Journal of Advanced Laboratory Research in Biology
E-ISSN: 0976-7614
Volume 12, Issue 2, April 2021
PP 14-23
https://e-journal.sospublication.co.in

Original Article | Open Access

Study of bacteria and yeast pathogens causing sore throat in a tertiary care center in Patna

Suprakash Das[1], Swati Suneha[2*], Sayan Bhattacharya[3] and Kranti Bhavana[4]

[1*]*Department of Microbiology, Hind Institute of Medical Sciences, Barabanki, U.P, India.*
[2,4]*Department of Otolaryngology, AIIMS, Patna, Bihar, India.*
[3]*Department of Microbiology, All India Institute of Hygiene and Public Health, Kolkata, India.*

Keywords

Sore throat
Group A Streptococci (GAS)
Antibiotic Resistance
Colonization

Abstract

Introduction- Sore throat is a common diagnosis in clinical practice and is mostly caused by viruses followed by bacteria and fungi. Proper management of sore throat depends on identification of the causative organism and judicious use of correct antimicrobials which is often ignored in daily clinical practice and is resulting in treatment failure and antimicrobial resistance. Healthy population carrying pathogenic organisms can be a significant source of sore throat, especially in the hospital environment.

Aim- The objective of this study was to determine the profile and antimicrobial susceptibility pattern of bacteria and yeast pathogens causing sore throat and to evaluate the colonization of such pathogens in an apparently healthy population.

Materials and Methods- This prospective study was conducted for 2 years among 2 groups: cases (103 clinically diagnosed patients) and controls (42 individuals with no clinical diagnosis of sore throat). 145 pairs of throat swab samples were collected and processed for staining, culture and antimicrobial susceptibility testing. An MS Excel spreadsheet (V. 2007) was used to perform the statistical calculations.

Results- The majority of the cases include adolescents and young adults aged 11-40 years, i.e. 55.3% with an overall male predominance with a male:female ratio of 2.1:1. The causative organisms vary in different age groups with gram-positive organisms being the most prevalent among children and young adults. Most of the cases (53.4%) have seen in months between November to April, indicating a seasonal variation in the incidence of sore throat. Clinically significant isolates from cases like *Staphylococcus aureus* showed 100% sensitivity against vancomycin and linezolid. Group A Streptococci showed good sensitivity (>80%) against penicillin and ampicillin. Gram-negative isolates showed good sensitivity (>50%) against amikacin, piperacillin-tazobactam and imipenem. *Neisseria gonorrhoeae* showed good sensitivity against ceftriaxone (100%).

Conclusion- Sore throat must be treated by identifying the causative pathogen based on the clinical and epidemiological profile of the patient. Surveillance of apparently healthy patient's throat flora in the hospital environment may reduce the spread of such organisms among the susceptible population and contribute to hospital infection control practices.

1 Introduction

Sore throat is one of the commonest complaints in general medicine and ENT OPDs [1]. Sore throat is caused by painful inflammation of the mucous membrane lining the pharynx. Sore throat can be due to various etiologies like infection caused by different viruses, bacteria, fungi or inflammation caused by allergic reactions, malignancy and airway obstruction [2].

The most prevalent bacteria causing sore throat in children is *Streptococcus pyogenes* or Group A Streptococcus (GAS) [3]. Other bacteria are causing sore throat like Group C and G Streptococcus (GCGS), *Corynebacterium diphtheriae, Haemophilus influenza*

*Corresponding Author: Swati Suneha 0000-0003-0711-4935
E-mail: sunehaswati@gmail.com
Phone No.: +918825304721

Received on: 28 April 2021
Accepted on: 27 May 2021

and *Neisseria gonorrhoeae*. Fungal pathogen like *Candida albicans* has also been found to be associated with sore throat [4]. In asymptomatic individuals who includes infants, children and adults, throat can be colonized by potential pathogenic organisms like *Staphylococcus aureus, Haemophilus influenzae, Streptococcus pyogenes,* etc., which may have a role in sore throat and other serious infections [5,6]. Such organisms may start colonization in the early neonatal period or in childhood, especially in developing countries where poor hygiene and overcrowding are very common. They can act as carriers and transmitting such pathogens to other healthy populations [7]. Culture of throat swabs is essential to detect such carriers, especially when *Staphylococcus aureus* or MRSA strains are suspected [8].

It is commonly observed in daily clinical practice that antibiotics are prescribed for sore throat without investigating the etiology and antimicrobial susceptibility pattern of such organisms. This has resulted in increased antimicrobial resistance (AMR) and subsequent treatment failures [9,10].

Considering the fact that sore throat can be caused by various pathogens and determining the correct etiology is important for appropriate therapy and reduction in increasing antimicrobial resistance. The present study aims to investigate various bacteria and yeast pathogens associated with sore throat along with their antimicrobial susceptibility pattern which will guide the clinicians to choose appropriate antimicrobial therapy for such cases. This study also aims to investigate the role of the apparently healthy asymptomatic population as a carrier of such pathogens with the potential to cause sore throat. To the best of our knowledge, no previous study has explored these aspects regarding sore throat cases in this region and will help in formulating appropriate treatment guidelines and infection control policies for such pathogens.

2 Methodology

2.1 Study Duration and Setting

The present prospective observational study was conducted in the Department of Microbiology at AIIMS, Patna for 2 years (November 2017 to October 2019), after obtaining ethical clearance from the institutional ethical committee (AIIMS/PAT/IEC/2017/192).

2.2 Study Population

The current study was carried out on two different groups of subjects. The first group, comprised of patients with a clinically diagnosed sore throat, is referred to as "Cases" and the second group comprised of individuals without any clinical manifestations of sore throat is referred to as "Controls". The controls were selected among the patients from ENT and general medicine wards admitted in AIIMS, Patna who didn't have symptoms or signs of sore throat and were selected randomly from all age groups and both sexes after obtaining proper consent.

Inclusion criteria for cases

Patients of all ages and sexes suffering from a clinically diagnosed sore throat.

Exclusion criteria for cases

Patients with sore throat due to etiology other than infections.

Inclusion criteria for controls

Patients of all ages and sexes who have no symptoms or signs of sore throat.

