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Efficiency of nasal and pharyngeal swabs in the identification of *Neisseria meningitidis* carriers

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INTRODUCTION AND PURPOSE

Neisseria meningitidis (NM) is an obligate, human-specific commensal and pathogen of importance due to its impact on human health. This organism can persist in the human nasopharynx without causing clinical symptoms in a state known as carriage. Invasion of the pharyngeal tissues and subsequent proliferation in the blood and cerebrospinal fluids are infrequent but lead to diseases, such as septicemia and meningitis, which are associated with significant levels of morbidity and mortality. Studies of meningococcal carriage are important in understanding the epidemiology of meningococcal disease and the impact of vaccination programme. The purpose of this study was to evaluate the effectiveness and efficiency of posterior pharyngeal and nasopharingeal swabs in identifying, quantifying and serogrouping meningococcal carriage by Taqman Real Time Polymerase chain reactions (RT-PCR).

METHODS

To identify the best suitable sampling approach, we compared the following site: a) posterior pharyngeal, swab of the posterior pharyngeal wall behind the uvula taken through the mouth; b) nasopharyngeal, swab of the posterior pharyngeal wall taken through the nose. The study population consisted of 584 young healthy adolescents aged 15–18 years. Eswab swab (kit Code 482CE Copan Italia, Brescia) was used, containing 1.0 mL of modified liquid Amies in 12x80 mm screw cap tube and a pernasal flocked swab with a moulded break.

DNA was extracted from both sampling site per carrier with NucliSens EasyMag automated extraction system (BioMerieux, Italia), using "Generic" protocol and 250uL sample input. NMs DNA were detected in two separate singleplex TaqMan RT-PCR by screening of two meningococcal genes, *ctrA* and SodC, and also serogrouped by RT-PCR using primers specific for capsular biosynthetic genes (*synD*, $siaD_B$, $siaD_C$, $siaD_{W-135}$, *and* $siaD_Y$ for serogroups A, B, C, W-135, and Y, respectively) or alleles of *ctrA* (X and Z), according to Thomas JD *et al.*, 2011 and Wang X *et al.*, 2012 protocols. The related NMs DNA of carriers were quantified by means of standard curves generated using genomic NM DNA extracted from clinical isolates, and DNA concentration adjusted to 1ng/uL; the DNA was then serially diluted in 10-fold increments and each NM DNA concentration was converted to it log_{10} [genome equivalent or genomic copies/mL] using a standard 2.2Mbp per *N. meningitidis* genome.

RESULTS

Nasopharyngeal and posterior pharyngeal swabs were used (Fig.1, 2) and a total of 564 healthy adolescents (106 males, 18.8%; mean age 16.7±1.4 years) were swabbed, 37 (6.6%) of whom were found to be *N. meningitidis* carriers (Table 1, 2). The most frequently carried serogroup was serogroup B (15 cases, 40.5 %); serogroups A, Y, X, W-135 and Z were found in, respectively, two (5.4%), five (13.5%), four (10.8%), three (8.1%) and one subject (2.7%). The serogroup was not identified in seven cases (Table 1, 2 and Fig.3, 4). The detection of carrier status was significantly more frequent using posterior pharyngeal swabs (5.3 % vs. 2.1 %; p=0.004), which also contained a significantly larger number of *N. meningitidis* genomic copies (4.91±1.39 vs. 2.50±0.8 log_{10} genomic copies/mL; p<0.001) (Table 2).

Fig.1: ESwab kit 482CE, Copan – Italy

Fig.2: Example of nasopharyngeal swab collection procedure

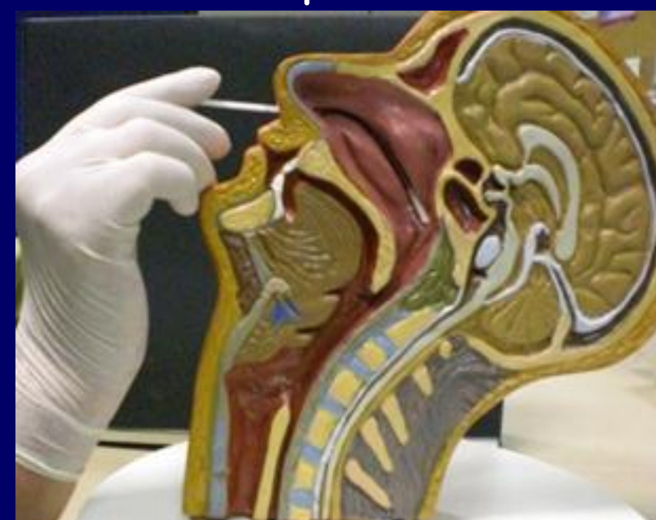


Table 1: General characteristics of the study population by *N. meningitides* carrier status

