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MAJOR ARTICLE

Pneumococci Can Become Virulent by Acquiring a New Capsule From Oral Streptococci

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(See the Editorial Commentary by Beall, on pages 343–6.)

Pneumococcal conjugate vaccines have been successful, but their use has increased infections by nonvaccine serotypes. Oral streptococci often harbor capsular polysaccharide (PS) synthesis loci (*cps*). Although this has not been observed in nature, if pneumococcus can replace its *cps* with oral streptococcal *cps*, it may increase its serotype repertoire. In the current study, we showed that oral *Streptococcus* strain SK95 and pneumococcal strain D39 both produce structurally identical capsular PS, and their genetic backgrounds influence the amount of capsule production and shielding from nonspecific killing. SK95 is avirulent in a well-established in vivo mouse model. When acapsular pneumococcus was transformed with SK95 *cps*, the transformant became virulent and killed all mice. Thus, *cps* from oral *Streptococcus* strains can make acapsular pneumococcus virulent, and interspecies *cps* transfer should be considered a potential mechanism of serotype replacement. Our findings, along with publications from the US Centers for Disease Control and Prevention, highlight potential limitations of the 2013 World Health Organization criterion for studying pneumococcal serotypes carried without isolating bacteria.

Keywords. pneumococcus; vaccine; serotype.

Streptococcus pneumoniae (the pneumococcus) is often carried in the nasopharynx without clinical symptoms but, in susceptible individuals, it can invade and cause serious infections such as bacteremia, meningitis, and pneumonia. Pneumococcal infections account for a large number of deaths among young children and elderly adults [1, 2]. A critical factor for development of invasive pneumococcal disease is the presence of a polysaccharide (PS) capsule, which protects against opsonophagocytosis. *S. pneumoniae* has a genetic locus (*cps* locus) composed of variable combinations of genes that encode approximately 100 antigenically distinct capsule types [3]. Antibodies against the specific PS capsule carried by the bacteria can provide effective protection to the host by opsonizing pneumococci for phagocytes. Current pneumococcal vaccines are designed to elicit antibodies to the capsule types that most commonly cause invasive diseases. Widely used pneumococcal conjugate vaccines (PCVs) contain 10–13 different capsule types, and their use has greatly reduced the occurrence of invasive pneumococcal diseases [4, 5].

Widespread use of PCVs has dramatically altered the pneumococcal serotypes now responsible for invasive pneumococcal diseases by reducing the carriage rates of and conferring resistance against the included vaccine capsule types, but with a concomitant increase in nonvaccine types [6, 7]. This phenomenon is called *serotype shift* and has been attributed to several mechanisms [8]. One mechanism is unmasking of the serotypes that were present as a minor subpopulation before the disappearance of the vaccine serotype that was dominant. The other is serotype switch as a result of capturing *cps* from another pneumococcus [9]. A third, but so far unobserved, possibility is that pneumococci may capture in part, or entirety, *cps* from other species [10]. In the latter circumstance, the most relevant donor species are oral streptococci, which are avirulent, genetically similar to pneumococci, and coexist with pneumococci in the nasopharynx [11].

Current evidence indicates that a majority of oral streptococci contain pneumococcus-like *cps* loci and actually produce capsular PSs that resemble pneumococcal capsule [12–15]. For instance, SK95 is an oral streptococcal isolate belonging to *Streptococcus oralis* subsp. *dentisani* and has a *cps* that is very similar to pneumococcal capsule type 2 (94% identity in the syntenic region of the capsule locus) [13]. Similarly, capsule type 5 may be among oral streptococci including *Streptococcus pseudopneumoniae* [16, 17]. Thus, if an interspecies genetic transfer occurs, it could significantly complicate serotype shift that follows the long-term use of PCVs.

Despite general similarity, oral streptococcal *cps* loci differ from pneumococcal *cps* by having *aliC*, *aliD*, and other genes in addition to random sequence differences [13]. Those small differences may have critical impacts on virulence because a

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small change in the control of capsule production can starkly reduce the capsule's role in increasing pneumococcal virulence [18, 19]. In addition, even a single nucleotide change can drastically alter the chemical structure of the capsule [3]. Therefore, we have directly investigated the impact of oral streptococcal *cps* on pneumococcal virulence, by examining whether SK95 produces capsular PS identical to pneumococcal serotype 2, and whether SK95 *cps* can convert a nonvirulent acapsular pneumococcus into a virulent encapsulated pneumococcus.

