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Description of an Unusual *Neisseria meningitidis* Isolate Containing and Expressing *Neisseria gonorrhoeae*-Specific 16S rRNA Gene Sequences

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An apparently rare *Neisseria meningitidis* isolate containing one copy of a *Neisseria gonorrhoeae* 16S rRNA gene is described herein. This isolate was identified as *N. meningitidis* by biochemical identification methods but generated a positive signal with Gen-Probe Aptima assays for the detection of *Neisseria gonorrhoeae*. Direct 16S rRNA gene sequencing of the purified isolate revealed mixed bases in signature regions that allow for discrimination between *N. meningitidis* and *N. gonorrhoeae*. The mixed bases were resolved by sequencing individually PCR-amplified single copies of the genomic 16S rRNA gene. A total of 121 discrete sequences were obtained; 92 (76%) were *N. meningitidis* sequences, and 29 (24%) were *N. gonorrhoeae* sequences. Based on the ratio of species-specific sequences, the *N. meningitidis* strain seems to have replaced one of its four intrinsic 16S rRNA genes with the gonococcal gene. Fluorescence *in situ* hybridization (FISH) probes specific for meningococcal and gonococcal rRNA were used to demonstrate the expression of the rRNA genes. Interestingly, the clinical isolate described here expresses both *N. meningitidis* and *N. gonorrhoeae* 16S rRNA genes, as shown by positive FISH signals with both probes. This explains why the probes for *N. gonorrhoeae* in the Gen-Probe Aptima assays cross-react with this *N. meningitidis* isolate. The *N. meningitidis* isolate described must have obtained *N. gonorrhoeae*-specific DNA through interspecies recombination.

Species of the genus *Neisseria* are well-known to be naturally transformable. Numerous studies describe lateral gene transfer between *Neisseria* spp. (1–3). Recombination appears to be frequent among *Neisseria* spp. that are closely related. Isolates of a human pathogen of the group, *Neisseria meningitidis*, have been shown to contain foreign gene sequences that were first identified in commensal strains, such as *Neisseria lactamica* or *Neisseria cinerea* (4–8). These data are based on multilocus sequence typing (MLST) of housekeeping genes (9–14) and multilocus enzyme electrophoresis (15). Conversely, very few recombinations between meningococci and gonococci have been reported, even though the two are very closely related and even could be considered a single species based on DNA-DNA hybridization findings (16, 17). Those two species historically were thought to be isolated populations (18). However, *N. meningitidis* is quite frequently described as the causative agent of urogenital infections (19–24), and there are numerous examples of *N. meningitidis* and *Neisseria gonorrhoeae* being coisolated either from the pharynx (25–27) or from urogenital sites (28–31). Consequently, Hodge et al. discovered a *Neisseria* strain that phenotypically resembles *N. meningitidis* but reacts with serological tests intended for gonococcal confirmation (32). A more-detailed study was able to show that this isolate is a meningococcus that has acquired a gonococcal protein IB (PIB) porin (33).

One common conclusion of these studies is that the boundaries between *Neisseria* spp. often are fuzzy and difficult to resolve due to the high degrees of relatedness and interspecies recombination. Many sequences need to be analyzed in concatenated data sets to improve the level of resolution (34). Likewise, confirmation of species allocations based on nucleic acid characteristics at at least two loci was recommended (35).

The biological concept of species has been discussed in great detail over the past years, with taxonomists using a variety of methods to define a bacterial species. One of the most widely used classification tools for cultured and uncultured bacteria is the 16S rRNA gene. It is present in all bacteria and contains conserved and variable sequence regions, allowing classification of both distant and closely related species. Previously, 16S rRNA gene typing proved to be very sensitive and specific in identifying *N. meningitidis* strains (36, 37). Despite the presence of four rRNA operons (38, 39), it was long thought that all 16S rRNA gene copies of *N. meningitidis* have identical sequences (40), whereas a more detailed analysis demonstrated low levels of variation among *N. meningitidis* isolates (41). However, that study was still able to distinguish *N. meningitidis* strains from *N. gonorrhoeae* based on 16S rRNA gene sequences. Smith et al. (42) performed extensive sequence analysis of a number of *Neisseria* spp. (*N. meningitidis* and *N. gonorrhoeae* strains as well as commensal *Neisseria* isolates) based on multiple genes, including 16S rRNA genes. High levels of variation and diversity among the strains analyzed were described, pointing to a high level of recombination, especially within the *N. lactamica* group. In that publication, no recombination events were reported for *N. gonorrhoeae*, which formed a distinct branch in all gene sequences analyzed.

