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# Characterization of Coagulase-Negative Staphylococci Isolated from Cases of Ostitis and Osteomyelitis

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A b s t r a c t

Coagulase-negative staphylococci (CoNS) are often responsible for cases of chronic ostitis and osteomyelitis, especially in patients with orthopedic prosthesis/implants. The aim of this study was to characterize CoNS isolated from ambulatory patients with chronic ostitis/osteomyelitis and to compare them by PFGE (pulsed-field gel electrophoresis). Out of 263 bacterial strains isolated from wounds/sinuses of patients with chronic ostitis/osteomylitis, 41 were identified as CoNS. Twenty methicillin-resistant strains were selected for this study. Our results confirm the superior performance of cefoxitin disk test to detect methicillin resistance in heterogenous population of CoNS. High level of antibiotic resistance was observed among the studied strains: majority of CoNS were resistant to tetracycline and erythromycin and also to clindamycin and ciprofloxacin. Importantly, in 15 out of 20 studied CoNS different phenotypes of macrolides, lincosamides and streptogramin – MLS resistance was suggested. Eight strains demonstrated resistance to both erythromycin and clindamycin, suggesting constitutive $MLS_B$ phenotype. Seven remaining strains presented resistance to erythromycin and susceptibility to clindamycin with negative D-test results, suggesting the presence of macrolides and streptogramines type A efflux pump. All studied strains were sensitive to vancomycin (MIC 0.75–2.0 μg/ml), teicoplanin (MIC 0.125–8.0 μg/ml), and quinupristin/dalfopristin (MIC 0.19–1.0 μg/ml). No clonal relatedness was observed in PFGE patterns.

K e y w o r d s: coagulase-negative staphylococci, ostitis/osteomyelitis, antibiotic resistance

## Introduction

Bacterial infections cause serious complications and constitute an important problem in orthopedic patients, especially in those after orthopedic surgery. The microorganisms most commonly causing deep wound infection in orthopedic patients are staphylococci (Wilk *et al*., 2004). Many publications describe cases of osteomyelitis caused by *Staphylococcus aureus* (Issartel *et al*., 2005). Coagulase-negative staphylococci (CoNS), however, are often responsible for the cases of chronic ostitis and osteomyelitis, especially in patients with orthopedic prosthesis and cause about 90% pin tract infections. Particular problems with correct obtaining samples for culturing exist for wounds that are in fact sinuses overlying a focus of chronic ostitis/osteomyelitis. For this reason, very often microbiological results are interpreted incorrectly.

CoNS are important pathogens especially in cases of sternal osteomyelitis following median sternectomy (Rupp and Archer, 1994). The vertebral bodies are a typical site of haematogenous osteomyelitis. Cases of spondylodiscitis following CoNS bacteremia have been also reported (Bucher *et al*., 2000). Of 32 CoNS species validly published, only half are seen in specimens of human origin. More recently, species of CoNS were isolated, which significantly differed from all other *Staphylococcus* spp. based on phenotypic characteristics and 16 rRNA gene sequencing and a novel *Staphylococcus pettenkoferi* species was proposed (Trulzsch *et al*., 2002; von Eiff *et al*., 2002). Methicillin resistance in both community- and hospital-acquired strains

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of staphylococci has emerged as an important and growing resistance threat (Pottumarthy *et al*., 2005). The aim of this study was to characterize CoNS isolated from specimens taken from ambulatory cared patients with chronic ostitis/osteomyelitis and to compare of the appropriate species representatives by PFGE.

## Experimental

### Materials and Methods

This study included group of 263 patients with chronic bone infections (ostitis/osteomyelitis) from different regions of Upper Silesia, who attended the Department of Medical Microbiology, Medical University of Silesia in Katowice from 2000 to 2005 to identify etiological agents of chronic infection. These patients were not related epidemiologically with each other and were not hospitalized at the same time in the same hospital ward. Patients were considered to this study if isolation of the same type of CoNS was confirmed at least twice and no other microorganism was isolated simultaneously.