Exclusion criteria for controls

Patients suffering from upper or lower respiratory tract infections and patients with intubation.

2.3 Sample size

The sample size was calculated based on the following formula [11]:

$$n= Z^2P (1-P)/d^2 \quad (1)$$

Where n = sample size; Z = 1.96 at 95% confidence interval; P = expected prevalence; d = precision (5% = 0.05).

According to a previously conducted study by Singh *et al.,* (2015), the prevalence of GAS among 63 symptomatic children was found to be 4.8% (3/63) and in 237 asymptomatic children, the carriage rate was 0.8% (2/237) [12]. The minimum sample size for cases was calculated as 96 and for control population it was 62. 103 cases and 42 controls were included in this study as the ratio of both cases and controls isn't of critical importance in a simple observational study.

2.4 Sample collection and processing

A total of 145 pairs of throat swab samples was collected from study subjects following signed and informed written consent, using 2 consecutive sterile swab sticks with proper aseptic precautions from general medicine and ENT OPDs and wards. Two smears are prepared using one of the throat swabs on two clean, grease free glass slides. One smear is stained with Gram's stain and the other with Albert's stain. Albert stain was employed in the case of suspected diphtheria or bacterial morphology appears similar to *Corynebacterium diphtheriae*. Another swab stick was used for culture on chocolate agar, blood agar, MacConkey agar and SDA plates based on gram stain morphology in stain smears. All swab culture plates were inoculated aerobically for 24-48 hours other than swabs, which showed gram-positive cocci in small chains or gram-negative diplococci in gram-stained smears. Those swab cultures were inoculated in a CO_2 incubator with 5% CO_2. The identification of organisms was done by standard microbiological procedures which include colony characteristics and biochemical tests [13,14]. All isolates were presumptively identified as *Streptococcus* spp. were further reconfirmed up to species level by KB005A HiStrep^{TM} Identification kit (Hi-Media, Mumbai) according to the manufacturer's directions (Fig-1 & 2). Antimicrobial susceptibility testing was performed by

Kirby-Bauer disc diffusion method in Mueller Hinton agar or MGM agar (in case of fungal isolates) using commercially available discs (Hi-Media, Mumbai) and zone size interpretations were done using the CLSI (2017) guidelines [15].

Fig-1: Bacitracin Sensitivity Test.

2.5 Statistical analysis

The statistical analysis was done using Microsoft Excel Sheet 2007 and quantitative data were represented as tables and graphs. The chi-square test was applied and $p \leq 0.05$ was considered statistically significant.

3 Results

This study comprised patients from children to the older age group and the age range of cases (n = 103) and controls (n = 42) were 6 months to 80 years and 9 months to 75 years respectively. The majority of the cases belong to the age group of 11-40 years (n = 57, 55.3%). Among the 103 cases, 70 were male and 33 were female with a male:female (M:F) ratio of 2.1:1. In the second control group, the male was 19 and females were 23 with a male:female (M:F) ratio of 1:1.2. The majority of control group participants were in the age group of 11-40 years (n = 21, 50%) (Table-1).

Out of the 103 cases, 31 (30.1%) were found clinically significant isolates with the potential to cause sore throat or oropharyngeal colonization, no growth was observed in 7 cases (6.8%) and in the rest of the 65 cases (63.1%) growth of normal oropharyngeal flora was seen. Among those clinically significant isolates obtained from cases, 18 were from male and 13 from female (Table-2, Fig-3).

In the second control group, 9 isolates (21.4%) were clinically significant as a potential pathogen [n = 5; *Streptococcus pyogenes* (n = 2), *Streptococcus dysgalactiae* (n = 2), *Neisseria gonorrhoeae* (n = 1)] and oropharyngeal colonizer [n = 4; *Staphylococcus aureus* (n = 1), *Staphylococcus epidermidis* (n = 1), *Pseudomonas aeruginosa* (n = 1), *Candida albicans* (n = 1)] and culture from 2 controls (4.8%) didn't grow any isolates and in rest 31 controls (73.8%) culture showed growth of normal oropharyngeal flora. Clinically significant isolates of control population have a male:female (M:F) ratio of 4:5 (Table-2, Fig-4).

Among the 31 clinically significant isolates from cases, 16 were found to be potential pathogens for causing sore throat, including 6 isolates of group A *streptococci* (*Streptococcus pyogenes*), 4 group C *streptococci* (*Streptococcus dysgalactiae*), 3 *Pseudomonas aeruginosa*, 3 *Neisseria gonorrhoeae* and 15 isolates were potential oropharyngeal colonizers including 6 isolates of *Staphylococcus aureus*, 2 *Staphylococcus epidermidis*, 3 *Escherichia coli*, 2 *Klebsiella pneumoniae*, 1 *Acinetobacter baumannii* and 1 *Candida albicans* (Table-2).

Fig-2: HiStrep ™ Identification Kit (KB005A) from HiMedia (Mumbai): A) Before inoculation of test strain, B) After inoculation of test strain.

Table-1: Age and Sex distribution among Cases and Controls.

Age (Years)	Cases-Male	Cases-Female	Total	Control-Male	Control-Female	Total
0-10	10	3	13	2	3	5
11-20	11	6	17	2	3	5
21-30	15	7	22	4	6	10
31-40	12	6	18	2	4	6
41-50	8	3	11	3	2	5
51-60	7	4	11	3	3	6
61-70	4	3	7	2	1	3
71-80	3	1	4	1	1	2
Total	**70**	**33**	**103**	**19**	**23**	**42**

Table-2: Distribution of isolates among Cases and Controls.

Organism	CASES			CONTROLS		
	Male	**Female**	**Total**	**Male**	**Female**	**Total**
Gram-positive cocci (n=24)						
***Streptococcus pyogenes* (n=8)**	4	2	6	1	1	2
***Staphylococcus aureus* (n=7)**	3	3	6	0	1	1
***Streptococcus dysgalactiae* (n=6)**	3	1	4	2	0	2
***Staphylococcus epidermidis* (n=3)**	1	1	2	0	1	1
Enterobacteriaceae (n=5)						
***Escherichia coli* (n=3)**	1	2	3			
***Klebsiella pneumoniae* (n=2)**	1	1	2			
Gram-negative non-fermenters (n=5)						
***Pseudomonas aeruginosa* (n=4)**	1	2	3	0	1	1
***Acinetobacter baumannii* (n=1)**	1	0	1			
Gram-negative cocci (n=4)						
***Neisseria* spp. (n=4)**	2	1	3	1	0	1
Fungus (n=2)						
***Candida albicans* (n=2)**	1	0	1	0	1	1
Total (n=40)	18	13	31	4	5	9

Fig-3: Distribution of culture isolates among cases.

