Aminoglycoside Resistance in Enterococci

Joseph W. Chow

High-level aminoglycoside resistance in enterococci is mediated generally by aminoglycoside-modifying enzymes, which eliminate the synergistic bactericidal effect usually seen when a cell wall–active agent is combined with an aminoglycoside. Clinical microbiology laboratories currently screen for aminoglycoside resistance in enterococci by testing gentamicin and streptomycin susceptibility. If the recently detected aminoglycoside resistance genes, $\text{aph(2')-Ib, aph(2')-Ic, and aph(2')-Id.}$ become more prevalent among clinical isolates, the approach for detecting susceptibility to aminoglycoside synergism in enterococci will require modification. More potent aminoglycosides need to be developed that will be resistant to modification by a broad spectrum of aminoglycoside-modifying enzymes present in enterococci.

Optimal antimicrobial therapy for serious enterococcal infections requires the use of synergistic combinations of a cell wall–active agent, such as a penicillin or a glycopeptide, with an aminoglycoside, which results in bactericidal activity against this organism. Enterococci have acquired aminoglycoside resistance genes that mediate production of aminoglycoside-modifying enzymes, which eliminate this synergistic bactericidal effect. Detection of these enzymes has resulted in the traditional approach for predicting bactericidal synergism in enterococci. The discovery of several new aminoglycoside resistance genes in enterococci requires a reevaluation of how prediction of synergism in enterococci should be done. A different approach for predicting aminoglycoside synergism will need to be put in place should these newly discovered genes become more prevalent among clinical enterococcal isolates.

Background

All enterococci have intrinsic low-level resistance to aminoglycosides, with minimal inhibitory concentrations (MICs) ranging from 4 $\mu$g/mL to as high as 256 $\mu$g/mL. The MIC of gentamicin, the most commonly used aminoglycoside against enterococci, typically ranges from 6 to 48 $\mu$g/mL. The facultative anaerobic metabolism of enterococci is thought to produce their low-level resistance to all aminoglycosides by limiting drug uptake, which is associated with the proteins involved in electron transport. The addition of an agent that interferes with cell wall synthesis, such as ampicillin (or vancomycin), markedly increases uptake of the aminoglycoside, greatly enhancing the killing of the enterococcus [1].

Enterococci have acquired aminoglycoside resistance genes that encode various aminoglycoside-modifying enzymes, which result in very high resistance to aminoglycosides (MICs usually $\geq 2000 \mu$g/mL), thereby eliminating the synergistic killing effect described above. The resistance genes found to date in enterococci that encode aminoglycoside-modifying enzymes are listed in table 1. The most clinically important of these is the bifunctional gene $\text{aac(6')-Ie-aph(2')-Ia,}$ which encodes the bifunctional enzyme Aac(6')-Ie-Aph(2')-Ia [2]. Enterococci that possess $\text{aac(6')-Ie-aph(2')-Ia}$ are resistant to virtually all of the clinically available aminoglycosides, including gentamicin, tobramycin, amikacin, kanamycin, and netilmicin, but not streptomycin [2, 3].

All Enterococcus faecium strains produce a chromosomally encoded aminoglycoside acetyltransferase, Aac(6')-II, which eliminates synergism between cell wall–active antimicrobials and the aminoglycosides tobramycin, kanamycin, netilmicin, and sisomicin [4, 5]. The $\text{apf(3')-IIIa}$ gene encodes the aminoglycoside phosphotransferase, Apf(3')-IIIa [6, 7], which confers high-level resistance to kanamycin. The $\text{ant(4')-Ia}$ gene, which encodes the aminoglycoside nucleotidyltransferase, Ant(4')-Ia, confers resistance to tobramycin, amikacin, and kanamycin [8, 9]. Although both $\text{apf(3')-IIIa}$ and $\text{ant(4')-Ia}$ mediate an amikacin MIC of only 64–256 $\mu$g/mL in enterococci, isolates that contain either gene are resistant to ampicillin-amikacin synergism [3, 9, 10, 11]. As seen in table 1, enterococci

