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Prevalence and Antibiotic Resistance Pattern of *Streptococcus* Genus and Other Pathogens Isolated from Throat Culture Samples of Patients in Fatemeh Al-Zahra Hospital of Sari, Iran

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A B S T R A C T

Introduction: Tracheal tubes are among the primary means of infection transmission in hospitals. Therefore, identifying microbial agents transmitted via this route is necessary to control and prevent these infections. This study aimed to investigate the prevalence of pharyngeal-contaminating microorganisms and their antibiotic resistance pattern. **Methods:** In this cross-sectional study, we used 117 pharyngeal swabs samples obtained from patients referred to Fatemeh Zahra Hospital of Sari, Iran, in 2018. The Samples were obtained using the sterile cotton swab from the throat and then cultured in the sheep blood agar. The positive colonies for the alpha-hemolytic test were subcultured on the Mueller-Hinton agar for further assays, including the susceptibility to optochin, catalase test, Gram's polychromatic stain, microscopic examination, pyrrolidonyl aminopeptidase (PYR) test, sensitization to bacitracin, and latex agglutination assay. The antibiotic susceptibility assay was performed using the agar disk diffusion method according to CLSI-2018 guidelines. The results were analyzed using SPSS 16.0 software and descriptive statistical methods. **Results:** The frequency of *Streptococcus pneumoniae* and *Streptococcus pyogenes* was 13.7% and 9.4%, respectively. However, the highest frequency belonged to *Pseudomonas aeruginosa* (14.5%), and the lowest to *Citrobacter* spp. (0.9%). **Conclusion**: Our results indicated increased antibiotic resistance in streptococcal strains due to inappropriate prescriptions and antibiotic misuse. Therefore, recognizing and controlling the contributing factors and the rational use of antibiotics can be very important.

INTRODUCTION

Attempts to rationalize drug use have always been a critical issue in drug policy. According to the World Health Organization (WHO), rational drug use can address patients' clinical needs with the lowest cost and side effects [1]. Despite the efforts made by many organizations and experts to reduce the excessive consumption of antibiotics in hospitals and communities, this trend is still growing [2]. Increased resistant bacteria and reduced efficacy of antibiotics have imposed considerable costs on the health systems. The high incidence of antimicrobial resistance in countries with the highest consumption shows an urgent need for new and more effective policies to prevent antibiotics overuse [3]. The prevalence of antibiotic resistance has reached dangerous levels in many health centers, and infectious diseases have become a severe threat to human health [4, 5]. Various factors, such as the antibiotics type, the frequency of prescription, prescribed dose, and usage method, contribute to increased antibiotic resistance [6].

In many countries, antibiotics contain 30-50% of prescription drugs among therapeutic agents. Although antibiotics prescription is essential in most bacterial infections, and avoiding them may endanger the patient's life, most studies have shown that 30%- 60% of the prescription drugs are inappropriate or wrong. Indeed, the physicians might make the wrong prescription, or people may use a kind of self-medication [7].

The upper respiratory tract infections affect different body parts such as the nose, sinuses, pharynx, and larynx. Respiratory system infections cause 4.5 million deaths among children annually, with deaths occurring mostly in developing countries [8-11]. Various agents can cause the upper respiratory tract infection, among which are members of the *Streptococcus* family. This family has several genera, including *Streptococcus pyogenes*, *Streptococcus agalactiae*, and *Streptococcus pneumonia* [12]. Over the past decade, *Streptococcus* bacteria have caused an average of 14.5 million severe infections per year worldwide, responsible for 11% of deaths in children below five years of age [13]. *S. pyogenes* is the primary cause of bacterial pharyngitis in children aged 3-15 years, and almost 15-30% of acute pharyngitis is in this age group. Infections caused by this bacterium include otitis of pneumonia, sinusitis, skin, and soft tissue infection, cardiovascular disease, and osteomyelitis encephalitis [14-16].

This study aimed to investigate the prevalence and antibiotic resistance pattern of the streptococcal family and other pathogens isolated from pharyngeal swab samples.

