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Bacterial attachment *in vivo* to epithelial cells of the nasopharynx during otitis media with effusion

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Abstract
Epithelial cells were obtained by swabbing the posterior wall of the nasopharynx (NPH) of 15 patients (age one to $6^{8}/_{12}$ years; eight males, seven females) undergoing ENT-surgery under general anaesthesia for otitis media with effusion (OME). Individually matched, ear healthy children served as controls. Bacteria attached to the non-ciliated cells were calculated according to a method described. Furthermore, quantitative and qualitative bacteriological analyses were performed on samples obtained from mucoid middle ear effusion material as well as from the NPH. All patients and controls harboured middle ear pathogens (*S. pneumoniae, H. influenzae, B. catarrhalis,* or *S. aureus*) in the NPH. Only 33 per cent of the patients harboured middle ear pathogens in the middle ear effusion and the same pathogen was invariably found in the corresponding NPH. Attachment of bacteria to the non-ciliated cells of the NPH diminished significantly with growing age in the ear healthy control group but not in the OME group. OME is closely correlated to the presence of middle ear pathogens in the NPH and to attachment of bacteria to the epithelial cells in the NPH.

Introduction

The bacterial flora of the nasopharynx (NPH) is extremely motley, containing both harmless non-pathogens as well as pathogens. Among the non-pathogens alpha-hemolytic streptococci, Corynebacterium spp. and Niesseria spp. are predominant. Though the etiopathogenesis of otitis media with effusion (OME) is far from being solved, it has become increasingly evident that microorganisms (*Streptococcus pneumoniae, Haemophilus influenzae, Branhamella catarrhalis,* Group A Streptococci, *Staphylococcus aureus,* coagulase-negative Staphylococci) harboured in the NPH are intimately involved in this disorder (Howie and Ploussard, 1971; Ingvarsson *et al.*, 1982; Prellner *et al.*, 1984). This assumption is based on the observation that the recovery rates of the above-mentioned pathogens of the NPH and of the effusion material aspirated from the middle ear cavity (MEC) during current disease are closely correlated. Thus, the nasopharyngeal recovery rate of some of these pathogens appears to be almost 100 per cent during acute otitis media (AOM) (Kamme, 1971; Schwartz *et al.*, 1979) and 80 per cent during OME (Sundberg *et al.*, 1981; Stenfors and Räisänen, 1989a).

In 1988, Lim *et al.*, stated that 'a fundamental understanding of bacterial adherence is important for understanding the pathogenesis of all mucosal infections'. Bacterial attachment to mucosal cells is of the utmost importance for bacterial colonization and in most cases a prerequisite for invasive disease. It must be mentioned that conventional bacterial culturing does not differentiate between attached and non-attached bacteria. Only few studies have been published concerning attached bacteria to epithelial cells of the NPH *in vivo* (Lundberg and Lönnroth, 1979; Lundberg *et al.*, 1982a; Lundberg *et al.*, 1982b). We have recently introduced a method for evaluating attached bacteria to epithelial cells *in vivo* (Stenfors and Räisänen, 1990a). In brief the method is based on acridine orange staining of cellular material and examination under a fluorescence microscope.

The purpose of the present study was to chart the bacterial attachment *in vivo* to non-ciliated epithelial cells of the posterior wall of the NPH, obtained by scraping, during current OME disease. Furthermore, standard bacterial culturing as well as quantification of the bacteria obtained from the MEC and the NPH was performed according to methods described recently (Stenfors and Räisänen, 1988, 1989b; 1990b; 1990c). The results are discussed in relation to bacterial involvement in the pathogenesis of OME as well as to what extent antibiotic treatment can be expected to influence the course of the disease.

