

BMJ Open Evaluation of swabbing methods for estimating the prevalence of bacterial carriage in the upper respiratory tract: a cross sectional study

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ABSTRACT

Objectives: Bacterial carriage in the upper respiratory tract is usually asymptomatic but can lead to respiratory tract infection (RTI), meningitis and septicaemia. We aimed to provide a baseline measure of *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Haemophilus influenzae* and *Neisseria meningitidis* carriage within the community. Self-swabbing and healthcare professional (HCP) swabbing were compared.

Design: Cross-sectional study.

Setting: Individuals registered at 20 general practitioner practices within the Wessex Primary Care Research Network South West, UK.

Participants: 10 448 individuals were invited to participate; 5394 within a self-swabbing group and 5054 within a HCP swabbing group. Self-swabbing invitees included 2405 individuals aged 0–4 years and 3349 individuals aged ≥5 years. HCP swabbing invitees included 1908 individuals aged 0–4 years and 3146 individuals aged ≥5 years.

Results: 1574 (15.1%) individuals participated, 1260 (23.4%, 95% CI 22.3% to 24.5%) undertaking self-swabbing and 314 (6.2%, 95% CI 5.5% to 6.9%) undertaking HCP-led swabbing. Participation was lower in young children and more deprived practice locations. Swab positivity rates were 34.8% (95% CI 32.2% to 37.4%) for self-taken nose swabs (NS), 19% (95% CI 16.8% to 21.2%) for self-taken whole mouth swabs (WMS), 25.2% (95% CI 20.4% to 30%) for nasopharyngeal swabs (NPS) and 33.4% (95% CI 28.2% to 38.6%) for HCP-taken WMS. Carriage rates of *S. aureus* were highest in NS (21.3%). *S. pneumoniae* carriage was highest in NS (11%) and NPS (7.4%). *M. catarrhalis* carriage was highest in HCP-taken WMS (28.8%). *H. influenzae* and *P. aeruginosa* carriage were similar between swab types. *N. meningitidis* was not detected in any swab. Age and recent RTI affected carriage of *S. pneumoniae* and *H. influenzae*. Participant costs were lower for self-swabbing (£41.21) versus HCP swabbing (£69.66).

Conclusions: Higher participation and lower costs of self-swabbing as well as sensitivity of self-swabbing favour this method for use in large population-based respiratory carriage studies.

Strengths and limitations of this study

- This study is the largest community-based swabbing study to date to compare carriage rates of multiple bacterial species simultaneously between self-swabbing and healthcare professional swabbing methods.
- This study provides important evidence for the use of nose swabs for detection of *Streptococcus pneumoniae* and other respiratory pathogens.
- Non-response bias needs to be considered within both self-swabbing and healthcare professional swabbing groups.

INTRODUCTION

The respiratory tract is host to a wide variety of commensal and pathogenic microorganisms, with approximately 250 species colonising the nasopharynx alone.¹ Asymptomatic carriage in the upper respiratory tract (URT) is the first stage in the process of respiratory tract infection (RTI), meningitis and sepsis. Carriage often occurs without disease but may also lead to serious invasive illness.^{2–3} In 2010, approximately 4.4 million deaths worldwide resulted from an RTI, most commonly in young children.⁴

Collecting samples from the URT enables the estimation of carriage rates of pathogenic organisms. The determination of carriage rates is essential for assessing circulating respiratory microbes which may go on to cause disease. A number of sites within the URT have been used to assess carriage, including the nasopharynx, oropharynx, nose and throat. Methods for assessing carriage have included swabbing, nose blowing and nasopharyngeal aspiration.^{5–12} However, no single study has evaluated the use of different swabbing methods using a large population-based sample. *Streptococcus pneumoniae* remains the

only bacterial species for which a WHO standard method has been established for detecting carriage.¹³ It is currently recommended to take a nasopharyngeal swab despite other sites being equally as effective, if not more sensitive, in assessing carriage of this organism.^{7 10} Self-swabbing has also been shown to be effective in assessing nasal carriage of *Staphylococcus aureus* and viruses and offers a cheaper alternative to more traditional healthcare professional (HCP) swabbing.^{12 14}

Most carriage studies have focused on a particular organism and participant age group. However, many microorganisms are thought to play a role in RTI development and carriage in all age groups is important in terms of understanding disease transmission and immunity against specific pathogens.¹⁵ Moreover, in the current vaccine era, we are likely to see an explosion of new vaccines during the coming decade that will affect the respiratory tract microbiota.^{16–20} This highlights the need for large population-based studies that include all age groups and aim to detect as many relevant microbial species as possible.

Our study aimed to provide a baseline measure for understanding multispecies bacterial carriage in the respiratory tract within the general population of one geographical area of the UK. The objectives were to assess the optimal sample collection method and site by comparing self-taken nose and mouth swabs with HCP-taken nasopharyngeal and mouth swabs; to gain an estimate of participant consent rates in both study groups and to test the feasibility of conducting a larger multisite investigation. Finally, the study aimed to estimate carriage rates of relevant URT bacterial species. This would help inform sample sizes for multicentre studies, particularly for use in prevaccine and postvaccine studies, as well as to aid in understanding the effects of demographic factors and deprivation on carriage.

METHODS

Sample size

This was a pilot study and not designed to have the power to detect non-inferiority of estimating carriage rates by HCP-administered versus self-administered swabs. Data from this study was predicted to inform sample sizes required for future large carriage studies. The sample size for this pilot study was based on the precision with which we can estimate true carriage rates. A 25% response rate among self-swabbing participants was assumed based on results from a previous staphylococcal carriage study.¹² A 25% response rate was also assumed for HCP-swabbing.

We estimated that by inviting 2020 children (101 from each general practitioner (GP) practice) aged 0–4 years and 3200 older children and adults (160 from each GP practice) to participate within each swabbing group, this would result in 505 children and 800 older children and adult responders within each swabbing group,

accounting for predicted lower carriage rates in older children and adults. A predicted carriage rate of 30% in 505 participating children would enable the determination of true carriage to within $\pm 4\%$ (95% confidence).²¹ A predicted carriage rate of 20% in 800 participating older children and adults would enable the determination of true carriage to within $\pm 2.8\%$ (95% confidence).⁹

Participant recruitment

Participants were selected from 20 GP practices within the Wessex Primary Care Research Network (PCRN) South West (East hub) area, in Southern England. GP practices were chosen to reflect a mix of urban/rural locations, practice sizes and area deprivation levels. Each GP practice produced a list of their entire patient cohort. Any individual deemed unfit for participation at the discretion of their GP, for example due to terminal illness or serious mental health problems, was removed from the list. From each GP list, 202 individuals aged 0–4 years and 320 individuals aged ≥ 5 years were randomly selected and allocated to one of two study groups using the *ralloc* command in Stata V.12. This resulted in approximately 101 individuals aged 0–4 years and 160 individuals aged ≥ 5 years within each swabbing group per GP practice.

