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Variable Sensitivity to Bacterial Methionyl-tRNA Synthetase Inhibitors Reveals Subpopulations of *Streptococcus pneumoniae* with Two Distinct Methionyl-tRNA Synthetase Genes

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As reported previously (J. R. Jarvest et al., J. Med. Chem. 45:1952-1962, 2002), potent inhibitors (at nanomolar concentrations) of *Staphylococcus aureus* methionyl-tRNA synthetase (MetS; encoded by *metS1*) have been derived from a high-throughput screening assay hit. Optimized compounds showed excellent activities against staphylococcal and enterococcal pathogens. We report on the bimodal susceptibilities of *S. pneumoniae* strains, a significant fraction of which was found to be resistant (MIC, ≥8 mg/liter) to these inhibitors. Using molecular genetic techniques, we have found that the mechanism of resistance is the presence of a second, distantly related MetS enzyme, MetS2, encoded by *metS2*. We present evidence that the *metS2* gene is necessary and sufficient for resistance to MetS inhibitors. PCR analysis for the presence of *metS2* among a large sample ($n = 315$) of *S. pneumoniae* isolates revealed that it is widespread geographically and chronologically, occurring at a frequency of about 46%. All isolates tested also contained the *metS1* gene. Searches of public sequence databases revealed that *S. pneumoniae* MetS2 was most similar to MetS in *Bacillus anthracis*, followed by MetS in various non-gram-positive bacterial, archaeal, and eukaryotic species, with streptococcal MetS being considerably less similar. We propose that the presence of *metS2* in specific strains of *S. pneumoniae* is the result of horizontal gene transfer which has been driven by selection for resistance to some unknown class of naturally occurring antibiotics with similarities to recently reported synthetic MetS inhibitors.

The development of antimicrobial compounds with novel modes of action is critical to the treatment of bacterial infections, which are increasingly showing broad resistance to the available agents used for therapy. Particularly promising bacterial targets are the aminoacyl-tRNA synthetases (13), which serve in protein synthesis for the attachment of an amino acid to its cognate tRNA. The natural product compound mupirocin (pseudomonic acid) is a specific inhibitor of bacterial isoleucyl-tRNA synthetase (6) and is used as a topical antibiotic against *Staphylococcus aureus* infections (15).

In our search for novel antibiotics effective against gram-positive coccal bacteria, we have undertaken high-throughput screening of small-molecule libraries for inhibitors of each aminoacyl-tRNA synthetase from *S. aureus*. We recently reported on a potent series of inhibitors of methionyl-tRNA synthetase (MetS; encoded by the gene *metS*) (11). This series of compounds exhibited activity against whole cells of a variety of gram-positive bacteria. Additionally, one compound was active in animal models. Whole-cell activity was shown by both physiological and genetic techniques to be due to MetS inhibition.

Although the MetS inhibitors are potent against a large number of strains of *S. aureus* and *Enterococcus* sp. isolates, the MICs of the MetS inhibitors for *Streptococcus pneumoniae*, as reported here, exhibited a broad distribution. We found that the MICs of a given compound could vary from 0.5 to >64 μg/ml for otherwise antibiotic-susceptible strains. Heterogeneous activity against strains of an important human pathogen such as *S. pneumoniae* is not a desirable trait for an antibiotic, so we embarked on a study to determine the cause of resistance. Here we show that resistance is due to the presence of a second MetS enzyme, MetS2, which is resistant to the compounds active against MetS1 and whose gene is widespread among clinical isolates of *S. pneumoniae.*

MATERIALS AND METHODS

Strains, media, and growth conditions. The *S. pneumoniae* strains used in the study described in this report were R6 (a commonly used laboratory strain), QA1442, and their derivatives. *S. pneumoniae* QA1442 was chosen for this study not only because of its resistance to MetS inhibitors but also because it is highly transformable. QA1442 is a member of the set of 40 strains originally tested for their sensitivities to MetS inhibitors. This set is from our Microbiology departmental strain collection and is used for routine profiling of antimicrobial compounds. Also used, where indicated, were clinical isolates collected as part of the Alexander Project, a global surveillance program for the monitoring of antibacterial resistance in key respiratory pathogens (5). *S. pneumoniae* was routinely propagated in THY medium (Todd-Hewitt medium supplemented with 0.5% yeast extract) at 37°C. MICs were determined by the broth microdilution method (11).

