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A Fusidic Acid-Resistant Epidemic Strain of *Staphylococcus aureus* Carries the *fusB* Determinant, whereas *fusA* Mutations Are Prevalent in Other Resistant Isolates

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Fusidic acid-resistant epidemic *Staphylococcus aureus* strains causing impetigo bullosa have been reported in Scandinavia. We show that these strains form part of a European epidemic clonotype that carries the *fusB* determinant. In contrast, resistance to fusidic acid in a collection of nonepidemic strains resulted primarily from mutations in *fusA*.

Fusidic acid inhibits bacterial protein synthesis by interfering with dissociation of elongation factor G (EF-G) from the ribosome (15). It is frequently employed as a topical agent for treatment of superficial staphylococcal skin infections, including impetigo and atopic dermatitis (2, 5). Limited data suggest that resistance to fusidic acid in clinical isolates of *Staphylococcus aureus* arises from mutations in the gene encoding EF-G (*fusA*) (1, 8) or by acquisition of a plasmid determinant (*fusB*) that encodes a poorly characterized resistance mechanism (3, 9, 12).

Recently, fusidic acid-resistant epidemic strains of *S. aureus* causing impetigo bullosa were reported in Sweden and Norway (11, 16). In this paper we demonstrate that resistant strains reported in these Scandinavian outbreaks are clonally related and exist in other European countries. Furthermore, we found that this clonotype carries the *fusB* resistance determinant. In contrast, fusidic acid resistance arising in nonepidemic strains was attributed, in several cases, to mutations in *fusA*.

Clinical *S. aureus* isolates in which the genetic basis of fusidic acid resistance was established are listed in Table 1. In addition to representatives of epidemic fusidic acid-resistant strains from Scandinavia and Ireland, resistant strains collected during a comparative phase III study (FCF0001 INT) and from a dermatology unit in Harrogate, United Kingdom (strain designations with an H prefix) (13), were examined. Detection of *fusA* mutations in these strains was performed by PCR amplification and sequencing, as previously described (10). The *fusB* determinant was detected by Southern hybridization (14) with the Alkphos Direct kit (Amersham Biosciences, Amersham, United Kingdom) using the entire *fusB* gene (A. J. O'Neill and I. Chopra, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1064, 2002) as a probe. Further isolates were screened by PCR amplification of a 292-bp fragment of the *fusB* gene using oligonucleotide primers FB(iii) (5′-ATTC AATCGGAAACCTATAATGATA) and FB(iv) (5′-TTATA TATTTCCGATTTGATGCAAG). Using an annealing temperature of 60°C, it was established that these primers generated an amplicon only from strains known to carry *fusB* (data not shown).

Pulsed-field gel electrophoresis analysis (7) of epidemic Fus^r strains from Norway, Sweden, Ireland, and the United Kingdom indicated that they constitute a single clonotype (Fig. 1). The clone exhibited low-level resistance to fusidic acid (MIC, ~4 μg/ml) (Table 1) and carried *fusB*. The *fusB* gene was detected in total DNA preparations from these strains (Fig. 2) but not in plasmid DNA preparations, indicating a chromosomal location for this resistance determinant.

The fusidic acid MIC for the epidemic strains (Table 1) was ~4-fold lower than that associated with strains carrying *fusB* on the archetypal fusidic acid resistance plasmid, pUB101 (MIC of 16 μg of fusidic acid/ml) (3). This is probably due to differences in resistance gene dosage, since chromosomal carriage likely involves a single copy of *fusB*, while pUB101 is typically present at 11 to 14 copies/cell (4).

Other Fus^r strains belonging to this clonotype from dermatology patients in Harrogate, United Kingdom (13), were tested for the presence of *fusB* by PCR. A 292-bp amplicon was generated from strains belonging to the epidemic clonotype (Fig. 1; strains with the prefix H), indicating the presence of *fusB* (data not shown).

The genetic basis of fusidic acid resistance was also examined in nonepidemic isolates recovered from patients undergoing topical fusidic acid treatment for atopic dermatitis. In instances where fusidic acid resistance arose during treatment, both initial (fusidic acid-susceptible) and final (fusidic acid-resistant) isolates were examined.

