



Original Article

Isolation, Identification, and Antimicrobial Susceptibility Testing of Bacterial Pathogens at Chronic Sore Throat Patients Attending Outpatient Department of a Tertiary Care Hospital

Md. Asadur Rahman,¹ Md. Khorshed Alam,² Nazmin Rahman,³ Ariful Alam,⁴ Faisal Alam,⁵ Anisur Rahman⁶

Abstract

Abstract: Sore throat manifested by throat pain is a troublesome issue or discomfort for which physicians are used to prescribing antibiotics, but most often, it is found that the patient is not responding to the drug, which gives rise to the question of drug-resistant or whether there is any infection at all? Nonspecific throat pain also imparts serious public health problems. Self-medication, inadvertent and inadequate doses of antibiotics frequently prescribed by both village doctors and registered MBBS doctors is really a public health problem in developing countries like Bangladesh.

Aims: The aims of this study are to isolate the common organisms in chronic throat infection and to find out the effectiveness of various commonly used antibiotics.

Methods: The samples were collected randomly from sore throat patients via a throat swab in 5 ml of 1% sterilized saline water, and the sample was brought to the laboratory and was inoculated within two hours in both blood agar and nutrient agar (NA) media and samples were incubated at 37°C overnight. Then pure colonies were transferred to Mueller Hinton agar, and antimicrobial susceptibility testing was done by the Kirby Bauer disc diffusion method.

Results: A total of 27(24%) out of 111 samples yield positive growth. In a large number of samples, 84 (76%) did not show any isolation of the pathogenic organism. The predominant organism is a *Staphylococcus aureus* (55.55%). Thereafter, streptococcus (18.51%), *Klebsiella pneumoniae* (14.81%) and *Pseudomonas* (11.11%). Amoxiclav is the most resistant drug, along with cefuroxime, azithromycin, and doxycycline, respectively. Levofloxacin and ciprofloxacin show intermediate sensitivity. The most sensitive drug used in chronic sore throat found is linezolid though not frequently used, and other sensitive drugs are moxifloxacin and ceftriaxone.

Conclusion: Throat pain or soreness does not always indicate that patient has been infected by bacteria. Rationale and ethical prescription, as well as microbiological sensitivity testing, are considered to avoid drug-resistant strains.

Keywords: Sore throat, antibiotics, drug sensitivity.

TAJ 2022; 35: No-1: 25-31

Introduction

Sore throat is one of the most common indicative reasons for seeking attention from a physician. In

the United States, 2.1% of ambulatory visits are due to sore throat.¹ On the other hand, in developing countries, the incidence is about 7.5%. Maximum sore throats are because by upper

¹ Associate Professor & Head, Department of ENT & HNS, Rajshahi Medical College, Bangladesh.

² Registrar, Department of ENT & HNS, Shaheed Ziaur Rahman Medical College Hospital, Bogura, Bangladesh.

³ Professor & Head, Department of Microbiology, Kushtia Medical College, Bangladesh.

⁴ Assistant Professor, Department of Surgery, Rajshahi Medical College, Bangladesh.

⁵ Professor & Head, Department of Microbiology, Shaheed Ziaur Rahman Medical College, Bogura.

⁶ Assistant Professor, Department of ENT & HNS, Shaheed Ziaur Rahman Medical College, Bogura.

respiratory tract viruses, for example, rhinovirus, coronavirus, and adenovirus.² The leading bacterial source of sore throat is group A β -hemolytic streptococci (GABHS), which is found in 15% to 36% of children and 5% to 17% of adults with a sore throat.³⁻⁹

Throat pain is a common complaint to ENT specialists. Chronic or recurrent throat pain is an illness that is caused by infection of the sinuses, nasopharynx, tonsils, pharynx, and larynx, even the upper part of the esophagus. So sore throat, tonsillitis, pharyngitis, and laryngitis are commonly caused by bacterial infection. Pain is a troublesome issue or discomfort for which patients seek advice. It is common practice for physician are used to prescribe antibiotic in judiciously, but most often, it is found that the patient does not respond, which give rise to the question of drug resistance or whether there is any infection at all? However, allergic pharyngitis and nonspecific throat pain also impart serious public health problems. Self-medication, inadvertent and inadequate doses of antibiotics prescribed by unregistered village doctors is really a burning problem in developing countries like Bangladesh. Even with a registered specialist, random choice of antibiotics and lack of proper patient counseling plays an important role in developing multi-drug-resistant organisms.