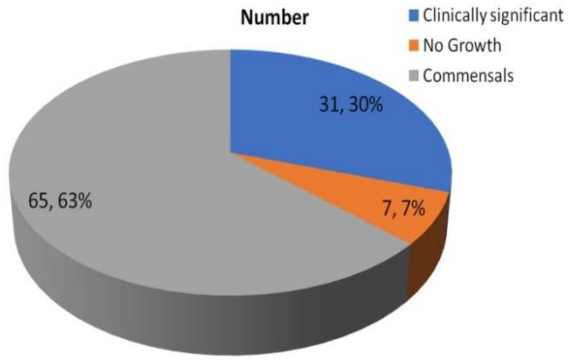
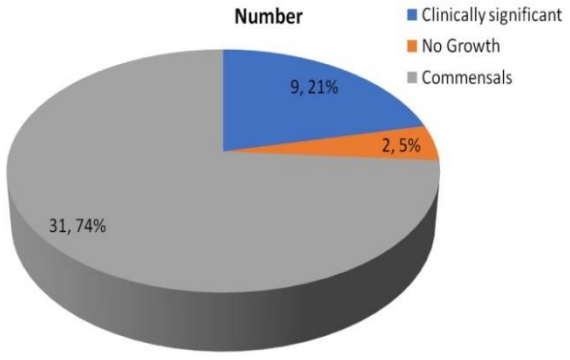


Fig-4: Distribution of culture isolates among controls.



Most of the clinically significant isolates obtained from cases (n = 20, 64.5%) and control group (n = 5, 55.6%) belong to the age group of 11-40 years (Table-3, Fig-5).

The majority of sore throat cases (n = 55, 53.4%) and most of the clinically significant isolates (n = 19, 61.3%) were recorded during the months of November to April (Fig-6 & 7).

Antimicrobial susceptibility testing of isolates of cases showed that *Staphylococcus aureus* isolates were highly resistant to antibiotics like ofloxacin (66.7%), cefepime (83.3%), ampicillin-clavulanate (66.7%) and erythromycin (66.7%). Better sensitivity was shown against gentamicin (50%), ciprofloxacin (50%) and tetracycline (50%). All the isolates were resistant to penicillin, but sensitive to vancomycin and linezolid. Amikacin (88.3%), netilmicin (88.3%), and clindamycin (66.7%) were some of the other antibiotics that showed higher sensitivity. Two MRSA isolates were found to be resistant to multiple groups of antibiotics.

Isolates of *Staphylococcus epidermidis* showed high resistance to antibiotics like cefepime (100%) and penicillin (100%). Most of the isolates were sensitive to amikacin (100%), ciprofloxacin (50%) and levofloxacin (50%). All of the isolates were sensitive to vancomycin, linezolid, netilmicin, tetracycline and co-trimoxazole (100% sensitivity).

Table-3: Age-wise distribution of isolates among Cases and Control.

Age Group	Organism	Cases			Control		
		Male	Female	Total	Male	Female	Total
0-10 (n=3)	*Streptococcus pyogenes*	1	0	2	1	0	1
	Staphylococcus aureus	1	0				
11-20 (n= 8)	*Streptococcus pyogenes*	2	1	7	0	1	1
	Staphylococcus aureus	1	1				
	Streptococcus dysgalactiae	1	0				
	Staphylococcus epidermidis	1					
21-30 (n= 9)	*Streptococcus pyogenes*	1	1	7			2
	Streptococcus dysgalactiae	2	1		1	0	
	Neisseria spp.	1	1				
	Staphylococcus aureus				0	1	
31-40 (n= 8)	*Staphylococcus aureus*	1	2	6			2
	Staphylococcus epidermidis	0	1				
	Streptococcus dysgalactiae				1	0	
	Escherichia coli	0	1				
	Neisseria spp.	1	0		1	0	
41-50 (n= 4)	*Escherichia coli*	1	1	3			1
	Klebsiella pneumoniae	1	0				
	Staphylococcus epidermidis				0	1	
51-60 (n= 4)	*Pseudomonas aeruginosa*	1	1	3			1
	Acinetobacter baumannii	1	0		0	1	
61-70 (n=2)	*Klebsiella pneumoniae*	0	1	2			0
	Pseudomonas aeruginosa	0	1				
71-80 (n=2)	*Candida albicans*	1	0	1	0	1	1
Total (n= 40)	18	13		31	4	5	9

Fig-5: Comparative age-wise distribution of clinically significant isolates (%) among cases and controls.

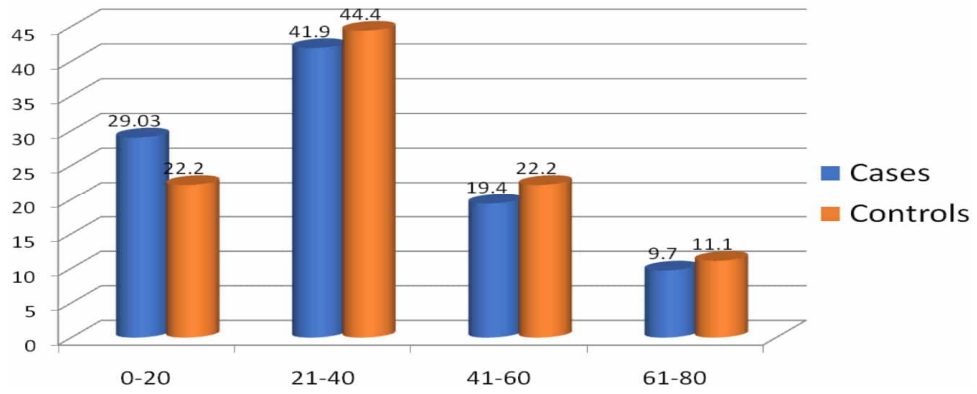


Fig-6: Month-wise distribution of cases.