Characteristic	Pharyngeal and/or nasopharyngeal swab positive for N. menngitidis (n=37)	Pharyngeal and nasopharyngeal swab negative for N. menngitidis (n=527)	P value*
No. of males (%)	9 (24.3)	97 (18.4)	0.37
Mean age ± SD, years	17.0 ± 1.1	16.7 ± 1.6	0.28
No. of Caucasians (%)	36 (97.3)	489 (92.8)	0.29
No. of smoking fathers (%)	5 (13.5)	97 (18.4)	0.45
No. of smoking mothers (%)	6 (16.2)	90 (17.0)	0.89
No. of siblings, mean ± SD	1.2 ± 0.8	1.1 ± 0.8	0.25
No. of subjects with chronic underlying disease (%)	2 (5.4)	35 (6.6)	1.00
No. of infections in previous 3 months (%)	3 (8.1)	40 (7.6)	0.76
No. of subjects receiving antibiotic therapy in previous 3 months (%)	4 (10.8)	60 (11.4)	1.00
No. of travellers to a foreign country in previous 3 months (%)	26 (70.3)	276 (52.4)	0.03
No. of smokers (%)	7 (18.9)	100 (18.9)	0.99
No. of subjects who had received meningococcal vaccination (%)	6 (16.2)	152 (28.8)	0.10
Meningococcal C conjugate vaccine, No. (%)	*5 (13.5)*	*134 (25.4)*	*0.33*
Meningococcal A, C, Y, W135 conjugate vaccine, No. (%)	*1 (2.7)*	*9 (1.7)*	
Experimental meningococcal B vaccine, No. (%)	*0 (0.0)*	*9 (1.7)*	

SD: standard deviation.
*P-values for comparison between groups, using Chi-square or Fisher's exact test, as appropriate, for categorical data and Student's test or Wilcoxon rank-sum test, as appropriate, for continuous variables.

Table 2: Comparison of pharyngeal and nasopharyngeal swab evaluations of *N. meningitidis* carrier state

	Pharyngeal swab (n=564)	Nasopharyngeal swab (n=564)	P value*
No. of N. meningitides-positive subjects (%)#	30 (5.3)	12 (2.1)	0.004
Detected serogroup			
Serogroup A, No.	*1*	*1*	
Serogroup B, No.#	*13*	*4*	
Serogroup Y, No.#	*3*	*3*	
Serogroup X, No.	*4*	*0*	
Serogroup W-135, No.#	*3*	*2*	
Serogroup Z, No.	*1*	*0*	
Other serogroups, No.	*5*	*2*	
Mean bacterial load ± SD, log10 genomic cp/mL	4.91 ± 1.39	2.50 ± 0.8	<0.001

SD: standard deviation.
*P-values for comparison between groups, using z-test for proportions and Student's test for continuous variable.
two, one and two cases of serogroup B, Y and W-135 respectively, are present in both pharyngeal and nasopharyngeal swab.

