Carriage of Neisseria meningitidis and Neisseria lactamica in northern Greece

Jenny Kremastinou a, Georgina Tzanakaki a,*, Stamanta Levidiotou b, Fani Markou c, Eleftheria Themeli b, Aliki Voyiatzi a, Eleni Psoma c, Maria Theodoridou d, C. Caroline Blackwell a,e

a National Meningitis Reference Laboratory, National School of Public Health, 196 Alexandras Ave., Athens, Greece
b Department of Microbiology, University of Ioannina, Medical School, Ioannina, Greece
c Department of Microbiology, Serres General Hospital, Serres, Greece
d First Department of Paediatrics, University of Athens, School of Medicine, Athens, Greece
e Immunology and Microbiology, University of Newcastle, Newcastle, NSW, Australia

Received 22 April 2002; received in revised form 11 April 2003; accepted 22 May 2003
First published online 14 June 2003

Abstract

In response to an increase in the number of cases of invasive meningococcal disease (IMD) in northern regions of Greece, a survey was carried out to determine if there was an increase in carriage of Neisseria meningitidis, particularly in areas where there have been increases in immigrant populations from neighbouring countries. The second objective was to determine if there was an increase in the serogroup C:2a:P1.5,2 phenotype associated with recent outbreaks or changes in antibiotic sensitivities. As carriage of Neisseria lactamica is associated with development of natural immunity to IMD, the third objective was to determine the carriage rate of N. lactamica in this population. Among 3167 individuals tested, meningococci were isolated from 334 (10.5%). Compared with our previous studies, the proportion of meningococcal carriers was significantly increased among children in secondary education (11.3%) (χ² = 9.67, P < 0.005) and military recruits (37.4%) (χ² = 21.11, P < 0.000). Only 5/334 (1.5%) isolates expressed the phenotype associated with the increase in IMD in Greece. N. lactamica was isolated from 146/3167 (4.6%) participants. It was isolated from 146/3167 (4.6%) children attending primary or nursery schools; however, the highest proportion of carriers (11.3%) was found in the boarding school for young Albanian men. In the 21–59-year age range, the majority of N. lactamica isolates (22/25, 88%) were from women, probably due to closer or more prolonged contact with children in the primary school age range. Smoking was significantly associated with isolation of meningococci from men but not from women. Penicillin-insensitive strains (25/334, 7.5%) were identified in all four regions examined; the majority (14/25, 56%) were obtained from military personnel. We conclude that there was a higher proportion of carriers in the population of northern Greece; however, the increase in carriage rate was not associated with the influx of immigrants from neighbouring countries, and there was not a higher incidence of the C:2a:P1.5,2 strain responsible for increased disease activity in Greece in either the immigrant or local populations.

IN) 2003 Federation of European Microbiological Societies. Published by Elsevier B.V. All rights reserved.

Keywords: Neisseria meningitidis; Neisseria lactamica; Northern Greece; Penicillin insensitivity

1. Introduction

Since the mid-1990s, there has been a steady rise in invasive meningococcal disease (IMD) due to serogroup C Neisseria meningitidis, particularly the C:2a:P1.5,2 phenotype [1]. In 1999, the numbers of cases of meningococcal disease reported to the National Meningitis Reference Laboratory from northern Greece increased to 28 from the 14 cases reported in 1998. In 1998 there were two cases among ethnic Albanians living in the area. As a consequence, a survey was initiated to assess carriage of meningococci in different age groups in northern Greece, particularly in areas in which there has been an increase in the numbers of immigrants from Albania (Ioannina and Florina), Bulgaria (Seres) and Bulgaria and Turkey (Evros).
The first objective of the survey was to determine if there was an increase in carriage of meningococci and the second objective was to determine if strains with the C:2a:P1.5,2 phenotype or increased antibiotic resistance were present in this area.

