Detection of Aminoglycoside Resistance Genes by Multiplex PCR among Coagulase Negative Staphylococci Isolated from Ventriculoperitoneal Shunt Infections

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ABSTRACT
A Ventriculoperitoneal (VP) shunt infection is a cause of significant morbidity causing shunt malfunction and chronic ill health. This study was carried out to detect the causative pathogens associated with VP shunt infections, to assess the frequency of Coagulase negative Staphylococci (CoNS) as well as study their phenotypic resistance pattern to methicillin and different aminoglycosides (AGs), and genotypic pattern for gene encoding aminoglycosides modifying enzymes (AMEs) by multiplex PCR. This study was carried out in Medical Microbiology & Immunology and Neurosurgery Departments, Faculty of Medicine, Zagazig University during the period from June 2008 to June 2010. During this period 240 shunt procedures were carried on 175 infants and children with hydrocephalus. CoNS were identified using standard microbiological laboratory techniques. Antimicrobial susceptibility testing was performed using disc diffusion method with 4 aminoglycosides and methicillin. Multiplex PCR assay was used to identify AMEs encoding genes. 58 out of 240 shunt procedures cases showed shunt infections, (64%) caused by CoNS, (28%) by Gram negative rods (5%) by Staph. aureus and (3%) by candida. 26 (70%) of CoNS isolates were methicillin resistant (MRCoNS). 25 (67.5%) isolates of CoNS were resistant to at least one of the tested AGs and the highest resistance was to gentamicin and tobramycin (67.5%) for both, followed by amikacin (27%) and streptomycin (19%). As regard results of multiplex PCR aac(6')-Ie+aph(2") gene encoding for the bifunctional enzyme was the most common (54%) followed by ant(4')-Ia gene encoding for the ANT(4') – Ia enzyme (46%) and the aph(3")-IIIa gene encoding APH(3") – IIIa enzyme (40.5%). In conclusion this study increased our knowledge about the causative pathogens of VP shunt infections, the phenotypic pattern of CoNS susceptibility to different AGs and the distribution of AMEs encoding genes in relation to methicillin susceptibility. The usage of appropriate antibiotic according to antimicrobial susceptibility testing at the probable time and the removal of the shunt apparatus are essential for successful treatment of VP shunt infection. Continued surveillance at both phenotypic and genotypic levels are recommended for monitoring the presence of other variant of the genes or new AGs resistance genes that may be produced within CoNS population.

INTRODUCTION
Ventriculoperitoneal shunt (VP) is one of the commonest procedures in neurosurgical practice. A significant problem encountered in shunt procedures is shunt infection (1). Despite improvements in surgical techniques and consistent use of preoperative antibiotics before shunt replacement, roughly 1 in 10 implanted VP shunts become infected in North America (2), with percentage range 5-27% of cases in most neurosurgical units throughout the world (3,4,5). Shunt infection is a common cause of shunt failure (6), leading to significant neurological morbidity in the pediatric population (7). Reduced intelligence quotient, psychomotor retardation and an increase risk of seizure disorders have all been associated with shunt infection. Moreover shunt infection is associated with 2 fold increase in the long – term mortality rate (7,8).

Risk factors for CSF shunt infection include causes of hydrocephalus (9,10), patient’s age (11,12,13,14), previous shunt failure (11) and duration of shunt surgery (15,16). Also, another study reported that premature birth, postoperative CSF shunt leak and intraoperatively breached gloves were independent risk factors for VP shunt infection (17).

Coagulase negative Staphylococci (CoNS), Staphylococcus aureus, variety of Gram-negative rods, Propionobacterium spp, and Enterococcus faecalis are important causes of shunt infection (11,13,18,19). CoNS were once considered the most common type of pathogen encountered in cases of CSF shunt infections, accounting for 36-80% of the episodes in different reported studies (20, 21, 22). The clinical significance of CoNS strains is also underscored by their resistance characteristics. CoNS strains have acquired resistance to methicillin and majority of them
are also resistant to almost all classes of antimicrobial agent\textsuperscript{(23)}.

