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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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ABSTRACT

This study compared flocked (nylon) swabs and (non-flocked) rayon swabs for the detection of *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis* in nasopharyngeal samples from 20 enrolled Indigenous children under the age of 6 years living in remote Australian Aboriginal communities, and determined which swab the child or parent perceived to be more comfortable. There was no evidence of a significant difference between flocked and rayon swabs in the recovery of common respiratory bacteria. Rayon swabs detected presence of *S. pneumoniae* (90% cf. 74%, $p = 0.375$), *H. influenzae* (79% cf. 74%, $p = 1.00$) and *M. catarrhalis* (79% cf. 74%, p = 1.00) at higher rates than the flocked swabs. Analysis of semi-quantitative growth scores also showed no significant differences in either the ranked distributions or medians. Rayon swabs median semi-quantitative growth scores were higher for *S. pneumoniae* (4 [IQR 1–5] cf. 3 [IQR 0–6], $p = 0.699$), and *H. influenzae* (2 [IQR1–5] cf. 1 [IQR0–5], $p = 0.946$). Sixty percent of participants preferred samples to be taken with flocked swabs. This study demonstrates that microbiological outcomes are not compromised when using flocked or rayon swabs in respiratory bacterial carriage studies in this population. Therefore, cost, methodological consistency across studies, and participant preference can be considered when choosing swab type.

1. Introduction

Accurate assessment of nasopharyngeal (NP) bacterial carriage is essential for clinical trials evaluating the impact of interventions (vaccines, antibiotics, hygiene practices) on carriage outcomes. Our studies of NP carriage in Australian Indigenous children at high risk of respiratory bacterial carriage and otitis media over the past two decades have used non-flocked rayon swabs (henceforth term rayon swabs). Use of flocked nylon swabs for the detection of respiratory viruses as well as some bacterial species, such as *Escherichia coli*, *Streptococcus agalactiae* (Nys et al., 2010) and methicillin-resistant *Staphylococcus aureus* (MRSA), (Smismans et al., 2009) have been reported in the literature as being more effective than rayon swabs. The World Health Organization working group recommends that globally standardised methods should be used wherever possible. However, whenever these standards change it is necessary to measure the potential impact of that change for local conditions (Satzke et al., 2013). There are few data comparing flocked and rayon swabs to support swab choice for isolation of respiratory bacteria. One study compared flocked nylon, dacron and rayon swabs for recovery of *Streptococcus pneumoniae* from mock samples and healthy children and found that flocked swabs absorbed significantly more secretions and increased entrapment of NP bacteria compared with dacron and rayon swabs (Dube et al., 2013). A non-randomised study of real time PCR positivity rates for *Bordetella pertussis* showed that flocked swabs in universal transport medium were non-inferior to rayon swabs in Amies gel with charcoal (Arbefeville and Ferrieri, 2014). Results from a 2014 study comparing different swabs to extract DNA (saliva, blood) from non-biological surfaces showed rayon swabs were more effective with respect to overall DNA quantity sampling and yield than flocked swabs (Verdon et al., 2014). Indigenous children in the Northern Territory (NT) of Australia continue to have very high rates of respiratory bacterial carriage and otitis media (Leach et al., 2016). There are no published data comparing the yield of common respiratory bacteria from flocked versus rayon swabs from Australian

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

	Swab type		
	Rayon % (n)	Flocked % (n)	McNemar *p*-value
Spn	90 (17)	74 (14)	0.375
Hi	79 (15)	74 (14)	1.000
Mc	79 (15)	74 (14)	1.000

Indigenous children in a clinical setting. This study aimed to determine if rayon swabs are comparable to flocked swabs in the detection of respiratory pathogens in NP swabs from Australian Indigenous children. We also determined which swab the child or parent preferred during the collection process.

2. Results

A higher proportion of rayon swabs were positive for *Streptococcus pneumoniae* (Spn), *Haemophilus influenzae* (Hi) and *Moraxella catarrhalis* (Mc) than flocked swabs (Table 1). However, using McNemar tests for paired data, we found no significant differences in the proportion of positive swabs between rayon and flocked for *S. pneumoniae* (90% cf. 74%, $p = .38$), *H. influenzae* (79% cf. 74%, $p = 1.00$) and *M. catarrhalis* (79% cf. 74%, p = 1.00). Using a Test of Proportions, we determined that a sample size of 47 would be sufficient to obtain a statistically significant difference ($p < 0.05$) in positive swabs between rayon and flocked swabs for *S. pneumoniae*, while a sample size of 500 would be required for *H. influenzae* and *M. catarrhalis* (assuming the proportions estimated in this study do not change with a larger sample).