Carriage of *Neisseria lactamica* was also assessed in the study. Evidence for its role as a natural immunogen against meningococcal disease has been reviewed recently [2]. Studies on the induction of bactericidal activity against meningococci found that *N. lactamica* strains from north and central Europe had a broader range of antigens cross-reactive with meningococcal strains of different phenotypes than *N. lactamica* strains from Greece [3]. The results indicate that there are major phenotypic differences among isolates from different regions which agrees with the genetic variation observed for *N. lactamica* isolates from children in a British survey [4]. The paper by Coen et al. [2] emphasised the need to assess the effects of immunisation against *N. meningitidis* in relation to changes in the prevalence of meningococci or *N. lactamica* as this might have considerable impact on the effectiveness of immunisation programmes. Early studies following introduction of polysaccharide vaccines among military recruits found that immunisation with polysaccharide antigens has been associated with reduced carriage of meningococci of the corresponding serogroup [5]. The effect of reduction of meningococcal carriage induced by immunisation might affect carriage rates of related species such as *N. lactamica* that have cross-reactive protein epitopes unrelated to the capsular antigen [6]. As the new conjugate vaccine for serogroup C meningococci was to be introduced into Greece in the winter of 2001, a third objective of the survey was to obtain baseline figures for the carriage rate of meningococci and *N. lactamica* in different age groups to assess the effects of the immunisation programme on carriage of these species.

2. Subjects and methods

2.1. Survey populations

Permission for the surveys was obtained from the Ministries of Health, Education and Defence. Residents of four regions in northern Greece were sampled (*n* = 3167): Ioannina (*n* = 1193); Evros (*n* = 690); Serres (*n* = 689); Florina (*n* = 595). Children in nursery (*n* = 144), primary (843), lower secondary (256) and upper secondary (584) schools were screened. This included a boarding school for young Albanian men (*n* = 57). Young adults in tertiary education (*n* = 85), 604 hospital personnel (504 in Ioannina, 22 in Serres, 31 in Evros and 47 in Florina), military personnel stationed in Serres (*n* = 223) and Florina (*n* = 154) and elderly people attending local social centres (*n* = 217) were also tested. The age ranges for the various groups are given in Table 1.

2.2. Isolation and characterisation of species

Throat swabs were plated directly onto Modified New York City medium (Oxoid) and incubated in 5% (v/v) CO₂ for 48 h. The colonies that grew on the selective medium were characterised by Gram stain, oxidase test and rapid carbohydrate utilisation test (Gallerie Pasteur, Pasteur-Merieux) as assessed in our previous studies [1,7–9].

2.3. Phenotypic characterisation of isolates for serogroup, serotype and subtype

Serogroups were determined by slide agglutination with polyclonal antisera to serogroups A, B, C, W-135, X, Y and Z (Abbot Diagnostics). Serotypes and subtypes were determined by whole-cell enzyme-linked immunoassay with monoclonal antibody reagents supplied by RIMV (Bilthoven, The Netherlands) [8–10].

2.4. Molecular assessment of isolates

The polymerase chain reaction (PCR) method was used to screen isolates that were non-groupable by conventional agglutination tests for serogroup [11]. DNA isolation for each meningococcal strain was carried out by making a suspension of the culture equal to 1.0 on the McFarland scale in 1 ml double-distilled sterile water and the samples were heated at 100°C for 20 min. The supernatant was removed after centrifugation for 10 min at 10 000×g and used in the PCR procedure.

Each sample was examined for species specificity with the IS1106 sequence. For serogroup prediction (A, B, C, W-135 and Y), the oligonucleotides in the siaD gene (serogroups B, C, W-135 and Y) and in orf-2 of a gene cassette required for the biosynthesis of the serogroup A capsule were used (Table 2). The sizes of the expected amplicons are 450 bp (serogroup B), 400 bp (serogroup A), 250 bp (serogroup C) and 120 bp (serogroups Y and W-135) [11].

The PCR reaction mix (50 µl) contained the following components: 3 µl MgCl₂ (20 mM); 5 µl of the PCR buffer composed of 750 mM Tris–HCl, 200 mM (NH₄)₂SO₄, and 0.1% Tween 20; 0.5 µl of each deoxynucleotide triphosphate (200 µM); 0.5 µl of the corresponding oligonucleotides (100 µM); 0.2 µl of 250 U Taq Polymerase (AB gene); 10 µl of template DNA.

The PCR reactions were performed in a PCR thermocycler (Robocycler, Gradient 96, Stratagene, USA) with 39 replications of the cycle: 25 s at 95°C, 40 s at 55°C and 1 min at 72°C.

The amplicons were separated by electrophoresis in 2% agarose gel and were visualised with a UV transilluminator following staining with ethidium bromide. Negative controls consisting of distilled water were used in each assay. Positive controls included standard strains used...
for species identification as well as strains of serogroups A, B, C, W-135 and Y.