Material and methods

Fifteen patients, of whom eight were male, and representing 24 ears affected with OME, comprised the study material. The ages ranged between one year and six years eight months (Table I). Individually age and sex matched children without any history of otitis media were used as controls. All OME patients had suffered at least three to five attacks of AOM or treatment-resistant OME and one to two myringotomies with subsequent aspiration of effusion material had been performed prior to the actual examination. All samples were taken while the subjects were under general anaesthesia for some ENT surgery. At the

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TABLE I

AGE, SEX AND AFFECTED MIDDLE EAR OF THE OME PATIENTS INVESTIGATED

Pat.no.	Age (years)	Sex	Side
1	1	Male	Bilateral
2	$1^{2}/_{12}$	Female	Bilateral
3	$1^{3}/_{12}$	Male	Dexter
4	$1^{4}/_{12}$	Female	Dexter
5	$1^{6}/_{12}$	Female	Bilateral
6	$1^{7}/_{12}$	Female	Bilateral
7	$2^{7}/_{12}$	Female	Bilateral
8	4	Male	Bilateral
9	$4^{5}/_{12}$	Male	Dexter
10	$4^{7}/_{12}$	Male	Bilateral
11	$4^{9}/_{12}$	Male	Bilateral
12	$5^{1}/_{12}$	Male	Bilateral
13	$5^{9}/_{12}$	Female	Dexter
14	6	Male	Dexter
15	$6^{8}/_{12}$	Female	Sinister

time of sampling, none of the patients or controls was suffering from AOM, acute sinusitis, or acute tonsillitis, as judged by the physician responsible for the sampling (L-E. S.) and the anaesthetist. No antibiotics had been administered within two weeks prior to the examination.

Samples from the middle ear cavity

Prior to aspiration of effusion material from the MEC, the external auditory canal was meticulously cleansed from wax and detritus and washed twice with 70 per cent alcohol solution. After puncturing the drum under an operating microscope, effusion material (roughly 0.2 ml) was aspirated into a sterile 1 ml syringe. All OME ears contained a thick, glue-like, extremely hydrophilic effusion material.

Samples from the nasopharynx

The patient's mouth was held open and the tongue depressed with a Boyle-Davis tongue depressor. Samples were obtained from two separate areas on the posterior wall of the NPH. The soft palate was lifted and a sterile glass cylinder (ID = 1.3cm) was pressed against the posterior wall of the NPH. Two ml of physiological saline was added and a sterile-packed, cotton wool tipped wooden swab was dipped into the cylinder and used to swab the mucosa in the area delineated by the glass cylinder. The swab was then placed in a separate test tube together with the remaining physiological saline, which was aspirated from the glass cylinder with a pipette. The test tubes with the swab material and the syringes containing middle ear effusion were immediately transferred to the clinical laboratory for further processing.

TABLE II

PROPORTIONS OF NON-CILIATED EPITHELIAL CELLS OF THE POSTERIOR WALL OF THE NPH WITH NO ADHERENT BACTERIA, 1–10 ADHERENT BACTERIA, 11–50 ADHERENT BACTERIA AND MORE THAN 50 ADHERENT BACTERIA DURING CURRENT OME

	No. of bacteria adhering			
Pat.no.	None (%)	1–10 (%)	11–50 (%)	>50 (%)
1	72	20	6	2
2	28	26	30	16
3	36	26	6	32
4	74	20	6	0
5	48	16	12	24
6	20	12	36	32
7	50	22	18	10
8	94	6	0	0
9	58	26	12	4
10	48	20	32	0
11	40	16	16	28
12	16	34	24	26
13	50	16	20	14
14	96	4	0	0
15	82	18	0	0
X	54.1	18.8	14.5	12.5

The middle ear effusions were analyzed with regard to bacterial species (inoculation on blood and chocolate agar plates) and to quantify the bacteria found (the method is described in detail in Stenfors and Räisänen, 1988, 1990b).

