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Comparison of nasopharyngeal aspirate and nasal swab specimens for detection of respiratory syncytial virus in different settings in a developing country

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Summary

OBJECTIVE To compare detection of respiratory syncytial virus (RSV) for diagnostic purposes using nasopharyngeal aspirate (NPA) and nasal swabs (NS) in different clinical settings in a community study in Guinea-Bissau.

METHOD During 1996–98 paired specimens were obtained from 635 children under 5 years of age (median: 274 days; interquartile range: 144–453 days) with symptoms of lower respiratory infections (LRI). The specimens were analysed by an enzyme-linked immunosorbent assay for RSV antigen in Guinea-Bissau and re-analysed in Denmark using the same assay. The gold standard for RSV antigen detection was defined as any test being positive.

RESULTS RSV antigen was detected in 84 (13%) children, the prevalence being 19% (41/219) among infants aged < 6 months, 12% (22/184) in infants aged 6–11 months, and 9% (21/230) in older children. Sensitivity of antigen detection was higher in NPA (92% in analyses in Guinea-Bissau and 98% in Denmark) than in NS (63% in analyses in Guinea-Bissau, 71% in Denmark). Specificity of RSV antigen detection was equally high in NPA and NS (99–100%). Time since onset of symptoms was significantly shorter in RSV antigen positive than negative samples. Sensitivity did not depend on clinical setting or age of the child.

CONCLUSION Using NS samples was associated with a 27–31% reduction in sensitivity compared with NPA specimens. As NPAs are costly and considered a nuisance by the population, it might be cost-effective in larger epidemiological studies to lose 25–30% in sensitivity but be able to collect samples from a much larger population.

keywords respiratory syncytial virus, nasopharyngeal aspirate, nasal swab

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Introduction

Respiratory syncytial virus (RSV) is one of the most common respiratory pathogens of infants and young children, causing yearly outbreaks of upper and lower respiratory infection (LRI) in both developed and developing countries (Glezen & Denny 1973; Selwyn 1990). Confirmation of viral aetiology is important before choosing therapy. RSV infection can be rapidly diagnosed with enzyme-linked immunosorbent assay (ELISA) for direct detection of RSV antigen or RSV specific antibodies (McIntosh *et al.* 1982; Hornsleth *et al.* 1986; Jensen *et al.* 1997). Earlier studies in developed countries have indicated that the source of specimens influences the likelihood of RSV detection (Hall & Douglas 1975; McIntosh *et al.* 1982; Treuhaft *et al.* 1985; Ahluwalia *et al.* 1987; Cruz *et al.* 1987; Frayha *et al.* 1989; Michaels *et al.* 1992). Most of these studies document the better sensitivity of nasopharyngeal aspiration (NPA) and nasal wash compared with nasal swab (NS), nasopharyngeal swab, or

throat swab techniques. Furthermore, detection of RSV antigen is more common in children with more severe disease and in samples taken as soon as possible after onset of symptoms (Hall *et al.* 1976; Glezen *et al.* 1981; Buckingham *et al.* 2000). To our knowledge, this has not been assessed in a developing country and in different clinical settings. In certain situations, a less sensitive test could be of interest if it were less unpleasant or costly.

The Bandim Health Project in Guinea-Bissau has conducted community studies of LRIs associated with RSV (Stensballe *et al.* 2000). As part of these studies we compared RSV detection using NPA and NS sampling in different clinical settings.

Subjects and methods

The Bandim Health Study conducts demographic and epidemiological surveillance in several districts in the capital of Guinea-Bissau, including Bandim 1, Bandim 2 and Belem (Aaby 1997). More than 3000 houses in the area are visited monthly to detect new pregnancies and to register newborns as soon as possible after birth. Children under 3 years of age are visited every 3 months to register growth, immunizations, morbidity and survival of the children.