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

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

3. Discussion

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

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

	Swab type SQ Score		
	Rayon Median (IQR)	Flocked Median (IQR)	Wilcoxon p-value
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

4. Materials and methods

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

4.1. Study design

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

4.2. Sample collection

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

Swabs were placed into tubes containing 1 ml of skim milk, tryptone, glucose, glycerol broth (STGGB), placed immediately into a liquid nitrogen dry shipper, transported frozen to the laboratory and transferred into a − 80 °C freezer.

4.3. Bacterial culture

After thawing, STGGB samples were vortexed to disperse organisms from the swab. Ten μL aliquots were inoculated onto chocolate agar, colistin nalidixic acid agar (CNA), and bacitracin vancomycin clindamycin chocolate agar (BVCCA) plates and incubated moist at 37 °C in 5% CO_2 overnight. Semi-quantitative density of *S. pneumoniae, H.*

influenzae and *M. catarrhalis* was estimated from quadrant streaking as follows: 0, no growth; 1, < 20 bacterial colonies; 2, 20–50 bacterial colonies; 3, 50–100 bacterial colonies; 4, confluent growth in the primary zone; 5, confluent growth in the primary zone and colonies in the secondary zone; 6, confluent growth in the secondary zone and colonies in the tertiary zone; and 7, confluent growth in the tertiary zone and colonies in final streak zone. Species confirmation was performed on a dominant morphological type and one other colony of any different morphological type if present.

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

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

4.4. Data analysis

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

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References

Arbefeville, S., Ferrieri, P., 2014. Comparison of rates of positivity for Bordetella pertussis by real-time PCR between specimens collected with rayon swabs on aluminum wire shaft in Amies gel with charcoal and specimens collected with flocked swabs in universal viral transport medium during an epidemic. J. Clin. Microbiol. 52, 2656–2658.

Dube, F.S., Kaba, M., Whittaker, E., Zar, H.J., Nicol, M.P., 2013. Detection of Streptococcus pneumoniae from different types of Nasopharyngeal Swabs in children. PLoS ONE 8, e68097.

Leach, A.J., Wigger, C., Beissbarth, J., Woltring, D., Andrews, R., Chatfield, M.D., Smith-Vaughan, H., Morris, P.S., 2016. General health, otitis media, nasopharyngeal carriage and middle ear microbiology in Northern Territory Aboriginal children vaccinated during consecutive periods of 10-valent or 13-valent pneumococcal conjugate vaccines. Int. J. Pediatr. Otorhinolaryngol. 86, 224–232.

Nys, S., Vijgen, S., Magerman, K., Cartuyvels, R., 2010. Comparison of Copan eSwab with the Copan Venturi Transystem for the quantitative survival of Escherichia coli, Streptococcus agalactiae and Candida albicans. Eur. J. Clin. Microbiol. Infect. Dis. 29, 453–456.

Satzke, C., Turner, P., Virolainen-Julkunen, A., Adrian, P.V., Antonio, M., Hare, K.M., Henao-Restrepo, A.M., Leach, A.J., Klugman, K.P., Porter, B.D., Sa-Leao, R., Scott, J.A., Nohynek, H., O'Brien, K.L., Group WHOPCW, 2013. Standard method for detecting upper respiratory carriage of Streptococcus pneumoniae: updated recommendations from the World Health Organization Pneumococcal Carriage Working Group. Vaccine 32, 165–179.

Smismans, A., Verhaegen, J., Schuermans, A., Frans, J., 2009. Evaluation of the Copan ESwab transport system for the detection of methicillin-resistant Staphylococcus aureus: a laboratory and clinical study. Diagn. Microbiol. Infect. Dis. 65, 108–111.

Verdon, T.J., Mitchell, R.J., van Oorschot, R.A., 2014. Swabs as DNA collection devices for sampling different biological materials from different substrates. J. Forensic Sci. 59, 1080–1089.