The samples obtained from the NPH were analyzed with respect to bacterial species and colony-forming units (CFU) per cm^2 of the posterior wall of the NPH using blood agar and chocolate agar plates (the method is described in detail elsewhere, Stenfors and Räisänen, 1989b; 1990c). For evaluation of bacteria attached to the epithelial cells of the NPH, any epithelial cells adhering to the cotton swab were loosened by rinsing the swab with 8 ml physiological saline. The samples were then homogenized by extruding the cell mixture twice through a 20 G needle in order to disrupt chains and clumps. Each sample, consisting of roughly 10 ml liquid, was then filtered through a filter, 5 µm pore size (Sartorius, Ministart,® NML). The squamous, non-ciliated epithelial cells fastened on the filter, whereas leukocytes, red cells, ciliated epithelial cells, unfixed bacteria and mucus passed through. The squamous, non-ciliated epithelial cells were removed from the filter with physiological saline and centrifuged for 10 min at 1500 rpm. The supernatants were discarded and the precipitates were adjusted to 0.5 ml with physiological saline. The cellular mixture was spread evenly over glass slides and allowed to dry. The slides were stained with acridine orange and examined under a fluorescence microscope (Leitz Labrolux microscope with standard fluorescence equipment).

Interpretation of bacteria attached to epithelial cells

Fifty squamous, non-ciliated epithelial cells were

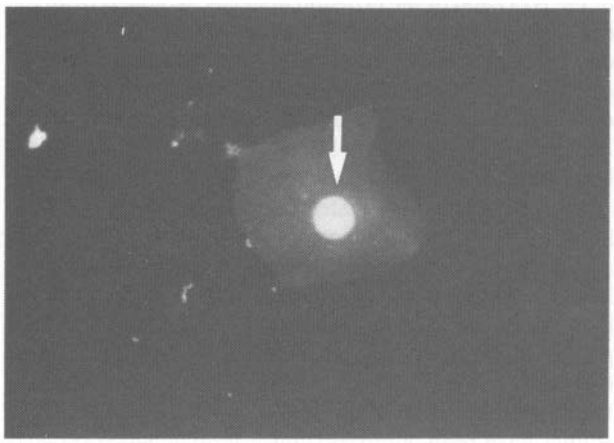

FIG. 1

Photograph of non-ciliated epithelial cell obtained by scraping the posterior wall of the NPH. The cell has no attached bacteria. Cellular nucleus (arrow). Acridine orange, ×1720.

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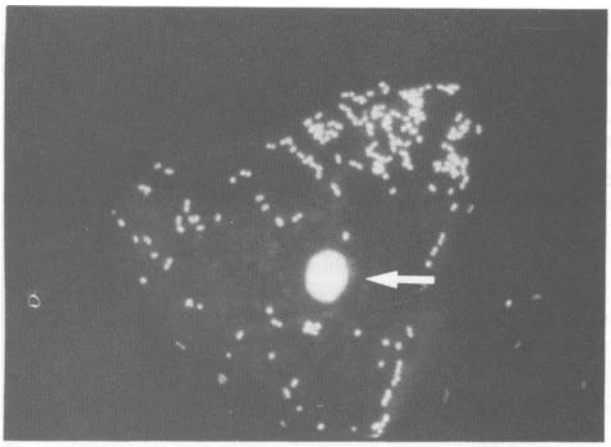

FIG. 2

Non-ciliated epithelial cell with 11–50 attached bacteria. Cellular nucleus (arrow). Acridine orange, ×2300.

examined and grouped according to the number of bacteria attached on a single cell, as follows: no adhering bacteria, 1–10, 11–50, more than 50 adhering bacteria. The method is described more in detail elsewhere (Stenfors and Räisänen, 1990a).

Statistical analysis

The findings on attached bacteria for OME patients *versus* controls were compared using the Wilcoxon signed rank test. To evaluate whether or not the number of attached bacteria depends on the subject's age, the Spearman rank order correlation method was used. p-Values below 0.05 were considered to be significant.

Results

Table II shows that 80 per cent (12/15) of the OME patients had non-ciliated epithelial cells on the posterior wall of the NPH, each with more than 10 attached bacteria. The corresponding figures for the individually matched ear healthy controls (Table IV) were 73 per cent (11/15). The difference was not significant ($p = 0.093$). Furthermore, 67 per cent (10/15) of the OME patients had epithelial cells with more than 50 attached bacteria. In the control group 60 per cent (9/15) reached these numbers. The difference was not significant. Fifty attached bacteria must be considered as representing a massive bacterial colonization on the cells. Patients nos. 3, 6, 11 and 12 had almost one third of the non-ciliated epithelial cells covered with bacteria (Fig. 3). On some of the non-ciliated epithelial cells, two or three morphologically different bacteria were seen. Thus both cocci and rods could be present on the same cell. The cocci-shaped bacteria could in some areas form microcolonies establishing clumps of bacteria. Patients nos. 8, 14 and 15 did not harbour any cells having more than 10 attached bacteria (Fig. 1).