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Received 14 March 2000; revised 22 May 2000; electronically published 7 September 2000.
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Clinical Infectious Diseases 2000; 31:586–9
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1058-4838/2000/3102-0032$03.00
that possess \(aac(6')-Ii\), \(aph(3')-IIIa\), or \(ant(4')-Ia\) remain susceptible to synergism with gentamicin, provided they do not also contain \(aac(6')-Ie-aph(2')-Ia\) (it is not uncommon for clinical enterococcal isolates to possess \(\geq3\) different aminoglycoside resistance genes). High-level resistance to streptomycin in enterococci can be ribosomal, arising from a single-step mutation of a ribosomal protein [12], or enzymatic, arising from production of \(Apt(6')-Ia\) [10, 13] or \(Apt(3')-Ia\) [14, 15].

Testing for high-level aminoglycoside resistance in enterococci has heretofore required only the use of gentamicin and streptomycin. Isolates resistant to high levels of gentamicin (MIC \(\geq500\ \mu\text{g/mL}\)) have been presumed to possess \(aac(6')-Ie-aph(2')-Ia\). Because the presence of \(aac(6')-Ie-aph(2')-Ia\) precludes the use of virtually all of the clinically available aminoglycosides, except for streptomycin, there has not been a need to screen for the presence of \(aph(3')-IIIa\), \(aac(6')-Ii\), or \(ant(4')-Ia\). In the absence of \(aac(6')-Ie-aph(2')-Ia\), gentamicin could be used in combination therapy, regardless of the presence or absence of \(aph(3')-IIIa\), \(aac(6')-Ii\), or \(ant(4')-Ia\). In the presence of \(aac(6')-Ie-aph(2')-Ia\), streptomycin could be used in combination therapy with a cell wall-active agent, provided the enterococcal isolate is not resistant to high levels of streptomycin (MIC \(\geq1000\ \mu\text{g/mL}\)).

**New Gentamicin Resistance Genes**

With the report of the new aminoglycoside resistance genes \(aph(2')-Ic\) [16], \(aph(2')-Id\) [17], and most recently, \(aph(2')-Ib\) [18], \(aac(6')-Ie-aph(2')-Ia\) is no longer the only aminoglycoside resistance gene in enterococci known to encode resistance to gentamicin, and a new approach to detecting resistance to aminoglycoside synergism may be required. The \(aph(2')-Ic\) gene was initially isolated from an *Enterococcus gallinarum* animal isolate but was subsequently also found in *E. faecium* and *Enterococcus faecalis* clinical isolates. Although the gentamicin MIC is 256–384 \(\mu\text{g/mL}\) for enterococci that possess \(aph(2')-Ic\), these isolates are nonetheless resistant to ampicillin-gentamicin synergism. Therefore, if gentamicin at 500 \(\mu\text{g/mL}\) continues to be used to detect gentamicin resistance (and hence resistance to synergism with a cell wall-active agent), isolates that possess \(aph(2')-Ic\) may be missed and falsely deemed susceptible to ampicillin-gentamicin synergism. On the other hand, because there is only a 2-fold dilutional difference between 256 and 500 \(\mu\text{g/mL}\), strains bearing \(aph(2')-Ic\) can at times show minimal growth in a screening test that uses 500 \(\mu\text{g/mL}\) gentamicin. These strains would be falsely assumed to possess \(aac(6')-Ie-aph(2')-Ia\) and thus falsely deemed resistant to multiple aminoglycosides (table 1), when they actually are potentially susceptible to the combination of ampicillin plus amikacin, netilmicin, or dibekacin [19].