Isolation of SB-362916-sensitive mutants. Strain QA1442 was mutagenized with 2% ethyl methanesulfonate. Mutagenized samples were subjected to three rounds of penicillin enrichment, as follows. Exponentially growing cells at an A_{600} of about 0.3 were treated with SB-362916 at a final concentration of 10 μg/ml, followed 30 min later by treatment with penicillin G at a final concentration

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of 100 μg/ml. After 2 to 4 h of treatment, the penicillin G was removed by extensive washing and a second round of enrichment was commenced. Alternatively, samples of enriched cultures were frozen at −80°C in the presence of 10% glycerol and regrown for further enrichment. Survivors were plated on Trypticase soy agar–5% sheep blood agar plates, and the colonies were scored by their ability to grow in THY medium containing 10 μg of SB-362916 per ml. After three rounds of enrichment, approximately 20% of the survivors were sensitive to SB-362916. Two isolates, named QS1 and QS2, were further used in this study.

Transformation of *S. pneumoniae*. A total of 10^6 *S. pneumoniae* R6 competent cells were incubated with DNA at 30°C for 30 min in the presence of 1 mg of competence-stimulating heptadecapeptide per ml by published methods (8) and transferred to 37°C for 90 min to allow expression of antibiotic resistance. The transformation mixtures were plated onto AGCH agar (12) containing antibiotic and were incubated at 37°C for 36 h under 5% CO_2.

Preparation of an enriched genomic library. Samples of genomic DNA digested to completion with different restriction enzymes were tested for their abilities to confer resistance to sensitive isolate QS1. It was found that *Hin*dIII-digested DNA was capable of transforming a sensitive strain, indicating that the target gene and sufficient flanking sequences for recombination lacked a *Hin*dIII restriction site. *Hin*dIII-digested DNA was then fractionated by sucrose density centrifugation, and fractions were tested for their abilities to transform QS1 to SB-362916 resistance. A fraction of *Hin*dIII-digested genomic DNA that conferred resistance (size range, 2 to 8 kb) was used to construct an enriched library in *S. pneumoniae-Escherichia coli* shuttle vector pDL278 (12a). Strain QS1 was then transformed with this library. The transformation mixture was plated onto medium containing either 25 or 40 μg of SB-362916 per ml (6 and 10 times the MIC, respectively).

Generation of *S. pneumoniae metS* allelic replacement mutants. Chromosomal DNA fragments (500 bp) flanking the genes of interest were amplified from *S. pneumoniae* QA1442 chromosomal DNA by PCR. Primers were designed so that flanking genes and potential promoters would remain intact in the deletion mutant to minimize polar effects. The fragments were used to make allelic replacement constructs in which they flanked the erythromycin resistance gene (*ermAM*) from pAMβ1 or the chloramphenicol acetyltransferase gene from pC194. *S. pneumoniae* QA1442 competent cells were prepared and transformed in the presence of 1 mg of competence-stimulating heptadecapeptide per ml by published methods (8).

To generate allelic replacement mutants, a total of 10^6 *S. pneumoniae* QA1442 competent cells were incubated with 500 ng of the allelic replacement construct at 30°C for 30 min and transferred to 37°C for 90 min to allow expression of antibiotic resistance. The transformation mixtures were plated in AGCH agar (12) containing 1 μg of erythromycin per ml or 2.5 μg chloramphenicol per ml and were incubated at 37°C for 36 h under 5% CO_2. Chromosomal DNA was prepared from the *metS1* deletion mutants and was used to transform *S. pneumoniae* QA1442, from which *metS2* was deleted, in the presence of 1 mg of competence-stimulating heptadecapeptide per ml. Similarly, DNA from the *metS2* deletion mutant was used to transform the *metS1* null strain. If no transformants were obtained in three separate transformation experiments with positive allelic replacement and transformation controls, the target gene was considered to be essential in vitro under the conditions chosen. Antibiotic-resistant *S. pneumoniae* colonies were picked and grown overnight in Todd-Hewitt broth (Difco) supplemented with 5% (wt/vol) yeast extract.

Chromosomal DNA from putative *S. pneumoniae* allelic replacement mutants was examined by both Southern blotting and diagnostic PCR analyses to verify that the appropriate chromosomal DNA rearrangement had occurred. By the former method, flanking DNA fragments labeled by using the enhanced chemiluminescence random-prime labeling kit (Amersham Pharmacia Biotech) were used as probes for chromosomal DNA restricted with appropriate enzymes and blotted by standard methods (14). By the latter method, DNA primers designed to hybridize within the antibiotic resistance determinant were paired with primers hybridizing to distal chromosomal sequences to generate DNA amplification products of characteristic sizes. DNA fragments were directly sequenced in both directions by using 3100 or 3700 automated sequencers (Applied Biosystems).

