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# Effectiveness of quadrivalent meningococcal conjugate vaccine against meningococcal carriage and genotype character changes: A secondary analysis of prospective cohort study in Korean military trainees

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ABSTRACT

*Objective:* We evaluated the changes and molecular epidemiology of meningococcal carriage in military recruits after quadrivalent meningococcal conjugate vaccines (MenACWY) vaccination.
*Methods:* Oropharyngeal swabs were obtained at the beginning and end of the 5-week training. Carriage rates before and after vaccination were compared to estimate vaccine effectiveness (VE). Cultured isolates were characterized by multi-locus sequence typing (MLST).
*Results:* Of 866 vaccinated participants, the overall carriage rate was 10.6% prior to MenACWY vaccination and it tended to decrease to 9.5% after 5 weeks of vaccination ($P = 0.424$). Carriage rate of serogroup ACWY decreased significantly after vaccination ($VE_{ACWY}$ = 72.6%, 95% CI: 36.3-88.2), and serogroup C was particularly reduced ($VE_C$ = 83.0%, 95% CI: 50.6-94.1), whereas non-groupable isolates increased significantly after vaccination ($VE_{NG}$ = -76.1%, 95% CI: -176.2 to -13.1). Among 99 carriage isolates with complete MLST profiles, 45 different sequence types with nine clonal complexes (CCs) were identified, and 35.3% of the carriage isolates belonged to hypervirulent strains such as CC-32, CC-41/44, and CC-269.
*Conclusions:* MenACWY vaccination in military recruits led to reduced carriage rates of serogroups C, W, and Y within a short 5-week period. However, serogroup B isolates belonging to the hypervirulent lineage remained after the implementation of MenACWY vaccination.



## Introduction

The human nasopharynx is the only reservoir for meningococcal colonization and the major source of transmission between humans, resulting in invasive diseases. Most meningococcal carriage isolates are not pathogenic. However, under certain circumstances, a minority of carried meningococci, regarded as hyperinvasive strains, can invade the bloodstream and cause Invasive meningococcal disease (IMD) [1]. Although the association between the carriage state and invasive disease is not fully elucidated, meningococcal carriage studies can improve our understanding of the epidemiology and pathogenesis of meningococcal disease and evaluate the effect of meningococcal vaccines on the carriage state, eventually measuring the effectiveness of meningococcal vaccines against carriage as a prerequisite for IMD development [2].

Monovalent meningococcal A and C conjugate vaccines have demonstrated significant reductions in meningococcal carriage for target serogroups, being included in vaccines, such that they provided herd protection in unvaccinated population [3,4]. According to a randomized controlled study, quadrivalent meningococcal conjugate (MenACWY) vaccine against serogroup ACWY led to a carriage reduction of 36.2% against serogroup CWY at 2 months after vaccination in UK students [5]. However, a meta-analysis study suggested that MenACWY vaccines did not demonstrate clear evidence for reducing meningococcal carriage [6].

In South Korea, military personnel, particularly new recruits, are at increased risk of meningococcal carriage and IMD. They have a high disease incidence of 0.8-3.3 cases per 100,000 persons per year, compared to an annual 0.01-0.08 cases per 100,000 persons

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in the general population [7]. They also have relatively high carriage rates (13.7-37.9%), although there has been a significant gap of carriage rate between study period [8,9]. Meningococcal vaccination program targeted for new recruits using MenACWY-CRM (Menveo®, GlaxoSmithKline) was introduced in late 2012 after the outbreak of meningococcal disease in a military training camp in which four cases of IMD with one fatal case were identified [10,11].

The effects of MenACWY vaccines on meningococcal carriage have not been sufficiently evaluated since its introduction. In addition, most previous studies only provided information on the cross-sectional or longitudinal state of carried meningococci under a high coverage rate of the MenACWY vaccine, rather than the change in the carriage state immediately after vaccination [12-14]. We previously reported a change in meningococcal carriage after the introduction of MenACWY vaccine [15]. However, this study did not evaluate the effectiveness of the MenACWY vaccine against vaccine serogroup carriage or the molecular epidemiology of the carriage isolates. To evaluate the appropriateness of vaccine use in serious but low-prevalence diseases such as IMD, we evaluated the effectiveness of the MenACWY vaccine against vaccine serogroup and non-vaccine serogroup carriages in military recruits and investigated the clonal complexes that remained as pathogenic strains associated with meningococcal disease after the implementation of MenACWY vaccination.

## Methods

### *Study design and participants*

We had conducted a prospective cohort study originally designed to describe the change in meningococcal carriage after the implementation of the MenACWY vaccine [15]. A secondary analysis of this prospective study was performed at a military training center in South Korea. Approximately 500 new military trainee recruits residing in a barrack, were enrolled, during the second week of March 2013 and 2014. The military trainees had been residing together for four days prior to their study enrollment. Because healthy men aged $\geq$ 18 years without significant underlying conditions can be members of military service, any new recruits who entered a military training center were eligible for this study. None of the new recruits had received vaccinations prior to enrollment. This study was approved by the Institutional Review Board of the Armed Forces Medical Command. All participants were enrolled after obtaining informed consent in accordance with the Declaration of Helsinki (1996) and the Guidelines for Good Clinical Practice.

