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The drug susceptibility of Staphylococcus aureus strains isolated from nose and pharynx

Lekowrażliwość szczepów *Staphylococcus aureus* izolowanych z wymazów z nosa i z gardła

For the first time, staphylococci were isolated from skin abscess by Pasteur in 1880, and in 1884 Rosenbach obtained them in pure culture and described. More than 100 years later *Staphylococcus aureus* remains a versatile and dangerous pathogen in human (3).

The serious staphylococcal infections have increased since the appearance of first antibiotics - resistant forms. The adaptive potential of this ubiquitous and virulent pathogen is unique. In 1942, when penicillin G was introduced into clinical use, the first resistant strains were found. In the next decade, some strains became resistant to chloramphenicol, erythromycin and the tetracyclines. In the early 60s, the production of β–lactamase resistant semisynthetic penicillins provided a temporary solution to that problem and ended with the emergence of methicillin–resistant *S. aureus* (MRSA). By the late 70s, the resistant strains of *S. aureus* established themselves in hospitals in the whole world. Additional forms of resistance developed toward all the new classes of antibiotics, including the quinolones (12, 7).

Among the antibiotics effective for methicillin–resistant isolates are glicopeptides – vancomycin and teicoplanin, inhibiting polymerisation of peptidoglycan and disturbing transpeptidation. They may be combined with fusidic acid, kotrimoxazol, fluoroquinolones or gentamicin to increase bacterial killing (12, 9, 5, 11, 6).

There are some mechanisms of resistance to antimicrobial agents. Penicillin is inactivated by β–lactamase, a serine protease, that hydrolyses β–lactam ring. The high level of resistance to methicillin requires the presence of the *mec* gene that encodes penicillin-binding protein (PBP 2a). The role of that protein consists in serving as peptidase during cell wall synthesising, when other PBPs are inactivated by β–lactam antibiotic. The ex-

pression of resistance also affects other staphylococcal genes, as *bla* (for β–lactamase) and *fem* (for factors essential for methicillin resistance) (5, 10, 9, 6, 11).

Resistance to methicillin in *S. aureus* may be homo– or heterogeneous, it means that during *in vitro* studying the level of that feature expression differs between strains. The percentage of bacterial population, expressing genes for resistance varies according to the environmental conditions.

The resistance identifications in homogenous strains are not difficult, but antimicrobial susceptibility testing of heterogeneous strains is often wrong interpreted. The problem of correct diagnosis for the methicillin resistance of staphylococci heterogenous strains is one of the most difficult in clinical microbiology (5, 10, 11, 9).

The cause of staphylococcal infections is the widespread carriage. The main reservoir and the source of spreading are the infected patient and carriers (the staphylococci mainly colonise the mucosal barrier of the nares). The bacteria are transmitted by contact with an infected patient or by an infected object, dust and environment, but these ways of transmission are less common. 30 – 50% of healthy persons are the carriers, and 10 – 20% are persistently colonised, by both MRSA and MSSA strains. Persons colonised with *S. aureus* are at an increased risk of subsequent infections. The high risk of infection is especially among patients undergoing hemodialysis, surgical patients, patients with type I diabetes and with acquired immunodeficiency syndrome. Patients with defects in leukocyte function, qualitative or quantitative, are also at an increased risk of staphylococcal disease. *Staphylococcus aureus* infection in that group of people is a major cause of skin, soft tissue, respiratory, bone, joint and endovascular disorders (5, 2).

Infections are initiated with breaking of the skin or mucosal barrier. Then staphylococci release a diverse arsenal of enzymes and toxins. The most important role in pathogenesis plays α hemolysin, responsible for pore formation and inducing proinflammatory changes in cells contributing, in consequence, to manifestations of the sepsis syndrome. The pyrogenic toxin, functioning as superantigen by binding to MHC class II proteins cause extensive T–cell proliferation and cytokine production. Some strains (about 20% of *S. aureus* isolates) produce enterotoxin. Different domains of the molecule are responsible for two diseases: toxic shock syndrome (TSST – 1) and food poisoning. Staphylococci produce toxins destroying leukocytes (leukocidin, α – and δ – toxin) and in that way they protect themselves against phagocitosis. The exfoliative toxins, including toxin A and B, cause scalded skin syndrome (3, 5, 8).

Staphylococci also release various enzymes, such as protease, lipase and hyaluronidase, which destroy tissue and facilitate the spread of infection to adjoining tissues. In the group of enzymes there are also: β–lactamase that inactivate penicillins and coagulase - a protrombin activator converting fibrinogen to fibrin (3, 5).

The presented above factors responsible for staphylococci virulence show the complexity of the infection process, and whether an infection is contained or spreads depends on a complex interplay between *S. aureus* virulence determinants and host defence mechanisms. Generally healthy human organism possess high resistance to infections.

This resistance is acquired as a result of the constant contact with pathogenic staphylococci.

The aim of the present study was to analyse the drug susceptibility of *Staphylococcus aureus* strains isolated from patients with chronic recurrences upper respiratory tract infections.

