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A comparison of flocked nylon swabs and non-flocked rayon swabs for detection of respiratory bacteria in nasopharyngeal carriage in Australian Indigenous children

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1 **Title**

2 A Comparison of Flocked Nylon Swabs and Non-Flocked Rayon Swabs for Detection of
3 Respiratory Bacteria in Nasal Carriage in Australian Indigenous Children

4

5 **Running title**

6 Swab Comparison of Nasal Bacteria in Children

7

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21

22 **ABSTRACT**

23 This study compared flocked nylon swabs and non-flocked rayon swabs for the detection of
24 *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* in nasopharyngeal
25 samples from Indigenous children under the age of 6 years living in remote Australian Aboriginal
26 communities, and determined which swab the child or parent perceived to be more comfortable.
27 There was no evidence of a significant difference between flocked and rayon swabs in the recovery
28 of common respiratory bacteria; however, rayon swabs detected presence of *S. pneumoniae* (89.5%
29 *cf.* 73.7%, $p=0.375$), *H. influenzae* (79% *cf.* 73.7%, $p=1.00$) and *M. catarrhalis* (79% *cf.* 73.7%,
30 $p=1.00$) at higher rates than the flocked swabs. Analysis of semi-quantitative growth scores also
31 showed no significant differences in either the ranked distributions or medians, though rayon
32 swabs median semi-quantitative growth scores were higher for *S. pneumoniae* (4 [IQR 1-5] *cf.* 3
33 [IQR 0-6], $p=0.699$), and *H. influenzae* (2 [IQR1-5] *cf.* 1 [IQR0-5], $p=0.946$). Participants
34 generally preferred samples to be taken with flocked swabs (60%). This study demonstrates that
35 microbiological outcomes are not compromised when using flocked or rayon swabs in this
36 population; therefore cost, methodological consistency across studies, and participant preference
37 can be considered when choosing swab type.

38

39 **KEYWORDS**

40 Flocked nylon swabs; Detection; rayon swabs; *Streptococcus pneumoniae*; *Haemophilus*
41 *influenzae*; *Moraxella catarrhalis*

42

43 **INTRODUCTION**

44 Accurate assessment of nasopharyngeal (NP) bacterial carriage is essential for clinical trials
45 evaluating the impact of interventions (vaccines, antibiotics, hygiene practices) on carriage
46 outcomes. Our studies of NP carriage in Australian Indigenous children at high risk of respiratory
47 bacterial carriage and otitis media over the past two decades have used rayon swabs. Use of flocked
48 nylon swabs for the detection of respiratory viruses as well as some bacterial species, such as
49 *Escherichia coli*, *Streptococcus agalactiae* (1) and methicillin-resistant *Staphylococcus aureus*
50 (MRSA) (2) have been reported in the literature as being more effective than rayon swabs. The
51 World Health Organization working group recommends that globally standardised methods should
52 be used wherever possible; however, whenever these standards change it is necessary to measure
53 the potential impact of that change for local conditions (3). There are few data comparing flocked
54 and rayon swabs to support swab choice for isolation of respiratory bacteria. One study compared
55 flocked nylon, dacron and rayon swabs for recovery of *Streptococcus pneumoniae* from mock
56 samples and healthy children and found that flocked swabs absorbed significantly more secretions
57 and increased entrapment of NP bacteria compared with dacron and rayon swabs (4). A non-
58 randomised study of real time PCR positivity rates for *Bordetella pertussis* showed that flocked
59 swabs in universal transport medium were non-inferior to rayon swabs in Amies gel with charcoal
60 (5). Results from a 2014 study comparing different swabs to extract DNA (saliva, blood) from
61 non-biological surfaces showed rayon swabs were more effective with respect to overall DNA
62 quantity sampling and yield than flocked swabs (6). Indigenous children in the Northern Territory
63 (NT) of Australia continue to have very high rates of respiratory bacterial carriage and otitis media
64 (7). There are no published data comparing the yield of common respiratory bacteria from flocked
65 versus rayon swabs from Australian Indigenous children in a clinical setting. This study aimed to
66 determine if rayon swabs are comparable to flocked swabs in the detection of respiratory pathogens

67 in NP swabs from Australian Indigenous children. We also determined which swab the child or
68 parent preferred during the collection process.

