INDEX COPERNICUS INTERNATIONAL

Multidrug-resistant strains of coagulase--negative staphylococci isolated from patients with chronic sinusitis – MDR, XDR, PDR strains

Authors' Contribution:
A – Study Design
B – Data Collection
C – Statistical Analysis
D – Data Interpretation
E – Manuscript Preparation
F – Literature Search
G – Funds Collection

Michał Michalik[1ABDE], Adrianna Podbielska-Kubera[1DEF], Alfred Samet[1DE], Wiesław Konopka[2ABDE]

[1]Medical Center MML, Warsaw, Poland; Head: Michał Michalik MD PhD
[2]Paediatric Teaching Unit at the Department of Paediatrics and Immunology of the Developmental Age, Medical University of Lodz, Poland, Head: prof. Wiesław Konopka MD PhD

Article history: Received: 22.08.2019 Accepted: 14.09.2019 Published: 14.10.2019

ABSTRACT:

Introduction: The development of resistance to multiple antimicrobial agents in pathogenic bacteria has become a threat to public health. Multidrug-resistant strains that are particularly dangerous include MDR, XDR and PDR strains.

Material and methods: Aspirate material from paranasal sinuses, obtained from patients with chronic sinusitis undergoing functional endoscopic sinus surgery (FESS) in Medical Center MML in Warsaw, was subjected to bacteriologic analysis. The isolated strains were identified to the species level and tested for antibiotic resistance. Then, minimal inhibitory concentration (MIC) was determined.

Results: The isolated strains of coagulase-negative staphylococci were resistant mainly to macrolides, aminoglycosides and tetracycline. Nine of the isolated strains exhibited multidrug-resistance.

Discussion: Bacteria causing chronic sinusitis are becoming increasingly resistant to antimicrobial agents. The diagnostic process for coagulase-negative staphylococci (CNS) is often limited to the identification of species, or even genus of the bacteria. The CNS strains are considered to be non-pathogenic and they are not subject to eradication. This may lead to erroneous therapeutic decisions and, consequently, to the development of antibiotic resistance. CNS infections are classified as nosocomial and therefore, appropriate epidemiological procedures have to be followed. The authors highlight the necessity to determine MIC values for antibiotics and to introduce personalized treatment.

KEYWORDS: antibiotic resistance, chronic sinusitis, coagulase-negative staphylococci, epidemiology, nosocomial infections, virulence factors

LIST OF ABBREVIATIONS

AM^R – aminoglycoside-resistant strains
CNS – coagulase-negative staphylococci
DA^R – clindamycin-resistant strains
ESBL – extended-spectrum beta-lactamases
KLOX^R – cloxacillin-resistant strains
LZD^R – linezolid-resistant strains
MAKRO^R – macrolides-resistant strains
MIC – minimum inhibitory concentration
MDR – multidrug resistance
MLSB – constitutive resistance to macrolide-lincosamide-streptogramin B
MRSA – methicyllin-resistant *Staphylococcus aureus*
MSB – macrolide and streptogramin B resistant strains
PDR – pandrug resistance (resistance to all approved antimicrobial agents)
QL^R – quinolone-resistant strains
STX^R – cotrimoxazole (trimethoprim-sulphamethoxazole) resistant strains
TET^R – tetracycline-resistant strains
VA^R – vancomycin-resistant strains
XDR – extensively drug-resistant

INTRODUCTION

Chronic sinusitis represents a spectrum of inflammatory and infectious processes involving the nose and paranasal sinuses. The disease significantly reduces the quality of life of patients and imposes a serious socio-economic burden.

The upper respiratory tract, including the nasal pharynx, is inhabited by a microbiome that constitutes potential pathogens capable of causing respiratory infections, such as sinusitis. During respiratory infections, the pathogens may spread from the nasopharynx contributing to the sinus infection [1]. It is unclear whether changes in the bacterial flora in patients with chronic sinusitis exacerbate the course of the disease or whether they are caused by the changing tissue environment in the inflamed sinuses [2]. In chronic sinusitis, the mucociliary clearance, responsible for the removal of micro-organisms from the sinuses, as well the host im-

 DOI: 10.5604/01.3001.0013.5258

Tab. I. Numerical distribution of bacteria isolated from patients with chronic sinusitis.