The HCP group involved participants being invited, via letter, to organise a swabbing appointment at their GP practice where nasopharyngeal (NPS) and whole mouth (WMS) swabs were taken by a registered HCP. Appointments were within normal surgery opening hours and at the individuals' GP practice (local to each participant). The self-swabbing group involved participants being sent a self-swabbing pack containing nose (NS) and WMS swabs by Danvers International (London, UK). Participants were not sent reminders. All swab heads were viscose (rayon). Nose and both WMS shafts were polystyrene whereas NP swab shafts were aluminium. Once taken, swabs were placed in polypropylene tubes containing amies transport medium with charcoal. HCP-taken swabs were returned for analysis on the day of swabbing by taxi or within 1–2 days by pre-existing National Health Service (NHS) delivery service. Self-taken swabs were returned by first-class freepost return (1–2 days).

Each participant was given an age-appropriate information sheet explaining the study aims, which aimed to motivate individuals to participate. Participants were asked to complete a consent form and questionnaire, provided either at their swabbing appointment or within their self-swabbing pack. The study questionnaire was identical for both study groups and requested the following details pertinent to bacterial carriage: participant age, recent use of antibiotics (within the past month), recent RTI (cold, flu, ear infection or chest infection within the past month) and vaccination status. Age was split into the following groups for analysis: 0–4, 5–17, 18–64 and 65 years and older due to the relevance of

each of these age groups in carriage of the different bacterial species. Recent use of antibiotics and recent RTI were split into the following groups for analysis: yes, no and do not know/missing. Vaccination status was split into the following groups for analysis: up-to-date, not up-to-date and do not know/missing. UK Index of Multiple Deprivation (IMD) 2010 scores were obtained for each GP practice based on the lower layer super output area (LSOA) it was located in and was used as a proxy for deprivation of each practices' patient population.²² UK IMD 2010 Score includes seven features of deprivation: income, education, employment, health, housing, crime and living environment. More deprived areas have lower levels of these seven features whereas less deprived areas have higher levels for the same seven features. This would enable the relationship between carriage and deprivation to be assessed, as in disease studies.²³ A total of 10 448 individuals were invited to participate in the study.

Sample collection and analysis

Self-swabbing packs were sent out to individuals between the 15 May and 23 July 2012 and samples were received between the 18 May and 31 August 2012. HCP swabbing appointments took place between 7 June and 28 August and samples were received between the 7 June and 31 August. On receipt, swabs were immersed in skim milk, tryptone, glucose and glycerine (STGG) storage media, vigorously rubbed against the side of the tube and vortexed to ensure transfer of bacteria into the STGG. Standard microbiology culture and identification techniques were used to analyse the swab contents for the presence of *S. pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *S. aureus*, *Pseudomonas aeruginosa* and *Neisseria meningitidis*. This was performed by transferring 10 µL STGG onto Columbia blood agar with horse blood (Oxoid, PB0124), Columbia blood agar with colistin and nalidixic acid (Oxoid, PB0308), Columbia blood agar with chocolate horse blood (Oxoid, PB0124), Columbia blood agar with chocolate horse blood and bacitracin (Oxoid, PB0220), *Pseudomonas* selective agar (Oxoid, PB0291) and lysed GC selective agar (Oxoid, PB0962). Identification of each bacterial species was undertaken according to methodology described in online supplementary table S1. After plating, the remaining swab content in STGG was then frozen for future use at -70°C .

Statistical analysis

Culture data and participant questionnaire information were tabulated into SPSS (V.20) for analysis. Missing or incomplete data was classed as missing within the SPSS variables window. Participation rates, the proportion of participants relative to total number of individuals invited, were calculated for each GP practice and age group. UK IMD 2010 scores for each GP practice area were examined in relation to participation rates using Pearson's Correlation. Swab positivity rates, the

proportion of swabs that isolated any of the target bacteria relative to total swab numbers, were calculated for each swab type. 95% CIs were calculated to assess reliability of participation and positivity rates.

Carriage rates, the proportion of a specific bacterial species relative to total number of swabs, were calculated according to swab type, age, recent RTI, recent antibiotic use, vaccination status, geographical location and deprivation. χ^2 and Fisher's Exact tests were used to determine any associations between carriage and these variables. Geographical mapping of carriage rates was performed using ArcGIS (ESRI, V.10.1).²⁴ Practices were grouped into geographical areas for statistical analysis based on proximity to one another. Finally, co-carriage rates, the proportion of samples containing multiple bacterial species relative to total number of swabs, were calculated according to swab type, age, recent RTI, recent antibiotic use, vaccination status and geographical location.

Study costs

Total costs associated with each swabbing method were calculated to allow cost comparisons between methods. Costs were calculated as total costs within a single swabbing group divided by the total number of responders from that swabbing group. This included swab packs sent out to individuals but not used. Costs were separated into laboratory consumables, printing, swabs, NHS Service Support Costs (additional healthcare costs due to the research taking place), transport and postage.

