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RESEARCH ARTICLE

An overview of the microflora of the patients admitted in an Ear Nose and Throat tertiary hospital from Romania

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Objective: The purpose of this retrospective study is to describe clinical, epidemiological and bacteriological findings on the Ear, Nose and Throat infections from patients admitted in a tertiary hospital from Romania, which might bring supportive data for the management of the ENT patients. **Methods**: Clinical data from 146 patients admitted in a clinic from Romania, between February 2016 and April 2018 were collected from the official registries of the Microbiology Laboratory, where the collected samples were processed by classical microbiological methods. **Results**: From 164 analyzed biological samples, 45.1% were suggestive for infection. Methicillin-sensitive Staphylococcus aureus was the most prevalent bacterial isolate (28.72%), followed by Pseudomonas aeruginosa (12.77%). There were 26 different species of microbes. Out of a total of 77 bacterial isolates, 62.33% were Gram positive and 37.66% Gram negative. The study showed that 11 ear discharge samples were positive in female and male patients. Upper respiratory tract exudates were positive on 3 females (21.4%) and on 17 males (28.8%). There were 7 positive pus samples from females, and from 25 males. **Conclusions**: Men were more affected by Ear Nose and Throat infections, caused by bacteria mostly from the genus Staphylococcus and Pseudomonas. More attention at the samples collection and a rigorous clinical examination should reduce the load on bacteriology laboratory. In specific cases, a negative bacteriological result can prove as an exclusion diagnostic.

Keywords: microbiology, bacteria, nosocomial, infection, ENT

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Introduction

The infections in the human body can be caused by endogenous microorganisms that are considered part of the commensal flora, found both inside the body (upper respiratory tract, digestive tract) or outside the body, especially from the skin, or by exogenous microorganisms from the environment (water, food, soil). The commensal microflora of the organism is composed of a large diversity of bacteria, which can be either Gram positive, Gram negative, aerobe or anaerobe, most of them presenting low pathogenicity [1,2].

The most common Ear, Nose and Throat (ENT) infections can be caused by either viruses, bacteria or fungi[1], but considering the routine diagnostic methods, viruses are mostly misdiagnosed. However, viruses such as adenovirus, rhinovirus, influenza, parainfluenza and, in the context of the current pandemic, coronavirus (SARS-CoV-2) can be found in the naso-pharynx and in the middle ear [3,4].

Along the upper respiratory tract, conditional pathogenic bacteria, like beta hemolytic *Streptococcus* spp. or *Staphylococcus aureus* can colonize some patients, without causing an infectious process. The oropharynx and nasopharynx can also host several bacteria like *Streptococcus pneumoniae* or *Haemophilus influenzae*, but in most of the cases without the association of an infection. Also, bacteria such as *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella oxytoca*, *Enterobacter cloacae* or *Pseudomonas aeruginosa*, are also associated with infectious processes in the ENT areas [1,2].

The external ear hosts an important part of the skin microflora (staphylococci, diphtherimorph bacteria, etc.), but also potential pathogens such as *Streptococcus pneumoniae* or *Pseudomonas aeruginosa*. As of fungal species, *Aspergillus niger* and *Candida albicans,* first described by Pound and Roscoe in 1901 are mostly identified in children and diabetic patients [1,5]. The middle ear and the internal ear are usually sterile [1,6], but considering the presence of the normal microflora in the proximal areas, in some patients with risk or favorable conditions, endogenous infections can develop. Predisposing factors can be anatomical, like the stenosis of the external auditory canal or exostoses, or the presence of excess hair on the external canal of the ear. Besides, skin diseases that usually associate with itching, environmental factors, habitual factors (wearing ear plugs and cotton buds, foreign bodies, constant manipulation and trauma to the ear canal), water in the ear canal (swimming) and stress can be factors that contribute to the development of endogenous infections of the external ear [2,7].

Community-acquired infections are considered those that are contacted by the patients outside the medical care units (hospitals or other care units). Nosocomial infections, also known as hospital-related infections, are found in patients who receive medical care in a hospital or any other health care unit, which were absent prior to admission [8]. Generally, artificial ventilation machines and catheters are

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frequently associated with this type of infection [9], but more specifically in ENT pathology, nosocomial infections can occur in oncologic patients hospitalized for head and neck surgery for a long period of time and patients who receive more than 48 hours of mechanical ventilation [10,11].

