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Rhinopharyngeal bacterial flora in 3 to 5 years old children from Yaoundé (Cameroon): Effects of extrinsic factors

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We studied the composition, the factors influencing bacterial carriage in children rhinopharynx, and the susceptibility to antibiotics of some isolated strains. Rhinopharyngeal swabs were collected from 150 pupils aged between 3 and 5 years and submitted to qualitative and quantitative analysis using standard methods. The antibiotic sensitivity of potential pathogenic species was evaluated using disc diffusion. Factors influencing bacterial carriage were determined, using many types of statistical tests. Unclassified *Streptococcus* were present in all the samples and at the highest concentration (2.17 10^8 CFU.ml^{-1}). *Staphylococcus epidermidis*, *Micrococcus spp*. and *Staphylococcus aureus* were found in 25 to 50% samples. Conversely, less than 5% of children carried either *Neisseria lactamica*, negative or positive Gram-bacilli. *S. aureus* carriage was significantly influenced by overcrowding and the frequent use of antibiotic while *S. epidermidis*, *Micrococcus spp.* and unclassified *Streptococcus* were affected by the children age and/or the season. *S. aureus* was sensitive to Oxacillin, Vancomycin, Gentamycin and Cotrimoxazole.

Key words: Rhinopharyngitis, commensal bacteria, children, antibiotics.

INTRODUCTION

Rhinopharyngitis are viral infections, resulting from the inflammation of the nasal and pharyngeal mucous membrane usually assimilated to global seizures of tracheo-bronchial mucous, sinusal membrane and middle ear (Kernbaun, 1998). They are frequently encountered in children under 6 years old in Western countries (Perronne, 1999). In Cameroon, rhinopharyngitis has become as population increases, a significant public health concern. Studies addressing the issue of rhinopharyngitis have been focused mainly on pathogenic bacteria, and so far, very little interest has been given to the commensal species. The 2003 annual report of the Cameroonian Mother and Child Health Centre's (CMCH) activities classified rhinopharyngitis as the first (15.2%) and second (18.6%) cases of emergencies and ambulatory respectively.

Generally, rhinopharyngitis are not severe except in the

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cases in which they are associated with secondary infections caused by the commensal bacterial flora from the rhinopharynx. The decrease of the host immunity in the course of a viral infection gives an opportunity to commensal bacteria to express their pathogenic potential, since these micro-organisms are able to migrate and to cause otitis, sinusitis, pneumonias or conjunctivitis. Therefore, it is important to know the composition of the rhinopharynx normal flora in order to facilitate the treatment of rhinopharyngitis and secondary infections (Perronne, 1999). The present study aimed at determining the qualitative and quantitative composition of the commensal bacterial flora that is found in the rhinopharynx of children as well as the factors influencing their distribution. In addition, owing to the fact that the comercialization and the consumption of antibiotics are widely spread and poorly controlled throughout the country, the antimicrobial susceptibility of potentially pathogenic strains of some species was also assessed.

MATERIALS AND METHODS

The studied population was recruited among children attending the Messa public nursery school in Yaoundé, after their agreement and consent of their parents or legal guardians; in compliance with administrative authorities and ethical considerations. The sample consisted of 150 pupils in good health, from 3 to 5 years old. The recruitment was conducted between January and May 2004. A questionnaire was distributed to their parents in order to obtain the following information: number of individuals in the household, number of rooms occupied by children, location of homes, feeding habits, medical history including details on drugs taken during rhinopharyngitis, availability of facilities such as running water, electricity, refrigerator, and home description. Children with any symptom of cold, nasal obstruction, sore throat, cough, fever or visible illness (whitlow, abscess, boil), as well as those who have received antibiotic treatment within the two weeks before enrolment were not eligible.

Collection and processing of samples

The rhinopharyngeal samples were collected via the pharyngeal cavity using cotton typed with flexible wire. The swabs were immediately transported to the laboratory, using a refrigerated box and processed.

Qualitative analysis

Each swab was agitated in 0.30 mL of sterile physiological saline solution before being plated on four types of selective media (chocolate agar enriched with polyvitex, a selective medium for several microorganisms; Chapman agar selective for *Staphylococcus* species; chocolate agar added with polyvitex and Vancomycin-Colimycin-Nystatin (VCN) selective for *Neisseria* species; blood agar added with Nalidixic Acid-Colimycin (NAC) selective for *Streptococcus*). The first two media were incubated aerobically at 37°C for 24 to 48 h, whereas the other media were incubated in a candle jar containing 5% CO_2 under the same conditions of time and temperature. Isolates were identified using colony morphology and conventional methods of determination (Api 20 strep (bioMerieux), Api *Neisseria-Haemophilus).* The percentage of bacteria's carriage in samples was calculated (Beytout, 1989).