RESULTS

Participation rates

Eighteen of the 20 GP practices participated in both self-swabbing and HCP-swabbing, one participated in self-swabbing only and one dropped out of the study. Participant characteristics are shown in table 1. Overall participation rates were higher in the self-swabbing group at 23.4% (n=1260; N=5395; 95% CI 22.3% to 24.5%) compared with the HCP group at 6.2% (n=314; N=5054; 95% CI 5.5% to 6.9%). Self-swabbing participation rates varied from 9.3% (n=27; N=290) to 33.1% (n=96; N=290) between practices whereas HCP participation rates varied from 1% (n=3; N=290) to 12.3% (n=34; N=277). Ten practices had participation rates $\geq 25\%$ in the self-swabbing group, which was the anticipated level of participation. There was a negative correlation between participation rate and IMD score in the self-swabbing group ($r=-0.473$, $p=0.041$) and the HCP group ($r=-0.417$, $p=0.085$), which was only significant in the former. Participation was higher in individuals aged ≥ 5 years at 27.8% (n=931; N=3349; 95% CI 26.8% to 29.3%) in the self-swabbing group and 8.2% (n=258; N=3146; 95% CI 7.2% to 9.2%) in the HCP group versus 0–4 years at 16.1% (n=329; N=2045; 95% CI 14.5% to 17.7%) in the self-swabbing group and 2.9% (n=56; N=1908; 95% CI 2.2% to 3.7%) in the HCP group. The

Table 1 Participant characteristics and study costs (in British Pounds) for self-swabbing and HCP swabbing

	Participant characteristics n(%) and costs per participant (£)	
	Self-swabbing	HCP swabbing
Age (years)		
Mean	37.42	50.09
Minimum	0	0
Maximum	94	88
0–4	329 (26.1)	56 (17.8)
5–17	137 (10.9)	24 (7.6)
18–64	465 (36.9)	89 (28.3)
65+	311 (24.7)	145 (46.2)
Missing	18 (1.4)	0 (0.0)
Recent antibiotic treatment		
Yes	101 (8.0)	26 (8.3)
No	1124 (89.2)	286 (91.1)
Unknown/missing	35 (2.8)	2 (0.6)
Recent respiratory infection		
Yes	365 (29.0)	61 (19.4)
No	860 (68.3)	250 (79.6)
Unknown/missing	35 (2.8)	3 (1.0)
Vaccination status		
Up-to-date	1022 (81.1)	270 (86.0)
Not up-to-date	40 (3.2)	10 (3.2)
Unknown/missing	198 (15.7)	34 (10.8)
Costs per participant (£)		
Laboratory consumables	8.06	8.47
Printing	2.14	7.23
Swabs and swab packs	17.08	9.65
Service support costs (SSC)	2.81	39.52
Transport (by taxi or internal mail)	0.00	4.78
Postage	11.12	0.00
Total	41.21	69.66

Costs (British Pounds) are per participant taking into account wastage of swabs and swab packs.
HCP, healthcare professional.

greatest number of responses received was from individuals aged 50–80 years, comprising 41.7% (n=656, N=1574) of total participants.

Swab positivity rates

Out of 1260 self-swabbing participants, 1254 returned both swabs with labels distinguishing nose from WMS but six individuals failed to label their swabs and thus were excluded from analyses. Out of 314 HCP swabbing participants, 309 had both swabs returned by their GP but five individuals were incorrectly swabbed by their GP and thus were excluded from analyses. Swab positivity rates were 35% (n=439; N=1254; 95% CI 32.4% to 37.6%) for NS, 19.1% (n=239; N=1254; 95% CI 16.9% to 21.3%) for self-taken WMS, 25.6% (n=79; N=309; 95% CI 20.7% to 30.5%) for NPS and 34% (n=105; N=309; 95% CI 28.7% to 39.3%) for HCP-taken WMS (see online supplementary figure S1). The NS and

HCP-taken WMS were most effective in detecting carriage of the target organisms. Positivity rates of NS were significantly higher than NPS ($\chi^2=9.974$, df=1, $p=0.002$). Positivity rates of HCP-taken WMS were significantly higher than self-taken WMS ($\chi^2=32.157$, df=1, $p<0.001$).

Bacterial carriage rates

Carriage rates within each swab type (figure 1) show few significant differences between self-swabbing and HCP swabbing. *S. pneumoniae* carriage was similar between NS and NPS ($\chi^2=3.403$, df=1, $p=0.075$) and between self-taken and HCP-taken WMS (test value=0.139, df=1, $p=0.661$). *M. catarrhalis* carriage was similar between NS and NPS ($\chi^2=3.757$, df=1, $p=0.058$) but significantly higher in HCP-taken WMS compared to self-taken WMS ($\chi^2=43.404$, df=1, $p<0.001$). *S. aureus* carriage was significantly higher in NS than NPS ($\chi^2=13.161$, df=1, $p<0.001$) but was similarly low in self-taken and HCP-taken WMS ($\chi^2=1.218$, df=1, $p=0.315$). *H. influenzae* carriage was similarly low in NS and NPS ($\chi^2=0.193$, df=1, $p=0.700$) as well as in self-taken and HCP-taken WMS (test value=2.888, df=1, $p=0.151$). *P. aeruginosa* carriage was similar in NS and NPS (test value=0.148, df=1, $p=1.000$) as well as in self-taken and HCP-taken WMS ($\chi^2=0.032$, df=1, $p=1.000$). *N. meningitidis* was not detected in any swab type used in this study.