METHODS

Capsular PS Isolation

Bacteria were grown overnight at 37°C in 1 L of chemically defined medium, as described elsewhere [20]. Bacteria were pelleted, washed, and treated with proteinase K. Capsular PS was released from the bacteria by an overnight incubation at 37°C with 2000 U of mutanolysin and 20 mg of lysozyme. The capsular PS was purified by means of anion exchange chromatography, and the PS-containing fractions were determined through an inhibition enzyme-linked immunosorbent assay (ELISA), which was performed as described elsewhere [21] using Hyp2M2 monoclonal antibody (mAb) [22]. The capsule was further purified by passing the pool through a size-exclusion column (Superose 6; GE Healthcare) and the fractions containing capsule were pooled, dialyzed, and lyophilized. (See Supplementary Material)

Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance (NMR) data were collected at 50°C on a Bruker Avance II spectrometer (^{1}H, 700.0 MHz) equipped with a cryogenic probe. Complete assignments of ^{1}H and ^{13}C signals were achieved by means of 2-dimensional double-quantum-filtered correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), nuclear overhauser effect spectroscopy (NOESY), multiplicity-edited ^{1}H-^{13}C heteronuclear single quantum correlation spectroscopy (HSQC), HSQC-TOCSY, and HSQC-NOESY. Characteristic ^{1}H and ^{13}C chemical shifts, described in previous studies [23, 24], were used to identify each sugar residue. MR data were

