

The Carrier Status of *Streptococcus pneumoniae* in a Multi-National Medical Student Population

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Parole chiave: *Streptococcus pneumoniae*, pneumococco, vaccinazione, portatore, studenti di medicina

Abstract

Background. Nasopharyngeal carriage of *Streptococcus pneumoniae* (*S. pneumoniae*) is a well-established risk factor for invasive disease. An unnecessarily high incidence of morbidity attributed to *S. pneumoniae* is vaccine-preventable. The objectives of this study were to determine the prevalence of *S. pneumoniae* carriage and to analyze the different vaccination schemes in a multinational population of final-year medical students.

Study Design. An observational cross-sectional study including final-year medical students attending their Infectious Diseases rotation.

Methods. One nasal and pharyngeal swab were collected from each participant who underwent a minimum eight-hour period of fasting and without rinsing or brushing teeth. Written informed consent was obtained from all participants. A univariate statistical analysis was carried out with statistical significance set at $p < 0.05$.

Results. Fifty students agreed to participate in this study (age range: 23-35, average: 24.86 ± 2.12 years). Six (12%) students had the pneumococcal vaccine: four (16%) from the foreign group and two (8%) from the Romanian group (OR: 2.19, 95% CI: 0.36-13.21, $p=0.39$). The carrier status for *S. pneumoniae* was 0%. A total of 12 (24%) specimens yielded microbial presence: *S. aureus* ($n=8$), *S. pyogenes* ($n=1$), Group C *Streptococcus* ($n=1$), Group G *Streptococcus* ($n=1$) and *K. pneumoniae* ($n=1$). All 13 (26%) specimens of alpha-hemolysis were identified from foreign students (OR: 55.08, 95% CI: 3.02-1003.75, $p=0.0068$), but had no clinical relevance, *S. pneumoniae* being ruled out.

Conclusion. The carrier status among vaccinated participants was 0%. The rate of microorganism isolation was twice that in Romanian students compared to foreign students.

Introduction

Nasopharyngeal carriage of *Streptococcus pneumoniae* (*S. pneumoniae*) represents the first phase of invasive pneumococcal disease.

Pathogenesis manifests initially, and primarily, in the form of pneumonia and may eventually lead to life-threatening meningitis and sepsis, especially in children, the elderly and the immune-compromised by evading opsonin-

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mediated phagocytosis. The pathogenic process is perpetuated by recombination and horizontal gene transfer with closely-related commensals of the nasopharyngeal flora, such as *S. mitis* and *S. pseudopneumoniae*. This is referred to as co-colonization (1-8).

However, pneumococcal morbidity is vaccine-preventable. The implementation of pneumococcal conjugate vaccines (PCV): PCV-7, PCV-10 and PCV-13 into vaccination schemes has decreased pneumococcal morbidity and carriage (2, 3, 5, 6, 9-13).

After the introduction of the heptavalent PCV-7 vaccine into the national Columbian immunization program, Parra et al. reported a decline of nasopharyngeal carriage rates in children from 55.7% to 44.2% (9). Moreover, Emgard et al. reported a more significant decrease in rates of pneumococcal carriage: 56% to 23% following PCV-13 vaccination of a paediatric Tanzanian cohort (10).

The objective of this study was to determine the prevalence of *S. pneumoniae* vaccination and carriage in a multi-national cohort of final-year medical students.

Methods

Study design

This observational, cross-sectional study was conducted in February 2016. A questionnaire containing four questions regarding demographics and 11 questions concerning vaccination schemes was distributed. After the questionnaires were completed, nasopharyngeal swabs were collected by nurses in the Infectious Diseases Department for further analysis. This study was carried out according to the STROBE checklist.

Study participants

The participants comprised final-year medical students from the “Carol Davila” University of Medicine and Pharmacy who were attending their Infectious Diseases rotation at the National Institute for Infectious

Diseases, a tertiary referral center in Bucharest. Participation in this study was open to all final-year medical students. Study design and requirements were explained to the participants by the corresponding author and her supervisor. Students who agreed to participate in the study signed consent forms for the participation and for the collection and storage of nasopharyngeal specimens. These were collected by trained nursing staff.

The consent rate was 28.1% (25/89) for the multi-national students and 25% (25/100) for Romanian students.

Participants were divided into two groups, the Foreign Group with multi-national students from the English Module and the Romanian Group. Both groups were balanced having 25 students each. The Foreign Group included students from European as well as non-European countries. All participants provided written informed consent prior to study commencement and were guaranteed anonymity.