Cocarrriage rates

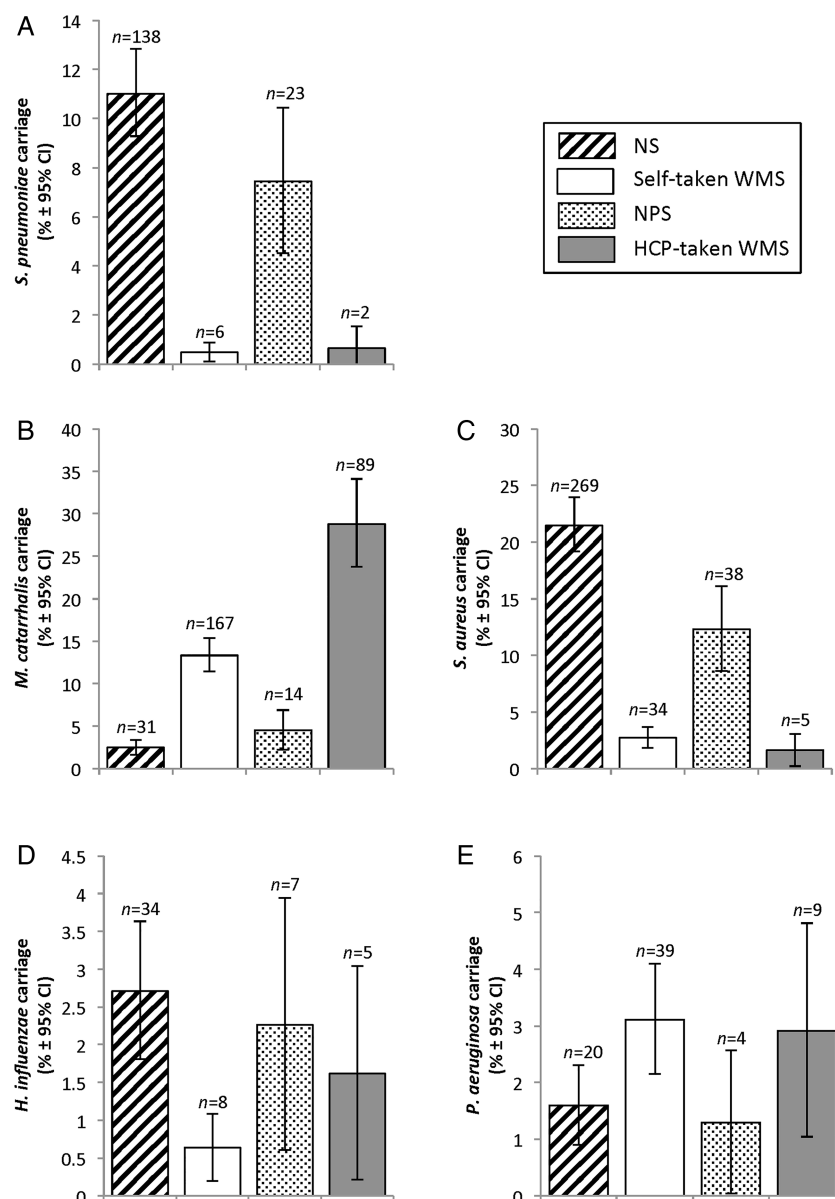
Overall cocarrriage rates were 3.9% (n=49; N=1219; 95% CI 2.8% to 5%) in NS, 1.1% (n=13; N=1219; 95% CI 0.5% to 1.7%) in self-taken WMS, 2.3% (n=7; N=307; 95% CI 0.6% to 4%) in NPS and 1.6% (n=5; N=307; 95% CI 0.2% to 3%) in HCP-taken WMS. In NS and NPS, cocarrriage rates were significantly higher in individuals aged 0–4 years (NS (9.1%; n=30; N=329; 95% CI 6% to 12.2%) and NPS (8.9%; n=5; N=56; 95% CI 1.4% to 16.4%)) versus ≥ 5 years (NS (2.1%; n=19; N=907; 95% CI 1.2% to 3%) and NPS (1.8%; n=2; N=253; 95% CI 0.2% to 3.4%)). Nose cocarrriage decreased with age, with 8% (n=11; N=137; 95% CI 3.5% to 12.5%) in individuals aged 5–17 years, 1.1% (n=5; N=464; 95% CI 0.2% to 2.1%) in individuals aged 18–64 years and 1% (n=3; N=306; 95% CI –0.1% to 2.1%) in those aged ≥ 65 years. The most common cocarrriage relationship in nose swabs was between *S. pneumoniae* and *H. influenzae* (50% (n=15; N=30) in 0–4 years, 26.3% (n=5, N=19) in ≥ 5 years).

Association between demographics and carriage

Participant age

Bacterial carriage was highly variable with age, in particular carriage of *S. pneumoniae*, *H. influenzae*, *M. catarrhalis* and *S. aureus* (tables 2 and 3). Carriage rates of *S. pneumoniae* and *H. influenzae* in both NS and NPS decreased with age, with 0–4-year-olds experiencing the highest carriage rates. *S. pneumoniae* carriage dropped off significantly after 5 years of age with $>2\times$ difference in NS and $>3\times$ difference in NPS between those aged 0–4 years and those aged 5–17 years. *S. pneumoniae* carriage in self-taken WMS

Figure 1 Bacterial Carriage Rates (%) of (A) *Streptococcus pneumoniae* (B) *Moraxella catarrhalis* (C) *Staphylococcus aureus* (D) *Haemophilus influenzae* (E) *Pseudomonas aeruginosa* by Swab Method and Site. Graphs are bar charts representing carriage frequencies as percentages. Error bars represent 95% CIs.



also showed higher carriage in the young (0–4 years and 5–17 years age groups) compared with adults. *H. influenzae* nasal carriage decreased more steadily with age. *M. catarrhalis* nose carriage was also highest in those aged 0–4 years but remained at lower levels in the other age groups. *S. aureus* nose carriage increased sharply after the age of 5 years but remained high in older children and adults. *S. aureus* nose carriage was >3× higher in participants aged 5–17 years when compared with participants 0–4 years. *P. aeruginosa* did not vary between the age groups in any swab type.

Participant questionnaire information

Higher nasal and NP carriage rates of *S. pneumoniae* and *H. influenzae* were observed in participants who had experienced a recent RTI. *S. pneumoniae* nose carriage was >3× higher in those with recent RTI versus those without recent RTI, using χ^2 ($\chi^2=66.408$, $df=1$, $p<0.001$).

H. influenzae nose carriage was also >2× higher in those with recent RTI versus those without recent RTI, using the χ^2 test ($\chi^2=12.533$, $df=1$, $p=0.001$). Recent antibiotic treatment was only significant in *P. aeruginosa* NP carriage, where recent antibiotics use was associated with increased carriage of this bacterium (test value=9.018, $df=1$, $p=0.037$). Vaccination status was not associated with significant changes in carriage of any of the target bacteria. Full results are shown in tables 2 and 3. In NS, recent RTI was also associated with higher co-carriage rates at 8% ($n=29$) when compared with no recent RTI at 2.2% ($n=19$). Recent antibiotic use, vaccination status and geographical location did not appear to affect co-carriage rates.