2.5. Antibiotic sensitivities

To test the antibiotic sensitivity, the strains were cultured on chocolate agar. E-test strips were applied to the inoculated plates and incubated at 5% (v/v) CO₂ for 24 h. The following breakpoints were used to define sensitivity or resistance: penicillin (≥ 0.125 mg l⁻¹); rifampicin (≥ 1 mg l⁻¹); cefaclor (≥ 4 mg l⁻¹); chloramphenicol (≥ 2 mg l⁻¹); ceftriaxone (≥ 1 mg l⁻¹); ciprofloxacin (≥ 1 mg l⁻¹); sulfamethoxazole (≥ 12 mg l⁻¹).

2.6. Statistical analyses

The M₂ test was used to assess the results (Minitab 13 for Windows).

3. Results

3.1. Carriage of N. meningitidis

Among the 3167 individuals screened, meningococci were isolated from 334 (10.5%). Compared to the proportions of carriers in the two groups in which there were no military recruits, Ioannina (77/1193, 6.4%) and Evros (58/690, 8.4%), the proportions of carriers were higher in Seres (115/689, 16.7%) and Florina (84/595, 14.1%). These figures were significantly higher due to the high proportion of isolates from military personnel, 39% and 35% respectively. The proportion of carriers in the civilian populations was similar to those in Ioannina and Evros: Serres = 28/595 (4.7%); Florina = 30/441 (6.8%) (M² = 56.09, P = 0.000) (Table 3).

Isolation of meningococci was low among the youngest children (age range 0–4 years) attending nursery school (2.1%) (Table 3) and increased with age to 4.7% among children in primary school, 13.9% and 10.3% among pupils in lower and upper secondary schools, 11.8% among students in tertiary education. The highest proportions of carriers were among ‘closed’ populations, a boarding school for Albanian teenagers (14%) and military personnel (37.4%) (Table 3).

Among adults (age range 21–59) working in hospitals, 26/604 (4.3%) were carriers of meningococci. The proportion of carriers among men was 12/140 (8.6%) compared with 14/464 (3%) for women (χ² = 8.05, P = 0.005). Among men in this group, mainly professional staff, 15 (10.7%) were smokers; however, among the 12 males from whom meningococci were isolated eight (66.6%) were smokers (χ² = 42.9, P = 0.000). Among women in this group, mainly nursing, technical and domestic staff, 178/464 (38.3%) were smokers and 8/14 women (57%) from whom meningococci were isolated were smokers (χ² = 2.15, P = 0.142).

Among people attending clubs for the elderly, 11/217 (5.1%) were carriers, 7/129 (5.4%) were men and 4/88 (4.5%) were women. Among men in this group, 27/129 (20.9%) were smokers and 4/7 (57%) men from whom meningococci were isolated were smokers (χ² = 5.87, P = 0.015). Only 2/88 (2.3%) of the women in this group were smokers and none of the women from whom meningococci were isolated were smokers.

3.2. Carriage of N. lactamica

N. lactamica was isolated from 146/3167 (4.6%) of the individuals tested. Isolation of N. lactamica ranged from 2.6 to 9.4% of pupils in nursery or primary schools; however, the highest proportion of isolates (10.5%) was found in the boarding school for Albanian teenagers. Lower proportions of N. lactamica were isolated from students in tertiary education (0%) and military recruits (1.3–3.6%). The isolation rate for the tertiary students and military recruits (10/462, 2.2%) was significantly lower than the overall figure of 136/2705 (5%) observed for the other groups (χ² = 7.34, P = 0.007).

Among 604 adults in the 21–59-year age range working in hospitals, 25 (4.1%) carried N. lactamica, and the majority of N. lactamica carriers were women (22/25, 88%). Among the three males from whom N. lactamica was isolated, two were smokers; among the 22 women from whom N. lactamica was isolated, the majority (16, 72%) were non-smokers. A similar carriage rate was noted for

Table 1

Age ranges of groups from northern Greece screened for carriage of N. meningitidis and N. lactamica