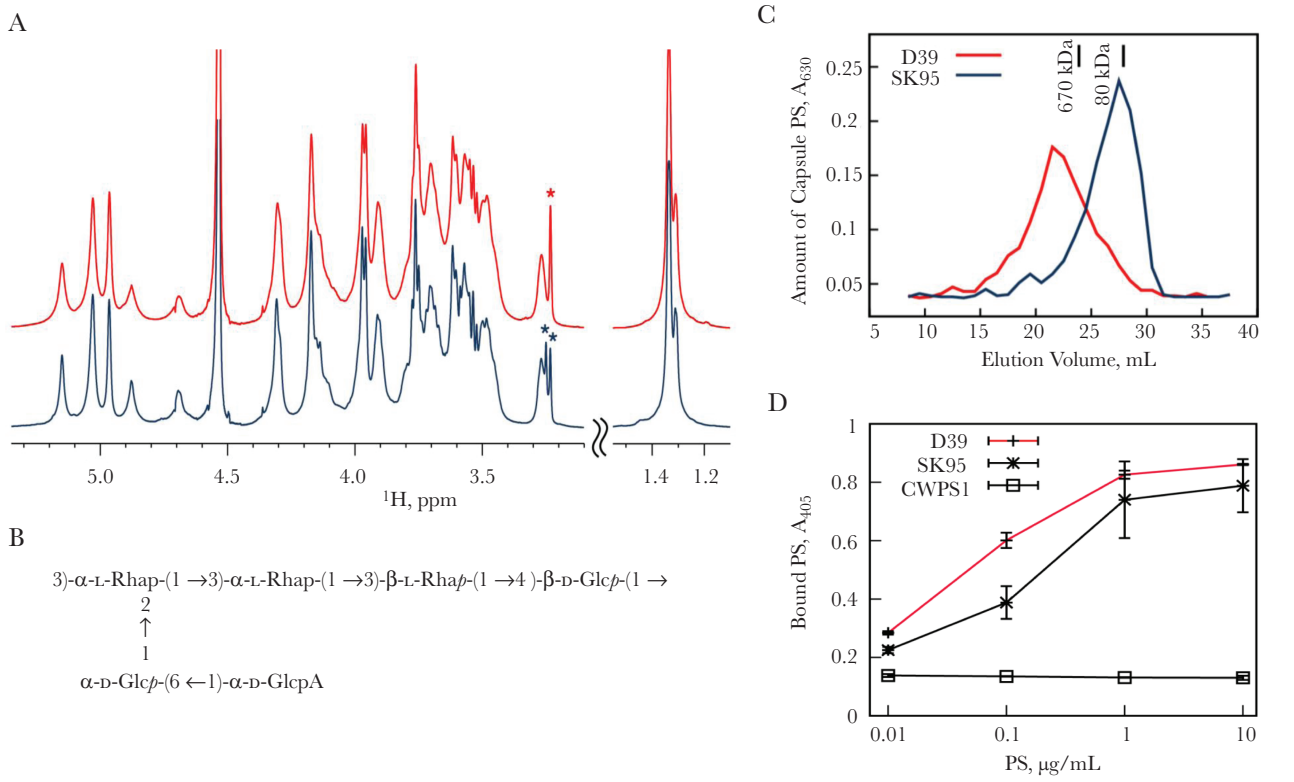

Figure 1. Structure of type 2 capsule polysaccharide (PS). *A,* Overlay of partial (^{1}H) Nuclear magnetic resonance (NMR) spectra of capsular PSs from type 2 *Streptococcus pneumoniae* (D39) (*red, top*) and *Streptococcus oralis* (SK95) (*blue, bottom*) recorded at 50°C. As shown, spectra are identical, but the signals of D39 are broader than those of SK95. Asterisks mark choline signals arising from teichoic acid. To facilitate presentation, the 2 spectral regions are plotted with different vertical scales. *B,* Structure of capsular PS from *S. oralis* (SK95) as determined with NMR spectroscopy. *C,* Amount of capsular PSs from SK95 (*blue*) and D39 (*red*) versus elution volumes of an S-500 column. Elution volumes of dextran molecular weight standards are indicated with vertical bars. *D,* Amount of type 2 capsule (y-axis) bound to the enzyme-linked immunosorbent assay (ELISA) plate at different amounts of PS (x-axis). ELISA wells were coated with antiphosphocholine antibody and captured PSs were determined with a monoclonal antibody (mAb) specific for type 2 PS (Hyp2M2). The mAb supernatant was diluted at 1:320, 1:160, and 1:80, respectively, for D39, SK95, and CWPS1 (teichoic acid) wells. Error bars at the data points indicate standard deviations (error bars for CWPS1 were too small to be seen). Abbreviations: A_{405}, absorbance at 405 nm; A_{630}, absorbance at 630 nm.

Table 1. Proton and Carbon Chemical Shifts of Capsular Polysaccharide From SK95

	Chemical Shift, ppm					
Sugar Residue	H1/C1	H2/C2	H3/C3	H4/C4	H5/C5	H6/CH^3
→3)-α-L-Rha*p*-(1→	5.13	4.29	4.09	3.69	3.89	1.32
2 ↑	104.6 [170 Hz][a]	81.1	83.1	76.3	74.1	21.2
→3)-α-L-Rha*p*-(1→	5.02	4.15	3.95	3.55	3.88	1.29
	106.8 [171 Hz]	74.7	83.1	76.1	73.9	21.3
→6)-α-D-Glc*p*-(1→	5.01	3.55	3.75	3.66	4.28	4.12, 3.71
	102.4 [171 Hz]	76.0	77.9	73.8	75.0	69.9
α-D-Glc*p*A-(1→	4.95 [2.4 Hz]	3.59	3.74	3.52	3.94	…
	102.9 [169 Hz]	76.2	77.7	76.8	77.0	181.1
→3)-β-L-Rha*p*-(1→	4.86	4.15	3.64	3.46	3.43	1.32
	105.2 [163 Hz]	75.2	85.1	76.1	76.7	21.4
→4)-β-D-Glc*p*-(1→	4.67 [6.3 Hz]	3.25	3.60	3.59	3.48	3.96, 3.78
	109.0 [161 Hz]	78.6	80.5	81.9	79.3	65.9

[a]Anomeric one-bond ^{13}C-^{1}H and resolved three-bond ^{1}H-^{1}H *J* couplings are given in brackets.

processed using Topspin version 3.6 or NMRPipe software [25] in combination with hmsIST programs to reconstruct non-uniform sampling (NUS) data [26] (see Supplementary Material)

Molecular Weight Determination

Purified capsule or dextran analytical standard samples were analyzed on a 40-mL chromatographic column packed with Sephacryl S 500 resin. The eluted fractions were analyzed for PS content by anthrone assay [27] (see Supplementary Material)