The purpose of this retrospective study is to describe clinical, epidemiological and bacteriological findings on the Ear, Nose and Throat infections from patients admitted in a tertiary hospital from Romania, which might bring supportive data for the management of the ENT patients.

Methods

Study group

Clinical data from 146 patients admitted in the ENT Clinic from the Clinical County Hospital Târgu Mureş, Romania, between February 2016 and April 2018 (27 months) were collected from the official registries of the Microbiology Laboratory, where the collected samples (nasal exudate, naso-pharyngeal exudate, pharyngeal exudate, ear discharge and pus from soft tissue lesions) were processed by classical microbiological methods. The data was statistically analyzed using GraphPad InStat and Spreadsheet software. Chi-Square test, Fisher's Exact Test and ANOVA test, were performed, considering a statistical significance if the p-value was less than 0.05.

Ethical statement

Ethical approval (No. 3405/07.02.2018) was obtained from the Emergency Clinical County Hospital Târgu Mureş, Romania according to the current legislative normative and the Ministry of Health of Romania regulations for retrospective studies. The patients' identification data were censored (stripped of the personal information).

Results

Data from 146 patients with the suspicion of an ENT infection was consolidated. The age ranged from 0.9 to 85 years (median 52). Most of the patients were male (68.03%), with a male:female ratio of 2.12:1.

Out of the total number of analyzed biological samples (n=164), 74 (45.1%) presented microbial growth suggestive for infection, consisting in either bacterial (n=67; 90.5%), fungal (n=5; 6.8%) or bacterial and fungal (n=2; 2.7%) etiology. Pus (46 positive samples) and ear discharge (28 positive samples) were the most common biological products that showed bacterial growth. Methicillin-sensitive *Staphylococcus aureus* (MSSA) was the most prevalent bacterial isolate (n=27; 28.72%), isolated mostly from pus samples (n=12; 44.4%). The next most frequent isolate was *Pseudomonas aeruginosa* (n=12; 12.77%). Details are presented in *Table I*.

The microbial diversity consisted of 26 different species, including Gram positive bacteria, Gram negative bacteria, and fungi. Out of a total of 77 bacterial isolates,

Table I. The distribution of microorganisms in the tested samples

Microorganism		Biological product					TOTAL (%)
		Pus	Ear discharge	Pharyngeal exudate	Nasal exudate	Naso-pharyngeal exudate	
Staphylococcus spp.	*Staphylococcus aureus*, MRSA		1	1			2.13
	Staphylococcus aureus, MSSA	12	6	3	4	2	28.72
	coagulase-negative *Staphylococcus*	1	1				2.13
Streptococcus alpha-hemolytic	*Streptococcus* alpha-hemolytic	6					6.38
Streptococcus beta-hemolytic	*Streptococcus pyogenes*	1	1	1			3.19
	Group C	1		2			3.19
	Streptococcus agalactiae	1					1.06
Enterobacterales	*Citrobacter freundii*			1		1	2.13
	Escherichia coli	3	1				4.26
	Klebsiella oxytoca	1					1.06
	Klebsiella pneumoniae	2	1	1			4.26
	Morganella morganii			1			1.06
	Proteus spp.		2				2.13
	Serratia marcescens	1					1.06
	Other Gram-negative bacilli	3					3.19
Non-fermenting bacteria	*Acinetobacter baumannii*			1			1.06
	Pseudomonas aeruginosa	6	5	1			12.77
	Stenotrophomonas maltophilia	1					1.06
Other bacteria	*Corynebacterium* spp.	1	5				6.38
	Enterococcus faecium	1					1.06
	Haemophilus influenzae	1					1.06
	Micrococcus spp.	1					1.06
	Moraxella catarrhalis				1		1.06
	Kocuria kristinae	1					1.06
Fungi	*Aspergillus* spp.		2				2.13
	Candida spp.	2	3				5.32
TOTAL		46	28	12	5	3	100
		94					

48 (62.33%) were Gram positive and 29 (37.66%) were Gram negative.

For each type of biological product except the ear discharge, more samples were collected from men than from women, as presented in *Table II*.

The samples were compared by their result, either negative – meaning that the sample was sterile or colonized with normal microflora, or positive – meaning that a clinically relevant pathogen was found during the sample analysis. Out of a total of 164 tested samples, 74 (45.1%) were found positive and 90 (54.9%) turned out to be negative. The detailed results are presented in *Table III*.