From July 1994 to the onset of the war June 1998 (Aaby *et al.* 1999), children under 5 years of age from the three study districts, who attended the outpatient clinic of the paediatric department of the national hospital in Bissau as well as the child clinic at the local health centre in Bandim, were screened systematically for LRIs to establish the incidence of RSV. In February 1995, we initiated a community cohort of all newborns in Bandim 1 and Bandim 2. The newborns were visited weekly by field assistants, who interviewed the mothers using morbidity questionnaires and clinically examined the babies for LRI. Sick children identified by a field assistant were referred to the hospital or visited on the same day by a project physician. The identification of LRI was based on an age-adjusted standard for respiratory frequency and chest in-drawings as recommended by World Health Organization (WHO) (Wright & Cutts 1996). There was no RSV diagnostic prior to these studies, and as they were interrupted by the war in 1998, there has not been any in Guinea-Bissau.

Sample collection and storage

From 1996 to 1998, paired specimens were obtained from 635 children with respiratory symptoms. NPA specimens were collected by suction of secretions from one nostril into mucous traps using a nasopharynx suction device (tracheal suction set manufactured by Kirudan, Denmark, for Maersk Medical) connected to a foot pump (Ambu® Twin Pump, Ambu International A/S). The catheter was pushed into the nasopharynx equivalent to the distance from the ear to the nostril of the patient. At this position suction was started while gently rolling and retracting the catheter. Secretions remaining in the catheter were obtained by washing with 5 ml isotonic saline. NS specimens were collected through the other nostril with a cotton-tipped swab (Culture Swab ™ Transport System, manufactured by Copan, Italia SpA, for DIFCO Laboratories). The swab was inserted 2–3 cm into the nostril and rotated thoroughly against the respiratory epithelial surface of the nasal cavity. The swab was then removed and placed in a container adding 5 ml of isotonic saline. Three Guinean physicians and three specially trained field assistants collected the samples.

Laboratory procedures

All specimens were delivered to the laboratory within a few hours and processed in an identical fashion irrespective of whether they derived from NPA or NS samples. Initially, 100 ml of the samples were examined for RSV antigen at the National Public Health Laboratory in Guinea-Bissau (Laboratório National de Saúde Pública, LNSP), with an antigen ELISA developed at Statens Serum Institut in Copenhagen. This antigen test covers both RSV type A and B. All samples were stored at –20 °C and subsequently transported to Copenhagen to be re-analysed by the same ELISA for RSV antigen (Obel *et al.* 1995). The RSV antigen re-testing served as a quality control of the ELISA test in Guinea-Bissau.

RSV antigen detection and definition of positive and borderline positive samples

As in previous studies and irrespective of the source of specimens, samples were considered positive if the optical density (OD) value was three times higher than the OD value of the unspecific control and higher than the OD of the negative control. In calculating the sensitivity and specificity, a gold standard RSV antigen positive child was defined as testing positive in one or more of the four tests of his or her samples. A sample was considered borderline positive if the OD value was between two and three times the OD value of the unspecific control and higher than the OD of the negative control. There were considerably more borderline values for the NS specimens. Virtually all of these borderline results were antigen positive in the NPA specimens. Including borderlines as positives increased sensitivity of the NS from 43 to 63% in Guinea-Bissau and

from 51 to 71% in Denmark without affecting specificity (data not shown). In further analyses, borderline positive samples were therefore regarded as RSV positives.

Statistical analyses

Standard statistical methods for analysing categorical and continuous data were used. Independent groups were compared using either chi-squared test, trend test or *T*-test as appropriate. Paired groups were compared using McNemar's test. 95% confidence intervals are given throughout.

Ethical approval and consent

The Ministry of Health in Guinea-Bissau and the Central Ethical Committee of Denmark (C-1994-27) approved the study. Inclusion of children in the study was based on informed consent of their mother.