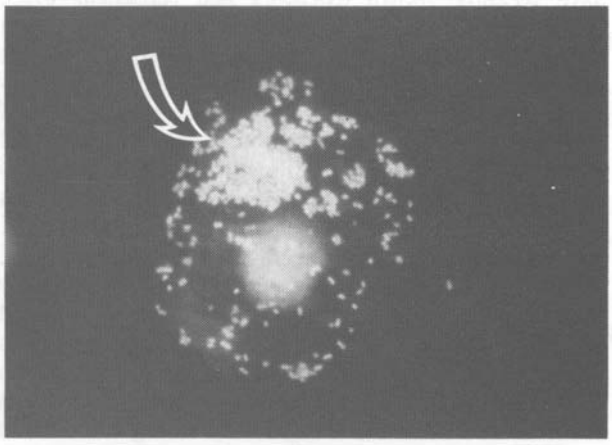

FIG. 3

Non-ciliated epithelial cell with >50 attached bacteria. Note the microcolony (open arrow) on the cell surface. Acridine orange, ×2300.

In the control group (Table IV) the attachment of bacteria decreased with the subject's increasing age. Thus, attachment of more than 10 bacteria per epithelial cell and especially of more than 50 bacteria per cell decreased significantly with advancing age ($p = 0.0026$ and $p = 0.001$). For the OME group there was no age-correlated reduction in bacterial adhesion to the epithelial cells of the NPH. ($p = 0.71$ for >10 attached bacteria per cell and $p = 0.34$ for >50 attached bacteria per cell, respectively).

According to Table III only 33 per cent (5/15) of the patients exhibited growth of pathogens in the middle ear effusion at culturing. When present in the middle ear cavity, the pathogens were rather few and did not exceed 2.0×10^5 bacterial per ml effusion material. The same bacterial species appeared in the NPH in every case. Even every individual of the control group harboured any of the main middle ear pathogens in their NPH.

Patient no. 8 showed no growth of the main middle ear pathogens (*S. pneumoniae, H. influenzae, B. catarrhalis*) in the NPH on culturing. In this case *S. aureus* was growing alongside non-pathogens. This patient did not harbour any non-ciliated cells with more than 10 bacteria attached. Usually only one pathogenic species was present in the NPH but patient 11 hosted three and patients 13 and 14 two different pathogens, simultaneously. Patient 15 had a massive growth of *B. catarrhalis* in the NPH and also growth of *B. catarrhalis* in the middle ear cleft simultaneously. However, no conclusive attachment of bacteria to the non-ciliated epithelial cells was noted.

The number of middle ear pathogens swabbed from the posterior wall of the NPH ranged between 2.2×10^6 and 1.1×10^9 colony forming units (CFU) per cm^2 of the nasopharyngeal area. Not a single case of bacterial attachment of ciliated cells of the NPH could be noted.

Discussion

It must be emphasized that conventional culture methods do not distinguish between attached and non-attached bacteria when swab samples are taken from mucosal membranes. Though middle ear pathogens were found at conventional culturing in all samples obtained from the NPH of both groups, it cannot be denied that some of the bacteria found attached to the epithelial cells were non-pathogens or anaerobic bacteria which do not grow on ordinary agar plates. For conclusive species identification of the attached bacteria, other methods must be used. Such studies are already in progress in our laboratories.

