

# Carriage of *N. lactamica* in a population at high risk of meningococcal disease

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## SUMMARY

Carriage of *Neisseria lactamica* among household contacts of meningococcal disease (MCD) cases was investigated during an epidemic in Auckland, New Zealand. The overall carriage rate for *N. lactamica* was 10·5% (95% CI 7·4–13·5%) with a peak carriage rate in 2-year-olds of 61·5% (95% CI 26·6–88·1%). Factors associated with a significant ( $P < 0\cdot05$ ) increase in the likelihood of carriage included runny nose, the number of people per bedroom and youth. Genetic analysis of isolates revealed a striking correlation of strains within the same household but a high level of diversity between households, suggesting that household contact is an important factor in acquisition. For household contacts aged less than 5 years, there was a higher rate of carriage amongst those in contact with MCD cases under 8 years old than for contacts of cases aged 8 years and over. It is likely that development of MCD is a reflection of the nature and intensity of the exposure to a virulent strain of *N. meningitidis*, coupled with an absence of host resistance among those individuals not carrying *N. lactamica*.

## INTRODUCTION

*Neisseria lactamica* is distinguished from *Neisseria meningitidis* by its ability to ferment lactose [1]. *N. lactamica* is an extremely rare cause of meningitis [2] or septicaemia [3] and the predominant site of human carriage is the nasopharynx [4]. *N. lactamica* differs from *N. meningitidis* in that it lacks capsular polysaccharide but does possess lipo-oligosaccharide epitopes [5]. The development of cross-reactive antibodies to *N. meningitidis* during nasopharyngeal carriage of *N. lactamica* has led to the belief that carriage of *N. lactamica* has a role in the development of natural immunity to invasive *N. meningitidis* infection [6]. Some evidence exists to support this theory, in that populations with high levels of

meningococcal disease (MCD) are reported to have lower rates of *N. lactamica* carriage than low incidence populations [7]. New Zealand has epidemic levels of MCD dating from 1991, predominantly due to a serogroup B *N. meningitidis* [8]. In 1997, the national incidence rate increased to 16·9 per 100 000 population [9] with the majority of cases occurring in Pacific and Maori children. In one Auckland suburb, the annual risk of MCD among Pacific children under 1 year of age was 1017 per 100 000. In this region of high incidence we studied carriage of *N. lactamica* among household contacts of MCD cases.

## METHODS

### Participants and recruitment

Participants were household contacts of MCD cases notified to Auckland health authorities between 6

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October 1996 and 14 September 1998, resident within the greater Auckland region. Households with MCD cases under 8 years of age were the subject of a case-control study into risk factors for MCD which commenced in April 1997. In order not to affect the validity of data collected in the case-control study, from 1 April 1997 contacts recruited to our study were only those of cases aged 8 years and over. Cases of MCD were included if either 'probable' or 'confirmed' according to the definition developed by a New Zealand MCD workshop [10]. A household contact was defined as a person sleeping in the same house as the case, for at least one night, in the 10 days prior to the onset of the case's illness. Those contacts currently on antibiotic medication or who had taken antibiotics in the preceding month were excluded from the study. Household contacts of MCD cases were identified and offered chemoprophylaxis within 24 h of notification of the index case, by a public health nurse. In order to determine the prevalence of nasopharyngeal carriage of *N. lactamica* among contacts, informed consent was sought from participants for a nasopharyngeal swab. A standardized questionnaire covering sociodemographic data and risk factors for carriage of *Neisseria* spp. was administered.

#### Throat swab

A per-oral nasopharyngeal specimen was obtained with a dry straight cotton-tipped swab. This was touched on each tonsillar bed and posterior nasopharynx behind the uvula. Swabs were streaked directly onto Modified Thayer–Martin Medium (Fort Richard Laboratories, Auckland, New Zealand) and transported to the laboratory in a candle jar.

