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Effect of Antibiotic Exposure on Upper Respiratory Tract Bacterial Flora

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Background: The human microbiota modulates the immune system and forms the surface flora. Antibiotic administration causes dysbiosis in the intestinal flora. It is not clear if antibiotic administration in the community effects the upper airway flora in the mid-term or long-term. This study aims to define long-term influence of antibiotics on upper airway flora.

Material/Methods: In this prospective study, aerobic microbiological analysis of nasal and nasopharyngeal surfaces was performed. Antibiotic administration history of the last 6 months was retrieved using the social insurance database. Culture results of antibiotic-treated and antibiotic-naïve subjects were compared by Pearson's chi-square test or Fisher's exact test.

Results: A total of 210 subjects were included in the study. Normal flora were documented in 86 nasal swabs and 99 nasopharyngeal swabs. Most of the remaining cases demonstrated gram-positive bacterial overgrowth. There were 113 subjects who did not receive any antibiotic, and 93% of the remaining 97 patients received broad-spectrum antibiotics. Statistical analysis showed that nasal and nasopharyngeal flora did not change upon antibiotic administration, but antibiotic administration during the last month caused increased methicillin resistance development of coagulase-negative *Staphylococcus* and *Staphylococcus aureus* microorganisms.

Conclusions: Antibiotic exposure did not lead to perturbations in general composition of upper airway flora within 6 months, although the incidence of methicillin resistance in coagulase-positive and -negative *Staphylococci* demonstrated significant increases when patients received antibiotic during the last month. This should be considered in case of broad-spectrum antibiotic administration, since methicillin resistance increases the morbidity and mortality of nosocomial *Staphylococcus* infections.

Keywords: Antibiotics M-4365 • Bacteriology • Paranasal Sinuses • Sinusitis

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Background

The human body is inhabited by a huge number of microorganisms inside and out, called the microbiota. Most of these organisms reside in the gastrointestinal system, and the upper aerodigestive tract, eyes, skin, and mucosal surfaces are colonized by their own microflora. There is a mutual symbiotic relationship between the host and the microbiota. The host provides a milieu for the microorganisms necessary for their survival. In turn, these commensal microbes contribute to the immune system and intestinal food absorption of their host [1]. Commensal microorganisms exert profound effects on the immune system [2]. Altered proportion and function of these organisms result in disease states such as infections, autoimmune diseases, atherosclerosis, and cancer [3]. The association between host and microbiota is so intermingled that they can be considered as an additional organ of the human body. Bacterial microbiota have an additional protective role against infections by preventing colonization by pathogenic organisms at different body sites [4]. Alteration of this protective barrier makes the host more vulnerable to harmful bacterial invasions. Maintainance of the integrity of the beneficial microbiota is crucial for avoidance of diseases.

The nasal cavity is an entry point of environmental microorganisms into the airway. Adjacent structures of the upper respiratory tract, including the nasal cavity, nasopharynx, and oral cavity, share some common pathogens, but they also have their own specific microorganisms [4]. Common bacteria isolated from the normal flora of the anterior nares include *Actinobacteria* (mainly *Propionibacterium* and *Corynebacterium* spp), coagulase-negative *Staphylococci*, and *Staphylococcus aureus* [4,5]. The middle meatus has a diverse community of microbes. Ramakrishnan et al evaluated the microbial composition of the middle meatus, finding that the predominating ones were *Staphylococcus aureus, Staphylococcus epidermidis,* and *Propionibacterium acnes* [6]. Chen et al analyzed 88 nasal samples, and the major microorganisms were *Corynebacterium* (21.53-48.60%), *Neisseria* (1.11-14.80%), *Staphylococcus* (6.12-9.61%), and *Streptococcus* (5.18-6.47%) [7]. The paranasal sinuses are theoretically sterile. Viridans group streptococci predominate in the nasopharynx, and *S pneumoniae, H influenzae, Neisseria meningitidis,* and *Moraxella catarrhalis* are also common. They are embedded in the harmless commensal microbiota, especially in children under the age of 2 years [8,9].