The values given for attached bacteria to non-ciliated epithelial cells of the NPH in the present study must be

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TABLE III

PROPORTION OF NON-CILIATED EPITHELIAL CELLS ON THE POSTERIOR WALL OF THE NPH WITH MORE THAN 10 BACTERIA ADHERING AND BACTERIAL FINDINGS IN THE MIDDLE EAR CAVITY (PATHOGEN, AND NUMBER/ML EFFUSION MATERIA) AND OF THE NPH (PATHOGEN, AND CFU/CM^2)

		Bacterial findings			
Pat. no.	Cells with >10 bact. (%)	Middle ear cavity (pathogen, bact/ml)		Nasopharynx (pathogen, CFU/cm^2)	
1	8	–		*B. catarrhalis*	4.5×10^8
2	46	–		*S. pneumnniae*	3.2×10^7
3	40	–		*S. pneumoniae*	5.0×10^8
4	6	–		*H. influenzae*	5.3×10^7
5	36	–		*S. pneumoniae*	6.0×10^7
6	68	dx. *H. influenzae*	2.0×10^5	*H. influenzae*	2.8×10^8
		sin. *H. influenzae*	1.0×10^5		
7	30	dx. *H. influenzae*	8.0×10^4	*H. influenzae*	5.0×10^7
		sin. *H. influenzae*	3.0×10^4		
8	0	–		*S. aureus*	3.2×10^6
9	16	–		*B. catarrhalis*	1.5×10^8
10	32	–		*S. pneumoniae*	1.1×10^9
11	44	dx. *S. pneumoniae*	2.0×10^4	*H. influenzae*	8.0×10^7
		sin.	–	*B. catarrhalis*	1.0×10^7
				S. pneumoniae	5.1×10^8
12	50	–		*B. catarrhalis*	2.2×10^6
13	34	*S. pneumoniae*	1.0×10^4	*B. catarrhalis*	1.2×10^8
				S. pneumoniae	5.1×10^7
14	0	–		*B. catarrhalis*	6.1×10^7
				H. influenzae	3.5×10^6
15	0	*B. catarrhalis*	4.5×10^4	*B. catarrhalis*	8.5×10^8

regarded as minimal values, as some of the bacteria must have been lost during the preparation procedure, ie during scraping of the nasopharyngeal mucosa, centrifugation of the cell mixture, etc. We regarded 10 bacteria as the minimum for true attachment, as values below ten may represent bacterial attachment to cells by pure chance.

The present study undoubtedly showed that OME is closely related to the presence of middle ear pathogens in the NPH and to the attachment of bacteria to non-ciliated epithelial cells of the NPH. We have recently shown that the occurrence of middle ear pathogens in the NPH (Stenfors and Räisänen, 1990c) as well as the attachment of bacteria to nasopharyngeal cells (Stenfors and Räisänen, 1991) diminish with growing age. However, only 33 per cent (5/15) of the patients harboured middle ear pathogens in middle ear effusions. This finding is very similar to results of other studies (Sundberg *et al.*, 1981; Stenfors and Räisänen, 1989a). The same bacterial species grew simultaneously both in the middle ear cavity and in the NPH. Against this background, it is arguable that the source of the pathogens found in the MEC is the NPH.

The relationship between the mucoid effusion material without live bacteria in the MEC, and the middle ear pathogens of the NPH was hitherto obscure. In our study, 93 per cent (14/15) of the patients harboured the main middle ear pathogens in their NPH, whereas only 33 per cent harboured them in the middle ear cavity. Mediators of inflammation such as histamine, kinins, proteases and prostaglandins have been demonstrated in middle ear effusions in addition to lysozyme, antibacterial antibodies of IgA, IgG, and IgM, complement activity and evidence of the presence of immunocomplexes (Veltri and Sprinkle, 1973; Virtanen and Lahikainen, 1979; Carlsson *et al.*, 1982; Juhn, 1982; Lim and DeMaria, 1982). We have recently shown (Stenfors and Räisänen, 1988, 1990b) that when middle ear pathogens are present in the MEC during a current OME, their number is scant, not exceeding 5×10^5 bacteria per ml effusion. These findings may give support to the belief that small numbers of middle ear pathogens continuously invade the MEC (Kamme and Nilsson. 1984), though this theory is contradicted by the difficulty of detecting bacterial constituents in thick, highly viscous mucoid effusion material.