69

70 **RESULTS**

71 A higher proportion of rayon swabs were positive for *Streptococcus pneumoniae* (Spn),
72 *Haemophilus influenzae* (Hi) and *Moraxella catarrhalis* (Mc) than flocked swabs (Table 1);
73 however, using McNemar tests for paired data, we found no significant differences in the
74 proportion of positive swabs between rayon and flocked for *S. pneumoniae* (89.5% cf. 73.7%,
75 $p=0.38$), *H. influenzae* (79% cf. 73.7%, $p=1.00$) and *M. catarrhalis* (79% cf. 73.7%, $p=1.00$). Using
76 a Test of Proportions, we determined that a sample size of 47 was sufficient to obtain a statistically
77 significant difference ($p<0.05$) in positive swabs between rayon and flocked swabs for *S.*
78 *pneumoniae*, while a sample size of 500 would be required for *H. influenzae* and *M. catarrhalis*,
79 assuming the proportions estimated in this study would not change with a larger sample.

80

81 As shown in Table 2, median semi-quantitative growth scores were higher in rayon swabs for *S.*
82 *pneumoniae* (4 [IQR 1-5] cf. 3 [IQR 0-6], $p=0.699$), and *H. influenzae* (2 [IQR1-5] cf. 1 [IQR0-5],
83 $p=0.946$), and the same for *M. catarrhalis* (3 [IQR 1-5] cf. 3 [IQR 0-4], $p=0.950$); however,
84 Wilcoxon Matched-pairs Signed-ranks Tests showed no significant differences between rayon or
85 flocked swabs for either *S. pneumoniae* ($p=0.70$), *H. influenzae* ($p=0.95$), and the same for *M.*
86 *catarrhalis* ($p=0.95$). All statistical tests were repeated for each outcome stratified by age (less
87 than 6 months ($n=10$), 6 months and older ($n=9$)), which showed no significant differences for
88 either positive swabs or semi-quantitative growth scores for the three pathogens.

89

90 Parents and children were asked about their sampling experience using the two different swabs.
91 Of the 20 sampled children, 12 (60%) preferred the flocked swab, 4 (20%) preferred the rayon
92 swab, and 4 (20%) were unsure or could not distinguish any difference between the two swabs.

93

94 **DISCUSSION**

95 In this study, presence and semi-quantitative growth scores of *S. pneumoniae* and *H. influenzae*,
96 and *M. catarrhalis* was higher for rayon swabs; however, these differences were not statistically
97 significant, indicating that flocked and rayon swabs are similarly effective in collecting and
98 preserving respiratory pathogens collected from children's noses in this population. This finding
99 should be interpreted cautiously given the small sample size (n=19). The lack of difference may
100 also reflect the high density of NP carriage common in remote Indigenous children at the time of
101 the study and as reported in earlier studies. We confirmed that when sampled at the same time, the
102 parent/carer or child themselves felt more comfortable receiving the flocked swab over the rayon
103 swab. Participants and their parents and researchers were not blinded to swab allocation and as
104 such, the rigidity and appearance of the rayon swab's shaft may have influenced their preference,
105 as would the carer's subjectivity in assessing comfort of swab type for their child. The
106 methodology of this study was acceptable to participants and can contribute to informing the
107 design and planning of future studies to verify our results. Our results suggest that cost,
108 methodological consistency across studies, and participant preference can be considered when
109 choosing swab type without compromising the microbiological outcomes in this population.

110

111 **MATERIALS AND METHODS**

112 This randomised trial compared flocked nylon swabs (Copan Italia FLOQ Technologies, Brescia,
113 Italy) on white pliable plastic shafts with non-flocked rayon bud applicators on aluminium wire
114 shafts (Copan Italia, Brescia, Italy) for the detection of *S. pneumoniae*, *H. influenzae* and *M.*
115 *catarrhalis* in NP specimens from Australian Indigenous children.