Although colonization by the normal flora is assumed to protect the mucosal surfaces, normal flora may be "potential pathogen" species. Potential pathogen microorganisms may turn into a causative agent for local and systemic infections. Garcia-Rodriguez et al found that children prone to recurrent otitis media and adults with chronic respiratory tract disease have higher nasopharyngeal carriage rates of potential pathogen species [10]. The impetus for this transformation may be the dysbiosis (abnormal distribution of the microorganisims) due to host and environmental factors [11]. Sakwinska et al compared the nasopharyngeal microbiota of pediatric pneumonia patients and control subjects, finding no significant differences in microbiota of the 2 groups. The only clear difference was the abundance of *S. pneumoniae, H. influenzae,* and *M. catarrhalis* in pneumonia patients [12]. Control subjects had more diverse and less numerous organisms.

Considering that a healthy microbiota is essential for our health, upper airway colonization and factors altering its composition should be taken into account. It is known that microbiota manifest marked changes with age, environmental factors, antibiotics, nutrition, and diseases [4,13]. Antibiotic exposure is known to alter gastrointestinal microbiota [14]. Dethlefsen et al investigated the distal gut bacterial communities of 3 healthy humans before and after treatment with ciprofloxacin. They demonsrated that about one-third of the bacterial taxa in the gut changed upon ciprofloxacin usage, so that the taxonomic richness, diversity, and evenness of the community changed. By 4 weeks, taxonomic composition reached nearly its pretreatment structure, but several taxa failed to recover within 6 months [15]. We think that antibiotic consumption may have similar deleterious short- and long-term effects on upper airway commensals. There are several articles supporting this opinion in the literature. One of these studies evaluated the maturation of nasal microbiota within the first 2 years of life and consequences of early antibiotic exposure [16]. They studied children exposed to systemic antibiotics during the first 2 months of life. Antibiotic administration during the first 2 months caused predominant age-discriminatory genera, including *Haemophilus*, while non-exposed children carried *Dolosigranulum* instead. Zeineldin studied the influence of parenteral antibiotic administration on the composition and diversity of nasal microbial flora in growing pigs [17]. Antimicrobials caused considerable changes in the nasal microbial populations. A single dose of parenteral antibiotic influenced nasal microbiota of growing pigs. Alteration of the upper airway flora may have clinical consequences. Dysbiosis of the nasal flora may play a role in the pathogenesis of chronic rhinosinusitis [18]. In a study comparing the sinonasal microbiome of chronic rhinosinusitis patients and healthy control sinuses, significant differences were seen between the 2 groups [19]. A retrospective case-control study investigated the association between antibiotic exposure and the risk of developing chronic rhinosinusitis, showing that use of antibiotics increased the risk of developing chronic rhinosinusitis within 2 years [20].

The aim of the present study was to evaluate the short-term or long-term effects of antibiotics on upper airway microbiota. The results may influence our approach to antibiotic

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prescription or management of acute and chronic upper airway diseases. To the best of our knowledge, no such study has been previously published.

Material and Methods

Experimental Design and Sampling

This prospective randomized clinical trial was approved by our local ethics committee (Clinical Research Ethics Committee; 2019-161). A priori power analysis was done to estimate the required sample size for the study by using G*Power 3.1 (Universität Düsseldorf, Düsseldorf). For a chi-square analysis in contingency tables with 4 degrees of freedom, with an alpha level of 0.05, power established at 0.95, and a moderate effect size of 0.3 (Cohen, 1988), it was found that 207 patients would be adequate.

The study included 210 adult subjects. All subjects over 18 years of age who were accepted to participate in the study and whose nasal and nasopharyngeal cultures could be taken without exclusion criteria were enrolled in the study. Subjects whose medical condition would alter the upper airway culture results were excluded. Exclusion criteria included subjects with upper airway diseases such as choanal atresia, acute upper airway infection, chronic sinusitis with or without nasal polyposis, dacryocystitis, inflammatory airway diseases, and systemic diseases such as immunodeficiencies, hematologic diseases, chronic renal diseases, cystic fibrosis, diabetes mellitus, and any disease influencing the immune system. Additional exclusion criteria were use of any kind of topical nasal drug or systemic corticosteroids and subjects who were not suitable for collecting nasopharyngeal culture, such as those with Mallampati 4 anatomy. Following adequate explanation of the study, volunteers signed the consent form.