### *Procedures*

Two rounds of oropharyngeal swabs from Korean military trainees were collected at the beginning and end of the basic training period, 5 weeks apart. Trained medical staff obtained oropharyngeal swabs from the posterior pharynx and tonsillar areas. The first swab was collected prior to a single vaccine dose of MenACWY-CRM (0.5 mg intramuscular); the second round was performed 5 weeks after vaccination. Together with oropharyngeal swabs, participants were given structured questionnaires covering personal characteristics, history of upper respiratory tract infection within 2 weeks, recent exposure to antibiotics within 4 weeks, smoking status, socioeconomic status, and place of residence before each swab sample was collected.

### *Culture and characterization of carriage isolates*

Immediately after specimen collection, swabs were placed in transport media (Culture SwabTM Plus Amies Gel with Charcoal; BD Diagnostic Systems), stored at room temperature, and transported within 4 h to the Neodin Medical Institute®. The swabs were immediately inoculated onto blood agar plates and Thayer-Martin (MTM) media, and the inoculated media were incubated for 72 h for cultivation. Suspected *N. meningitidis* colonies were initially selected by Gram staining and morphology, such as smooth, round, moist, and uniform large grey/brown colonies with glistening surfaces. They were confirmed as *N. meningitidis* using conventional methods such as oxidase tests, carbohydrate fermentation tests, 30% catalase tests, and API NH kits (bioMérieux, Durham, U.S.). In addition, molecular confirmation of meningococcal isolates was conducted through polymerase chain reaction (PCR) amplification of the conserved crgA gene [16].

### *Serogrouping of isolates*

Serogroups were initially identified by slide latex agglutination with polyclonal antisera (Difco, Becton, Dickinson, and Company, Franklin Lakes, NJ, USA). As for isolates yielding inconclusive or non-reactive results by latex agglutination test, confirmatory PCR analysis was subsequently done. Multiplex PCR was performed with oligonucleotide primers from *sia D* gene to detect serogroups B, C, W, and Y and with the *orf*-2 gene cassette for serogroup A. For the detection of serogroups E, X, and Z, the *ctrA* gene was chosen as the PCR target, as described by Bennett et al. [17].

### *Multi-locus sequence typing analysis for isolates*

Cultured isolates were further analyzed by multi-locus sequence typing (MLST) using fragments from seven housekeeping genes (*abcZ, adk, aroE, fumC, gdh, pdhC*, and *pgm*) to determine the epidemiological characteristics of carriage isolates. PCR-amplified gene fragments were commercially sequenced by Cosmo Genetech Inc. (Seoul, Korea). Sequences were compared with existing alleles on the Neisseria MLST website (http://pubmlst.org/neisseria/) to identify allele numbers, sequence types (STs), and clonal-complexes (CCs) of each isolate. New alleles and STs were submitted to the MLST website for assignment. GrapeTree based on the seven-locus MLST scheme was generated by using an analysis plugin from pubMLST website. A minimum-spanning tree diagram was created to illustrate the phylogenetic relationships among *N. meningitidis* strains using MLST analysis, which is based on sequence variation in seven specific housekeeping genes.

### *Statistical analysis*

Comparisons were made using the chi-squared test, Fisher's exact test, or Student's t-test, as appropriate. Vaccine effectiveness (VE) against carriage was calculated as changing of carriage rate after MenACWY vaccination using following formula: (1-odds ratio [ORs]) $\times$ 100%. Logistic regression analysis was used to evaluate risk factors for new meningococcal carriage. Risk factors were first assessed using univariate analysis. Variables associated with meningococcal carriage in the univariate analysis ($P$ <0.10) or those likely to be clinically relevant were included in the final multivariate model. All statistical analyses were performed using the SPSS version 25 (IBM Corp., Armonk, NY, USA).

## Results

### *Baseline characteristics of army recruits*

A total of 872 participants were enrolled in the study. Two cross-sectional swabs and questionnaire surveys were collected from all participants. Study participants were newly recruited each year, and there were 428 participants in 2013, and 444 in 2014.

**Table 1**
Demographic and clinical characteristics of military recruits.

| | Case (%[a]) Total No. = 866 |
|---|---|
| Age (mean ± SD), years | 20.7 ± 1.5 |
| Underlying conditions, *n* (%) | |
| No underlying condition | 775 (89.6) |
| Allergic rhinitis | 51 (5.9) |
| Asthma | 7 (0.8) |
| Hypertension | 29 (3.3) |
| Others[b] | 4 (0.5) |
| Prior URI[c] before first swab sampling, *n* (%) | 435 (50.2) |
| URI history[d] after first swab sampling, *n* (%) | 770 (88.9) |
| Taking antibiotics after first swab sampling, *n* (%) | 194 (22.4) |
| Smoking | 407 (47.0) |
| Education levels | |
| <High school | 132(15.2) |
| ≥University | 734(84.8) |
| Household monthly income (Korean won) | |
| <3,000 x $10^3$ | 320 (37.0) |
| ≥3,000 x $10^3$ and <5,000 x $10^3$ | 327 (37.7) |
| ≥5,000 x $10^3$ | 219 (25.3) |

[a] Percentage means the proportion of the number of cases per total number of cases ($n$ = 872).
[b] Others included arrhythmia and thyroid disease.
[c] Recent URI was defined as URI within 2 weeks of vaccination.
[d] URI history was defined as experiencing URI after sampling first swab and before sampling second swab.URI, upper respiratory infection.

The participants were mostly healthy participants with a mean age of 20.7 ± 1.5 years, and 89.6% (775 participants) had no underlying diseases (Table 1).

