Fusidic acid binds to elongation factor G (EF-G), preventing its release from the ribosome, thus stalling bacterial protein synthesis. In staphylococci, high-level fusidic acid resistance is usually caused by mutations in the gene encoding EF-G, \textit{fusA}, and low-level resistance is generally caused by the horizontally transferable mechanisms \textit{fusB} and \textit{fusC} that have a putative protective role on EF-G. In addition, \textit{fusD} is responsible for intrinsic resistance in \textit{Staphylococcus saprophyticus}, and alterations in the L6 portion of \textit{rplF} (\textit{fusE}) have a role in fusidic acid resistance. Fusidic acid has been used in Europe and Australia for decades. More recently, it has also been used in other countries and regions, but not in the United States. Worldwide fusidic acid resistance has been slow to develop, and the level of resistance and genetic mechanisms responsible generally reflect the time since introduction, indications for treatment, route of administration, and prescribing practices.

**MECHANISM OF ACTION**

Fusidic acid was isolated in 1962 from \textit{Fusidium coccineum} [4]. In the same report, fusidic acid was shown to inhibit gram-positive organisms (including 21 strains of \textit{Staphylococcus aureus}, \textit{Neisseria gonorrhoeae}, \textit{Neisseria meningitidis}, and \textit{Mycobacterium tuberculosis}, but not Enterobacteriaceae, \textit{Pseudomonas aeruginosa}, yeast or fungi [4]. The chemical structure of fusidic acid (Figure 1) was determined and found to be chemically related to 2 other steroidal compounds, cephalosporin \textit{P}, and helvolic acid [5, 6].

In 1965, Yamaki [7] first reported fusidic acid as an inhibitor of protein synthesis. Further study by Harvey et al [8] in 1966 excluded direct binding of fusidic acid to the ribosome, or other known constituents necessary for protein synthesis, and they reasoned that the agent prevented peptide bond formation. Subsequently, it was confirmed that susceptibility to fusidic acid was not determined by the ribosome but was associated with G factor (now known as elongation factor G [EF-G]) [9].

During protein synthesis, the ribosome transiently interacts with soluble EF-G, a GTPase, that catalyzes the complete translocation of the mRNA-tRNA complex in the final step of peptide chain elongation [10]. This process frees the aminoacyl site (A site) to accept the
Fusidic acid resistance determinants have been reported; however, it is only recently that most of these mechanisms have been fully characterized. For several years, mutations in fusA (the gene encoding EF-G) were postulated to be the primary cause of resistance to this antibiotic [3]. However, plasmid-mediated fusidic acid resistance has been demonstrated, and genes encoding proteins that play a protective role in EF-G were most recently identified [12]. These genes, known as fusB, fusC, and fusD, are very prevalent among several staphylococcal species [13–16].

Mutations in the riboprotein L6 operon within rplF were recently associated with fusidic acid resistance in S. aureus laboratory mutants and rare clinical strains with reduced fitness cost [17]. In addition, alteration in permeability and enzymatic inactivation by group I chloramphenicol acetyl transferase were also associated with fusidic acid resistance in a few isolates of Staphylococcus species and Enterobacteriaceae without other resistance determinants being detected [3].

Alteration of Binding Site
More than 30 fusA mutation types have been described [18]; however, most amino acid alterations were detected among laboratory mutants that were exposed to fusidic acid, and only a few of these were actually experimentally confirmed as a cause of resistance to this compound [19]. Mutations in fusA have been experimentally documented to increase S. aureus MIC values, and site-directed mutagenesis has concluded that mutations L461K, H457Y and P406L elevate the MIC of fusidic acid by at least 32-fold [19].

In a study that evaluated the crystal structure of EF-G, S. aureus mutants were selected in the presence of fusidic acid and structural analysis determined the location of these mutations [20]. The majority of alterations on EF-G (10 of 16 strains analyzed) induced by the presence of fusidic acid were located in the domain III or aminoacid positions 404–483, with rarer mutations affecting domain I and V (amino acids 1–280 and 606–693, respectively). Furthermore, mutations in domain V were observed to be associated with small colony variants (SCV) of S. aureus [17].