SPECIES/GENUS OF BACTERIA	NUMBER OF STRAINS
S. epidermidis	507
S. aureus	307
Coagulase-negative staphylococci	73
Corynebacterium spp.	37
β-hemolytic streptococci	33
Pseudomonas spp.	32
Escherichia coli	32
Klebsiella spp.	32
Enterobacter spp.	24
Streptococcus pneumoniae	24
Enterococcus spp.	22
Citrobacter spp.	22
Candida spp.	20
Streptococcus viridans spp	16
Moraxella spp.	14
Proteus spp.	14
Acinetobacter spp	9
Serratia spp.	6
Morganella spp.	5
Haemophilus spp.	3

mune system, are impaired to such an extent that the sinuses are often colonized by the nasal microflora [3].

Bacterial infections are responsible for 60–90% of all cases of sinusitis. One of the most common Gram-positive bacteria are *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Staphylococcus epidermidis* and other coagulase-negative staphylococci [4].

Empiric antibiotic therapy is often used for sinusitis. On the other hand, the emergence of antibiotic resistance has increased the failure rate of this approach [5]. In clinical practice, there is a significant population of patients with chronic sinusitis who remain resistant to treatment despite strict regimens, including surgical procedures and prolonged antibiotic therapy. Strains isolated from these patients require the determination of antibiotic resistance genes. It is essential to personalize treatment on the basis of pharmacokinetic and pharmacodynamic properties of the drugs.

Multidrug-resistant strains have acquired resistance to at least one agent in three or more antimicrobial categories. MDR infections may result in insufficient or delayed antimicrobial therapy, more frequent exacerbations of the disease and worse treatment outcomes [6]. They can also be a gateway to infection for predisposed patients, e.g. after transplantation. Individuals from whom MDR strains have been isolated are often characterized by longer disease duration and sometimes higher mortality rates. XDR strains are resistant to most antimicrobial agents, but they are susceptible to at least one agent in all but two or fewer antimicrobial categories. PDR is non-susceptible to all approved antimicrobial agents [6].

Currently available drugs include agents effective in MDR infections. However, there are only few options available for the treatment of XDR infections. Almost no option is available for the treatment of infections with PDR strains [6].

Currently, research is ongoing to find new therapeutic options for PDR strains. The efficacy studies will include the best synergistic combination of two or more antibiotics.

MATERIAL AND METHODS

The material was obtained from patients treated at the Medical Center MML in Warsaw between January 2017 and December 2017. Patients with chronic sinusitis were enrolled on the basis of their medical history, physical examination, endoscopy and computed tomography.

ISOLATION AND IDENTIFICATION OF STAPHYLOCOCCI

The study material consisted of aspirates taken from maxillary, frontal and ethmoid sinuses. Samples obtained from 380 patients were subjected to bacteriologic analysis. The material was collected during FESS surgery performed due to chronic sinusitis and delivered to the laboratory within one hour. Preliminary isolation and identification was performed with the use of bioMerieux solutions. The material was identified to the species level. In some cases, such an identification was not possible due to taxonomic discrepancies. The semi-automated Vitek 2 system was used to identify and evaluate antibiotic susceptibility and to determine the MIC value. If the antibiogram had to be expanded to include additional drugs, a disk diffusion test (Kirby-Bauer disc test) was used. The results were entered to the laboratory computer system and delivered to clinicians.

RESULTS

A total of 1232 strains were isolated from the examined material throughout one year (Tab. I., Fig. 1.). 580 of isolated strains were coagulase-negative staphylococci, including 507 *S. epidermidis*. Tab. II. and Fig. 2. show the numerical distribution of particular species of coagulase-negative staphylococci.