Another new gentamicin resistance gene recently found in enterococci is \(aph(2')-Id\). This gene was isolated initially from an *Enterococcus casseliflavus* blood isolate. All subsequent detection of \(aph(2')-Id\) among clinical isolates has been in vancomycin-resistant *E. faecium*. The \(aph(2')-Id\) gene encodes an aminoglycoside phosphotransferase, \(Aph(2')-Id\), which modifies gentamicin, tobramycin, kanamycin, netilmicin, and dibekacin. The MICs of these 5 aminoglycosides in enterococci possessing \(aph(2')-Id\) have all been \(\geq2000\ \mu\text{g/mL}\). Enterococci that possess only \(aph(2')-Id\) should be susceptible to ampicillin-amikacin synergism. Under the current screening protocol, these isolates would be deemed mistakenly to contain \(aac(6')-Ie-aph(2')-Ia\) and so could be falsely deemed resistant to amikacin synergism.

The most recent gene encoding high-level resistance to gentamicin detected in enterococci is \(aph(2')-Ib\) (S.J. Kao, personal communication). This gene also mediates high-level resistance in enterococci to tobramycin, kanamycin, netilmicin, and dibekacin (table 1). Enterococci that contain \(aph(2')-Ib\) should theoretically be susceptible to ampicillin-amikacin synergism. However, all 6 *E. faecium* isolates found to date to contain \(aph(2')-Ib\) have been resistant also to amikacin (MIC \(\geq512\ \mu\text{g/mL}\)); this resistance is probably due to the presence of a separate aminoglycoside resistance gene that encodes amikacin resistance.

Only relatively small surveys have been done to determine the prevalence of the new aminoglycoside resistance genes \(aph(2')-Ib\), \(aph(2')-Ic\), or \(aph(2')-Id\). Screening by use of PCR primers specific for the individual genes can be as effective as the more laborious technique of using probes of these genes to hybridize to Southern blots of total cellular DNA (J.W. Chow, unpublished data). Of 121 enterococci with high-level genta-

### Table 1. Susceptibility profiles of genes that mediate resistance to aminoglycoside synergism in enterococci.

<table>
<thead>
<tr>
<th>Resistance gene</th>
<th>Gentamicin</th>
<th>Tobramycin</th>
<th>Amikacin</th>
<th>Kanamycin</th>
<th>Netilmicin</th>
<th>Dibekacin</th>
<th>Streptomycin</th>
<th>Arbekacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>(aac(6')-Ie-aph(2')-Ia)</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S*</td>
</tr>
<tr>
<td>(aph(2')-Ib)</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>(aph(2')-Id)</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>(aph(3')-IIIa)</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>(aac(6')-Ii)</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>NT</td>
<td>S</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>(ant(3')-Ia)</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>(ant(4')-Ia)</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>NT</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>(ant(6')-Ia)</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

**NOTE.** NT, not tested; R, resistant to synergism; S, susceptible to synergism.

* Forty percent of isolates tested susceptible to synergism.
micin resistance, 96 isolates (79%) were found to contain \( \text{aac}(6^\prime)\cdot\text{Ie-aph}(2^\prime)\cdot\text{Ia} \), 6 isolates (5%) contained \( \text{aph}(2^\prime)\cdot\text{Ib} \), 2 isolates (1.6%) contained \( \text{aph}(2^\prime)\cdot\text{Ic} \), and 17 isolates (14%) contained \( \text{aph}(2^\prime)\cdot\text{Id} \) [17, 18]. None of these isolates possessed more than 1 of these 4 gentamicin resistance genes. A multi-center study of enterococcal bacteremia found that, of 179 enterococcal blood isolates for which the gentamicin MIC was \( \geq 256 \, \mu \text{g/mL} \), 168 isolates (93.8%) contained \( \text{aac}(6^\prime)\cdot\text{Ie-aph}(2^\prime)\cdot\text{Ia} \), 6 isolates (3.4%) contained \( \text{aph}(2^\prime)\cdot\text{Ib} \), 2 isolates (1.1%) contained \( \text{aph}(2^\prime)\cdot\text{Ic} \), and 3 isolates (1.7%) contained \( \text{aph}(2^\prime)\cdot\text{Id} \) (J.W. Chow, unpublished data). In 3 of the 7 participating hospitals, \( \text{aac}(6^\prime)\cdot\text{Ie-aph}(2^\prime)\cdot\text{Ia} \) was the only gentamicin resistance gene detected in enterococci. Thus, the prevalence of gentamicin-resistant enterococci that do not contain the \( \text{aac}(6^\prime)\cdot\text{Ie-aph}(2^\prime)\cdot\text{Ia} \) gene, but instead contain 1 of the other 3 resistance genes, was 21% in 1 small survey but only 6% in the larger survey. The difference may be a function of the geographic location of the participating hospitals, and possibly the results from the first survey were skewed because it included a hospital that, in retrospect, appeared to have had an outbreak of vancomycin-resistant \( E. \text{faecium} \) that contained the \( \text{aph}(2^\prime)\cdot\text{Id} \) gene (M.J. Zervos, personal communication).