Sandwich Immunoassay

To test linkage between capsular PS and teichoic acid, a sandwich-type assay was performed, as described elsewhere [20]. Briefly, ELISA plate wells were coated with an mAb antiphosphocholine antibody (HPCG2b; 1 µg/mL in phosphate-buffered saline) and tested with various dilutions of D39 capsular PS, SK95 capsular PS, and teichoic acid (CWPS1 [3]; Staten Serum Institute). The bound serotype 2 capsule was detected with a type 2–specific mAb, Hyp2M2 [28]. All reactions were done in triplicate.

Construction of Isogenic Strains

Isogenic strains were constructed using the Janus cassette (JS) [29, 30]. Briefly, the TIGR-JS strain containing the Janus cassette [29, 31] was mixed with D39 or SK95 cell lysates, in competence medium (Todd-Hewitt broth plus 0.5% yeast extract, 0.2% bovine serum albumin, 0.01% calcium chloride, and competence stimulating peptide 1 at 50 ng/µL). Transformants were then selected on Todd Hewitt broth plus 0.5% Yeast extract (THY) agar plates containing streptomycin and were backcrossed 3 times with TIGR-JS. Each transformant was tested for serotype using agglutination with type-specific antiserum [32], surface phenotype by flow cytometry [21] with type 2–specific immunoglobulin M mAb (Hyp2M2) [28], and genetic markers using polymerase chain reaction targeting *cpsA*/*wzg, cpsB*/*wzh,* and *cpsD*/*wze* genes.

Nonspecific Killing Assay

The well-characterized University of Alabama at Birmingham opsonophagocytic killing assay [33] (described in detail elsewhere [34]) was adapted to determine nonspecific killing. Briefly, 30 µL of bacteria suspended in opsonization buffer b (OBB; Hanks buffer supplemented with 0.1% gelatin and 5% fetal calf serum) was mixed with 10 µL of baby rabbit serum (BRS) of specified concentration, and 40 µL of differentiated HL60 cells (1.0×10^7 cells/mL) in OBB. To determine whether the bacteria are killed by phagocytosis, some HL60 cells were preincubated with 10 µmol/L cytochalasin D (Sigma-Aldrich) for 30 minutes at room temperature. The mixture was incubated with shaking (700 rpm) for 45 minutes at 37°C with 5% carbon dioxide. Then 10 µL of reaction mixture was plated on THY agar plates, and, after an overnight incubation, the bacterial colonies were counted. Nonspecific killing was calculated by the following formula: nonspecific killing (%) = [1 − (colony-forming units [CFUs] in control B/CFUs in control A)] × 100, where control A contains heat-inactivated BRS and control B, active BRS. All reactions were done in triplicate.