Aminoglycoside antibiotics play an important role in the therapy of staphylococcal infections despite reports of increased resistance to these drugs in Europe\textsuperscript{(24,25,26)}. Aminoglycosides are potent bactericidal agent, inhibiting protein synthesis by binding to the 30S ribosomal subunit. Gentamicin and tobramycin are the most active against staphylococci and are often used in combination with either \beta-lactam or glycopeptides\textsuperscript{(27)}.

The main mechanism of aminoglycoside resistance is drug inactivation by aminoglycoside – modifying enzymes (AMEs) encoded within mobile genetic elements\textsuperscript{(28,29)}. Clinically the most important of these are that encode acetyltransferase (AAC), adenylyltransferase (ANT), or phosphotransferase (APH) enzymes. Aminoglycosides modified at amino groups by AAC enzymes or at hydroxyl groups by ANT or APH enzymes, lose their ribosome-binding ability and thus no longer inhibit protein synthesis\textsuperscript{(30)}.

In the present study, the objectives were to determine the rate of VP shunt infections with CoNS, detect the phenotypic antibiotic resistance pattern of isolated CoNS to different aminoglycosides and to investigate the prevalence of the clinically important aminoglycoside resistant genes: \textit{aac(6\textsuperscript{'})}-\textit{le}+\textit{aph(2\textsuperscript{''})}, \textit{ant(4\textsuperscript{'})}-\textit{Ia} and \textit{aph(3\textsuperscript{'})}-III\textit{a}.

**SUBJECTS, MATERIAL & METHODS**

This study was carried out in Medical Microbiology and Immunology and Neurosurgery Departments, Faculty of Medicine, Zagazig University during the period from June 2008 to June 2010.

**Subjects:**

Over this period 240 shunt procedures were carried on 175 infants and children with hydrocephalus, the infection of 58 shunts were suspected by two or more of the following clinical findings: fever, recurrent vomiting, poor feeding, depressed consciousness, irritability, seizures, bulging tense anterior fontanel, increased white blood cells and wound breakdown involving the shunt\textsuperscript{(31)}.

**Clinical specimen:**

CSF samples and sometimes the tip of removed VP shunt catheter were obtained. Samples were plated without delay on blood agar (Oxoid, UK) and incubated at 37\degree C for 24 hours. CoNS were identified by colony morphology, Gram staining, positive catalase test, and negative results to the tube coagulase and DNase tests\textsuperscript{(32)}.

**Antimicrobial susceptibility testing:**

All CoNS isolates were tested using disc diffusion method for susceptibility to gentamicin, tobramycin, amikacin and streptomycin using Muller Hinton agar (Oxoid, UK) with commercial antibiotic discs (Oxoid) according to Clinical and Laboratory Standard Institute (CLSI) guidelines for susceptibility testing\textsuperscript{(33)}.

Screening for methicillin resistance among all CoNS isolates was done by oxacillin (Oxoid) disc diffusion testing.

**Multiplex PCR:**

All CoNS isolates were screened for the presence of the three AMEs genes of interest (Table 1).