<table>
<thead>
<tr>
<th>Category</th>
<th>Age (years)</th>
<th>Ioannina</th>
<th>Evros</th>
<th>Serres</th>
<th>Florina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nursery</td>
<td>1–6</td>
<td>144</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>5–14</td>
<td>326</td>
<td>272</td>
<td>139</td>
<td>106</td>
</tr>
<tr>
<td>Secondary</td>
<td>12–19</td>
<td>77</td>
<td>387</td>
<td>233</td>
<td>143</td>
</tr>
<tr>
<td>Tertiary</td>
<td>19–28</td>
<td>85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albanian school</td>
<td>15–21</td>
<td>57</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital personnel</td>
<td>21–59</td>
<td>504</td>
<td>31</td>
<td>22</td>
<td>47</td>
</tr>
<tr>
<td>Elderly</td>
<td>65–98</td>
<td></td>
<td>72</td>
<td>145</td>
<td></td>
</tr>
<tr>
<td>Military</td>
<td>18–33</td>
<td></td>
<td>223</td>
<td>154</td>
<td></td>
</tr>
<tr>
<td>Total = 3167</td>
<td></td>
<td>1193</td>
<td>690</td>
<td>689</td>
<td>595</td>
</tr>
</tbody>
</table>
the elderly population, 9/217 (4.1%), 6/129 (4.5%) among the males and 9/88 (3.4%) among females. Smoking was not associated with isolation of these bacteria in the elderly.

3.3. Phenotypic characteristics of the isolates

The majority of meningococcal isolates (204/334, 61%) were non-groupable by the conventional agglutination technique. The serogroup of each of the strains that could be typed by the conventional agglutination test was in agreement with the results obtained by the molecular typing methods for A, B, C, W135 and Y: A = 4 (1%); B = 67 (20%); C = 4 (1%); W-135 = 13 (3.9%); Y = 34 (10%); X = 1 (0.3%); and Z = 7 (2%). In addition, the molecular method classified 92 of 204 (45%) of the strains that were non-agglutinable into serogroups B = 61 (29.9%), C = 17 (8.3%), and Y = 14 (6.9%); 112/204 (55%) isolates remained non-groupable. No additional isolates of serogroup A were identified.

Although there was a significant increase in disease caused by strains of serogroup C between 1993 and 1997, only 21/334 (6.3%) isolates from carriers were serogroup C by conventional and/or molecular methods.

Three isolates could not be revived from storage for serotype and subtype testing. Of the 331 isolates tested for serotype and subtype, the majority (246, 74.3%) did not react with any of the current serotype reagents. The most common serotype was 14 (31/331, 9.4%) followed by 4 (21/331, 6.3%). Serotype 2a, which was predominant among serogroup C isolates from patients during the 1993-1998 period, was identified for 5/331 (1.5%) of the isolates. Only 1% of the isolates reacted with the type 22 monoclonal antibody.

Of the 330 isolates tested for subtype, only 77 (23.3%) did not react with the subtype reagents. The most common subtype was P1.14 (85/330, 25.7%) followed by P1.5 (42/330, 12.7%). Subtype P1.2, associated with some of the serogroup C strains isolated from patients, was found on 18/330 (5.4%) of the carrier isolates.

There were five strains expressing the 2a:P1.5,2 phenotype. Three were non-groupable with the polyclonal antiserum but were group C by PCR. No strains with this phenotype were isolated from the Albanian pupils or from subjects in Florina.

Of the eight isolates obtained from Albanian children, the agglutination method identified two as group B, two as group Y, and one as group Z. Three were non-groupable by the serological method but the molecular method identified two of these as group B.

Table 2
Oligonucleotide sequences used in the PCR assays

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Gene amplified (serogroup)</th>
<th>Amplicon length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5'-attatttcagaccgcggcag-3'</td>
<td>IS1106</td>
<td>650</td>
</tr>
<tr>
<td>5'-cgcataacctaggatatctc-3'</td>
<td>orf2 (A)</td>
<td>400</td>
</tr>
<tr>
<td>5'-cgtatagtttctgtatctctc-3'</td>
<td>siaD (B)</td>
<td>450</td>
</tr>
<tr>
<td>5'-cggatcctccgatggactaat-3'</td>
<td>siaD (C)</td>
<td>250</td>
</tr>
<tr>
<td>5'-ctaactcctgaaaatccata-3'</td>
<td>siaD (W-135)</td>
<td>120</td>
</tr>
<tr>
<td>5'-caggaagtggatgctcctc-3'</td>
<td>siaD (Y)</td>
<td>120</td>
</tr>
</tbody>
</table>

Table 3
Isolation of N. meningitidis and N. lactamica from different populations in northern Greece