A Preliminary Alternative Approach for Predicting Synergism

If the prevalence of the \( \text{aph}(2^\prime)\cdot\text{Ib} \), \( \text{aph}(2^\prime)\cdot\text{Ic} \), or \( \text{aph}(2^\prime)\cdot\text{Id} \) genes is found to be high in more extensive studies, then the current method for predicting synergism with an aminoglycoside would have to be modified to test for more than susceptibility to gentamicin and streptomycin. There are no established guidelines for how this should be done. What follows is a preliminary approach based on the limited published data. To detect isolates that contain \( \text{aph}(2^\prime)\cdot\text{Ic} \), the threshold for determining resistance to gentamicin synergism might be lowered from a gentamicin concentration of 500 \( \mu \text{g/mL} \)-256 \( \mu \text{g/mL} \). In general, isolates that do not grow in the presence of gentamicin at 256 \( \mu \text{g/mL} \) would be assumed to be susceptible to ampicillin-gentamicin or vancomycin-gentamicin synergism, provided they are not highly resistant to ampicillin (\( \geq 256 \, \mu \text{g/mL} \)) or vancomycin. If the isolate is resistant to gentamicin at 256 \( \mu \text{g/mL} \) but susceptible to streptomycin (MIC <500 \( \mu \text{g/mL} \)), then streptomycin can be used in combination with a cell wall-active agent. However, there are some exceptions to this rule. There exist rare strains of enterococci for which the gentamicin MIC is 256 \( \mu \text{g/mL} \) but that are susceptible to ampicillin-gentamicin synergism [19]. These strains can thus be falsely deemed resistant to gentamicin synergism. On the other hand, there are also rare strains for which the gentamicin MIC is very low (8–16 \( \mu \text{g/mL} \)) but that are resistant to ampicillin-gentamicin synergism via an as-yet-unknown mechanism [20–22]. These strains would be falsely assumed to be susceptible to gentamicin synergism. Gentamicin MICs cannot be used to detect these strains, because the majority of enterococci for which the gentamicin MIC is 16 \( \mu \text{g/mL} \) are susceptible to gentamicin synergism.

If the enterococcal isolate is resistant to both gentamicin and streptomycin, then MICs should be determined for netilmicin, dibekacin, and amikacin. If the isolate is \( E. \text{faecalis} \), the netilmicin MIC is \( \leq 32–64 \, \mu \text{g/mL} \), and the dibekacin MIC is \( \leq 128 \, \mu \text{g/mL} \), then the isolate probably contains \( \text{aph}(2^\prime)\cdot\text{Ic} \), and either of the aminoglycosides could potentially be used for synergism [19]. (Because all \( E. \text{faecium} \) possess the \( \text{aac}(6^\prime)\cdot\text{Ii} \) gene, they are probably all resistant to netilmicin synergism, regardless of the netilmicin MIC [5].) If the isolate’s netilmicin MIC is \( \geq 128 \, \mu \text{g/mL} \) and the dibekacin MIC is \( \geq 256 \, \mu \text{g/mL} \), but the amikacin MIC is \( \leq 256 \, \mu \text{g/mL} \), amikacin could potentially be used (see table 1). However, because the use of amikacin is precluded if the \( \text{aph}(3^\prime)\cdot\text{IIIa} \) or \( \text{ant}(4^\prime)\cdot\text{Ia} \) gene is present, the presence of these 2 genes would first need to be ruled out by use of a method such as PCR. Furthermore, as mentioned above, the 6 \( E. \text{faecium} \) isolates found to date to contain \( \text{aph}(2^\prime)\cdot\text{Ib} \) are all resistant to amikacin, and the few \( E. \text{faecium} \) isolates containing \( \text{aph}(2^\prime)\cdot\text{Id} \) that have been tested to date have been relatively resistant to ampicillin-amikacin synergism [17]. This resistance to amikacin is probably due to the presence of \( \text{aph}(3^\prime)\cdot\text{IIIa} \) or \( \text{ant}(4^\prime)\cdot\text{Ia} \) or both or to the presence of an as-yet-undiscovered aminoglycoside resistance gene in these isolates.