**Table (1): Genes encoding aminoglycoside-modifying enzymes, primer sequences and expected corresponding phenotype**

<table>
<thead>
<tr>
<th>Aminoglycoside resistance gene</th>
<th>Amplicon size (bp)</th>
<th>Primer sequence (5\textsuperscript{'} – 3\textsuperscript{‘})</th>
<th>AME</th>
<th>Expected phenotype\textsuperscript{(35)}*</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{aac(6\textsuperscript{'})}-\textit{le}+\textit{aph(2\textsuperscript{''})}-Ia</td>
<td>348</td>
<td>CAGAGGCTTGGGAAGATGAAG CTCCTGTTAATTCTCATGTTCGGC</td>
<td>AAC(6\textsuperscript{'})/ PH(2\textsuperscript{''})</td>
<td>GEN-R, NET-r, TOB-R, AMK-r, KAN-R, STREP-r</td>
</tr>
<tr>
<td>\textit{ant(4\textsuperscript{'})}-Ia</td>
<td>294</td>
<td>CAAACTGCTAAATCGGTAGAAGCC GGAAAGTTGACCAGACATTACGAACT</td>
<td>ANT(4\textsuperscript{'})-I</td>
<td>GEN-S, NET-S, TOB-R, AMK-R, KAN-R, STREP-S</td>
</tr>
<tr>
<td>\textit{aph(3\textsuperscript{'})}-III\textit{a}</td>
<td>523</td>
<td>GGCTAAAATGAGAATATCACGG CTTTTAAATCACATCAGCTGCGG</td>
<td>APH(3\textsuperscript{'})-III</td>
<td>GEN-S, NET-S, TOB-S, AMK-R, KAN-R, STREP-S</td>
</tr>
</tbody>
</table>

AME, aminoglycoside modifying enzymes; GEN, gentamicin; TOB, tobramycin; NET, netilmicin; AMK, amikacin; KAN, kanamycin; STREP, streptomycin.S, susceptible; R, resistant; r, reduced zones but likely to remain susceptible at BSAC breakpoints.

BSAC: British society for antimicrobial chemotherapy.

* Variations in AME substrate specificity explain differences in antibacterial activity among aminoglycosides\textsuperscript{(38)}.
Primers specific for conserved regions of the staphylococcal 16S rRNA gene 5’-294-GCCGGTGGAGTAACCTTTAGGAC; 3’-1522-AGGAGGTGATCCAACCGCA were used as additional internal control\(^{[36]}\). The primers were supplied by (Qiagen, USA). Template DNA was extracted using E.Z.N.A DNA isolation commercial kit (Omega- Biotek Doraville, USA) according to manufacturer’s instructions.

Multiplex PCR amplifications were carried out using pureTaq ready to go PCR Beads (Amersham, UK) and thermal cycler (Biometra, Germany).

Samples were denatured at 94°C for 1 min followed by 30 amplification cycles using the following parameters: 95°C for 30 sec, 58°C for 10 sec and 72°C for 30 sec. A final extension cycle of 72°C for 3 min was used \(^{[37]}\). The amplified products were electrophoresed in 1.5% agarose gel. A DNA molecular weight marker (100 to 1500 bp DNA ladder) (Bioron-Germany) was run in parallel. The gel were stained with ethidium bromide and visualized under ultraviolet transilluminator (Fig. 1).

![Fig. (1): Agarose gel showing amplified products. Lane 1 and 8 show DNA marker (100 to 1500 bp DNA ladder). Lane2 is the negative control. Lanes 3 to 7 show bands of internal control at 1228 bp. Lane 3 shows bands at 523 of \textit{aph(3’)-IIIa}, at 348 of \textit{aac(6’)-le-aph(2’’)-Ia} and at 294 of \textit{ant(4’)-I}. Lane 4 shows bands of \textit{aph(3’)-IIIa} and \textit{ant(4’)-I}. Lane 5 shows no bands for genes of AMEs. Lane 6 shows bands of \textit{aac(6’)-le-aph(2’’)-Ia} and \textit{ant(4’)-I}. Lane 7 shows a band of \textit{aac(6’)-le-aph(2’’)-Ia}.](image)

**RESULTS**

240 VP shunt procedures were carried on 175 infants and children with hydrocephalus. 58 cases showed shunt infection (24 %) and a single pathogen was found in each infection episode. 37 cases were infected by CoNS (64%), 16 (28%) by Gram negative rods, 3 (5%) cases by \textit{Staph. aureus} and 2 (3%) cases by Candida spp. (Table 2).
Table (2): Number and percentage of different pathogens causing shunt infection.

