medical interventions in last two years.

Nasal swabs (collected separately from both anterior nostrils) were obtained on the day of admission and on 8<sup>th</sup> day of hospital stay. They were inoculated on blood agar and mannitol salt agar and incubated in aerobic conditions for 24–48 hours. Identification of isolated strains

included: fermentation of mannitol, coagulase production, other biochemical properties (API Staph BioMerieux). Susceptibility to antibiotics was performed by disc-diffusion method; resistance to methicillin (MR) was detected by the use of cefoxitin 30 µg disc. MLSB phenotype was assessed by the use of the following discs: lincomycin 15 µg, erythromycin 15 µg and clindamycin 2 µg according to the recommendations of the National Reference Committee for Antimicrobial Susceptibility (7).

## RESULTS

*Staph. epidermidis* was present in all but one admission culture, in 8-day cultures this species was isolated from 33 patients. In every performed culture 1 to 3 different strains of *Staph. epidermidis* were isolated. In 6 patients no changes in *Staph. epidermidis* strains were noticed comparing the first and second culture, in other 7 total change of isolated strains occurred during hospital stay. In the next 8 patients only several new strains appeared. In 1 subject eradication of 2 strains was observed but no new strains appeared. Twenty-two patients were hospitalized in the last two years, 7 of them were operated, 7 subjects had diabetes mellitus.

We did not notice any influence of previous surgery or the presence of diabetes mellitus on the occurrence of resistant (possessing at least one examined resistance mechanism) strains of *Staph. epidermidis* in the admission culture. In previously hospitalized patients 10 resistant strains and in non-hospitalized ones 7 resistant strains were isolated, but taking in account the most important resistance mechanisms, namely MR (methicillin resistance) and MDR (multidrug resistance), there were no differences between these two groups. In both of them 5 MDR and 5 MR strains occurred. As a total, 17 resistant strains were brought into the hospital, among them 10 MR, 10 MDR and 6 MLSB (some resistance mechanisms were present in the same strain simultaneously). During hospital stay the examined patients were colonized with 12 resistant strains of *Staph. epidermidis*, among them 11 MR, 8 MDR, 4 MLSB.

As a total 73 strains of *Staph. epidermidis* were isolated, 26 strains were present in both cultures, 23 strains were acquired in the hospital, 24 strains were lost during stay at the hospital.

Table 1. *Staph. epidermidis* strains isolated from the examined group

<i>Staph. epidermidis</i> strains which survived hospital stay	<i>Staph. epidermidis</i> strains eradicated during hospital stay	<i>Staph. epidermidis</i> strains acquired during hospital stay
26 strains (4 MRSE, 2 MLSB, 4 MDR)	24 strains (4 MRSE, 2 MLSB, 4 MDR)	23 strains (11 MRSE, 4 MLSB, 8 MDR)

21 out of 73 strains (28.8%) appeared to be resistant to methicillin (MRSE). Figures 1 and 2 show the susceptibility of isolated MSSE and MRSE to antibiotics.

Among isolated MSSE strains full susceptibility to gentamicin and amoxicillin/clavulanic acid was observed. Also ciprofloxacin and co-trimoxazole proved to be highly active against this group of *Staph. epidermidis* strains. Relatively high percentage of MSSE strains turned out to be resistant to penicillin, tetracyclin, lincomycin, clindamycin, and erythromycin.

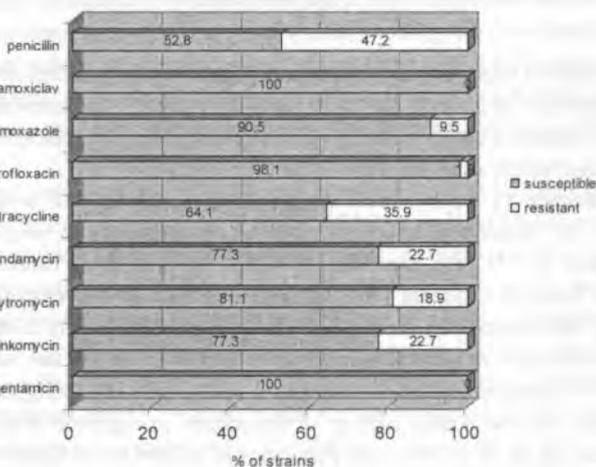


Fig. 1. Susceptibility to antibiotics of isolated MSSE strains (%)

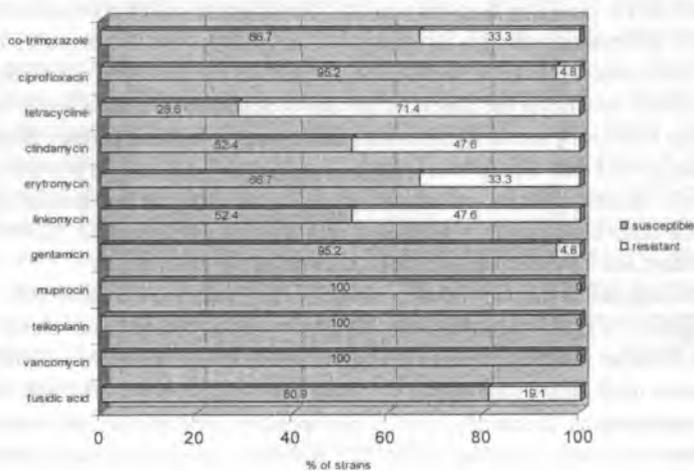


Fig. 2. Susceptibility to antibiotics of isolated MRSE strains (%)