Sequence analysis. We found other methionyl-tRNA synthetase sequences in public databases, including partial genome sequences (National Center for Biotechnology Information), using *S. pneumoniae* MetS2 as the query sequence. Searches for both translated open reading frames and complete DNA sequences were conducted by using the programs BLASTP and TBLASTN, respectively (1). Preliminary sequence data for *Bacillus anthracis* (Ames strain) were obtained from The Institute for Genomic Research through its website (http://www.tigr.org). The sequences were initially aligned by using the CLUSTALW program (version 1.8) (2) and were then manually refined by using the SEQLAB program of the GCG package (version 10.2; Genetics Computer Group, Madison Wis.). Sequence comparisons are based on the BLOSUM62 weighting matrix and the lengths of the shorter sequence without gaps.

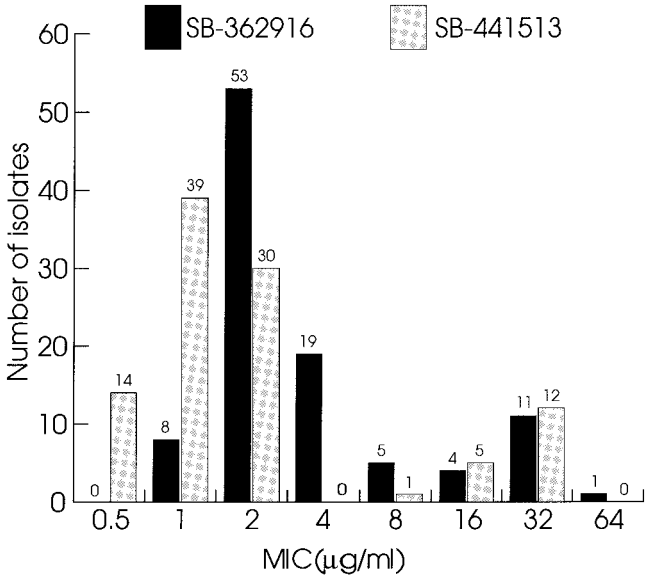


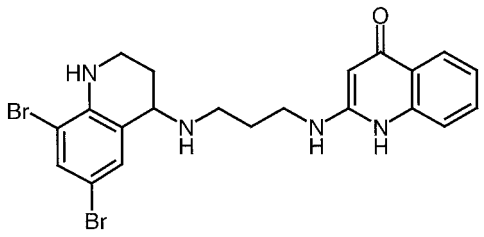
FIG. 1. Distribution of resistance in 101 *S. pneunomiae* isolates to three synthetic small-molecule inhibitors of MetS. Resistance to MetS inhibitors SB-362916, SB-430537, and SB-441513 is measured as the MIC. The absolute numbers of isolates are shown above each bar.

Identification of *metS2* among clinical isolates. *S. pneumoniae* isolates were obtained from the collections of the Alexander Project (5). The earliest (1992) to the most recent (1999) isolates ($n = 315$) available from a wide geographical range were selected. Detection of the two loci in different strains was done by separate amplification reactions by PCRs with primers specific for *metS1* (primer metS1F [5′-CATATCGGTTCTGCCTACACAACTAT-3′] and primer metS1R [5′-CTCGATGAAGTTGCGTAGCATTTCATT-3′]) and *metS2* (primer metS2F [5′-GCAAACGGTTCGTTACATATTGGTCA-3′] and primer metS2R [5′-CTCAGGAGCATTTATTGTTAGGAAGTA-3′]), which amplified DNA fragments of 565 and 1,054 kb, respectively. Colonies on blood agar were transferred directly to PCR mixtures (PCR SuperMix High Fidelity; Gibco-BRL), which were set up according to the conditions recommended by the vendor. An initial cycle of 3 min of 94°C was followed by 35 cycles of 30 s of 94°C, 45 s of 50°C, and 1 min of 72°C, with a final cycle of 2 min of 72°C. All experiments were run in duplicate with positive and negative amplification controls. Following electrophoresis on a 1.0% agarose gel, the gels were evaluated for the presence or absence of DNA fragments of the proper length. DNA fragments from 10 randomly selected experiments were sequenced to confirm that the *metS1* and *metS2* genes were the proper amplification products.

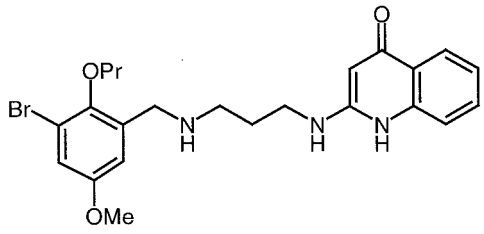
Nucleotide sequence accession number. The *S. pneumoniae metS2* gene is available from GenBank under accession number AY198311.