*Effectiveness of MenACWY vaccine against meningococcal carriage*

Effectiveness of MenACWY vaccine was evaluated in 866 vaccinees excluding six unvaccinees. *N. meningitidis* was carried by 92 participants prior to MenACWY vaccination, with an overall carriage rate of 10.6%. Overall carriage rate tended to decrease to 9.5% ($VE_{total}$ = 12.0%, 95% CI: -20.4 to 35.7), and carriage rate of serogroup ACWY decreased significantly after vaccination ($VE_{ACWY}$ = 72.6%, 95% CI: 36.3-88.2) (Table 2). Particularly, serogroup C were reduced significantly after vaccination ($VE_C$ = 83.0%, 95% CI: 50.6-94.1) whereas non-groupable meningococci increased significantly after vaccination ($VE_{NG}$ = -76.1%, 95% CI: -176.2 to -13.1). In 2013, overall carriage rate of *N. meningitidis* was significantly higher after vaccination than before vaccination ($VE_{total}$ = -62.6, 95% CI: -149.5 to -6.1). Increased meningococci carriage after vaccination included non-groupable isolates ($VE_{NG}$ = -239.0, 95% CI: -528.4 to -82.8). Meningococci carriage for serogroup ACWY was decreased from seven isolates (1.6%) to two isolates (0.5%) without statistical significance ($VE_{ACWY}$ = 71.8%, 95% CI: -36.7 to 94.2) after vaccination. On the contrary, in 2014, the overall carriage rate of *N. meningitidis* was significantly lower after vaccination than before vaccination ($VE_{total}$ 61.6%, 95% CI: 35.6-77.1). Both carried meningococci of serogroups ACWY, and non-vaccine serogroups significantly decreased 5 weeks after vaccination (4.1% vs. 1.1%, $VE_{ACWY}$ 73.1%, 95% CI: 26.8-90.1; 8.0% vs. 3.9%, $VE_{NV}$ 53.5%, 95% CI 15.7-74.4).

*Serogroups and sequence types (STs) of carriage isolates*

Serogroup identification by antisera and PCR was done in 174 meningococcal carriage isolates. The predominant serogroups were serogroup B (25.3%) and serogroup C (15.5%), while 50.0% of the isolates were categorized as non-groupable. In terms of the change in meningococcal serogroup, 0.8% (7/866 participants) newly acquired or persistent carriage of serogroup ACWY 5 weeks after MenACWY vaccination, compared to the carriage state at baseline (Supplementary Table 1). Among the isolates with newly acquired or persistent carriage after vaccination, 55 non-groupable isolates were the most common, followed by serogroup B (15 isolates), and serogroup X (5 isolates). Meanwhile, of the 92 participants with meningococci carriage at pre-vaccination, 77.2% (71 participants) changed to a negative conversion.

Among the 174 carriage isolates, complete sequence data for seven housekeeping genes were identified in only 99 carriage isolates. Using MLST analysis, 45 different sequence types (STs) were identified among the 99 carriage isolates with complete MLST profiles. The relationships of the carriage isolates using the seven-locus MLST alleles clustered 85.9% (85/99 isolates) into nine previously known clonal complexes (CCs), and 14 isolates were not assigned to previously known CCs (Figure 1 and Table 3). The most common ST was ST-2397, which was identified in 16 isolates and belonged to clonal complex (CC)-178. CC-178 was the most common genotype in 36 isolates (36.4%) being classified to seven different STs, followed by CC-32 with seven different STs in 15 isolates (15.2%), and CC-41/44 (13.1%) with eight different STs in 13 isolates. Of 99 genotypable isolates, 35.3% (35/99 isolates) of the carriage isolates belonged to CCs regarded as hypervirulent strains, such as CC-32, CC-41/44, and CC-269. Figure 2 shows the association between serogroups and CC strains. Most serogroup C carriage isolates belonged to CC-32. Although serogroup B isolates were associated with more diverse CCs than serogroup C isolates, CC-41/44 and CC-269 were the primary genotypes of serogroup B meningococci carriage.

*Risk factors for carried meningococci after vaccination*

We assessed the social and behavioral risk factors for meningococcal carriage after vaccination. Shared living spaces in military units C and D were likely to affect carriage acquisition (adjusted odd ratio [OR] = 2.72, 95% CI: 1.31-5.63 in unit C and adjusted OR = 2.77, 95% CI: 1.34-5.69 in unit D). Although febrile illness and antibiotic use tended to lower carriage rate (unadjusted OR = 0.51, 95% CI: 0.29-0.89 and unadjusted OR = 0.45, 95% CI: 0.23-0.90), these factors were not significantly associated with carriage status after vaccination (adjusted OR = 0.57, 95% CI: 0.32-1.02 and adjusted OR = 0.54, 95% CI: 0.27-1.08) in multivariable analysis. Shared living space was a significant risk factor of meningococcal carriage (Supplementary Table 2).

## Discussion

We found that MenACWY vaccination in military recruits led to a significant reduction of 72.6% in serogroup ACWY carriers 5weeks after vaccination, which was predominantly driven by serogroup C. This effect of MenACWY on vaccine serogroup carriage is consistent with the results of previous observational studies on meningococcal conjugate polysaccharide vaccines [3,18]. In U.K. meningococcal vaccination program for students aged 15-19 years, MenACWY vaccine displayed a carriage reduction of 80% against serogroup CWY, and monovalent serogroup C conjugate vaccine demonstrated vaccine effectiveness of 75% against serogroup C carriage. A randomized controlled trial reported that MenACWY conjugate vaccine could result in significantly lowering carriage rate of 36.2% (95% CI: 15.6-51.7) against serogroup CWY during 12 months after vaccination even though there was no difference at 1 month between MenACWY vaccine and control groups [5]. However, a meta-analysis study suggested that MenACWY vaccine was effective at reducing IMD but had no effect on vaccine serogroup carriage (relative risk = 0.88, 95% CI: 0.66-1.88) [6]. These inconsistent findings were not surprising. In the meta-analysis, higher weighted studies were performed in the popula-