MATERIAL AND METHODS

BACTERIAL STRAINS

The materials for our study were nasal and pharyngeal swabs from 300 patients with chronic recurrence infections of upper respiratory tract, repeatedly undergoing antibiotic therapy.

MICROBIOLOGICAL STRAINS IDENTIFICATIONS

The materials were incubated onto blood agar and Chapman agar with mannitol. All cultures were incubated at 37°C for two days and examined daily for evidence of growth. After that time the genus of bacteria was first identified. When the growth of *Staphylococcus* was suspected another differentiation was performed to distinguish the pathogenous species from nonpathogenous. The strains identified as *S. aureus* were tested for the coagulase, using the vial method. The data were examined after 2, 4 and 6 h of incubation. The microscopical examination was also performed using the Gram method.

Gram-positive cocci were identified as *S. aureus* in accordance with their characteristic growth on mannitol-salt agar and positive results of coagulase.

DETECTION OF SUSCEPTIBILITY TO ANTIMICROBIAL AGENTS

Susceptibility of antistaphylococcal drugs was tested by disc-dffusion method on Mueller-Hinton agar. The dilution trays were inoculated with an inoculum 0.5 U using Mc Farland's scale, during 15 min. The inoculum was cultured on MHA agar. Then, the discs with antibiotics were put on a surface of agar and incubated at 35°C.

The drugs susceptibility was detected according to NCCLS recommendations. The organism was considered resistant, intermediate susceptible or susceptible to antibiotics following the criteria below:

	resistant	interm. susc.	susceptible
- 1μg oxacillin	≤ 10 mm	11-12 mm	≤ 13 mm
- 10U penicillin	≤ 28 mm	-	≤ 29 mm
- 15μg erythromycin	≤ 13 mm	14-22 mm	≤ 23 mm
- 2μg clindamycin	≤ 14 mm	15-20 mm	≤ 21 mm
- 30μg tetracycline	≤ 14 mm	15-18 mm	≤ 19 mm
- 5μg ciprofloxacin	≤ 15 mm	16-20 mm	≤ 21 mm
- 1.25 kg / 23.75 μg trimethoprim--sulfamethoxazole	≤ 10 mm	11-15 mm	≤ 16 mm

The results were analysed after 24 h detecting the zone size of growth inhibition.

DETECTION OF DRUG SUSCEPTIBILITY IN MRSA STAPHYLOCOCCI

The discs with antibiotics for MRSA strains were put on MHA agar cultured as earlier. The antibiotics were:

	resistant	interm. suscept.	susceptible
- 30 μg teicoplanin	to 10 mm	11-13 mm	from 14 mm
- 30 μg tetracycline	to 14 mm	15-18 mm	from 19 mm
- 30 μg vankomicin	to 9 mm	10-11 mm	from 12 mm
- 200 μg mupiracin	-	-	from 10 mm
- 10 μg fusidic acid	to 14 mm	15-21 mm	from 22 mm

The results were analysed after 24h detecting the zone size of growth inhibition.

RESULTS AND DISCUSSION

145 *Staphylococcus ssp.* strains were found in 300 clinical specimens. In 145 (70%) cases *S. aureus* was present and in 46 (30%) cases, other species of coagulase–negative staphylococci. Among 145 *S. aureus* strains 7 (5%) were methicillin–resistant (MRSA) and 138 (95%) were methicillin susceptible (MSSA). The results of drug susceptibility in *S. aureus* strains are shown in Table 1 a, b.

Table 1. The susceptibility of *Staphylococcus aureus* to antimicrobial agents

a) MSSA strains

Antibiotics	Number of MSSA strains (138)	
	S (%)	R (%)
Penicillin	18 (13)	120 (86.9)
Erythromycin	93 (67.3)	45 (32.6)
Clindamycin	126 (91)	12 (8.6)
Tetracycline	70 (50.7)	68 (49.2)
Ciprofloxacin	137 (99.2)	1 (0.8)
Trimethoprim--sulfamethoxazole	135 (97.8)	3 (2.1)
Oxacilin	138 (100)	0 (0)

S – susceptible, R - resistant

b) MRSA strains

Antibiotics	Number of MRSA strains (7)	
	S (%)	R (%)
Erythromycin	3 (42.9)	4 (57.1)
Clindamycin	7 (100)	0 (0)
Tetracycline	3 (42.9)	4 (57.1)
Ciprofloxacin	6 (85.7)	1 (14.2)
Trimethoprim--sulfamethoxazole	6 (85.7)	1 (14.2)
Vancomycin	7 (100)	0 (0)
Teicoplanin	7 (100)	0 (0)
Tetracycline	3 (42.9)	4 (57.1)
Mupirocin	7 (100)	0 (0)
Fusidic acid	7 (100)	0 (0)

S – susceptible, R – resistant

All MRSA strains produced β-lactamase and 4 were also resistant to macrolides and tetracyclines. Our findings confirm the susceptibility to vancomycin, teicoplanin and fusidic acid in MRSA strains. 100% of MRSA isolates were susceptible to those antibiotics.