In addition to its function as a taxonomic tool in the bacterial world, rRNA can be used to detect and to identify bacterial species. Diagnostic tests such as the Gen-Probe AccuProbe culture identification tests and the Aptima line of amplified assays take advantage of the high rRNA copy numbers in bacterial cells, resulting in

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very high sensitivity for detection. The Aptima Combo 2 and Aptima GC assays for the detection of gonococci are characterized by specificities of 97.5% to 100% for urogenital samples, depending on the type of sample (43). Cross-reactivity with *N. meningitidis* was reported recently by Tabrizi et al. (44), whereas a follow-up study did not confirm those initial data (45).

Here, we describe an isolate that was characterized as *N. meningitidis* based on phenotypic and serological characteristics but was identified as positive for *N. gonorrhoeae* with Aptima assays. Amplification and sequencing of single copies of its 16S rRNA gene revealed that this isolate contains 16S rRNA genes from both *N. meningitidis* and *N. gonorrhoeae*, which led to the hypothesis that the isolate laterally acquired a gonococcal 16S rRNA gene. We were able to show with fluorescence *in situ* hybridization (FISH) that both meningococcal and gonococcal rRNA genes are expressed in this isolate.

(Part of this work, including Fig. 2, was presented at the European Congress of Clinical Microbiology and Infectious Diseases, 2011.)

MATERIALS AND METHODS

Isolation, cultivation, and identification. The isolate described herein was isolated from a urethral swab sample on Thayer-Martin agar and subsequently cultured on chocolate agar. Probe specificity for FISH was verified with 5 strains of both *N. meningitidis* and *N. gonorrhoeae* (strains ATCC 13077, ATCC 13090, ATCC 35560, ATCC 35562, and ATCC 43744 for *N. meningitidis* and ATCC 19424, ATCC 27633, ATCC 31426, ATCC 49226, and ATCC 53420 for *N. gonorrhoeae*). The strains were cultured on chocolate agar (Hardy Diagnostics, Santa Maria, CA) at 37°C in 5% CO_2.

Biochemical identification. The clinical isolate was characterized by biochemical methods. Both the Vitek system (bioMérieux, Marcy l'Etoile, France) and Biolog MicroStation system (Biolog, Inc., Hayward, CA) were used for identification according to the manufacturer's instructions.

DNA probe assay identification. Patient samples as well as pure cultures were characterized by Gen-Probe Aptima GC and Aptima Combo 2 assays for the detection of *N. gonorrhoeae*. Bacterial cells from either cultures or urethral swab samples were lysed and assayed according to the manufacturer's instructions.

Serological identification. Single colonies of this isolate were tested with specific antibodies for serological classification. Serological tests were performed with the following Becton Dickinson *Neisseria meningitidis* antisera: X, Y, Z, Z′ (E), W135 (W), Poly (against serogroups A, B, and C), and Poly 2 (against serogroups X, Y, and Z). All tests were performed and recorded according to the manufacturer's instructions. The specificities of the sera were confirmed with strains affiliated with known *N. meningitidis* serogroups, i.e., serogroups A (ATCC 13077), X (ATCC 35560), Z (ATCC 35562), and W (ATCC 43744), and *Neisseria gonorrhoeae* (ATCC 31426).

Sequence analysis. (i) PCR. The 16S rRNA gene was amplified from whole cells. A cell suspension from a single colony was prepared in specimen transport medium (Gen-Probe) and diluted 1:100 in sterile water. One microliter of this dilution was mixed with 49 μl of PCR master mix containing 25 μl 2× HotStarTaq master mix (Qiagen, Valencia, CA) and 400 nM concentrations of each forward and reverse primer. The primers used for amplification of the 16S rRNA gene were the universal bacterial primers 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 1492R (5′-TAC GGY TAC CTT GTT ACG ACT T-3′). The 16S rRNA gene was amplified with an initial denaturation step at 94°C for 10 min, followed by 35 cycles of denaturation (94°C for 45 s), annealing (55°C for 45 s), and elongation (72°C for 1 min), with a final elongation step at 72°C for 10 min.