Quantitative analysis

The sample bacterial concentration was determined according to Fauchère and April (2002). The results were expressed as colony forming unit per milliliter (CFU/ml).

Antibiogramme

The antimicrobial sensitivity of potentially pathogenic species was studied using standardized disc diffusion method with Mueller-Hinton agar (bioMerieux), in conformity with the National Committee for Clinical Laboratory's (NCCL) standard guidelines. Susceptibility to seven antibiotics were tested, namely penicillin G, oxacillin, gentamycin, vancomycin, erythromycin, tetracycline and cotrimoxazole.

Data analysis

The obtained data were compared, using Chi-square ($\chi 2$) and Wilcoxon tests. To compare more than two values, either one-way ANOVA or Kruskal-Wallis was used, following by Student or Mann-Whitney tests.

RESULTS

Qualitative and quantitative analysis of bacterial flora

Unclassified *Streptococcus* was found in all the 150 children sampled in the study. Among them, 77 (51.3%), 55 (36.6%) and 38 (25.3%) harbored *S. epidermidis, Micrococcus spp*. and *S. aureus* respectively. Three other taxa were found, but at relatively low frequencies namely Gram-negative bacilli, *Neisseria lactamica* and Gram-positive bacilli in 6 (4%), 4 (2.6%) and 3 (2%) children respectively.

The unclassified *Streptococcus* mean concentration on swabs ($2.17x\ 10^8 \pm 8.55x\ 10^8$ CFU/ml), was 2 to 3 fold higher compared to *S. epidermidis* ($0.94 \times 10^8 \pm 9.29 \times 10^8$), *Micrococcus spp*. ($0.83 \times 10^8 \pm 2.48 \times 10^8$ CFU /mL) and *S. aureus* ($0.66 \times 10^8 \pm 1.27 \times 10^8$ CFU/ml) as shown in Table 1. The other species were quite rare to be counted.

Effects of factors on the bacterial carriage and concentrations

Age

Gram-positive bacilli and *N. lactamica* were not recorded in 3 years old children. Unclassified *Streptococcus* was observed at similar rates (100%) in children from 3 to 5 years old. For *S. aureus*, *S. epidermidis* and *Micrococcus spp*., the tendency was the reduction of the carriage with age (Table 2). There was a significant relation between age and concentration for unclassified *Streptococcus* and *Micrococcus spp.* ($p < 0.05$), (Table 3).

Gender

No significant difference ($p > 0.05$) was found between gender and bacterial carriage (Table 4) nor gender and

Table 1. Bacterial carriage and concentrations.

Specie	n	M ±SD x 10^8
Unclassified *Streptococcus*	150	2.17 ±8.55
Staphylococcus epidermidis	77	0.94 ±9.29
Micrococcus spp.	55	0.83 ±2.48
Staphylococcus aureus	38	0.66 ±1.27

M: Mean concentration in CFU/ml; n: number of children carrying the bacterial species; SD : standard deviation.

Table 2. Relation between age and bacterial carriage.

Specie	N	Age			Difference between age
		3	4	5	
		19	46	85	
S. aureus	n	7	12	19	NS
	%	36.8	26.1	22.4	
S. epidermidis	n	13	24	40	NS
	%	68.4	52.2	47.1	
Micrococcus spp.	n	9	17	27	NS
	%	47.4	37	31.1	
Unclassified *Streptococcus*	n	19	46	85	NS
	%	100	100	100	
Gram negative Bacilli	n	3	2	1	ND
	%	15.8	4.43	1.2	
N. lactamica	n	-	2	2	ND
	%	-	4.3	2.4	
Gram positive bacilli	n	-	2	1	ND
	%	-	4.3	1.2	

N: Number of children at a certain age; n: number of children at a certain age carrying a given bacteria; ND: not determined; NS: statistically non significant; %: percentage.

bacterial density, (Table 5).

Bed-sharing

The bacterial carriage rate was not influenced by bed sharing for all the isolates except for *S. aureus* that was found to be significantly more frequent in children sharing the bed ($p < 0.05$, Table 4). This factor modulated significantly only, the concentration of *S. epidermidis*, (Table 5).

Season

Staphylococcus epidermidis was significantly more isolated during the dry season, and *Micrococcus spp.* during the rainy one ($p<0.05$) (Table 4). Quantitatively, there was a trend for *S. aureus*, *S. Epidermidis, Micrococcus spp.* and unclassified *Streptococcus* to be present in higher concentrations in nasopharyngeal swabs during the rainy season (Table 5).