<table>
<thead>
<tr>
<th>Type of organism</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoNS</td>
<td>37 (64%)</td>
</tr>
<tr>
<td>Gram negative rods</td>
<td>16 (28%)</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>3 (5%)</td>
</tr>
<tr>
<td>Candida spp.</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Total</td>
<td>58 (100%)</td>
</tr>
</tbody>
</table>

CoNS, coagulase negative staphylococci

Out of a total of 37 CoNS isolates, 26 (70%) isolates were methicillin resistant coagulase negative Staphylococci (MRCoNS). In this study 25 CoNS out of 37 (67.5%) isolates were resistant to at least one of the tested aminoglycosides, 21 (80.7%) and 4 (36.3%) isolates were of MRCoNS and MSCoNS respectively, while the remaining 12 (32.4%) isolates of CoNS were fully susceptible to all of the tested aminoglycosides (Table 3).

Table (3): Distribution of aminoglycosides resistance among CoNS isolates in relation to methicillin resistance.

<table>
<thead>
<tr>
<th>Resistance to individual aminoglycoside</th>
<th>MRCoNS (no=26)</th>
<th>MSCoNS (no=11)</th>
<th>Total (no=37)</th>
<th>X² P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin (no=25)</td>
<td>21 (80.7)*</td>
<td>4 (36.3)</td>
<td>25 (67.5)</td>
<td>6.96 0.03</td>
</tr>
<tr>
<td>Tobramycin (no=25)</td>
<td>21 (80.7)*</td>
<td>4 (36.3)</td>
<td>25 (67.5)</td>
<td>6.96 0.03</td>
</tr>
<tr>
<td>Amikacin (no=10)</td>
<td>9 (34.6)</td>
<td>1 (9)</td>
<td>10 (27)</td>
<td>2.6 0.27</td>
</tr>
<tr>
<td>Streptomycin (no=7)</td>
<td>6 (23)</td>
<td>1 (9)</td>
<td>7 (19)</td>
<td>0.99 0.61</td>
</tr>
<tr>
<td>Sensitive to all AGs</td>
<td>5 (19.2)*</td>
<td>7 (63.6)</td>
<td>12 (32.4)</td>
<td>6.96 0.03</td>
</tr>
</tbody>
</table>

* Significant value. MRCoNS, methicillin resistant CoNS. MSCoNS, methicillin susceptible CoNS

The distribution of aminoglycosides resistance in CoNS isolates showed that the highest resistance rates were to both gentamicin and tobramycin 25 (67.5%) isolates with significant P value, followed by amikacin 10 (27%) and then streptomycin 7 (19%). On the other hand only 5 of 26 (19.2%) MRCoNS were susceptible to the aminoglycosides tested compared to 7 of 11 (63.6%) MSCoNS isolates with a significant difference (P<0.05) (Table 3).

Table (4): The distribution of aminoglycoside resistance genes among CoNS isolates in relation to methicillin resistance, aminoglycosides susceptible and aminoglycosides resistance.

<table>
<thead>
<tr>
<th>Resistance genes</th>
<th>MRCoNS (No=26)</th>
<th>MSCoNS (No=11)</th>
<th>Total</th>
<th>P value</th>
<th>AR (No=25)</th>
<th>AS (No=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (%)</td>
<td>No (%)</td>
<td>No %</td>
<td></td>
<td>No (%)</td>
<td>No (%)</td>
</tr>
<tr>
<td>aac(6')-Ie+aph(2'')</td>
<td>+ve 18 (69.2)</td>
<td>2 (18)</td>
<td>20 (54)</td>
<td>0.004**</td>
<td>20 (80)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>-ve 8 (30.8)</td>
<td>9 (82)</td>
<td>17 (46)</td>
<td></td>
<td>5 (20)</td>
<td>12 (100)</td>
</tr>
<tr>
<td>ant(4')-Ia</td>
<td>+ve 14 (53.8)</td>
<td>3 (27.3)</td>
<td>17 (46)</td>
<td>0.13</td>
<td>17 (68)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>-ve 12 (46.2)</td>
<td>8 (72.7)</td>
<td>20 (54)</td>
<td></td>
<td>8 (32)</td>
<td>12 (100)</td>
</tr>
<tr>
<td>aph(3')-IIIa</td>
<td>+ve 11 (42.3)</td>
<td>4 (36.4)</td>
<td>15 (40.5)</td>
<td>1.0</td>
<td>15 (60)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>-ve 15 (57.7)</td>
<td>7 (63.6)</td>
<td>22 (59.5)</td>
<td></td>
<td>10 (40)</td>
<td>12 (100)</td>
</tr>
<tr>
<td>No detected genes</td>
<td>6 (19)</td>
<td>7 (63.6)</td>
<td>13 (35)</td>
<td>0.02*</td>
<td>1 (4)</td>
<td>12 (100)</td>
</tr>
</tbody>
</table>