The 23S rRNA gene was amplified with the universal primers 129F (5′-CYG AAT GGG GVA ACC-3′) and 2241R (5′-ACC GCC CCA GTH AAA CT-3′), using the following protocol. After an initial denaturation step at 94°C for 15 min, the 23S rRNA gene was amplified with 35 cycles of denaturation (94°C for 1 min), annealing (57°C for 1 min), and elongation (72°C for 2 min), with a final elongation step at 72°C for 10 min. Positive (*Escherichia coli* cell suspension) and negative (water only) controls were included.

(ii) Single-copy 16S rRNA gene PCR. DNA was isolated using the DNeasy Blood & Tissue minikit (Qiagen, Valencia, CA) and was fragmented with the restriction enzyme BbsI, according to the manufacturer's instructions. BbsI was chosen since it does not have a restriction site within the *Neisseria* 16S rRNA gene. An analysis of published genomes for both *N. meningitidis* and *N. gonorrhoeae* revealed that the average size of all fragments generated by BbsI digestion is approximately 1,600 bp and the fragments containing 16S rRNA genes are approximately 5 kb long. Restriction digestion with BbsI generates 1,350 to 1,400 fragments from whole-genome *Neisseria* DNA.

The fragmented DNA was diluted to generate suspensions containing approximately 25 to 50 fragments/μl. Statistically, a maximum of one 16S rRNA gene fragment is present when 1 μl of this dilution is used in a PCR. The PCR cycle number was increased to 45 for better sensitivity due to the single target molecule. For a higher specificity, and to avoid contaminated negative-control samples during PCR, a *Neisseria* genus-specific forward primer (NeissPCR2F, 5′-CGG GTG AGT AAC ATA TCG G-3′) was used in conjunction with the universal 1492R reverse primer. Due to the risk of contaminating the *Neisseria*-specific single-copy PCRs, no positive-control reactions were performed concurrently for this set of experiments. All PCR products were analyzed on an agarose gel and purified using Zymo DNA Clean & Concentrator-5 columns (Zymo Research, Irvine) for further analysis.

(iii) Direct sequencing. Purified PCR products were used for direct sequencing. Two microliters of template was mixed with 8 μl of BigDye Terminator v3.1 ready reaction mix (Applied Biosystems) and 3.2 pmol of one primer, in a total reaction volume of 20 μl. The 16S rRNA gene was sequenced with the following four primers: 27F or NeissPCR2F (for single-copy PCR products), BacA519+ (5′-CAG CAG CCG CGG TAA TAC-3′), EcoA806− (5′-CTA CCA GGG TAT CTA ATC-3′), and 1492R. The 23S rRNA gene was sequenced with the following five primers: 129F, EubB801+ (5′-GAT AGC TGG TTC TCC CCG AAA-3′), EubB1059+ (5′-GTT GGC TTA GAA GCA GCC A-3′), EubB1601+ (5′-GTA CCC CAA ACC GAC ACA GGT-3′), and 2241R.

Sequencing reactions consisted of 25 cycles of denaturation (96°C for 30 s), annealing (50°C for 15 s), and elongation (60°C for 4 min). The reaction products were purified with a DyeEx 2.0 spin kit (Qiagen), dried in a SpeedVac concentrator for 20 min, and resuspended in 10 μl Hi-Di formamide (Applied Biosystems). Samples were denatured at 96°C for 1 min, cooled on ice, and analyzed with an ABI 3100 genetic analyzer (Applied Biosystems). The sequences obtained were manually proofread and annotated with Gene Codes Sequencher 4.7 software.

(iv) Cloning and sequencing. Purified PCR products were ligated into the vector pCR2.1 and transformed into *E. coli* Top10 cells using the TOPO TA cloning kit (Life Technologies, Carlsbad, CA), according to the manufacturer's instructions. Overnight cultures from clones were used to isolate plasmids (QIAprep spin miniprep kit; Qiagen). Four microliters of the purified plasmids was used for sequencing reactions (see above) with primer 27F.