**Table 2**
Effectiveness of MenACWY vaccine against meningococcal carriage in military recruits.

| | 2013 | | | | 2014 | | | | 2013 + 2014 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-vaccination | 5 weeks after vaccination | OR (95% CI) | VE (95% CI) | Pre-vaccination | 5 weeks after vaccination | OR (95% CI) | VE (95% CI) | Pre-vaccination | 5 weeks after vaccination | OR (95% CI) | VE (95% CI) |
| | No. of carriage isolates (%[a]) | | | | No. of carriage isolates (%[a]) | | | | No. of carriage isolates (%[a]) | | | |
| Serogroup A | 0 (0) | 0 (0) | NA | NA | 0 (0) | 0 (0) | NA | NA | 0 (0.0) | 0 (0.0) | NA | NA |
| Serogroup C | 6 (1.4) | 0 (0) | NA | NA | 17 (3.8) | 4 (0.9) | 0.23 (0.08 to 0.68) | 77.2 (31.6 to 92.4) | 23 (2.7) | 4 (0.5) | 0.17 (0.06 to 0.49) | 83.0 (50.6 to 94.1) |
| Serogroup W | 0 (0) | 2 (0.5) | NA | NA | 1 (0.2) | 1 (0.2) | 1 (0.06 to 16.04) | 0 (-1503.8 to 93.8) | 1 (0.1) | 3 (0.3) | 3.01 (0.31 to 28.96) | -200.7 (-2796.4 to 68.8) |
| Serogroup Y | 1 (0.2) | 0 (0) | NA | NA | 0 (0) | 0 (0) | NA | NA | 1 (0.1) | 0 (0.0) | NA | NA |
| Serogroup ACWY | 7 (1.6) | 2 (0.5) | 0.28 (0.06 to 1.37) | 71.8 (-36.7 to 94.2) | 18 (4.1) | 5 (1.1) | 0.27 (0.10 to 0.73) | 73.1 (26.8 to 90.1) | 25(2.9) | 7(0.8) | 0.27 (0.12 to 0.64) | 72.6 (36.3 to 88.2) |
| Serogroup B | 18 (4.2) | 9 (2.1) | 0.49 (0.22 to 1.10) | 51.1 (-10.2 to 78.3) | 11 (2.5) | 6 (1.4) | 0.54 (0.20 to 1.47) | 46.1 (-47.1 to 80.2) | 29 (3.3) | 15 (1.7) | 0.51 (0.27 to 0.96) | 49.1 (4.4 to 72.9) |
| Serogroup E | 0 (0) | 0 (0) | NA | NA | 5 (1.4) | 0 (0) | NA | NA | 5 (0.7) | 0 (0.0) | NA | NA |
| Serogroup X | 0 (0) | 5 (1.2) | NA | NA | 1 (0.2) | 0 (0) | NA | NA | 1 (0.1) | 5 (0.6) | 5.02 (0.59 to 43.09) | -402.3 (-4208.5 to 41.4) |
| Non-groupable | 14 (3.3) | 44 (10.3) | 3.39 (1.83 to 6.28) | -239.0 (-528.4 to -82.8) | 18 (4.1) | 11 (2.5) | 0.60 (0.28 to 1.29) | 39.9 (-28.8 to 71.9) | 32 (3.7) | 55 (6.4) | 1.77 (1.13 to 2.76) | -76.1 (-176.2 to -13.1) |
| Non-vaccine serogroups | 32 (7.5) | 58 (13.6) | 1.94 (1.23 to 3.06) | -94.1 (-205.7 to -23.2) | 35 (8.0) | 17 (3.9) | 0.47 (0.26 to 0.84) | 53.5 (15.7 to 74.4) | 67 (7.7) | 75 (8.7) | 1.13 (0.80 to 1.60) | -13.1 (-59.5 to 19.8) |
| Total | 39/426 (9.2) | 60/426 (14.1) | 1.63 (1.06 to 2.50) | -62.6 (-149.5 to -6.1) | 53/440 (12.0) | 22/440 (5.0) | 0.38 (0.23 to 0.64) | 61.6 (35.6 to 77.1) | 92/866 (10.6) | 82/866 (9.5) | 0.88 (0.64 to 1.20) | 12.0 ( -20.4 to 35.7) |

[a] Percentage means the proportion of the number of carriage cases per total number of cases in each column.NA, not available; OR, odds ratio; VE, vaccine effectiveness.

