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## The Emergent Invasive Serotype 4 ST10172 Strain Acquires *vanG*-Type Vancomycin-Resistance Element: A Case of a 66-Year-Old With Bacteremic Pneumococcal Pneumonia

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### Abstract

We report a single case of invasive pneumococcal disease (IPD) by serotype 4, multilocus sequence type 10172 (ST10172) isolate with *vanG*-type resistance genes and reduced vancomycin susceptibility. The isolate was recovered during 2022 from a 66-year-old resident with bacteremic pneumococcal pneumonia within a Centers for Disease Control and Prevention Active Bacterial Core surveillance (ABCs) site hospital. The patient had received 23-valent pneumococcal polysaccharide vaccine and there was no evidence of concurrent or prior receipt of vancomycin in the previous year. Serotype 4/ST10172 IPD has shown increases within western ABCs sites, and the recent acquisition of a *vanG* element warrants close monitoring of this lineage.

### Keywords

*Streptococcus pneumoniae*; invasive pneumococcal disease; antibiotics; vancomycin resistance; emergent lineages

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Vancomycin binds to D-Ala-D-Ala ends of peptidoglycan subunits to prevent cell wall synthesis [1] and is commonly used in combination with other antibiotics for suspected severe pneumococcal disease, often before bacteriologic results are available. Vancomycin-resistant pneumococci have not been reported in the United States (US), and before 2014 vancomycin nonsusceptibility had not been detected within the 3 major streptococcal pathogens (*Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Streptococcus agalactiae*) monitored through the Centers for Disease Control and Prevention's (CDC) Active Bacterial Core surveillance (ABCs) [2]. In 2014 we reported characterization of 2 vancomycin-nonsusceptible *S agalactiae* isolates that carried *vanG* elements, which confer low-level vancomycin resistance through the activity of the *vanG*-encoded D-Ala–D-Ser ligase [2].

Marked increases in serotype 4 invasive pneumococcal disease (IPD) cases were reported within the western US through ABCs before the coronavirus disease 2019 pandemic, and adults experiencing homelessness (AEH) were disproportionately affected [3]. This increase has been associated with this serotype's propensity to display geographically and temporally related genomic clusters, comprised of IPD isolates sharing near or complete genomic identity. Besides a general increase of serotype 4 IPD, the genomic lineages within serotype 4 have undergone a genomic shift during recent years, with multilocus sequence type ST10172 increasing from about 31% (11/36) during 2015 to 78% (59/78) of serotype 4 IPD isolates during 2019. During 2015–2019, >90% of serotype 4 cases occurred within 3 of the 10 ABCs sites that included the Denver area in Colorado, the state of New Mexico, and the San Francisco area in California [3]. Here we describe the first known pneumococcal isolate carrying a *vanG* element (strain 20238042), a recently occurring IPD isolate of the emergent serotype 4/ST10172 lineage.

## METHODS

IPD cases were identified through ABCs, an active, laboratory- and population-based program, and defined through isolation of *S pneumoniae* from a normally sterile site in a surveillance area resident [4]. ABCs data were reviewed by CDC and deemed not to be research, and the study was conducted consistent with applicable federal law and CDC policy.

All ABCs isolates received since January 2015 are subjected to short read sequencing with genome sequences deposited in the National Center for Biotechnology Information Sequence Read Archive (BioProject PRJNA284954). Isolates were assigned serotypes, multilocus sequence types (STs), and resistance determinants employing whole-genome sequencing [5]. Selected isolates, including 2 control serotype 4/ST10172 isolates, were subjected to phenotypic antibiotic susceptibility testing employing broth microdilution and Etest (bioMérieux, Durham, North Carolina) as previously described [2].

The standard PacBio Microbial Multiplexing procedure was implemented (Pacific Biosciences, Menlo Park, California) for the single *vanG*-positive serotype 4/ST10172 isolate (laboratory isolate 20238042, GenBank accession number [CP1545950](#)) [6].

Sequence analysis was employed as previously described [6]. In brief, Prokka version 1.13.5 was used to annotate open reading frames. The *vanG* regions of representative pneumococcal, *S. agalactiae*, and *Enterococcus faecalis* strains were aligned to generate figures using Easy Fig 2.2.3. Core genomic maximum parsimony genomic trees were generated from pneumococcal assemblies with  $\leq 200$  contigs employing kSNP3.0 with a kmer size of 19. The core.tre file generated from kSNP3.0 was used to generate a phylogenetic diagram and pairwise single-nucleotide polymorphism (SNP) comparisons employing MEGA7. A circular genomic map of *vanG*-positive strain 20238042 was generated employing Proksee.