Staphylococci were the most commonly isolated bacteria. *S. epidermidis* prevailed, followed by *S. aureus* and other coagulase-negative staphylococci (except *S. epidermidis*). The following strains of CNS were dominant: *S. hominis* (20/73), *S. warneri* (19/73), *S. haemolyticus* (19/73). *S. lentus* (2/73) and *S. auricularis* (1/73) were rarely isolated.

The isolated strains were resistant to various groups of antibiotics. Tab. III. and Fig. 3. present antibiotic susceptibility test results for coagulase-negative staphylococci. All isolates of coagulase-negative staphylococci were susceptible to vancomycin and linezolid. Quinolones were also very effective – only 3 CNS strains were resistant to this group.

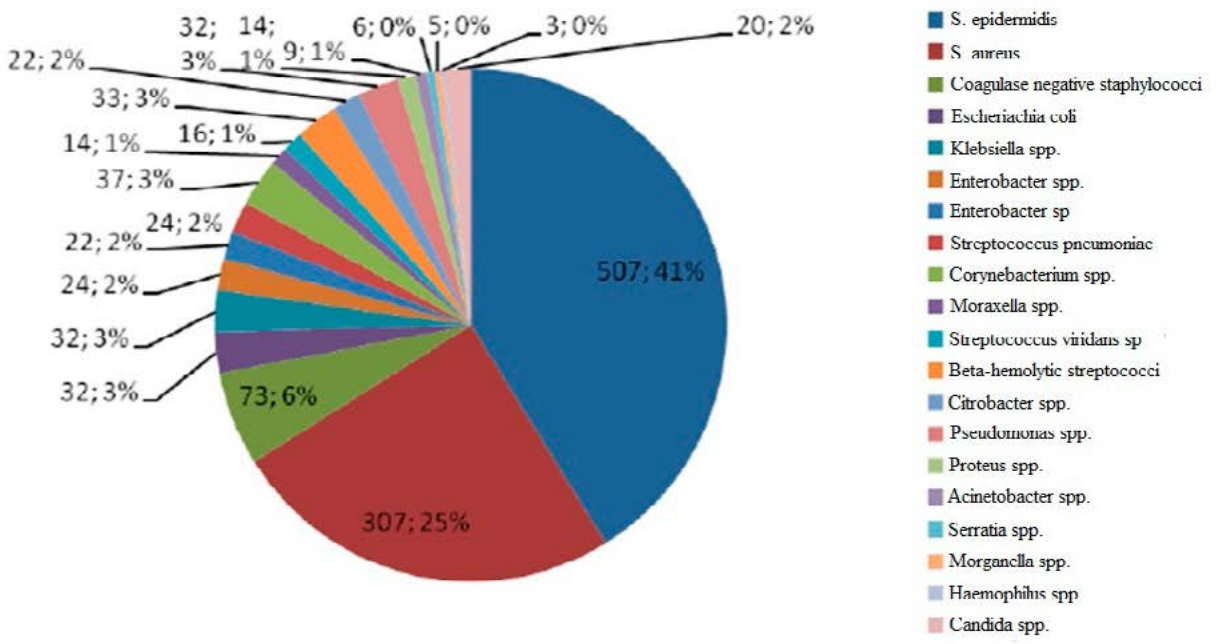

Fig. 1. Percentage distribution of bacterial species isolated from maxillary sinuses.

The isolated strains of coagulase-negative staphylococci were resistant mainly to macrolides (50.7%), aminoglycosides (27.4%) and tetracycline (26%).

The research showed that 20.5% of coagulase-negative staphylococci were methicillin-resistant. 20.5% of coagulase-negative staphylococci strains were characterized by the MLSB resistance mechanism and 31.5% by the MSB resistance mechanism. Nine of the isolated strains exhibited multidrug resistance.

Three strains of *S. haemolyticus* were characterized by resistance to several groups of antibiotics: macrolides, aminoglycosides, quinolines, tetracyclines. They showed MLSB resistance phenotype. Another three strains of *S. haemolyticus* were resistant to macrolides, aminoglycosides and cloxacillin and showed MSB resistance phenotype. Among multidrug resistant strains there was also *S. lungdynensis*, resistant to macrolides, aminoglycosides and tetracyclin, with MSB resistance mechanism, and one strain of *S. hominis* and *S. warneri*, both resistant to macrolides and aminoglycosides.