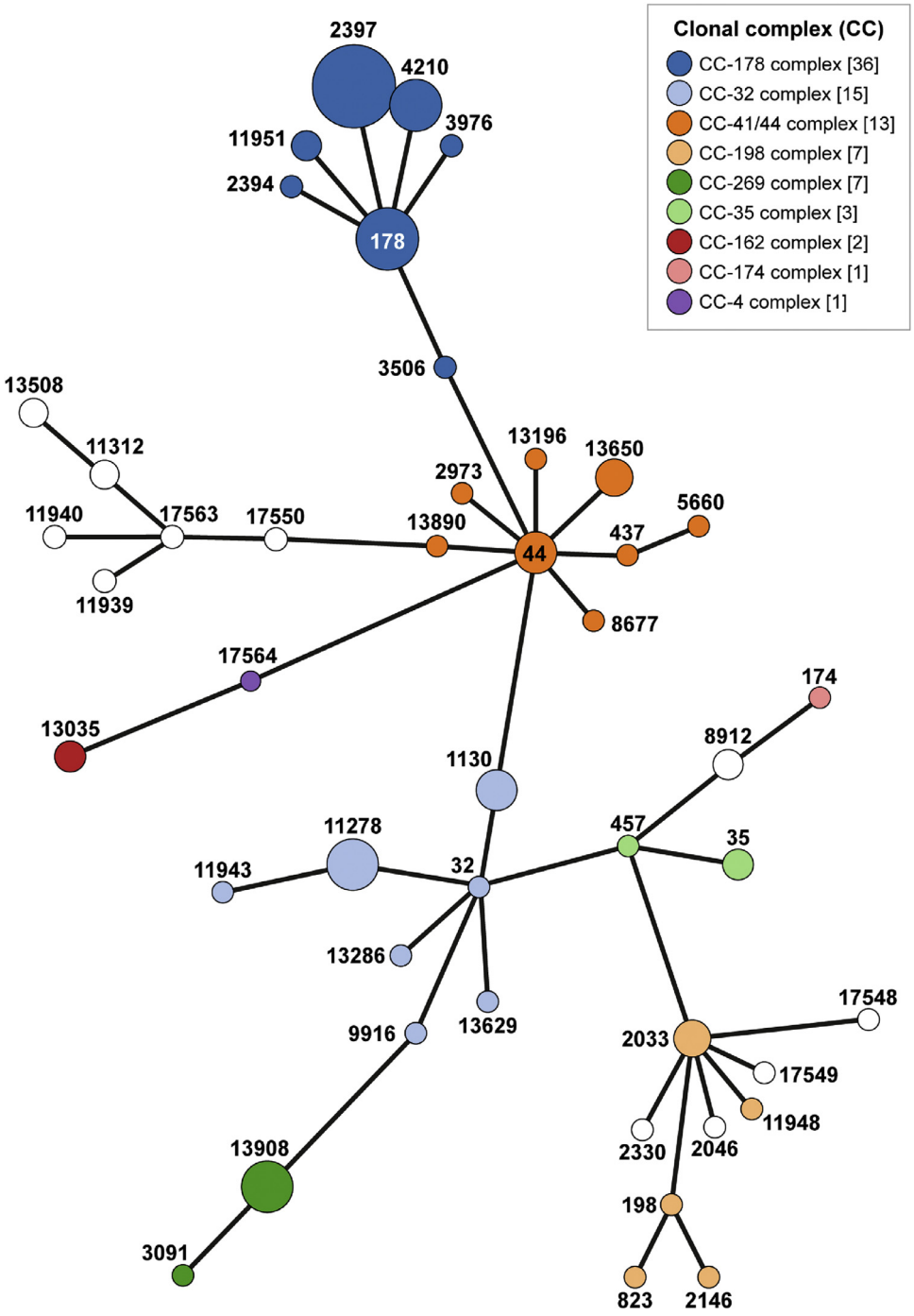


**Figure 1.** GrapeTree for *N. meningitidis* carriage isolates using seven-locus multi-locus sequence typing. The sequence type (ST) numbers are represented with circles. Same clonal complexes (CCs) were designated by same colored circles, while unassigned CCs were represented by open circles. The node size in the GrapeTree diagram is proportional to the number of bacterial strains sharing the same ST, and the length of edges between nodes are proportional to genetic distances.

tions with >80% MenACWY vaccine [12,14,19] or low meningococcal carriage rate of approximately 3% [13]. In this study, we compared the carriage rate at 5 weeks post-vaccination with that at pre-vaccination and found a significant reduction in carriage in a short period after MenACWY vaccination. These findings are contrary to those of previous randomized controlled trials in which vaccine serogroup carriage did not differ between the MenACWY vaccine and control groups 1 month after vaccination. This may be attributed to the different study populations and living conditions used in the two studies. Nevertheless, this study implies that MenACWY vaccine could be effective for vaccine serogroup carriage reduction, especially serogroup C during the early post-vaccination period.

In this study, the overall carriage rate of *N. meningitidis* was decreased after vaccination, although the decrease was not significant (10.6% vs. 9.5%, $P = 0.424$). Carriage reduction primarily resulted from a significant decrease in serogroup B and serogroup ACWY carriages after vaccination. The reduction in serogroup B carriers was unexpected. Meningococcal carriage studies have reported that carriage and transmission generally increase after beginning living conditions in a closed or semi-closed group, such as military barracks or dormitory [8,20-23]. A longitudinal study in Korea