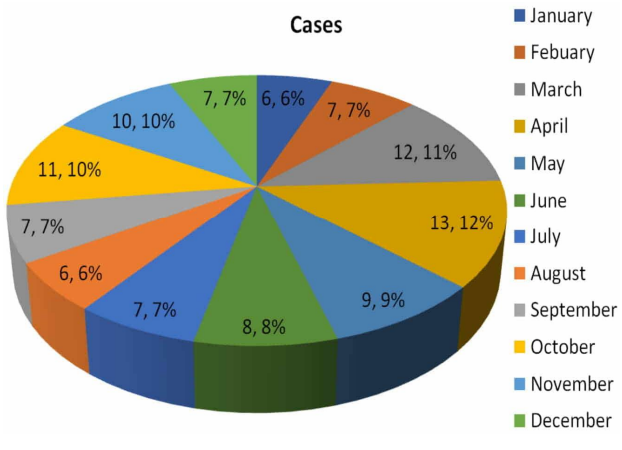
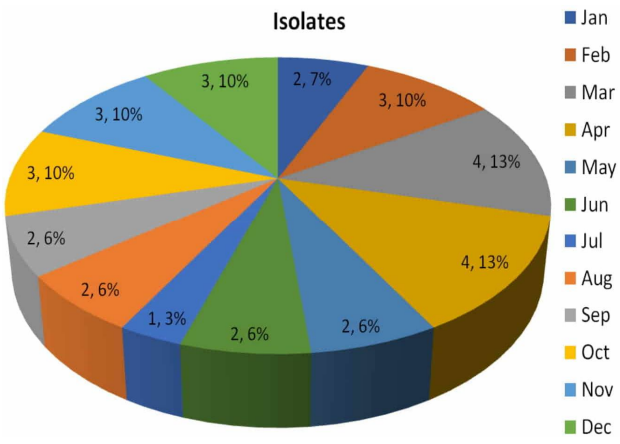


Fig-7: Month-wise distribution of clinically significant isolates from cases.



The group A streptococci (*Streptococcus pyogenes*) showed 100% sensitivity to amikacin, cefepime, netilmicin, tetracycline, clindamycin, co-trimoxazole, linezolid and vancomycin.

Ciprofloxacin showed relatively less sensitivity (66.7%). With a single exception, all the isolates were sensitive to penicillin (88.3%).

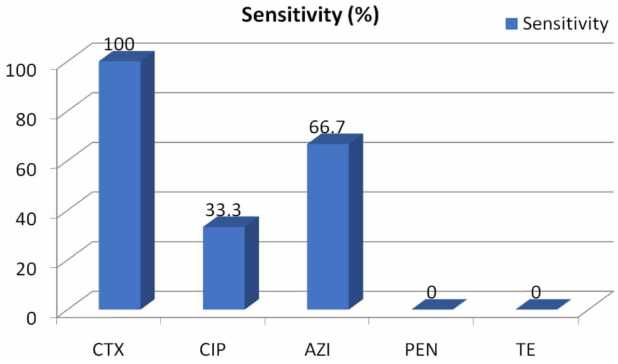
The group C streptococci (*Streptococcus dysgalactiae*) showed 100% sensitivity to multiple groups of antibiotics, but only 75% sensitivity to ceftazidime, cefepime, and tetracycline (Table-4).

Gram-negative non-fermenters like *Pseudomonas aeruginosa* and *Acinetobacter baumannii* showed less sensitivity to multiple groups of antibiotics such as piperacillin-tazobactam (50%), ciprofloxacin (50%), levofloxacin (50%), ceftazidime (25%), cefepime (25%), and ceftriaxone (50%). They showed high sensitivity to amikacin (100%), imipenem (75%), co-trimoxazole (75%), netilmicin (75%) and meropenem (100%).

Isolates of the Enterobacteriaceae family, such as *Escherichia coli* and *Klebsiella pneumoniae* were resistant to ciprofloxacin (40%), levofloxacin (60%), ceftazidime (60%), cefepime (80%) and ceftriaxone (60%). They showed high sensitivity to amikacin (80%), piperacillin-tazobactam (60%), co-trimoxazole (80%), imipenem (80%), meropenem (60%), cefoperazone-sulbactam (60%) and netilmicin (80%) (Table-5).

All isolates of *Neisseria gonorrhoeae* were susceptible to ceftriaxone, while 2 of them were resistant to ciprofloxacin (66.7%), 1 to azithromycin (33.3%) and all to penicillin and tetracycline (100%) (Fig-8).

Fig-8: Antimicrobial Sensitivity (%) of *Neisseria gonorrhoeae.*



There was a single isolate of *Candida albicans* which showed sensitivity to amphotericin B, and fluconazole.

4 Discussion

In the present study, 103 clinically diagnosed cases of sore throat were included, showed a male predominance with a Male:Female (M:F) ratio of 2.1:1, which is consistent with a study done by Yadav *et al.*, (2015) where 57 patients out of 100 were male with a Male:Female (M:F) ratio of 1.3:1.

Most of the cases (55.3%) belong to the age group of 11-40 years, which includes the adolescent and young age group population. This is in contrast to previous studies where patients of the paediatric age group and cases below ten years were considered [16,17]. In this study, patients of all age groups were included and it was found that the adolescent and adult population may be a major contributor (55.3%) of sore throat cases, but when compared to other age groups, there was no statistically significant difference ($p = 0.218997$, i.e. ≥ 0.05) in the isolation of clinically significant isolates. A study conducted by Humair *et al.*, (2006) showed a similar age distribution among adult patients with a sore throat, where 37.4% of patients were in the age group of 26-35 years and 37.6% of patients were culture positive for GAS [18].

The culture results of the swab samples collected from cases showed growth of clinically significant isolates in 30.1% of the cases and the growth of commensal oropharyngeal flora in 63.1% of the cases. This finding is consistent with the results of previous studies, which found the growth of pathogenic isolates in 35%, 37.6% and 42.4% of cases [1,18,19].