Among MSSA strains we found resistance to tetracycline (49%) and erythromycin (32.7) and lack of susceptibility to penicillin, caused by β-lactamase production, was observed in 85% of cases. The level of resistance to penicillin among MSSA strains corresponded to the level described by other authors, but resistance to tetracycline is on much higher level, than in European studies (about 1%). Such high level of resistance to tetracycline among Polish *S. aureus* isolates is probably the result of frequent application of them in 70s (4).

Staphylococcus aureus resistance to methicillin is an important problem with microbiological, epidemiological and antimicrobial therapy point of view.

MRSA strains in hospital infections are found in about 30 – 40% [9, 11]. Among the tested in our study *S. aureus* strains in outpatients, MRSA were found in 5% of cases. The probable cause of these results is that all isolates were obtained from nasal and pharyngeal swabs, and MRSA strains are mainly present in surgical and critical care areas (up to 60%) (4).

The anterior nares were the typical site of *S. aureus* carrying (colonized in 51%). The pharynx infection was probably the result of carrying staphylococci in the nose. Such situation was observed in 30% of patients.

In accordance with the fact that all species of genus *Staphylococcus* possess similar mechanisms of resistance to β-lactam antibiotics, the results presented in our study should be spread beyond the species *Staphylococcus aureus*. The infection of upper respiratory tract is mainly caused, instead of staphylococci, by other bacteria and rarely by fungi or protozoas (1). Our study of *S. aureus* was also connected with description of bacterial flora in staphylococcal infections.

Table 2. Percentage of microorganisms isolated from nose and pharynx (except staphylococci)

swabs	HI	HPI	SP	Sβ	MC	PG(-)	CS
nose	1%	7%	4%	1%	4%	5%	3%
pharynx	4%	20%	5%	8%	3%	7%	8%

HI – *Haemophilus influenzae,* HPI – *Haemophilus parainfluenzae,* SP – *Streptococcus pneumoniae,* Sb - *Streptococcus* b - *haemolitic,* MC – *Moraxella catarralis,* PG(-) – G (-) baccilii, CS – *Candida* ssp.

As the table shows, the most frequent strains isolated from pharynx were *Haemophilus parainfluenzae* (20%) and at lower percentage, *Moraxella catarralis* (3%), *Haemophilus influenzae* (4%) and *Streptococcus pneumoniae* (5%).

During the nasal swabs analysis we have observed that the most frequently accompanying strains of *S. aureus* infections were *Haemophilus parainfluenzae* (7%) and G (-) bacilli – *Proteus* and *Pseudomonas* – 5%, at lower percentage - *Streptococcus* b (1%) and *Haemophilus influenzae* (1%). These findings overlap with Białecka et al. report (1). In her study, the haemophilus bacilli isolated from the accessory sinuses of the nose were the major etiological agents of respiratory tract infections.

CONCLUSIONS

On the basis of the obtained results, we demonstrated data of *S. aureus* resistance of both MRSA and MSSA strains to antibiotics commonly used in staphylococcal infections therapy. The resistance to antibiotics routinely applied during the upper respiratory tract infections was significantly higher in the group of β-lactams and tetracyclines. It is a serious epidemiologic and therapeutic problem. The presence of multiple microorganisms with *S. aureus* responsible for infections occurred as the etiological agent of respiratory tract diseases and resistance occurring among them should be taken into consideration (1). These facts unequivocally emphasise the necessity of detection of susceptibility to antimicrobial agents among the virulence strains that should proceed antimicrobial therapy.

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STRESZCZENIE

Niniejsza praca miała na celu ocenę wrażliwości na wybrane antybiotyki szczepów *Staphylococcus aureus* izolowanych od pacjentów z przewlekłymi, nawracającymi zapaleniami górnych dróg oddechowych, wielokrotnie leczonych antybiotykami. Materiał do badań stanowiły wymazy z nosa i z gardła.

Z 300 próbek materiału klinicznego wyhodowano 145 szczepów gronkowców, z czego w 99 (70%) przypadków obecny był *S. aureus*, a w 46 (30%) inne gatunki gronkowca kg (-). Spośród 99 szczepów *S. aureus* 7 (5%) określono jako MRSA, a 138 (95%) jako MSSA. Wszystkie szczepy MRSA były oporne na penicyliny, a 4 dodatkowo wykazywały oporność na makrolidy i tetracyklinę. Wszystkie były natomiast wrażliwe na wankomycynę, tejkoplaninę i kwas fusydowy. Przebadane szczepy MSSA wykazywały oporność na tetracyklinę, erytromycynę i penicyliny.

Czynnikami etiologicznymi w obrębie dróg oddechowych, obok *S. aureus*, są również inne drobnoustroje. W niniejszej pracy najczęściej izolowano *Haemophilus parainfluenzae*, a zaraz za nim *Streptococcus* b (z gardła) i pałeczki G (-) (z nosa). Poważnym problemem terapeutycznym i epidemiologicznym jest fakt narastania oporności na antybiotyki również wśród nich.