Animal Infection

Female, 5–6 week-old BALB/cJ mice (Jackson Laboratory) were challenged with 1.0×10^3 *S. pneumoniae* (D39), 1.0×10^4 *S. oralis* (SK95), 1.0×10^5 acapsular pneumococcus (TIGR-JS), or isogenic strains (FG34, FG44, FG3, and FG4) in 100 µL of phosphate-buffered saline, by intraperitoneal infection. Bacteremia was assessed with a tail bleed after 6 or 24 hours, and animal status/health was checked every 6 hours by assessing the body score index. When mice were deemed moribund, they

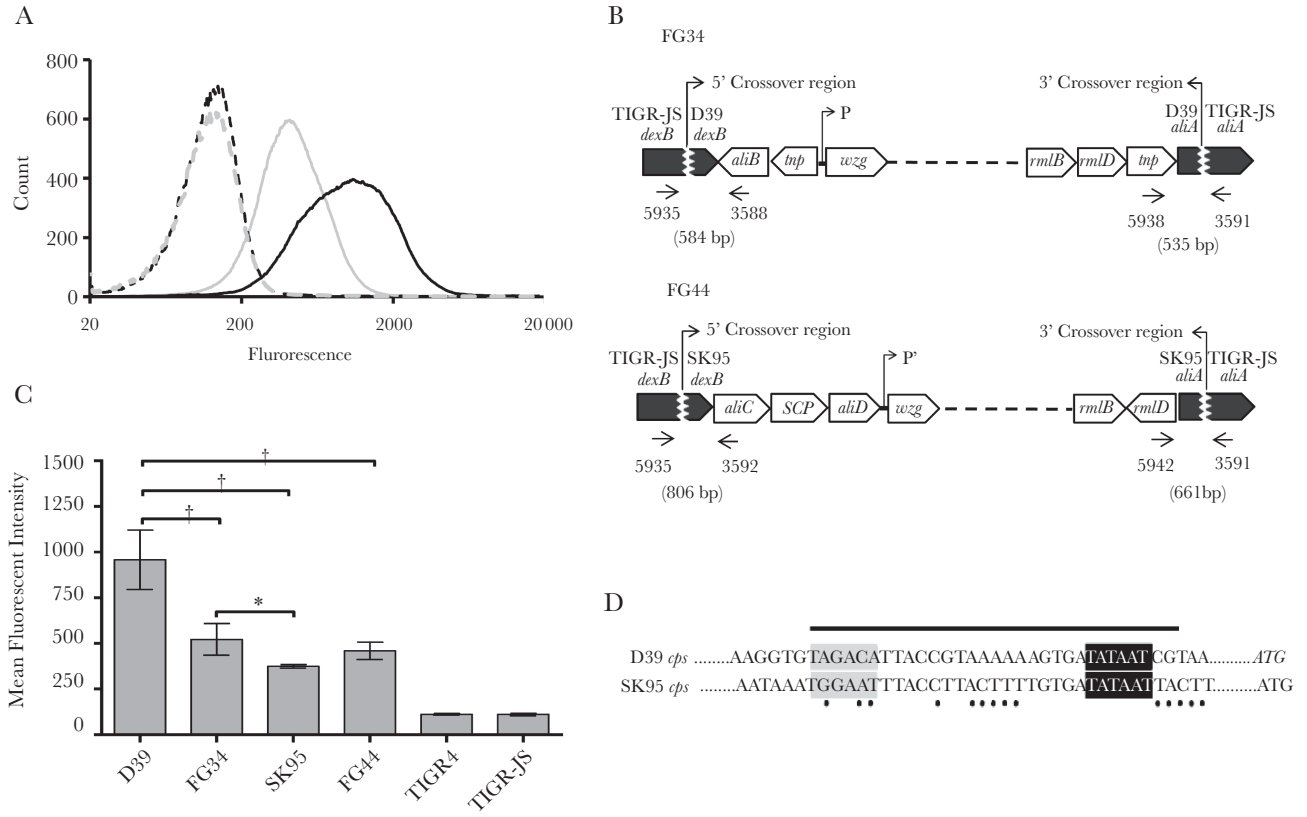

Figure 2. Type 2 capsule expression in wild type and isogenic strains. *A,* Flow cytometric analysis of capsule expression. Histograms depict binding of monoclonal antibody (mAb) Hyp2M2 to strains D39 (*solid black line*) and SK95 (*solid gray line*). The corresponding dashed lines show control binding (secondary antibody alone). *B,* Genetic map of the *cps* region of FG34 (TIGR-JS transformant, with D39 *cps*) and FG44 (TIGR-JS transformant, with SK95 *cps*). The 5′ crossover points for FG34 and FG44 are between bases 316624–316729 and 316754–316814 of the TIGR4 genome, respectively (see Supplementary Table 2 for details). Arrows and 4-digit numbers indicate binding positions of the polymerase chain reaction (PCR) primers (Supplementary Table 1); numbers in parentheses, expected sizes of the PCR products; and P and P′, promoter locations. *C,* Mean fluorescent intensity of type 2 capsule expression for indicated strains. Experiments were performed 6 times, and error bars represent standard errors. **P* < .01; †*P* < .001. *D,* Sequences of the core promoter regions (black bar) of D39 and SK95 (GenBank accession nos. CP000410.2 [41] and AFUB01000057.1 [13]). Gray- and black-shaded regions indicate core promoter motifs at positions –35 and –10, respectively; *ATG,* translation start codons of *cps*A; and black dots, nucleotide mismatch in the core promoter region between D39 and SK95 *cps*.

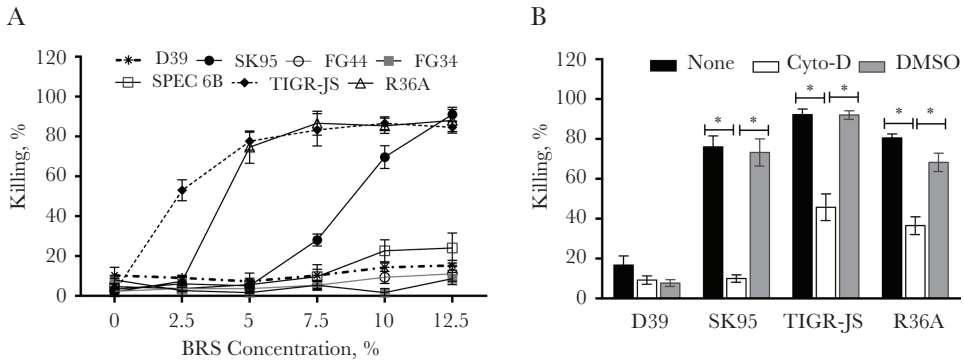

Figure 3. Nonspecific killing (NSK) and inhibition of phagocytosis. *A,* NSK of various bacterial strains in the presence of different baby rabbit serum (BRS) concentrations. TIGR-JS and R36A are nonencapsulated control strains. FG34 and FG44 are TIGR-JS transformants with D39 *cps* and SK95 *cps,* respectively. SPEC1, TIGR4, and TREP19A are encapsulated pneumococcal isolates [33], and their killing curves lie between FG34 and SPEC6B, and are not shown for the purpose of clarity. *B,* NSK of bacterial isolates (labeled on x-axis) with HL60 cells treated with OBB alone (*white bar*), OBB with 10 µmol/L cytochalasin D (Cyto-D) (*black bar*), or OBB with 1% dimethyl sulfoxide (DMSO) control (*gray bar*) at 10% BRS. Error bars indicate means of 3 replicates with standard errors. **P* < .001.