Bacterial resistance to antibiotics in infection processes has been increasing in recent years and has become a serious public health problem.¹⁰

According to the World Health Organization (WHO), bacterial resistance to antibiotics is one of the main health problems worldwide, and no novel antibiotic has been discovered since 1987. Antibiotic resistance prolongs patients' hospital length of stay, increases the costs of treatment, and increases mortality related to infectious diseases. According to the WHO, inappropriate antibiotic use is considered the main reason for the generation of bacterial resistance to antibiotics.¹¹ The abusive and indiscriminate use of antibiotics on a global scale has led to a growing concern in

all health area sectors. As a result, we are on the verge of experiencing a new era where banal infections can no longer be treated with antibiotics¹².

Methodology

This prospective, observational study was conducted in collaboration with one of the specialized microbiology lab and Ear, Nose & Throat outpatient centers in Kushtia Medical College Hospital, Bangladesh, during the period from Jan2020 to Dec 2020.

Materials and Methods

Specimens were collected by a trained medical technologist in the microbiology department. A sterile cotton-wool swab was rubbed over each tonsil, over the back wall of the pharynx, and over any other inflamed area. Two swabs were taken from the same areas. One was used to prepare a smear, while the other was placed into a plastic container. The specimens were processed within two hours. Smear by gram staining was done. The specimens were inoculated on Nutrient Agar, Blood Agar, and MacConkey's Agar media. Identification of the growth was made by colony morphology, gram staining, and relevant biochemical tests. Antimicrobial susceptibility testing of the isolates was performed by Kirby-Bauer modified disc-diffusion technique using Mueller Hinton agar following Clinical and Laboratory Standards Institute (CLSI) 2020 guideline¹³ with commercial discs (Oxoid, UK). The antibiotic discs used in antibiogram were amikacin (10 μ g), amoxicillin-clavulanic acid (20/10 μ g), ampicillin (10 μ g), azithromycin (15 μ g), cefepime (30 μ g) cefixime (30 μ g), cefoxitin (30 μ g), ceftazidime (30 μ g), ceftriaxone (30 μ g), cefuroxime (30 μ g), ciprofloxacin (5 μ g), clindamycin (2 μ g), cotrimoxazole (25 μ g), doxycycline (30 μ g), gentamicin (10 μ g), levofloxacin (5 μ g), linezolid (30 μ g), meropenem (10 μ g), moxifloxacin (5 μ g), penicillin (10 unit), piperacillin-tazobactam (100/10 μ g).

Results

A total of 111, with 56 female and 55 male cases of chronic throat pain not responding to antibiotics, were taken into this study. The culture and sensitivity test was done in all positive cases. Out of the 27 (24.3%), cases show positive results. A large number of patients, 84 (75.6%), did not show any growth. Among the positive group, 18 were female, and 09 were male, and the mean age was between 26 to 45 years. The most common organism isolated is *Staphylococcus aureus* (55.55%) though the primary pathogen of pharyngitis is *Streptococcus. Pyogenes*. The result shows *Streptococcus* (18.51%), *Klebsiella pneumonia*(14.81%), and *Pseudomonas* (11.11%).

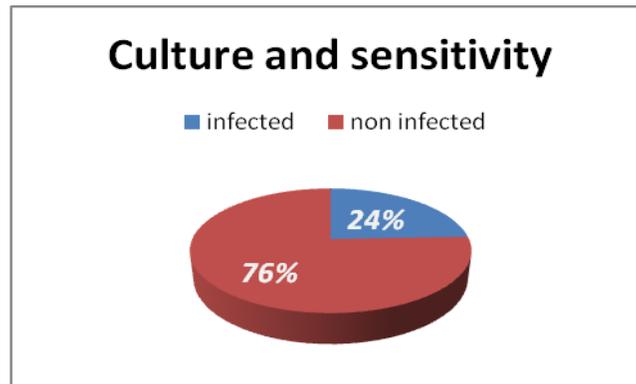


Table 1: Drug history before culture and sensitivity.