By comparing the positivity rate on the different biological samples, it was shown that the chance of a patient to get a positive pharyngeal exudate result is 5.6 times greater than a positive result from ear discharge (p<0.001, OR=5.64, 95% CI 2.2-14.4). All the other associations were not statistically significant.

Distribution by genders showed that the most positive samples were from pus and from male patients (n=25), while that the most negative results came from pharyngeal exudates also from male patients (n=31). Naso-pharyngeal samples were collected only from men. More details can be found in *Figure 1*.

Further classification of the biological samples into three main categories (ear discharge, upper respiratory tract exudates and pus) showed that each 11 ear discharge samples were positive in female (57.9% positivity rate) and male patients (68.8% positivity rate). Upper respiratory tract exudates came out positive on 3 female patients (21.4% positivity rate) and on 17 male patients (28.8% positivity rate). Positive results on pus samples from female patients were 7 (38.9% positivity rate), as opposed to those from 25 male patients (65.8% positivity rate). When comparing the ear discharge samples versus pus, male patients presented a 3.5 times higher chance to develop an infection which leads to pus formation (p=0.042, OR=3.57, 95% CI 1.0-11.6). Also, male patients presented a 5.5 times higher chance to develop an upper respiratory tract infection than an ear infection (p=0.023, OR=5.66, 95% CI 1.2-25.0).

Further studying of the data showed that although the number of infections on male patients was greater, men

Table II. The distribution of samples by gender

Gender	Ear discharge	Nasal exudate	Naso-pharyngeal exudate	Pharyngeal exudate	Pus
Female	19	3	0	11	18
Male	16	8	10	41	38

Table III. The positive and negative results for each biological product

Result	Ear discharge (N=35)	Nasal exudate (N=11)	Naso-pharyngeal exudate (N=10)	Pharyngeal exudate (N=52)	Pus (N=56)
Positive	22	5	3	12	32
Negative	13	6	7	40	24