Fig.3: Distribution (%) of *N. meningitides*-positive swabs

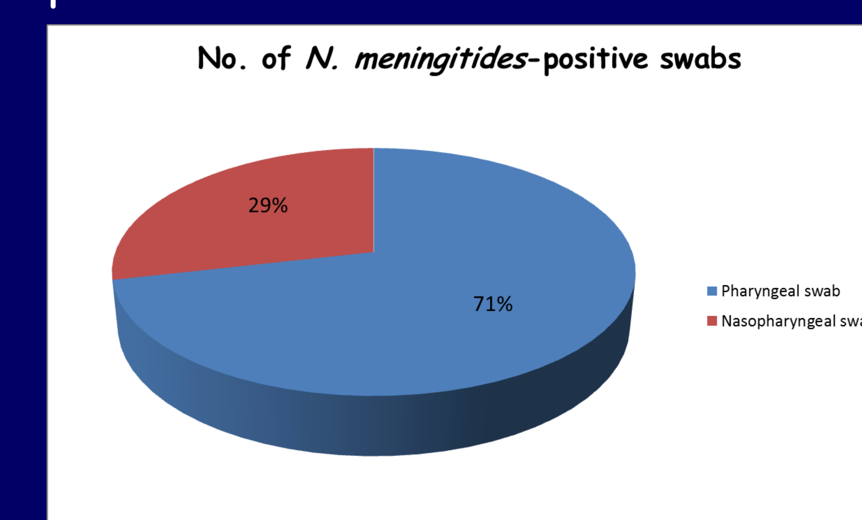
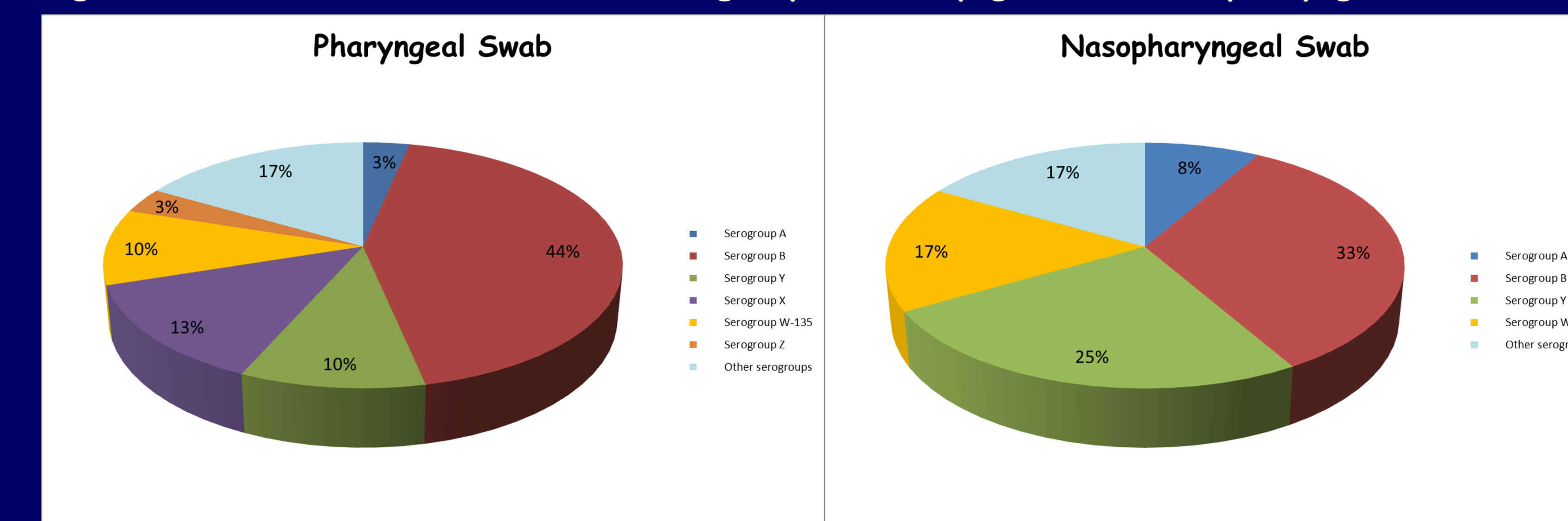


Fig.4: Distribution (%) of detected serogroups in Pharyngeal and Nasopharyngeal swab



CONCLUSIONS

The findings of this study indicate that posterior pharyngeal swabs are significantly better than nasopharyngeal swabs for evaluating *N. meningitidis* carrier status. The number of subjects carrying the pathogens and the amount of recovered bacterial DNA were both significantly larger when the respiratory secretions were collected from the posterior pharyngeal wall through the mouth than when they were collected in the pharynx through the nose.

The bacterial load estimated on the basis of the number of *N. meningitidis* genomic copies was also significantly higher using posterior pharyngeal wall swabs, thus, confirming their greater efficiency and supporting their use. Studies of the genetic characteristics of *N. meningitidis* can identify the strains with hyper-invasive lineages and which carriers seem to be at higher risk of developing invasive *N. meningitidis* disease, but require a considerable amount of bacterial DNA, and posterior pharyngeal wall swabs seem to provide this.

The *N. meningitidis* serogroup B was the most frequently identified pathogen. This is not unexpected because this serogroup has been the most frequent cause of disease in Italy for some years. Interestingly, we did not find any member of serogroup C; this is in line with what has been reported by other Italian authors studying carrier status in a different region of Italy and seems to be independent of the use of vaccine. Furthermore, the Italian National Institute of Health has reported a significant reduction in diseases due to *N. meningitidis* serogroup C over recent years. In conclusion, posterior pharyngeal wall swabs seem to be the best means of collecting samples for large-scale epidemiological studies of *N. meningitidis* carrier status.