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New Pneumococcal Serotype 15D

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The pneumococcal serogroup 15 comprises four capsular polysaccharide serotypes (15A, 15B, 15C, 15F) that collectively account for an impactful disease burden (1, 2). These four serotypes are defined by Quellung reaction typing, employing specific serologic factor sera to visualize the capsule (3) (Table 1). Each of these four serotypes shares an identical pentasaccharide repeat unit that forms an identical linear structure in types 15A and 15F, and a distinct branched structure in types 15B and 15C (4). The two distinct repeat oligosaccharide polymerase genes, *wzy15BC* and *wzy15AF* (4), provide the basic structural difference between these two pairs of highly related serotypes (Fig. 1). The difference between serotypes 15B and 15C is dictated by *O*-acetylation of the polysaccharide in serotype 15B by the *wciZ*-encoded enzyme, with the two serotypes frequently interconverting due to reversible frameshifting of a TA repeat within *wciZ* (5). Similarly, the *wciZ* gene in serotype 15A contains multiple inactivating mutations compared to its functional counterpart in serotype 15F (4, 6). At the end of the *cps15F* operon is another putative *O*-acetyltransferase gene, *wcjE* (4), that potentially contributes to the serologic difference between 15A and 15F strains. The *wzy* primary target, combined with the *wciZ* secondary target, distinguishes these four serotypes in our whole-genome sequence-based pneumococcal bioinformatic pipeline (1). Note that the incomplete rhamnose biosynthetic apparatus (*rmlB* and *rmlD*) and the *glf* gene that encodes UDP-galactopyranose serve no apparent functions in serotype 15F (Fig. 1), since serogroup 15 capsules do not contain rhamnose or galactofuranose (7, 8).

We recently encountered two strains (invasive U.S. blood isolate 84245 recovered during 2018 and Mozambique carriage isolate MZ877 recovered during 2019) that our bioinformatics pipeline identified as serotype 15A due to an identical *wzy15AF* target combined with a divergent *wciZ15F* target sequence (92% sequence identity). Strains 84245 and MZ877 shared near-identity over the full-length *wciZ* gene (977/978 identical bp) but had only 2 to 3 conservative amino acid substitutions compared to the WciZ15F protein (not shown). Strains 84245 and MZ877 also lacked the *wcjE* gene present in *cps15F* (Fig. 1). Consistent with their unique *cps* operons, these two strains displayed unique reactivity with serogroup 15 serotyping factors (Table 1). To summarize, this new serotype, designated 15D, is potentially predicted by the unique combination of *wzy15AF*-encoded polymerase activity, *wciZ*-encoded *O*-acetyltransferase activity, and lack of a *wcjE*-encoded *O*-acetyltransferase.

Strain 84245 (BioSample accession no. SAMN14150919), described during US invasive pneumococcal disease surveillance (2), was predicted to have low-level penicillin resistance and macrolide resistance, while strain MZ877 (SAMN17684515) was predicted to have reduced penicillin susceptibility combined with co-trimoxazole-resistance. Strain 84245 had a new multilocus sequence type (ST15307) that is a single locus variant of ST9692, associated with serotype 15A strains recovered in Kenya, while

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TABLE 1 Unique serogroup 15 factor reactivity of new serotype 15D employing CDC *Streptococcus* lab typing antisera and SSI[a] typing antisera

Pneumococcal strain	CDC or SSI 15 pool[a]	CDC factors 15bf[b]	CDC factors 15de[c]	CDC factor 15g[d]	CDC or SSI factor 15e[e]	CDCor SSI factor 15h[f]	SSI factor 15b[g]	SSI factor 15c[g]
15A strain 389/39	+	−	+	+	−	−	−	+
15F strain 688/63	+	+	−	−	−	−	+	+
15B strain 7904/39	+	+	+	−	+	+	+	−
15C strain 553/62	+	−	+	−	+	−	−	−
15D invasive US 84245	+	+	−	+	−	−	+	+
15D carriage Mozambique MZ877	+	+	−	+	−	−	+	+

[a]Pooled antiserum prepared against serotypes 15A, 15F, 15B, and 15C. Identical results were obtained using CDC and commercial antiserum from Serum Staten Institut (SSI, Copenhagen Denmark) where indicated. For CDC purposes, pooled antiserum, factor 15bf, factor 15de, and factor 15g routinely used to resolve serogroup 15 strains into its 4 different serotypes (and now can be used for identifying 5 different serotypes with the inclusion of 15D). The reagents were prepared as described (3).
[b]Factor 15bf consists of antiserum prepared against serotype 15F strain followed by absorbing with serotype 15A strain.
[c]Factor 15de consists of antiserum prepared against serotype 15C strain followed by absorbing with serotype 15F strain.
[d]Factor 15g consists of antiserum prepared against serotype 15A strain followed by absorbing with serotype 15F strain and serotype 15C strain. We highlight this result since it is the only serologic difference shown in the table between serotypes 15F and 15D.
[e]Factor 15e consists of antiserum prepared against serotype 15C strain followed by absorbing with serotype 15A strain.
[f]Factor 15h consists of antiserum prepared against serotype 15B strain followed by absorbing with serotype 15F strain and 15C strain.
[g]Factors 15b and 15c were obtained from SSI (Serum Staten Institut).