Geographical location

Carriage rates of the target bacterial species showed some differences according to practice location (see

Table 2 Bacterial nose and nasopharyngeal carriage rates of *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, *Haemophilus influenzae* and *Pseudomonas aeruginosa* by participant age group, recent RTI, recent antibiotic treatment and vaccination status

Category	Participants (N)		Carriage of bacterial species within nose and nasopharyngeal swabs in different participant categories % (n) (95% CI)									
	SS	HCP	<i>S. pneumoniae</i>		<i>H. influenzae</i>		<i>M. catarrhalis</i>		<i>S. aureus</i>		<i>P. aeruginosa</i>	
			Nose	NP	Nose	NP	Nose	NP	Nose	NP	Nose	NP
Age (years)												
0–4	329	56	32.8 (108) (27.7 to 37.9)	33.9 (19) (21.5 to 46.3)	7.3 (24) (4.5 to 10.1)	10.7 (6) (2.6 to 18.8)	5.8 (19) (3.3 to 8.3)	10.7 (6) (2.6 to 18.8)	9.7 (32) (6.5 to 12.9)	5.4 (3) (–0.5 to 11.3)	2.7 (9) (1.0 to 4.5)	1.8 (1) (–1.7 to 5.3)
5–17	137	22	13.1 (18) (7.5 to 18.8)	9.1 (2) (–2.9 to 21.1)	5.1 (7) (1.4 to 8.8)	0.0 (0) NA	0.7 (1) (–0.7 to 2.1)	4.5 (1) (–4.1 to 13.2)	35.0 (48) (27.0 to 43.0)	13.6 (3) (–0.7 to 27.9)	0.7 (1) (–0.7 to 2.1)	0.0 (0) NA
18–64	464	88	1.1 (5) (0.2 to 2.1)	0.0 (0) NA	0.2 (1) (–0.2 to 0.6)	1.1 (1) (–1.1 to 3.3)	1.5 (7) (0.4 to 2.6)	3.4 (3) (–0.4 to 7.2)	24.8 (115) (20.9 to 28.7)	11.4 (10) (4.8 to 18.0)	1.3 (6) (0.3 to 2.3)	1.1 (1) (–1.1 to 3.3)
65+	306	143	2.0 (6) (0.4 to 3.6)	1.4 (2) (–0.5 to 3.3)	0.7 (2) (–0.2 to 1.6)	0.0 (0) NA	1.3 (4) (0.0 to 2.6)	2.8 (4) (0.1 to 5.5)	23.2 (71) (18.5 to 27.9)	15.4 (22) (9.5 to 21.3)	1.0 (3) (–0.1 to 2.1)	1.4 (2) (–0.5 to 3.3)
p Value			<0.001*	<0.001	<0.001	<0.001	0.001	0.100	<0.001*	0.263	0.288	1.000
Recent respiratory tract infection												
Yes	363	59	22.3 (81) (18.0 to 26.6)	15.3 (9) (6.1 to 24.5)	5.2 (19) (2.9 to 7.5)	6.8 (4) (0.4 to 13.2)	3.6 (13) (1.7 to 5.5)	3.4 (2) (–1.2 to 8.0)	19.3 (70) (15.2 to 23.4)	6.8 (4) (0.4 to 13.2)	2.2 (8) (0.7 to 3.7)	3.4 (2) (–1.2 to 8.0)
No	856	247	6.3 (54) (4.7 to 7.9)	5.7 (14) (2.8 to 8.6)	1.6 (14) (0.8 to 2.4)	1.2 (3) (–0.2 to 2.6)	2.1 (18) (1.1 to 3.1)	4.9 (12) (2.2 to 7.6)	22.3 (191) (19.5 to 25.1)	13.8 (34) (9.5 to 18.1)	1.3 (11) (0.5 to 2.1)	0.8 (2) (–0.3 to 1.9)
p Value			<0.001*	0.023	0.001*	0.028	0.163*	1.000	0.253*	0.188*	0.310*	0.169
Recent use of antibiotics												
Yes	101	26	5.9 (6) (1.3 to 10.5)	3.8 (1) (–3.6 to 11.2)	1.0 (1) (–0.9 to 2.9)	0.0 (0) NA	1.0 (1) (–0.9 to 2.9)	3.8 (1) (–3.6 to 11.2)	15.8 (16) (8.7 to 22.9)	0.0 (0) NA	1.0 (1) (–0.9 to 2.9)	7.7 (2) (–2.6 to 18.0)
No	1118	281	11.5 (129) (9.6 to 13.4)	7.8 (22) (4.7 to 10.9)	2.9 (32) (1.9 to 3.9)	2.5 (7) (0.7 to 4.3)	2.7 (30) (1.8 to 3.7)	4.6 (13) (2.2 to 7.1)	21.7 (243) (19.3 to 24.1)	13.5 (38) (9.5 to 17.5)	1.5 (17) (0.8 to 2.2)	0.7 (2) (–0.3 to 1.7)
p Value			0.097*	0.706	0.515	1.000	0.508	1.000	0.203*	0.056	1.000	0.037
Vaccinations up-to-date												
Yes	1017	265	12.8 (130) (10.8 to 14.9)	8.7 (23) (5.3 to 12.1)	3.0 (31) (2.0 to 4.1)	2.6 (7) (0.7 to 4.5)	2.8 (28) (1.8 to 3.8)	4.5 (12) (2.0 to 7.0)	20.4 (207) (17.9 to 22.9)	13.2 (35) (9.1 to 17.3)	1.6 (16) (0.8 to 2.4)	1.5 (4) (0.0 to 3.0)
No	40	10	5.0 (2) (–1.8 to 11.8)	0.0 (0) NA	2.5 (1) (–2.3 to 7.3)	0.0 (0) NA	2.5 (1) (–2.3 to 7.3)	10.0 (1) (–8.6 to 28.6)	25.0 (10) (11.6 to 38.4)	0.0 (0) NA	2.5 (1) (–2.3 to 7.3)	0.0 (0) NA
p Value			0.219	1.000	1.000	1.000	1.000	0.389	0.548*	0.621	0.484	1.000

χ^2 (indicated by *) and Fisher's exact tests for independence were used to determine significant differences between bacterial carriage rates in different age groups, with/without recent RTI, with/without recent antibiotic treatment and with/without an up-to-date vaccination status. p Values are 2-tailed. 95% CI are written as (upper CI to lower CI).

NP, nasopharyngeal swab; NA, not applicable.