The pattern of drug intake	No. of the patient(n=111)	Percentage
Regular intake	25	22.52%
Irregular intake of the drug	26	23.42%
Self-medication of antibiotic/ drug abuse	30	27.02%

Table 2: Organism isolated (n=27)

Name of organism	No of patient	Percentage
S. aureus	15	55.55%
Streptococci	05	18.51%
Klebsiellapneumone	04	14.81%
Pseudomonas	03	11.11%

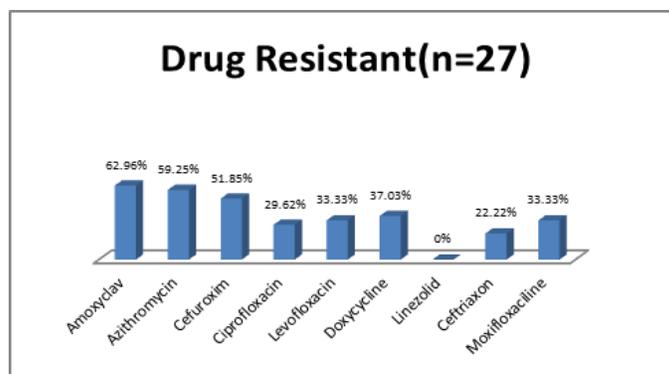


Table 3: General performance of the antibiotics (n = 27)

Name of drug	Resistant(n=27)	Sensitivity(n=27)	Percentage
Amoxiclav	17 (62.96%)	10	37.03%
Azithromycin	16 (59.25%)	11	40.74%
Cefuroxime	14 (51.85%)	13	48.14%
Ciprofloxacin	08 (29.62%)	19	70.37%
Levofloxacin	09 (33.33%)	18	66.66%
Doxycycline	10 (37.03%)	17	(62.96%)
Linezolid	0 (0%)	0	0%
Ceftriaxon	06 (22.22%)	21	77.77%
Moxifloxaciline	05 (33.33%)	22	81.48%

Result & Discussion

Observational studies conducted in the UK have exposed that a huge number of patients with sore throats require to be treated with antibiotics to inhibit a single complication of infection.^{14, 15}Evidences have revealed that one in five patients taking broad-spectrum antibiotics and one in twelve taking narrow-spectrum antibiotics suffer side effects, for example, a rash or gastrointestinal trouble,¹⁶ at the same time, as observational studies with routine data have connected antibiotic use straight to drug resistance at international and local levels.^{17, 18}

In this study, the culture and sensitivity test shows that 84(75.67%) patients did not have any growth of the organism. Robinson AC et al., in their series, also show a large number none grown pathogenic organisms in their study¹⁹. Study

shows in both culture-positive and culture-negative group, females are more affected than male.²⁰study Moirangthem et al. were culture positive in males is 48.78% and females is 51.36%.²¹(Rewrite sentences and correct tense)

Our study shows 27(24.32%) patients having growth of various species of bacteria. The most common organism identified is staphylococcus aureus 15 (55.55%) is similar to A. Moirangthem et al., where a study showed staphylococcus aureus is (56.75%), but PT wakodel et al. reported staph. aureus 25.25%, followed by Streptococcus pyogenes (1.05%).^{21,22} In our study Streptococcus pyogenes 5 (18.51%), Klebsiellapneumone 4 (14.81%) and pseudomonas 3(11.11%).

Regarding age & sex distribution, those who are above 26 years are more susceptible to throat infection. Though usually, children are more

susceptible to throat infections. Females (66.66%) are more victims of bacterial infection than males (33.33%), but opposite to Muhammad Ali et al. where the male is 57.2% & female 42.2%. No male&female difference by sobhanNandiet al.^{23, 24}Female of the lower-class group are frequently affected may be a lack of personal hygiene. On the other hand, a study shows that the incidence of non-infective throat pain is higher in males than females.

For evaluation of the factors regarding drug-resistant history, the pattern of drug consumption by patients was taken and showed that 25(29.76%) have a regular intake of antibiotics, 26(23.42%)have irregular intake, and 30(35.71%) have a history of self-medication. Moreover, frequent use of broad-spectrum antibiotics leaves narrow-spectrum, giving rise to the development of drug-resistant. (Rewrite)

Information from Survey shows that commonly used drugs in throat pain are cefuroxime, azithromycin, amoxiclav, and doxycycline. The result of this study shows that Amoxyclav is the most resistant drug, followed by azithromycin cefuroxime and doxycycline, respectively. Levofloxacin and ciprofloxacin are still less resistant drugs. The most sensitive drug used in chronic throat pain is linezolid though it is not regularly used, and the second sensitive drug is ceftriaxone and moxifloxacin. In upper respiratory tract infection, the commonly chosen drug is amoxiclav, which shows the highest resistance level. The second popular drug preferred by the physician is cefuroxime axetil which also showed moderate resistance.

If we analyze the data, the isolated organism study shows *staphylococcus is* resistant to Amoxyclav11(73.33%), azithromycin 10(66.66%), cefuroxime 9(60.00%), doxycycline 5(33.33%) respectively. Highest sensitivity performed by linezolid 22(100%), Moxifloxacin 12(81.81)% doxycycline10(66.66%).The lowest sensitivity is seen by Amoxyclav 04(26.66%) and azithromycin5(33.33%). Levofloxacin9(60.00%) and ciprofloxacin9(60.00%) are still less resistant drugs.