FISH. A single colony of a *Neisseria* culture was resuspended in phosphate-buffered saline (PBS) and fixed by addition of 3 volumes of a 4% paraformaldehyde solution and incubation at 4°C for 3 h (46). Fixed cells were washed once with PBS and stored in a 50% ethanol-PBS mixture at −20°C. Sufficient amounts of cells were transferred onto gelatin-coated glass slides, dried, and dehydrated by serial immersion of the slides in increasing ethanol concentrations (47). FISH was carried out with a formamide concentration of 30%, as described previously (48). In brief, immobilized cells were incubated for 2 h at 46°C in hybridization buffer (0.9 M NaCl, 20 mM Tris-HCl [pH 7.2], 0.01% SDS, 30% formamide) containing 1 μM labeled probe and then were washed by immersion in wash buffer (0.1 M NaCl, 20 mM Tris-HCl [pH 7.2], 0.01% SDS, 0.5 mM

EDTA) for 15 min at 48°C. The following probes were used in conjunction for hybridization of *N. meningitidis* and *N. gonorrhoeae*: NeMe183-FAM, 5′-CCT GCT TTC TCT CTC AAG A-3′; NeGo183-Cy3, 5′-CCT GCT TTC CCT CTC AAG A-3′ (48). The probes acted as competitors for each other to ensure specificity and to distinguish the single-base mismatch.

RESULTS

The phenotypic identity of clinical isolate CI5240 is *N. meningitidis*. Three single colonies of the isolate were selected for phenotypic identification with the bioMérieux Vitek system (bioMérieux) and the Biolog MicroStation system (Biolog, Inc.). All three colonies were identified as *N. meningitidis* with 98 to 99% confidence values with both systems.

Clinical isolate CI5240 reacts positively with the Aptima series of tests. Pure cultures picked from single colonies of the clinical isolate CI5240, identified as *N. meningitidis*, tested positive for *Neisseria gonorrhoeae* with the Gen-Probe Aptima Combo 2 and Aptima GC assays.

***N. meningitidis* CI5240 is classified in serogroup Z.** Serological testing confirmed the identity of CI5240 as *Neisseria meningitidis*. A detailed analysis with several antisera proved that this isolate is categorized as *N. meningitidis* serogroup Z. Pure cultures demonstrated strong agglutination reactions with antisera for *N. meningitidis* groups X, Y, and Z as well as with a specific antiserum for serogroup Z.

***N. meningitidis* CI5240 contains at least one copy of the *N. gonorrhoeae* 16S rRNA gene.** To confirm the identity of the *N. meningitidis* clinical isolate, we sequenced the 16S rRNA gene, which is commonly used for phylogenetic classification of bacterial strains. Also, since the Aptima Combo 2 and Aptima GC assays both target 16S rRNA for capture and detection, the sequence obtained should provide clarification on why the isolate tested positive for *N. gonorrhoeae*. Interestingly, direct sequencing of PCR products produced double peaks, which indicates the presence of at least two types of 16S rRNA gene sequences in the reaction mixture. These mixed bases are in signature positions used to discriminate between *N. meningitidis* and *N. gonorrhoeae*, suggesting the presence of both *N. gonorrhoeae* and *N. meningitidis* 16S rRNA gene sequences. The two peaks for all mixed bases found were base transitions (A to G or C to T).

We cloned the PCR products to isolate the different sequence types and retrieved almost-full-length high-quality sequences for 44 clones. Thirty-three sequences matched one of two sequence types that were clearly affiliated with either *N. gonorrhoeae* or *N. meningitidis*. Eleven chimeric sequences, with nine different chimeric patterns in the signature regions, also were detected (data not shown).

It is highly unlikely that all of these sequences represent individual 16S rRNA genes. Members of the genus *Neisseria* contain four rRNA operons. Therefore, the 11 different sequence types detected (nine chimeric types and two species-specific types) cannot be explained by operon-to-operon variations. The appearance of chimeric sequences obviously can be explained by PCR and sequencing bias (49, 50), but it is very unlikely that this would have resulted in chimeras in 25% of the clones analyzed. However, these numerous sequence types could have been generated by heteroduplex formation during PCR cycling. When transformed into *E. coli*, the host's intrinsic MutHLS-mediated mismatch repair system randomly repairs mismatched bases on both strands, creating random chimeras that are detected when the plasmid inserts are sequenced (51–53).

To overcome this PCR and cloning bias, a single-copy PCR method was developed using fragmented genomic DNA dilutions. The PCR products were again sequenced directly. Two sequence types were obtained from 119 unambiguous sequences with this method. Of those, 91 (76%) were *N. meningitidis* specific, and 28 (24%) were *N. gonorrhoeae* specific. The two sequence types are deposited under GenBank no. KC561932 and KC561933. Their affiliations with *N. meningitidis* (accession no. KC561933) and *N. gonorrhoeae* (accession no. KC561932) are shown in the dendrogram in Fig. 1.