A study conducted by Rao Sadanand and Shanker Venkatesh (2018) showed a 26% prevalence of group A streptococci between 3-15 years of age group, which is significantly higher than the prevalence of 13.3% found in this study [20].

Gram-positive cocci such as *Streptococcus pyogenes* (GAS) and *Staphylococcus aureus* constitute the majority of the clinically significant isolates (n = 12/31, 38.7%), which is consistent with the findings of a study conducted by Yadav *et al.*, (2015), who found that these 2 organisms constitute 29% of pathogenic isolates [1]. Another study conducted by Mokkapati *et al.*, (2013) showed that *S. pyogenes* and *S. aureus* constitute 59.69% of all bacterial isolates, which is a higher percentage of isolates obtained and correlates with the current study [19]. Two of the *S. aureus* isolates were methicillin-resistant (MRSA). According to a study conducted by Thirumazhisi Sachithanandam (2014), MRSA isolates induce an increase in pharyngeal infection [21].

Table-4: Antimicrobial Sensitivity (%) of Gram-Positive Pathogenic isolates from Cases.

Organism	AK	GEN	CIP	LE	CAZ	CPM	NET	TE	CD	E	AMC	P	COT	LZ	VAN
S. aureus	88.3	50	50	33.3	33.3	16.7	88.3	50	66.7	33.3	33.3	0	83.3	100	100
S. epidermidis	100	100	50	50	50	0	100	100	50	50	100	0	100	100	100
S. pyogenes	100	83.3	66.7	83.3	83.3	100	100	100	100	83.3	50	83.3	100	100	100
S. dysgalactiae	100	100	100	100	75	75	100	75	100	100	100	100	100	100	100

AK: Amikacin; **GEN**: Gentamicin; **CIP**: Ciprofloxacin; **LE**: Levofloxacin; **CAZ**: ceftazidime; **CPM**: Cefepime; **NET**: Netilmicin; **TE**: Tetracycline; **CD**: Clindamycin; **E:** Erythromycin**; AMC**: Ampicillin-Clavulanate; **P**: Penicillin; **COT**: Co-Trimoxazole; **LZ**: Linezolid; **VAN**: Vancomycin

Table-5: Antimicrobial Sensitivity (%) of Gram-Negative Pathogenic isolates from Cases.

Organism	AK	GEN	PT	IMP	MRP	CPM	CTX	CAZ	CFS	CIP	LE	NET	COT
Enterobacteriaceae													
E. coli + K. pneumoniae	80	60	60	80	60	20	40	40	60	60	40	80	80
Gram-negative Non-fermenters													
P. aeruginosa + A. baumannii	100	50	50	75	100	25	50	25	50	50	50	75	75

AK: Amikacin; **GEN**: Gentamicin; **PT**: Piperacillin-Tazobactam; **IMP**: Imipenem: **MRP**: Meropenem; **CPM**: Cefepime; **CTX**: Ceftriaxone; **CAZ**: ceftazidime; **CFS**: Cefoperazone-Sulbactam; **CIP**: Ciprofloxacin; **LE**: Levofloxacin; **NET**: Netilmicin; **COT**: Co-Trimoxazole

Our results are in agreement with previous studies that showed 3 isolates of *Pseudomonas aeruginosa* (9.7%) were isolated from cases that are potential colonizers of throat and may cause sore throat in patients with cystic fibrosis [22,23].

A single isolate of *Acinetobacter baumannii* was isolated from cases, that may represent a colonizer rather than the actual pathogen causing sore throat [24,25].

In the present study, 3 isolates of *Escherichia coli* (9.7%) were isolated from cases that are a potential colonizer mostly in debilitated patients and responsible for different respiratory infections, including pneumonia, which is consistent with the findings of Wang *et al.*, (2000) and de Lastours *et al.*, (2015) [26,27].

Two isolates of *Klebsiella pneumoniae* (6.5%) were isolated from cases and it is best known as a pathogen causing various respiratory infections including sore throat [28]. According to Mokkapati *et al.*, (2013), *K. pneumoniae* was detected in 23.88% percent of bacterial isolates from sore throat patients, showing its significance as a respiratory pathogen [19].

As shown in a study by Fredheim *et al.*, (2015), two isolates of *Staphylococcus epidermidis* (6.5%) isolated from cases may represent colonizers rather than causative organism for sore throat [29].

In this study, four isolates of *Streptococcus dysgalactiae* (13%) were isolated from cases. Studies carried out by various authors revealed their role as an emerging cause of pharyngitis which was previously considered as commensal or laboratory contaminants [30,31].

A single isolate of *Candida albicans* has been isolated from a case and a study done by Southern *et al.*, (2008) showed that *Candida* spp. can be a potential colonizer of mucosal surfaces in patients with debilitating conditions and may be associated with chronic pharyngitis. This is in line with the findings of the current study, in which the isolate was obtained from an elderly patient [32].

Three isolates of *Neisseria gonorrhoeae* were isolated from young and sexually active cases. *Neisseria gonorrhoeae* is an obligate human pathogen and may cause a sore throat. A study conducted by Morris *et al.*, (2006) showed that the prevalence of pharyngeal gonorrhoeae was 5.5% among sexually active MSM population and positively associated with the younger population. These findings are consistent with the results of the present study [33].

In this study, it was found that there was a seasonal variation in the incidence of sore throat as most of the cases were in the months of winter and springtime (n = 55, 53.4%). A similar observation was seen in studies conducted by Peter *et al.*, (1977) and Nandi *et al.*, (2001), where the prevalence of pharyngitis was highest in the spring and winter months, while other authors observed the highest prevalence in summer or rainy seasons [34,35,36].

A study conducted by Eccles *et al.*, (2002) suggested a possible explanation for this seasonal variability, which is due to exposure and inhalation of cold air, resulting in cooling of the nasal epithelium and subsequent inhibition of defense mechanisms such as mucociliary clearance and phagocytic activity of leucocytes [37].

The control population was selected to find out the role of colonizing or commensal organisms in the development of sore throat and other infections. The study also provides information about the incidence of throat colonizers with the potential to cause sore throat in an apparently healthy population. The control patients were selected with similar age ranges to the cases for a better comparison and understanding.