Study setting

This study took place at the National Institute for Infectious Diseases, a tertiary referral center in Bucharest, Romania.

Source of data

Participants were contacted by the corresponding author during the Infectious Diseases rotation regarding their willingness to participate in this study. Laboratory results were held in confidence and were disclosed to each student individually by the course director.

Study procedure

One nasal and one pharyngeal swab were collected from each participant with a flexible wire with a Dacron/Rayon tip by the same two full-time laboratory nurses working exclusively at the Institute. Swabs were collected without brushing or rinsing teeth in the morning before classes and after an 8-hour fasting period. All patients were afebrile at the time of sampling

and presented without symptoms of any infection.

After sampling, swabs were placed in MW 173 Amies transport media and then plated on Columbia agar with 5% sheep blood as well as on Chocolate Agar medium (Biomerieux, Marcy, L'Etoile, France) in normal atmosphere in a 5% CO₂ environment.

All cultures underwent visual inspection and a further agglutination test if *Staphylococcus aureus* was suspected. A positive agglutination test warranted an anti-biogram (performed on Mueller-Hilton agar (Biomerieux, Marcy, L'Etoile, France) using a 0.5 McFarland inoculum with a bacterial density of 1.5 x 10⁸ colony-forming units per millilitre). In the case of alpha-hemolysis, cultures were treated with optochin to confirm the presence of *S. pneumoniae*.

Statistical Analysis

Statistical analysis encompassed both descriptive and analytical statistics with parametric as well as non-parametric tests. The descriptive statistical analysis was performed for numerical parameters using Microsoft Excel 2010 functions including mean, median, range (minimum-maximum) and standard deviation. The analytical statistical analysis included univariate tests comprising Chi-square and subsequent *p*-value for parametric variables with statistical significance set at *p* < 0.05. Statistical analysis was undertaken with the programme Sofa Stats.

Ethical Approval

All participants provided written informed consent for study participation and specifically for the processing and storage of their bacteriological isolates. This study was discussed with the hospital's ethics committee. The committee advised that since the medical students were aware of the specimen collection/storage process and since they provided written consent on the hospital's official forms, an ethics committee approval was not necessary.

Results

A total of 50 final-year medical students participated in the study: 25 foreign students and 25 Romanian students. Age ranged between 23-35 years (average: 24.86 ± 2.12 years). Males outnumbered females in the Foreign Group, accounting for 60% (n=15) of the population. In the Romanian group males accounted for only 16% (n=4) of the cohort (*p*=0.0017). Foreign students were somewhat older (range: 23-35 years, average: 25.4 ± 2.78 years) than Romanian students (range: 23-27, average: 24.28 ± 0.89).

The Foreign Group was highly heterogeneous with 25 students from 15 different countries, the highest number of students being from Israel (n=4, 16%). Almost one-quarter (n=6) of foreign students resided in countries where they were not born, as illustrated in Table 1.

The mandatory vaccination scheme in Romania comprises vaccines for: measles-mumps-rubella (MMR), diphtheria-tetanus-pertussis (DTP), Bacille Camille Guerin (BCG), hepatitis B virus (HBV), *Haemophilus influenzae* type B (Hib) and the inactivated polio vaccine (IPV).

Four students (16%) from the Foreign Group and two students (8%) from the Romanian Group received the PCV-13 and PCV-7 pneumococcal vaccine, respectively. Although the rate of *S. pneumoniae* vaccination among foreign students was twice as high as in Romanian students, statistical significance was not obtained (OR: 2.19, 95% CI: 0.36-13.21, *p*=0.39). The rate of carrier status for *S. pneumoniae* was 0% as the germ was not isolated in any of the six vaccinated students.