Data Collection and Processing

Nasal and nasopharyngeal cultures were taken from all enrolled subjects by a single ENT physician. Nasal swabs were obtained with sterile dacron-tipped swab rods from the nasal cavity just anterior to the middle turbinate. The sampling site was chosen by comparison of the left and right nares, and the one appropriate for sampling without any contamination was preferred. A sterile swab was rotated 5 full turns. Nasopharyngeal swabs were collected through the oral cavity, similarly avoiding any contamination and turning it 5 full turns. Materials were then sent to the microbiology laboratory for culturing. Samples were inoculated onto 5% sheep blood agar (RTA, Kocaeli, Turkey), chocolate agar (RTA, Kocaeli, Turkey), eosin methylene blue agar (RTA, Kocaeli, Turkey), and Sabouraud dextrose agar (RTA, Kocaeli, Turkey) plates, and then these plates were incubated aerobically for 24-48 h at 37°C. After 24-48 h of incubation, all isolates were identified by standard microbiological procedures; colonies were differentiated by color, form, alpha, beta, or gamma haemolysis, and consistency and were counted semiquantitatively by macroscopic inspection. Gram-staining characteristics, potassium hydroxide, cytochrome-oxidase, catalase-testing, and further biochemical tests were used for the identification of these bacteria. The chemicals used to carry out these tests were purchased from Merck India Limited. Bacterial identification was performed according to microbial examination standards for categorization; a small bacterial population of common nasal cavity was categorized as normal flora. Any large bacterial population including typically normal residents were regarded as pathogenic flora. Pathogenic strains that were not identified by standard microbiological procedures were identified using the BD Phoenix (Becton Dickinson, MD, USA) fully automated system, and antibiotic susceptibilities of isolates was determined using the Kirby-Bauer disc diffusion method. Antimicrobial susceptibilities of strains were interpreted according to the European Committee on Antimicrobial Susceptibility Testing standards [21]. We tested ampicillin, penicillin, cefoxitin, clindamycin, daptomycin, erythromycin, fusidic acid, levofloxacin, linezolid, mupirocin, nitrofurantoin, ofloxacin, teicoplanin, tetracycline, tigecycline, trimethoprim-sulfamexazole, and vancomycin. The disc diffusion test was performed with Mueller-Hinton agar using discs from Oxoid® England.

Data Acquisition

Antibiotic administration data of the subjects were obtained from the prescription history in the medical records of the official social insurance database for the last 6-months period. Subjects were classified as antibiotic-naïve group or antibiotic-treated. The antibiotic-treated group was further investigated with respect to the time of use and antibiotics used. Data on antibiotics prescribed within the last 6 months were retrieved and documented based on 3 time periods: within the last month, within previous second and third months, and within 4 to 6 months. Antibiotics were categorized into 4 groups according to antimicrobial profile as antibiotics effective against: (1) gram-positive organisms (fusidic acid, penicillin G benzathine, phenoxymethyl penicillin); (2) both gram-positive and gram-negative organisms (broad-spectrum antibiotics such as ampicillin, amoxycillin, cephalosporins such as cefprozil, cefuroxime, cefpodoxime, ceftriaxone, cefixime, cefdinir, cephalexin, quinolones such as gemifloxacin, moxifloxacin, ofloxacin, macrolides including clarithromycin, azithromycin, dirithromycin); (3) gram-negative organisms (ciprofloxacin): and (4) anaerobes (clindamycin, methronidazole, ornidazole).

We evaluated nasal and nasopharyngeal culture results for the antibiotic-naïve and antibiotic-treated groups. We compared the

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culture results of the antibiotic-naïve group with the antibiotic-treated group and subgroups one by one. Antibiotic-treated groups were not compared with each other, and nasal cultures were not compared with nasopharynx cultures. Methicilline resistance of *Staphylococcus spp.* and *S. Aureus* were compared between the antibiotic-naïve group and the antibiotic-treated within the last month and within the previous 6 months.

Data Analysis

If expected counts were below 5, Pearson's chi-square test or Fisher's exact test was used to compare the groups. Fisher's exact test was performed on 2×2 contingency tables with SPSS v26 (IBM, Inc., Chicago, IL, USA) statistical software, and on larger than 2×2 contingency tables with a web calculator (http://www.physics.csbsju.edu/stats/). Relative risk (RR) and 95% confidence intervals (CI) were calculated. All comparisons were 2-tailed and a *P* value less than 5% was considered statistically significant. Since there was no continuous variable obtained by measurement in the study, normality control was not performed with the Kolmogorov-Smirnov test. Also, no statistical test was used that required a normal distribution assumption.

