Aminoglycosides resistance in clinical isolates of Staphylococcus aureus from a University Hospital in Białystok, Poland

Tomasz Hauschild¹, Paweł Sacha², Piotr Wieczorek², Marta Zalewska², Katarzyna Kaczyńska³, Elżbieta Tryniszewska²

¹Department of Microbiology, Institute of Biology, University of Białystok, Poland
²Department of Microbiological Diagnostics, Medical University of Białystok, Poland
³Department of Microbiological Diagnostics, University Hospital of Białystok, Poland

Abstract: Staphylococcus aureus obtained from a University Hospital in Poland were characterized in relation to resistance to aminoglycoside antibiotics and the distribution of the genes encoding the most clinically relevant aminoglycoside modifying enzymes (AMEs). Of a total of 118 S. aureus, 45 (38.1%) isolates were found to be resistant to at least one of the tested antibiotics. All aminoglycoside resistant isolates except one 44 (97.8%) were resistant to kanamycin. The majority of strains 37 (82.2%) and 32 (71.1%) expressed resistance to neomycin and tobramycin, respectively. Eleven strains (24.4%) were resistant to gentamicin or amikacin. All S. aureus strains were sensitive to netilmicin. The most prevalent resistance gene was aac(6')-Ie+aph(2') found in 13 (28.9%) strains and 12 (26.7%) isolates carried ant(4')-Ia gene, whilst aph(3')-IIIa gene was detected in only 7 (15.6%) isolates. Additionally, the ant(6)-Ia and str genes were detected in 14 (31.1%) and 2 (4.4%) strains, respectively. Ten (22.2%) strains resistant to amikacin, tobramycin, kanamycin or neomycin did not harbor any of the above-noted genes.

Key words: Aminoglycoside - Aminoglycoside modifying enzymes (AME) - Streptomycin - Staphlococcus aureus

Introduction

Staphylococcus aureus is a major cause of hospital- and community-acquired infections, and can result in serious consequences. Hospital infections caused by S. aureus include those affecting the bloodstream, lower respiratory tract, skin and soft tissues, as well as ventilator-assisted pneumonia and central venous catheter-associated bacteraemia. The importance of S. aureus as a human pathogen, apart from its ability to cause a diverse range of life-threatening infections, is its extraordinary potential to develop antimicrobial resistance [7].

One of the class of antibiotics playing an important role in the therapy of serious staphylococcal infections are aminoglycosides despite reports of increased resistance to these drug in many countries of the Europe [11]. The main mechanism of aminoglycoside resistance is drug inactivation by aminoglycoside-modifying enzymes (AMEs) encoded within mobile genetic elements [14]. The following three AMEs are of particular significance among staphylococci since they modify and thereby inactivate the traditional aminoglycosides of therapeutic importance: aminoglycoside-6'-N-acetyltransferase/2''-O-phosphoryltransferase [AAC(6')/APH(2'')], aminoglycoside-4''-O-nucleotidyltransferase I [ANT(4'')-I] and aminoglycoside-3''-O- phosphoryltransferase III [APH(3'')-III]. Resistance to gentamicin and concomitant resistance to tobramycin and kanamycin in staphylococci are mediated by bifunctional enzyme displaying AAC(6') and APH(2'') activity encoded by aac(6')-Ie+aph(2'') gene. Resistance to neomycin, kanamycin, tobramycin and amikacin is mediated by an ANT(4'')-I enzyme encoded by ant(4'')-Ia gene. The APH(3'')-III enzyme, which inactivates kanamycin and neomycin is encoded by aph(3'')-IIIa gene [12, 14]. Resistance to strepto-
mycin in staphylococci is associated with the enzymes ANT(6)-I and APH(6)-I encoded by ant(6)-Ia and str genes, respectively [11,12,14].

As aminoglycosides resistance and the distribution of the genes encoding aminoglycoside-modifying enzymes has not been well characterized or documented in large collection of S. aureus in Poland, we investigated S. aureus strains isolated from a University Hospital, in order to gain some insight into the nature of the resistance to this class of antibiotics.

Materials and methods

Bacteria. A total of 118 S. aureus isolates obtained during 2002-06 at the University Hospital were included in this study. All isolates were identified as S. aureus by ID 32 Staph (bioMerieux, France) according to the manufacturer instructions. Pure culture were preserved in 30% glycerol at -80°C and were subcultured in brain heart infusion broth, and incubated at 37°C prior to further testing. Multiple isolates of the same patient were excluded.