RESULTS AND DISCUSSION

In our initial survey, 11 of 40 *S. pneunomiae* isolates were significantly more resistant to a class of synthetic MetS inhibitors (MICs, 8 μg/ml or higher). An extended profile was initiated to get a better indication of the distribution of the MICs. Of 101 clinical isolates recently collected from different clinics worldwide, about 20% were resistant to MetS inhibitors (as defined by MICs ≥8 μg/ml) (Fig. 1). Resistance to antimicrobial compounds is frequently due to target-based mutations. However, analysis of the sequences of the *metS1* genes from a number of resistant isolates failed to reveal any consistent differences from those of the *metS1* genes of sensitive strains (data not shown), making a target-based mechanism of resis-



SB-362916



SB-441513

FIG. 2. Structures of SB-362916 and SB-441513. Me, methyl; Pr, propyl.

tance unlikely. For those strains the profiles of resistance to known antibiotics were not correlated with resistance to MetS inhibitors, which led us to conclude that the observed MICs were not the result of some multidrug resistance mechanism. These findings strongly suggested that the resistance mechanism was novel and unique to MetS inhibitors. A genetic approach was therefore undertaken to identify it.

The strategy that we devised to identify the gene or genes required for resistance is based on the genetic transformation of a sensitive strain to resistance with DNA isolated from a resistant strain. Compound SB-362916 (Fig. 2), typical of the class of MetS inhibitors derived from the high-throughput screening effort, was used throughout this study. First attempts at transformation of sensitive strain R6 with genomic DNA from a resistant strain failed. This raised the possibility that the resistance determinant was part of an island of DNA not found in the genome of the sensitive strain; thus, DNA fragments with resistance factors could not recombine with the genome of the sensitive host. To address this, we isolated SB-362916-sensitive mutants of the normally resistant strain *S. pneumoniae* QA1442. The MICs for eight SB-362916-sensitive isolates were 4 μg/ml, whereas the MIC for the parent strain was 128 μg/ml. One of these sensitive isolates, QS1, was selected for further studies. Genomic DNA from QA1442 was capable of transforming QS1 to resistance, suggesting that the determinant is carried on a single locus (or on multiple tightly linked loci). To identify the allele responsible, an enriched library was constructed as described in Materials and Methods and was used to isolate clones capable of conferring SB-362916 resistance to QS1 (the scheme used to identify the resistance locus is outlined in Fig. 3). Screening of eight SB-362916-resistant colonies revealed that the sizes of the plasmid inserts were 1.5 to 4 kb. Five of the recombinant plasmids containing approximately 3-kb inserts were selected and used to transform *S. pneumoniae* R6. Of the clones tested, four were capable of transforming laboratory strain *S. pneumoniae* R6 to SB-362916 resistance. Sequence analysis of two of the plasmids that conferred SB-362916 resistance showed that they contained identical *Hin*dIII fragments. Further analysis of the 3-kb insert showed that it contained only one complete open reading frame, which encodes a novel MetS protein. To distinguish the two enzyme-encoding genes and their products, the previously identified gene is defined as *metS1* and encodes MetS1. The second gene is designated *metS2* and encodes the putative drug-resistant synthetase MetS2 (see below).

To confirm that the *metS*2 gene encodes an enzyme (MetS2) resistant to SB-362916, we constructed deletion mutants of strain *S. pneumoniae* QA1442 from which both *metS1* and *metS2* were deleted by allelic replacement. The ability to isolate deletion mutants from which each gene by itself is deleted indicates that both genes are functional and can complement each other, and consequently, neither gene is essential for viability in this strain background. A double-deletion mutant could not be generated, consistent with the expectation that no other source of MetS activity is present. We could not isolate a *metS1* deletion mutant in laboratory strain *S. pneumoniae* R6, strongly suggesting that *metS1* is essential in strains in which *metS2* is lacking.