Results

Of the 210 enrolled subjects, 112 (53%) were female and 98 (47%) were male, with a mean age of 45.97±16.69 years. When we analyzed culture results, 86 nasal swabs and 99 nasopharyngeal swabs met the definition of normal flora. Most of the remaining cases demonstrated overgrowth, mainly of gram-positive bacteria, while there were fewer gram-negative microorganisms and fungi (**Table 1**). Microorganisms cultured from nasal swabs and their frequency (in parenthesis) were as follows: *Staphylococcus aureus* (46), *Coagulase-negative Staphylococcus* (21), *Staphylococcus epidermidis* (25), *Streptococcus species* (8), *Staphylococcus haemolyticus* (5), *Corynebacterium pseudodiphtheriticum* (6), *Staphylococcus hominis* (2), *Arcanobacterium haemolyticum* (1), *Streptococcus pneumoniae* (2), *Klebsiella aerogenes* (1), *Staphylococcus hominis* (2), *Morganella morganii* (1), *Pseudomonas putida* (1), *Proteus mirabilis* (1), *Staphylococcus equorum* (1), *Acinetobacter species*(1), *Gemella morbillorum* (1), *Klebsiella oxytoca* (1), *Corynebacterium amycolatum* (1), gram-positive bacilli (2), and *Candida species* (1). Nasopharyngeal culture results yielded following microorganisms and frequencies: *Staphylococcus aureus* (40), *Coagulase-negative Staphylococcus* (7), *Streptococcus species* (43), *Staphylococcus hominis* (1), *Arcanobacterium haemolyticum* (1), *Streptococcus pneumoniae* (6), *Klebsiella aerogenes* (1), *Pseudomonas putida* (1), *Pseudomonas aeruginosa* (1),*Proteus mirabilis* (1), *Enterococcus* (2), *Acinetobacter species*(1),*Klebsiella oxytoca* (1), *Klebsiella pneumoniae* (1), *Streptococcus anginosus* (1),

Table 1. Culture results of the nasal and nasopharyngeal swabs of subjects.

	Nose	Nasopharynx
Culture result		
Normal flora	86	99
Gram positive bacteria	117	90
Gram positive and negative bacteria	0	5
Gram negative bacteria	6	16
Fungus	1	0
Total	**210**	**210**

Streptococcus salivarius (1), *Streptococcus oralis* (1), *Enterobacter cloacae* (4), *Burkholderia cepacia* (1), *Rothia dentocariosa* (2), *Corynebacterium propinquum* (1), *Moraxella catarrhalis* (1), and *Streptococcus anginosus* (1). Next, we categorized antibiotics according to antimicrobial profiles to assess the possible influence on nasal and nasopharyngeal flora. In all groups, most of the subjects had not been treated with any antibiotic (n: 113; 53.80% for 0-6 months). Most of the antibiotic-treated subjects had been treated with antibiotics effective against both gram-positive and gram-negative bacteria (n: 93; 44.28% for 0-6 months). During the previous 6 months, 2 patients received antibiotics effective against only gram-positive bacteria and 6 received antibiotics effective against only anaerobic bacteria. None of the subjects had been treated with an antibiotic effective against only gram-negative organisms. We concluded that for the last 6 months, 93% of prescribed antimicrobials were broad-spectrum antibiotics effective against gram-positive and gram-negative bacteria. So, we decided not to take into account the antimicrobial spectrum when evaluating the culture results.

Recovered microorganisms were categorized as normal flora, gram-positive overgrowth, gram-negative overgrowth, and fungal growth. Isolates of the antibiotic-naïve group were considered as the baseline, and these values were compared with that of antibiotic-treated subjects. Statistical comparisons were made to reveal the probable influence of the antibiotics. Documentation of these evaluations is shown in **Table 2** according to selected time period. Of the 113 antibiotic-naïve subjects, 50 (44.2%) nasal isolates and 56 (49.6%) nasopharyngeal isolates had normal flora. In this group, 59 (52.2%) nasal cultures and 44 (38.9%) nasopharyngeal cultures were positive for gram-positive bacteria. Gram-negative bacteria and fungi were identified in a small percentage of subjects. Five subjects had both gram-positive and gram-negative overgrowth in the nasopharynx.