One nonepidemic strain (CS642) carried *fusB* (Fig. 2) and exhibited low-level resistance (Table 1). As for the epidemic strains, *fusB* appeared to be chromosomally located in strain CS642. Higher-level fusidic acid resistance (MIC > 12 μg/ml) in the nonepidemic strains resulted exclusively from nucleotide substitutions in *fusA* (Table 1). In addition, two low-level Fus^r strains (CS1116 and CS957) were also resistant as a result of mutation in EF-G (Table 1). All mutations identified in EF-G save one (A71T) have previously been shown to confer fusidic

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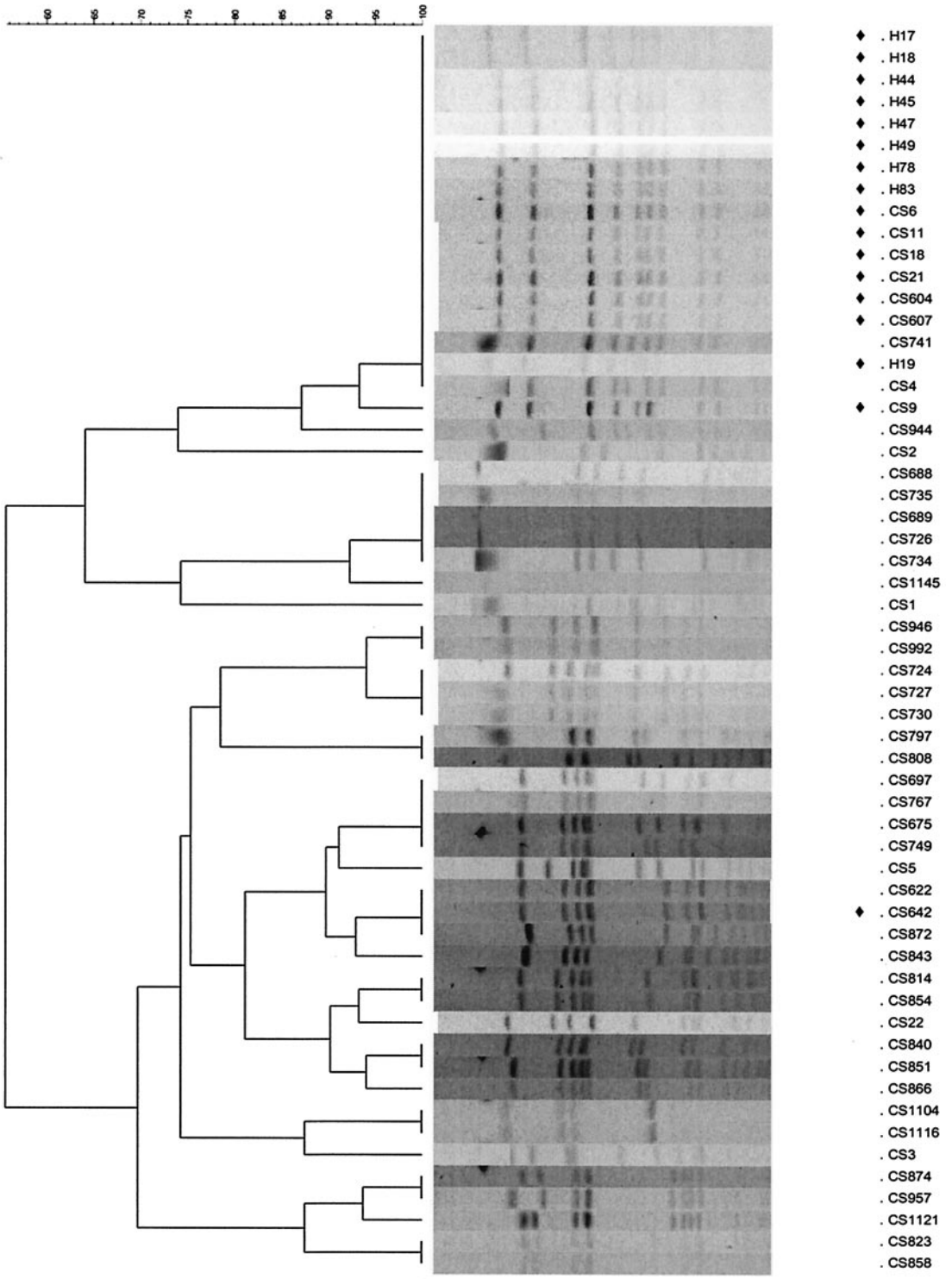

FIG. 1. Dendrogram and individual PFGE profiles showing relationships between *S. aureus* strains employed in this study. The cluster representing the epidemic European clonotype is at the top of the figure. Strains carrying the *fusB* determinant are indicated by the diamonds.