## RESULTS

A pneumococcal isolate of serotype 4, multilocus sequence type 10172 (serotype 4/ST10172), positive for a *vanG*-type resistance determinant, was recovered from a blood culture collected from a 66-year-old resident in New Mexico (not recorded as experiencing homelessness) with bacteremic pneumococcal pneumonia in 2022. The patient had a history of tobacco smoking, chronic obstructive pulmonary disease, and poorly controlled diabetes. There was no evidence of concurrent or prior receipt of vancomycin in the previous year. The patient had received the 23-valent pneumococcal polysaccharide vaccine (PPSV23, which contains serotype 4) 3 years prior to illness onset but not the pneumococcal conjugate vaccine (PCV). The patient was discharged home after 19 days in the hospital without any stay in the intensive care unit.

Repeated broth microdilution testing of the *vanG*-positive isolate consistently yielded a minimum inhibitory concentration (MIC) of 0.5–1.0  $\mu\text{g/mL}$ , while all controls tested at  $\leq 0.25$ –0.5  $\mu\text{g/mL}$ . Etest provided a consistent difference between the *vanG*-positive isolate and controls, invariably providing MICs of 1.0  $\mu\text{g/mL}$  and 0.38–0.5  $\mu\text{g/mL}$ , respectively. Controls included closely related serotype 4/ST10172 isolates.

To find evidence of the approximate timeframe when strain 20238042 acquired the *vanG* element and evidence of where it might have likely occurred, we performed phylogenetic analysis of the core genomes from 477 of the 501 available (477 had  $\leq 200$  contigs) serotype 4/ST10172 isolates recovered during 2015–2023 (partial 2023) (Supplementary Figure 1). Only 5 of the 501 available serotype 4/ST10172 isolates from 4 ABCs sites (Minnesota, Connecticut, Georgia, Maryland) were recovered from cases outside of the 4 western ABCs sites (Colorado, New Mexico, Oregon, California) during this period. Of the 477 isolates subjected to phylogenetic analysis, 470 were from ABCs (33 from 2023) and 7 from AEH in the non-ABCs El Paso County, Colorado, during late 2022. Most of the isolates were phylogenetically distributed according to the state of isolation (Supplementary Figure 1). Strain 20238042 was a genomically clustering isolate, defined as sharing  $\leq 10$  SNPs with 1 or more IPD isolates within ABCs [3]. Specifically, strain 20238042 differed by no more than 7–10 SNPs from 7 serotype 4/ST10172 IPD isolates, all recovered in New Mexico during 2017–2021 (Supplementary Figure 1) in individuals residing in Bernalillo (5 isolates) and San Miguel (1 isolate) counties. The single isolate recovered during 2021 and strain 20238042 were from individuals residing in Valencia County (Bernalillo and Valencia are contiguous counties). Serotype 4/ST10172 isolates were generally tightly genomically

clustered, despite outliers, with an overall mean distance between isolates of 65 SNPs, which is consistent with the relatively recent first discovery of this strain in ABCs during 2013 and its subsequent recorded emergence since 2015 [3, 7].

We found that strain 20238042 carried a *vanG*-type resistance operon that shared 99.8% identity with the 6925 base *vanG* operon from *E faecalis* strain N03–0233 [8] (Figure 1A), including complete sequence identity with the promoter region upstream of *vanU*. The *vanG* operon was inclusive of the *vanG* D-Ala-D-Ser ligase gene, the *vanXY* D, D-carboxypeptidase/D, D-peptidase gene, and the *vanT* serine racemase gene. The pneumococcal element was inserted within the *rumA* gene, known to be a genomic “hotspot” for resistance element insertion within gram-positive bacteria, as reported for *vanG* element insertions from 2 invasive *S agalactiae* strains recovered through ABCs and a *Streptococcus anginosus* strain [2]. Instead of fusing at *rumA* base 1328 (as seen with the *vanG* element insertions in the *S agalactiae* and *S anginosus rumA* homologs), with a resultant AG dinucleotide repeat flanking the insertion, the pneumococcal 46 190 bp insertion was fused to base 1322 of the pneumococcal *rumA* gene with resultant CA dinucleotide repeats (Figure 1B) at nucleotide positions 924 790 and 970 980 on the circular genomic map (Figure 2). In contrast to the near identity (99.8%/6925 bp) exhibited between the pneumococcal and enterococcal *vanG* operon sequences, inclusive of identical upstream putative promoter, the *S agalactiae vanG* operon was comparatively divergent (approximately 70% identity) and also contained a *vanY* gene, predicted to encode a D, D-carboxypeptidase (Figure 1A and 1B). The rest of the pneumococcal *vanG* element shared variable homology to both the enterococcal and the *S agalactiae* elements, with the most pronounced difference shown between the predicted recombinases at the element 3′ ends (about 54% identity to the enterococcal *vanG* element recombinase gene and less than 30% identity to the *S agalactiae* counterpart).