**Table 3**
Distribution of carriage isolates' sequence types within clonal complexes.

| Clonal complex (CC) | Sequence type (ST) | Number of isolates | Pre-vaccination | Post-vaccination |
|---|---|---|---|---|
| **CC-178** | 178 | 9 | E (3) | NG (5), X (1) |
| | 2394 | 1 | | X (1) |
| | 2397 | 16 | | NG (14), B (1), X (1) |
| | 3506 | 1 | | X (1) |
| | 3976 | 1 | E (1) | |
| | 4210 | 6 | NG (2), E (1) | NG (3) |
| | 11951 | 2 | | NG (2) |
| **CC-32** | 1130 | 4 | B (1), C (1) | NG (1), C (1) |
| | 9916 | 1 | C (1) | |
| | 11278 | 6 | C (5) | C (1) |
| | 13286 | 1 | NG (1) | |
| | 11943 | 1 | | C (1) |
| | 13629 | 1 | C (1) | |
| | 32 | 1 | B (1) | |
| **CC-41/44** | 44 | 4 | B (1) | B (2) |
| | 437 | 1 | | NG (1) |
| | 2973 | 1 | B (1) | |
| | 5660 | 1 | | B (1) |
| | 8677 | 1 | | NG (1) |
| | 13196 | 1 | | B (1) |
| | 13650 | 3 | B (2) | NG (1) |
| | 13890 | 1 | NG (1) | |
| **CC-269** | 13908 | 6 | B (1), C (1) | B (4) |
| | 3091 | 1 | | B (1) |
| **CC-198** | 198 | 1 | NG (1) | |
| | 823 | 1 | NG (1) | |
| | 2033 | 3 | NG (2) | NG (1) |
| | 2146 | 1 | NG (1) | |
| | 11948 | 1 | | NG (1) |
| **CC-35** | 35 | 2 | B (1) | B (1) |
| | 457 | 1 | B (1) | |
| **CC-162** | 13035 | 2 | B (1) | B (1) |
| **CC-174** | 174 | 1 | | NG (1) |
| **CC-4** | 17564[a] | 1 | NG (1) | |
| **Unassigned** | 2046 | 1 | NG (1) | |
| | 2330 | 1 | | NG (1) |
| | 8912 | 2 | W (1) | W (1) |
| | 11312 | 2 | NG (1) | NG (1) |
| | 11939 | 1 | | NG (1) |
| | 11940 | 1 | NG (1) | |
| | 13508 | 2 | NG (1) | NG (1) |
| | 17548[a] | 1 | X (1) | |
| | 17549[a] | 1 | NG (1) | |
| | 17550[a] | 1 | | NG (1) |
| | 17563[a] | 1 | NG (1) | |

[a] Newly assigned sequence types: ST-17564, ST-17548, ST-17549, ST-17550, ST-1756.

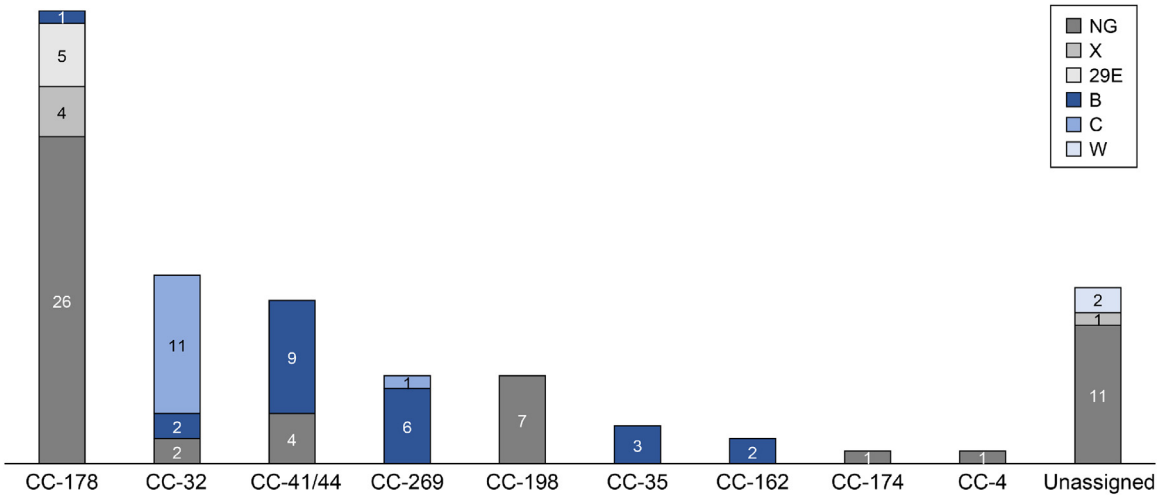


**Figure 2.** Association between serogroups and clonal complexes of meningococcal isolates.

showed that meningococcal carriage rate increased from 2.7% to 11.8% within the first 3 months stay in dormitory students due to the spread of identical genotypes [21]. Finnish military study reported an increase in carriage rate from 2.2% at the beginning of military service to 18.5% at the end of service [22]. The decrease in serogroup B carriage may have been partially caused by the administration of antibiotics. Meningococcal carriage was associated with antibiotic use in the risk factor analysis of carriage acquisition or persistence after vaccination (Supplementary Table 2). However, this change in serogroup B carriage cannot be completely explained by antibiotic use. In contrast to serogroup B, non-groupable meningococcal carriage increased and could be eliminated by antibiotic administration. A similar study in Korean military recruits showed an increase in the meningococcal carriage rate with predominant serogroup B carriage in a high antibiotic prescription setting [8]. Further research and surveillance for serogroup B carriers after the implementation of the MenACWY vaccination will be required.

Molecular epidemiological studies showed that 99 isolates fell into 9 previously known CCs, with the remaining 14 unassigned isolates. Of these, the ST-8912 serogroup W, CC-32 serogroup C, CC-41/44, and CC-269 serogroup B carriage isolates remained problematic after MenACWY vaccination. These strains were associated with disease strains from IMD in Korea between 2010 and 2016 [24]. In Korea, serogroup Y CC-23 strain was primarily identified in IMD cases during the early 2000s [25]. However, frequent CC and related serogroups found in IMD cases were CC-41/44 and CC-269 serogroup B, CC-32 serogroup C, and ST-8912 serogroup W in the early 2010s. Particularly, CC-269 and CC-41/44 associated with serogroup B were common carriage strains in Korean adolescents [21,26]. These strains are endemic and responsible for sporadic infections across Europe and the United States [27]. CC-41/44, which is associated with serogroup B, is a prevalent disease strain in Northeast Asia [28,29]. Therefore, further surveillance is required for circulating strains from disease and carriage isolates, and the introduction of the meningococcal B vaccine should be considered for military recruits.