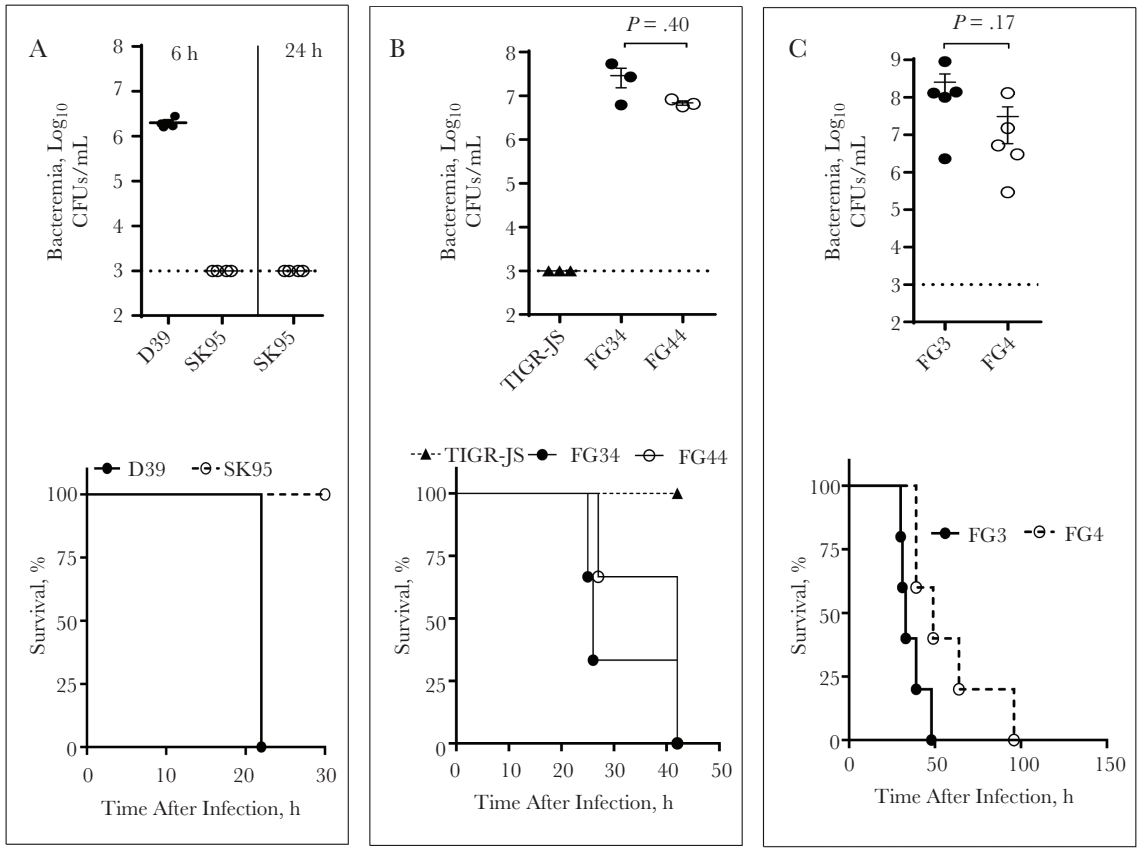

Figure 4. In vivo virulence study of D39 and SK95 *cps*. *A,* Bacteremia at 6 and 24 hours, and survival of BALB/cJ mice that were intraperitoneally infected with either D39 or SK95 strains (4 mice per group). *B,* Bacteremia and survival of mice after intraperitoneal infection with acapsular pneumococcus (TIGR-JS, 4 mice/group), pneumococcus strain TIGR4-JS with D39 *cps* (FG34; 3 mice per group), and TIGR4-JS with SK95 *cps* (FG44; 3 mice per group). *C,* Bacteremia and survival of mouse after intraperitoneal infection with TIGR-JS with D39 *cps* (FG3) or with SK95 *cps* (FG4). Each group has 5 mice. Infection doses per mice were 10^3 colony-forming units (CFUs) of D39, 10^4 CFUs of SK95, or 10^5 CFUs of TIGR-JS, FG34, FG44, FG3, and FG4. *B, C,* For bacteremia, errors bars represent standard errors of the mean, and *t* test were used. Survival curves were compared using log-rank (Mantel-Cox) tests: $P = .045$ for D39 versus SK95 (*A*); $P = .01$ for both TIGR-JS versus FG34 and TIGR-JS versus FG44 (*B*); $P = .02$ for FG34 versus FG44 (*C*). h, hours.

were then checked every 30–60 minutes to assess the time of death. All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee at The University of Alabama at Birmingham (protocol IACUC-20175). Animal care and experimental protocols adhered to public law 89–544 (Animal Welfare Act) and its amendments, Public Health Services guidelines, and the *Guide for the Care and Use of Laboratory Animals* (US Department of Health and Human Services).

RESULTS

S. oralis SK95 Capsule Structure Is Identical to Pneumococcal Serotype 2 Capsule

To elucidate the chemical basis for the serologic cross-reactivity to type 2 capsule for SK95 [13], we used MR spectroscopy to determine the structure of SK95 capsular PS. The ^{1}H MR spectra of purified capsular PS from SK95 and D39 were identical (Figure 1A), suggesting that SK95 and D39 produce identical capsular PS. Both spectra showed ^{1}H signals arising from choline (chemical shifts at 3.23 and 3.25 ppm, marked with an asterisk in Figure 1A), suggesting the presence of teichoic acid. Teichoic acid is often present in pneumococcal capsule preparations because both the capsule and teichoic acid are covalently linked to peptidoglycan [35].

To completely delineate SK95 capsular PS structure, the ^{1}H and ^{13}C chemical shifts of SK95 PS were obtained using a set of 2-dimensional MR studies (Table 1). When all the chemical shifts were identified, the SK95 capsule structure was found to be identical to the published structure of type 2 capsular PS (Figure 1B) [36]. The D39 capsule structure was partially determined because the MR signals of D39 capsule were noticeably broader [37]. The partial structure of D39 was identical to the published structure of serotype 2 [36], and also to the SK95 structure. The broad MR signals suggest that the D39 capsule is longer than the SK95 capsule (ie, consisting of more identical oligosaccharide repeats). Indeed, the molecular weight of the purified D39 capsule was much larger than that from SK95 (Figure 1C).

To examine whether the SK95 capsule is linked to teichoic acid, as pneumococcal capsule is, we performed a sandwich-type ELISA, which determined the amount of type 2 capsule captured with antiphosphocholine antibody. The ELISA captured type 2 PS from D39 and SK95 capsules in a dose-dependent manner (Figure 1D). In contrast, our negative control of purified teichoic acid (CWPS1) did not produce any signals. Thus, SK95 capsule is anchored to teichoic acid and to peptidoglycan.