Results

We collected paired samples from 635 children. Eighty-four samples were RSV antigen positive in at least one test. The prevalence was highest among samples from children under 6 months (18.7%; 41/219) compared with infants aged 6 months to 1 year (12.0%; 22/184), and children aged 1 year and older (9.1%; 21/230) (trend test $P = 0.003$) (Table 1). The prevalence of positive samples was virtually the same for boys (13.5%; 46/342) and girls (12.8%; 37/290). For 531 children, there was information available on the delay between the onset of respiratory symptoms and the collection of the samples (Table 2). Missing information was usually because of the mother being away at the time of the interview. For samples positive according to our gold standard, the mean (SD) interval was 4.3 (2.1) days compared with 6.0 (5.0) days for negative samples, a highly significant difference (*T*-test with correction for unequal variances $P = 0.0001$) (data not shown).

As seen in Table 3, sensitivity obtained with NPA analyses was equally good in Guinea-Bissau (91.7%, CI: 83.6–96.6) and Denmark (97.6%, CI: 91.7–99.7). Nor was there any difference in the sensitivities of NS samples analysed in Guinea-Bissau (63.1%, CI: 51.9–73.4) and Denmark (71.4%, CI: 60.5–80.8).

Compared with the sensitivity of the NPA specimens, NS samples were 31% [1 – (63.1%/91.7%)] less sensitive when analysed in Guinea-Bissau and 27% [1 – (71.4%/97.6%)] in Denmark (McNemar's test $P < 0.0001$ in both cases). The difference between sensitivities of NPA and NS was the same when analysed in Guinea-Bissau (29%, CI: 19–38%) or Denmark (26%, CI: 16–36%).

Specificity was high for both NPA and NS specimens (Table 3). No significant differences were found. Analysed according to setting, specificities remained high, the lowest occurring in NS specimens from the health centre analysed in Guinea-Bissau (97.3%, CI: 93.8–99.1).

There was no significant difference in sensitivity for NS according to age [data not shown, Denmark: $P = 0.99$ (chi-square test); Guinea-Bissau: $P = 0.54$ (chi-square test)]. For samples analysed in Guinea-Bissau and stratified

Table 1 Prevalence of respiratory syncytial virus (RSV) antigen positive samples according to age and sex among children with lower respiratory infections. Guinea-Bissau, 1995–96

	No. of RSV antigen positives (%)		
Age	Boys	Girls	All children
Age < 6 months	24/127 (18.9%)	16/91 (17.6%)	41/219 (18.7%)
6 months ≤ age < 1 year	11/100 (11.0%)	11/84 (13.1%)	22/184 (12.0%)
Age ≥ 1 year	11/115 (9.6%)	10/114 (8.8%)	21/230 (9.1%)
	46/342 (13.5%)	37/290 (12.8%)	84/635 (13.2%)

The final column and row include two children with unknown age and three children with unknown gender, respectively.

Table 2 Sample positivity in relation to days after onset of symptoms

	Days after onset of symptoms							
	0–1 day	2 days	3 days	4 days	5 days	6 days	7 days	8–30 days
Positive samples/ total samples	1/18, 5.6%	3/26, 11.5%	13/98, 13.3%	4/58, 6.9%	2/36, 5.6%	1/25, 4%	1/48, 2.1%	1/36, 2.8%

Table 3 Sensitivity and specificity according to clinical setting, mode of sample collection, and place of analyses

Setting	No. of positive samples/ total no. of samples (%)	Sensitivity (positive samples identified) NPA-DK*	NPA-GB*	NS-DK*	NS-GB*
Hospital	19/162 (11.7%)	100% (19)	94.7% (18)	73.7% (14)	73.7% (14)
Health centre	28/213 (13.1%)	100% (28)	92.9% (26)	75.0% (21)	64.3% (18)
Community	37/260 (14.2%)	94.6% (35)	89.2% (33)	67.6% (25)	56.8% (21)
Total	84/635 (13.2%)	97.6% (82) (CI: 91.7–99.7)†	91.7% (77) (CI: 83.6–96.6)†	71.4% (60) (CI: 60.5–80.8)†	63.1% (53) (CI: 51.9–73.4)†
	No. of negative samples	Specificity (negative samples identified)			
		NPA-DK	NPA-GB	NS-DK	NS-GB
Total	551	98.9% (545) (CI: 97.7–99.6)†	98.9% (545) (CI: 97.6–99.6)†	99.5% (548) (CI: 98.4–99.9)†	98.4% (542) (CI: 96.9–99.3)†