#### Laboratory procedures for culture and isolation of *Neisseria lactamica*

Plates were incubated at 36 °C in 5% CO<sub>2</sub> and examined at 24, 36, 48 and 60 h. Colonies resembling *Neisseria* spp. underwent oxidase testing and, if oxidase-positive, were then Gram stained. Three colonies of Gram-negative diplococci were selected and each was subcultured onto an individual sheep blood agar plate to provide pure cultures for identification and further testing. Cultures were incubated for 18–24 h at 36 °C in 5% CO<sub>2</sub>. *Neisseria* spp. were further identified using a rapid carbohydrate degradation test (RapID NH System™, Diagnostic Systems

Inc., Norcross, GA, USA). A heavy suspension of each isolate was made into 1 ml of glycerol broth and stored at –70 °C.

#### Genetic typing

The interrelationship of carriage between and within households was investigated by genetic typing. Macrorestriction analysis (restriction fragment length polymorphism) of isolates using *Sfi*I was based on the method of Bygraves and Maiden [11]. Chromosomal DNA (*Saccharomyces cerevisiae*) was used in gels as a band migration size reference, and restricted DNA from one control strain was loaded into a lane on each gel to control pattern reproducibility. Gel patterns were resolved using pulsed-field gel electrophoresis.

#### Statistical analyses

The Epi-Info statistical programme [12] (CSAMPLE) was used to determine point estimates of carriage prevalence and 95% CIs relating to the clustered sample. In order to identify factors predicting carriage of *N. lactamica* within households, a generalized linear mixed model logistic regression approach was used, incorporating the cluster variable of case as a random effect. The multivariate logistic regression analysis included the explanatory variables of respiratory symptoms – runny nose, sore throat, cough, personal smoking and passive smoking, number of people per bedroom, ethnicity, age, sex, education of the head of the household and income per householder (total income of all household contacts of a single case, divided by the number of household contacts). Ethnic-specific carriage prevalences were directly age-standardized in six age bands to the Greater Auckland population as recorded in the 1996 National Census.

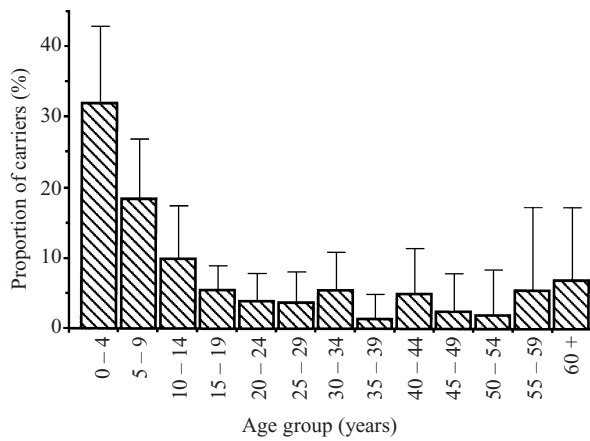
## RESULTS

#### Response rates

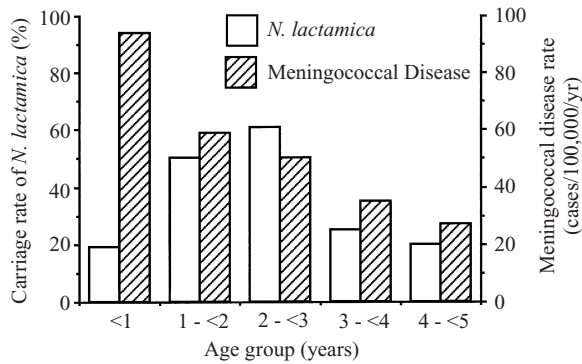
Nine hundred and fifty-four of the 1041 invited household contacts agreed to a throat swab, a response rate of 91.6%. A total of 288 subjects were contacts of MCD cases aged less than 8 years, and 666 were contacts of cases aged 8 years and over.

#### Carriage rates

The overall carriage rate of *N. lactamica* among household contacts of 160 MCD cases was 10.5%



**Fig. 1.** Carriage of *N. lactamica* among household contacts of meningococcal disease cases, Auckland, New Zealand Sept. 1996–Nov. 1998 (upper 95% confidence limits).