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>N. meningitidis n</th>
<th>%</th>
<th>N. lactamica n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nursery</td>
<td>144</td>
<td>3</td>
<td>2.1</td>
<td>18</td>
<td>8.3</td>
</tr>
<tr>
<td>Primary</td>
<td>843</td>
<td>40</td>
<td>4.7</td>
<td>53</td>
<td>6.1</td>
</tr>
<tr>
<td>Lower secondary</td>
<td>256</td>
<td>35</td>
<td>13.9</td>
<td>5</td>
<td>1.9</td>
</tr>
<tr>
<td>Upper secondary</td>
<td>584</td>
<td>60</td>
<td>10.3</td>
<td>20</td>
<td>3.4</td>
</tr>
<tr>
<td>Tertiary</td>
<td>85</td>
<td>10</td>
<td>11.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Boarding school</td>
<td>57</td>
<td>8</td>
<td>14</td>
<td>6</td>
<td>10.5</td>
</tr>
<tr>
<td>Military</td>
<td>377</td>
<td>141</td>
<td>37.4</td>
<td>10</td>
<td>2.9</td>
</tr>
<tr>
<td>Hospital staff</td>
<td>604</td>
<td>26</td>
<td>4.3</td>
<td>25</td>
<td>4.1</td>
</tr>
<tr>
<td>Elderly</td>
<td>217</td>
<td>11</td>
<td>5.1</td>
<td>9</td>
<td>4.1</td>
</tr>
</tbody>
</table>
None of the *N. lactamica* isolates reacted with any of the serogroup, serotype or subtype reagents.

3.4. Antibiotic sensitivities

No strains resistant to rifampicin, cefaclor, chloramphenicol, ceftriaxone or ciprofloxacin were detected. Reduced sensitivity to penicillin (0.125–0.25 mg l\(^{-1}\)) was observed for strains from Ioannina (2/67, 3%), Serres (3/115, 2.6%), Evros (1/57, 1.8%) and Florina (19/84, 22.6%). Sulphonamide resistance (≥ 12 mg l\(^{-1}\)) was found only among strains from Serres (12/115, 10.4%) and Evros (7/57, 12.3%). There were no particular phenotypes associated with increased resistance to sulphonamide or reduced sensitivity to penicillin. The majority of the isolates with reduced sensitivity to penicillin (14/25, 56%) were obtained from military personnel, 11/54 (20%) from Florina and 3/87 (3.5%) from Serres.

4. Discussion

The first objective was to determine if there was an increase in the carriage of meningococci in different age groups in northern Greece that might account for the increase in disease activity due to serogroup C. The proportions of carriers among children attending nursery schools and primary schools was similar to that found previously for primary schools in Athens (2–5%) in 1990–1991 [12]. In this study, immigrant children made up less than 10% of the populations of the primary schools tested, and none of the immigrant children in the primary schools were carriers of either meningococci or *N. lactamica*. This is in contrast to our study carried out in 1995. The isolation rate for meningococci (13.1%) was higher among primary school pupils in an area of Athens in which 64% of the children were from families of ethnic Greeks who had recently immigrated from Russia. This was not a closed community comparable to boarding or military institutions; many of the families worked as market traders and had regular contact with the general population [8].

The boarding school for Albanian men was the only group in which there was a clearly defined immigrant population. There were no other areas identified in which there were large numbers of Albanians, Turks or Bulgarians.

For children in secondary education, the proportion of carriers (11.3%) was significantly higher than for secondary pupils in Athens during 1990–1991 (6.7%) (χ\(^2\) = 9.66, \(P < 0.005\)) [9], however, this increase was not associated with a higher proportion of immigrants. The highest proportion of carriers was among pupils of the Albanian boarding school (14%).

Among military recruits in the region, the proportion of carriers (37.4%) was significantly higher compared to our previous study during 1990–1991 which included recruits from all areas of Greece (25%) (χ\(^2\) = 21.11, \(P < 0.000\)).

These figures were, however, similar to those found previously for recruits from northern Greece: eastern Macedonia (30%); western Macedonia, 33%; Thrace (36%); Epirus (30%) [7,9]. Among hospital personnel, the carriage rate was 4.3% and a similar figure (5.1%) was observed for the subjects attending centres for the elderly.

The higher proportion of carriers of meningococci in northern Greece was not associated with the higher carriage of meningococci among immigrant groups. Dispersal of the immigrant families in the community might account for the absence of schools with unusually high proportions of immigrant children (64%) as was observed in Athens. The proportion of carriers among nursery and primary school pupils in this study was similar to those found for primary school pupils in 1991 for schools in Athens in which pupils came from families in which the parents were born in Greece. The social or environmental factors associated with the significantly higher carriage rate among secondary school pupils in northern Greece need to be examined further.