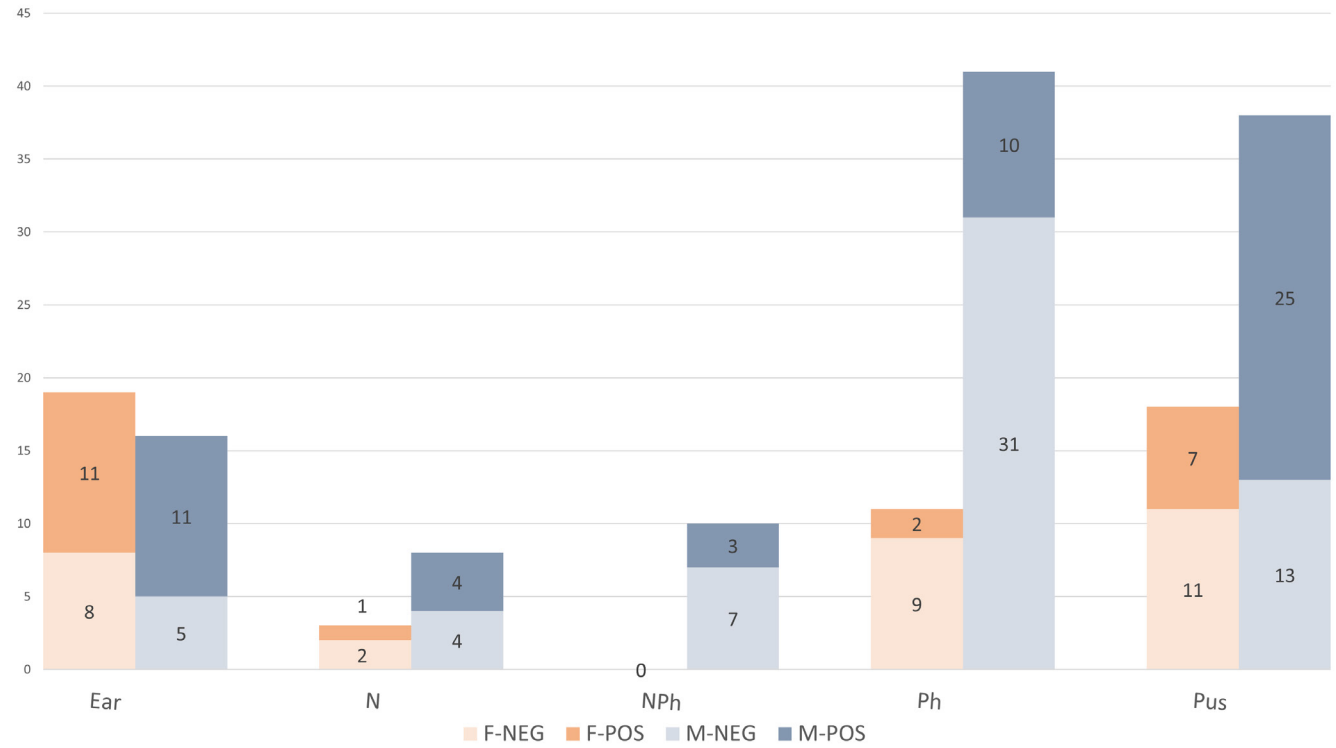

Fig. 1. A comparison between the positive and negative samples by gender. M-POS=Male, positive sample, F-POS=Female, positive sample, M-NEG=Male, negative sample, F-NEG=Female, negative sample, Ear = ear discharge, N = nasal exudate, NPh = Naso-pharyngeal exudate, Ph = Pharyngeal exudate

have a higher chance to contact other infections than ear infections. In spite of the fact that the percentage of ear discharge and pus positive samples from males was higher, female patients were more likely to develop ear infections, when relating to naso-pharyngeal infections (p=0.05), but not as much as when relating to purulent infections (p=0.08).

The numbers were also scrutinized depending on the age groups of the patient, resulting in three groups: pediatric patients (0-18 years old), adults (19-60 years old) and elders (above 61 years old), with the number of samples tested detailed in *Table IV*. In these groups, the harvesting of the pathological products from the ENT area was not homogenous, the results showing that more pharyngeal exudates than ear discharges were collected from elders, if compared to the adult population (p=0.002, OR=5.99, 95% CI 1.8-19.9) or pediatric population (p=0.0007, OR=16.69, 95% CI 3.0-90.8). Other associations were not statistically significant.

The data showed an increase of sampling towards the end of each year, but also a steep drop in the summer months, with a peak in November (n=26) and a low in June (n=6). Positive samples also followed the sampling pattern (*Figure 2*).

The seasonal overview of total and positive samples showed a peak of ear discharge samples tested in Autumn time (n=17), with more than half of them being positive (n=9). As for pharyngeal exudates, the peak was in winter, with 20 samples sent to the laboratory, out of which, only 2 came back positive. The extended data can be found in *Table V*.

Discussions

According to our study, ENT infections were common to both men and women and spread along all age groups, with a tendency to mostly affect the men. However, the gender difference in ENT patients and their associated infections were poorly-investigated, with the exception of oncologic-

Table IV. Samples tested from each age group

Age group	Ear discharge (N=35)	Pus (N=56)	Naso-pharyngeal exudate (N=10)	Nasal exudate (N=11)	Pharyngeal exudate (N=52)
Pediatric	8	7	2	2	3
Adult	23	33	8	6	24
Elder	4	16	0	3	25

Table V. Seasonal overview of the total and positive samples

	Season	Ear discharge	Nasal exudate	Naso-pharyngeal exudate	Pharyngeal exudate	Pus	TOTAL
Total Samples	Winter	5	1	3	20	11	40
	Spring	9	4	1	14	18	46
	Summer	4	2	3	3	14	26
	Autumn	17	4	3	15	13	52
Positive Samples	Winter	4 (80%)	0	1 (33%)	2 (10%)	5 (45%)	12 (33%)
	Spring	8 (89%)	1 (25%)	0	4 (29%)	8 (45%)	21 (46%)
	Summer	1 (25%)	1 (50%)	1 (33%)	0	9 (65%)	12 (46%)
	Autumn	9 (53%)	3 (75%)	1 (33%)	6 (40%)	10 (77%)	29 (56%)