Interestingly enough, a recent study by Granfors *et al.* (1989) demonstrates the presence of chlamydia and/or yersinia antigens in synovial tissue and synovial-fluid cells during reactive arthritis. In these cases the infectious focus was not known. If parallels are to be drawn between this study and the OME disease, however, the infectious focus in the latter must be regarded as being the NPH. In these cases middle ear pathogens, firmly attached to the epithelial cells, are no doubt of the utmost importance as these bacteria, in particular, are capable of colonizing, releasing toxins and spreading. Otitis media in general is a

TABLE IV

PROPORTIONS OF NON-CILIATED EPITHELIAL CELLS OF THE POSTERIOR WALL OF THE NPH WITH NO ADHERENT, 1–10 ADHERENT, 11–50 ADHERENT BACTERIA AND MORE THAN 50 ADHERENT BACTERIA IN THE INDIVIDUALLY MATCHED HEALTHY PERSON

	No. of bacteria adhering			
Indiv. no.	None (%)	1–10 (%)	11–50 (%)	>50 (%)
1	42	30	6	22
2	52	30	10	8
3	14	18	26	42
4	32	20	24	24
5	60	16	10	14
6	28	28	30	14
7	10	10	16	64
8	86	14	0	0
9	86	10	4	0
10	46	44	2	8
11	80	8	6	6
12	94	6	0	0
13	92	8	0	0
14	96	4	0	0
15	90	8	2	0
X	60.5	16.9	9.1	13.5

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disease closely associated with the young. This particular phenomenon must be closely correlated to the adherence potential of these microorganisms to the epithelial cells. Further evidence in this direction offered the present study. Attachment of bacteria to the epithelial cells of the NPH diminished significantly with growing age in the healthy control group but not in the OME group.

The rationale for antimicrobial therapy in OME is based on the assumption that this disease is caused by bacterial infection. The present investigation and other studies (Sundberg *et al.*, 1981; Stenfors and Räisänen, 1989a) have shown that bacteria can be found in the effusion material in roughly 30 per cent of the cases. However, the incidence of middle ear pathogens in the NPH is much greater (Stenfors and Räisänen, 1990c), though this incidence is highly age-dependent. The benefit of antibiotics in children with OME as seen in some double-blind, randomized, placebo-controlled studies (Principi *et al.*, 1989; Thomsen *et al.*, 1989) must be attributed mainly to effects upon the bacterial flora of the NPH. However, the antibiotic action seems to be only partial and extremely short-lived. This circumstance may be due to the fact that some nasopharyngeal bacteria are only present in the mucus film covering the epithelium and not attached to epithelial cells at all. Our findings suggest that *B. catarrhalis* may well be such a microorganism. It is obvious that more penetrating studies regarding the true effects of antibiotic treatment on bacterial adherence to epithelial cells need to be performed. Such studies are already in progress in our laboratories.

References

Carlsson, B., Lundberg, C., Ohlson, K. (1982) Granulocyte proteases in middle ear effusions. *Annals of Otology, Rhinology and Laryngology,* **91:** 76–79.

Granfors, K., Jalkanen, S., Von Essen, R., Lahesmaa-Rantala, R., Isomäki, O., Pekkola-Heino, K., Merilahti-Palo, R., Saario, R., Isomäki, H., Toivanen, A. (1989) Yersinia antigens in synovial-fluid cells from patients with reactive arthritis. *New England Journal of Medicine,* **320:** 216–221.

Howie, V. M., Ploussard, J. H. (1971) Simultaneous nasopharyngeal and middle ear cultures in otitis media. *Pediatric Digest,* **2:** 31–35.

Ingvarsson, L., Lundgren, K., Ursing, J. (1982) The bacterial flora in the nasopharynx in healthy children. *Acta Otolaryngologica,* **Supplement 386:** 94–96.

Juhn, S. (1982) Studies on middle ear effusions. *Laryngoscope,* **92:** 287–291.

Kamme, C. (1971) The aetiology of acute otitis media in children and the relationship between bacterial characters and the clinical course in penicillin V therapy. Thesis, Lund, Sweden.

Kamme, C., Nilsson, N.-I. (1984) Secretory otitis media. Microbiology of the middle ear and the nasopharynx. *Scandinavian Journal of Infectious Diseases,* **16:** 291–296.