Tab. II. Numerical distribution of particular species of bacteria in the group of coagulase-negative staphylococci (except *S. epidermidis*).

COAGULASE-NEGATIVE STAPHYLOCOCCI	COAGULASE-NEGATIVE STAPHYLOCOCCI
S. hominis	20
S. warneri	19
S. haemolyticus	19
S. lugdunensis	12
S. lentus	2
S. auricularis	1

DISCUSSION

CNS strains can contribute to the development of infections, especially in patients with implants, catheters, artificial heart valves, endocarditis, atopic dermatitis, wounds, immunosuppression, as well as in cancer patients, premature newborns, elderly and patients with chronic conditions. The colonization of skin and mucous membranes of hospitalized patients with multi-resistant CNS strains and their transfer through hospital staff classify CNS strains as hospital pathogens [7]. The development of antibiotic resistance is largely influenced by the widespread use of broad-spectrum antibiotics, both in humans, veterinary medicine and agriculture. Antibiotics added to animal feed contribute to successful breeding, but they may also be responsible for the development of resistance as animals become reservoirs of antibiotic resistance genes. Most resistance genes are found on mobile genetic elements, facilitating transposition between organisms through gene transfer levels [8].

Pathogens causing chronic sinusitis, especially Gram-positive bacteria, are becoming more resistant to antibiotics, especially to beta-lactams. MRSA or ESBL strains, responsible for chronic sinusitis, are becoming increasingly common. Moreover, antibiotic resistance affects the effectiveness of various generations of cephalosporins in the treatment of chronic sinusitis [5]. Rezai et al. report that bacteria isolated from patients with chronic sinusitis are susceptible to 8–10 antibiotics only in 9% of cases, whereas 76% of pathogens is resistant to at least three groups of antibiotics [5].

Infections with coagulase-negative staphylococci are usually treated with macrolides, lincozamides and streptogramin B. This group of antimicrobial agents is considered the drug of choice for diseases of skin and soft tissue caused by CNS infections and as a substitute drug in patients intolerant to penicillin. Moreover, due to its good oral bioavailability, it is the preferred option for outpatients and as a continuation of intravenous injections [7].

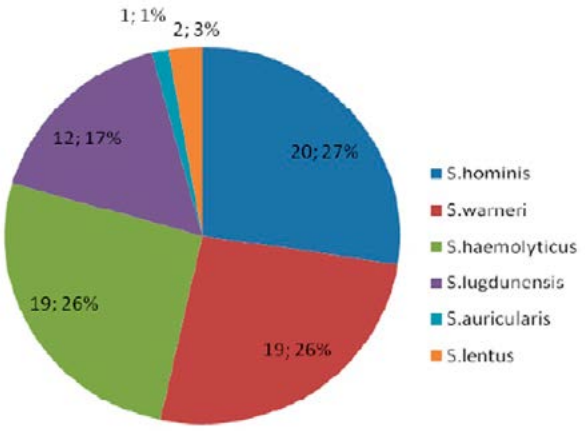

Fig. 2. Numerical and percentage distribution of particular coagulase-negative staphylococci species (except *S. epidermidis*).

Tab. III. Numerical distribution of particular species of bacteria in the group of coagulase--negative staphylococci (except *S. epidermidis*).

TYPE OF RESISTANCE	NUMBER OF RESISTANT STRAINS	PERCENTAGE OF RESISTANT STRAINS
MAKRO^R	37	50,7
MSB	23	31,5
AM^R	20	27,4
TET^R	19	26,0
MRCNS	15	20,5
MLSB	15	20,5
KLOX^R	14	19,2
DA^R	13	17,8
QL^R	3	4,1
VA^R	0	0,0
LZD^R	0	0,0

CNS strains are often resistant to many antibiotics, especially beta-lactams, with methicillin resistance observed in 60–70% of all isolates [9]. Methicillin resistance is often associated with resistance to fluoroquinolones, clindamycin and rifampicin, the first line of oral antibiotics used in bone and joint infections. New agents, including linezolid, daptomycin and tigecycline, have been developed as alternatives to glycopeptides against multi-resistant strains [9].