* P < 0.05 (Significant value), ** P < 0.01 (highly significant). AR, aminoglycoside resistance. AS, aminoglycoside susceptible. MRCoNS, methicillin resistant CoNS. MSCoNS, methicillin susceptible CoNS
AME genes were detected in 24 of 37 (65%) isolates while, none of these genes were detected in 13 (35%) isolates with a significant difference. *Aac(6')-Ie+aph(2'')* gene, *ant(4')-Ia* gene and *aph(3')-IIIa* gene were encountered in 20 (54%), 17 (46%) and 15 (40.5%) of CoNS isolates respectively. However the *aac(6')-Ie+aph(2'')* gene was found with a significant difference in 18 (69.2%) of MRCoNS in comparison to only 2 (18%) of MSCoNS. On the other hand, the 20 (76.9%) of MRCoNS harbored at least one of AMEs genes while 4 (36.4%) of *ant(4')-Ia* gene and *aph(3')-IIIa* gene were present in higher percentage in MRCoNS than in MSCoNS, 14 (53.8%) and 11 (42.3%) respectively but statistically non significant. Aminoglycosides resistance genes were not detected in all aminoglycosides susceptible isolates 12 out of 12 (100%) and in only one (4%) of aminoglycosides resistant isolates (Table 4).

### Table (5): Distribution of AMEs genes by multiplex PCR and phenotypic resistance to different aminoglycosides.

<table>
<thead>
<tr>
<th>Phenotypic resistance to</th>
<th>Multiplex PCR results</th>
<th>Gentamicin (25)</th>
<th>Tobramycin (25)</th>
<th>Amikacin (10)</th>
<th>Streptomycin (7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminoglycosides by disc</td>
<td>+ve -ve</td>
<td>+ve -ve</td>
<td>+ve -ve</td>
<td>+ve -ve</td>
<td></td>
</tr>
<tr>
<td>Gentamicin (25)</td>
<td>20(80%) 5(20%)**</td>
<td>17(68%)* 8(32%)*</td>
<td>15(60%)* 10(40%)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tobramycin (25)</td>
<td>20(80%) 5(20%)**</td>
<td>17(68%)* 8(32%)*</td>
<td>15(60%)* 10(40%)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amikacin (10)</td>
<td>8(80%) 2(20%)</td>
<td>7(70%) 3(30%)</td>
<td>8(80%) 2(20%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptomycin (7)</td>
<td>6(86%) 1(14%)</td>
<td>5(71%) 2(29%)</td>
<td>6(86%) 1(14%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As regard distribution of AME genes among aminoglycosides, resistant CoNS phenotypes, *aac(6')-Ie+aph(2'')* gene was found in 20 (80%), 20 (80%), 8 (80%) and 6 (86%) of gentamicin, tobramycin, amikacin and streptomycin resistant isolates respectively. *Ant(4')-Ia* gene was found in 17 (68%), 17 (68%), 7 (70%) and 5 (71%) of gentamicin, tobramycin, amikacin and streptomycin resistant isolates respectively. The results of *aac(6')-Ie+aph(2'')* gene and *ant(4')-Ia* gene showed statistically significant differences in isolates resistant to gentamicin and tobramycin. While the presence of *aph(3')-IIIa* gene in isolates resistant to different AGs was statistically non significant (Table 5).