Amount of Capsule Produced by SK95 and D39 *cps* Loci Is Dependent on Genetic Background

The amount of capsule anchored to SK95 and D39 was then compared by means of flow cytometry. D39 was found to display more capsule on its surface than SK95 in multiple experiments (Figure 2A). To investigate whether the amount of capsule is determined by *cps* or other genes, we transferred D39 or SK95 *cps* into TIGR-JS, a noncapsulated variant of TIGR4. Resulting transformants with D39 *cps* or SK95 *cps* were named FG34 or FG44, respectively. Genetic mapping showed that the transformants acquired the entire *cps* flanked by *dexB* and *aliA* (Figure 2B and Supplementary Tables 1 and 2). In the case of FG44, it acquired *aliC*, a gene encoding a SCP domain protein, and *aliD* genes in addition to the 17 genes needed to produce type 2 capsule. FG34 displayed much less capsule than D39 ($P < .001$) and FG44 also displayed measurably more capsule than SK95, although the difference was not statistically significant ($P = .07$) (Figure 2C). In contrast, FG34 and FG44 had similar amounts of capsule on their surface ($P = .17$). Taken together, within these transformants, the amount of capsule production was more influenced by the genetic background (eg, TIGR-JS background vs D39) than the species origin of *cps* loci.

S. oralis cps Can Provide Shielding From Nonspecific Phagocytosis to Noncapsulated Pneumococci

A major function of capsule is to shield bacteria from nonspecific complement deposition and opsonophagocytosis. Indeed, noncapsulated pneumococci (TIGR-JS and R36A) could be killed, essentially completely (about 80% killing), by phagocytes in the presence of 5% of BRS (Figure 3A). In contrast, only 10% of D39 were killed, even when BRS levels were raised to 12.5%. SK95 was more resistant to killing than noncapsulated pneumococci, but about 60% was killed with 10% BRS (Figure 3A). The killing of SK95 is dependent on phagocytosis, because this killing could be almost completely abrogated with 10 μmol/L cytochalasin D (Figure 3B). Heat-inactivated BRS did not kill any bacteria (data not shown). Thus, even though SK95 produces type 2 capsule, similar to D39, SK95 is susceptible to nonspecific complement-mediated phagocytosis. Just like other encapsulated pneumococcal strains, <10% of both FG34 and FG44 were killed, even at 12.5% BRS (Figure 3A). Thus, SK95 *cps* was as effective as D39 *cps* in shielding pneumococci from phagocytes.

S. oralis cps Can Make Nonvirulent Noncapsulated Pneumococci Virulent

To directly test the virulence of the transformants in vivo, we intraperitoneally infected young female BALB/cJ mice with either 10^3 CFUs of D39 or 10^4 CFUs of SK95 per mouse. Mice are highly susceptible to this route of challenge, and this is a long-established model of pneumococcal bacteremia and sepsis. Within 6 hours of infection, SK95 was undetectable (<10^3 CFUs/mL) in the blood, but D39 caused a high level (approximately $2x10^6$ CFUs/mL) of bacteremia. Within 24 hours, D39 infection killed all mice but SK95 infection had killed none ($P = .045$) (Figure 4A). The difference in virulence was even more striking when considering that SK95 challenged mice were injected with 10 times more CFUs than D39 challenged animals.

To directly compare the impact of SK95 *cps* on virulence, we infected mice with 10^5 CFUs of noncapsulated pneumococcus (TIGR-JS), TIGR-JS transformed with D39 *cps* (FG34), and TIGR-JS transformed with SK95 *cps* (FG44). As shown in Figure 4B, TIGR-JS killed no mice but both FG34 and FG44 killed all animals within 2 days ($P < .01$ for both). To confirm that the experimental result was not specific to a single transformation event, this experiment was repeated using clones from a different transformation experiment (FG3 and FG4). Again, the results showed that both FG strains have comparable virulence in causing bacteremia and killing the infected mice (Figure 4C). For both experiments (Figure 4B and 4C), D39 and SK95 *cps* showed similar bacteremia levels, but D39 *cps* killed slightly faster than SK95 *cps*. Nevertheless, it is clear that SK95 *cps* has the potential to make acapsular pneumococcus become virulent.

DISCUSSION

Although capsules ("coaggregation receptor PSs") of oral streptococci were described some time ago [12] and have been proposed to be involved in adherence to other bacteria [38], there is now an increasing appreciation of the capsular PSs produced by oral streptococci [13–15]. More recently, genomic studies have shown that >74% of oral *Streptococcus* isolates have pneumococcuslike *cps*, except for *S. pseudopneumoniae* [13]. Serologic similarities between pneumococcal and oral streptococcal capsular PSs have been shown elsewhere [13, 15–17, 39]. However, it was unknown whether oral streptococcal capsule was chemically identical to that of pneumococci. In the current study, we show that SK95 produces capsular PS that is chemically identical to highly virulent, pneumococcal serotype 2 capsule, although it is shorter than the D39 capsule. Although the SK95 capsule is anchored to peptidoglycan, as pneumococcal capsule is, SK95 produces less capsule than D39, consistent with previously reported thin capsules of oral streptococci [40] with inadequate shielding [13]. Studies with FG44 indicate that

genetic background (ie, non-*cps* genes) significantly influences the amount of capsule production.