Antimicrobial agents and susceptibility testing. Antimicrobial susceptibility testing was performed using disk diffusion method on Mueller-Hinton agar plates. S. aureus ATCC 29213 was used as quality control strain for in vitro susceptibility testing. The antibacterial agents tested were: gentamicin (10 µg), amikacin (30 µg), netilmicin (30 µg), tobramycin (10 µg), kanamycin (30 µg) and neomycin (30 µg). Susceptibility to aminoglycosides was interpreted according to document M2-A8 of the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards, NCCLS) [9].

PCR amplification of aminoglycosides resistance genes. All isolates demonstrating resistance to at least one of the aminoglycoside antibacterial agent were screened for the presence of the aac(6')-Ie/aph(2") and str genes. Genomic DNA as a template for PCR assay was extracted by incubating with lysostaphin followed by purification with a commercially available purification kit (Eurx, Poland).

The aac(6')-Ie/aph(2") and str genes were detected by previously described primers [2, 5, 11]. PCR was performed in a Perkin-Elmer DNA thermal cycler, for the first of fourth genes at 94°C for 10 min, then 35 cycles at 94°C for 45 s, 60°C for 60 s, and 72°C for 60 s. A final extension cycle of 60 s, then 30 cycles of 94°C for 45 s, 55°C for 45 s, and 72°C for 60 s, and final extension at 72°C for 10 min.

The Gene Ruler 100 bp DNA Ladder Plus (MBI Fermentas, Lithuania) was used as a molecular size marker on the gels. All isolates were tested at least twice at independent occasions before being considered as positive.

Results

Of total 118 S. aureus isolates included in this study, 45 (38.1%) were resistant to at least one of the tested aminoglycoside antibacterial agents (Table 1). All isolates except one (97.8%) were resistant to kanamycin. The majority of strains 37 (82.2%) and 32 (71.1%) expressed resistance to neomycin and tobramycin, respectively. Eleven strains (24.4%) were resistant to gentamicin or amikacin. The most active aminoglycosidal agents against S. aureus was netilmicin. All isolates were sensitive to this drug.

With regard to resistance phenotypes, only 3 (6.7%) isolates were resistant to the greatest number of antibiotics tested, and were sensitive only to netilmicin. The great number of S. aureus isolates 13 (28.9%) were resistant to tobramycin, kanamycin and neomycin followed by resistance to kanamycin and neomycin in 12 (26.7%) isolates. Resistance to amikacin, tobramycin, kanamycin and neomycin was observed in 7 (15.6%) isolates, and the same number of strains were resistant to gentamicin, tobramycin and kanamicin. The other three resistance phenotypes: gentamicin/tobramycin/kanamycin/neomycin, amikacin/kanamycin/neomycin or tobramycin were observed in one (2.2%) isolate.

All of aminoglycosides resistant S. aureus were screened for the presence of three genes encoding the most clinically relevant aminoglycoside modifying enzymes (Table 1). The most prevalent resistance gene was aac(6')-Ie+aph(2") found in 13 (28.9%) strains. Twelve (26.7%) isolates carried ant(4')-Ia gene, whilst aph(3')-IIIa gene was detected in only 7 (15.6%) isolates. Additionally, the ant(6)-Ia and str genes were detected in 14 (31.1%) and 2 (4.4%) strains, respectively. Ten (22.2%) strains resistant to amikacin, tobramycin, kanamycin or neomycin did not harbor any of the above-noted genes.

Discussion

Aminoglycoside resistance is common in S. aureus isolated from different countries, and especially gentamicin resistance, is of clinical importance because it can compromise the therapeutic effectiveness of these antibacterial agents [15].

Since PCR is a reliable tool for the identification of aminoglycoside modifying enzyme gene in staphylococci [5,11], it was used in this study to detect the aac(6')-Ie+aph(2") and str genes in the S. aureus tested, and, hence the enzymes they encode.

The incidence of ANT(4') in this study was higher than that reported in other studies were the AAC(6')-APH(2") enzyme has been found to be the most common AME in S. aureus [3,10,15]. The higher incidence of the ANT(4') enzyme was because it included isolates that were resistant to kanamycin, gentamicin susceptible and contained genes for only the ANT(4') enzyme. The results were in agreement with results of antibiotic resistance testing which demonstrated that 44 of the 45 isolates were kanamycin resistant, and with results aminoglycoside resistance in S. aureus isolated in Kuwait hospitals [13]. Similarly, the study carried out in Japan reported much higher prevalence of ANT(4') enzyme than that of the other two AME.
enzymes [6]. However, insignificant frequently was detected genes for AAC(6')-APH(2") enzyme, which is similar to other reports that studies S. aureus [4,8,10,12,15,16]. Although, it have been demonstrated an upward in the proportions of AAC(6')-APH(2") and ANT(4') in aminoglycoside resistant S. aureus in our study in comparison with other European hospitals [11]. In contrast, netilmicin had excellent activity against all isolates. Similarly, Vanhoof et al. [15] reported a low incidence of netilmicin resistance among S. aureus in Belgium hospitals. Because of its activity against the all isolates, a combination of netilmicin and tobramycin has been advocated for the treatment of infections caused by aminoglycoside resistant strains producing AAC(6')-APH(2") enzyme since the combination acts synergistically [1].

