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Original Research Article

Pattern of antibiotic resistance of various strains of bacteria causing acute tonsillitis

N. Jyothsna*, A. Ramya, Kotti Abhilash Reddy, Bathsa Liza Johnson, Kavya Sree Bospali, Ramu G., Sree Vyshnavi A.

Department of Pharmacy Practice, Bharat Institutions, Rangareddy, Telangana, India

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***Correspondence:**
N. Jyothsna,
E-mail: jyothsnanedhnuri@gmail.com

ABSTRACT

Background: Our study was done to determine the pattern of antibiotic resistance of various strains of bacteria causing acute tonsillitis.
Methods: the study was a randomized cross sectional study. Patients matching the inclusion criteria were included. Duration of study was 6 months.
Results: Out of 120 cases, 46 cases showed no bacterial growth (NBG) and 74 cases showed bacterial growth. 42 cases were gram-negative bacterial strain and 32 cases were positive bacterial strain out of 72 bacterial grown cases. A list of 25 antibiotic drugs in gram-negative and 31 drugs in gram-positive strain, their sensitivity and resistance were taken and noted. Among gram-negative bacteria imipenem (71.4%) showed highest sensitivity. Highest antibiotic resistance was seen in ampicillin (85.71%). Least sensitivity is observed in clindamycin, amoxicillin+clavulanic acid with 2.38%. Among gram-positive bacteria, highest sensitivity was noted in cefotaxime (75%). Highest antibiotic resistance was seen in cotrimoxazole (46.8%). Least sensitivity is observed in netilmicin, sulbactam with 3.12%.
Conclusions: The number of drugs resistant to the gram-positive bacteria are lesser than number of drugs sensitive, which showed significant difference ($p<0.05$). Significant difference of antibiotic drugs was not found in gram-negative bacteria. Our study findings helped in appropriate and guarded use of the antibiotic drugs in acute tonsillitis, minimizing the exposure of individuals to antibiotic resistance by choosing an appropriate sensitive drug, therefore improving the quality of therapy.

Keywords: Antibiotic resistance, Tonsillitis, Gram-positive, Gram-negative, Resistance, Sensitive

INTRODUCTION

Tonsillitis

Tonsils are the two lymph nodes which are situated in between the posterior and anterior pillars of the oropharynx. The primary function is the first-line defense against illness by producing WBC and act as an immune defense mechanism by antigen presentation. Macrophages B and T lymphocytes are produced. They are first to reach MALT (mucosa-associated lymphoid tissue) system. Palatine tonsils are physiologically hyperplastic until the age of 6 as it is the main phase of immune acquisition, blood flow is from the ascending pharyngeal artery, lingual artery, ascending and descending palatine arteries.

Tonsillitis is more commonly seen in children, which is caused commonly by *Streptococcal bacteria*, *Staphylococci*, *Pneumococci* or *H. influenza* and

Corynebacterium diphtheria.[1,2] The most common bacterium causing tonsillitis is group A beta-hemolytic *Streptoc*occus. The spread is through an infected person who sneezes or talks, germs in the droplet get released, where they can settle on the mucosa of individuals where the multiplication of germs starts.[2]

Most common signs and symptoms include erythema, enlarged lymphoid nodes, whitish membrane on the surface area, throat pain and difficulty in swallowing. Atypical symptoms include stomach ache, nausea and vomiting.[2] Complications with acute tonsillitis are rare, peritonsillar abscess or quinsy, rheumatic fever and acute glomerulonephritis.[2]

It can be diagnosed if the tonsils are swollen and coated and the patient is with fever >38.3˚C and no cough mostly it can be bacterial tonsillitis. A throat swab test can be done to collect the secretions from tonsils and blood test is rarely done.[1-3]

General treatment measures are antibiotics, NSAIDs and decongestants. Betadine mouth gargle in 1:1 dilution and throat lozenges are suggested[2]

Antibiotic resistance

It is capacity developed by the bacteria to defeat the antibiotic, so the bacteria can grow proportionally and does not get killed. Antibiotics are generally over-prescribed by medical professionals and overused by the public. Bacterial or fungal infections caused to the people are threatened for the effectiveness in preventing and treatment due to antimicrobial resistance (AMR).[2,4] Here antibiotic resistance means the bacteria are becoming resistant to the drugs but not the body that is the individual who is taking the drug, bacteria which has developed resistance can also be called super bugs.[5,6]

AMR in bacterial pathogens is a world-wide challenge leading to high mortality and morbidity in health care systems. Multi-drug microbial resistance patterns of both gram-negative and positive bacteria do not get killed by the current antibiotics.[7]

The pattern of antimicrobial resistance is usually seen due to the lack of the following measures: etiological identification of microbes like bacteria or fungus and its anti-microbial sensitivity in patients with bacterial infections, unneccessary usage of broad-spectrum antibiotics, new drug development lacks.[4]