MATERIALS AND METHODS

Isolation and Identification of isolates. We obtained 117 tracheal throat samples from Fatemeh Zahra Hospital of Sari, Iran, in 2018. Demographic characteristics such as gender, admission ward, antibiogram pattern, and the type of microorganisms were registered. Sampling was performed using swabs from the throat through an endotracheal tube (ETT). The swabs were then placed in the Amies transport medium and transported to the Department of Microbiology, Tehran University of Medical Sciences. All samples were cultured on the blood agar medium (Merck) containing 5% sheep blood. The plates were incubated in a jar containing 5% carbon dioxide at 37°C for 48 h and then examined by alpha hemolysis for the growth of the grayish colonies. The positive colonies for the alpha-hemolytic test were isolated and subcultured on the Mueller-Hinton agar for further investigations, including the susceptibility to optochin, catalase test, Gram's polychromatic stain, microscopic examination, pyrrolidonyl aminopeptidase (PYR) test, sensitization to bacitracin, and latex agglutination assay.

Determination of antibiotic resistance patterns. Antibiotic susceptibility testing was performed by the disk diffusion method with 5% carbon dioxide under anaerobic conditions [17]. Antibiotic disks used for this purpose included Ampicillin, Ceftazidime, Ceftriaxone, Cefixime, Colistin, Cephalexin, Cefixime Ciprofloxacin, Cefotaxime, Gentamicin, Imipenem, Norfloxacin, Cotrimoxazole, Vancomycin, Penicillin, Framycetin, Tobramycin, Nalidixic Acid, Oxacillin, Sulfadiazine, Tetracycline, and Novobiocin. (Himedia). The antibiotic susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI-2018) [18]. The results were analyzed using SPSS 16.0 software according to the Mann-Whitney U and Chi-Square tests, the statistical expert's opinion, and the randomness of the collected data.

RESULTS

Isolation and Identification of microorganisms. Our results showed that most samples were from the Intense Care Unit (ICU) section, and very few samples from the outpatient department (OPD) and post-take ward. Statistical analysis based on gender indicated that there was no significant difference between males and females. Table 1 shows the association of gender with the infection in different wards of the hospital.

Table 1. Association of gender to the infection in different wards of the hospital

	Gender		Admission ward				
	Male	Female	ICU	CCU	Post-take	OPD	Urgency
Frequency (No.)	54	58	60	37	2	2	2
Frequency (%)	46.2	49.6	51.3	31.6	1.7	1.7	1.7
Missing system	4.3% (5/117)						
Total	95.7% (112/117)		88.0% (103/117)				
	All samples were related to the throat and throat culture						
	Frequency (No.)		**Percent**				
Valid ETT	114		97.4				
Missing System	3		2.6				

In this study, the frequency of *S. pneumonia* and *S. pyogenes* was 13.7% and 9.4%, respectively. However, the highest frequency belonged to *pseudomonas aeruginosa* (14.5%), and the lowest to *Citrobacter* spp. (0.9%) (Table 2).

Antibiotic susceptibility pattern. According to antibiotic susceptibility patterns, the highest susceptibility belonged to nalidixic acid, ampicillin, and ciprofloxacin. On the other hand, the lowest susceptibility belonged to tetracycline, sulfadiazine, vancomycin, and norfloxacin (Table 3).

DISCUSSION

Streptococcal sore throat is a common disease of autumn and winter that mainly affects children. Typical symptoms are sore throat and fever; in this case, people usually think they have a cold. Therefore, they go for treatment by drinking warm liquids, eating light foods, relaxing, and possibly using multiple doses of antipyretics and pain relievers. Meanwhile, the lack of timely treatment of this sore throat may cause a heart disease named rheumatic fever, which may proceed to cardiac complications. Therefore, early and timely diagnosis and its correct treatment can prevent the onset of rheumatic fever and cardiac complications [19-21]. *S. pneumoniae* is part of the normal microbiota of the upper respiratory tract. Like many microbiotas, it can become pathogenic under specific conditions, especially when the host immune system is suppressed [22]. This bacterium is typically infrequent. However, it can be pathogenic for humans and causes severe diseases such as streptococcal pharyngitis, rheumatic fever, rheumatic heart disease, and scarlet fever [23].

Table 2. The frequency of various microorganisms isolated from throat swab samples.