* NPA-DK nasopharyngeal aspirate specimens analysed in Denmark; NPA-GB nasopharyngeal aspirate specimens analysed in Guinea-Bissau; NS-DK nasal swab specimens analysed in Denmark; and NS-GB nasal swab specimens analysed in Guinea-Bissau.
† Clopper-Pearson 95% confidence intervals for binomial distribution.

according to setting, the loss in sensitivity using NS instead of NPA was 27% [1 – (68%/94%)] for samples collected in a clinical setting (hospital and health centre) and 36% [1 – (57%/89%)] for samples collected in the community. This difference was not significant ($P = 0.49$).

Discussion

Comparing NS with NPA or nasal wash, previous studies from developed countries (Hall & Douglas 1975; McIntosh *et al.* 1982; Treuhaft *et al.* 1985; Ahluwalia *et al.* 1987; Cruz *et al.* 1987; Frayha *et al.* 1989; Michaels *et al.* 1992) have found between 9 and 65% reduction in sensitivity, the large variation presumably being because of the limited size of many studies and variation in how the swab specimens were collected. In a developing country, Guinea-Bissau, the reduction in sensitivity of NS specimens compared with NPA specimens for RSV antigen detection by ELISA was 25–30%. Specificity only differed slightly, NS being the most specific. However, in comparing the two techniques, it should be taken into consideration that NPA requires a vacuum system, a suction catheter, a mucus trap, and a transport medium, whereas NS only needs a swab and a transport medium. In our opinion and the opinion of the mothers, the NS was also less traumatic to the children. NS samples are much easier and therefore cheaper to collect, while the NPA technique usually requires more time and better trained personnel. In epidemiological studies or vaccination trials where repeated samples have to be taken, some reduction in sensitivity could be acceptable if it were outweighed by facility, lower cost, and better acceptability among parents. Using NS one might be able to cover a much larger population and/or collect samples more frequently.

The proportion of borderline positive values to positive values was higher in NS than NPA specimens. This might be because of differences in the quantity of specimens, as the much smaller amount of nasal secretion obtained by NS was also diluted with 5 ml isotonic saline. Besides, samples are not routinely microscoped before an ELISA procedure and the cellular context of the samples is unknown. As the catheter used for NPA covers a larger area of airway epithelium, it is likely that the content of RSV infected cells is higher in NPA than NS samples.

To test whether severity of disease would influence the sensitivity of antigen detection, the data were analysed according to the setting in which the samples had been collected and according to the age of the children as primary infection in the youngest cases are usually most severe. Children going to hospital or the health centre were presumed to be more severely ill than cases only identified during home visits. But neither setting nor age had any major effect on the sensitivity.

In 2001 prices a tracheal suction set with catheter was nine times as costly as a swab (17/1.8 DKr) without freight. The usefulness of the less sensitive NS depends on considerations of costs, ease of collection, and acceptability of repeated sampling. These considerations may be important in studies needing a large number of RSV cases.

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References

Aaby P (1997) An unplanned longitudinal study. In: *Prospective Community Studies in Developing Countries*, 1st edn (eds M Das Gupta *et al.*) Oxford University Press/Clarendon Press, Oxford, pp. 276–296.

Aaby P, Gomes J, Fernandes M, Djana Q, Lisse I & Jensen H (1999) Nutritional status and mortality of refugee and resident children in a non-camp setting during conflict: follow up study in Guinea-Bissau. *British Medical Journal* **319**, 878–881.

