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Nasopharyngeal versus Oropharyngeal Sampling for Isolation of Potential Respiratory Pathogens in Adults

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The optimal methodology for the identification of colonization by potential respiratory pathogens (PRP) in adults is not well established. The objectives of the present study were to compare the sensitivities of sampling the nasopharynx and the oropharynx for identification of PRP colonization and to compare the sensitivities of samples from the nasopharynx by swab and by washing for the same purpose. The study included 500 participants with a mean age of 65.1 ± 17.8 years. Of these, 300 patients were hospitalized for acute febrile lower respiratory tract infection and 200 were controls. Each participant was sampled by oropharyngeal swab (OPS), nasopharyngeal swab (NPS), and nasopharyngeal washing (NPW). The samples were tested by conventional bacteriological methods to identify *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*. OPS detected colonization by *S. pneumoniae* in 30% of the subjects compared with 89% by NPS and NPW ($P < 0.000001$). The corresponding rates for *H. influenzae* were 49% and 64%, respectively (no significant difference [NS]), and for *M. catarrhalis* were 72% and 46%, respectively ($P < 0.0004$). NPS identified 61% of the cases of colonization with *S. pneumoniae*, compared with 76% by NPW (NS). The corresponding rates for *H. influenzae* were 31% and 56%, respectively ($P < 0.04$), and for *M. catarrhalis* were 39% and 33%, respectively (NS). We conclude that the sensitivities of nasopharyngeal and oropharyngeal sampling for identification of PRP colonization in adults are different for each of the three bacteria in this category. The combined results of sampling from both sites are necessary to obtain a true picture of the rate of colonization. NPW is superior to NPS.

Colonization of the mucosal membrane of the nasopharynx and the oropharynx by the potential respiratory pathogens (PRP), *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*, has several clinical manifestations. In most cases colonization causes only a carrier state without producing clinical symptoms, but, if a change occurs in the host's condition, the PRP can cause disease (3). In addition, the bacteria that are carried in the upper respiratory tract can spread from person to person (6, 7, 10), which is why their presence can provide useful and important information on the emergence of resistance in clinical isolates (8, 11).

Age is the most influential factor in the rate of colonization by PRP in healthy individuals. The maximal rates seen in preschool children decline during the school years and decline sharply in adults (5, 6). The high colonization rates in the pediatric age group are the reason that the vast majority of studies on this issue were conducted on this age group, while the number of studies that evaluated PRP colonization in adults is small (3). In these circumstances, it is not surprising that even the basic methodology to test for colonization in adults is not well established. The present study was designed to deal with one of the aspects of this issue.

The two techniques that are commonly used to test for PRP are sampling using nasopharyngeal and oropharyngeal swabs. The sensitivities of these two methods for identification of colonization by PRP in adults were compared in several earlier studies, particularly regarding *S. pneumoniae*, but the results were not consistent (1, 2, 4, 6, 9, 12). The nasopharyngeal wash technique, which is commonly used to isolate viruses in children, has not gained popularity as a method to test for PRP colonization in adults.

In light of the above we designed a study to assess colonization by all three PRP bacteria in a study population that included adult patients with acute respiratory diseases and adult controls of all ages. The objectives of the study were to compare the sensitivities of samples from the oropharynx and the nasopharynx for identification of colonization by the three bacteria and to compare the sensitivities of nasopharynx sampling by swabs and washings for the same purpose.

MATERIALS AND METHODS

The study population. The study population comprised two groups of subjects, patients hospitalized with lower respiratory tract infection and controls. The study was approved by the Helsinki Committee for research on human beings of the Soroka Medical Center, and all participants gave signed informed consent to participate. Recruitment of patients and controls was conducted in the same time period between 1 November 2004 and 15 March 2005.

The patient groups included patients who were hospitalized from the community in one of the internal medicine departments of the Soroka Medical Center and who fulfilled the following four criteria: (i) over 18 years of age, (ii) had an acute febrile illness of at least 1 week's duration, (iii) had a cough that appeared or worsened over the week prior to hospitalization, and (iv) in the week prior to hospitalization experienced (a) appearance or worsening of shortness of breath, (b) sputum production, (c) wheezing, or (d) chest pain or discomfort. None of the patients was recruited from a nursing home to preserve, as much as possible, the uniformity of the study population in terms of the basic bacterial flora of their upper respiratory tracts.

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In accordance with accepted criteria the patients were subclassified into three groups: community-acquired pneumonia, nonpneumonic lower respiratory tract infection, and acute exacerbation of chronic obstructive pulmonary disease.