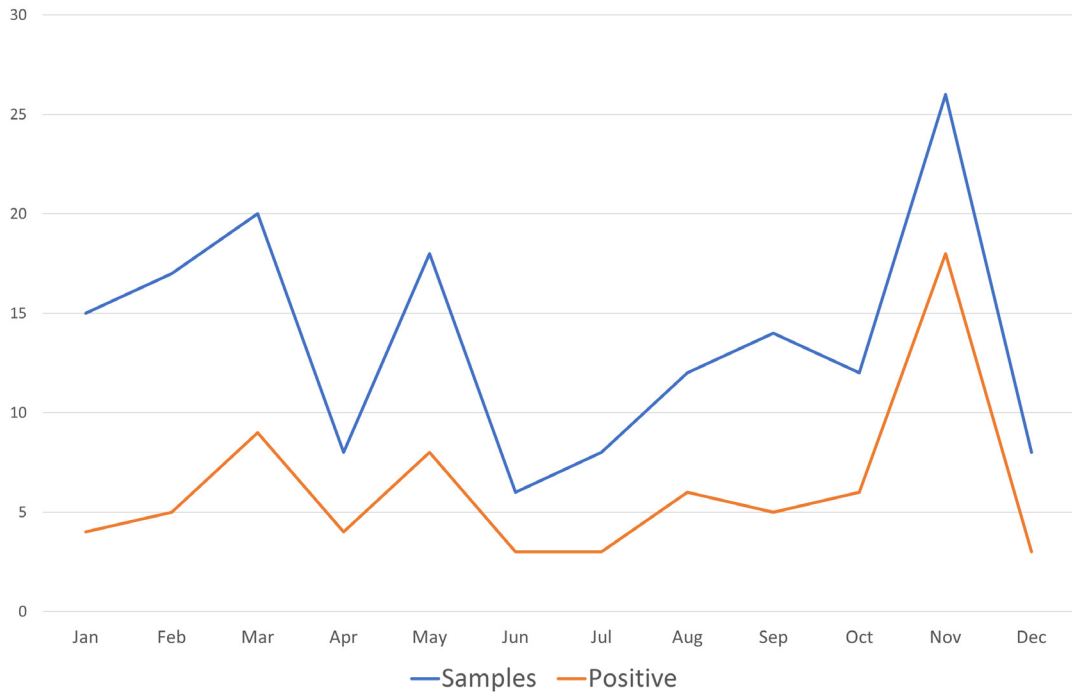

Fig. 2. Monthly evolution of the tested and positive samples

related patients [12]. According to a study conducted by Klug in 2014 in Denmark on peritonsillar abscess, the incidence of the infectious disease has a peak in teenagers and is gradually decreasing until older ages. There were more females under the age of 14, but as the age of the patients increased, men were more affected than women, in the age groups of 20-29 years and 40-49 years [13]. In another study performed in Poland between 2003 and 2013, Mazur et al. revealed that out of 111 patients admitted with peritonsillar abscess more than half were males, showing once again a certain prevalence of this gender in these infections [14]. Also, a study performed in Craiova, Romania in 2019 by Anghelina et al. on 82 patients, revealed that 63.4% were males. Male patients tended to be more likely to develop complications from bacterial upper respiratory tract infections, and complications are more likely to occur in adults than in children [15]. The reasons for the gender difference remains unknown, however it can be addressed to the better oropharyngeal hygiene, a better health care and health concern of women, or habitual factors like smoking and alcohol consumption in men [16,17].

Another factor which led to these results can be related to the fact that many of the patients admitted in the ENT Clinic from Târgu Mureș, Romania were oncologic patients, thus a prevalence of male patients; high prevalence of men in oncology field was also presented by Popescu et al, in 2010, showing that hypopharynx cancer develops in the second half of life, mostly in males. Tobacco consumption, early debut of smoking, combined with alcohol consumption raise the risk of developing cancer in the upper digestive-respiratory tract up to 100 times higher than patients who do not smoke or drink [18].

When analyzing the data in terms of age differences between the tested patients, the results showed that pharyngeal exudates during hospitalization were more likely to be performed on elders (age 61 and higher) . This result can also be related to the fact that most oncologic patients are in the second half of their lives, as Popescu C.R. et al suggested in their study [18].

By the nature of their pathology, ENT oncologic patients are predisposed to long-term hospitalization and long-term post-surgery medical care, oncologic treatment (radiotherapy and chemotherapy) and frequent readmissions, thus a long-term contact with the hospital environment. Due to these factors, oncologic patients have a greater chance of developing nosocomial infections. In a review published by Dustin A. et al in 2020, infections with *Pseudomonas aeruginosa* and *Staphylococcus aureus* predominated. Risk factors such as smoking, malnutrition, and the presence of a tracheostomy tube are significant for healthcare-associated infections in these patients [19].