Regular use of antibiotics

The overuse of antibiotics enhanced the carriage of *S. aureus* ($p < 0.05$), (Table 4). Quantitatively, there was a trend for children who did not take antibiotics frequently to concentrate more bacteria than those who did (Table 5); these differences were significant for *Micrococcus spp.* and unclassified *Streptococcus* ($p < 0.05$).

Table 3. Relation between age and bacterial concentrations.

Specie	N	Age			Difference between age
		3	4	5	
		19	46	85	
S. aureus	n		1	8	ND
	M ± SD x 10^8	-	0.07	1.05±2.43	
S. epidermidis	n	5	11	24	NS
	M ± SD x 10^8	2.71±3.83	0.28±0.32	0.70±2.63	
Micrococcus spp.	n	6	9	15	S
	M ± SD x 10^8	0.94±1.46	1.32± 1.80	0.15±0.37	
Unclassified *Streptococcus*	n	14	33	65	S
	M ± SD x 10^8	2.55±5.22	2.16±5.64	2.10±10	

M : Mean concentration in CFU/ml; N: number of children at a certain age; n: number of children at a certain age carrying a given bacteria; ND: not determined; NS : statistically non significant; S : statistically significant; SD : standard deviation.

Table 4. Relations between risk factors and bacterial carriage**.**

Specie	N	Sex		Bed sharing		Season		ATB use	
		Ma	Fe	Yes	No	Dry	Rainy	Yes	No
		81	69	116	34	75	75	31	94
S. aureus	n	17	21	35	3	21	17	26	5
	%	21	30.4	30.2	8.8	28	22.6	83.9	5.31
	Difference	NS		S		NS		S	
S. epidermidis	n	37	40	60	17	52	25	19	53
	%	45.7	58	51.7	50	69.3	33.3	61.3	56.3
	Difference	NS		NS		S		NS	
Micrococcus spp.	n	35	20	45	10	16	39	10	37
	%	43.2	29	38.8	29.4	21.3	52	32.2	39.3
	Difference	NS		NS		S		NS	
Unclassified *Streptococcus*	n	81	69	116	34	75	75	31	94
	%	100	100	100	100	100	100	100	100
	Difference	NS		NS		NS		NS	
Gram negative bacilli	n	6	-	4	2	1	5	6	-
	%	7.4	-	3.4	5.9	1.3	6.6	19.3	-
	Difference	ND		ND		ND		ND	
N. lactamica	n	3	1	3	1	1	3	-	4
	%	3.7	1.4	2.6	2.9	1.3	4	-	4.2
	Difference	ND		ND		ND		ND	
Gram positive bacilli	n	1	2	3	-	-	3	2	-
	%	1.2	2.9	2.6	-	-	4	6.4	-
	Difference	ND		ND		ND		ND	

N: Number of children with a certain risk factor; n: number of children with a certain risk factor carrying a given bacteria; ND: not determined; NS: statistically non significant; %: percentage; S: statistically significant.

Table 5. Relations between risk factors and bacterial concentrations

Parameter		*Staphylococcus aureus*		*Staphylococcus epidermidis*		*Micrococcus spp.*		Unclassified *Streptococcus*	
		n	M±SDx10^8	n	M ±SDx10^8	n	M±SDx10^8	n	M±SDx 10^8
Gender	Male	3	2.4 ±4.0	18	1.6 ±3.6	23	0.48±1.1	60	1.7 ±5.1
	Female	6	0.23±0.43	22	0.22±0.28	7	1.2 ±1.7	52	2.8 ±11
	Difference		NS		NS		NS		NS
Bed sharing	Yes	8	1.1 ±2.4	31	1.0 ±2.8	25	0.69 ±1.3	85	2.33 ±9.52
	No	1	0.02	9	0.11±0.24	5	0.53 ±0.65	27	1.02 ±2.39
	Difference		NS		S		NS		NS
Season	Dry	4	0.28 ±0.55	34	0.3±0.67	6	0.004 ±0.01	46	0.77±4.57
	Rainy	5	1.5 ±3.1	6	3.9±0.57	24	0.8 ±1.4	66	3.2 ±10
	Difference		NS		NS		NS		NS
ATB use	Yes	9	0.94±2.29	6	0.13±0.16	6	0.038 ±0.05	16	0.035±0.05
	No	-	-	20	1.48 ±3.42	18	7.68 ±18.8	25	2.24±4.14
	Difference		-		NS		S		S

ATB: Antibiotic; M: mean concentration in UFC/ml; n : number of children at a certain risk factor ; NS : non significant ; S : significant; SD : standard deviation.

Antibiotic sensitivity of *S. aureus*

The majority of *S. aureus* isolates was sensitive to vancomycin (100%), Oxacillin (86.96%), gentamycin (86.96%) and to a lesser extent to cotrimoxazole (69.56%). It was resistant to erythromycin (52.17%) and to tetracyclin (73.91). All the isolates were resistant to Penicillin G.