Regarding,*strepto.pyogen*,cefuroxime4(80%),amoxyclav3(60%),azithromycin3(60%)resistant. Whereas.ceftriaxon5(100%),moxifloxacin5(100%),linezolid5(100%), Levofloxacin4(80%) and ciprofloxacin4(80%) sensitive.

Staphylococci are known to produce multidrug-resistant as it has the ability to produce penicillinase, which is usually determined by the presence of the plasmid. Whereasstreptococcus is a delicate organism but resistant to erythromycin Borbeau E et al. s (1982), Finland et al. 6(1976) and Seppala H. et al. v (1992) and azithromycin.^{25, 26}

Out of four Klebsiella species, 3(75%) show sensitivity to ciprofloxacin same for levofloxacin 3(75%) and two species (50%) %)for cefuroxime axetil, doxycycline, and ceftriaxon3(75%) closely matches with a study by Muhammad Ali et al. where Klebsiella showed high susceptibility to levofloxacin, Ciprofloxacin (83.3%) each, Gentamicin, Ofloxacin and Ceftriaxone (75%) each and relatively sensitive to Tobramycin (66.7%).

For 3(11.11%) Pseudomonasaureginosa Cipro and levofloxacin and moxifloxacin show 100% sensitivity supported by P.T.Wakode et al., whereas ceftriaxone show 1 (33.33%)sensitivity.²⁷This organism shows resistance to cefuroxime, azithromycin amoxiclav doxycycline. In this study, all of the organisms show resistance to more than one antibiotic, except no organism found resistant to linezolid.

Antibiogram also reveals the sensitivity of various drugs by analyzing the disc diffusion assay, where the degree of inhibition at a specific concentration(1mg/ml) of a particular antibiotic against the test organism is done. Organism shows the highest level of inhibition with linezolid and second inhibition with ceftriaxone.

Antibiotic resistance has the potential to affect people at any stage of life, as well as the healthcare, veterinary, and agriculture industries, making it one of the world's most urgent public health problems. The widespread use of antibiotics kills the susceptible cells of microbial organisms, leaving the rare resistant species to survive and

repopulate the ranks. Using an antimicrobial drug for any infection, in any dose, and over any period of time forces microbes either to adapt or to die; microbes that adapt carry genes for drug resistance that are then passed on. When an antimicrobial is not used appropriately, for the correct period of time, correct dose, at adequate potency, or for the right disease, microbes are more likely to develop resistance to that drug.

Conclusion

Non-infective throat pain or sore throat is not infrequent. Reasons for exploring non-infective sore throat is not the aim of this study due to lack of financial funds. Here result from this study inform the clinicians' current trends of effective antibiotics for sore throat and help them to choose proper antibiotics in the proper dose. Screening before prescribing antibiotics is also important. The selection of appropriate antibiotics, in turn, reduces the risks of antibiotic resistance. Microbiological sensitivity and resistance tests must be considered in order to avoid drug overuse and the development of drug-resistant strains.