Much more extensive synergism studies of cell wall-active agents combined with various aminoglycosides against enterococci that possess different combinations of aminoglycoside resistance genes must be done before a more definitive approach than that outlined above can be proposed. The use of PCR to screen for the presence of all of the aminoglycoside resistance genes in table 1 would enhance the interpretation of aminoglycoside MICs and their implications for synergism. However, PCR, or even Southern blot hybridization with gene probes, is not free of problems, because partial deletions that inactivate the genes might not be detected. Tests of synergistic killing that combine ampicillin or vancomycin with various aminoglycosides against each gentamicin- and streptomycin-resistant clinical isolate would provide a much more definitive answer in selecting combination therapy for that isolate. However, this would be very laborious and costly and therefore of doubtful practical use.

Development of New Aminoglycosides Needed

With the increased prevalence of aminoglycoside resistance genes, in particular \( \text{aac}(6^\prime)\cdot\text{Ie-aph}(2^\prime)\cdot\text{Ia} \), among clinical enterococcal isolates, the choice of aminoglycoside for synergistic combination therapy has been very limited. In many tertiary care hospitals, the majority of enterococcal isolates have high-level resistance to gentamicin. In addition, the vast majority of those often are also highly resistant to streptomycin [23]. In these cases, optimal therapy cannot be achieved, because the enterococcal isolate is resistant to virtually all other clinically available aminoglycosides. This problem will remain until newer aminogly-
cosides are developed that are more resistant to modification by the enzymes mediated by the genes listed in table 1. One somewhat promising newer aminoglycoside is arbekacin. Arbekacin is a derivative of dibekacin and is currently available only in Japan, where it is used to treat gentamicin- and methicillin-resistant Staphylococcus aureus infections [24]. Arbekacin is modified by the bifunctional enzyme Aac(6’)-Ie-Aph(2’)-Ia at a slower rate than gentamicin and is thus may prove useful against some enterococci that possess aac(6’)-Ie-aph(2’)-Ia. Ampicillin combined with arbekacin has produced in vitro synergistic killing against 40% of enterococci that possess the aac(6’)-Ie-aph(2’)-Ia resistance gene [25]. In a rabbit model of infective endocarditis caused by an E. faecalis isolate containing aac(6’)-Ie-aph(2’)-Ia, ampicillin plus arbekacin was significantly more effective than ampicillin alone in decreasing the number of bacteria in the cardiac vegetations [26]. In preliminary studies, ampicillin plus arbekacin produced in vitro synergistic killing against 8 of 13 vancomycin-, ampicillin-, and gentamicin-resistant E. faecium isolates that possess the aph(2’)-Id gene [27]. Thus, arbekacin could be potentially a useful addition to the armamentarium of antimicrobial agents against enterococci, but resistance to this agent among a significant percentage of enterococcal isolates remains a problem. More potent, yet less toxic, aminoglycosides need to be developed if effective combination therapy for serious enterococcal infections such as endocarditis is not to become a thing of the past.

References