### Table (6): The presence of AME genes, by multiplex PCR, in relation to different aminoglycosides phenotypic resistance patterns.

<table>
<thead>
<tr>
<th>Resistance phenotypes (No=25)</th>
<th>AMEs genes</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>CN, TOB (No=15)</td>
<td><em>aac(6')-Ie+aph(2''), ant(4')-Ia &amp; aph(3')-IIIa</em></td>
<td>5</td>
</tr>
<tr>
<td>CN, TOB (No=3)</td>
<td><em>aac(6')-Ie+aph(2'')</em></td>
<td>5</td>
</tr>
<tr>
<td>CN, TOB, AK (No=3)</td>
<td><em>aac(6')-Ie+aph(2'') &amp; ant(4')-Ia</em></td>
<td>2</td>
</tr>
<tr>
<td>CN, TOB, AK, STREP (No=7)</td>
<td><em>ant(4')-Ia &amp; aph(3')-IIIa</em></td>
<td>2</td>
</tr>
<tr>
<td>CN, TOB, AK, STREP (No=7)</td>
<td><em>ant(4')-Ia</em></td>
<td>1</td>
</tr>
<tr>
<td>CN, TOB, AK, STREP (No=7)</td>
<td>No genes detected</td>
<td>1</td>
</tr>
</tbody>
</table>

CN: Gentamicin, AK: Amikacin, TOB: Tobramycin, STREP: Streptomycin.
The 25 aminoglycosides resistant CoNS isolates showed 3 different phenotypic resistance patterns (Table 6).

**DISCUSSION**

VP shunt infection is a common cause of shunt failure\(^{(6)}\). It occurred in most neurosurgical units throughout the world with percentage range from 5-27\%\(^{(3,4,5)}\). In this study, we reported shunt infection in 58 out of 240 (24\%) VP shunts procedures. Despite the improvements in surgical techniques and consistent use of preoperative antibiotics before shunt replacement, our infection rate is still in the high rate which may reflect the improper infection control policies in our hospital.

CoNS in this study represented 64\% of VP shunt infections, followed by Gram negative rods 28\%. This result was in compatible with that of Wang et al.\(^{(38)}\) and Sarguna and Lakshmi\(^{(39)}\) who reported that CoNS and Gram negative rods were the most prevalent causes of VP shunt infections. This high incidence of VP shunt infections by CoNS may be due to direct wound contamination with skin commensals.\(^{(6)}\) As, the next involved pathogens were Gram negative rods this may be due to either skin colonization, which is less common cause, or due to "Retrograde" infection mechanism, in which an asymptomatic perforation of bowel will lead to distal contamination of VP shunt and retrograde progression of the infection\(^{(39)}\).

We found that 26 out of 37 (70\%) CoNS isolates causing VP shunts infections were methicillin resistant CoNS. Singhall et al.\(^{(40)}\) found that 62.7\% of CoNS isolated from tertiary care hospital were MRCoNS. Higher percentage of MRCoNS (84\%) was found by Karchmer et al.\(^{(41)}\) in their study on Staph. epidermidis causing prosthetic valve endocarditis. All these studies reflected the high percentage of MRCoNS infection in the hospitals all over the world. In our study we found that 25 out of 37 (67.5\%) CoNS were resistant to at least one of the tested aminoglycosides. The highest resistant was to gentamicin and tobramycin (67.5\% for each) then amikacin (27\%) and streptomycin (19\%). Our results were in agreement with that of Klingenberg et al.\(^{(37)}\) who reported CoNS resistance rates to gentamicin, tobramycin and amikacin of 66\% 68% and 38 \% respectively.