Studies by Lourtet-Hascoët J. et al. [9] showed that 52.1% of CNS strains isolated from patients with orthopaedic infections were resistant to methicillin. None of the CNS strains was resistant to linezolid or daptomycin. *Some S. capitis* isolates were proved to be resistant to oxacylin, erythromycin and clindamycin but susceptible to glycopeptides. Differences in antimicrobial resistance were observed among CNS strains. MDR isolates were more frequent in *S. hominis* and *S. haemolyticus* than in *S. capitis* and *S. warneri.* In a study of neonatal sepsis caused by *S. capitis* resistance to vancomycin was diagnosed in all 9 cases [10].

Bora P. et al. [7] showed that following strains are predominant in isolates obtained in clinical conditions: *S. epidermidis* (56.6%), *S. haemolyticus* (21.6%) and *S. lugdunensis* (11.6%). Other authors obtained similar results in their studies of blood, pus and urine isolates. Bora P. et al. reported resistance to penicillin (96.5%), ciprofloxacin (57%) and kotrimoxazole (44.5%). They also confirmed 100% sensitivity to vancomycin, theikoplanine and linezolid. These drugs should only be used as the last-line therapy in specific clinical cases [7].

The objective of the study conducted by Pedroso et al., was to characterize resistance aspects of CNS isolated from patients with bloodstream infections acquired in hospitals in Brazil. Strains showed resistance to the following antimicrobials: benzylpenicillin (100%), oxacillin (93.1%), gentamicin (36.3%), ciprofloxacin (63.7%), norfloxacin (81.0%), erythromycin (86.2%), clindamycin (75.8%), linezolid, teicoplanin and vancomycin (1.7%), tigecycline (0%), fusidic acid (10.35%), rifampicin (13.7%) and trimethoprim/sulfamethoxazole (46.5%). To conclude, the strains investigated in this study were multidrug resistant and carried multiple antibiotic resistance genes [9].

CNS strains (except *S. epidermidis*) isolated from patients treated in the Medical Center MML were characterized by the constitutive mechanism of resistance to macrolides, linkosamides and streptogramine B MLSB (9%), macrolides and streptogramine B MSB (15%) and methicillin (9%). Moreover, strains resistant to clindamycin constituted 8% of CNS strains and strains resistant to aminoglycosides – 13%.

Among CNS, *S. haemolyticus* has the highest tendency to develop resistance to multiple antibiotics. *S. hominis* isolates display lower virulence than *S. haemolyticus* and are less frequently recognized as significant human pathogens. However, there are reports indicating that *S. hominis* can be responsible for nosocomial outbreaks [11]. Research results show that *S. hominis* is often resistant to erythromycin and most of its strains show inducible MLSB resistance phenotype. One case of a nosocomial sinus infection with multidrug resistant *S. hominis* was observed among patients treated at the Medical Center MML.

Orthopaedic treatment was performed in a patient with a long history of recurrent shin and foot inflammation and atopic dermatitis. Postoperative wound infection with multidrug resistant *S. hominis* strain developed. The patient began to suffer sinus symptoms. Multidrug resistant *S. hominis* strain was isolated with the same susceptibility to antibiotics as the strain previously isolated from the wound infection. E. Szczuka et al. isolated 55 strains of *S. hominis* from blood samples and surgical wounds of hospitalized patients. In *S. hominis* strains, 75 and 84%, respectively, were erythromycin resistant and clindamycin susceptible. Among erythromycin-resistant *S. hominis* isolates, 68% of strains showed the inducible MLSB phenotype [11].

