



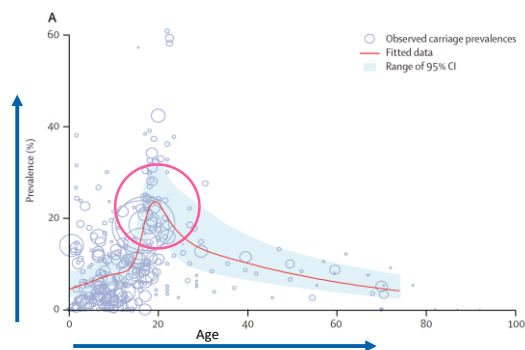
'B Part of It study': Carriage of *Neisseria meningitidis* in first year university students in South Australia; a longitudinal study

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seekLIGHT

Carriage of *N. meningitidis*



Christensen H. Lancet Infectious Diseases 2010;10(12):853-61. Epub 2010/11/16. doi: 10.1016/S1473-3099(10)70251-6.

Background on testing for *N. meningitidis* carriage

- Oro-pharyngeal swabs direct plated on selective agar culture media is considered the gold standard method to detect meningococcal carriage.
- Freezing oro-pharyngeal samples prior to plating is necessary when direct plating is not practical.
- The time from swab collection to freezing is likely to be an important factor in the isolation of *Neisseria* species.
- No data exist for samples frozen later than 3 hours.
- The addition of saliva testing to carriage studies is being considered.

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Aims/Objectives

Primary Objective

- Estimate the carriage prevalence of all genogroups of *N. meningitidis* in South Australian first year university students.

Secondary Objectives

- Identify risk factors associated with carriage
- Estimate the change in carriage prevalence at baseline (first week of university) and 3 months later.
- Estimate any difference in culture and PCR positivity of isolates at different freezing times post swab collection.
- Estimate the reliability of saliva testing compared to throat swabs for the detection of *N. meningitidis*. (Add on study at visit 2)

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Methods

Visit 1

- Recruitment during orientation week (20th to 24th of February 2017), The University of Adelaide
- First year university students aged 17 to 25 were eligible to participate.
- Oro-pharyngeal swabs from the posterior pharynx.
- Swabs were placed in liquid transport medium skim milk, tryptone, glucose, and glycerin (STGG) for transport to SA Pathology.



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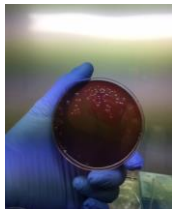
Methods

Based on UKMencar4 study questionnaire <http://www.ukmencar4.org/>

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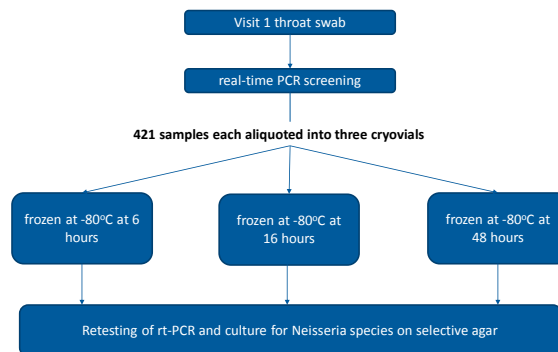
Laboratory process

- All specimens were subjected to real-time PCR (rt-PCR) prior to freezing.
- Further rt-PCR analysis was used on positive specimens to determine the genogroup (A, B, C, W, X, Y).
- PCR samples were also thawed and cultured on selective agar.
- Whole genome sequencing analysis of *N. meningitidis* isolates was performed.



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Time to freezing



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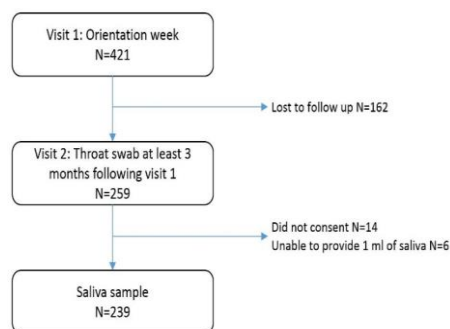
Methods

Visit 2

- Students were contacted via email and phone to return for a repeat swab after 3 months.
- At this visit students were also asked if they consented to providing a saliva sample.
- For those that consented, 1ml of saliva was drooled into an empty sterile container and immediately syringed into a vial containing 1ml of STGG transport media.