The control group comprised ambulatory patients who came to one of the outpatient clinics of the Soroka Medical Center, agreed to participate in the study, and fulfilled all of the following three conditions: (i) over 18 years of age, (ii) by medical documentation had, and in response to a direct question reported, no evidence of a known chronic lung disease or a state of immunosuppression, and (iii) by response to a direct question reported that, in the month prior to hospitalization, they had not had (a) febrile illness, (b) a cough, (c) a throat ache, (d) hoarseness, or (e) a running nose and that they had not taken antibiotic medications or had evidence of being definitely or possibly pregnant (in the case of women).

For each of the participants in both groups we collected data concerning age, sex, smoking habit, and vaccination status. In addition, for each of the hospitalized patients we collected data on antibiotic therapy prior to hospitalization and length of time (in hours) of antibiotic therapy during their hospital stay before the naso- and oropharyngeal samples were taken.

Sampling. Three physicians, who were trained specifically for the task, took all the samples from the patients and controls. In all hospitalized patients the samples were taken as close as possible to the time of admission to the hospital, and in no case more than 24 h later. Three consecutive samples were taken from each participant in the following order: oropharyngeal swab, nasopharyngeal swab, and nasopharyngeal washing. The oropharyngeal swab was taken, under direct observation of the posterior throat and tonsil area, using a commercial rigid cotton-tipped swab applicator (blue cap; Copan Venturi Transystem; Copan Italia Spa, Brescia, Italy). The nasopharyngeal swab was taken using the same type of rigid swab applicator, which was introduced directly into the depth of the inferior meatus of one of the nostrils until resistance was felt. After sampling, both swab applicators were placed into tubes containing Amies agar gel transport medium. The nasopharyngeal washing was obtained by instilling 2.5 ml of a sterile physiological saline solution into one of the patient's nostrils with the patient lying down. The instilled water was then gently suctioned out through a delicate tube that was introduced deep into the nostril and emptied into a special collection container (mucous trap; Unomedical A/S, Lynge, Denmark) that was connected to the portable suction equipment (Easy Go Vac aspirator; Precision Medical, Northampton, PA). The raw washing matter and the two swabs were all processed within 3 hours in the clinical microbiology laboratory of the Soroka Medical Center.

Bacteriology. To facilitate detection of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*, respiratory tract specimens were seeded on (i) Trypticase soy agar with added 5% sheep blood and 5.0 μg/ml gentamicin, (ii) chocolate agar, and (iii) Trypticase soy agar with added 5% sheep blood and 2.0 μg/ml vancomycin, respectively. Inoculated media were incubated aerobically at 35°C for 48 h in a CO_2-enriched atmosphere.

For the detection of *S. pneumoniae*, swabs and the nasopharyngeal wash fluid were inoculated onto Columbia agar with added 5% sheep blood and 5.0 μg/ml of gentamicin and incubated aerobically at 35°C for 48 h in a CO_2-enriched atmosphere. *S. pneumoniae* was identified by colony morphology, alpha-hemolysis, and inhibition by optochin and confirmed by a positive slide agglutination test (Phadebact; Pharmacia Diagnostics, Uppsala, Sweden).

Identification of *H. influenzae* was based on Gram stain, growth on chocolate agar medium, failure to grow on Trypticase soy agar with added sheep blood, and nutritional requirements for both hemin and nicotine adenine nucleotide. Organisms that failed to agglutinate with antisera to *H. influenzae* groups a to f (Phadebact; Pharmacia Diagnostics, Uppsala, Sweden) were considered untypeable.

M. catarrhalis was identified by colony morphology, Gram stain, a positive cytochrome oxidase test, and hydrolysis of butyrate.

Data analysis. Data were recorded and analyzed using the Epi Info, version 3.3.2, software. Rates between samples were compared using the χ^2 test with Yates correction or Fisher's exact test, as appropriate. Statistical significance was set at a P value of <0.05 throughout.

RESULTS

The study population consisted of 300 hospitalized patients and 200 controls. The mean age of the patients was 66.4 ± 19.0 years (range, 19 to 99 years), and 145 (48.3%) were women. Ninety-eight of the patients were diagnosed as having community-acquired pneumonia, 167 as having nonpneumonic lower respiratory tract infection, and 35 as having acute exacerbation of chronic obstructive pulmonary disease. Eighty-four patients (28.0%) were treated with at least one oral antibiotic preparation during the week before they were hospitalized. Two hundred sixty-one patients (87.0%) were treated with an antibiotic during the time between their admission to the hospital and the collection of the naso- and oropharyngeal samples (mean time, 11.2 ± 6.7 h; range, 1 to 23 h). Of these, 235 (90.0%) received the antibiotic treatment by the intravenous route.

The mean age of the control patients was 63.0 ± 15.7 years (range, 19 to 93 years), and 112 (56.0%) were women. The mean age of the entire study population of 500 was 65.1 ± 17.8 years (range, 19 to 99 years), and 257 (51.4%) were women.

