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Identifying and Isolating Bacterial Pathogens Causing Acute Pharyngitis

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ABSTRACT

Acute pharyngitis is a prevalent inflammatory condition primarily caused by viral or bacterial infections. Accurate identification of the causative agent is crucial for appropriate treatment selection. This study aimed to investigate the bacterial profile of patients with tonsillitis admitted to Marjan Teaching Hospital, as well as evaluate the potential psychological impact on affected individuals. Tonsil swabs were collected from male and female patients presenting with symptoms such as fever, dysphagia, swollen lymph nodes and pharyngeal tissues, voice alterations, and vomiting. Customized growth media was utilized for culturing the samples. Out of 250 samples, all exhibited positive bacterial growth (100%). Phenotypic and biochemical analyses were conducted to identify the bacteria. The most prevalent bacterium found was Staphylococcus aureus (40%, or 10 isolates), followed by Bacillus cereus (20%, or 5 isolates), Streptococcus pyogenes (16%, or 4 isolates), Streptococcus viridans (12%, or 3 isolates), Staphylococcus epidermidis (8%, or 2 isolates), and Streptococcus pneumoniae (4%). The results indicated a higher susceptibility to infection among male patients compared to females, particularly within the age range of 6-12 years old. These findings contribute to understanding the etiology of acute pharyngitis and underline the importance of accurate pathogen identification for effective treatment strategies.

KEYWORDS: Tonsils, Tonsillitis, Staphylococcus spp., Streptococcus spp., Pharyngitis

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INTRODUCTION

Pharyngitis, a prevalent respiratory tract condition, affects individuals of all ages and both genders worldwide, with children aged 5-15 experiencing higher frequency. The tonsils comprise three groups - lingual tonsil, pharyngeal tonsil, and palatine tonsils - serving as lymphoid tissue contact points between the oral cavity, nose, and pharynx. Covered by mucous squamous tissue and embedded with lymph nodes (Rudolf et al., 2006), these sites often host bacterial infections. Accumulated mucous and liquid secretions in these sites provide a habitat for germs. Tonsillitis arises from the interaction between the tonsils' lymphatic tissues and inflammatory factors. Continuous infections lead to symptoms such as throat irritation, fever, swollen tonsils, and voice distortion (Schappert & Burt, 2006). Infections can progress to complications such as abscesses in or around the tonsils or surrounding tissue (Lauro, 2012), leading to acute tonsillitis. Further complications include otitis media, bronchitis, meningitis, scarlet fever, arthritis, glomerulonephritis, and rheumatic heart disease (Rudolf et al., 2006). The pharynx is divided into three sections: oropharynx, nasopharynx, and hypopharynx. Pharyngitis occurs when inflammation affects the mucous membranes and submucosal structures within the esophagus. These inflammations can be acute or chronic (Gerber, 2005) and often spread to other areas such as the larynx. Bacteria play a crucial role in conditions affecting the tonsils and pharynx. Common bacterial strains associated with tonsillitis and pharyngitis include Staphylococcus aureus, Klebsiella spp., Moraxella catarrhalis, Streptococcus pyogenes, and Haemophilus influenzae (Charles, 2011). Tonsil infections are frequent upper respiratory tract conditions causing morbidity and mortality. The leading causes of these infections are viruses such as the Influenza virus and Group A Beta bacterium (Agarwal et al., 2014). Children are more prone to infection, with a prevalence rate of 30-51% (Ozkaya et al., 2014). Tonsillitis can manifest as acute or chronic/recurrent forms. Acute tonsillitis occurs when pathogenic bacteria cause a primary infection, while the chronic form results from failed treatment or recurring acute