Sterile swabs were used to obtain material for culturing of aerobic/anaerobic microorganisms. Swabs obtained from deeper part of wounds/sinuses were cultured by using routine microbiological techniques (Baron *et al*., 1999). Isolated colonies were identified by colony morphology, Gram staining, catalase activity, determination of clumping factor (Slidex Staph-Kit, bioMerieux, Marcy I'Etoile, France) and coagulase activity by using rabbit plasma. Biochemical identification was performed by using ID-32 Staph panels (bioMerieux, Marcy I'Etoile, France). Each isolated strain was tested for methicillin sensitivity with oxacillin (1 μg) and cefoxitin (30 μg) disks, according to CLSI guidelines. All isolates were tested for the *mecA* gene by PCR (Murakami *et al*., 1991).

Antibiotic susceptibility testing was done by disk-diffusion methods for trimethoprim/sulfamethoxazole; chloramphenicol; tetracycline; erythromycin; clindamycin; gentamicin; ciprofloxacin; fusidic acid; moxifloxacin; quinupristin/dalfopristin; and linezolid (Oxoid, Basingstoke, UK), inhibition zone diameters were interpreted according to ranges recommended by CLSI.

MICs for vancomycin, teicoplanin and quinupristin/dalfopristin were determined by E-test (AB Biodisk, Solna, Sweden). Pulsed-field gel electrophoresis (PFGE) was performed using CHEF DRII apparatus (Bio-Rad Laboratories, Hercules, CA, USA), according to Chung and coworkers (Chung *et al*., 2000). PFGE patterns were compared with the use of Molecular Analyst software, version 1.12 (Bio-Rad, CA, USA).

## Results and Discussion

Out of 263 bacterial isolates 41 were identified as strains of CoNS (Table I). Twenty strains were methicillin-resistant (MRCoNS) and were selected for this study. Among these MRCoNS: 6 strains of *Staphylococcus epidermidis*, 7 strains of *Staphylococcus haemolyticus*, 3 strains of *Staphylococcus simulans*, and single strains of *Staphylococcus sciuri*, *Staphylococcus cohnii*, *Staphylococcus hominis* and *Staphylococcus lentus* were identified. Clinical and microbiological data concerning these isolates are presented in Table II.

Comparison of methicillin susceptibility testing by oxacillin and cefoxitin disks demonstrated non-concordance (oxacilline-resistant/cefoxitin susceptible) in 5 cases (strains no 3, 13, 16, 19 and 20). However the presence of *mecA* gene was not demonstrated in these strains. These results confirm the superior performance of cefoxitin disk test to detect methicillin resistance in heterogenous population of CoNS. Similar observations in studied population of coagulase-positive and coagulase-negative staphylococci were published by other authors (Pottumarthy *et al*., 2005; Sharp *et al*., 2005). All studied strains were sensitive to vancomycin (MIC 0.75–2.0 μg/ml), teicoplanin (MIC 0.125–8.0 μg/ml), and quinupristin/dalfopristin

Table I
Sources of coagulase-negative staphylococci

| Strains of CoNS | Ostitis/Osteomyelitis | Endoprosthesis | Sternal osteomyelitis |
|---|---|---|---|
| *S. epidermidis* | 7 | 3 | 2 |
| *S. haemolyticus* | 11 | 2 | – |
| *S. simulans* | 4 | – | – |
| *S hominis* | 2 | 1 | 1 |
| *S. warneri* | 1 | 2 | – |
| *S. sciuri* | – | 1 | – |
| *S. cohnii* | 2 | – | – |
| *S. lentus* | 1 | – | – |
| *S. chromogenes* | 1 | – | – |
| Total | 29 | 9 | 3 |