Because of the recent increase in the disease due to serogroup C [1], the second objective of the study was to determine if the phenotypes of the isolates or antibiotic sensitivities of meningococci in northern Greece were different from those in our previous studies. Strains expressing the C:2a:P1.5,2 phenotype were isolated from only five subjects; therefore, increased carriage of this phenotype was not associated with increased levels of IMD reported in the area. As in our other studies, the majority of strains were non-groupable by serological reagents. The proportion of serogroup A (1%) was lower than in previous surveys of recruits (4%) or Russian immigrants (28%) [7,8]. The proportion of group B (20%) was similar to that found previously for recruits, but higher than that among Russian immigrants (14.2%). By the agglutination test, 1% of the isolates in this study (1999) were serogroup C which was lower than the proportion identified in previous studies in which the agglutination technique was the only method available for detection of serogroup: Russian immigrants, 4.8% in 1995; school children in Athens, 10.5% in 1990–1991; recruits, 5% in 1990–1991. The proportions of serotypes observed might reflect changes noted for children 0–13 years of age hospitalised in the Athens area between 1 January 1999 and 31 December 2000. There was a sharp decrease in serogroup C from 19% of cases in 1999 to 3% in 2000 (\(P = 0.013\)). Of note was the emergence of serogroup A responsible for 7% of the cases [13].

As with our earlier studies on phenotypes of carrier isolates, the majority did not react with the serotype monoclonal antibodies (74%) compared with 65% for recruits and 44% of those from Russian immigrants. Serotype 2a was present in only 1% of isolates, similar to that observed for the earlier study of recruits. Serotypes 4 (6.3%), 14 (9.4%) and 15 (4.2%) were the most common. These did not differ significantly from results of the study...
of recruits, but the proportion of isolates reactive with these monoclonal reagents was higher among the isolates from Russian immigrants: 4 (13.4%); 14 (11%); 15 (18.3%). Only 1% of isolates in this study reacted with the type 22 reagent compared to 13.4% of those from the Russian immigrants [8].

The only notable difference between subtypes was a smaller proportion of P1.2 among isolates in the current study (5.4%) compared with 16% for the recruit study and a higher proportion of P1.14 (25.7%) compared with 2% of isolates from recruits in the early 1990s [9].

Overall isolation of *N. lactamica* (4.6%) was lower than that found for our study of Russian immigrants (17.3%) [8]. In both surveys, *N. lactamica* was isolated more frequently from children in the primary school age range; however, in the current study, the highest proportion of carriers was found in the boarding school for young Albanian men. In no subject did we detect both species in any of our studies. Among the hospital personnel the proportions of meningococcal isolates from men and women were 12/26 (46%) and 14/26 (54%) respectively. In comparison, for the *N. lactamica* isolates, 23/25 (88%) were obtained from women compared with 3/25 (12%) from men. The higher proportion of *N. lactamica* isolates among women probably reflects longer or closer contact with children in the primary school age range as has been observed in studies in England [14]. There were no significant differences in isolation of *N. lactamica* from men and women in the elderly group who were less likely to have regular and prolonged contact with young children.

The isolates with reduced sensitivity to penicillin were not distributed evenly among the age ranges or areas examined. The majority were among isolates from Florina (19/25, 76%). Antibiotic usage is higher among young children, but the highest proportion of penicillin-insensitive isolates were obtained from older age groups. Over half the 25 isolates with reduced sensitivity to penicillin (14, 56%) were from recruits, eight (32%) from school children. In Greece, antibiotics are available without prescription. These differences in distribution of the penicillin-insensitive isolates might reflect more respiratory illness among military personnel and children and should be further examined.

In conclusion, the study reported here indicates that there is a higher carriage rate of meningococci among individuals in northern Greece compared with previous studies among school children in Athens and recruits from all areas of Greece [8,12]; however, there was no evidence that this increase in carriage rate was associated with the influx of immigrants from neighbouring countries and there was not a higher incidence of the C:2a:P1.5,2 strain responsible for increased disease activity in Greece [1] in either the immigrant or local populations. Carriage of *N. lactamica* among the different groups was in the ranges expected, except for the group of young Albanian men which was similar to the proportion of isolates observed among the Russian immigrant population [8]. The high proportion of penicillin-insensitive isolates in the Florina region, particularly among military personnel, needs to be further investigated.

Acknowledgements

This work was supported by a grant from the European Community Initiative ‘Interreg II’ for Public Health in the Balkans through the Greek Ministry of Health. C.C.B. was supported by a grant from the Meningitis Association of Scotland.

References