Conflict of interest: None declared

References

- Woodwell DA. National Ambulatory Medical Care Survey: 1998 Summary. Hyattsville, Md: National Center for Health Statistics; 2000. Advance Data From Vital and Health Statistics, No. 315.
- Gwaltney Jr JM, Bisno AL. Pharyngitis. In: Mandell GL, Bennett JE, Dolin R, eds. *Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases*. 5th ed. Philadelphia, Pa: Churchill Livingstone; 2000:656-662.
- Mclsaac WJ, White D, Tannenbaum D, Low DE. A clinical score to reduce unnecessary antibiotic use in patients with sore throat. *CMAJ*. 1998; 158:75-83.
- Mclsaac WJ, Goel V, To T, Low DE. The validity of a sore throat score in family practice. *CMAJ*. 2000; 163:811-815.
- Komaroff AL, Pass TM, Aronson MD. et al. The prediction of streptococcal pharyngitis in adults. *Journal of General Internal Medicine*, volume 1 (Jan/Feb), 1986; 1:1-7.
- Walsh BT, Bookheim WW, Johnson RC, Tompkins RK. Recognition of streptococcal pharyngitis in adults. *Arch Intern Med*. 1975; 135:1493-1497.
- Kljakovic M. Sore throat presentation and management in general practice. *The New Zealand Medical Journal*, 01 Sep 1993, 106(963):381-383
- Kaplan EL, Top Jr FH, Dudding BA, Wannamaker LW. Diagnosis of streptococcal pharyngitis: differentiation of active infection from the carrier state in the symptomatic child. *The Journal of Infectious Diseases*, Vol. 123, No.5. May 1971; 123:490-501.
- Poses RM, Cebul RD, Collins M, Fager SS. The accuracy of experienced physicians' probability estimates for patients with sore throats: implications for decision making. *JAMA*. 1985; 254:925-929.
- US CDC, "Antibiotic resistance threats in the United States," *Centers Dis. Control Prev.*, 2019
- Harbarth S, Balkhy H.H., Goossens H, et al., "Antimicrobial resistance: One world, one fight! Harbarth et al. *Antimicrobial Resistance and Infection Control* (2015) 4:49
- Snow V., Mottur-Pilson C., Cooper R. J., et al. "Principles of appropriate antibiotic use for acute pharyngitis in adults," *Ann Intern Med*. 2001;134:506-508.
- Bauer, A.W., Kirby, W.M.M., Sherris, J.C. & Turok, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *The American Journal of Clinical Pathology*, 45:493-496.
- Petersen I, Johnson AM, Islam A et al. Protective effect of antibiotics against serious complications of common respiratory tract infections: retrospective cohort study with the UK General Practice Research Database. *BMJ*. 2007; 335:982.
- Little P, Gould, Williamson. Reattendance and complications in a randomised trial of prescribing strategies for sore throat: the medicalising effect of prescribing antibiotics. *BMJ*. 1997; 315: 350-2.
- Lode H. Safety and tolerability of commonly prescribed oral antibiotics for the treatment of respiratory tract infections. *The American Journal of Medicine*, Vol 123, No 4A, April 2010 S26-38.
- Editorial. Antimicrobial resistance: a global threat. *Essential Drugs Monitor*, Issue – No 28 & 29 (2000).
- Thapa B. Antimicrobial resistance: a global threat. *Int J Infect Microbiol* 2012;1(2):41-42
- Robinson A C , Hamf J, Dumbreck LA, Pnchard AJ, Manners BT (1997) Throat swabs m chronic tonsfllms - a time honoured practice best forgotten *Pediatr Infect Dis J* 16 (7) 651-6
- Ataelmanan A. E., Abakar A. D., Hamdan E. M., Isolation and identification of the causative agent of bacterial throat infection according to a response to commonly antibiotic, *GSC Biological and Pharmaceutical Sciences*, 2020, 12(01), 267-272.
- Moirangthem A., Gurung K., Bacteriological Analysis And Its Antibiogram Profile Of Pharyngitis Cases From The Patients Attending Referral Hospital, Sikkim, India. *Bali Medical Journal (BMJ)* 2013, Volume 2, Number 1: 10-13 p-issn.2089-1180, e-ISSN.2302-2914

22. Wakode P. T., Gawarle S.H., Joshi S.V., Bajoriya R. Throat swab-culture and sensitivity reports an overview. Indian Journal of Otolaryngology and head and neck surgery (April-June). Vol.55. No.2. 2003
23. Nandi S., Kumar R., Ray P., Vohra H. &Ganguly N. K. Group A streptococcal sore throat in a periurban population of northern India:a one year prospective study. Bulletin of the World Health Organization, 2001, 79 (6), P:528- 533.
24. Ali M., Mu'azu L., Abdallah M. S. Isolation, Identification and Determination of Antibiotic Susceptibility Pattern of Some Bacteria Associated with Upper Respiratory Tract Infections. Archives of Pulmonology and Respiratory Medicine ISSN: 2639-362X Volume 3, Issue 1, 2020, PP: 10-16
25. Bourbeau P, Campos JM (1982) Current antbloucsusceptibility of group A b hemolytic streptococci. The Journal of Infectious Diseases. Vol. 145, No.6. June 1982, P-916
26. Finland M., Garner C., Wilcox C., et al. Susceptibility of Beta-Hemolytic Streptococci to 65 Antibacterial Agents. Antimicrobial Agents and Chemotherapy, Jan. 1976, Vol. 9, No. 1, p. 11-19.
27. Gul A. A., Rahim E., Ali L., Et Al. Chronic Suppurative Otitis Media; Frequency Of Pseudomonas Aeruginosa In Patients And Its Sensitivity To Various Antibiotics. Professional Med J Sep 2007; 14(3): 411-415.

All correspondence to
Dr Md. Asadur Rahman
Associate Professor & Head,
Department of ENT & HNS
Rajshahi Medical College
Email: dr.asad65@gmail.com