In this study, smoking, a well-known risk factor for meningococcal carriage, was not associated with carriage after vaccination. Instead, shared residency in military unit C and D had a significant impact on carriage status. This finding suggests that the baseline carriage prevalence in closed population can be a major driver in the spread of *N. meningitidis* [30].

Our study had some limitations. First, the number of serogroup W carriers in this study was so small that we could not evaluate the effect of MenACWY vaccine on serogroup W carriers. Since the introduction of the MenACWY vaccination program in U.K. adolescents, serogroup W has substantially increased despite high vaccine coverage [31]. This suggests that vaccine-induced immunity may have different effects depending on the vaccine serogroup [32]. Second, MLST analysis could only be completed in 99 of the 174 carriage isolates because some of the isolates were unexpectedly exposed to suboptimal condition during repeated transport, prolonged storage, and repeated freeze and thaw cycles. The collected isolates were stored in deep freezers at −70°C or below prior to analysis. Multiplex PCR for serogroups was successfully performed within 6 months of isolation, while MLST was performed 4 years after the stored samples had been transported three times between laboratory centers. These unfavorable conditions may affect DNA degradation over time. Furthermore, sequencing failures were observed for one to three loci in some isolates. This could potentially be attributed to genetic variations in primer binding sites or sequence homology with other genomic regions. Technical issues with sample processing during PCR may have led to the sequencing failure observed for certain housekeeping gene.

In conclusion, MenACWY vaccination in Korean military recruits is effective in reducing vaccine serogroup carriages, especially serogroup C, within a short 5-week period. However, serogroup B isolates belonging to the hypervirulent lineage remained after the implementation of the MenACWY vaccination program. These findings highlight the effectiveness of the MenACWY vaccination in meningococcal disease, which results in serious outcomes but has a low prevalence. Further studies are required to evaluate the long-term efficacy of the MenACWY vaccination.

## Data availability

Supplementary materials are available at online database. Primary analysis study is not provided by online journal, but it will be available on reviewer's or reader's request.

## Declarations of competing interest

All authors have no conflicts of interest.

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## Ethical approval

This study was approved by the Institutional Review Board of the Armed Forces Medical Command. All participants were enrolled after obtaining informed consent in accordance with the Declaration of Helsinki (1996) and the Guidelines for Good Clinical Practice.

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## Author contributions

Study concept and design: JYH, OHS. Data acquisition and analysis: YRK, JYH. All authors contributed to the interpretation of data. Drafting of the manuscript: YRK, JYH. All authors critically revised the manuscript for intellectual content and approved the final draft for submission.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2024.107150.

## References

[1] Stephens DS, Greenwood B, Brandtzaeg P. Epidemic meningitis, meningococcaemia, and *Neisseria meningitidis*. *Lancet* 2007;**369**(9580):2196–210.
[2] Caugant DA, Tzanakaki G, Kriz P. Lessons from meningococcal carriage studies. *FEMS Microbiol Rev* 2007;**31**(1):52–63.
[3] Maiden MC, Ibarz-Pavon AB, Urwin R, Gray SJ, Andrews NJ, Clarke SC, et al. Impact of meningococcal serogroup C conjugate vaccines on carriage and herd immunity. *J Infect Dis* 2008;**197**(5):737–43.