## DISCUSSION

Evidence from ABCs over the past several years indicates that societal issues, especially lack of housing, are important risk factors for serotype 4 IPD [3]. Until 2017, the primary causes of serotype 4 IPD in ABCs were the closely related ST244 and ST695 lineages [3, 7], although all serotype 4 lineages were disproportionately causes of IPD among AEH. For several years there have been more cases of serotype 4 IPD among AEH than for any other serotype [3], and such isolates are frequently within genomic clusters. In fact, 1 isolate from AEH is depicted within the Figure 1B phylogeny that differed by only 7 SNPs from the *vanG*-positive isolate 20238042.

While the patient presented in this report was not reported as an AEH, the patient had multiple underlying conditions that are known to increase the risk of pneumococcal disease [9] and resided in the West, where serotype 4 IPD is emergent [3]. In recent years, increase in serotype 4 IPD cases has been reported through routine surveillance in North America and in Europe, mainly affecting adults aged <65 years, including those who are not AEH [10–12]. The reasons for the recent surge in adult serotype 4 IPD cases is not yet understood. Of note, in a study of adults hospitalized with community-acquired pneumonia in Georgia and Tennessee during 2018–2022, serotype 4 detection by serotype-specific urinary antigen

test was infrequent [13]. Serotype 4 is included in PPSV23 and all licensed PCVs except 21-valent PCV, approved for US adults in June 2024. In populations where emergence of serotype 4 IPD cases is a concern, use of a pneumococcal vaccine that includes serotype 4 may be prudent. Interestingly, the patient in our report had received PPSV23 3 years prior to illness (no history of PCV receipt); however, presence of multiple comorbidities and the limited protection of PPSV23 against mucosal disease could have made the patient susceptible to the development of IPD.

Besides its increasing incidence in IPD within western ABCs sites, we have noted 2 highly unusual occurrences associated with the serotype 4/ST10172 lineage. Besides its unprecedented acquisition of a *vanG* element within this species, we observed a highly unusual genomic cluster during late 2022–early 2023 associated with 10 IPD cases in Oregon that arose through recombination of a serotype 4/ST10172 donor with a serotype 12F/ST220 genetic recipient (manuscript submitted for publication). The appearance of this serotype-switch variant progeny strain in Oregon during 2022 coincided with the sudden appearance of the donor strain serotype 4/ST10172 genomic clusters within Oregon. In the report we have noted a pronounced increase of serotype 4/ST10172 within New Mexico during 2022, which coincided with the discovery of *vanG*-positive strain 20238042 within a genomic cluster in New Mexico. What both events share in common is an increased prevalence of the serotype 4/ST10172 genetic substrate that increased the probability of encountering a second genomic substrate. Serotype 4/ST10172 served reverse roles, as a genetic recipient for the *vanG* element and as a genetic donor for the serotype-switch event.

We are faced with the unlikely hypothesis that the genomic insertion of the *vanG* element within pneumococcal strain 20238042 may have resulted from contact with a *vanG* carrying bacterial strain in the upper respiratory tract. The reported occurrence of *vanG* elements within clinical isolates of group B streptococci and other streptococcal species has been extremely rare [2]. It must be noted, however, that there is frequent genetic exchange between pneumococci and the related nonpneumococcal mitis group within the upper respiratory tract. In addition, an *S. anginosus* strain was discovered carrying a *vanG* element [2]. *Streptococcus anginosus* strains have not been extensively studied and are commonly found in the upper respiratory tract [14]. The nonpneumococcal streptococcal genetic reservoir in the upper respiratory tract is demonstrably vast [15], yet only a relatively small subset of strains has been studied.