Table 3 Bacterial self-taken and HCP-taken whole mouth swab carriage rates of *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, *Haemophilus influenzae* and *Pseudomonas aeruginosa* by participant age group, recent RTI, recent antibiotic treatment and vaccination status

Category	Participants (N)		Carriage of bacterial species within mouth swabs in different participant categories % (n) (95% CI)									
	SS	HCP	<i>S. pneumoniae</i>		<i>H. influenzae</i>		<i>M. catarrhalis</i>		<i>S. aureus</i>		<i>P. aeruginosa</i>	
			Self-taken WMS	HCP-taken WMS	Self-taken WMS	HCP-taken WMS	Self-taken WMS	HCP-taken WMS	Self-taken WMS	HCP-taken WMS	Self-taken WMS	HCP-taken WMS
Age (years)												
0–4	329	56	1.2 (4) (0.0 to 2.4)	3.6 (2) (–1.3 to 8.5)	1.2 (4) (0.0 to 2.4)	5.4 (3) (–0.5 to 11.3)	11.9 (39) (8.4 to 15.4)	35.7 (20) (23.2 to 48.3)	2.4 (8) (0.8 to 4.1)	0.0 (0) NA	4.9 (16) (2.6 to 7.2)	3.6 (2) (–1.3 to 8.5)
5–17	137	22	1.5 (2) (–0.5 to 3.5)	0.0 (0) NA	0.0 (0) NA	0.0 (0) NA	11.7 (16) (6.3 to 17.1)	27.3 (6) (8.7 to 45.9)	4.4 (6) (1.0 to 7.8)	4.5 (1) (–4.2 to 13.2)	3.6 (5) (0.5 to 6.7)	0.0 (0) NA
18–64	464	88	0.0 (0) NA	0.0 (0) NA	0.9 (4) (0.0 to 1.8)	1.1 (1) (–1.1 to 3.3)	15.3 (71) (12.0 to 18.6)	22.7 (20) (14.0 to 31.5)	3.0 (14) (1.5 to 4.6)	2.3 (2) (–0.8 to 5.4)	1.7 (8) (0.5 to 2.9)	2.3 (2) (–0.8 to 5.4)
65+	306	143	0.0 (0) NA	0.0 (0) NA	0.0 (0) NA	0.7 (1) (–0.7 to 2.1)	13.1 (40) (9.3 to 16.9)	30.1 (43) (22.6 to 37.6)	1.6 (5) (0.2 to 3.0)	1.4 (2) (–0.5 to 3.3)	2.9 (9) (1.0 to 4.8)	3.5 (5) (0.5 to 6.5)
p Value			0.006	0.063	0.204	0.159	0.476*	0.390*	0.361	0.377	0.079	0.910
Recent respiratory tract infection												
Yes	363	59	0.8 (3) (–0.1 to 1.7)	1.7 (1) (–1.6 to 5.0)	0.8 (3) (–0.1 to 1.7)	1.7 (1) (–1.6 to 5.0)	10.5 (38) (7.4 to 13.7)	25.4 (15) (14.3 to 36.5)	2.5 (9) (0.9 to 4.1)	0.0 (0) NA	3.9 (14) (1.9 to 5.9)	5.1 (3) (–0.5 to 10.7)
No	856	247	0.4 (3) (0.0–0.8)	0.4 (1) (–0.4 to 1.2)	0.6 (5) (0.1 to 1.1)	1.6 (4) (0.0 to 3.2)	14.7 (126) (12.3 to 17.1)	29.6 (73) (23.9 to 35.3)	2.7 (23) (1.6 to 3.8)	1.6 (4) (0.0 to 3.2)	2.8 (24) (1.7 to 3.9)	2.4 (6) (0.5 to 4.3)
p Value			0.370	0.349	0.701	1.000	0.054*	0.632*	0.850*	1.000	0.368*	0.382
Recent use of antibiotics												
Yes	101	26	0.0 (0) NA	0.0 (0) NA	0.0 (0) NA	0.0 (0) NA	14.9 (15) (8.0 to 21.8)	23.1 (6) (6.9 to 39.3)	3.0 (3) (–0.3 to 6.3)	3.8 (1) (–3.6 to 11.2)	2.0 (2) (–0.7 to 4.7)	3.8 (1) (–3.6 to 11.2)
No	1118	281	0.5 (6) (0.1 to 0.9)	0.7 (2) (–0.3 to 1.7)	0.7 (8) (0.2 to 1.2)	1.8 (5) (0.3 to 3.4)	13.4 (150) (11.4 to 15.4)	29.2 (82) (23.9 to 34.5)	2.6 (29) (1.7 to 3.5)	1.4 (4) (0.0 to 2.8)	3.2 (36) (2.2 to 4.2)	2.8 (8) (0.9 to 4.7)
p Value			1.000	1.000	1.000	1.000	0.761*	0.652*	0.744	0.360	0.764	0.554
Vaccinations up-to-date												
Yes	1017	265	0.6 (6) (0.1 to 1.1)	0.8 (2) (–0.3 to 1.9)	0.6 (6) (0.1 to 1.1)	1.9 (5) (0.3 to 3.5)	13.7 (139) (11.6 to 15.8)	29.4 (78) (23.9 to 34.9)	2.8 (28) (1.8 to 3.8)	1.5 (4) (0.0 to 3.0)	3.2 (33) (2.1 to 4.3)	3.0 (8) (1.0 to 5.1)
No	40	10	0.0 (0) NA	0.0 (0) NA	2.5 (1) (–2.3 to 7.3)	0.0 (0) NA	5.0 (2) (–1.8 to 11.8)	50.0 (5) (19.0 to 81.0)	2.5 (1) (–2.3 to 7.3)	0.0 (0) NA	2.5 (1) (–2.3 to 7.3)	0.0 (0) NA
p Value			1.000	1.000	0.237	1.000	0.153*	0.175	1.000	1.000	1.000	1.000

χ^2 (indicated by *) and Fisher's exact tests for independence were used to determine significant differences between bacterial carriage rates in different age groups, with/without recent RTI, with/without recent antibiotic treatment and with/without an up-to-date vaccination status. p Values are 2-tailed. 95% CI are written as (upper CI to lower CI). NA, not applicable; WMS, whole mouth swab.

online supplementary figure S2). Overall bacterial carriage was significantly different by geographical area in NS ($\chi^2=11.609$, $df=5$, $p=0.04$) and self-taken WMS ($\chi^2=13.900$, $df=5$, $p=0.02$) but not in either HCP swab. However, individual bacteria carriage rates were not significantly different between geographical areas.

Deprivation

Participants attending practices in less deprived locations had slightly higher bacterial carriage rates, except for *P. aeruginosa*, suggesting a possible negative relationship between deprivation score and bacterial carriage. However, the differences observed were not statistically significant.

Study costs

Overall, total costs per participant were over a third lower in the self-swabbing group at £41.21 (\$67.92) versus the HCP group at £69.66 (\$114.82; table 1). NHS service support costs made up a large proportion of the difference between the two study groups, representing 56.7% (£39.52/person) of costs in the HCP group but only 6.8% (£2.81/person) of costs in the self-swabbing group.

