Characterization of a Staphylococcal Plasmid Related to pUB110 and Carrying Two Novel Genes, vatC and vgbB, Encoding Resistance to Streptogramins A and B and Similar Antibiotics

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We isolated and sequenced a plasmid, named pIP1714 (4,978 bp), which specifies resistance to streptogramins A and B and the mixture of these compounds. pIP1714 was isolated from a Staphylococcus cohnii subsp. cohnii strain found in the environment of a hospital where pristinamycin was extensively used. Resistance to both compounds and related antibiotics is encoded by two novel, probably cotranscribed genes, vatC, encoding a 212-amino-acid (aa) acetyltransferase that inactivates streptogramin A and that exhibits 58.2 to 69.8% aa identity with the Vat, VatB, and SatA proteins, and (ii) vgbB, encoding a 295-aa lactonase that inactivates streptogramin B and that shows 67% aa identity with the Vgb lactonase. pIP1714 includes a 2,985-bp fragment also found in two rolling-circle replication and mobilizable plasmids, pUB110 and pBC16, from gram-positive bacteria. In all three plasmids, the common fragment was delimited by two direct repeats of four nucleotides (GGGC) and included (i) putative genes closely related to repB, which encodes a replication protein, and to pre(mob), which encodes a protein required for conjugative mobilization and site-specific recombination, and (ii) sequences very similar to the double- and single-strand origins (dso, sso₉₈) and the recombination site, RS₈. The antibiotic resistance genes repB and pre(mob) carried by each of these plasmids were found in the same transcriptional orientation.

Streptogramins and related antibiotics are produced by streptomycetes and are classified as A and B compounds according to their basic primary structure (9). Compounds of the A group, including streptogramin A (SgA), pristinamycin IIA (PIIA), virginiamycin M, mikamycin A, and synergistin A, are polyunsaturated cyclic macrolactones. Compounds of the B group, including streptogramin B (SgB), pristinamycin IB (PIB), virginiamycin S, mikamycin B, and synergistin B, are cyclic peptide macroactones. A and B compounds are bacteriostatic when used separately, but they can act in synergy to become bactericidal, mainly against gram-positive bacteria. Natural mixtures such as pristinamycin (Pt), synergistin, virgiamycin, and mikamycin are used orally and topically. A semisynthetic injectable streptogramin (Synercid), consisting of a mixture of derivatives of A and B compounds (dalfopristin and quinupristin, respectively), is currently undergoing in vivo clinical trials and evaluation by the U.S. Food and Drug Administration (see the entire volumes of the Journal of Antimicrobial Chemotherapy [volume 30, Suppl. A, 1992, and volume 39 Suppl. A, 1997]). In this study, pristinamycins (PIIA, PIB, and Pt) were used to evaluate the levels of resistance to A and B compounds and to the synergistic mixtures of these compounds. The MICs of dalfopristin and quinupristin, which are the derivatives of PtB, are similar to those of PIIA and PIB, respectively.

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MATERIALS AND METHODS

Bacterial strains and plasmids. S. cohnii subsp. cohnii BM10711 was isolated from a cupboard in Douera Hospital in Algiers, Algeria (23). It is resistant to macrolides, lincosamides, streptogramins A and B and their mixture, β-lactams due to penicillinase production and the presence of the mecA gene, tetracycline, trimethoprim, sulfonamide, cadmium salts, sodium arsenate, ammonium bromide, and ethidium bromide. The 53 staphylococcal isolates resistant to A components screened for the presence of vatC and vgbB genes include BM10711 (23) and the 52 isolates described previously (1) (S. aureus, 32 isolates; Staphylococcus epidermidis, 14 isolates; Staphylococcus haemolyticus, 4 isolates; Staphylococcus simulans, 1 isolate; S. cohnii subsp. urealeticum, 1 isolate). S. aureus RN4220 (22) and Escherichia coli TG1 (16) were used as recipients.