Yildirim et al. bacteriologically evaluated 48 culture-positive patients with chronic sinusitis who had been medically treated for at least 3 months and had undergone sinus surgery. Specimens were obtained during the surgery. The most common isolates were coagulase-negative staphylococci (45.8%) followed by *S. pneumoniae* (16.7%), *Enterobacteriaceae* (16.7%), *S. aureus* (10.4%) and *P. auruginosa* (10.4%). Despite the significant predominance of coagulase-negative staphylococci, for many years they have been assumed to be contaminants and their presence in bacterial cultures was neglected [12].

Results of a study performed by Rezai et al. showed the increased role of MRSA and ESBL-producing bacteria in chronic sinusitis. Total of 47.61% of *Staphylococcus* strains were MRSA and 33.33% of Enterobacteriaceae isolates were ESBL-producing bacteria. The authors reported that 60% of MRSA strains and 20% of MSSA strains were resistant to vancomycin. Manarey et al. found that 9.28% of chronic sinusitis cases is caused by MRSA. Motamedi et al. and Dibah et al. reported the incidence rate of MRSA *S. aureus* of 25% and 46%, respectively. The data illustrate a significant increase in the rate of MRSA in patients with chronic sinusitis [5]. Antibiotic susceptibility test showed that 73.46% of the Gram-positive isolates studied by Rezai et al. were MDR. The authors also confirmed high prevalence of MDR bacteria among *Enterobacteriaceae* family, especially *K. pneumoniae* strains. In the study, the rate of MDR for *E. coli* was over 76% and for *Enterobacter* over 70% [5].

The role of skin staphylococci in chronic sinusitis is very important. Multidrug resistant strains are more frequently alert pathogens. They are found in patients who have never been hospitalized. Due to the historical approach to CNS as a commensal organism, it appears that the diagnostic process is too often limited to the identification of species, or even genus of the bacteria. Coagulase-negative staphylococci cannot be classified as physiological flora. Accurate identification of CNS strains and evaluation of their susceptibility to antimicrobials will enable clinicians to determine the role of these bacteria as significant pathogens. High prevalence of CNS not only reduces therapeutic options but also enables the transfer of resistance mechanisms to other staphylococcal strains in the hospital environment, including virulent pathogens such as *S. aureus*.

The development of resistance to multiple antimicrobial agents in pathogenic bacteria has become a threat to public health as there are less and less antimicrobials effectively treating these infections. The problem is constantly growing, and precise definitions are needed in order to describe and classify multidrug resistant bacteria so that reliable epidemiological data can be collected and compared between healthcare settings of different countries.

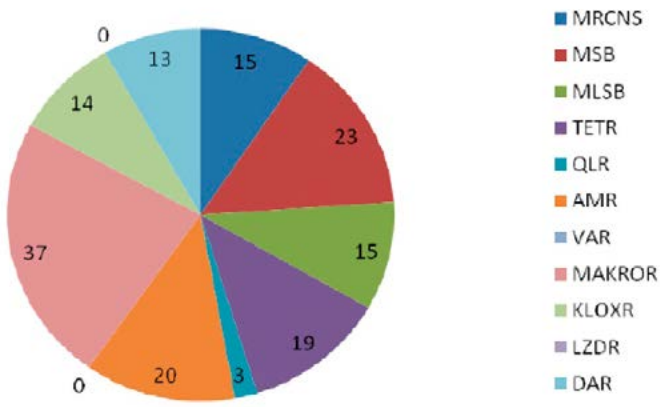

Fig. 3. Resistance to antibiotics in coagulase-negative staphylococci (except *S. epidermidis*) – numerical values.

The authors highlight the need to eradicate multidrug resistant strains and determine genome of the strain. The authors performed genome analysis of *E. coli* strains isolated from patients with chronic sinusitis [13]. Moreover, underestimated role of chronic sinusitis in the development of nosocomial infections in orthopaedics, cardiosurgery and transplantology should be considered. Early identification and surveillance of MDR, XDR and PDR strains should be considered and implemented in all laboratories. Personalized treatment and appropriate epidemiological procedures will help to control the spread of multidrug resistant strains and prevent nosocomial infections.