**Fig. 2.** Comparison of age-specific carriage of *N. lactamica* among household contacts of meningococcal disease cases with meningococcal disease rates, Auckland, New Zealand, Sept. 1996–Nov. 1998.

(100/954, 95% CI 7.4–13.5%). This compared to a carriage rate of 20.8% (198/954, 95% CI 17.3–24.2%) for *N. meningitidis*. Age-specific carriage of *N. lactamica* was highest in those under 5 years of age (Fig. 1). Carriage of *N. meningitidis* in children under 5 years of age was low at 5.8% (7/120, 95% CI 1.3–10.4%). The peak carriage of *N. lactamica* was 61.5% (8/13, 95% CI 26.6–88.1%) in 2-year-olds. Children aged less than 1 year old had the lowest carriage rate, of any year under 5, at 18.5% (5/27, 95% CI 14.8–33.3%). Although the rates of carriage were highest among Pacific people in most age groups, the differences were not great. When allowing for age alone, there was a probability of 0.05 for an effect of ethnicity on carriage. In the under 5 years age group, 35.2% (25/71, 95% CI 21.7–48.7%) of Pacific children carried *N. lactamica* followed by European/other at 30.0% (6/20, 95% CI 5.8–54.2%) and Maori at 24.1% (7/29, 95% CI 3.2–45.1%). Age-

standardized carriage rates were 11.3% (95% CI 8.5–14.0%) in Pacific contacts, 6.5% (95% CI 3.6–9.3%) in Europeans/others and 5.8% (95% CI 2.6–8.9%) in Maori. There were no gender differences, with 10.3% (50/486, 95% CI 6.9–13.7%) carriage in males and 10.7% (50/468, 95% CI 6.8–14.6%) in females. Figure 2 compares the rate of meningococcal disease among the Auckland population aged less than 5 years, to the carriage rate of *N. lactamica* among their household contacts of similar age. While MCD rates were highest in those under 1 year of age, in whom carriage of *N. lactamica* was lowest, incidence remained elevated in those aged 1–3 years despite the carriage of *N. lactamica* of between 50 and 60% among household contacts. Only 3% of the 100 (95% CI 0–6.1%) *N. lactamica* carriers also carried *N. meningitidis*, while 23% of those without *N. lactamica* carried *N. meningitidis* ( $\chi^2 = 21.4$ ,  $P < 0.0001$ ). When the entire sample of 954 contacts was considered, the prevalence of simultaneous carriage of the two species was 0.3% (95% CI 0–0.7%) compared to the 2.2% which would be expected if the occurrence of the two organisms was independent of one another and all contacts had the same chance of carriage. Two of the three contacts carrying both species harboured the epidemic strain of *N. meningitidis* (B:4:P1.4), the third a serogroup 29E organism (29E:1:P1.5,2).

***N. lactamica* carriage and case age**

An assessment was made of *N. lactamica* carriage in contacts of cases aged less than 8 years versus cases 8 years or older. Household contacts (all ages) of cases aged less than 8 years were significantly more likely ( $P = 0.0001$ ) to carry *N. lactamica* than contacts of cases 8 years and over. If the MCD cases are divided into those aged less than 8 years or 8 years and over, for cases less than 8 years of age, contacts under 5 years were significantly more likely ( $P = 0.04$ ) to carry *N. lactamica* than contacts aged under 5 years of cases aged  $\geq 8$  years.