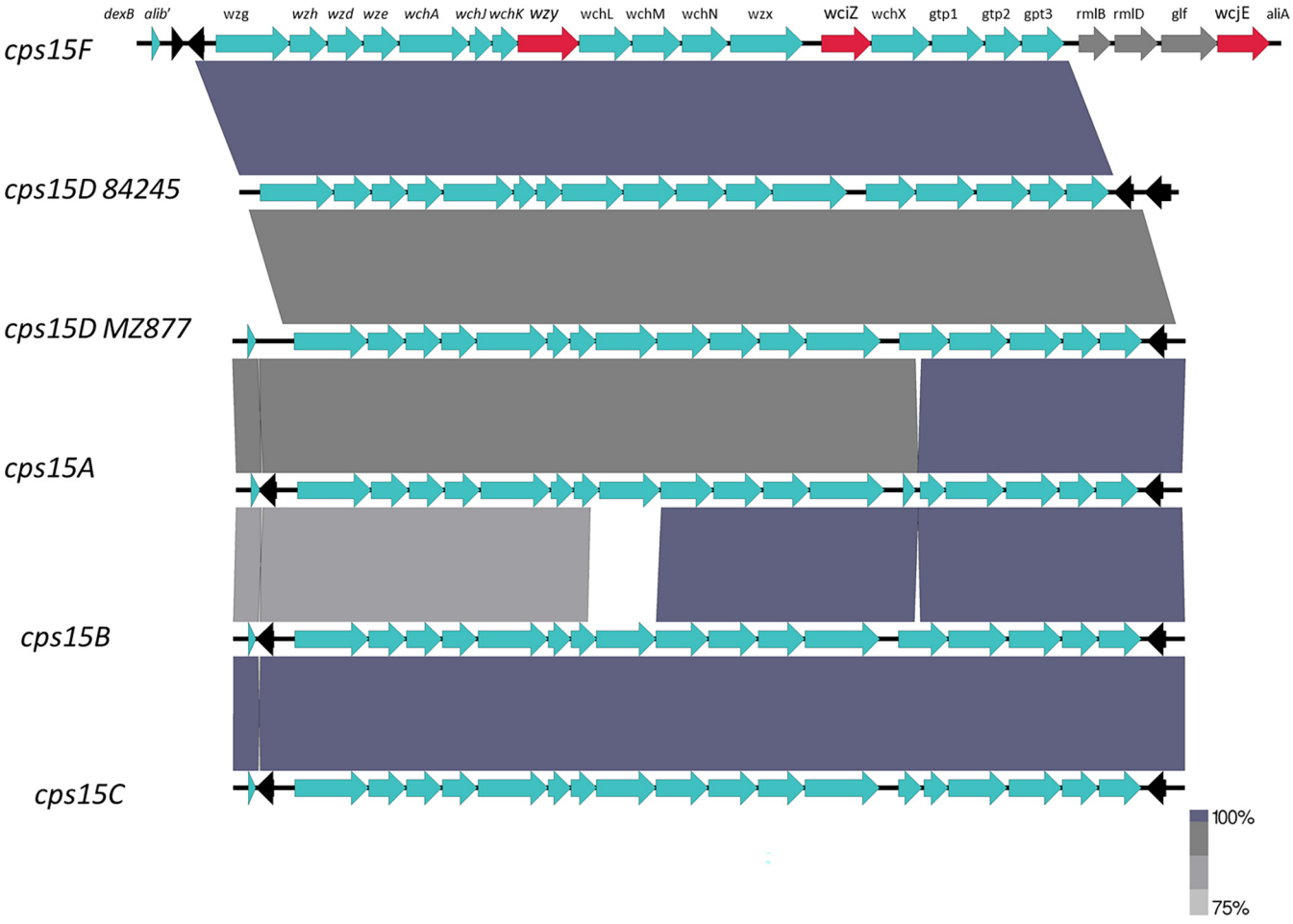

FIG 1 Alignment of *cps* operons from the four known serogroup 15 serotypes and from the two isolates expressing the new serotype 15D. The three genes indicated in red provide basis by which to resolve the five different serotypes shown. The gray open reading frames encode enzymes that have functions unrelated to serogroup 15 polysaccharide structure. The short black open reading frames are transposase gene remnants. The two serotype 15D strains were subjected to whole-genome sequencing as previously described (1, 2). Sequences encompassing the *dexB* 3′ end and the *aliA* 5′ end were extracted from these two strains and were aligned with published GenBank sequences for *cps15F* (CR931666), *cps15A* (CR931663), *cps15B* (CR931664), and 15C (CR931665) (see references 4 and 6). The extracted sequences and published serogroup 15 *cps* operons were subjected to Prokka to identify and annotate open reading frames (9). The resultant annotated sequences were analyzed by BLAST and the homolog *cps* locus regions were aligned into figures using EasyFig version 2.2.3 software (10). Strains 84245 and MZ877 were assigned BioSample accession numbers SAMN14150919 and SAMN17684515, respectively, under project number PRJNA697931.

MZ877 has the unrelated genotype ST10654, reported from 15A strains recovered in South Africa (https://pubmlst.org/organisms/streptococcus-pneumoniae). Our finding is conceivably important for future pneumococcal vaccine formulations. We were originally mistaken in assigning these two strains the serotype 15A. It is conceivable that other researchers have also mistakenly assigned serotype 15A or 15F to strains that actually express serotype 15D.

Data availability. Strains 84245 and MZ877 were assigned BioSample accession numbers SAMN14150919 and SAMN17684515, respectively, under project number PRJNA697931.

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