The presence of *S. aureus* was suspected on visual inspection in eight (16%) samples, which was confirmed by a positive agglutination test in all eight specimens. Of these eight cases of *S. aureus*, five (62.5%) were isolated from the nose and three (37.5%) from the pharynx. The proportion of *S.*

Table 1 - Countries of Birth and Residence in the Foreign Student Group

Country of birth	Number	Percentage	Country of residence	Different country of residence
Afghanistan	1	4%	Pakistan	Y
Austria	1	4%	Austria	N
Canada	1	4%	Canada	N
Germany	3	12%	2: Germany, 1: Australia	Y (1/3)
Iran	2	8%	1: Iran, 1: Germany	Y (1/2)
Israel	4	16%	Israel	N
Japan	1	4%	Japan	N
Jordan	1	4%	Jordan	N
Malaysia	1	4%	Malaysia	N
Morocco	1	4%	Morocco	N
Nigeria	1	4%	Nigeria	N
Pakistan	1	4%	Canada	Y
Romania	1	4%	U.S.A.	Y
Somalia	1	4%	U.K.	Y
Turkey	3	12%	Turkey	N
U.S.A	2	8%	U.S.A.	N
Total	25	100%		6/25 (24%)

aureus isolated from foreign and Romanian students was equal, with four cases in each group. Figures 1 and 2 exemplify the relationship between the suspected *S. aureus* based on visual inspection and the positive agglutination test results from an unvaccinated participant enrolled in this study.

All eight samples of *S. aureus* proved to be methicillin-sensitive *Staphylococcus*

aureus (MSSA), as illustrated in Figure 3. The figure also shows the inhibition zones of Fox (Cefoxitine) to measure 28 mm and of VA (Vancomycin) to be 18mm, compared to a 34 mm zone for RA (Rifampicin) from an unvaccinated participant enrolled in this study.

Overall, a total of 12 (24%) specimens showed microbial invasion. The



Figure 1 - *S. aureus* culture from an unvaccinated participant enrolled in the study

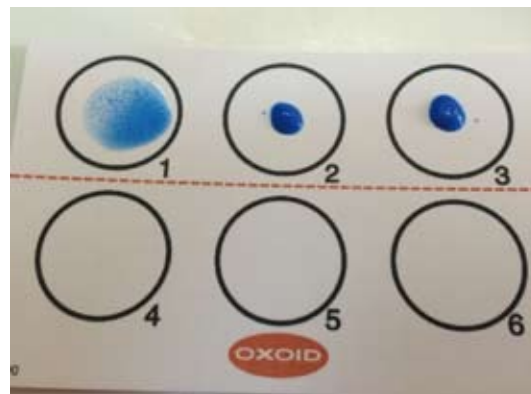


Figure 2 - Positive agglutination test from an unvaccinated participant enrolled in the study

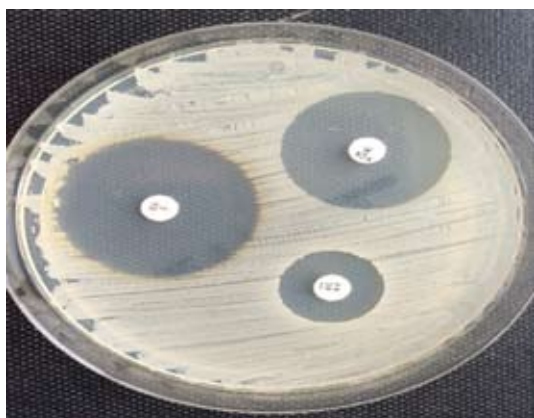


Figure 3 - Antibiogram inhibition zones of MSSA from an unvaccinated participant enrolled in the study

microorganisms comprised *S. aureus* (n=8), *S. pyogenes* (n=1), *Group C Streptococcus* (n=1), *Group G Streptococcus* (n=1) and *K. pneumoniae* (n=1). The last four microorganisms were isolated from pharyngeal swabs from females in the Romanian Group.

Isolation of pathogenic microorganisms was twice as common in the Romanian Group as in the Foreign Group. Microbial isolation from the Foreign Group was present in four (16%) students compared to eight (32%) in the Romanian Group (OR: 0.40, 95% CI: 0.10-1.57, $p=0.19$).

An equal proportion of microorganisms was isolated from the nose and the pharynx with six specimens from each group. Moreover, microbial isolation from the pharynx of Romanian students was twice as high compared to foreign students (8 cases vs. 4 cases). Positive nasal swabs were equal in terms of frequency in both groups, with three cases each (OR: 2.00, 95% CI: 0.19-20.61, $p=0.56$).

Discussion and Conclusions

The overall percentage of pneumococcal vaccination in this cohort was 12% (6/50). Foreign students were twice as likely to

opt for vaccination, however, statistical significance was not attained ($p=0.39$). The carrier status among all vaccinated students was 0%. The low rate of vaccination could be attributed to age-based recommendations for pneumococcal vaccinations: namely age < 5 years, age > 65 years or immunocompromised status (14), none of which applied to this study.