REFERENCES

1. Pedroso S.H.S.P., Sandes S.H.C., Filho R.A.T., Nunes A.C., Serufo J.C. et al.: Coagulase-Negative Staphylococci Isolated from Human Bloodstream Infections Showed Multidrug Resistance Profile. Microb. Drug Resist., 2018; 24(5): 635–647.
2. Hoggard M., Biswas K., Zoing M., Wagner Mackenzie B., Taylor M.W. et al.: Evidence of microbiota dysbiosis in chronic rhinosinusitis. Int. Forum Allergy Rhinol., 2017; 7(3): 230–239.
3. Brook I.: Microbiology of chronic rhinosinusitis. Eur. J. Clin. Microbiol. Infect., 2016; 35(7): 1059–1068.
4. Becker K., Heilmann Ch., Peters G.: Coagulase-Negative Staphylococci. Clin. Microbiol. Rev., 2014; 27(4): 870–926.
5. Rezai M.S., Pourmousa R., Dadashzadeh R., Ahangarkani F.: Multidrug resistance pattern of bacterial agents isolated from patient with chronic sinusitis. Caspian J. Intern. Med., 2016; 7(2): 114–119.
6. Guilbert J., Meau-Petit V., de Labriolle-Vaylet C., Vu-Thien H., Renolleau S.: Coagulase-negative staphylococcal osteomyelitis in preterm infants: a proposal for a diagnostic procedure. Arch Pediatr., 2010; 17(10): 1473–1476.
7. Bora P., Datta P., Gupta V., Singhal L., Chander J.: Characterization and antimicrobial susceptibility of coagulase-negative staphylococci isolated from clinical samples. J. Lab. Physicians., 2018; 10(4): 414–419.
8. Podkowik M., Bania J., Schubert J.: Gronkowce koagulazo-ujemne: nowe zagrożenie dla zdrowia publicznego? Życie Weterynaryjne, 2014; 89(1); 60–66.
9. Lourtet-Hascoët J., Félicé MP., Bicart-See A., Bouige A., Giordano G.: Species and antimicrobial susceptibility testing of coagulase-negative staphylococci in periprosthetic joint infections. Epidemiol Infect., 2018; 146(14): 1771–1776.
10. Brooks D., Thomas V., Snowden J.: Staphylococcus capitis Osteomyelitis: Case Report. Glob Pediatr Health., 2019; 6: 2333794X19833736.
11. Szczuka E., Makowska N., Bosacka K., Słotwińska A., Kaznowski A.: Molecular basis of resistance to macrolides, lincosamides and streptogramins in Staphylococcus hominis strains isolated from clinical specimens. Folia Microbiol (Praha), 2016; 61(2): 143–147.
12. Wei H.Z., Li Y.C., Wang X.D., Lu X.X., Hu C.H. et al.: The microbiology of chronic rhinosinusitis with and without nasal polyps. Eur Arch Otorhinolaryngol., 2018; 275(6): 1439–1447.
13. Michalik M., Samet A., Marszałek A., Krawczyk B., Kotłowski R. et al.: Intra-operative biopsy in chronic sinusitis detects pathogenic Escherichia coli that carry fimG/H, fyuA and agn43 genes coding biofilm formation. PLoS One., 2018; 13(3): e0192899.

Word count: 2930 Tables: 3 Figures: 3 References: 13

DOI: 10.5604/01.3001.0013.5258 Table of content: https://otolaryngologypl.com/issue/12703

Competing interests: The authors declare that they have no competing interests.

Corresponding author: Adrianna Podbielska-Kubera; Medical Center MML, Warsaw, Poland; Bagno Str. 2, 00-112 Warsaw, Poland; Phone: +48 508 613 946; E-mail: adrianna.podbielska@mml.com.pl

Cite this article as: Michalik M., Podbielska-Kubera A., Samet A., Konopka W.: Multidrug-resistant strains of coagulase-negative staphylococci isolated from patients with chronic sinusitis – MDR, XDR, PDR strains; Otolaryngol Pol 2020; 74 (2): 36-41