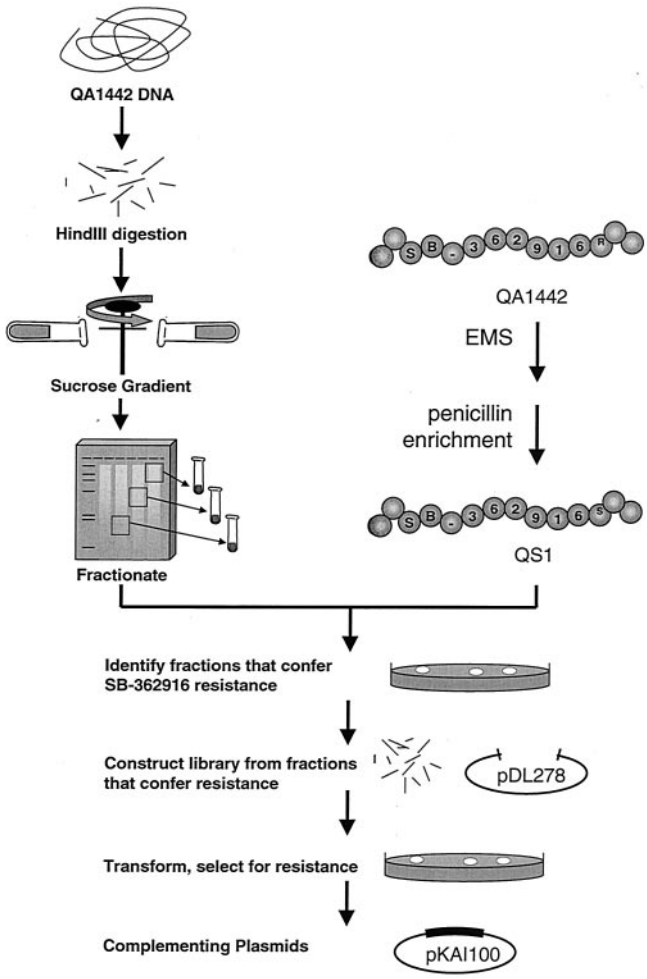


FIG. 3. Scheme used to identify the resistance locus. EMS, ethyl methanesulfonate