Among the participants, the rate of microbial isolation other than *S. pneumoniae* was 24% (n=12), with *S. aureus* (n=8, 66.7%) being most prominent. The rate of microorganism isolation was twice as high in Romanian students compared to foreign students from the 15 different countries. This clearly contradicted our initial hypothesis that the widely diverse Foreign Group would have a more diverse microbial flora than the geographically more uniform Romanian Group. An equal proportion of positive samples were collected from both the nasal and pharyngeal swabs. Microorganism isolation from pharyngeal swabs was greater in the Romanian Group, however without statistical significance ($p=0.56$).

A comprehensive literature search on Medline revealed that this is the first study of its kind that compares *S. pneumoniae* carriage between medical students from a geographically confined area and international students from 15 different countries.

The limitations of this study include a single-centre setting, cross-sectional design without serotyping of isolates due to the lack of polymerase chain reaction (PCR). The small sample size allowed only univariate statistical analyses.

The efficacy of PCV-7 has been questioned in a serotypical study of nasopharyngeal isolates from a cohort of 1,315 Italian children. This study alluded that there is an initial decrease in rates of *S. pneumoniae* carriage within the first year following the vaccination. However, there is a subsequent increase in the rates of carriage after the one year of vaccination (3).

As an incentive for vaccination, high-income European countries offer pneumococcal vaccinations free of charge. However, the persisting low participation rates remain a public health issue (15-18).

Childhood immunisation was introduced to create herd immunity as a preventive medicine strategy. Furthermore, it was found that herd immunity also decreased the rate of carriage, thereby improving public health (19, 20). The concept of herd immunity is especially pertinent to low-income countries where high density living propagates increased rates of pneumococcal infections (21).

Vadlamudi et al. demonstrated that an increased single dose of PCV-13 elicited a superior immune response compared to a single dose of PCV-23. It also has a comparable safety profile. The only advantage identified in five randomized controlled trials of the PCV-23 vaccine was higher tolerability as evidenced by lower rates of local reactions (22). Following vaccination, the next public health priority is long-term immunity via immunological memory (10).

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Riassunto

Lo stato di portatore di *Streptococcus pneumoniae* in una popolazione multinazionale di studenti di Medicina

Premessa. Lo stato di portatore nasale di *Streptococcus pneumoniae* (*S. pneumoniae*) rappresenta un ben documentato fattore di rischio di malattia invasiva, e la vaccinazione può evitare un'elevata quota della relativa morbosità. Scopo di questo lavoro è stato quello di determinare la prevalenza di portatori di *S. pneumoniae* e di analizzare i differenti schemi di vaccinazione in una

popolazione multi-etnica di un corso dell'ultimo anno di una Facoltà di Medicina.

Disegno dello studio. Osservazionale trasversale su studenti dell'anno finale partecipanti a rotazione al Servizio di Malattie Infettive.

Materiali e metodi. Sono stati prelevati un tampone nasale ed uno faringeo da soggetti che avevano firmato il consenso informato, che da almeno 8 ore avevano mantenuto il digiuno ed avevano evitato di lavarsi i denti o sciacquare la bocca. È stata effettuata un'analisi statistica univariata con livello di significatività pari a $p < 0,05$.

Risultati. Hanno partecipato 50 studenti (età 23-35, media $24,86 \pm 2,12$ anni). Sei (12%) avevano ricevuto il vaccino pneumococcico: 4 (16%) erano stranieri e 2 (8%) rumeni (OR: 2,19, 95% IC: 0,36-13,21, $p=0,39$). Lo stato di portatore di *S. pneumoniae* è risultato pari allo 0%. Un totale di 12 campioni (24%) ha evidenziato altra presenza microbica: *S. aureus* (n=8), *S. pyogenes* (n=1), *Streptococcus* di Gruppo C (n=1), *Streptococcus* di Gruppo G (n=1) e *K. pneumoniae* (n=1). Tutti i 13 (26%) casi di alfa-emolisi sono stati rilevati tra gli studenti stranieri (OR: 55,08, 95% IC: 3,02-1003,75, $p=0,0068$), ma senza rilevanza clinica, essendo stato escluso lo *S. pneumoniae*.

Conclusioni. Nessuno dei soggetti vaccinati è risultato portatore di *S. pneumoniae*, mentre la frequenza di isolamento di altri microorganismi è risultata doppia negli studenti romeni rispetto agli stranieri.

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