In this study, gram-positive cocci of *Streptococci* spp. and *Staphylococci* spp. were found to be the predominant pathogens or colonizers (n = 14/16, 87.5%) among the control population aged < 30 years. Several studies have reported that these organisms are potential colonizers with the ability to cause a sore throat and other infections [7,8]. This study confirms these findings.

Neisseria gonorrhoeae was isolated from young sexually active control individual, which coincides with cases and indicates the asymptomatic carriage of *N. gonorrhoeae* in the young sexually active population. A study conducted by Budkaew *et al.*, (2019) showed a high oropharyngeal carriage rate of about 28% in the MSM population [38].

Palmer *et al.*, (2001) isolated 3 isolates of *Pseudomonas aeruginosa* and 15 of *Candida albicans* from elderly institutionalized patients, both of which are potential throat colonizers [39].

The incidence of clinically significant isolates in healthy controls were (9/42, 21.4%) and cases (31/103, 30.1%) (*p*-value is = 0.39, i.e. > 0.05). Gunnarsson *et al.*, (2001) found a statistically significant difference in isolation of pathogenic bacteria between healthy populations and patients with sore throat [40]. In this

study, there was no statistically significant difference found between these two groups, which may be due to a smaller number of controls or a higher colonization rate in a control population. An increasing rate of colonization by potential pathogens among the healthy population in the hospital or community level may pose a significant risk for the further spread of these organisms and the development of sore throat or other infections [7].

The antimicrobial susceptibility test showed that gram-positive isolates were susceptible to amikacin, vancomycin, linezolid, netilmicin, tetracycline and co-trimoxazole. Staphylococcal isolates, including MRSA isolates, showed higher resistance to multiple classes of antimicrobials. Almost all Streptococcal isolates were susceptible to penicillin and most antibiotic classes. Yadav *et al.*, (2015) and Jadhav *et al.*, (2013) reported similar results, except higher resistance to amikacin in *Staphylococcus* isolates [1,36].

Gram-negative isolates of both the Enterobacteriaceae family and non-fermenters showed good sensitivity to amikacin, meropenem, imipenem and co-trimoxazole and also resistance to several commonly used antibiotic classes such as ciprofloxacin. Isolation of such organisms necessitates antimicrobial therapy, which should be based on antimicrobial susceptibility testing. The results of this study are in accordance with the study conducted by Philpot *et al.*, (1980) and Cooper *et al.*, (2001), who found that gram-negative organisms are the most common cause of sore throat and that antibiotic treatment in such patients without determining the actual causative agent increases therapeutic failure and antimicrobial resistance [41,42].

Phouangsouvanh *et al.*, (2018), found that ceftriaxone and azithromycin had the highest sensitivity against *Neisseria gonorrhoeae* [43], which is consistent with the findings of this investigation.

Although *Candida albicans* isn't a recognised cause of sore throat, an antifungal susceptibility test was performed to detect antifungal drug resistance, which is increasing among such isolates, as previously described [44]. The isolate was susceptible to fluconazole and amphotericin-B, which are still the most commonly used antifungal drugs with good sensitivity against *C. albicans* [45].

5 Limitations

This study did not consider the anaerobic flora that causes sore throat. Molecular epidemiological methods are required to determine clonal relationships between isolates obtained from cases and control. The pathogenic potential of colonizing organisms to cause sore throat has yet to be determined.

6 Conclusion

Sore throat is a common clinical condition that affects both children and adults. Sore throat can be caused by a variety of microorganisms, the most common of which are bacteria. Gram-positive cocci are the most significant human bacterial pathogens causing sore throat in children and adults followed by gram-negative organisms. A person's age and associated clinical conditions make them more susceptible to a specific group of bacteria or fungus that might cause sore throat. Both gram-positive and gram-negative microorganisms associated with sore throat showed drug resistance. For drug-resistant cases and to monitor increasing drug resistance, proper identification of causative agents, along with antimicrobial susceptibility testing, may be required. In a hospital environment, apparently healthy individuals may be carriers of clinically significant microorganisms that might colonize other patients or cause sore throat and other infections. A healthy population is predisposed to becoming a carrier of pathogens due to age and other associated clinical conditions. Surveillance of a healthy individual's throat flora during hospital admission may help to prevent colonisation and subsequent development of sore throat, as well as the transmission of such organism among susceptible individuals.

Conflict of Interest - The authors declare that there is no conflict of interest.

References

[1]. Yadav, J.K., Farooq, U., Begum, R., Tak, V., Yadav, S.K., Kushwaha, D.K.S. & Pandey R.K. (2015). Isolation and Identification of Organism from Throat Swab Along with Sensitivity Pattern from the Patients in North India. *Ann. Int. Med. Den. Res.*, *1*(3): 2395-2814.

[2]. Wessels, M.R. (2011). Streptococcal pharyngitis. *N. Engl. J. Med.*, *364*(7): 648–655. https://doi.org/10.1056/NEJMcp1009126.

[3]. Bisno, A.L. (2001). Acute pharyngitis. *N. Engl. J. Med.*, *344*(3): 205–211. https://doi.org/10.1056/NEJM200101183440308.

[4]. Sykes, E.A., Wu, V., Beyea, M.M., Simpson, M. & Beyea, J.A. (2020). Pharyngitis: Approach to diagnosis and treatment. *Can. Fam. Physician*, *66*(4): 251–257.

[5]. Bhatta, D.R., Hamal, D., Shrestha, R., Parajuli, R., Baral, N., Subramanya, S.H., Nayak, N. & Gokhale, S. (2018). Nasal and Pharyngeal Colonization by Bacterial Pathogens: A Comparative Study between Preclinical and Clinical Sciences Medical Students. *Can. J. Infect. Dis. Med. Microbiol.*, 2018, 7258672. https://doi.org/10.1155/2018/7258672.

[6]. Berkovitch, M., Bulkowstein, M., Zhovtis, D., Greenberg, R., Nitzan, Y., Barzilay, B. & Boldur, I. (2002). Colonization rate of bacteria in the throat of healthy infants. *Int. J. Pediatr. Otorhinolaryngol.*, *63*(1): 19–24. https://doi.org/10.1016/s0165-5876(01)00635-8.