**Factors predicting carriage**

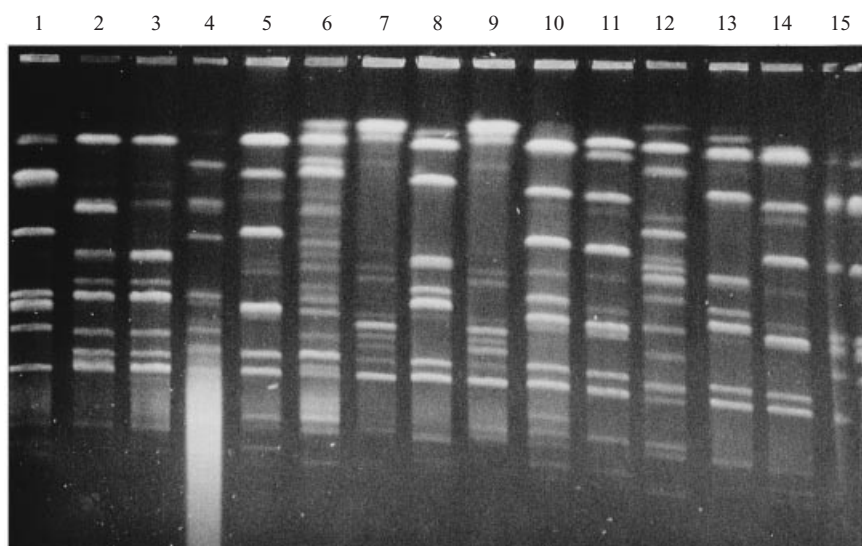
Factors predictive of carriage of *N. lactamica* among householders are shown in Table 1. Factors associated with increased odds of carriage included runny nose, cough, the number of people per bedroom, smoking by someone else in the house and age. Although the overall effect of smoking for carriage was significant ( $P = 0.003$ ), there was no evidence of an effect of

Table 1. *Multivariate logistic regression for factors associated with carriage of N. lactamica (n = 864)*

Factor	Frequency in carriers (%)	OR	95% CI	P value, B = 0
Sore throat	7.8	0.38	0.18–0.79	0.01
Running nose	48.5	1.80	1.09–2.95	0.02
Cough	43.4	1.49	0.88–2.52	0.14
Smoking in house				0.003
Smoke personally	10.2	0.63	0.29–1.34	
Other member smokes	53.1	1.92	0.99–3.71	
No smoking	36.7	1.00		
Number persons/bedroom	*	1.49	1.02–2.17	0.04
Ethnicity				0.73
Maori	17.0	0.71	0.26–1.78	
Pacific	67.0	0.68	0.26–1.78	
Other†	16.0	1.00		
Age (years)	*	0.97	0.95–0.98	0.0001
Sex (male:female)	50.0:50.0	1.43	0.95–2.14	0.09
Educational level	*	1.18	0.86–1.62	0.29
Income per householder	*	1.00		0.78

\* Continuous variables.

† Includes all other ethnic groups.



**Fig. 3.** Lanes 1 and 15 internal reference control; lanes 2 and 3 each represent different members, same household; lanes 4–6 each represent different members, same household; lanes 7–14 each represent a different household.

personal smoking compared to non-smoking and only weak evidence of an increased chance of carriage if other household members smoked when compared to non-smoking. Age was a strong predictive factor for carriage. The multivariate analysis was able to adjust for a number of confounders simultaneously and the apparent association between Pacific ethnicity and carriage disappeared once person per bedroom was included in the analysis. There was a positive association between odds of carriage and the number

of people per bedroom. The only factor associated with reduced odds of carriage was the reported presence of a sore throat.

### Genetic typing

Thirty distinct macrorestriction types were identified among the 64 *N. lactamica* isolates available for typing, from a total of 35 case-household groupings. There was striking correlation between genetic types

within the same household grouping and considerable pattern difference from household to household. These differences are shown in Figure 3. The probability that the observed distribution of household macrorestriction patterns could have occurred by chance alone is  $< 10^{-10}$ .