Plasmids pIP1714 and pIP1742 (this study) were isolated from BM10711. The shuttle vector pOXS7, also named pOXS300 (14), consisting of pUC18 and pBluescript II. The underlined sequence is the BamHI site); oligonucleotide Q, 5′-CTGATCTGAATC-3′ (the underlined sequence is the PstI site); oligonucleotide R, 5′-GTGGGAGAAGCATACC-3′, 5′-GGTCTATGTTGAGATATTCGCAAAATCAGCAAGG-3′ (the underlined sequence is the EcoRI site). Primer pair O-P was designed to amplify a 581-bp fragment of pIP1714 as described previously (2). The samples were loaded onto the sequencer and run for 12 h in a 4.5% denaturing acrylamide gel.

For the sequencing of vatC, the following primers were used: 5′-GAATTCGCAAAATCAGCAAGG-3′, 5′-ATGCCTAGTTGAGATATTCGCAAAATCAGCAAGG-3′, 5′-CA GGCATGTTGAGATATTCGCAAAATCAGCAAGG-3′, and 5′-CTGATCTGAATC-3′. For the sequencing of vgbB, the following primers were used: 5′-GTTCCTATGTTGAGATATTCGCAAAATCAGCAAGG-3′, 5′-GTCGTT TGTATATCAGTTGAGATATTCGCAAAATCAGCAAGG-3′, 5′-TCTGATCTGAATC-3′, and 5′-TGTCAGTTGAGATATTCGCAAAATCAGCAAGG-3′.

Sequence analysis. The aa sequence was analyzed with the Genetics Computer Group package. The aa sequences of vatC and vgbB were compared by using the program FASTA with those deduced from nucleotide sequences in the GenBank-EMBL Data Library. The aa sequences were aligned according to the algorithms in the Clustal V package.

Nucleotide sequence accession number. The sequence of plasmid pIP1714 described in this paper has been deposited in the GenBank-EMBL Data Library under accession no. AF015628.

RESULTS AND DISCUSSION

Isolation of plasmids pIP1714 and pIP1742 harbored by S. cohnii subsp. cohnii BM10711. Two plasmids harbored by BM10711 were introduced separately by electroporation into recipient strain S. aureus RN4220. pIP1714 was found in a transformant selected on brain heart infusion agar supplemented with 10 μg of PIIA per ml and pIP1742 was found in a transformant selected on brain heart infusion agar supplemented with 20 μg of PIB per ml. Plasmid pIP1714 conferred resistance to PIIA (MIC, 3 μg/ml) and PIB (MIC, 1 μg/ml), whereas plasmid pIP1742 conferred constitutive resistance to macrolides, lincosamides, and PIB (MIC, 64 μg/ml). The ability of the transformants carrying each of these two plasmids to inactivate PIIA and PIB was tested by the microbiological test described by Gots (17). The transformant harboring pIP1714 inactivated PIIA and PIB, whereas neither of these two antibiotics was inactivated by the transformant containing pIP1742.

Sequence of pIP1714. Cleavage of pIP1714 with HindIII generated a single fragment of 5 kb which was ligated into the HindIII site of pOX7 (14), giving pIP1715. The sequence of the HindIII insert (4,978 nucleotides [nt]) in pIP1715 was determined (Fig. 1) and is registered in the GenBank-EMBL Data Library under accession no. AF015628. Four putative genes were detected, and all were in the same transcriptional orientation. Two of these genes are the same as the repB and pre(mob) genes in pUB110 (24) and pBC16 (29). One other gene is similar to the gene vgbB (69.5% nt sequence identity) and is named vgbB, and the fourth gene is similar to the genes vat, vatB, and vatA (71.7, 62.2, and 64.1% nt sequence identities, respectively) and was named vatC.

DNA sequencing. An automated 373A DNA sequencer (Applied Biosystems, Inc.) and the protocol described by the manufacturer were used for sequencing. The sequencing reaction was performed by PCR amplification in a final volume of 20 μl with 500 ng of plasmid DNA, 10 pmol of primer, and 9.5 μl of a dye terminator premix. After heating at 94°C for 2 min, the reaction was carried out as follows: 25 cycles of 30 s at 94°C and 30 s at 55°C and then 4 min at 60°C (9600 thermal cycles; Perkin-Elmer). Excess dye terminators were removed with Quick Spin columns (Boehringer Mannheim). The samples were dried in a vacuum centrifuge and dissolved in 4 μl of a deionized mixture (5/1; vol/vol) of formamide and 50 mM EDTA (pH 8). The samples were loaded onto the sequencer and run for 12 h in a 4.5% denaturing acrylamide gel.