The increase in vancomycin MIC in this *vanG*-positive isolate is modest compared to the nonpneumococcal streptococcal species reported in 2014 [2]. Nonetheless, increases in pneumococcal MICs for this important antibiotic could conceivably evolve toward further MIC increases and selective advantage in carriage or disease persistence. Invasive disease is a relatively rare occurrence for this opportunistic pathogen that normally resides in the upper respiratory tract. This case of bacteremic pneumonia caused by *vanG*-positive strain 20238042 suggests that it had sufficient fitness to first multiply in the upper respiratory tract, and possibly be transmitted to other hosts, before penetrating the respiratory epithelium to further multiply in human blood. Previous experience with  $\beta$ -lactams showed that it is important to monitor for the appearance and emergence of potential pneumococcal threats against commonly utilized antimicrobials.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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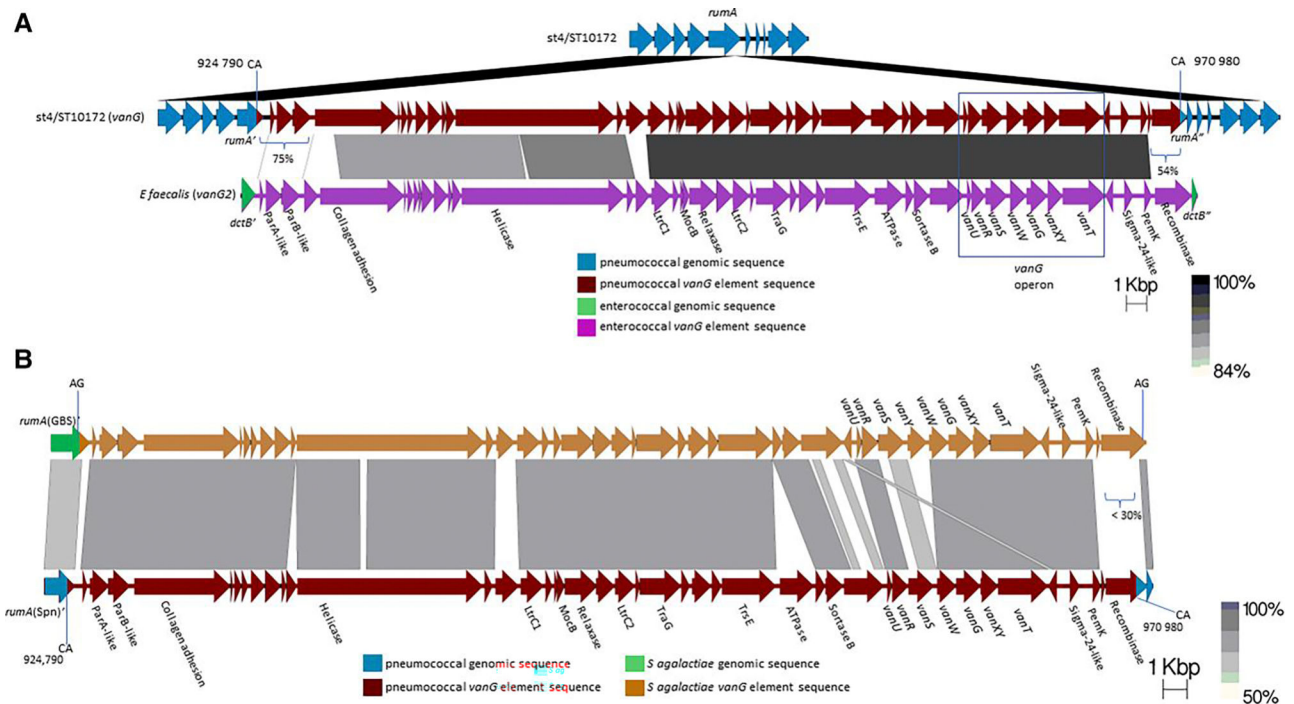
## Data sharing.

Whole-genome sequencing data for the study isolates have been deposited in the National Center for Biotechnology Information Sequence Read Archive under BioProject number PRJNA284954 and are publicly accessible upon manuscript publication.