DISCUSSION

Our study demonstrates that self-swabbing is as effective in detecting bacterial pathogens in the respiratory tract as HCP swabbing and that nose swabs could be used more routinely to detect the presence of bacterial pathogens *S. pneumoniae*, *H. influenzae*, *S. aureus* and *P. aeruginosa*. WMS, on the other hand, are the most sensitive swab for detection of *M. catarrhalis*. The swabs used in this study were not sensitive for detection of *N. meningitidis*.

Higher participation rates within the self-swabbing group compared with the HCP group highlight the willingness of individuals to participate in such studies when the process is facilitated. The very low participation rate of the HCP group would render this method invalid for large-scale studies. While the responsiveness of the self-swabbing group was higher, it was still less than the anticipated 25%, meaning there will always be a problem of non-response bias. However, similar carriage rates were observed in our study when compared with previous swabbing studies, demonstrating that our sample size is large enough to overcome differences that may result from non-response bias. Barriers to participation in the HCP group might include the amount of time required for organising and attending swabbing appointments and the slight discomfort experienced during nasopharyngeal swabbing. Self-swabbing overcame many of these barriers by offering a relatively straightforward, rapid and easy alternative. High participation rates in elderly participants might be a result of their increased availability for participation and their increased chance of exposure to RTI allowing them to

relate to the study aims. Parents may also be reluctant to swab their children if they are very young. The negative correlation between participation rates and deprivation highlights certain barriers associated with high levels of deprivation, which have been observed in other studies.²⁵

Swab positivity rates and bacterial carriage rates indicate that self-swabbing is as effective as HCP swabbing in sampling microbial species within the airways of the general population within our large population-based study. Higher positivity rates in NS versus NPS and higher carriage of *S. aureus* within NS versus NPS demonstrate the potential for using a self-taken NS rather than HCP-taken NPS to detect respiratory pathogens. Higher positivity rates in HCP-taken WMS versus self-taken WMS and higher carriage of *M. catarrhalis* within HCP-taken WMS demonstrate the sensitivity of HCP-swabbing. However, lower participation rates with fewer children and more elderly participants within HCP swabbing have most probably resulted in reduced carriage rates within NPS. Self-swabbing allowed the recruitment of a greater spread of age groups, which is essential for obtaining a true estimate of carriage. Very low participation in the HCP group is problematic for assessing carriage within the general population as fewer numbers of samples can be obtained and the cost of obtaining them is high. In order to obtain the same spread of ages as the self-swabbing group, a much larger number of individuals would need to be invited. The high costs of HCP swabbing are mainly due to the operation of swabbing clinics. In order to increase participation, healthcare providers could undertake verbal encouragement or study advertisement in practice. WMS were efficient in isolating *M. catarrhalis* and *P. aeruginosa*, however, large amounts of background flora within this site and low isolation levels for the other bacteria render this swab less efficient on the whole. The lack of isolation of *N. meningitidis* may be due to the type of swabs used, as oropharyngeal swabs are often preferred.²⁶ Low response rates from teenagers, the most frequent carriers of *N. meningitidis*, may also have caused the lack of isolation of this species.²⁷

Carriage rates of five out of the six target organisms follow previously observed patterns with *S. pneumoniae* and *H. influenzae* being carried predominantly in young children and *S. aureus* being carried more in older children and adults.^{12 28 29} *M. catarrhalis* and *P. aeruginosa* carriage rates were constant across all age groups demonstrating that carriage of these organisms is unaffected by age. *N. meningitidis* carriage did not follow previously observed patterns as no isolates were detected. However, the number of participants in the study may not have been large enough to detect any isolates with 95% confidence. Furthermore, swab types used and turn-around times from swabbing to sample processing may not be optimal for *N. meningitidis* recovery. The effect of recent RTI on carriage of *S. pneumoniae* and *H. influenzae* is one that might be expected as colds and flu weaken host

immunity allowing for carriage by these organisms.³⁰ The lack of an apparent effect of vaccination status is potentially due to herd immunity, as unvaccinated people benefit from protection from disease as a result of a largely vaccinated population.³¹ However, further details of vaccines received via access to individual participant immunisation records in future studies might enable improved assessment of the effects of immunisation on carriage of target and non-target bacteria.

This pilot study has also enabled all aspects of study set-up through to completion to be tried and tested, which will be essential for setting up larger swabbing studies. Study documentation, study protocol, ethics application and sample size calculations have been trialled and alterations can now be performed on further studies in order to improve outcomes and efficiency. Limitations, including numbers of non-responses, can be improved in further studies in order to increase confidence in study outcomes. The results from this pilot study have allowed the comparison of swabbing methodologies for determining carriage of the targeted bacterial species within the respiratory tract. The advantages of self-swabbing are evident with higher responsiveness and lower costs than HCP swabbing. Further assessment will determine whether our findings are applicable to other geographical locations, over time and to a wider array of bacterial species. Such assessment would help to refine methodologies, which will be key to obtaining a precise understanding of bacterial carriage in the respiratory tract.

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Contributors ALC was involved in study set-up, data collection, data analysis and writing. RNW was involved in study set-up, data collection and proofreading of manuscript. NB and RA were involved in data collection, proof-reading of manuscript. AT was involved in study design, data collection and proof-reading of manuscript. SNF, JMJ, HMY, PJR, MAM and MVM were involved in study design, data analysis, proofreading of manuscript. SCC was involved in study design, data collection, data analysis and proof-reading of manuscript.