Table II
Characteristics of coagulase-negative staphylococci

| No | Identification | Health-Care Unit[1] | Ox[2] | Fox[2] | [3]*mecA* | STX | C | T | E | CC | Ge | Cip | Fa | MXF | Syn | LZD | [4]PFGE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *S. haemolyticus* | A | $R^{6}$ | $R^{22}$ | + | S | S | R | S | R | S | S | S | S | S | S | A1 |
| 2 | *S. haemolyticus* | F | $R^{14}$ | $R^{24}$ | + | S | R | R | R | R | S | S | S | S | S | S | B1 |
| 3 | *S. haemolyticus* | U | $R^{17}$ | $S^{25}$ | – | S | R | R | R | S | S | S | S | S | S | S | C1 |
| 4 | *S. haemolyticus* | U | $R^{6}$ | $R^{24}$ | + | R | S | R | R | S | S | S | S | S | S | S | D1 |
| 5 | *S. haemolyticus* | E | $R^{6}$ | $R^{20}$ | + | R | S | S | R | S | R | S | S | S | S | S | E2 |
| 6 | *S. haemolyticus* | E | $R^{6}$ | $R^{14}$ | + | R | S | S | R | S | R | R | S | S | S | S | F1 |
| 7 | *S. haemolyticus* | Z | $R^{6}$ | $R^{17}$ | + | R | R | R | R | S | S | R | S | S | S | S | G1 |
| 8 | *S. epidermidis* | R | $R^{6}$ | $R^{14}$ | + | S | R | R | R | R | R | R | S | S | S | S | H1 |
| 9 | *S. epidermidis* | E | $R^{6}$ | $R^{11}$ | + | R | S | R | R | R | S | R | S | S | S | S | I1 |
| 10 | *S. epidermidis* | T | $R^{6}$ | $R^{22}$ | + | R | S | R | R | R | R | S | S | S | S | S | J1 |
| 11 | *S. epidermidis* | B1 | $R^{6}$ | $R^{15}$ | + | S | S | R | R | S | R | S | S | S | S | S | K1 |
| 12 | *S. epidermidis* | B1 | $R^{6}$ | $R^{12}$ | + | S | S | R | R | R | R | R | S | S | S | S | L1 |
| 13 | *S. epidermidis* | E | $R^{17}$ | $S^{27}$ | – | S | S | R | R | R | S | R | S | S | S | S | M1 |
| 14 | *S. simulans* | F | $R^{12}$ | $R^{24}$ | + | S | R | R | S | S | S | S | S | S | S | S | N1 |
| 15 | *S. simulans* | E | $R^{6}$ | $R^{15}$ | + | R | R | R | R | R | R | R | S | R | S | S | O1 |
| 16 | *S. simulans* | S | $R^{17}$ | $S^{26}$ | – | S | S | S | R | R | S | R | S | R | S | S | P1 |
| 17 | *S. cohnii* | B | $R^{6}$ | $R^{14}$ | + | S | S | R | R | S | S | S | S | S | S | S | R1 |
| 18 | *S. sciuri* | E | $R^{13}$ | $R^{24}$ | + | S | S | S | S | S | S | S | S | S | S | S | S1 |
| 19 | *S. hominis* | B1 | $R^{17}$ | $S^{28}$ | – | S | S | R | S | S | S | S | S | S | S | S | T1 |
| 20 | *S. lentus* | U | $R^{17}$ | $S^{29}$ | – | S | S | S | S | S | S | S | S | S | S | S | U1 |
| Total | | 10 | R – resistant S – susceptible | | 15/20 | 7/20 | 6/20 | 15/20 | 15/20 | 9/20 | 7/20 | 8/20 | 0/20 | 2/20 | 0/20 | 0/20 | 20 |