Table 1 shows the distribution of isolation of *S. pneumoniae* by the types of sampling and the various sampling combinations, for the study and control groups and for the merged study population of 500. For the entire study population sampling of the nasopharynx identified 89% of the colonization by *S. pneumoniae* (by at least one of the nasopharyngeal methods) compared to only 30% for the oropharyngeal sampling ($P <$ 0.000001). There was no significant difference in the rates of detection of colonization by this bacterium between nasopharyngeal swabs and washings.

Table 2 shows the distribution of isolation of *H. influenzae* by the types of sampling and the various sampling combinations, for the study and control groups and for the entire study population of 500. All *H. influenzae* isolates were untypeable. For the entire study population sampling of the nasopharynx identified 64% of the colonization by *H. influenzae* (by at least one of the nasopharyngeal methods) compared to 49% for the oropharyngeal sampling (no significant difference). Nasopharyngeal washing swabs identified more colonization than was identified by swabbing ($P < 0.04$).

Table 3 shows the distribution of isolation of *M. catarrhalis* by the types of sampling and the various sampling combinations, for the study and control groups and for the entire study population of 500. For the entire study population sampling of the nasopharynx identified only 47% colonization by *M. catarrhalis* (by at least one of the nasopharyngeal methods) compared to 72% for the oropharyngeal sampling ($P < 0.0004$). There was no significant difference in the rates of detection

TABLE 1. Distribution of the number of *S. pneumoniae* isolates by single sampling method and combinations of sampling methods, in the two patient groups and for the merged group

Sampling method(s)[a]	No. of subjects positive for *S. pneumoniae*		
	Controls (n = 200)	Patients (n = 300)	Merged data (%[b]) (n = 500)
OPS	3	13	16 (30)
NPS	4	29	33[c] (61)
NPW	4	37	41 (76)
NPS and/or NPW	6	42	48[d] (89)
OPS and/or NPS	6	36	42 (78)
OPS and/or NPW	6	41	47 (87)
OPS and/or NPS and/or NPW	8	46	54 (100)

[a] OPS, oropharyngeal swab; NPS, nasopharyngeal swab; NPW, nasopharyngeal washing.
[b] Percentage of isolates from the group of subjects positive for *S. pneumoniae* by at least one of the three sampling methods.
[c] Nonsignificant compared to NPW.
[d] $P < 0.00001$ compared to OPS.

TABLE 2. Distribution of the number of *H. influenzae* isolates by single sampling method and combinations of sampling methods, in the two patient groups and for the merged group

Sampling method(s)[a]	No. of subjects positive for *H. influenzae*		
	Controls (n = 200)	Patients (n = 300)	Merged data (%[b]) (n = 500)
OPS	6	13	19 (49)
NPS	4	8	12[c] (31)
NPW	5	17	22 (56)
NPS and/or NPW	5	20	25[d] (64)
OPS and/or NPS	9	20	29 (74)
OPS and/or NPW	10	26	36 (92)
OPS and/or NPS and/or NPW	10	29	39 (100)

[a] OPS, oropharyngeal swab; NPS, nasopharyngeal swab; NPW, nasopharyngeal washing.
[b] Percentage of isolates from the group of subjects positive for *H. influenzae* by at least one of the three sampling methods.
[c] $P < 0.04$ compared to NPW.
[d] Nonsignificant compared to OPS.

TABLE 3. Distribution of the number of *M. catarrhalis* isolates by single sampling method and combinations of sampling methods, in the two patient groups and for the merged group

Sampling method(s)[a]	No. of subjects positive for *M. catarrhalis*		
	Controls (n = 200)	Patients (n = 300)	Merged data (%[b]) (n = 500)
OPS	30	43	73 (72)
NPS	12	28	40[c] (39)
NPW	10	24	34 (33)
NPS and/or NPW	14	33	47[d] (46)
OPS and/or NPS	36	65	101 (99)
OPS and/or NPW	35	60	95 (93)
OPS and/or NPS and/or NPW	36	66	102 (100)

[a] OPS, oropharyngeal swab; NPS, nasopharyngeal swab; NPW, nasopharyngeal washing.
[b] Percentage of isolates from the group of subjects positive for *M. catarrhalis* by at least one of the three sampling methods.
[c] Nonsignificant compared to NPW.
[d] $P < 0.0004$ compared to OPS.

of colonization by this bacterium between nasopharyngeal swabs and washings.

There was no significant difference in the sensitivity rates (the percentages of isolates among the overall population of carriers) in any of the three sampling types for any of the three bacteria between the patient and control groups (nine comparisons). Similarly, there was no significant difference in any sensitivity rates (the percentages of isolates among the overall population of carriers) in any of the three sampling types for any of the three bacteria between patients who were treated with antibiotics prior to hospitalization and those who were not, or between those who were treated, after admission, with antibiotics for more than 12 h before sampling compared with those who were treated for less than 12 h previously.