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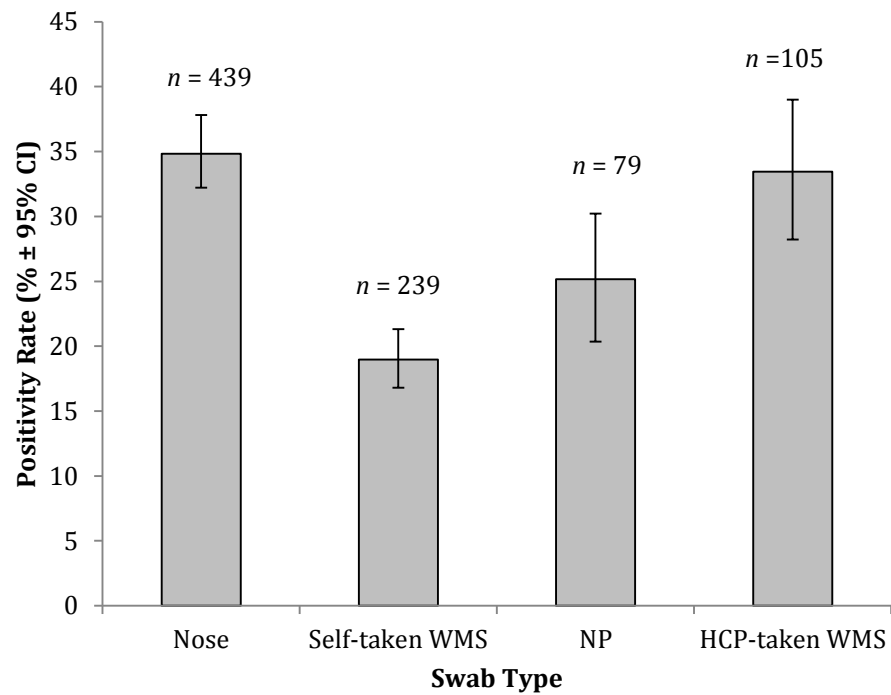
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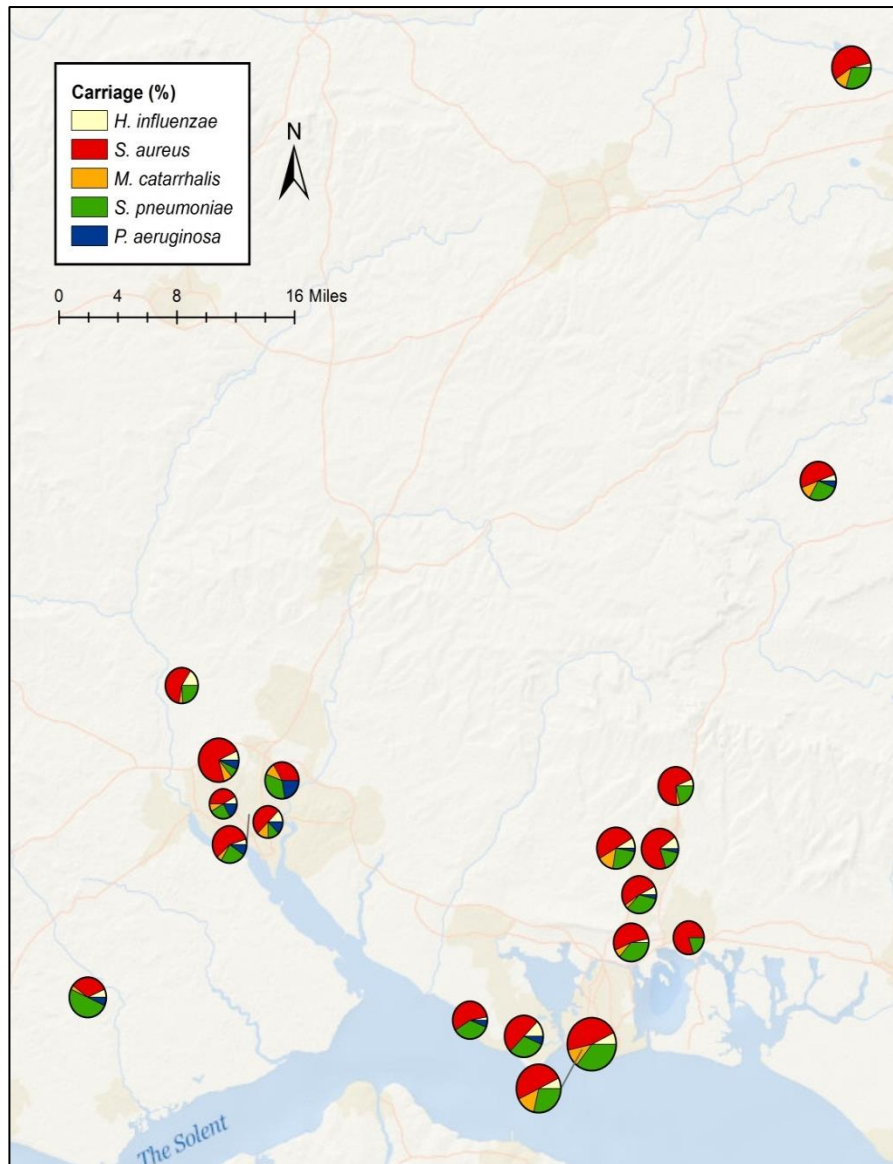
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Supplementary File



Supplementary Figure 1. Total Positivity Rates of the Four Swab Types

Bar chart showing positivity rates for all four swab types. WMS = whole mouth swab, NP = nasopharyngeal swab. Error bars represent 95% confidence intervals. Numbers of positive swabs are shown above each bar.



Supplementary Figure 2. Total Bacterial Carriage Rates of *S. pneumoniae*, *M. catarrhalis*, *P. aeruginosa*, *S. aureus* and *H. influenzae* in all swab types by geographical location of practices

Pie charts represent total bacterial carriage rates (%) in each GP practice with size representing proportionate amounts of bacterial carriage. Percentages of each bacterium within the total carriage rate are represented by coloured sections within each pie chart. Red lines are major roads, blue lines are rivers and darker areas of land represent cities.

Supplementary Table 1. Microbiology Identification Techniques for the Six Target
Bacterial Species

Bacterial species	Identification Technique
<i>S. pneumoniae</i>	Characteristic gram-positive alpha-haemolytic optochin-sensitive colonies growing on blood agar with nalidixic acid.
<i>H. influenzae</i>	Characteristic small gram-negative colonies requiring X+V factors growing on chocolate blood agar with bacitracin.
<i>M. catarrhalis</i>	Characteristic gram-negative tributyrin test-positive, DNase-positive diplococci growing on blood agar.
<i>P. aeruginosa</i>	Characteristic gram-negative oxidase-positive green colonies growing on <i>Pseudomonas</i> -selective CFC agar.
<i>S. aureus</i>	Characteristic gram-positive coagulase-positive colonies growing on blood agar.
<i>N. meningitidis</i>	Characteristic oxidase-positive gram-negative diplococci growing on <i>Neisseria</i> -selective GC agar and matching the correct API NH profile.