Similarly to MSSE group, a high number of isolated MRSE strains proved to be resistant to tetracyclin, lincomycin, clindamycin, and erythromycin. All isolated strains in this group were fully susceptible to vancomycin, teicoplanin, and mupirocin. Gentamicin, ciprofloxacin, and fusidic acid appeared to be highly active against MRSE. 10 out of 73 (13.7%) isolated *Staph. epidermidis* strains possessed MLSB resistance mechanism (3 of inducible type and 7 of constitutive one). six of them were brought into the hospital, 4 were acquired during hospital stay. In 3 of them MLSB was present as a single resistance mechanism, 4 were simultaneously MRSE, and 7 proved to be MR strains. 18 strains (24.7%) were multiresistant (i.e. resistant to more than 3 different groups of antibiotics), 13 of them were simultaneously methicillin-resistant.

## DISCUSSION

Coagulase negative staphylococci constitute an important component of normal flora of the skin and mucous membranes. In the past, they were rarely the cause of significant infections, but with the increasing use of implanted catheters and prosthetic devices, they have emerged as important agents of hospital-acquired infections (1, 10, 17).

Nasal colonization by staphylococci among patients and hospital personnel constitutes an important source of nosocomial infections, particularly dangerous in surgical departments and intensive care units (6, 13). It is considered to be one of the leading factors associated with the complications of hospital treatment (13). Its presence frequently deteriorates the course of the disease, prolongs the hospital stay and increases the cost of treatment (5). It should be remembered that patient-to-patient transmission of staphylococci should occur via the hands, instruments and perhaps the air (15). Hospital epidemics caused by *S. aureus* are a continuing and recurrent problem, associated in many cases with nasal carriage of this species among patients and hospital personnel, estimated to be as high as 10 to 70% in the population of healthy individuals (1, 17).

The perils of staphylococci are mostly due to their increasing resistance to many different antimicrobials. In particular, resistance to methicillin (MR) and multi-drug resistance (MDR) create an enormous problem in clinical practice, limiting the choice of antibiotics, which can be used in the treatment of infections, caused by these bacteria (12). New mechanisms of resistance are still detected, among them MLSB-cross resistance to all macrolides, lincosamides and streptogramin B (7).

Hospital environment influences bacterial colonization of patients. After a few days of staying in the hospital bacteria colonizing the patient on the day of admission are replaced by hospital strains, which have been selected by the use of antibiotics and frequently resistant to many antimicrobials (5). In the examined group a total change in *Staph. epidermidis* nasal colonization was noted in 7 patients, in 8 of them new strains appeared and coexisted with the old ones, in one subject only old strains were lost, in the other 6 no change in isolated strains was observed. Besides, 52.4% of all isolated methicillin-resistant and 44.4% of MDR strains were acquired in the hospital.

Methicillin-resistant (MRSA – methicillin-resistant *Staph. aureus*, MRCNS – methicillin-resistant coagulase negative staphylococci, MRSE – methicillin-resistant *Staph. epidermidis*) staphylococci create an alarming problem in hospital infections (6). Resistance to methicillin means clinical resistance to all beta-lactam antibiotics, namely penicillins, penicillins with beta-lactamases inhibitors, cephalosporins (frequently used in perioperative prophylaxis) and carbapenems, thus eliminating these antibiotics from clinical use (12). In Poland, similarly to other European countries, MRSA and MRCNS are isolated in every hospital, because hospital environment enhances selection and survival of resistant strains (12). The rates of methicillin resistance vary among strains isolated in different hospitals or even in different departments of the same hospital. In our research 28.8% of isolated *Staph. epidermidis* proved to be methicillin-resistant. 47.6% of all isolated MRSE strains were brought to the hospital and 52.4% of them were acquired in the hospital.

Methicillin-resistant strains of staphylococci create even more severe problem, because they are frequently simultaneously resistant to many different antibiotics and disinfectants (17). Recently, VISA (vancomycin intermediate *Staph. aureus*) and VRSA (vancomycin-resistant *Staph. aureus*) strains were isolated in Japan and in the USA. VISA strains were also detected in Poland (9). Among MRSE isolated in our study, a high number of strains proved to be resistant to tetracyclin, lincomycin, clindamycin, and erythromycin. All isolated strains in this group were fully susceptible to vancomycin, teicoplanin, and mupirocin. Gentamycin, ciprofloxacin, and fusidic acid appeared to be highly active against MRSE colonizing our patients.