The results from the present study showed the presence of hospital-related bacteria like *Pseudomonas aeruginosa*, *Acinetobacter baumannii* or *Enterococcus faecium*, which confirms that part of the infections found in the ENT spectrum can be related to the risk factors presented above. Other epidemiological relevant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Stenotrophomonas maltophilia* or *Morganella morganii*, were found in small percentages and not directly relevant to the clinical status of the patient, as they were isolated from non-sterile sites (pharyngeal exudate and ear discharge).

Further analysis of the results concluded that the majority of the tested samples turned out to be negative at the bacteriological examination. One reason could be the fact that many of the middle ear infections (acute otitis media – AOM) are caused by respiratory viruses. Approximately 35% of upper respiratory tract infections are complicated with AOM [20]. Respiratory viruses like adenoviruses, coronaviruses, enteroviruses, influenza viruses, parainfluenza viruses, syncytial respiratory virus, or rhinoviruses are known to cause AOM [20]. The positive ear discharge samples revealed several bacteria that could originate from a pre-existing colonization of the naso-pharynx (such as methicillin-sensitive *Staphylococcus aureus*). When expressing cold symptoms caused mainly by viruses, an inflammation of the nasopharynx and Eustachian tube is initiated and the negative pressure from the middle ear allows bacteria to migrate upwards and cause secondary bacterial AOM [21]. In addition, fungal infections with *Candida* spp. and *Aspergillus spp.* were found, these fungi being known to cause otomycosis (fungal infection of the outer ear canal) [22]. The cause of these fungi appearances in the ear discharge (which is a fluid from the middle ear) was probably a contamination during the sample collection. In the ENT clinics, the middle ear fluid can be obtained in three ways: intraoperative, by tympanocentesis, or during a spontaneous fistulization of the tympanic membrane [23–25]; in our case, the latter case lead most probably to the contamination of the ear discharge with outer ear canal flora.

Concerning the monthly distribution of the samples analyzed versus the positive samples, a pattern of increasing and decreasing number is revealed as the year goes by, with a higher absolute peak in November and an absolute low in June. There can be several reasons implied. Firstly, the seasonality of infections could be a factor that can influence the sampling behavior during the year. Moriyama et al related in their study in 2020 that external factors like temperature, absolute humidity, sunlight and Vitamin D status can affect respiratory virus transmission. For example, influenza virus, human coronavirus or respiratory syncytial virus are predominant in winter time. Adenovirus, parainfluenza virus, human metapneumovirus, and rhinovirus can be detected throughout the year [26]. This can lead to a higher number of patients with respiratory symptoms and a higher rate of pharyngeal exudate sampling, but not necessarily to a high positivity percentage on bacteriological testing. As shown, only 10% of the tested pharyngeal exudates in winter were positive. Another number that draws attention is the number of ear discharge samples analyzed in the Autumn, which might be associated with the end of Summer vacation and the beginning of school/

kindergarten [27]. Roughly 50% samples were positive for bacterial and fungal etiology; assuming that the testing was requested by clinical evidence (patients showing symptoms of middle ear infection), it can be concluded that probably viruses were an important cause in these infections. Secondly, the pattern of sampling could also have been affected by the vacation schedules in the clinic. During the summertime and around Christmas holidays, due to staff shortage, the doctors in the clinic tend to admit fewer non-emergency patients and thus, a lower number of patients reflect a lower rate of testing [28].

Conclusions

Although both men and women develop infections in the Ear, Nose and Throat area, men were more affected. Upper respiratory tract infections and acute otitis media were caused by bacteria, mostly from the genus *Staphylococcus* and *Pseudomonas*. A careful collection of the samples is important to reduce the contamination with commensal microflora, in order to avoid false positive bacteriological results. Because viruses are not easily detectable in ENT by routine methods, an attentive clinical examination has to be followed. In addition, a careful assessment of the clinical status of the patients, especially for signs of viral etiology will reduce the load on bacteriology laboratory. Nevertheless, in specific cases, a negative bacteriological result can prove as an exclusion diagnostic.

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Conflict of interest

None to declare.

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