DISCUSSION

The present study on PRP colonization in adults is original and unique in several respects. The study objectives focused exclusively on the methodological aspect of identification of colonizing bacteria and purposely ignored other aspects of this issue that require a different size and composition of the study population. Although the comparative sensitivities of samples from the nasopharynx and the oropharynx have been studied before, nasopharyngeal swabs and washings have not been compared before in adults. The evaluation of all three PRP bacteria is also a unique and original aspect of this study. The age distribution of the study population covers the entire range of the adult population, with a particularly high mean age that reflects the enlistment of a large number of elderly patients. In terms of clinical characteristics the study population included a broad spectrum of acute respiratory disease and a control group with a similar age distribution. The patients in the patient group were all hospitalized, but the sampling was done close to the time of their admission to the hospital with the aim of averting the effect of hospital-based colonization.

Two methodological issues related to the study require clarification. The first is the merging of the two study groups, patients and controls, for the purpose of data analyses. This action is, at first glance, problematic in light of the differences in the rates of colonization in the two groups. However, in this study we purposely ignored the question of the colonization rates in the study groups and focused only on the methodological issue of the relative sensitivities of the three sampling techniques for identification of colonization. The sensitivities for the three methods were compared for each of the three bacteria between the study groups, and no significant differences were found between them. In light of this result we believe that it was justified to merge the two populations for further data analyses.

The second issue is the use of the rigid swab applicator to sample the nasopharynx, as was done in some earlier studies (1, 6), and not the flexible swab applicator, as was done in others (4, 9, 12). At the preliminary stage of this study the investigators tested both types of applicators for nasopharyngeal sampling. The investigators' impression was that adult patients suffer much less with the rigid applicator than the flexible one, so the rigid applicator was used for all nasopharyngeal samplings in this study. This choice would not necessarily be the case in the pediatric population, in which sampling is usually conducted with the child being held by the parents or the staff.

The sensitivity for identification of colonization of *S. pneumoniae* by isolation from the nasopharynx in this study was three times higher than from the oropharynx. Sampling the throat yielded only an additional 11% of positive patients who were not identified by nasopharyngeal sampling. Corresponding results from previous studies that evaluated this question for adults reflect a range from a clear advantage to the nasopharynx (2, 12) to no advantage to either site (4, 9) to a clear advantage to the oropharynx (1, 6). The explanation for these discrepant differences in results is probably in the patient characteristics of the populations that were sampled in the various studies in terms of age and perhaps the specific technique used for sampling the nasopharynx.

In contrast to the differences found for *S. pneumoniae*, we found no significant differences in sensitivity for the identification of colonization by *H. influenzae* between nasopharyngeal and oropharyngeal samples. This finding contrasts with two previous studies of adults that showed a significant advantage to the isolation of this bacterium by sampling the oropharynx (4, 9). Further analysis of the sensitivities of the three techniques for isolation of this bacterium shows an advantage

to the oropharyngeal sampling by swabbing in our study as well, but the addition of the isolates received from the nasopharyngeal washings led to the nonsignificant comparison between the two sites.

In the absence of studies that compared the sensitivities of identification of colonization by *M. catarrhalis* by isolates from the nasopharynx and oropharynx we cannot compare the significant advantage that we found for oropharyngeal samplings with any previous reports.

Interpretation of the study results in terms of the three bacteria shows a shift in which there is a sharp advantage to sampling of the nasopharynx for identification of colonization by *S. pneumoniae*, equality between sampling of the nasopharynx and the oropharynx for *H. influenzae*, and an advantage to sampling of the oropharynx for the identification of colonization by *M. catarrhalis*. It is reasonable to assume that the grounds for this shift are the pathophysiological factors that enable each of these bacteria to be dominant in one of the areas of the pharynx. Clarification of this pathophysiological explanation is beyond the scope of this study and needs to be addressed by a study specifically designed to do so.

A unique and original subject of this study is the comparison of sensitivities between nasal swabs and washings for the identification of colonization by each of the three bacteria. For two of the bacteria, *S. pneumoniae* and *M. catarrhalis*, there was no significant difference between the two methods, but there was a significant advantage to washing over swabs for the identification of *H. influenzae*. For all three bacteria the combination of swabs and washings identified a higher rate of colonization than either of the methods alone. The absence of a significant difference in sensitivity for two of the bacteria demonstrates, in our opinion, that use of the rigid applicator instead of the flexible one did not have a significant effect on the study results, since the washings were conducted with a flexible tube, which did not give this technique any advantage for the two bacteria.

We conclude that the sensitivities of nasopharynx versus oropharynx sampling for the identification of PRP colonization are different for the three bacteria included in this category. The only way to get a true picture of this colonization is to merge the results of sampling from the two sites. Sampling of the nasopharynx by washing is superior to swabbing.

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