DISCUSSION

According to our results, many opportunistic bacteria commonly found in the nasal and pharyngeal membrane could be responsible of a range of harmful diseases (otitis, sinusitis, pneumonias or conjunctivitis) which frequently occur with rhinopharyngitis (Garcia–Rodriguez and Martinez, 2002; Lieberman et al., 2006). The isolated bacteria have been already identified from young children worldwide (Wolf et al., 1999; Chien et al., 2013)). The different rates of some bacterial carriage were previously reported (*S. aureus* isolated in 28.4% of Turkish infants (Ciftci et al., 2007), and Gram negative bacilli found in 50% of Brazilian, 57% of Angolan, and 4% of Dutch children (Wolf et al., 1999)). It should be noticed that, most of the studies carried out in rhinopharyngeal bacterial flora were focused on the carrier rate of the main potentially pathogenic species because of their clinical impact. So, *Haemophilus influenza*, *S. pneumonia* and *Moraxella catarrhalis* were isolated *S. pneumonia* by Cohen et al. (2012) and Xu et al. (2012). The absence of these bacteria in this study could be due to the fact that the children were healthy. In this study an unfair association of bacteria species was noticed in children; an average of 21% of the studied population was carrying unclassified *Streptococcus* and *Micrococcus spp.* or *S. epidermidis,* whereas the proportion of children carrying both *Micrococcus spp.* and *S. epidermidis*, *S. aureus and S. epidermidis*, or more than two species was below 10%. This result suggests that unclassified *Streptococcus* better tolerate cohabitation with other bacterial species. It can also be hypothesized that some bacterial species occupy well defined localizations in the rhinopharynx. This issue has been insufficiently explored, and should be monitored with a large sample. In cases of bispecific association between *S. aureus* and *S. epidermidis*, it has been mentioned an inhibition of *S. aureus* whenever *S. epidermidis* concentration increases above a certain threshold. Lina *et al*. (2003) noticed a 6 to 10 fold decrease of *S. aureus* concentration when *S. epidermidis* density increased in the medium from 10^3 to 10^5 CFU/ml. In this work, the threshold was determined to be a concentration equal or more than 5.10^4 CFU/ml. The production of bacteriocins, the suppression of adhesion and/or the reduction of nutrients in the medium may explain this phenomenon.

The analysis of conditions susceptible to influence the acquisition or carriage of bacteria by children revealed no effect of age on the presence of unclassified *Streptococcus*, *N. lactamica* and Gram positive bacilli while carriage of *S. aureus*, *S. epidermidis*, *Micrococcus spp.* and Gram-negative bacilli decreased with age. This fact could be explained by the maturation of the children immunelogical system as they are growing, that makes more difficult to potential pathogens to establish in the rhinopharyngeal epithelial cells. The gender of children did not interact with bacterial carriage as shown by Hilty et al.

(2012). Overcrowding was facilitating horizontal transfer of bacteria among children. It was found that kids who shared their bed with other children carried more often *S. aureus* than those who slept alone. The carriage of *Micrococcus spp.* and *S. epidermidis* varied with the seasons. *S. epidermidis* was more frequent in children during the dry season. A similar variation had been previously reported by Lagrange (1989) who observed that about 50% of children carried this bacterium during the summer period and only 21% in winter. *Micrococcus spp.* was in contrast more frequent during the rainy season. It is possible that some viral infections which prevail in the rainy season boost the carriage of this species. The regular use of antibiotics appeared to increase significantly the carriage of some species namely Gram negative bacilli and *S. aureus*. Species with lower pathogenic potential (*S. epidermidis*, *Micrococcus spp.* and unclassified *Streptococcus*) were in contrast frequent in children who took rarely antibiotics. A high antibiotics pressure resulting from a regular absorption of drugs is able to destroy or modify the normal sensitive bacterial flora of the rhinopharynx, favoring the multiplication of some resistant bacteria.

Conclusion

Out of the seven bacteria that were isolated in the rhinopharyngeal flora of children in Yaoundé, Unclassified *Streptococcus* was the more frequent, followed by *S. epidermidis, Micrococcus spp*. and *S. aureus.* The concentration of these bacteria was proportional to the carriage rate. The age of the children had an influence on the carriage of the majority of bacteria species; the influence of season was restricted to *Micrococcus spp*. and *S. epidermidis,* while the carriage of *S. aureus* was also conditioned by socio-economic determinants (the regular use of antibiotics and bed sharing). Potentially pathogenic species as *S. aureus* remained sensitive to the most antibiotics tested.

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