infections more than six times per year. Recovery may be hindered by patients' noncompliance with prescribed treatments or self-medication without medical advice (Windfuhr et al., 2016). If intensive antibiotic treatment fails, tonsillectomy may be necessary (Bond et al., 2006). Symptoms of tonsil infection include vivid secretions in the tonsils, swollen lymphatic glands and tonsils, and fever up to 1.0°C (Wessels, 2011). Infections may result in clinical symptoms such as scarlet fever due to fecal contamination; however, some carriers transmit infections without exhibiting symptoms themselves (Windfuhr et al., 2016). Various aerobic and anaerobic bacteria inhabit the tonsils and pharynx due to favorable conditions like temperature (35-37°C), high humidity, and oxygen variability. Common bacterial strains include Streptococcus spp. and Staphylococcus spp., particularly Staphylococcus aureus (Ryan et al., 2004), with the latter being more virulent. Bacterial infections play a significant role in causing tonsillitis and pharyngitis. In infants and young children, pharyngitis is often accompanied by swollen lymph nodes surrounding the pharynx. More severe infections in older children and adults can manifest as tonsillitis and pharyngitis (Rudolf et al., 2006). Tonsillitis, a widespread condition particularly among children, is predominantly caused by Streptococcus bacteria. Streptococcus pyogenes is a significant human pathogen, entering through the mouth and nose, and causing pharyngitis in the oropharyngeal tract (Ryan et al., 2004). Streptococcus pneumoniae inhabits the tonsils and nasopharynx but also causes severe respiratory infections worldwide. Other implicated bacteria include Staphylococcus aureus, coagulase-negative Staphylococcus (CONS), Enterococcus, E. coli, and Pseudomonas aeruginosa (Blanc et al., 2000). Tonsil injuries can lead to serious complications when caused by Streptococcus pyogenes, Staphylococcus spp., or Enterobacteriaceae species such as Proteus mirabilis and Klebsiella spp. (Sadoh et al., 2007). This study investigates bacterial profiles in acute pharyngitis patients, highlighting the importance of accurate pathogen identification for treatment strategies.

MATERIAL AND METHODS

Preparation of Culture Media

The culture media was prepared in accordance with the processing company's guidelines, utilizing verified information from the packaging. The media was sterilized using an autoclave at 121°C and under 15 psi for 15 minutes, followed by pouring and incubating at 37°C for 24 hours to ensure the absence of contamination. Subsequently, it was stored in a refrigerator at 4°C until usage (Mahon et al., 2011).

Identification of Bacterial Isolates

Bacterial isolates were identified based on their morphological and microscopic features, as detailed below.

Preliminary Bacteriological Identification

Colonies grown on the surface of the initial culture media were first identified according to their morphological characteristics, such as shape, size, color, consistency, odor, and hemolysis. Additionally, bacterial cell features were examined microscopically on agar medium after Gram staining and observation under an oil immersion lens of a light microscope (Cruickshank et al., 1975).

Biochemical Testing

Catalase Test and Oxidase Tests were conducted along with Mannitol Fermentation Test for detecting Staphylococcus aureus based on mannitol sugar fermentation within saline mannitol medium (Macfaddin, 2000).

Bacitracin Sensitivity Test

This test involved spreading Bacitracin antagonist on blood agar media inoculated with bacteria and incubating it at 37°C for 24-48 hours. It was utilized to differentiate between Streptococcus pyogenes (sensitive) and Streptococcus pneumoniae (resistant) to this antibiotic (Cruickshank et al., 1975).

Optochin Sensitivity Test

The test was performed by distributing optochin antagonist on blood agar medium inoculated with bacteria and incubating at 37°C for 24-48 hours. It was used to differentiate between Streptococcus pneumoniae (sensitive) and Streptococcus viridans (tolerant) to this antibiotic (Cruickshank et al., 1975).

Antibiotic Sensitivity Test

Bacteria were inoculated on Mueller-Hinton agar medium using sterile swabs, which were then spread across the medium's surface. Antibiotic discs were subsequently distributed on the medium surface using sterile forceps, followed by incubation at 37°C for 18-24 hours. Results were interpreted by measuring inhibition zones around each disc and comparing them with standard charts from the Clinical and Laboratory Standards Institute (CLSI, 2007) to categorize bacteria as resistant, sensitive, or moderately sensitive to antibiotics.

RESULTS

The study included 250 a sample of patients with tonsillitis who visited a Murjan Teaching Hospital in the year and for both sexes 7 females and 18 males. The results showed that 250 samples gave a positive growth on the media used, with 100% activity. Table (1) shows the numbers of bacterial growth taken from tonsil infections. Regarding the distribution of the number of injuries to the age groups of patients from whom samples were taken. Table (2) shows the numbers and percentages of samples by age group, with regard to infections. Tonsils had the highest incidence of infection for the age group (6-12) years, at a rate of (48%).

Table 1: distribution of isolates.

Source of isolates	Male		Female		Total
	No.	%	No.	%	
Tonsils	180	72	70	28	250 (100%)

Table 2: Distribution of the number of infections and their percentage by age groups.