[7]. Robinson, J. (2004). Colonization and infection of the respiratory tract: What do we know? *Paediatr. Child Health*, *9*(1): 21–24. https://doi.org/10.1093/pch/9.1.21.

[8]. Mertz, D., Frei, R., Jaussi, B., Tietz, A., Stebler, C., Flückiger, U. & Widmer, A.F. (2007). Throat swabs are necessary to reliably detect carriers of Staphylococcus aureus. *Clin. Infect. Dis.*, *45*(4): 475–477. https://doi.org/10.1086/520016.

[9]. Agarwal, M., Raghuwanshi, S.K. & Asati, D.P. (2015). Antibiotic Use in Sore Throat: Are We Judicious? *Indian J. Otolaryngol. Head Neck Surg.*, *67*(3): 267–270. https://doi.org/10.1007/s12070-015-0864-1.

[10]. Bhardwaj, N., Mathur, P., Behera, B., Mathur, K., Kapil, A. & Misra, M.C. (2018). Antimicrobial resistance in beta-haemolytic streptococci in India: A four-year study. *Indian J. Med. Res.*, *147*(1): 81–87. https://doi.org/10.4103/ijmr.IJMR_1517_16.

[11]. Pourhoseingholi, M.A., Vahedi, M. & Rahimzadeh, M. (2013). Sample size calculation in medical studies. *Gastroenterol. Hepatol. Bed Bench*, *6*(1): 14–17.

[12]. Singh, A.K., Kumar, A., Agarwal, L., Agarwal, A. & Sengupta, C. (2015). Prevalence of group A streptococcal pharyngitis among schoolchildren of Barabanki district, Uttar Pradesh, India. *J. Acad. Clin. Microbiol.*, *17*(2): 110. http://dx.doi.org/10.4103/0972-1282.171893.

[13]. Traub, W.H., Raymond, E.A. & Linehan, J. (1970). Identification of Enterobacteriaceae in the clinical microbiology laboratory. *Appl. Microbiol.*, *20*(3): 303–308. https://doi.org/10.1128/am.20.3.303-308.1970.

[14]. Kharangate, N.V., Pinto, M.J., Rodrigues, S. & Verenkar, M.P. (2001). Characterization of nonfermenters from clinical samples. *J. Assoc. Physicians India*, *49*: 324–326.

[15]. CLSI (2017). Performance standards for antimicrobial susceptibility testing; approved standard; 27th informational supplement. CLSI document M100-S27. Clinical and Laboratory Standards Institute, Wayne, PA.

[16]. Thattil, S.J., Santhosh, S. & Ajith, T.A. (2018). *Staphylococcus* associated acute throat infection among children presented to a tertiary care hospital. *Int. J. Res. Med. Sci.*, *6*(10): 3287–3292. https://dx.doi.org/10.18203/2320-6012.ijrms20184034.

[17]. Dajani, A., Taubert, K., Ferrieri, P., Peter, G. & Shulman, S. (1995). Treatment of Acute Streptococcal Pharyngitis and Prevention of Rheumatic Fever: A Statement for Health Professionals. *Pediatrics*, *96*(4): 758–764.

[18]. Humair, J.P., Revaz, S.A., Bovier, P. & Stalder, H. (2006). Management of acute pharyngitis in adults: reliability of rapid streptococcal tests and clinical findings. *Arch. Intern. Med.*, *166*(6): 640–644. https://doi.org/10.1001/archinte.166.6.640.

[19]. Mokkapati, A. & Yalamanchili, M. (2013). Bacterial isolates causing pharyngitis. *Int. J. Biol. Med. Res.*, 4(2): 3118- 3120.

[20]. Rao Sadanand, L.N. & Shanker Venkatesh, B.M. (2018). A Study of Group A Streptococcal Pharyngitis among School Children (3–15-Year) of Urban Community. *Sch. J. App. Med. Sci.*, *6*(11): 4269-4274.

[21]. Thirumazhisi Sachithanandam, S. (2014). Rising Methicillin-Resistant *Staphylococcus aureus* Infections in Ear, Nose, and Throat Diseases. *Case Rep. Otolaryngol.*, 2014, 253945. https://doi.org/10.1155/2014/253945.

[22]. Pradeepan, S. & Wark, P. (2018). Pseudomonas pharyngitis in a cystic fibrosis patient. *Respirol. Case Rep.*, *6*(5): e00325. https://doi.org/10.1002/rcr2.325.

[23]. Seidler, D., Griffin, M., Nymon, A., Koeppen, K. & Ashare, A. (2016). Throat Swabs and Sputum Culture as Predictors of *P. aeruginosa* or *S. aureus* Lung Colonization in Adult Cystic Fibrosis Patients. *PloS one*, *11*(10): e0164232. https://doi.org/10.1371/journal.pone.0164232.

[24]. Anstey, N.M., Currie, B.J., Hassell, M., Palmer, D., Dwyer, B. & Seifert, H. (2002). Community-acquired bacteremic *Acinetobacter* pneumonia in tropical Australia is caused by diverse strains of *Acinetobacter baumannii*, with carriage in the throat in at-risk groups. *J. Clin. Microbiol.*, *40*(2): 685–686. https://doi.org/10.1128/JCM.40.2.685-686.2002.

[25]. Martín-Aspas, A., Guerrero-Sánchez, F.M., García-Colchero, F., Rodríguez-Roca, S. & Girón-González, J.A. (2018). Differential characteristics of *Acinetobacter baumannii* colonization and infection: risk factors, clinical picture, and mortality. *Infect. Drug Resist.*, 11: 861–872. https://doi.org/10.2147/IDR.S163944.

[26]. Wang, J.Y., Hsueh, P.R., Wang, J.T., Lee, L.N., Yang, P.C. & Luh, K.T. (2000). Recurrent infections and chronic colonization by an *Escherichia coli* clone in the respiratory tract of a patient with severe cystic bronchiectasis. *J. Clin. Microbiol.*, *38*(7): 2766–2767. https://doi.org/10.1128/JCM.38.7.2766-2767.2000.