Regarding the frequency of aminoglycosides modifying enzymes genes, we found that aac(6\()-le+aph(2\')) gene was the most common among CoNS isolates, it was found in 20/37 isolates (54\%) followed by ant(4\()-Ia gene which was found in 17/37 isolates (46\%) then aph(3\()-IIIa gene which was detected in 15/37 (40.5\%). Our results were in agreement with previous reports from Europe\(^{(28,37)}\) who reported that aac(6\()-le+aph(2\')) gene was the most prevalent AME gene, encountered in more than two-thirds of all isolates.

One isolate in this study showed resistance to all aminoglycosides tested while none of the three AMEs encoding genes was found in that isolate. A similar observation was mentioned by Hauschild et al.\(^{(42)}\) and Shaheen et al.\(^{(43)}\) who reported that 10 out of 45 isolates and 8 out 31 isolates of Staph. aureus respectively showed phenotypic resistance without carrying any AMEs genes. This finding may be due to the presence of other resistance mechanisms like modification of the target by mutation of the genes encoding ribosomal proteins, alteration of membrane permeability or active efflux\(^{(44)}\), or may be due to production of new aminoglycosides resistance genes\(^{(42)}\).

As regard the aac(6\()-le+aph(2\')) gene encoding for the bifunctional enzyme which is responsible for resistance to gentamicin, tobramycin, and amikacin, there was statistical significance agreement between gentamicin resistance and the presence of this gene. However this gene was absent in 5 isolates that show phenotypic resistance to gentamicin. Udo and Dashti\(^{(45)}\) found that this gene was absent in 2 gentamicin resistant isolates, this observation may be due to the presence of another variant of the enzyme that could not be detected. However, a substantial proportion of isolates carrying aac(6\()-le+aph(2\')) gene, but still classified as susceptible to amikacin (12 out of 25 (48\%)) and streptomycin (14 out of 25(56\%)). A similar observation was reported by Klingenberg et al.\(^{(37)}\) who found 28 out of 59 CoNS carrying the aac(6\()-le+aph(2\')) gene still susceptible to amikacin. This is in agreement with other studies\(^{(28,45)}\), that amikacin is more resistant to the substrate-inactivating effect of the aac(6\()-le+aph(2\')) enzyme than gentamicin.

We detected aac(6\()-le+aph(2\')) gene with a significant prevalence in 18/26 (69.2\%) of MRCoNS in comparison to only 2/11 (18\%) of MSCoNS. Also the ant(4\()-Ia gene was detected with higher prevalence in MRCoNS in comparison to MSCoNS (14/26 (54\%) versus 3/11 (27.3\%)) respectively. A similar finding was reported from a Japanese study, it was
presumed to be due to the adjacent locations of ant(4′)-Ia gene and mecA. However, this association shows statistically a non-significance difference, in our study, may be due to small number of isolates. In another study from Korea, the prevalence of ant(4′)-Ia gene was 27% among CoNS and they believed that this gene was not strongly associated with mecA, but frequently combined with aac(6′)-Ie+aph(2′′) gene carriage. However, these observations may explain the close correlation between aminoglycoside resistance and methicillin resistance found both in our results and in other studies.

We conclude that 24(65%) of CoNS isolated from VP shunts infections carried AME genes either alone or in combination. Gentamicin was the preferred substrate for phenotypic detection of aac(6′)-Ie+aph(2′′) gene. Among traditional aminoglycosides, streptomycin and amikacin showed the highest in vitro susceptibility rates.

This study has increased knowledge of the prevalence and organisms causing VP shunt infections and also the distribution of AME genes in CoNS isolated from VP shunt infections.

We recommend that the strict sterile techniques and infection control measures must be applied during shunt procedures, as in majority of cases the causative organism was present at operative site prior to and during surgery.

We suggest more studies on aminoglycosides resistant CoNS and gene encoding other variants of the genes or new resistance genes in CoNS.