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University cohort results



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Results: Participant characteristics (visit 1)

| Participant characteristics | N (%) |
|--|-------------|
| Age (years): mean (sd) | 18.5 (1.4) |
| Female | 237 (56.4%) |
| Previous verified Men B vaccination | 6 (1.4%) |
| Current cold or sore throat | 54 (12.8%) |
| Current smoker | 8 (1.9%) |
| E-cigarette use in the last week | 1 (0.2%) |
| Water pipe use in the last month | 14 (3.3%) |
| 1 or more nights out in the last week | 203 (49.5%) |
| 1 or more people kissed in the last week | 135 (33.2%) |
| Current partner | 115 (28.2%) |
| Household size >=10 | 16 (3.9%) |
| Ethnicity | |
| Aboriginal | 4 (1.0%) |
| Caucasian | 269 (65.1%) |
| Asian | 111 (26.9%) |
| Other | 29 (7.0%) |

In a 2016 SA survey for 15 to 29 year olds, the numbers smoking was 10.5%

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Carriage prevalence at visit 1

Table: Proportion of *N. meningitidis* carriage at visit 1

| Genogroup | Visit 1 swab (n=421) | |
|---------------|----------------------|--------------|
| | N (%) | (95% CI) |
| Group Y | 12 (2.9%) | 1.6% to 5.0% |
| Group B | 7 (1.7%) | 0.8% to 3.5% |
| Non-groupable | 6 (1.4%) | 0.6% to 3.1% |
| Group W | 3 (0.7%) | 0.2% to 2.2% |
| Total | 26* (6.2%) | 4.2% to 8.9% |

* No A, C, or X genogroups were identified.

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Association with carriage (Univariable) at visit 1

| Effect | Reference | Detected N (%) | Odds Ratio (95% CI) | P-value |
|-------------------------|--|-----------------------|-----------------------------|------------------|
| Age | Reference: 17 year olds | 5/66 (7.6%) | | |
| | 18 years | 11/223 (4.9%) | 0.63 (0.21 to 1.89) | 0.41 |
| | 19 years | 7/67 (10.5%) | 1.42 (0.43 to 4.73) | 0.57 |
| | > 20 years | 3/65 (4.6%) | 0.59 (0.14 to 2.58) | 0.48 |
| Sex | Reference: Male | 14/183 (7.7%) | | |
| | Female | 12/237 (5.1%) | 0.64 (0.29 to 1.42) | 0.28 |
| Sore throat/Cold | Reference: No cold | 20/367 (5.5%) | | |
| | Current cold or sore throat | 6/54 (11.1%) | 2.17 (0.83 to 5.67) | 0.11 |
| Party, or bar | Reference: None last week | 5/207 (2.4%) | | |
| | One visit in the last week | 8/118 (6.8%) | 2.94 (0.94 to 9.20) | 0.06 |
| | Two or more in the last week | 13/85 (15.3%) | 7.29 (2.51 to 21.18) | <0.001 |
| Kissing | Reference: No kissing last week | 7/272 (2.6%) | | |
| | One or more person | 17/135 (12.6%) | 5.45 (2.20 to 13.50) | <0.001 |

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Association with carriage at visit 1

Table: Prevalence *N. meningitidis* pharyngeal carriage from oro-pharyngeal swab, at visit 1, by kissing, and social visits per week.

| People kissed last week | Number of times going out to pubs and clubs in the last week | PorA PCR | | Total | P-value |
|-------------------------|--|--------------|------------|-------|---------|
| | | Not Detected | Detected | | |
| No kissing | No going out | 152 (98.7%) | 2 (1.3%) | 154 | 0.134 |
| | Went out once | 71 (97.3%) | 2 (2.7%) | 73 | |
| | Went out two or more times | 42 (93.3%) | 3 (6.7%) | 45 | |
| Total | | 265 (97.4%) | 7 (2.6%) | 272 | |
| Kissing >=1 person | No going out | 50 (98.0%) | 1 (2.0%) | 51 | 0.004 |
| | Went out once | 38 (86.4%) | 6 (13.6%) | 44 | |
| | Went out two or more times | 30 (75.0%) | 10 (25.0%) | 40 | |
| Total | | 118 (87.4%) | 17 (12.6%) | 135 | |

Multivariable logistic regression

- out to a bar or pub one night a week OR 4.14 [95% CI, 1.06 to 16.15], p=0.04
- two or more nights a week OR 9.26 [95% CI, 2.51 to 34.08], p=0.001
- one or more persons kissed in the last week OR 4.13 [1.63 to 10.45], p=0.014

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Carriage prevalence at visit 1 and visit 2

Table: Proportion of *N. meningitidis* carriage at visit 1 and visit 2

| Genogroup | Visit 1 swab in those lost to follow up (n=162) | | Visit 1 swab in those who returned for visit 2 (n=259) | | Visit 2 swab (n=259) | |
|----------------------|---|---------------|--|--------------|----------------------|--------------|
| | N (%) | 95% CI | N (%) | 95% CI | N (%) | 95% CI |
| Total PCR +ve | 16 (9.9%) | 6.1% to 15.6% | 10 (3.9%) | 2.1% to 7.1% | 16 (6.2%) | 3.8% to 9.9% |

Those that did not return were more likely to have visited a pub or club (p<0.001), compared to those that returned.

- The carriage prevalence in the 259 students who did return after 3 months increased from 3.9% to 6.2%.

Multiple imputation was performed to impute carriage at visit 2. Visiting pubs and clubs, and kissing were included in the model.

- Visit 1 (6.2%) vs Visit 2 (8.6%), OR 1.42 (95% CI 0.91 to 2.20), p = 0.12.