The presence of those two types of species-specific sequences supports the hypothesis that the gonococcal 16S rRNA gene was laterally transferred and integrated into the genome of the clinical isolate described herein. Furthermore, based on the relative ratio of *N. meningitidis*-specific sequences to *N. gonorrhoeae*-specific sequences, one of the intrinsic 16S rRNA genes was most likely replaced with the gonococcal gene.

The 23S rRNA gene sequence data for *N. meningitidis* CI5240 do not suggest mixed template sequences. The analysis described above clearly demonstrated the presence of 16S rRNA gene sequences from both *N. meningitidis* and *N. gonorrhoeae*, but it was unknown if strain CI5240 horizontally acquired only a gonococcal 16S rRNA gene or if it integrated a full rRNA operon. For this reason, its 23S rRNA gene was amplified with the universal primers 129F and 2241R (54) and sequenced. No mixed bases or mismatches with known *N. meningitidis* strains were observed (data not shown), suggesting that the whole-genome template DNA (containing all four 23S rRNA genes) did not have sequence variabilities. A comparison of publicly available GenBank sequences for meningococci and gonococci did not allow clear discrimination of the two species based on 23S rRNA gene sequences, due to the presence of many intraspecies differences and very few interspecies differences, which makes this gene unsuitable for further analysis.

No similar 16S rRNA gene sequence heterogeneity can be found in publicly available *N. meningitidis* or *N. gonorrhoeae* sequences. In addition to the 16S rRNA gene sequences derived from the clinical isolate described herein, we analyzed a total of 836 *N. meningitidis* and 5 *N. gonorrhoeae* 16S rRNA gene sequences from GenBank as well as 48 *N. gonorrhoeae* sequences from in-house clinical isolates. All sequences were at least 1,350 bp long.

Interestingly, we observed very high levels of intraspecies heterogeneity within the 16S rRNA gene sequences for both species. Among the 53 *N. gonorrhoeae* sequences analyzed, we identified 13 different alleles. Likewise, 105 different alleles were observed for all *N. meningitidis* sequences. For this study, an allele was defined when at least three different strains of the 889 strains analyzed showed the same mismatch to a reference sequence. Mismatches that were less frequent could be caused by PCR and/or sequencing errors and therefore were omitted from the analysis. We found 45 positions in the almost-full-length 16S rRNA gene sequences analyzed that met the criteria for an allele. Of those 45 positions, only 11 were clear signature regions in which all *N. meningitidis* sequences differed from all *N. gonorrhoeae* sequences, allowing differentiation between the two species.

None of the 889 sequences displayed rRNA gene sequences or mixed bases similar to those of the clinical isolate described