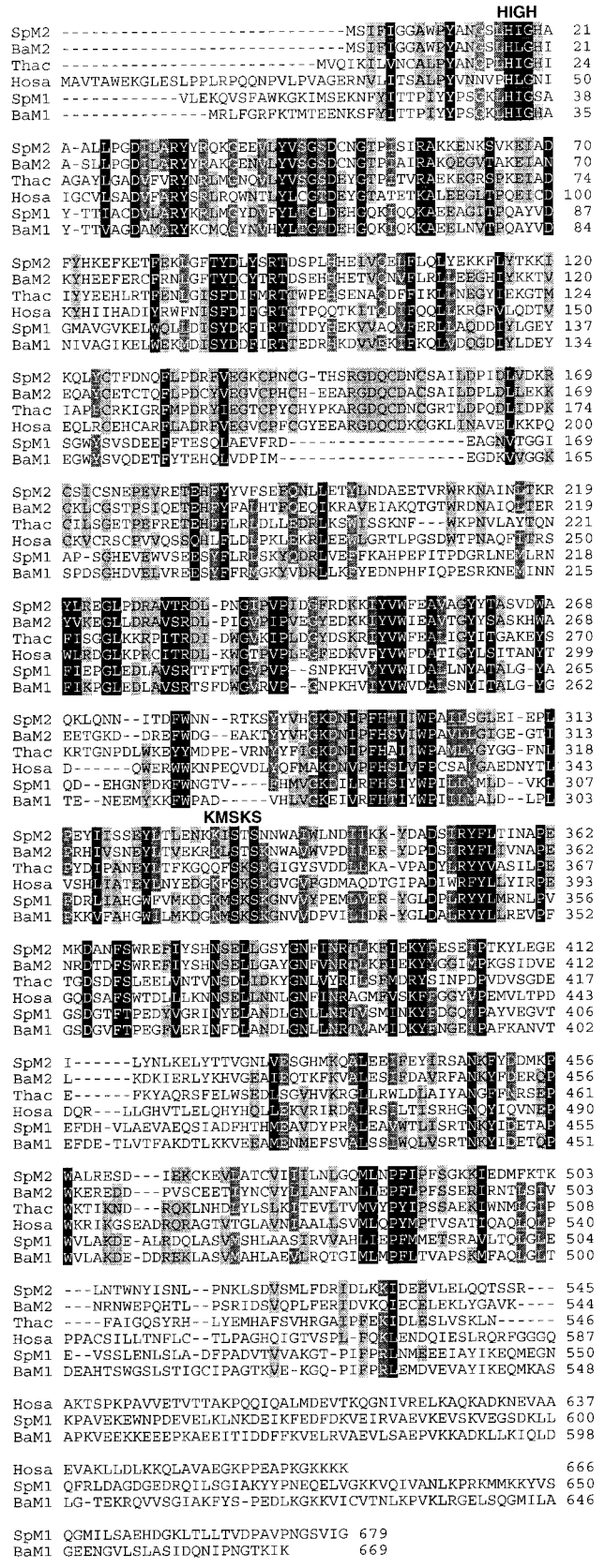


FIG. 4. Multiple-sequence alignment of MetS. The sequences shown are those of *S. pneumoniae* MetS1 (SpM1) and MetS2 (SpM2) and examples of closely related orthologs in public genome databases, *B. anthracis* MetS1 (BaM1) and MetS2 (BaM2), *Thermoplasma acidophilum* (Thac), a member of the domain *Archaea*, and *Homo sapiens* (Hosa). The locations of the conserved amino acid motifs HIGH and KMSKS, which are signatures for class I aminoacyl-tRNA, are indicated above the sequences. The dark, medium, and light shadings represent high (100%), medium (80%), and low (60%) levels of amino acid conservation, respectively. The figure was prepared with GeneDoc software (version 2.6002; K. B. Nicholas and H. B. Nicholas, Jr., 2000 [distributed by the authors at www.psu.edu/biomed/genedoc]).

TABLE 1. MICs of various compounds for *S. pneumoniae* QA1442 and its mutants

Strain	MIC (μg/ml)			
	SB-362916	Tetracycline	Chloramphenicol[a]	Erythromycin
QA1442	>64	0.5	4	0.125
QA1442 Δ*metS1*	>64	0.5	4	>64
QA1442 Δ*metS2*	4	0.5	32	0.125
QA1442 QS1	4	0.5	4	0.125
QA1442 QS2	4	0.5	4	0.125

[a] The increased MICs of erythromycin and chloramphenicol for QA1442 Δ*metS1* and QA1442 Δ*metS2,* respectively, are due to the resistance cassette used for gene disruption.

MIC analysis was performed with the QA1442 *metS1* and *metS2* deletion mutants. As shown in Table 1, deletion of *metS2* completely abolished resistance to SB-362916, while deletion of *metS1* had no effect on the MIC. We also sequenced *metS2* from strains QS1 and QS2, sensitive variants of QA1442, and found alterations in one of two highly conserved residues, R35H and P487S. Taken together with the observed MICs of SB-362916 for these two sensitive mutants, which were identical to the MIC for the *metS2* deletion mutant, we conclude that these mutants are likely *metS2* null strains. Further evidence that MetS2 is responsible for the observed resistance is the finding that the recombinant MetS2 enzyme is resistant to MetS1 inhibitors in vitro (data not shown).

The amino acid sequence of *S. pneumoniae* MetS2 is highly distinctive from those of the MetS1 proteins typically found in *S. pneumoniae* and other gram-positive bacteria (Fig. 3). Searches of public sequence databases for sequences homologous with the MetS2 sequence revealed that the closest homologs to MetS2 were MetS from members of the domain *Archaea*, eukaryotes, and non-gram-positive bacteria such as *Chlamydia* and spirochetes, with one exception. Interestingly, *B. anthracis* has a MetS2 protein that was similar (65%) to that of *S. pneumoniae*. In addition to various amino acid substitutions, all MetS2-type proteins, including those of *B. anthracis* and *S. pneumoniae*, differ from MetS1-type proteins by having an inserted sequence of 17 or 18 amino acids between residues F173 and N192 of the human MetS protein (Fig. 4). Furthermore, *B. anthracis* has a second, more typical *metS1* locus which is similar to those found in other *Bacillus* species as well as *S. pneumoniae metS1*. A comparison of the amino acid sequence similarities suggests that *metS2* did not arise from a recent gene duplication event after the speciation of *S. pneumoniae*. We suggest that *metS2* was probably acquired by *S. pneumoniae* from a distantly related species via horizontal gene transfer. A more extensive evolutionary analysis of MetS will be presented elsewhere.