Among clinical S. aureus strains, the substitution L461K seems to be most prevalent. It encodes high-level fusidic acid resistance [14, 15, 19]. Other alterations in the same position (L461S and L461F) have been described but have been associated with lower fusidic acid resistance levels, compared with L461K [14, 15, 19, 21]. Among other EF-G alterations, substitutions at position 457 (H457Y and H457Q) were previously described to be present in clinical strains. These substitutions, along with those detected in amino acid 461, were considered important for in vivo resistance [19]. Strains harboring multiple EF-G amino acid substitutions showing low fusidic acid MIC values may have additional compensatory mutations and/or have polymorphisms that are not related to fusidic acid resistance [21]. These compensatory mutations may overcome the loss of fitness due to the mutations in EF-G that cause resistance [19].

Alterations in the L6 portion of rplF have been reported to encode fusidic acid nonsusceptibility among SCV S. aureus, indicating that fusidic acid could have a secondary site of action in this ribosomal region, named fusE [17]. Strains that were found to harbor these mutations present with MIC values of 4–32 μg/mL; in most cases, these alterations are deletions of multiple base pairs or encode for stop codons in the protein (Table 1) (unpublished data from the SENTRY Program). These alterations are likely to increase the fitness cost in the bacterial cell and lead to the observation of reduced colony size on agar plates.

Acquired Fusidic Acid Resistance Genes
Acquired fusidic acid resistance mechanisms that have a putative protective role on EF-G have been described in the past 10 years, and fusB and fusC have been characterized in various clinical strains [13]. These genes can be chromosome or plasmid mediated, and fusB was reported to be carried in pUB101 [22], a ubiquitous plasmid [3]. The fusB gene encodes an EF-G–binding protein that protects the staphylococcal translation...
Table 1. Recent Studies Evaluating Fusidic Acid Resistance Mechanisms in *Staphylococcus* Species

<table>
<thead>
<tr>
<th>Reference (year)</th>
<th>Characteristics of the FA-R* staphylococcal clinical strains</th>
<th>No. of isolates analyzed</th>
<th>fusA mutations (types [no. of isolates])</th>
<th>fusB</th>
<th>fusC</th>
<th>fusE mutations (types [no. of isolates])</th>
</tr>
</thead>
<tbody>
<tr>
<td>[15] (2010) FA-R <em>Staphylococcus aureus</em> and CNS from surveillance study evaluating 28 European hospitals (13 countries)</td>
<td>443</td>
<td>56 (V90I [2], L461K [32], V90A [1], A376V [1], P404L [2], H457Y [3], L461S [10], T387/I449K [1], D189V/L430S [1], A70V/A160V/H457Y [1], F441Y [1], V90I/H457Q/L461K [1])</td>
<td>123 positive</td>
<td>95 positive</td>
<td>1 (Q140L [1])</td>
<td></td>
</tr>
<tr>
<td>[14] (2010) FA-R <em>S. aureus</em> and CNS from surveillance in USA, Australia and Canada</td>
<td>87</td>
<td>3 (M433I [2], H457Q [1])</td>
<td>46 positive</td>
<td>33 positive</td>
<td>0 positive</td>
<td></td>
</tr>
<tr>
<td>[33] (2010) FA-R <em>S. aureus</em> collected during 2002–2007 in a hospital located in Taiwan, categorized by MSSA and MRSA (71 total)</td>
<td>45 MRSA</td>
<td>38 (P404L [1], P406L [1], E444K [1], G451V [2], M453I [2], H457Q [1], H457Y [5], L461K [6], L461S [2], M16/I/H457Y [3], A67T/H457Y [3], A70V/H457Y [1], A71V/P404L [1], R76C/H457Y [1], A376V/L461F [1], E444V/L461F [1], H457Q/L461F [4], H457Q/L461K [1], L461K/C473S [1])</td>
<td>0 positive</td>
<td>7 positive</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>26 MSSA</td>
<td>3 (P404L [1], P478S [1], M651I [1])</td>
<td>15 positive</td>
<td>8 positive</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>[16] (2008) FA-R <em>Staphylococcus epidermidis</em> from 1 UK hospital and other European countries</td>
<td>23</td>
<td>3 (L461K [3])</td>
<td>18 positive</td>
<td>2 positive</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>[13, 29] (2007) FA-R <em>S. aureus</em> recovered from impetigo patients in Scandinavia</td>
<td>41</td>
<td>13 (V607I [1], H457Y [5], G451V [1], G452S [1], A71T/P404L/L461S [1], A67T/H457Y [2], L461K [2])</td>
<td>15 positive (14 clonal)</td>
<td>13 positive</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>[17] (2007) FA-R SCV <em>S. aureus</em> from reference laboratory</td>
<td>20</td>
<td>10 (T385N [1], P404L [2], G452S [2; 1 SCV], G452C [1], L456F [1], H457Y [1], R464S [1], R464H [1])</td>
<td>NA</td>
<td>NA</td>
<td>5, all SCV (Stop at 229 [1], stop at 241 [1], CTG at 1 [1]; deletion 203-231 [1], deletion 404-427 [1])</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. CNS, coagulase-negative staphylococci; FA-R, fusidic acid resistance (FA-R); MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; NA, not available; SCV, small colony variant.