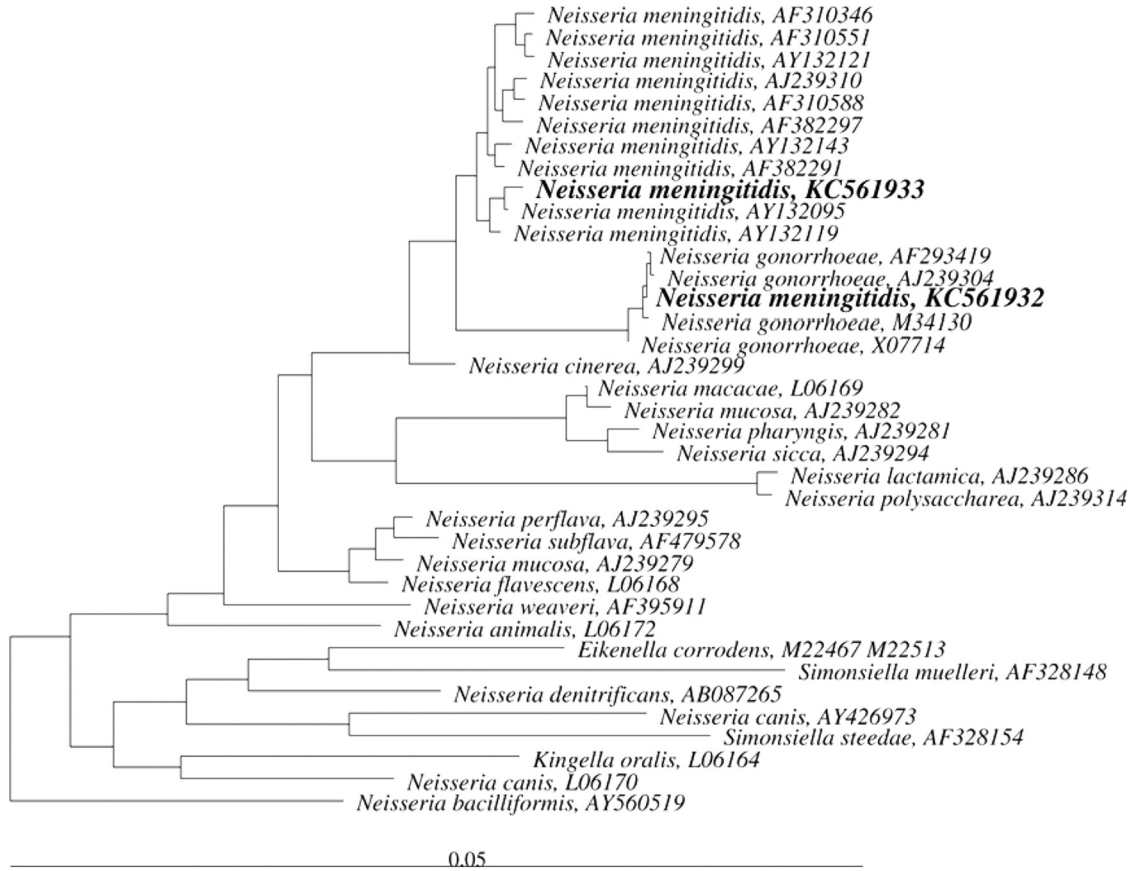

FIG 1 Dendrogram showing the affiliations of the two 16S rRNA gene sequence types found in the clinical isolate described. The two sequence types are highlighted in bold and are clustered with *N. meningitidis* (GenBank accession no. KC561933) and *N. gonorrhoeae* (GenBank accession no. KC561932) sequences. Outgroups are not shown. The scale bar represents 5% distance between sequences.

herein. Based on the GenBank sequences analyzed, none of the 836 *N. meningitidis* strains would have resulted in a false-positive cross-reaction with the *N. gonorrhoeae*-specific probes in the Gen-Probe Aptima assays.

***N. meningitidis* CI5240 expresses the foreign gonococcal 16S rRNA gene.** To confirm that both 16S rRNA gene sequences were derived from a pure culture of *N. meningitidis* CI5240 and to determine the expression patterns of both rRNA genes, fluorescence *in situ* hybridization (FISH) was performed in order to achieve single-cell resolution. Two probes were used for the detection of both *N. meningitidis*-specific (NeMe183, labeled with fluorescein) and *N. gonorrhoeae*-specific (NeGo183, labeled with Cy3) rRNA types (slight modification of the method described by Poppert et al. [48]). We confirmed the specificities of both probes with five *N. meningitidis* and five *N. gonorrhoeae* strains obtained from the ATCC. Interestingly, both *N. meningitidis*-specific and *N. gonorrhoeae*-specific rRNA sequences are expressed in this clinical isolate, as shown in Fig. 2. Not all cells of the isolate expressed both sequence types equally. This can be seen by the different intensities of green and red fluorescence, as well as by the color resulting from the overlay of red and green. Equal signal intensities from the red and green fluorescently labeled probes would result in a yellow color, whereas the green (*N. meningitidis*-specific) signals prevailed over the red (*N. gonorrhoeae*-specific) signals in many single cells, suggesting that the *N. meningitidis* type of rRNA was expressed in greater amounts. Although not quantitative in nature, this finding corresponds well with the number of *N. meningitidis* versus *N. gonorrhoeae* 16S rRNA genes present in the strain.

DISCUSSION

The discrepancy between Aptima assay and conventional identification results is explained by horizontal gene transfer. The clinical isolate described here has been identified with Gen-Probe Aptima assays for the detection of *N. gonorrhoeae* and phenotypic identification using automated systems. It was identified as *N. gonorrhoeae* by positive Aptima Combo 2 and Aptima GC test results, whereas the phenotypic identification was *N. meningitidis*. The discrepancy in those test results led us to investigate this isolate in further detail. Since the target sequence of the Gen-Probe Aptima assays is bacterial 16S rRNA, we investigated the specificity of the assay through sequence analysis of the 16S rRNA gene.