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Table 2. Comparison of the nasal and nasopharyngeal culture results of the antibiotic naïve group with antibiotic consumed patients with respect to antibiotic prescription periods.

		Antibiotic naïve		Antibiotic exposed: 0-1 month			Antibiotic exposed: 2-3 months			Antibiotic exposed: 4-6 months			Antibiotic exposed: 0-6 months		
		n	%	n	%	p	n	%	p	n	%	p	n	%	p
Nose	Normal flora	50	44.3	12	42.9		20	42.6		16	33.3		36	37.1	
	Gram+	59	52.2	15	53.6		27	57.4		30	62.5		58	59.8	
	Gram-	4	3.5	0	0	0.169	0	0.0	0.396	2	4.2	0.436	2	2.1	0.432
	Fungus	0	0.0	1	3.6		0	0.0		0	0.0		1	1.0	
	Total	**113**	**100**	**28**	**100**		**47**	**100**		**48**	**100**		**97**	**100**	
Nasopharynx	Normal flora	56	49.6	11	39.3		16	34		27	56.3		43	44.3	
	Gram+	44	38.9	16	57.1		26	55.3		17	35.4		46	47.4	
	Gram-	8	7.1	1	3.6	0.271	5	10.6	0.093	4	8.3	0.458	8	8.2	0.135
	Gram+ & Gram-	5	4.4	0	0.0		0	0.0		0	0.0		0	0.0	
	Total	**113**	**100**	**28**	**100**		**47**	**100**		**48**	**100**		**97**	**100**	

n – number. Each *p* value represents the statistical comparison of antibiotic naïve group with corresponding antibiotic exposure group.

Table 3. Comparison of the methicillin resistance of the nasal and nasopharyngeal Staphylococcus between the antibiotic naïve group and antibiotic consumed patients.

		Antibiotic naïve		Antibiotic exposed: 0-1 month			Antibiotic exposed: 0-6 months		
		n	%	n	%	P	n	%	P
Nose	MRS	12	23.5	9	64.3		16	31.4	
	MSS	39	76.5	5	35.7	**0.008**	35	68.7	0.375
	Total	51	100	14	100		51	100	
Nasopharynx	MRS	3	12	3	42.9		4	17.4	
	MSS	22	88	4	57.1	0.101	19	82.6	0.696
	Total	25	100	7	100		23	100	

MRS – methicillin resistant strains; MSS – methicillin sensitive strains. Each *p* value represents the statistical comparison of antibiotic naïve group with corresponding antibiotic exposure group.

Overall, antibiotic exposure during the previous 6 months did not show a statistically significant effect on the nasal and nasopharyngeal flora. *P* values were clearly bigger than 0.05 irrespective of the evaluated time period (*P*=0.169; *P*=0.271; *P*=0.396; *P*=0.093; *P*=0.436; *P*=0.458; *P*=0.432; *P*=0.135). Therefore, we concluded that antibiotic treatment during the previous 6 months did not influence the nasal or nasopharyngeal flora.

We documented the coagulase-negative *Staphylococcus* (CNS) (eg, *S. epidermidis, S. haemolyticus, S. capitis)* colonization rates and their methicillin susceptibility results. Ratios of methicillin-resistant strains (MRS) to methicillin-sensitive strains (MSS) were evaluated. Subjects who did not receive any antibiotic were compared with ones who received antibiotic during the last month or within the previous 6 months, separately (**Table 3**). Statistical analysis showed that antibiotic consumption within the last month conferred approximately 4 times greater likelihood of carrying nasal methicillin-resistant CNS instead of methicillin-sensitive CNS (RR(CI): 3.77(1.44-9.87); *P*=0.008). MRS/MRS+MSS ratios of the nasopharyngeal swabs in this period (*P*=0.101) and MRS/MRS+MSS ratios of the nasal cavity and the nasopharynx during the last 6 months were not significantly different (*P*=0.375 and *P*=0.696, respectively).

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Table 4. Comparison of the methicillin resistance of the nasal and nasopharyngeal S. aureus between the antibiotic naïve group and antibiotic received patients.