116

117 **Study Design.** Indigenous children aged 0-6 years living in remote NT Aboriginal communities
118 who were enrolled in ear health studies in 2012 were asked to contribute an additional swab for
119 comparison. The study was approved by the Human Research Ethics Committee of the Northern
120 Territory (HREC2010-1395 and 08-83) and written informed consent was obtained from
121 parents/guardians. In the absence of any published data for estimating effect size, we randomised
122 a total of 20 children for this pilot study. Stata software (version 12: Stata Corp, College Station,
123 TX) was used to generate a random sequence and allocated participants 1:1 to have the flocked
124 swab collected either before or after the rayon swab collection from each participant. The
125 randomisation sequence was stratified by age (0 to 6 months, n=10, and >6 months to 6 years,
126 n=10).

127

128 **Sample Collection.** Two swabs were collected from each child by trained research nurses. The
129 swab randomly allocated to be collected first was via the right nostril. The second swab was then
130 taken via the left nostril. Collection quality was recorded for each swab as: i) Good: swab inserted
131 at least half the measured distance from earlobe to anterior nostril and rotated, leaving the swab in
132 place for a count of five (classified as a NP swab) or, ii) Fair: swab inserted into the nose for the
133 length of the bud and rotated, leaving the swab in place for a count of five (classified as a nasal
134 swab). If swab quality was less than fair (not inserted far enough or for too short a time) for either

135 swab, another child would be selected for randomisation and the swabs collected from the first
136 child contributed only to the primary study.

137

138 Swabs were placed into tubes containing 1ml of skim milk, tryptone, glucose, glycerol broth
139 (STGGB), placed immediately into a liquid nitrogen dry shipper, transported frozen to the
140 laboratory and transferred into a -80°C freezer. After thawing, STGGB samples were vortexed to
141 disperse organisms from the swab. Ten μL aliquots were inoculated onto chocolate agar, colistin
142 nalidixic acid agar (CNA), and bacitracin vancomycin clindamycin chocolate agar (BVCCA)
143 plates and incubated at 37°C in 5% CO_2 overnight. *S. pneumoniae*, *H. influenzae* and *M.*
144 *catarrhalis* were identified using standard methods.

145

146 To assess participants' swab preference for comfort, the researcher asked the child
147 immediately after both swabs were taken, 'which swab was better, the first or the second one?' To
148 ensure the question was understood, the question would be reworded to 'which swab did you like
149 more?' If the researcher deemed the child too young to answer the question themselves, the parents
150 was asked to answer, based on their evaluation of the child's reaction to the procedure.

151

152 Twenty paired samples were collected; 10 paired samples in 0 to 6 months age group and 10 paired
153 samples in children over 6 months to 6 years old. All swabs collected had visible nasal secretions.
154 For this analysis nineteen pairs were included and one paired sample was excluded due to
155 collection error (two swabs were placed in one broth).

156

157 **Data Analysis.** Statistical analyses were performed using Stata software (version 15: Stata Corp,
158 College Station, TX). McNemar tests for paired samples were used to compare the proportion of
159 swabs culture-positive for *S. pneumoniae*, *H. influenzae* or *M. catarrhalis*. Given the small sample
160 size, we used a Test of Proportions to determine if increasing the sample size would change the
161 significance of any results. The non-parametric Wilcoxon Matched-pairs Signed-ranks Test was
162 used to assess differences in the ranked distribution (and medians) of semi-quantitative growth
163 scores for each pathogen.

164

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167 Research Council (NHMRC 545232). The funders had no role in study design, data collection and
168 interpretation, or the decision to submit the work for publication.

169

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174

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200 consecutive periods of 10-valent or 13-valent pneumococcal conjugate vaccines. *Int J*
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202

203 **Table 1. Positive culture by swab type, n=19**

	Swab type		McNemar p-value
	Rayon	Flocked	
	% (n)	% (n)	
Spn	89.5 (17)	73.7 (14)	0.375
Hi	79.0 (15)	73.7 (14)	1.000
Mc	79.0 (15)	73.7 (14)	1.000

204

205 **Table 2. Semi-quantitative scores by swab type, n=19**

	Swab type SQ Score		Wilcoxon p-value
	Rayon	Flocked	
	Median (IQR)	Median (IQR)	
Spn	4 (1-5)	3 (0-6)	0.699
Hi	2 (1-5)	1 (0-5)	0.946
Mc	3 (1-5)	3 (0-4)	0.950

206

207

208