[27]. de Lastours, V., Malosh, R.E., Aiello, A.E. & Foxman, B. (2015). Prevalence of *Escherichia coli* carriage in the oropharynx of ambulatory children and adults with and without upper respiratory symptoms. *Ann. Am. Thorac. Soc.*, *12*(3): 461–463. https://doi.org/10.1513/AnnalsATS.201412-586LE.

[28]. Yeh, C.F., Li, W.Y. & Hsu, Y.B. (2014). *Klebsiella pneumoniae* pharyngitis mimicking malignancy: a diagnostic dilemma. *Infection*, *42*(6): 1047–1050. https://doi.org/10.1007/s15010-014-0643-z.

[29]. Fredheim, E., Flægstad, T., Askarian, F. & Klingenberg, C. (2015). Colonisation and interaction between *S. epidermidis* and *S. aureus* in the nose and throat of healthy adolescents. *Eur. J. Clin. Microbiol. Infect. Dis.*, *34*(1): 123–129. https://doi.org/10.1007/s10096-014-2197-5.

[30]. Shah, M., Centor, R.M. & Jennings, M. (2007). Severe acute pharyngitis caused by group C streptococcus. *J. Gen. Intern. Med.*, *22*(2): 272–274. https://doi.org/10.1007/s11606-006-0049-4.

[31]. Kłos, M. & Wójkowska-Mach, J. (2017). Pathogenicity of Virulent Species of Group C

Streptococci in Human. *Can. J. Infect. Dis. Med. Microbiol.*, 2017, 9509604. https://doi.org/10.1155/2017/9509604.

[32]. Southern, P., Horbul, J., Maher, D. & Davis, D.A. (2008). *C. albicans* Colonization of Human Mucosal Surfaces. *PLoS One*, *3*(4): e2067. https://doi.org/10.1371/journal.pone.0002067.

[33]. Morris, S.R., Klausner, J.D., Buchbinder, S.P., Wheeler, S.L., Koblin, B., Coates, T., Chesney, M. & Colfax, G.N. (2006). Prevalence and incidence of pharyngeal gonorrhea in a longitudinal sample of men who have sex with men: the EXPLORE study. *Clin. Infect. Dis.*, *43*(10): 1284–1289. https://doi.org/10.1086/508460.

[34]. Peter, G. & Smith, A.L. (1977). Group A streptococcal infections of the skin and pharynx (second of two parts). *N. Engl. J. Med.*, *297*(7): 365–370. https://doi.org/10.1056/NEJM197708182970706.

[35]. Nandi, S., Kumar, R., Ray, P., Vohra, H. & Ganguly, N.K. (2001). Group A streptococcal sore throat in a periurban population of northern India: a one-year prospective study. *Bull. World Health Organ.*, *79*(6): 528–533. https://apps.who.int/iris/handle/10665/74774.

[36]. Jadhav, P.E., Meshram, S.A., Borkar, R.S., Satyanarayana & Hemanthrao, M. (2013). A study on pattern of organisms in throat swab culture and their sensitivity to antibiotics in patients of rims, Adilabad, Andhra Pradesh. *Int. J. Biol. Med. Res.*, *4*(1): 2915-2919.

[37]. Eccles, R. (2002). An explanation for the seasonality of acute upper respiratory tract viral infections. *Acta Otolaryngol.*, *122*(2): 183–191. https://doi.org/10.1080/00016480252814207.

[38]. Budkaew, J., Chumworathayi, B., Pientong, C. & Ekalaksananan, T. (2019). Prevalence and factors associated with gonorrhea infection with respect to anatomic distributions among men who have sex with men. *PLoS One*, *14*(4): e0211682. https://doi.org/10.1371/journal.pone.0211682.

[39]. Palmer, L.B., Albulak, K., Fields, S., Filkin, A.M., Simon, S. & Smaldone, G.C. (2001). Oral clearance and pathogenic oropharyngeal colonization in the elderly. *Am. J. Respir. Crit. Care Med.*, *164*(3): 464–468. https://doi.org/10.1164/ajrccm.164.3.2008149.

[40]. Gunnarsson, R.K., Holm, S.E. & Söderström, M. (2001). The prevalence of potential pathogenic bacteria in nasopharyngeal samples from individuals with a respiratory tract infection and a sore throat--implications for the diagnosis of pharyngotonsillitis. *Fam. Pract.*, *18*(3): 266–271. https://doi.org/10.1093/fampra/18.3.266.

[41]. Philpot, C.R., McDonald, P.J. & Chai, K.H. (1980). Significance of enteric gram-negative bacilli in the throat. *J. Hyg.*, *85*(2): 205–210. https://doi.org/10.1017/s0022172400063233.

[42]. Cooper, R.J., Hoffman, J.R., Bartlett, J.G., Besser, R.E., Gonzales, R., Hickner, J.M. & Sande, M.A. (2001). Principles of appropriate antibiotic use for acute pharyngitis in adults: background. *Ann. Intern. Med.*, *134*(6): 509–517. https://doi.org/10.7326/0003-4819-134-6-200103200-00019.

[43]. Phouangsouvanh, S., Mayxay, M., Keoluangkhot, V., Vongsouvath, M., Davong, V. & Dance, D.A.B. (2018). Antimicrobial susceptibility of Neisseria gonorrhoeae isolates in Vientiane, Lao PDR. *J. Glob. Antimicrob. Resist.*, *13*: 91–93. https://doi.org/10.1016/j.jgar.2017.12.001.

[44]. Whaley, S.G., Berkow, E.L., Rybak, J.M., Nishimoto, A.T., Barker, K.S. & Rogers, P.D. (2017). Azole Antifungal Resistance in *Candida albicans* and Emerging Non-albicans Candida Species. *Front. Microbiol.*, *7*: 2173. https://doi.org/10.3389/fmicb.2016.02173.

[45]. Bhattacharjee, P. (2016). Epidemiology and antifungal susceptibility of Candida species in a tertiary care hospital, Kolkata, India. *Curr. Med. Mycol.*, *2*(2): 20–27. https://doi.org/10.18869/acadpub.cmm.2.2.5.