During this analysis, it was determined that the *Neisseria* strain possesses a copy of a *N. gonorrhoeae* 16S rRNA gene. Sequencing of 16S rRNA gene PCR products from a single gene copy using dilutions of fragmented genomic DNA revealed 76% pure *N. meningitidis* and 24% pure *N. gonorrhoeae* sequences, an approximate ratio of 3:1. The presence of the *N. gonorrhoeae* gene can be explained by lateral gene transfer between the two species. Furthermore, considering the fact that the genomes of all *Neisseria* spp. contain four rRNA operons, it seems that this isolate also replaced

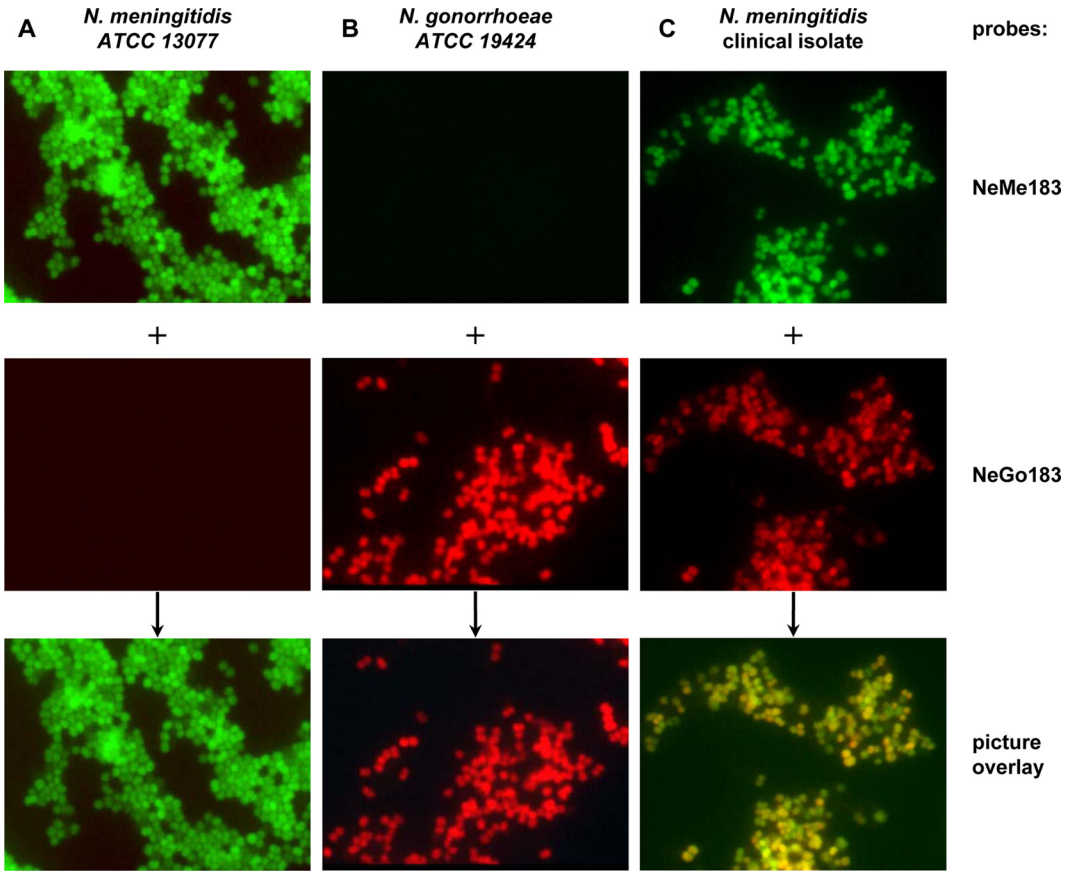

FIG 2 FISH results for the detection of *N. gonorrhoeae*-specific and *N. meningitidis*-specific rRNA. The probes used were NeMe183 (specific for *N. meningitidis* and labeled with fluorescein isothiocyanate [green]) (first row) and NeGo183 (specific for *N. gonorrhoeae* and labeled with Cy3 [red]) (second row); the probes were used in conjunction as competitors to each other. The overlay of red and green signals results in a yellow color (third row). The specificities of the two probes were determined with a panel of 5 *N. meningitidis* control strains and 5 *N. gonorrhoeae* control strains. (A) One *N. meningitidis* control strain. (B) One *N. gonorrhoeae* control strain. (C) Probe signals detected with both probes in single cells of clinical isolate CI5240. All images were recorded with an exposure time of 40 ms.

one of its own 16S rRNA genes with the foreign gene, according to the ratio of individual meningococcal 16S rRNA gene sequences to gonococcal 16S rRNA gene sequences obtained in this study.