## DISCUSSION

There have been a number of studies of *N. lactamica* carriage in countries including USA [6], Denmark [7], Nigeria [13], Norway [14], England [15, 16], Germany [17] and Spain [18]. Many were cross-sectional surveys in communities with outbreaks or epidemics of MCD. With the exception of the study by Olsen and others [7], they reveal overall carriage rates of 2.9–6.3%, much lower than the 10.5% found in this study. Although the sample for our study was much younger than the general population with a median age of 21 years, with few exceptions, age-specific carriage rates were also higher in our study compared with those reported in the international literature. The study of Olsen and others, over-sampled 0- to 4-year-old children, with the effect that this age group represented approximately 30% of the total study sample size compared to 12.5% for our study. When this difference is taken into account, the carriage rates for the two studies would be similar. There is some evidence at least at the ecological level [7] that communities with high rates of MCD have lower rates of carriage of *N. lactamica* and vice versa. However, if the absolute risk of MCD among the Auckland population is considered, then children aged less than 5 years old had a risk of 365/100000 per year, with a risk for all ages of 52/100000 per year for the duration of the survey. Pacific children under 5 years old, not only had the highest carriage rate for *N. lactamica* of all ethnic groups (37.3%, 95% CI 21.7–48.7%), but also the highest rate of MCD (525/100000 per year) compared with 210/100000 per year for Maori and 49/100000 per year for the European/other ethnic groups. This suggests that the absolute risk of developing invasive MCD does not have a simple inverse relationship with *N. lactamica* carriage at the population level. However, in individuals, the low rate of carriage of *N. meningitidis* among carriers of *N. lactamica* (3%) confirms the findings of others [6, 7]. The fact that the two species infrequently coexist in the nasopharynx would be consistent with the hypothesis that *N. lactamica* has a role in preventing nasopharyngeal colonization by strains of *N.*

*meningitidis* and potentially the development of MCD in an individual. One further explanation for the co-existence of high carriage rates of *N. lactamica* and high rates of invasive MCD among subgroups of the population, is that ‘risk factors’ for carriage of the two organisms at the individual level may differ. The effect of this would be that the same individual is unlikely to carry both species, but those able to be colonized with *N. meningitidis* have a high risk of MCD. Genetic typing of *N. lactamica* isolates, hitherto unreported in the literature, demonstrated a wide diversity of macrorestriction types between households but a striking correlation of specific strains within households. This suggests a strong influence of household contact on nasopharyngeal colonization by *N. lactamica*. Factors predicting carriage of *N. lactamica* identified by other investigators include age, active and passive smoking [16], respiratory viral infection and kissing contact [19]. Age, as in other studies, was confirmed as a strong predictive factor for carriage. The positive association between odds of carriage and the number of people per bedroom has biological plausibility. In this context, carriage may reflect close contact and prolonged nocturnal exposure to an organism capable of spread by respiratory droplets. Nevertheless, there should be some caution in interpreting the effect of number of people per bedroom as this factor is likely to be confounded by a number of other factors also relating to the carriage of *N. lactamica* such as communal living and sharing of food among householders. The association of personal smoking with carriage is consistent with other studies. However, the differing direction of association of personal smoking versus another family member smoking in the household is difficult to explain with the latter association possibly due to chance. The result that those reporting sore throat had a reduced odds of carriage was unexpected. Recent or current treatment of sore throat with antibiotics could not explain this finding as these contacts were excluded from the study. It is possible that viral or other bacterial pathogens causing a sore throat may interrupt carriage of *N. lactamica* but this is purely speculative and the fact that runny nose and cough, common accompaniments of sore throat were associated with increased odds of carriage cannot be explained easily. The finding that *N. lactamica* carriage rates were higher among householders under 5 years of age who were contacts of cases under 8 when compared to contacts of similar age of cases 8 years and over, suggests that the higher risk of

MCD in the young is not simply explained by the lower rates of *N. lactamica* carriage in their households. However, these findings should be interpreted with caution. The study lacked a suitable control sample frame such as households without a meningococcal case with which to compare carriage in the under 5 years old contacts. The use of MCD case householders as the sample population, introduces a selection bias such that the results cannot be applied to the general population. Nevertheless, the observed high rate of carriage of *N. lactamica* among household MCD contacts, a population at high risk of MCD, is not necessarily inconsistent with a role for this organism in protecting against MCD. The high rates of MCD could simply reflect individual host factors and the nature and frequency of exposure to a virulent strain of *N. meningitidis* among a young population, most of whom are not carrying *N. lactamica*. Further research is required on this enigmatic organism, particularly concerning the immunogenicity of *N. lactamica* and its role, if any, in protection against MCD.

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