Microorganism	Frequency (No.)	Frequency (%)
Yeast and Candida	10	8.5
Staphylococcus epidermidis	7	6
S. pneumonia	16	13.7
S. pyogenes	11	9.4
Staphylococcus aureus	8	6.8
P. aeruginosa	17	14.5
***Acinetobacter* spp.**	3	2.6
***Citrobacter* spp.**	1	0.9
***Klebsiella* spp.**	5	4.3
Other	34	29.1
Missing data	5	2.5
Total	117	100

Table 3. Antibiotic susceptibility pattern of the isolates to different antibiotics by disk diffusion method.

Antibiotics abbreviation*	Valid			Missing System (%)
	S	R	I	
NA	23.1%	26.5%	6.0%	44.4%
AMP	23.1%	26.5%	6.0%	44.4%
CAZ	2.6%	29.1%	-	68.4%
CRO	9.4%	37.6%	4.3%	48.7%
CFM	4.3%	9.4%	2.6%	83.8%
CT	12.8%	26.5%	39.3%	60.7%
CN	7.7%	10.3%	-	82.1%
CFM	4.3%	24.8%	-	70.9%
CP	23.1%	35.9%	1.7%	39.3%
CC	8.5%	12.8%	21.4%	78.6%
GM	17.9%	38.5%	0.9%	42.7%
IPM	17.9%	29.9%	3.4%	48.7%
NOR	1.7%	1.7%	-	96.6%
SXT	8.5%	29.9%	0.9%	60.7%
VA	1.7%	-	-	98.3%
P	2.6%	12.8%	-	84.6%
FM	3.4%	12.8%	0.9%	82.9%
TOB	4.3%	2.6%	1.7%	91.5%
NA	3.4%	9.4%	-	87.2%
OX	4.3%	7.7%	-	88.0%
S	1.7%	2.6%	-	95.7%
TE	1.7%	2.6%	-	95.7%
NB	7.7%	0.9%	-	91.5%

*AMP, ampicillin CAZ, ceftazidime; CRO, ceftriaxone; CFM, cefixime; CT, colistin; CN, cephalexin; CFM, cefixime; CP, ciprofloxacin; CC, cefotaxime; GM, gentamicin; IPM, imipenem; NOR, norfloxacin; SXT, cotrimoxazole; VA, vancomycin; P, penicillin; FM, framycetin; TOB, tobramycin; NA, nalidixic acid; OX, oxacillin; S, sulfadiazine TE, tetracycline; NB, novobiocin

In this study, the prevalence of pneumococcal bacteria was 13.7% for *S. pneumonia* and 9.4% for *S. pyogenes*. This result differed significantly from previous similar studies conducted in Iran. For example, the prevalence of this bacterium was 10.9% in Gorgan [24],78.8% in kindergartens of Mashhad [25], 37.5% in Yazd, and 44.4% in Tehran [26]. These differences might be due to public health status, sample collection method, and the dose of teken antibiotics.

In Turkey, the prevalence of *S. pneumoniae* was 13.9% among healthy children (6-13 years old) [27]. In a study carried out in Belgium, the prevalence of *S. pneumoniae* was 69% [10]. The results obtained from this study showed that the asymptomatic carriers of *S. pneumoniae* in comparison with other countries were low. In addition to geographical differences, this information also indicates the proper health care conditions in this city.

In the present study, the resistance to the examined antibiotics showed a different pattern compared to the previous studies conducted in Iran and other parts of the world. In Mashhad and Yazd, the number of cotrimoxazole-resistant isolates was much higher than in our study; this result can be due to the over-the-counter sales of antibiotics [25]. In our study, 1.30% of the isolates were not resistant to any of the six tested antibiotics. In another study in Spain, 95% of the isolated *S. pneumoniae* showed resistance to amoxicillin/clavulanic acid due to the high use of this antibiotic in Spain over the last few decades [26]. In Taiwan, ceftriaxone resistance was significantly higher than in our study. This difference in the level of resistance and type of resistant antibiotics in different regions of the world could be due to the different types of antibiotics used in different parts of the world [14]. The treatment of *S. pneumoniae* infections should be according to the geographical location of infection and illness. One of the reasons for the high resistance of *S. pneumoniae* to these antibiotics is excessive use of these combinations in pulmonary, sinusitis and middle ear infections and over-the-counter sales in pharmacies.

Based on the results achieved from this study, the resistance to some antibiotics has increased, and it is necessary to take efficient steps to correct the consumption and prescription of antibiotics.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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