		Antibiotic naïve		Antibiotic exposed: 0-1 month			Antibiotic exposed: 0-6 months		
		n	%	n	%	P	n	%	P
Nose	MRSA	2	8.7	5	62.5	**0.006**	6	26.1	0.243
	MSSA	21	91.3	3	37.5		17	73.9	
	Total	23	100	8	100		23	100	
Nasopharynx	MRSA	3	13.6	2	33.3	0.286	3	16.7	0.789
	MSSA	19	86.4	4	66.7		15	83.3	
	Total	22	100	6	100		18	100	

MRSA – methicillin resistant *S. areus*; MSS – methicillin sensitive *S. areus*. Each *p* value represents the statistical comparison of antibiotic naïve group with corresponding antibiotic exposure group.

We performed a similar assessment of *Staphylococcus aureus* colonization, which is the major species of coagulase-positive *Staphylococcus*. Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA) ratios of the antibiotic-naïve group were compared with the subjects who received antibiotic during the last month or within the previous 6 months (**Table 4**). MRSA/MRSA+MSSA ratios of the nasal cavity were significantly (*P*=0.006) different between antibiotic-naïve subjects and antibiotic-treated subjects within the last month. Antibiotic prescription within the last month increased the incidence of MRSA colonization by approximately 6 times (RR(CI): 5.71(1.80-18.18)). We found no significant difference in nasopharyngeal MRSA/MRSA+MSS ratio for the same period (*P*=0.286), or upon comparison of the nasal cavity and nasopharyngeal flora of subjects treated with an antibiotic within the last 6 months (*P*=0.243 and *P*=0.789, respectively).

When some potential pathogenic microorganisms were scrutinized, they were not suitable for statistical analysis because of small number of cases. Five nasopharyngeal cultures were positive for *Streptococcus pneumoniae*. Three of these cases did not receive any antibiotic; 2 of them were penicillin-sensitive strains and 1 was penicillin-resistant. The remaining 2 subjects had received antibiotic; one was penicillin-resistant and the other was penicillin-sensitive. The nasal culture result of one subject was positive for *Pseudomonas putida* and the other subject was positive for *Pseudomonas aeruginosa*, and one nasopharynx was positive for *Pseudomonas putida*. None of these 3 cases had been treated with any antibiotic within the last 6 months. *Burkholderia cepacia* was identified in the nasopharynx of 1 subject and this subject did not have a history of antibiotic treatment.

Discussion

Antibiotics are commonly prescribed drugs that are used to combat many infections. They are chosen according to the characteristics of the pathogenic microorganisms identified. Each antibiotic has a well-known spectrum of action. However, this spectrum of action is not restricted to the pathogenic organisms, and they show variable influences on normal microbiota and flora. As seen in the Results section above, broad-spectrum antimicrobials constitute the majority of prescribed antibiotics, increasing the likelihood of affecting other than targeted organisms and normal flora.

Commensal microorganisms constituting the microbiota participate in immune system development and metabolism of nutrients. The composition of these microbial communities is influenced by environmental exposures such as via diet and antibiotics. A systematic review investigating the effect of antibiotics on intestinal microbiota was performed by Zimmermann and Curtis [22]. Their review included 129 studies, 2076 participants, and 301 controls. They concluded that antibiotics have profound effects on the intestinal microbiota. The impacts on bacterial diversity and resistance were influenced by the dose and class of the antibiotic, formulation of the drug (syrup or tablet), duration of treatment, and timing of sampling. Multiple studies reported that antibiotics affect the microbiota and immune system in both the short- and long-term [3,23].

The URT has indigenous flora, and there is no published study evaluating the long-term effect of antibiotics on URT flora. Several articles evaluated the nasal microbial composition on different clinical situations such as rhinosinusitis cases or on short-term drug exposures, such as antibiotic consumption during the last few weeks. These studies did not aim to assess the mid-term or long-term influence of these drugs on upper airway flora. Maintaining the normal upper airway flora is important with respect to upper and lower airway diseases.