TABLE 2. Occurrence of *metS2* loci in *S. pneumoniae* clinical isolates

Country	Yr of isolation	No. of isolates in which *metS2* is[a]:			Frequency of *metS2*
		Present	Absent	Total	
France	1992	3	8	11	0.27
	1993	9	11	20	0.45
	1994	7	7	14	0.50
	1995	9	4	13	0.69
	1996	7	6	13	0.54
	1997	6	9	15	0.40
	1998	4	11	15	0.27
Germany	1992	1	3	4	0.25
	1994	0	5	5	0.00
	1997	2	1	3	0.67
	1998	1	5	6	0.17
Italy	1994	1	1	2	0.50
	1995	3	2	5	0.60
	1996	3	7	10	0.30
	1997	3	4	7	0.43
	1998	0	3	3	0.00
Spain	1992	2	3	5	0.40
	1993	3	4	7	0.43
	1994	6	1	7	0.86
	1995	9	1	10	0.90
	1996	4	1	5	0.80
	1997	6	2	8	0.75
United Kingdom	1992	2	2	4	0.50
	1993	1	7	8	0.13
	1994	1	2	3	0.33
	1995	10	2	12	0.83
	1996	6	2	8	0.75
	1997	7	3	10	0.70
	1998	21	38	59	0.36
United States	1992	0	1	1	0.00
	1997	7	10	17	0.41
Hong Kong	1997	0	5	5	0.00
Total		144	171	315	0.46

[a] The isolates were evaluated for the presence or absence of *metS2* by PCR amplification experiments.

Database searches failed to reveal other examples of *metS2* in gram-positive coccal species; thus, we assume that this locus is specific to particular *S. pneumoniae* strains. In order to evaluate the global distribution of *metS2*, we surveyed 315 clinical *S. pneumoniae* isolates collected as part of the Alexander Project surveillance program for the occurrence of *metS1* and *metS2* using PCR with specific DNA primer pairs. These isolates were obtained from various clinics in seven different countries between 1992 and 1998. We found the *metS2* locus to be very widespread in terms of both geography and time (Table 2), being detected in 46% of all isolates tested. Insufficient sample size is the likely explanation for the few missing occurrences of *metS2* in particular country and year combinations.

The rate of occurrence of *metS2*, as determined by PCR, in clinical isolates is higher (46%) than that which would be predicted from MIC data collected in earlier studies (20%). Larger sample sizes might result in more consistent results between the two methods. Also, there could be variability with respect to resistance to MetS inhibitors among *metS2* gene products, or *metS2* may not be expressed in all strains that possess it. More extensive MIC profiling and PCR surveys would be required to test these alternative hypotheses.

All Alexander Project isolates have been tested for resistance to at least 15 commercially available antibiotics (5). We did not find any trend in the occurrence of *metS2* and resistance or susceptibility to existing classes of antibiotics. Therefore, *metS2* does not appear to be associated with known mechanisms of drug resistance or temporal or geographical segregation. Interestingly, the typical *metS1* locus was found in all isolates surveyed, which supports the hypothesis that *metS1* was present in the ancestor of *S. pneumoniae*, while *metS2* was horizontally transferred into specific *S. pneumoniae* strains.

Dual copies of a few types of aminoacyl-tRNA synthetases have been reported for some bacteria, such as tyrosyl-, threonyl-, and histidyl-tRNA synthetases in the gram-positive bacterium *Bacillus subtilis*, all of which are duplications within the domain *Bacteria* and were not acquired by horizontal transfer from an archaebacterium or eukaryote (for reviews, see references 2 and 17). An exception is the acquisition of plasmids with eukaryote-like isoleucyl-tRNA synthetases by certain strains of *S. aureus* (3). Similar to MetS, this divergent isoleucyl-tRNA synthetase conveys resistance to an antibiotic, pseudomonic acid (mupirocin), which is the derivative of a natural product (4, 7, 9). However, unlike mupirocin, recently developed MetS inhibitors are totally synthetic compounds and do not resemble any known classes of natural antibiotics (11). Thus, bacteria have never been exposed to these compounds in either clinical or natural environments. The widespread occurrence of the *metS2* gene suggests that it must contribute some selective advantage to those strains harboring it. It is therefore possible that MetS is inhibited by some unknown class of natural product antibiotics and that *S. pnuemoniae* acquired *metS2* as a countermeasure to such compounds.

Our study shows the great importance of understanding intraspecific genomic variation in pathogenic bacteria. Although the complete genome sequences of two different *S. pneumoniae* strains are available, neither had MetS2 or any indication of duplicate aminoacyl-tRNA synthetases (10, 16). As witnessed here, a large number of *S. pneumoniae* strains are capable of incorporating genes from diverse exogenous species. This gene incorporation introduces considerable intraspecific genetic variation and, potentially, seemingly novel modes of resistance. Thus, in the development of novel antimicrobial compounds, it is critical to extensively survey clinical bacterial isolates for unusual patterns of resistance. Any resistant strains that are observed should be subjected to a thorough molecular biological analysis in order to understand the underlying mechanisms of resistance and intraspecific genetic variation.