Since Gen-Probe Aptima assays target 16S rRNA rather than genomic DNA, it was important to determine if the isolate expresses gonococcal RNA. We were able to prove the purity of this isolate as well as the expression of gonococcal rRNA by *in situ* hybridization using specific probes for the two sequence types (Fig. 2).

Interoperon variability of 16S rRNA has been described previously. Numerous publications over the past 2 decades described 16S rRNA gene sequence variability when comparing the individual genes from each rRNA operon of a single bacterial strain. Variability in the small rRNA subunit of representative bacteria has been shown across several bacterial phyla (55–60). Clear determination of species affiliations was still possible in all of those studies. The frequency of this variability might even be underestimated, considering the fact that direct amplification and sequencing often do not deliver the necessary level of resolution to reveal intrastrain rRNA gene sequence variability, due to the fact that base-calling algorithms often cannot detect mixed bases when minor alleles account for only a small percentage of the target sequence (61, 62). Single copies must be separated first to show sequence variations. Expression of the different rRNA gene sequences has not been investigated in these studies.

Lateral gene transfer of rRNA genes between *Neisseria* spp. is an apparently uncommon event. Horizontal gene transfer between *Neisseria* spp. has been described previously. It is well-known that this genus is naturally competent (6, 63, 64). Many mosaic sequences from *N. meningitidis* or *N. gonorrhoeae* and other commensal nonpathogenic members of this genus have been found (5, 65–69). To our knowledge, none of these studies described rRNA gene transfer between two *Neisseria* species. In order to explore the frequency of interspecies rRNA gene exchange between gonococci and meningococci, all *N. meningitidis* and *N. gonorrhoeae* 16S rRNA gene sequences publicly available at the time of the study were investigated. GenBank sequences might not be entirely representative of all sequences circulating clinically; therefore, the possibility of false-positive Aptima assay results cannot be ruled out. However, it is still interesting to see that none of the almost 900 sequences analyzed featured anomalies similar to those of the isolate described herein, such as mixed bases in signature regions or a chimeric 16S rRNA gene sequence that would point toward mixed genes in the genome. A detailed analysis of the binding regions of all publicly available *N. meningitidis* rRNA gene se-

quences for the Aptima assay probe proved that none of the corresponding isolates would have resulted in a positive reaction with the *N. gonorrhoeae* probe (data not shown).

It was noted that most base differences between the two species are also present in sequences from different strains of the same species. Discrimination between *N. meningitidis* and *N. gonorrhoeae* using 16S rRNA gene sequences is still possible but must be based on the 11 signature regions described herein.

To our knowledge, this is the first description of a *N. meningitidis* strain possessing and also expressing gonococcal 16S rRNA. It is generally assumed that highly conserved genes that are already present in a host's genome, such as the 16S rRNA gene, are not easily transferable between different species (70, 71). It should be mentioned that there are few examples of lateral gene transfer of 16S rRNA genes between different strains of the same species, e.g., *Bartonella henselae* (72), *Vibrio parahaemolyticus* (73), and *Campylobacter hyointestinalis* (58), or between different species of the same genus, e.g., *Veillonella* spp. (74). These are rare examples of lateral rRNA gene transfer, which has not yet been described between *N. meningitidis* and *N. gonorrhoeae*.

Summary. Based on the results of this study, *N. meningitidis* isolate CI5240 appears to have obtained *N. gonorrhoeae*-specific DNA containing the 16S rRNA gene through interspecies recombination. Based on the observed 1:3 ratio of gonococcal sequences to meningococcal sequences and on the fact that the genomes of *Neisseria* spp. contain four rRNA operons, the *N. meningitidis* isolate described seems to have replaced one of its four intrinsic 16S rRNA genes with the gonococcal gene. We have also shown that this gonococcal 16S rRNA gene is expressed, which explains why the probe for *N. gonorrhoeae* in the Gen-Probe Aptima assays cross-reacts with this *N. meningitidis* isolate. Lateral gene transfer of gonococcal rRNA genes is a potential source of false-positive *N. gonorrhoeae* results with the Aptima Combo 2 assay. Additional studies will be required to determine if other clinical isolates of *N. meningitidis* were incorrectly identified using 16S rRNA probe-based assays as a result of similar lateral gene transfer and integration events. To our knowledge, this is the first description of a *Neisseria meningitidis* isolate that has acquired a gonococcal rRNA gene, has replaced one of its own intrinsic 16S rRNA genes with the gonococcal one, and even expresses the acquired 16S rRNA.

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