Ahluwalia G, Embree J, McNicol P, Law B & Hammond GW (1987) Comparison of nasopharyngeal aspirate and nasopharyngeal swab specimens for respiratory syncytial virus diagnosis by cell culture, indirect immunofluorescence assay, and enzyme-linked immunosorbent assay. *Journal of Clinical Microbiology* **25**, 763–767.

Buckingham SC, Bush AJ & Devincenzo JP (2000) Nasal quantity of respiratory syncytical virus correlates with disease severity in hospitalized infants. *Pediatric Infectious Diseases* **19**, 113–117.

Cruz JR, Quinonez E, de Fernandez A & Peralta F (1987) Isolation of viruses from nasopharyngeal secretions: comparison of aspiration and swabbing as means of sample collection. *Journal of Infectious Diseases* **156**, 415–416.

Frayha H, Castriciano S, Mahony J & Chernesky M (1989) Nasopharyngeal swabs and nasopharyngeal aspirates equally effective for the diagnosis of viral respiratory disease in hospitalized children. *Journal of Clinical Microbiology* **27**, 1387–1389.

Glezen P & Denny FW (1973) Epidemiology of acute lower respiratory disease in children. *New England Journal of Medicine* **288**, 498–505.

Glezen WP, Paredes A, Allison JE, Taber LH & Frank AL (1981) Risk of respiratory syncytial virus infection for infants from low-income families in relationship to age, sex, ethnic group, and maternal antibody level. *Journal of Pediatrics* **98**, 708–715.

Hall CB & Douglas RG (1975) Clinically useful method for the isolation of respiratory syncytial virus. *Journal of Infectious Diseases* **131**, 1–5.

Hall CB, Douglas RG & Geiman JM (1976) Respiratory syncytial virus infections in infants: quantitation and duration of shedding. *Journal of Pediatrics* **89**, 11–15.

Hornsleth A, Friis B & Krasilnikof PA (1986) Detection of respiratory syncytial virus in nasopharyngeal secretions by a biotin–avidin ELISA more sensitive than the fluorescent antibody technique. *Journal of Medical Virology* **18**, 113–117.

Jensen IP, Thisted E, Glikmann G *et al.* (1997) Secretory IgM and IgA antibodies to respiratory syncytial virus in nasopharyngeal aspirates: a diagnostic supplement to antigen detection. *Clinical Diagnosis in Virology* **8**, 219–226.

McIntosh K, Hendry RM, Fahnestock ML & Pierik LT (1982) Enzyme-linked immunosorbent assay for detection of respiratory syncytial virus infection: application to clinical samples. *Journal of Clinical Microbiology* **16**, 329–333.

Michaels MG, Serdy C, Barbadora K, Green M, Apalsch A & Wald ER (1992) Respiratory syncytial virus: a comparison of diagnostic modalities. *Pediatric Infectious Diseases* **11**, 613–616.

Obel N, Andersen HK, Jensen IP & Mordhorst CH (1995) Evaluation of Abbott Test Pack RSV and an in-house RSV ELISA for detection of respiratory syncytial virus in respiratory tract aspirates. *APMIS* **103**, 416–418.

Selwyn BJ (1990) The epidemiology of acute respiratory tract infection in young children: comparison of findings from several developing countries. *Reviews in Infectious Diseases* **12** (**Suppl. 8**), S870–S888.

Stensballe LG, Kofoed PE, Nante EJ, Sambo M, Jensen IP & Aaby P (2000) Duration of secretory IgM and IgA antibodies to respiratory syncytial virus in a community study in Guinea-Bissau. *Acta Paediatrics* **89**, 421–426.

Treuhaft MW, Soukup JM & Sullivan BJ (1985) Practical recommendations for the detection of pediatric respiratory syncytial virus infections. *Journal of Clinical Microbiology* **22**, 270–273.

Wright PF & Cutts FT (1996) *Generic Protocol to Examine the Incidence of Lower Respiratory Infection (LRI) Due to Respiratory Syncytial Virus (RSV) in Children Less Than Five Years of Age*. World Health Organization, Geneva.