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The vgbB gene (885 nt) is delimited by a potential ATG start codon at nt 399 to 401 and a stop codon at nt 1284 to 1286. The potential start codon is preceded by a 5-nucleotide ATTCAATGG and a potential stop codon at nt 1284 to 1286.
putative ribosome-binding site (RBS; GGAGG). The free energy of association of the most stable structure between the putative RBS and the 3′ terminus of the 16S rRNA calculated as described by Tinoco et al. (34) is −60.2 kJ/mol. The G+C content of the gene is 38.9%. It encodes a 295-aa protein with a calculated molecular mass of 32.7 kDa. VgbB is very similar to the staphylococcal Vgb lactonase which inactivates B components (5) (67.0% identical aa and 81.9% similar aa). The common aa are distributed throughout the peptide chain (data not shown). No significant similarities were detected between the two SgB lactonases, Vgb and VgbB, and other peptide sequences in data banks. The molecular masses of Vgb and VgbB are similar to that of a lactonase in Actinoplanes missouriensis which inactivates virginiamycin B components (35 kDa) (20).

The putative gene vatC (636 nt) extends from an ATG start codon at nt 1307 to 1309 to the stop codon at nt 1943 to 1945. The start codon is preceded 10 nt upstream by an 8-nt putative RBS (TGGGAGTG). The free energy of association of the most stable structure between the putative RBS and the 3′ terminus of the 16S rRNA calculated as described by Tinoco et al. (34) is −44.3 kJ/mol. The G+C content of the gene is 36.3%. It encodes a 212-aa protein with a calculated molecular mass of 23.6 kDa; the sequence of this protein is similar to those of the three known SgA acetyltransferases with similar masses: Vat (24.3 kDa; 69.8% identical aa and 83.5% similar aa), and SatA (23.3 kDa; 66.0% identical aa and 80.1% similar aa). These four SgA acetyltransferases have in common 44.3% identical aa and a repeated sequence of an isoleucine patch (13) also found in the peptide sequences of several homotrimer acetyltransferases which modify various substrates (data not shown). The presence of highly related plasmids in staphylococci, bacilli, and L. monocytogenes is indicative of recent horizontal spread, probably facilitated by the high frequency of homologous and illegitimate recombination (18). Various pieces of evidence led Novick (26) to suggest that pUB110 is native to bacilli: its higher degree of stability and higher copy number in various gram-positive genera (Bacillus, Lactococcus, Streptococcus).

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ick (29) have reported that pBC16-like plasmids are widely distributed among aerobic spore-forming bacilli. Thus, it is likely that pPI1714 is also native to bacilli but that the genes encoding resistance to streptogramins may have been acquired from another source.

Distribution of the vgbB and vatC genes among 53 independent wild-type isolates resistant to the synergistic mixtures of A and B compounds. Plasmids pPI1740 and pPI1741 were used as probes in hybridization experiments under stringent conditions. pPI1740 is pUC18 carrying, between the EcoRI and SacI sites, a vatC intragenic fragment of 581 bp amplified with the primer pair O-P and cleaved with the same enzymes. pPI1741 consists of the vgbB intragenic fragment of 729 bp amplified with the primer pair Q-R and cleaved with XbaI and BamHI inserted between the XbaI and BamHI sites of pUC18. The 53 staphylococcal isolates analyzed included S. cohnii subsp. cohnii BM10711 harboring pPI1714, which was used as a positive control. Both probes gave a strong positive signal with BM10711, whereas none of the 52 other isolates contained hybridizing nucleotide sequences.

The habitat of S. cohnii subsp. cohnii is human skin, where it produces small and transient populations (21). The presence of BM10711 on the top of a cupboard in a trauma ward of the Douera Hospital in Algiers therefore probably resulted from pPI1714 on the top of a cupboard in a trauma ward of the hospital. S. cohnii BM10711, whereas none of the 52 other isolates contained pIP1741 encoding a putative ABC transporter conferring resistance to streptogramin A and related compounds. Gene 202:133–138.


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