Streptomycin resistance is mainly associated with ant(6)-Ia, str, or ant(3')-Ia genes [14]. Within the S. aureus population under study, resistance to this antibiotic was mainly encoded by ant(6)-Ia. Comparative analysis of our results is difficult due to poorly data on resistance to streptomycin in S. aureus.

The strains harboring aac(6')-Ie+aph(2') gene encoding AAC(6')-APH(2") enzyme are considered to be resistant to gentamicin and even to all aminoglycosides. However, in our study this gene was detected in two gentamicin susceptible isolates. In some studies have also reported similar findings [10, 13, 15]. The detection of resistance genes in antibiotic susceptible isolates may be due to the amplification of repressed antibiotic resistance gene [10] or AME of these strains display lower enzymatic activity, detected also in other study of S. aureus [15].

It is noteworthy to mention that in the 10 isolates demonstrating phenotypic resistance to any one of the antibiotics tested, we did not detect a known gene encoding an aminoglycoside modifying enzyme which could account for the phenotype. Because it is possible to deduce the type of enzyme present from the patterns of resistance (resistance to amikacin, tobramycin, kanamycin and neomycin, and susceptibility to gentamicin), these strains pointed toward the presence of the enzymes encoded by ant(4')-Ia or aph(3')-IIIa genes. The failure to detect these two genes may be due to either the presence of an ant(4')-Ia or aph(3')-IIIa variant gene that cannot be detected with the primers used or this suggests that new aminoglycoside resistance genes are circulating within the S. aureus population.

DNA methods that detect resistance genes appear to be very sensitive in detecting resistance mechanism even when the resistance is not expressed. This is significant because exposure of these organism to the antibiotics at a later date will result in the expression of full resistance if the genes are present, and can influence proper prescription, and use of appropriate agents for therapy.

**Table 1.** Aminoglycoside resistance genes in S. aureus with different aminoglycoside resistance phenotypes.

<table>
<thead>
<tr>
<th>Resistance phenotypesa</th>
<th>AMEs</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gm, Ak, Tob, K, N</td>
<td>AAC(6')-APH(2&quot;)+ANT(6)</td>
<td>1</td>
</tr>
<tr>
<td>Gm, Ak, Tob, K, N</td>
<td>AAC(6')-APH(2&quot;)+ANT(4')</td>
<td>1</td>
</tr>
<tr>
<td>Gm, Ak, Tob, K, N</td>
<td>AAC(6')-APH(2&quot;)</td>
<td>1</td>
</tr>
<tr>
<td>Gm, Tob, K, N</td>
<td>AAC(6')-APH(2&quot;)</td>
<td>1</td>
</tr>
<tr>
<td>Gm, Tob, K</td>
<td>AAC(6')-APH(2&quot;)+ANT(6)</td>
<td>6</td>
</tr>
<tr>
<td>Gm, Tob, K</td>
<td>AAC(6')-APH(2&quot;)+APH(6)</td>
<td>1</td>
</tr>
<tr>
<td>Ak, Tob, K, N</td>
<td>ANT(4')</td>
<td>5</td>
</tr>
<tr>
<td>Ak, Tob, K, N</td>
<td>ANT(6)</td>
<td>2</td>
</tr>
<tr>
<td>Ak, K, N</td>
<td>ANT(6)</td>
<td>1</td>
</tr>
<tr>
<td>Tob, K, N</td>
<td>AAC(6')-APH(2&quot;)+ANT(4')</td>
<td>1</td>
</tr>
<tr>
<td>Tob, K, N</td>
<td>AAC(6')-APH(2&quot;)+ANT(6)+APH(6)</td>
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</tr>
<tr>
<td>Tob, K, N</td>
<td>ANT(4')</td>
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</tr>
<tr>
<td>Tob, K, N</td>
<td>APH(3')</td>
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<tr>
<td>K, N</td>
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<tr>
<td>K, N</td>
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</tr>
<tr>
<td>K, N</td>
<td>ANT(6)</td>
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<tr>
<td>Tob</td>
<td>ANT(4')</td>
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</tbody>
</table>

Abbreviations: *Gm*, gentamicin; Ak, amikacin; Tob, tobramycin; K, kanamycin; N, neomycin.

References


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