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Changes between visit one and two

- Seven out of the 10 students who were PCR positive at visit one were positive again at visit two. All carrying the same genotype:
 - 2 Y
 - 3 B
 - 1 W
 - 1 non-groupable.
- Three students were PCR positive at visit one were negative at visit 2, all were genogroup Y.
- Nine students who were negative at visit one were positive at visit 2:
 - 4 Y
 - 5 non-groupable

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Visit 2 saliva sample

Table: Proportion of *N. meningitidis* carriage from visit 2 saliva sample.

| Genogroup | Visit 2 saliva sample (n=239) | |
|----------------------|-------------------------------|--------------|
| | N (%) | 95% CI |
| Group B | 3 (1.3%) | 0.4% to 3.8% |
| Group W | 0 (0%) | |
| Group Y | 5 (2.1%) | 0.9% to 5.0% |
| Non-groupable | 6 (2.5%) | 1.1% to 5.5% |
| Total | 13 (5.4%) | 3.2% to 9.2% |

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Saliva testing vs throat swab

Table: Cross tabulation of *N. meningitidis* carriage identified by throat swab vs saliva sample.

| PCR throat swab | PCR saliva | | Total |
|-----------------|--------------|----------|-------|
| | Not detected | Detected | |
| Not Detected | 220 | 4 | 224 |
| Detected | 6 | 9 | 15 |
| Total | 226 | 13 | 239 |

unweighted Kappa = 0.62 (95% CI, 0.39 to 0.85).

- In this relatively small sample (n=239) saliva collection, identified an additional four participants with carriage; one group B, and 3 non-groupable.

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N. meningitidis identification after freezing at 6, 16, and 48 hours

Table: Proportion of *N. meningitidis* isolates grown on selective agar from samples frozen at 6 hours, 16 hours, and 48 hours

| Freeze time (hours) | N (%) | P-value |
|---------------------|---------------|---------|
| 6hrs | 24/26 92.3% | |
| 16hrs | 23/26 88.5% | 0.56 |
| 48hrs | 14/26 53.9% ↓ | 0.007 |

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N. meningitidis identification after freezing at 6, 16, and 48 hours

Table: PCR Cycle thresholds in samples frozen at 6, 16, and 48 hours compared to pre-freezing, using a linear mixed effects model.*

| Freeze time | n | Mean | SE | P-value |
|-------------|----|-------|------|---------|
| Baseline | 26 | 31.18 | 0.77 | |
| 6hrs | 26 | 31.00 | 0.89 | 0.51 |
| 16hrs | 26 | 31.44 | 0.74 | 0.08 |
| 48hrs | 26 | 31.87 | 0.83 | 0.01 |

* One sample was allocated a Ct of 46 (threshold +1) as it was not detected in the 6 and 48 hr frozen samples.

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Whole genome sequencing

| Serogroup(s) (% of isolates) | Clonal complex(CC) | Number of isolates | MLST (% of isolate) | PorA type (% of isolates) | FetA | fHbp | BAST |
|---------------------------------|-----------------------|-----------------------|---|--|------------------------|--|---------|
| W (100) | 11 | 3 | ST-11 (33) ST-1287 (67) | P1.5,2 (100) | F1-1 (100) | 22 (100) | 2 (100) |
| Y (100) | 23 | 13 | ST-23 (62) ST-1655 (38) | P1.5-1,10-1 (38) P1.5,2 (23) P1.5-2,10-1 (31) P1.5-2,10-29 (8) | F4-1 (92) F5-12 (8) | 25 (100) 221 (38) 427 (23) 228 (31) 1212 (8) | U (100) |
| B (100) | 32 | 1 | ST-32 (100) | P1.7,16-26 (100) | F3-3 (100) | 224 (100) | U (100) |
| B (83) NG (17) | 41/44 | 6 | ST-44 (50) ST-1097 (17) ST-6058 (17) ST-13605 (17) | P1.18,25-19 (33) P1.18-1,34 (33) P1.17-1,1 (17) P1.19-1,26-4 (17) | F1-5 (83) F1-7 (17) | 19 (100) 315 (33) U (67) | |
| B (100) | 213 | 1 | ST-213 (100) | P1.22,14 (100) | F5-36 (100) | 45 (100) | U (100) |
| E (100) | 1157 | 1 | ST-1157 (100) | P1.21-7,16 (100) | F5-36 (100) | 13 (100) | U (100) |

- One MenW with sequence type 11 was identified. This is the most common W isolate causing disease in Australia.²
- None of the most common disease causing serogroup B PorA type was identified (P1.7-2,4).³

² Invasive Meningococcal Disease National Surveillance Report – December 2017. Australian Government Department of Health, 2017

³ Lefina MM, Enayati RP. Australian Meningococcal Surveillance Programme annual report, 2015. Communicable diseases intelligence quarterly report 2016;40(6):E503-E11.

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Conclusion

- Overall rates of carriage (6.2%) were lower than anticipated (8%), possibly due to low rates of smoking.
- Risks of carriage were similar to overseas studies and were driven by social behaviour.
- Freezing of oro-pharyngeal samples within 16 hours is an acceptable timeframe if direct plating or freezing within 6 hours is not possible.
- Saliva sampling in addition to oropharyngeal swabs improves sensitivity in meningococcal carriage studies.

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B Part of it Team

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