We were surprised to find that SK95 *cps* is as effective as is D39 *cps* in increasing virulence of acapsular pneumococci. Our finding was unexpected because prior studies have demonstrated that a slight difference in the control of capsule production greatly reduced resistance to in vitro phagocytosis or to the in vivo virulence of a pneumococcus strain. For instance, a slight sequence variation in *cpsE*/*wchA* [19] or small changes in the *cps* core promoter [18] starkly reduces in vivo virulence, and the core promoter region is highly conserved among pneumococcal isolates [41]. Although similar, oral streptococcal and pneumococcal *cps* loci do have significant differences. The 2 species have different core promoter sequences (Figure 2D). In addition, oral streptococcal *cps* has *aliC* and *aliD* genes, and occasionally other genes, such as a gene encoding a SCP domain protein in SK95, which are absent in most pneumococcal *cps*. Thus, in view of the differences, it is surprising that SK95 *cps* was as effective as D39 *cps* in increasing virulence. This finding is important, because it indicates that oral streptococci, even though nonpathogenic, can serve for *S. pneumoniae* as genetic reservoirs of the *cps*, the key virulence factor.

The potential of such an interspecies *cps* transfer, perhaps facilitated by PCV usage, is important for several reasons. First oral streptococci have been considered a genetic reservoir of allelic variants of virulence genes for pneumococci [10], including the antibiotic resistance genes that appeared in pneumococci after long-term use of antibiotics [42, 43]. Even though interspecies *cps* transfer has not been observed yet, it may occur, because their *cps* loci share similar genomic architecture in general, and are often syntenic. Second, interspecies *cps* transfers should be considered in interpreting epidemiologic observations. For instance, serotype 2 infections have appeared in several areas after a long global absence [44, 45]. One should consider whether the recent epidemics are from pneumococcal acquisition of type 2 *cps* from oral streptococci. Third, oral streptococci are genetically more diverse than pneumococci, and oral streptococci should harbor many unique and distinct *cps* that can be potentially transferred to pneumococci [10, 13]. We need not only to continue monitoring serotype shift but also to expect the appearance of unusual new serotypes and increase our knowledge of capsule types produced by oral streptococci as a part of our long-term strategy of using PCVs.

Conversely, immunizations with PCVs may reduce the prevalence of oral streptococci expressing the vaccine capsule type and the coaggregating bacteria, thereby leading to changes in the oral microbiome [46]. Several studies reported that adults who were vaccinated with 13-valent PCV were less likely to have pneumococci vaccine-type genes detected in their specimens than those who were unvaccinated [15, 47, 48]. In a response to serotype replacement, many companies are developing PCVs with 15 [49], 20 [50], or more serotypes. As we anticipate deployment of these next-generation PCVs, this converse possibility is a matter of increasing interest. The long-term consequence of oral microbiome change for oral health is unclear and may have important health implications. As such, the impact of PCV usage on the oral microbiome requires study.

Finally, our finding has another important implication for studying nasopharyngeal carriage of pneumococci. The current World Health Organization recommendation for carriage studies states that nasopharyngeal swab samples can be directly examined for certain pneumococcal genes (including capsule genes) without first obtaining bacterial isolates [51]. Based on the recommended criterion, a study found that PCV may reduce pneumococcal carriage of vaccine type strains among adults [47]. However, because oral streptococci can have pneumococcuslike *cps*, the PCV may have reduced carriage of oral streptococci expressing vaccine serotypes instead of pneumococci. Several studies from the US Centers for Disease Control and Prevention have noted the presence of pneumococcuslike *cps* among oral streptococci and have cautioned against using the 2013 World Health Organization recommended approach [14–17]. Our findings further support the caution from the Centers for Disease Control and Prevention, and future studies of pneumococcal carriage should consider the possibility that oral streptococci not only can have pneumococcuslike *cps* but also can produce pneumococcal capsule itself. Moreover, because oral streptococci and pneumococci are so similar in capsule production, the source of a pneumococcal capsule would not be identified without examining the bacterial isolates.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. M. H. N. supervised the group, devised and executed the project, had full access to all study data, and takes responsibility for the integrity and accuracy of the data analysis, and contributed to drafting and revision of the manuscript. M. K., C. J. O., and J. S. S. helped with the conceptualization, experimental design, and critical revision of the manuscript. T. B., J. V., and F. G. performed the experiments, performed data analysis and interpretation, and contributed to the critical revision of the manuscript.

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