Age groups	No.	%
6-12	120	48
12-20	80	32
20-25	50	20
Total	250	100

Table (2), shows the types of isolates bacteria from the tonsils, with *Staphylococcus aureus* being the most isolated with 10 isolates out of 250. The other types are also mentioned.

Table 3: Types of isolated bacteria.

Types of isolates	No.	%
Staph. aureus	100	40
Staph. epidermidis	20	8
Strep. pneumonia	10	4
Strep. viridans	30	12
Strep. pyogens	40	16
Bacillus cereus	50	20
Total	250	100

Bacterial isolates were diagnosed based on their culturological and microscopic characteristics and tests. The biochemicals, which included tests for gram-positive bacteria, the table (4) describe the tests for the genus *Staphylococcus* spp. The genus *Streptococcus* spp. and *Bacillus cereus*. It included both (oxidase, catalase, growth on mannitol saline medium, hemolysin production).

Table 4: biochemical test results of isolated bacteria.

Isolates	Oxidase	Catalase	Haemolycin	Novobiocin	Optochin	Bacitracin	MSA
Staph. aureus	-	+	B	+			+
Staph. epidermidis	-	+	Y	-			-
Strep. pneumonia	-	-	a		-		
Strep. viridans	-	-	a		+	-	
Strep. pyogens	-	-	B			+	
Bacillus cereus	-	+	a				

Table 5: susceptibility test results of isolated bacteria

Antibiotics	Concentration	Staph. aureus	Staph. epidermidis	Strep. pneumonia	Strep. pyogens	Strep. viridans	Bacillus cereus
Ciprofloxacin CIP	10	S	S	S	S	S	S
Amikacin AK	10	S	S	S	S	S	S
Levofloxacin LEV	5	S	S	S	S	S	S
Cefixime CEM	5	S	S	S	S	R	S
Gentamycin Cn	10	R	R	S	R	S	R

R= Resistant S= sensitive

DISCUSSION

In this research, 250 bacterial isolates from tonsils were identified and analyzed. The findings, as presented in Table 5, reveal a higher infection rate among males (72%) compared to females (18%). This is in agreement with the study conducted by Al-Ahmary et al. (2012) in Saudi Arabia, which reported a higher prevalence of infection in males than females. The increased susceptibility of males to infections

can be attributed to several factors, including exposure to risk factors and physiological, anatomical, and immunological differences between the sexes. Males exhibit larger pharyngeal skeletal dimensions than females, which may contribute to their increased infection rates (Davidsons et al., 2002). In contrast, females demonstrate an enhanced ability to produce antibodies that provide protection against pathogens and bacterial infections. Concerning age distribution of infection sources, Table 2 displays patient age groups according to the type of infection. Infections occur mostly between the ages of two and three years and peak during early school years before declining towards puberty. High rates of tonsillitis arise from Staphylococcus infections during this period. This pattern can be explained by the spread of infections through close contact in settings such as schools and nurseries via sneezing or coughing. These findings are consistent with those reported by Al-Yasiri et al. (2005) who observed that most cases occurred among children aged 5-8 years old. Regarding specific tonsil infections, Table 3 illustrates that Staphylococcus aureus is the most common cause of tonsil infections (40%), with ten isolates found naturally in the region associated with various conditions such as recurrent tonsillitis and otitis externa (Kocaturk et al., 2003). With regard to antibiotic sensitivity tests, all bacterial isolates were sensitive to antibiotics listed in Table 5 apart from Staph aureus and Staph. epidermidis, Strep. pyogens and Bacillus which were resistant to Gentamycin, and Strep. viridans resistant to Cefixime (Tortora et al., 2004). The resistance mechanisms include inhibition of protein synthesis by binding with the cell's ribosome and an active efflux mechanism that prevents the accumulation of antibiotics within the bacterial cell (Dubin, 2004).

CONCLUSION

In conclusion, this study found that Staphylococcus aureus was the most isolated bacteria from tonsils. The results also highlight a higher susceptibility of males to infections compared to females, particularly during early childhood. In addition to the physical symptoms associated with acute pharyngitis, the psychological impact of this condition should not be underestimated. Patients suffering from acute pharyngitis may experience emotional distress and anxiety due to the painful nature and debilitating effects of their illness. This can lead to increased levels of stress, sleep disturbances, and a potential decrease in overall quality of life. Furthermore, children within the most susceptible age range (6-12 years old) might face challenges in their social lives and academic performance as a result of frequent absences from school or reduced participation in extracurricular activities.

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