TABLE 1. Mechanisms of fusidic acid resistance in clinical isolates of *S. aureus*[a]

Strain	Country of origin	Reference or source	Fusidic acid MIC (μg/ml)[b]	Mechanism of fusidic acid resistance		
				fusB	Polymorphism(s) in EF-G	Other (undefined)
CS944	Denmark	FCF0001 INT	0.047	−	−	
CS734	Finland	FCF0001 INT	0.094	−	−	
CS675	Belgium	FCF0001 INT	0.125	−	−	
CS823	Spain	FCF0001 INT	0.125	−	−	
CS840	Denmark	FCF0001 INT	0.125	−	−	
CS726	Germany	FCF0001 INT	0.125	−	−	
CS689	Spain	FCF0001 INT	0.19	−	−	
CS749	Belgium	FCF0001 INT	0.19	−	V607I	
CS1121	Denmark	FCF0001 INT	0.47	−	−	
CS1116	Belgium	FCF0001 INT	1.5	−	H457Y (mixed population)	
CS992	Denmark	FCF0001 INT	2	−	−	+
CS642	Germany	FCF0001 INT	2	+	−	
CS730	Finland	FCF0001 INT	3	−	−	+
CS808	France	FCF0001 INT	3	−	−	+
CS866	Denmark	FCF0001 INT	3	−	−	+
CS957	Denmark	FCF0001 INT	3	−	G451V	
CS18	**Norway**	**16**	**3**	**+**	**−**	
CS607	**Denmark**	**FCF0001 INT**	**4**	**+**	**−**	
CS11	**Norway**	**16**	**4**	**+**	**−**	
CS21	**Ireland**	**LEO Pharma Ireland**	**4**	**+**	**−**	
CS6	**Sweden**	**11**	**4**	**+**	**−**	
CS9	**Sweden**	**11**	**4**	**+**	**−**	
H18	**UK**	**13**	**4**	**+**	**−**	
H19	**UK**	**13**	**4**	**+**	**−**	
H44	**UK**	**13**	**4**	**+**	**−**	
H45	**UK**	**13**	**4**	**+**	**−**	
H47	**UK**	**13**	**4**	**+**	**−**	
H49	**UK**	**13**	**4**	**+**	**−**	
H83	**UK**	**13**	**4**	**+**	**−**	
H78	**UK**	**13**	**6**	**+**	**−**	
H17	**UK**	**13**	**8**	**+**	**−**	
CS767	Belgium	FCF0001 INT	12	−	G452S	
CS858	Spain	FCF0001 INT	48	−	H457Y	
CS688	Spain	FCF0001 INT	64	−	H457Y	
CS697	Belgium	FCF0001 INT	64	−	H457Y	
CS735	Germany	FCF0001 INT	96	−	H457Y	
CS741	Finland	FCF0001 INT	96	−	A71T, P404L, L461S	
CS1145	Denmark	FCF0001 INT	>256	−	A67T, H457Y	
CS854	Denmark	FCF0001 INT	>256	−	A67T, H457Y	
CS872	Denmark	FCF0001 INT	>256	−	L461K	
CS874	Denmark	FCF0001 INT	>256	−	L461K	

[a] Representatives of the epidemic clonotype are in bold.
[b] The resistance breakpoint for fusidic acid recommended by the British Society for Antimicrobial Chemotherapy is 2 μg/ml (6).

acid resistance or to participate in compensatory adaptation to the costs of fusidic acid resistance (8, 10). Since an alternative Fus^r substitution at the A71 locus (A71V) has been described (8), the A71T mutation discussed above is likely to confer resistance to fusidic acid. Several mutations (P404L, G451V, and G452S) previously identified only in Fus^r strains derived in vitro (8, 10) were detected here for the first time in clinical *S. aureus* isolates (Table 1).

In four nonepidemic strains exhibiting relatively low-level resistance (CS992, CS730, CS808, and CS866), neither *fusB* nor polymorphic variations in *fusA* were detected. This suggests that while mutations in *fusA* and possession of *fusB* are common routes to fusidic acid resistance in clinical isolates, other mechanisms may also exist.

In conclusion, we have demonstrated that several fusidic acid-resistant clinical isolates of *S. aureus* recovered from patients with impetigo bullosa in European countries represent a

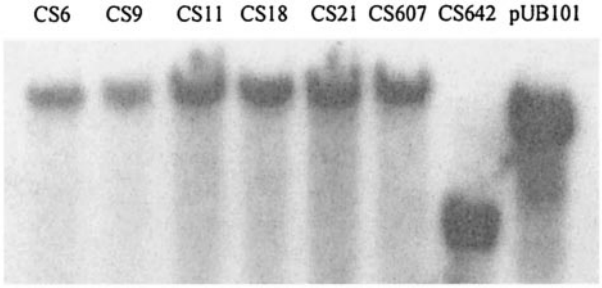

FIG. 2. Detection of *fusB* in total DNA from clinical fusidic acid-resistant *S. aureus* strains. Southern hybridization with EcoRI-digested total DNA derived from six representatives of the epidemic clonotype (CS6 to CS607) and a distinct strain (CS642). Plasmid pUB101 is the positive control.

clonal epidemic strain carrying the *fusB* determinant on the chromosome. Mutations in *fusA* were identified in nonepidemic fusidic acid-resistant strains, but such mutations were not identified in the clonal epidemic strain. The factors favoring dissemination of the epidemic clonal strain have yet to be identified.

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