MLSB, another recently detected resistance mechanism, means cross resistance to all macrolides, lincosamides and streptogramin B (7). It occurs frequently among strains isolated from outpatients. In our study 13.7% of isolated *Staph. epidermidis* strains possessed MLSB resistance mechanism. Six of them were brought into the hospital, 4 strains were acquired during hospital stay. In 3 of them MLSB was present as a single resistance mechanism, 4 were simultaneously MRSE, and 7 proved to be MR strains.

MDR strains are also frequently found among currently isolated staphylococci. In our study 18 out of 73 isolated *Staph. epidermidis* were MDR. 55.5% of isolated MDR strains were brought by patients, 44.5% of them were acquired in the hospital. Antibiotics select resistant strains of bacteria not only in hospitals but also among outpatients. Thus, resistance and even multi-resistance are now the features of both hospital and non-hospital strains (9, 12). In our research no difference was noted between the number of MR and MDR strains isolated in the first culture from previously hospitalized patients and the ones who were hospitalized for the first time. Furthermore, 47.6% of isolated methicillin-resistant and 55.5% of MDR strains were brought into the hospital.

Bacterial colonization is associated with: 1) infection of the surgical site – in about 90% of cases caused by the patient's own strain, 2) expansion of bacteria in the hospital environment (16). Hence, an important question is: how to decrease the risk of surgical wound infection and to limit the expansion of resistant *Staph. epidermidis* in vascular surgery departments? There is the evidence that eradication of *Staph. aureus* nasal colonization in patients prepared for cardiovascular surgery decreases the risk of surgical site infection (8, 11). Several sets of investigators have shown that preoperative mupirocin decreased *Staph. aureus* surgical site infections (14). Similar procedure, attempting to eradicate resistant *Staph. epidermidis* strains by analogy with recommendations aimed at preventing the dissemination of MRSA, should be considered before routine vascular surgery (15). Taking in account that all MRSE strains isolated from patients hospitalized in the Department of Vascular Surgery and Angiology were fully susceptible to mupirocin, this antimicrobial could be used in the attempt to eradicate MRSE strains before the routine vascular reconstruction and, all the same, to limit the expansion of resistant strains in this ward. Besides, the knowledge about bacteria colonizing a given patient on the day of admission to the hospital can supply an important hint for targeted, individualized, more efficient antibiotic prophylaxis. Microbiological examination on the last day of hospital stay may bring important information about changes in patient's colonization, help to follow-up the patient and prevent possible infective complications.

## CONCLUSIONS

Taking into consideration a high rate of resistant strains among *Staph. epidermidis* isolated from patients prepared for routine vascular reconstructions it seems useful to evaluate nasal colonization in every subject before and after such intervention:

- 1) to have the opportunity to verify perioperative antibiotic prophylaxis in selected cases and to prevent possible complications,
- 2) to limit the expansion of resistant strains of *Staph. epidermidis* in the hospital.

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#### SUMMARY

The aim of our study was to examine *Staph. epidermidis* colonization in patients qualified for routine vascular reconstructions and to evaluate antimicrobial susceptibility of isolated strains. The study included 35 patients with PAOD. Nasal swabs were obtained on the day of admission and on 8<sup>th</sup> day of hospital stay. Classification to the species was done on the ground of the biochemical properties of isolated strains. Susceptibility to antibiotics was assessed by disc-diffusion method. In 6 patients no change in *Staph. epidermidis* nasal colonization was noticed comparing first and second culture, in other 7 a complete change of isolated strains occurred during hospital stay. As a total, 17 resistant strains were brought into the hospital. During hospital stay the examined patients were colonized with 12 resistant strains of *Staph. epidermidis*.

Kolonizacja opornymi szczepami *Staph. epidermidis* u chorych  
poddawanych planowym operacjom naczyniowym

Celem pracy była ocena kolonizacji przedsionka nosa szczepami *Staph. epidermidis* u chorych kwalifikowanych do planowych operacji rekonstrukcyjnych oraz określenie lekowrażliwości izolowanych szczepów tego gatunku. W badaniu brało udział 35 chorych z miażdżycą tętnic kończyn dolnych. Wymazy z przedsionka nosa pobierano od każdego chorego dwukrotnie: w dniu przyjęcia do szpitala oraz w ósmej dobie pobytu na oddziale. Izolowane szczepy kwalifikowano do gatunku na podstawie ich cech biochemicznych. Wrażliwość na antybiotyki badano metodą dyfuzyjno-krażkową. Analizując szczepy izolowane w pierwszym i drugim posiewie, stwierdzono, że u sześciu chorych szczepy *Staph. epidermidis* kolonizujące nozdrza przednie nie uległy zmianie podczas pobytu w szpitalu, u siedmiu zaś nastąpiła całkowita wymiana szczepów. Ogółem „wniesiono” na oddział 17 opornych szczepów *Staph. epidermidis*. Podczas pobytu w szpitalu badani chorzy zostali skolonizowani łącznie przez 12 opornych szczepów tego gatunku.