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REFERENCES

1. **Altschul, S. F., T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman.** 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25:**3389–3402.
2. **Brown, J. R.** 1998. Aminoacyl-tRNA synthetases. Evolution of a troubled family, p. 217–230. *In* J. Wiegel and M. W. W. Adams (ed.), Thermophiles: the keys to molecular evolution and the origin of life? Taylor and Francis Ltd., London, United Kingdom.
3. **Brown, J. R., J. Zhang, and J. E. Hodgson.** 1998. A bacterial antibiotic resistance gene with eukaryotic origins. Curr. Biol. **8:**R365–R367.
4. **Dyke, K. G. H., S. P. Curnock, M. Golding, and W. C. Noble.** 1991. Cloning of the gene conferring resistance to mupirocin in *Staphylococcus aureus*. FEMS Microbiol. Lett. **77:**195–198.
5. **Felmingham, D., and J. Washington.** 1999. Trends in the antimicrobial susceptibility of bacterial respiratory tract pathogens—findings of the Alexander Project 1992–1996. J. Chemother. **11:**5–21.
6. **Fuller, A. T., G. Mellows, M. Woolford, G. T. Banks, K. D. Barrows, and E. B. Chain.** 1971. Pseudomonic acid: an antibiotic produced by *Pseudomonas fluorescens*. Nature **234:**416–417.
7. **Gilbart, J., C. R. Perry, and B. Slocombe.** 1993. High-level mupirocin resistance in *Staphylococcus aureus*: evidence for two distinct isoleucyl-tRNA synthetases. Antimicrob. Agents Chemother. **37:**32–38.
8. **Havarstein, L. S., G. Coomaraswamy, and D. A. Morrison.** 1995. An unmodified heptadecapeptide pheromone induces competence for genetic transformation in *Streptococcus pneumonia*e. Proc. Natl. Acad. Sci. USA **92:** 11140–11144.
9. **Hodgson, J. E., C. P. Curnock, K. G. Dyke, R. Morris, D. R. Sylvestor, and M. S. Gross.** 1994. Molecular characterization of the gene encoding high-level mupirocin resistance in *Staphylococcus aureus* J2870. Antimicrob. Agents Chemother. **38:**1205–1208.
10. **Hoskins, J., W. E. Alborn, Jr., J. Arnold, L. C. Blaszczak, S. Burgett, B. S. DeHoff, S. T. Estrem, L. Fritz, D. J. Fu, W. Fuller, et al.** 2001. The genome of the bacterium *Streptococcus pneumoniae* strain R6. J. Bacteriol. **183:**5709–5717.
11. **Jarvest, J. R., J. M. Berge, V. Berry, H. F. Boyd, M. J. Brown, J. S. Elder, A. K. Forrest, A. P. Fosberry, D. R. Gentry, M. J. Hibbs, D. D. Jaworski, P. J. O'Hanlon, A. J. Pope, S. Rittenhouse, R. J. Sheppard, C. Slater-Radosti, and A. Worby.** 2002. Nanomolar inhibitors of *Staphylococcus aureus* methionyl tRNA synthetase with potent antibacterial activity against gram-positive pathogens. J. Med. Chem. **45:**1952–1962.
12. **Lacks, S.** 1966. Integration efficiency and genetic recombination in pneumococcal transformation. Genetics **53:**207–235.

12a. **Leblanc, D. J., L. N. Lee, and A. Abu-Al-Jaibat.** 1992. Molecular genetic, and functional analysis of the basic replicon of pVA380–1, a plasmid of oral streptococci origin. Plasmid **28:**130–145.

13. **Payne, D. J., N. B. Wallis, D. R. Gentry, and M. Rosenberg.** 2000. The impact of genomics on novel antibacterial targets. Curr. Opin. Drug Disc. Dev. **3:** 177–190.
14. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
15. **Sutherland, R., R. J. Boon, K. E. Griffin, P. J. Masters, B. Slocombe, and A. R. White.** 1985. Antibacterial activity of mupirocin (pseudomonic acid), a new antibiotic for topical use. Antimicrob. Agents Chemother. **27:**495–498.
16. **Tettelin, H., K. E. Nelson, I. T. Paulsen, J. A. Eisen, T. D. Read, S. Peterson, J. Heidelberg, R. T. DeBoy, D. H. Haft, R. J. Dodson, et al.** 2001. Complete genome sequence of a virulent isolate of Streptococcus pneumoniae. Science **293:**498–506.
17. **Woese, C. R., G. J. Olsen, M. Ibba, and D. Söll.** 2000. Aminoacyl-tRNA synthetases, the genetic code, and the evolutionary process. Microbiol. Mol. Biol. Rev. **64:**202–236.