a. FA-R was defined and/or all isolates had minimum inhibitory concentrations >1 μg/mL.
apparatus from inhibition by fusidic acid.[12] *FusC* was inferred to have a similar role as *fusB* on the basis of the homology of the genes. [13] In addition, *fusD* is responsible for “intrinsic fusidic acid resistance” among *S. saprophyticus* [13].

**Other Resistance Mechanisms**

Other resistance mechanisms, such as alteration in permeability and enzymatic inactivation by group I chloramphenicol acetyltransferase, were also associated with fusidic acid resistance in a few isolates of *Staphylococcus* species and Enterobacteriaceae without other resistance determinants being demonstrated [3]. In addition, chloramphenicol acetyltransferase type I found in Enterobacteriaceae inactivates fusidic acid by competitively binding to this molecule and sequestering it [3]; however, this mechanism does not seem to play an important role in staphylococcal fusidic acid resistance.

Although β-haemolytic streptococci usually cause a small number (<10%) of SSSIs, optimal therapy should include coverage of these virulent organisms. [23] The fusidic acid MIC distribution for β-haemolytic streptococci is 1–8 mg/L [24]. The mechanism of the lower susceptibility remains unknown. However, no instances of resistance due to EF-G mutations have been reported in β-haemolytic streptococci [25–27], and β-haemolytic streptococcal infections have been responsive to treatment with fusidic acid [28].

**EPIDEMIOLOGY OF FUSIDIC ACID RESISTANCE MECHANISMS**

Recent surveillance studies evaluating fusidic acid resistance mechanisms in *Staphylococcus* species are summarized in Table 1. Since the early 1970s, horizontal acquisition of genes has been postulated to be an important mechanism encoding fusidic acid resistance in *S. aureus* clinical strains,[16], despite early studies suggesting that alterations in EF-G were the primary fusidic acid resistance mechanisms. Two of the earlier studies (from 2003 and 2007) listed in Table 1 investigated EF-G mutations alone; thus, the presence of *fusB* and *fusC* is not known in these isolates. [17, 19] O’Neill et al [29] suggested that among acquired resistance genes in European countries, *fusB* was the most prevalent fusidic acid resistance mechanism in *S. aureus* and *Staphylococcus epidermidis* clinical strains. Upon discovering the *fusC* gene, however, O’Neill and colleagues reported the presence of the *fusC* gene in all strains in the previous study that tested negative for the *fusB* gene and *fusA* mutations [13]. It is extremely important, therefore, to understand that any studies prior to 2007 and/or studies that did not investigate all known resistance mechanisms may not reflect the true prevalence of these mechanisms.

The prevalence of fusidic acid resistance mechanisms in *S. epidermidis* was evaluated by McLaws et al [16] among 23 fusidic acid resistant isolates collected in 20 European hospitals. The *fusB* determinant was very prevalent, being detected among 41.8% of the tested sample. The remaining isolates harbored *fusC*, and 3 carried a L461K *fusA* mutation [16]. In a recent study published in 2009, *fusA* mutations were demonstrated to be as frequently detected as the presence of *fusB* and *fusC* among invasive *S. aureus* strains isolated from hospitals in Denmark [18].