[1] 10 Health Care Units in region of Upper Silesia, from where patients were directed to the Department of Medical Microbiology Medical University of Silesia in Katowice
R – resistant; S – sensitive
[2] Ox (1 μg) – oxacillin and [2]Fox (30 μg) – cefoxitin; index demonstrates the zone in mm of bacterial growth inhibition in disk diffusion test
[3]*mecA* – gene was determined by Murakami *et al*. (1991).
SXT (1.25/23.75 μg) – trimethoprim/sulfamethoxazole; C (30 μg) – chloramphenicol; T (30 μg) – tetracycline; E (15 μg) – erythromycin; CC (2 μg) – clindamycin; GM (10 μg) – gentamicin; CIP (5 μg) – ciprofloxacin; FA (10 μg) – fusidic acid; MXF (5 μg) – moxifloxa cin; SYN (15 μg) – quinupristin/dalfopristin; LZD (30 μg) – linezolid
[4] PFGE – Pulsed-field gel electrophoresis performed according to Chung *et al*. (2000).

(MIC 0.19–1.0 μg/ml) in E-test method. Majority of CoNS (15/20) were resistant to tetracycline and erythromycin. About half of them were resistant to clindamycin (9/20) and ciprofloxacin (8/20). Seven out of 20 studied strains were resistant to gentamycin and trimethoprim/sulfamethoxazole. Only 6 strains demonstrated resistance to chloramphenicol and 2 strains – to moxifloxacin. No resistance was observed to fusidic acid, linezolid and quinupristin/dalfopristin. Eight strains demonstrated resistance to erythromycin and clindamycin, suggesting constitutive resistance to $MLS_B$ agents. Macrolides and lincosamides are commonly used antibiotics in treatment of staphylococcal infections, particularly skin and soft tissue infections, ostitis and osteomyelitis and also as alternatives in penicillin-allergic patients. Seven strains demonstrated resistance to erythromycin and susceptibility to clindamycin, however D-test results were negative (data not shown). These strains were considered to be negative for inducible resistance, but could have an active efflux pump. In these cases according to Polish recommendations for susceptibility testing to antimicrobial agents of selected bacterial species (Hryniewicz *et al*., 2005) 14–15-carbon containing macrolides and streptogramins B are not recommended for treatment. Although clindamycin is a good alternative for treatment of methicillin-resistant/susceptible staphylococcal infections, results of antibiotic susceptibility testing to erythromycin and clindamycin should be analyzed very carefully to avoid therapeutic failures. Attention should also be drawn to the uncommon lincosamindes modification by 3-lincomycin,4-clindamycin O-nucleotidyl-transferase encoded by *linA*/*linA*' genes in staphylococci (Lina *et al*., 1999). Inducible $MLS_B$

resistance is not recognized by standard susceptibility testing, including standard broth-based or agar dilution tests. When staphylococci appear to be resistant to erythromycin and susceptible to clindamycin by routine tests it is very important to perform the D-test to demonstrate the occurrence of inducible resistance to clindamycin. To detect lincosamide resistance, susceptibility to lincomycin and clindamycin should be checked, because *in vitro* high levels of lincomycin resistance and susceptibility to clindamycin simultaneously have been described (Leclercq *et al*., 1987). Our CoNS strains resistant to erythromycin and susceptible to clindamycin, demonstrated susceptibility to lincomycin in disk diffusion test (data not shown), confirming the presence of active efflux pump mechanism. Susceptibility to quinupristin/dalfopristin is caused by the dalfopristin component, which is a streptogramin A (quinupristin is a streptogramin B) (Azap *et al*., 2005). Among 20 studied strains no clonal relatedness was observed in PFGE patterns, although some strains were cultured from patients treated in the same health care unit, but not during the same period of time (no 3 and 4; no 5, 6, 9 and 13; no 11 and 12 in Table II). Importantly, in 15 out of 20 studied CoNS different phenotypes of $MLS_B$ resistance were demonstrated. This is alarming news, especially for treatment strategies of ostitis/osteomyelitis due to CoNS. Further studies on a larger number of strains are required for detailed characterization of CoNS – causative agents of chronic ostitis and osteomyelitis.

## Literature


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