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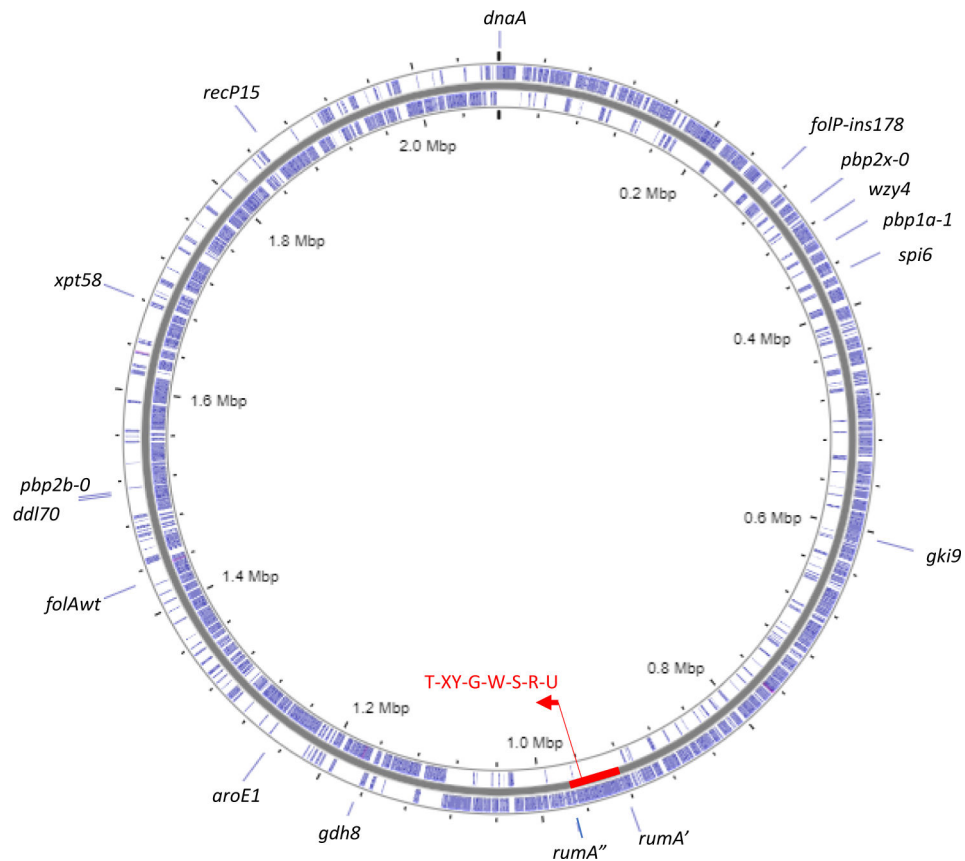
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**Figure 1.**

A, Alignment of wild-type serotype 4/sequence type 10172 (st4/ST10172) strain *rumA* gene region (top) with corresponding *vanG*-positive st4/ST10172 strain (middle), which is aligned with *vanG*-positive *Enterococcus faecalis* strain NO3-0233 described in reference [8]. The dinucleotide repeat CA flanks the insertion where the *vanG* element bisects the pneumococcal *rumA* gene. B, Alignment of *vanG* element from group B streptococcal (GBS) strain NY-1 (from [2]) with that of *vanG*-positive pneumococcal strain 20238042. While both elements intersect the *rumA* genes that share approximately 70% sequence identity, the pneumococcal *vanG* element is fused at base 1322 while the GBS *vanG* element is fused at base 1328. This explains the different dinucleotide repeats, since both the relative positions of both dinucleotides are conserved at bases 1321–1322 (the CA) and at bases 1327–1328 (the AG). Although the *rumA* 3' end is homologous to the 3' end of the GBS element, the actual GBS 3' end lies a further 44.7 kb downstream, flanking a second integrative conjugative element–insertion as described in [2].



**Figure 2.**

Circular 2 075 787 bp genome of pneumococcal *vanG*-positive strain depicting resistance pipeline core genomic targets (3 *pbp* genes, *folA*, and *folP*) and the *vanG* operon resistance determinants (red). Intermediate cotrimoxazole resistance is inferred from the insertion within *folP* and the unlinked wild-type *folA* gene. Susceptibility to  $\beta$ -lactams is inferred from the highly conserved *pbp1a-1*, *pbp2b-0*, and *pbp2x-0* sequence translations. The 7 multilocus sequence typing alleles are depicted with the ST10172 allelic profile. The origin region of chromosomal replication is shown by the position of the *dnaA* gene.