REFERENCES


تحديد الجينات المقاومة للأمينوجليكوسيديات باستخدام تفاعل إنزيم البيلمهر المتعدد في المكورات العنقودية سالبة التخثر المعزولة من حالات العدوى في جهاز تصفية السائل النخاعي من بطينات الدم إلى الغشاء البريتوتي

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تعتبر العدوى التي تحدث في جهاز تصفية الدم (التحويل) الطفل النخاعي من بطينات الدم من أهم الأسباب التي تؤدي إلى إعداد صحة الأطفال الذين يتم إجراء هذه العملية بما يؤدي إلى حدوث بعض الوفيات بين هؤلاء الأطفال. ويفيد هذا العمل إلى البحث عن الميكروبات المسببة لهذا النوع من العدوى. وتوجد دراسات مؤشرات المكورات العنقودية سالبة التخثر ودراسات أخرى تشير أن المكورات العنقودية المقاومة للإمينوجليكوسيديات والامينوجليكوسيديات وعلاقة هذه الأمراض بين تفاعل إنزيم البيلمهر المتعدد للكشف عن الجينات المقاومة للأمينوجليكوسيديات.

أجريت هذه الدراسة في قسم علم الميكروبيولوجيا الطبية والمناعة وقسم جراحة الدم والاعتماد، كلية الطب، جامعة الزقاق خلال الفترة من يناير ٢٠٠٨ إلى فبراير ٢٠١٠. وقد أجريت خلال هذه الفترة ٢٣٠ عملية تركيب أو استبدال لجهاز تصفية الدم سالب النخاعي من بطينات الدم إلى هناك العينة على أجساد الدمslide بعد ١٤ يومًا من علاج الأمراض. تم استخراج عينة مصدر ذاتي من وظائف المكورات المسببة لعدوى العضوب من الأمينوجليكوسيديات وتحديد من خلال تفاعل إنزيم البيلمهر المتعدد والتفاعلpond نوع فاصل، كما أجري لإجراء التفاعل المتعدد للكشف عن الجينات المقاومة للأمينوجليكوسيديات.

أسفرت النتائج هذه البحث عن وجود عدوى تم لها تركيب أو استبدال لجهاز تصفية الدم سالب النخاعي. وكان أكثر المكورات المسببة للعدوى العنقودية سالب النخاعي (٢٤ %) بيئة العصويات سالب الجرام (٢٤ %) والمكورات العنقودية المختصرة (٥ %). وكانت ٢٧ (١٠ %) عينة من المقاومة للإمينوجليكوسيديات، كما أرسلت النتائج ٢٦ (١٠ %) عينة من المقاومة للميكروبيويديات، وكانت أعلا مقاومة للميكروبيويديات، وكانت أعلا مقاومة للميكروبيويديات، وكانت أعلا مقاومة للميكروبيويديات للكلا ميكروب (٢٧ %) والامينوجليكوسيديات (١٩ %).

كما أصبحت نتائج إنزيم البيلمهر المتعدد وجدت الجينات المسئولة عن تفاعل الأدوية المقاومة للأمينوجليكوسيديات (أحد هذه الجينات أو كلاهما) في ٢٤ عينة من أصل١٠٠ عينة أظهرت مقاومة شارحية للأمينوجليكوسيديات. وقد أوضحت الدراسة هذه الحالة وثيقة بين متكمني الميكروبيويديات وعالية هذه الجينات كما أصبحت أيضا وجود لداء بين متكمني الميكروبيويديات ومتفاعلة الميكروبيويديات.

وتعليق السيناريوه هذا النوع من العدوى يوصى بالاستخدام الأمثل للمضادات الحيوية في الوقت المناسب. وأتبااع مجاوز تصفية الدم النخاعي (التحويل) إذا لم الزر أمر. يوصى هذه الدراسة إلى أهمية البحث الدائم للملاحظات المستمرة على المستوى الظاهري والجني

للكشف عن ظهور أنواع جديدة من الجينات المسئولة عن مقاومة المكورات العنقودية سالب النخاعي للأمينوجليكوسيديات.