[4] Kristiansen PA, Diomande F, Ba AK, Sanou I, Ouedraogo AS, Ouedraogo R, et al. Impact of the serogroup A meningococcal conjugate vaccine, MenAfriVac, on carriage and herd immunity. *Clin Infect Dis* 2013;**56**(3):354–63.
[5] Read RC, Baxter D, Chadwick DR, Faust SN, Finn A, Gordon SB, et al. Effect of a quadrivalent meningococcal ACWY glycoconjugate or a serogroup B meningococcal vaccine on meningococcal carriage: an observer-blind, phase 3 randomised clinical trial. *Lancet* 2014;**384**(9960):2123–31.
[6] McMillan M, Chandrakumar A, Wang HLR, Clarke M, Sullivan TR, Andrews RM, et al. Effectiveness of meningococcal vaccines at reducing invasive meningococcal disease and pharyngeal *Neisseria meningitidis* carriage: a systematic review and meta-analysis. *Clin Infect Dis* 2021;**73**(3):e609–ee19.
[7] Heo JY, Choe K-W, Yoon C-G, Jeong HW, Kim WJ, Cheong HJ. Vaccination policy in Korean armed forces: current status and future challenge. *J Korean Med Sci* 2015;**30**(4):353–9.
[8] Hwang IU, Lee HK, Seo MY, Kim JP, Seo YB, Bang YJ. The changes of meningococcal carriage rate and the serogroup in Korean army recruits. *J Korean Mil Med Assoc* 2010;**41**:188–99.
[9] Kim JH, Jun YH, YS J. Study on pharyngeal *Neisseria meningitidis* carrier rate of Korean army recruits. *J Korean Mil Med Assoc* 1990;**21**:35–47.
[10] Lee SO. Commencement of the meningococcal vaccination for the republic of Korea army. *Infect Chemother* 2013;**45**(1):113–15.
[11] Jo YM, Bae SM, Kang YH. Cluster of serogroup W-135 meningococcal disease in 3 military recruits. *J Korean Med Sci* 2015;**30**(5):662–5.
[12] Breakwell L, Whaley M, Khan UI, Bandy U, Alexander-Scott N, Dupont L, et al. Meningococcal carriage among a university student population—United States, 2015. *Vaccine* 2018;**36**(1):29–35.
[13] Harrison LH, Shutt KA, Arnold KE, Stern EJ, Pondo T, Kiehlbauch JA, et al. Meningococcal carriage among Georgia and Maryland high school students. *J Infect Dis* 2015;**211**(11):1761–8.
[14] McNamara LA, Thomas JD, MacNeil J, Chang HY, Day M, Fisher E, et al. Meningococcal carriage following a vaccination campaign with MenB-4C and MenB-FHbp in response to a university serogroup B meningococcal disease outbreak—Oregon, 2015-2016. *J Infect Dis* 2017;**216**(9):1130–40.
[15] Heo JY, Bae SM, Cheong HJ, Kim WJ, Kim MY, Na W, et al. Impact of quadrivalent meningococcal conjugate vaccine on carried meningococci in Korean military trainees. *J Korean Mil Med Assoc* 2014;**45**(1):33–42.
[16] Taha MK. Simultaneous approach for nonculture PCR-based identification and serogroup prediction of *Neisseria meningitidis*. *J Clin Microbiol* 2000;**38**(2):855–7.
[17] Bennett DE, Mulhall RM, Cafferkey MT. PCR-based assay for detection of *Neisseria meningitidis* capsular serogroups 29E, X, and Z. *J Clin Microbiol* 2004;**42**(4):1764–5.
[18] Carr JP, MacLennan JM, Plested E, Bratcher HB, Harrison OB, Aley PK, et al. Impact of meningococcal ACWY conjugate vaccines on pharyngeal carriage in adolescents: evidence for herd protection from the UK MenACWY programme. *Clin Microbiol Infect* 2022;**28**(12):1649.e1–1649.e8.
[19] Soeters HM, Whaley M, Alexander-Scott N, Kanadanian KV, MacNeil JR, Martin SW, et al. Meningococcal carriage evaluation in response to a serogroup B meningococcal disease outbreak and mass vaccination campaign at a college—Rhode Island, 2015-2016. *Clin Infect Dis* 2017;**64**(8):1115–22.
[20] Yazdankhah SP, Caugant DA. *Neisseria meningitidis*: an overview of the carriage state. *J Med Microbiol* 2004;**53**(Pt 9):821–32.
[21] Choi H, Lee HM, Lee W, Kim JH, Seong H, Kim JH, et al. Longitudinal study of meningococcal carriage rates in university entrants living in a dormitory in South Korea. *PLoS One* 2021;**16**(1):e0244716.
[22] Jounio U, Saukkoriipi A, Bratcher HB, Bloigu A, Juvonen R, Silvennoinen-Kassinen S, et al. Genotypic and phenotypic characterization of carriage and invasive disease isolates of *Neisseria meningitidis* in Finland. *J Clin Microbiol* 2012;**50**(2):264–73.
[23] Neal KR, Nguyen-Van-Tam JS, Jeffrey N, Slack RC, Madeley RJ, Ait-Tahar K, et al. Changing carriage rate of *Neisseria meningitidis* among university students during the first week of term: Cross Sectional Study. *BMJ* 2000;**320**(7238):846–849.
[24] Lee H, Seo Y, Kim K-H, Lee K, Choe K-W. Prevalence and serogroup changes of *Neisseria meningitidis* in South Korea, 2010–2016. *Sci Rep* 2018;**8**(1):1–7.
[25] Bae SM, Kang YH. Serological and genetic characterization of meningococcal isolates in Korea. *Jpn J Infect Dis* 2008;**61**(6):434–7.
[26] Kim HW, Lee S, Kwon D, Cha J, Ahn JG, Kim KH. Characterization of oropharyngeal carriage isolates of *Neisseria meningitidis* in healthy Korean adolescents in 2015. *J Korean Med Sci* 2017;**32**(7):1111–17.
[27] Brehony C, Jolley KA, Maiden MC. Multilocus sequence typing for global surveillance of meningococcal disease. *FEMS Microbiol Rev* 2007;**31**(1):15–26.
[28] Takahashi H, Morita M, Kamiya H, Fukusumi M, Sunagawa M, Nakamura-Miwa H, et al. Genomic characterization of Japanese meningococcal strains isolated over a 17-year period between 2003 and 2020 in Japan. *Vaccine* 2023;**41**(2):416–26.
[29] Zhu B, Shi F, Zhang A, Sun X, Xu Z, Xu L, et al. Prevalence and genetic characteristics of 4CMenB and rLP2086 vaccine candidates among *Neisseria meningitidis* serogroup B strains, China. *Vaccine* 2018;**36**(15):1983–9.
[30] MenAfriCar C. Household transmission of *Neisseria meningitidis* in the African meningitis belt: a longitudinal cohort study. *Lancet Glob Health* 2016;**4**(12):e989–95.
[31] Oldfield NJ, Cayrou C, AlJannat MAK, Al-Rubaiawi AAA, Green LR, Dada S, et al. Rise in group W meningococcal carriage in university students, United Kingdom. *Emerg Infect Dis* 2017;**23**(6):1009–11.
[32] Oldfield NJ, Green LR, Parkhill J, Bayliss CD, Turner DPJ. Limited impact of adolescent meningococcal ACWY vaccination on *Neisseria meningitidis* serogroup W carriage in university students. *J Infect Dis* 2018;**217**(4):608–16.