A European fusidic acid-resistant impetigo clone (EEFIC) has been described in the United Kingdom, Ireland, and France[30]. This clonal *S. aureus* strain, also described in Scandinavia and sporadically reported in other countries, is characterized by *agr* allelic group IV, *spa* type 171 (or single locus variants t408, t659, t874, and t875), sequence types 123 (clonal complex 121), the presence of exfoliative toxin A (*etaA*) and often *etaB*, and low-level fusidic acid resistance due to the presence of chromosomal *fusB* and strong association with impetigo [29, 31]. In a contemporary published survey of fusidic acid resistance mechanism prevalence in Europe, *S. aureus* strains harboring *fusB* were detected. The majority were recovered from blood cultures, but small numbers were collected from skin infections or wounds of hospitalized patients [15]. Four *fusB* carrying strains recovered from these infection sites were *spa* typed to investigate the correlation with EEFIC. These strains, isolated in French, Irish, and UK hospitals, displayed *spa* types t261, t189, t1546, and the novel variant t5876, being unrelated to the clonal EEFIC strain [15]. These results may indicate a recent decrease in the prevalence of the EEFIC clone or a low number of impetigo-like cutaneous infections that were received from hospital-based laboratories in this survey.

The need for epidemiological data on fusidic acid resistance mechanisms has been recently highlighted in the literature [1, 16], and although there has been an increase in the understanding of these genetic determinants over the past 2 years, only data from small-sample subsets are currently available in the medical microbiology literature. In our recent studies, we evaluated a large global collection of staphylococcal strains using a strategy that minimized the cost of molecular surveillance by using a multiplex polymerase chain reaction approach to detect *fusB*, *fusC*, and *fusD*, followed by amplification and sequencing of *fusA* and *fusE* to detect mutations [14, 15, 24]. This approach is supported by the observation from O’Neill and Chopra [12] that the insertion of *fusB* in isolates already carrying *fusA* mutations did not increase fusidic acid resistance levels, indicating that the combination of mechanisms offer no clear advantage to the bacterial cell and, therefore, would rarely emerge in clinical strains. In addition, a rapid method for detection of alteration L461K was developed to optimize the detection of this prevalent mutation, which encodes high-level fusidic acid resistance. This strategy allowed our group—and, we hope, will allow other researchers—to produce epidemiologically and clinically significant information to guide therapy.
DISCUSSION

Fusidic acid has been used for the treatment of staphylococcal infections in European countries for almost 50 years, mainly as a topical agent to treat impetigo [29]. In addition, this antibiotic has been used in Australia generally in combination with rifampicin as oral therapy against difficult-to-treat methicillin-resistant Staphylococcus aureus infections [32]. Overall, resistance rates in most countries surveyed to date remain at low levels (ie, the rate of S. aureus infection is less than the rate of coagulase-negative staphylococcal infection), contradicting the early beliefs that widespread fusidic acid resistance would rapidly emerge after clinical availability of this compound [3].

Elevated fusidic acid resistance rates observed in some countries (eg, Greece and Ireland), with a concurrent high prevalence of fusA mutations, may indicate a distinct use pattern (possibly topical) of this antimicrobial agent that exerts more selective pressure causing mutations to arise followed by clonal expansion [15]. In contrast, fusidic acid has not been used in clinical practice in the United States, and resistance in S. aureus has been reported to be both rare (0.3%) and low-level (MIC, 4-16 mg/mL), with fusC being most responsible [14]. However, the presence of the mobile fusB and fusC genes in staphylococcal isolates from a country such as the United States, where no selective pressure is exerted, demonstrates the potential for global expansion of these resistance genes due to coselection by use of other antimicrobial classes, because these genes are plasmid mediated and these genetic elements can harbor other resistance determinants.

In conclusion, the prevalence of mechanisms responsible for fusidic acid resistance appears to be distributed geographically, driven by the time since introduction, indications for treatment, route of administration, and prescribing practices. There is also evidence that mobile genes can spread into geographical regions independent of these cited parameters. There is a paucity of literature from longitudinal studies surveying the molecular epidemiology and genetics of fusidic acid resistance. Clearly, additional surveillance of resistance prevalence and the mechanisms responsible are necessary to better understand the emergence and spread (or lack of) fusidic acid resistance among staphylococci, especially in a nation such as the United States, where staphylococcal pathogens are naive to fusidic acid exposure.

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References