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We consider the upper airway flora as an important aspect of human well-being, similar to microbiota of the gastrointestinal system. Several studies investigated the alterations of upper airway flora with disease states. A review performed by Esposito and Principi evaluated the upper respiratory tract microbiota and respiratory tract infections in pediatric patients [24]. They reported that upper respiratory tract microbiota determines respiratory health in children. Modulation of beneficial commensal colonization may reduce the risk of disease. Profound change of the nasopharyngeal microbiota is associated with respiratory tract diseases. They recommended the administration of pre- and probiotics to modulate and support the colonization of beneficial commensals. Abreu et al compared microbiome profiles of CRS patients and healthy controls. Microbiota of CRS patients had lower bacterial diversity than healthy controls [25]. They concluded that normal mucosal microbiota is necessary to protect against pathogenic microbiota. Hauser et al examined the nasal bacterial population of 13 endoscopic sinus surgery for CRS patients. Surgery and perioperative antibiotics shifted the ethmoid microbiota temporarily, with subsequent return to baseline levels within 6 weeks [26]. Choi et al assessed the nasal lavage fluid samples of patients with chronic rhinosinusitis without nasal polyps (CRSsNP), chronic rhinosinusitis with nasal polyps (CRSwNP), and non-chronic rhinosinusitis controls [27]. They analyzed the bacteria composition using 16S-rDNA pyrosequencing. Patients with chronic rhinosinusitis (CRS) had greater bacterial abundance and lower bacterial diversity. It is not clear if this altered microbiota is the result of the infection or predisposing factor for CRS. In a study performed in 1970, Aly et al studied the influence of 12 days of oral cephalexin treatment on nasal vestibular skin flora (the outer half-inch of the nostril). Coagulase-positive cocci and diphtheroids were the most sensitive and reduced microrganisms. After 36 days, total count returned to the original number except for diphteroids, which were replaced by coagulase-negative cocci [28]. In infancy, nasopharyngeal bacterial pathogens at the time of viral upper respiratory tract infections have been proposed to determine the risk for spread of the infection to the lower airways [29]. In our study, we documented that broad-spectrum antibiotics effective against both gram-positive and gram-negative microorganisms constitute the majority of the prescribed antimicrobials. This increases the theoretical risk of dysbiosis in the upper respiratory tract upon antibiotic treatment. However, our study demonstrated that antibiotic treatment does not have any influence on the general composition of the upper airway flora, and these drugs did not change the upper airway flora within 6 months period. Patients who received antibiotics did not demonstrate any shift in gram-positive or gram-negative composition or increase of potential pathogenic bacteria.

Staphylococci are among the commensal microorganisms of the human body, but they are also potential pathogens and can cause serious infections when human defense mechanisms are impaired. Carriage of *S. aureus*, a normal inhabitant of the anterior nares in 20% of humans, has been considered a risk factor for nosocomial or community-acquired infections. *S. aureus* can cause severe and fatal infections. MRSA colonization poses additional risk of problematic infection because of drug resistance [30,31]. A meta-analysis performed on *Staphylococcus aureus* bacteremia patients compared mortality rates of MSSA and MRSA cases. Bacteremia mortalities associated with MRSA were significantly higher than that of MSSA patients [32]. In our study, antimicrobial profile data revealed that antibiotic exposure within the last month increases the incidence of MRS and MRSA in the nasal flora. This distortion in nasal microbiota can increase the risk of antibiotic-resistant infections, especially in the hospital setting. Hospitalized patients receiving broad-spectrum antimicrobials should be evaluated for developing resistant MRSA infections.

One limitation of our study is that we did not determine the anaerobic microorganism composition of the upper airway. In this study, we evaluated the upper airway flora using culture-based microbiological techniques under aerobic conditions. This technique gives us the opportunity to evaluate a large number of subjects and define antimicrobial sensitivity of microbial communities. However, precise identification of all aerobic and anaerobic microorganisms is not possible with this method. Alternatively, a wide range of organisms of the upper airway microbiota could be documented by the culture-independent 16S rRNA gene sequencing approach. This technique can detect broader microbiological diversity, including anaerobes, as well as infrequent bacteria and fungi. One main drawback of gene sequencing is the high cost of this technique. Secondly, antimicrobial sensitivity detected by genotype analysis may not reflect the true antimicrobial phenotype of these microorganisms [33-35].

Conclusions

Antibiotic exposure did not lead to perturbations in general composition of upper airway flora during the last 6 months, although the incidence of methicillin resistance in coagulase-positive and -negative *Staphylococci* demonstrated significant increases if patients received antibiotic during the last month. Broad-spectrum antibiotics constituted the majority of the prescribed antimicrobials in our study. This should be considered in hospitalized patients because these patients frequently receive broad-spectrum antibiotics and methicillin resistance increases the morbidity and mortality of nosocomial *Staphylococcus aureus* infections.

Department and Institution Where Work Was Done

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