Lim, D. J., DeMaria, T. (1982) Pathogenesis of otitis media. Bacteriology and immunology. *Larynoscope,* **92:** 278–286.

Lim, D. J., DeMaria, T. F., Bakaletz, L. O. (1988) Current concepts of pathogenesis of otitis media: A review. *Acta Otolaryngologica,* **Supplement 458:** 174–180.

Lundberg, C., Lönnorth, J. (1979) Bacterial adherence to epithelial cells in the nasopharynx in children. *Acta Otolaryngologica,* **88:** 438–442.

Lundberg, C., Lönnroth, J., Nord, C. E. (1982a) Adherence in the colonization of Streptococcus pneumoniae in the nasopharynx in children. *Infection,* **10:** 63–66.

Lundberg, C., Lönnorth, J., Nord, C. E. (1982b) Identification of attached bacteria in the nasopharynx of the child. *Infection,* **10:** 58–62.

Prellner, K., Christensen, P., Hovelius, B., Rosén, C. (1984) Nasopharyngeal carriage of bacteria in otitis-prone and non-otitis prone children in day-care centres. *Acta Otolaryngologica,* **98:** 343–350.

Principi, N., Marchisio, P., Massironi, E., Grasso, R. M., Filiberti, G. (1989) Prophylaxis of recurrent acute otitis media and middle-ear effusion. *American Journal of Diseases of Children,* **143:** 1414–1418.

Schwartz, R., Rodriguez, J., Mann, R., Khan, W. (1979) The nasopharyngeal culture in acute otitis media. A reappraisal of its usefulness. *Journal of American Medical Association,* **241:** 2170–2173.

Stenfors, L.-E., Räisänen, S. (1988) Quantification of bacteria in middle ear effusions. *Acta Otolaryngologica,* **106:** 435–440.

Stenfors, L.-E., Räisänen, S. (1989a) Colonization of middle ear pathogens in the nasopharyngeal opening of the Eustachian tube during secretory otitis media. *Acta Otolaryngologica,* **107:** 104–110.

Stenfors, L.-E., Räisänen, S. (1989b) The bacterial flora of the nasopharynx, with special reference to middle ear pathogens. A quantitative study in 20 children. *Acta Otolaryngologica,* **108:** 122–125.

Stenfors, L.-E., Räisänen, S. (1990a) Age-dependent changes in bacterial adherence to epithelial cells of nasopharynx in vivo. *Acta Otolaryngologica,* **110:** 292–299.

Stenfors, L.-E., Räisänen, S. (1990b) Quantitative analysis of the bacterial findings in otitis media. *Journal of Laryngology, and Otology,* **104:** 749–757.

Stenfors, L.-E., Räisänen, S. (1990c) Occurrence of middle ear pathogens in the nasopharynx of young individuals. A quantitative study in four age groups. *Acta Otolaryngologica,* **109:** 142–148.

Stenfors, L. -E., Räisänen, S. (1991) Is attachment of bacteria to the epithelial cells of the nasopharynx the key to otitis media? *International Journal of Pediatric Otorhinolaryngology,* **22:** 1–8.

Sundberg, L., Cederberg, Å, Edén, T., Ernstson, S. (1981) Bacteriology in secretory otitis media. *Acta Otolaryngologica,* **Supplement 384:** 18–25.

Veltri, R., Sprinkle, P. (1973) Serous otitis media. Immunoglobulin and lysozyme levels in middle ear fluids and serum. *Annals of Otology, Rhinology and Laryngology,* **82:** 297–301.

Virtanen, S., Lahikainen, E. (1979) Lysozyme activity and immunoglobulins in middle ear effusion fluids in acute purulent otitis media and otitis media with effusion. *Scandinavian Journal of Infectious Diseases,* **11:** 63–67.

Thomsen, J., Sederberg-Olsen, J., Balle, V., Vejlsgaard, R., Stangerup, S.-E, Bondeson, G. (1989) Antibiotic treatment of children with secretory otitis media. A randomized, double-blind, placebo-controlled study. *Archives of Otolaryngology, Head and Neck Surgery,* **115:** 44–51.

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