The rate of antibiotic resistance continues due to overuse or misuse of the drugs.[2,7] The CDC determines the AMR pattern by the seven factors. They are transmissibility, effective antibiotics availability, prevention barriers, economic impact factor, clinical impact, incidence. urgent, concerning or serious is the threat level classification.[8,9]

Epidemiological data from the past 20 years explains multi-drug resistance (MDR) has reached a pandemic level.[10] Estimates of CDC are 23000 deaths out of 2 million in the US.[11] 10 million annual deaths are predicted by Britain.[6]

Among gram-positive bacteria more American's are being killed due to methicillin-resistant staphylococcus aureus (MRSA) than due to HIV and other complicated diseases.[4,6] Among gram-negative bacteria recreation of reminiscent situations of the pre-antibiotic era.[8]

Mechanism of occurrence of antibiotic resistance is the drug degradation enzymatically, change or altering of the bacterial proteins which have particular drug targets in individuals, alteration in the drug permeability and receptor changes.[12] Resistance is also observed when bacteria share their genetic material with other bacteria.[13]

Preventive measures are patients should not force medical care professionals to prescribe antibiotics and should not use antibiotics that are not prescribed by medical professionals.[5] Importance of antibiotic sensitivity test is it confirms sensitivity, detects the resistance and guides the health care professionals in the selection of drugs for effective patient therapy.[14] This testing results can be combined clinically to suggest the appropriate alternative antibiotic.[15] Testing should be done if the individual doesn't respond to the given antibiotic.[5,16]

Procedure and report

Sensitivity analysis is done by taking the throat swab sample for tonsillitis patients at the infected area, which is swollen and whitish membrane can be seen with yellowish purulent. After this the swab is sent to the clinical laboratory, where culture sensitivity producer is done, there they spread on a medium, growth of bacteria will be seen which is called as culture, which will multiply and form colonies. These colonies are exposed to the antibiotic drugs and results are interpreted.

The results will be in the form of susceptible, resistance. These are in response to antibiotic drugs. Susceptible means the antibiotic drug is effective, resistant means antibiotic drug is not effective and the growth of bacteria is continued in the treatment also.

Objectives

The pattern of antimicrobial resistance against different strains of bacteria causing acute tonsillitis is needed to find out antibiotic sensitivity/susceptibility pattern against microbial pathogens in acute tonsillitis to maintain safe and planned use of antibiotics, to avoid the emergence of resistance of bacteria threaten the profits of health. To decrease the overuse and misuse of drugs, to decrease the antimicrobial resistance, to reduce the development of drug resistance and health care cost, creating awareness to patients regarding the proper use of antibiotics.

METHODS

Institutional study

This study was conducted in the department of otolaryngology (ENT), Durgabai Deshmukh hospital and research centre, a 300 bedded multispecialty hospital, Vidhya Nager, Hyderabad.

Study duration

This study was conducted for 6 months from August 2019 to February 2020.

Study design

The study design was a cross sectional study.

Sample size

The sample size for the study was 120.

Ethical statement

The study was conducted only after the approval of the hospital ethical committee.

Tools

The tools used to conduct the study were patient case sheets and reports of culture sensitivity testing.

Study criteria

Inclusion criteria

People of age 1 to 90 years of either sex (males and females), patients presenting with clinical symptoms and diagnosed with tonsillitis and patients with bacterial tonsillitis were included in the study.

Exclusion criteria

Chronically ill and immune-compromised patients, pregnancy and lactating patients, patients with viral tonsillitis were excluded from the study.

Efficacy assessment

The efficacy assement was to assess people who are resistant to different strains of bacteria in tonsillitis disease.

Data collection

All the relevant and necessary data was collected from patient case sheets and reports of culture sensitivity testing.

Method of study

Antibiotic resistance of various strains of bacteria in acute tonsillitis patients was assessed in this study. The samples from patients meeting the inclusion criteria was then collected. Microscopic studies, culture sensitivity test was done to assess antibiotic resistance/susceptibility. All the patient's details were collected in a suitable data collection form. Results obtained were then evaluated and analyzed statistically.

Evaluation parameters

The various evaluation parameters were sensitive, resistance and their percentage of resistance and sensitivity to bacteria.

Statistical tools

Appropriately suitable tests of significant ANOVA, f test, variants ratio, t test and the normal test were applied. Multi-variant logistic regression analysis, discriminate functional analysis were also used.

Sampling technique

The sampling technique used was randomized.

RESULTS

The study was aimed at finding the pattern of antibiotic resistance in various strains of bacteria causing acute tonsillitis. Patients were categorized based on age, sex and presence of bacterial growth. The culture and sensitivity test results were used for finding the bacteria resistance and sensitivity pattern of gram-positive bacteria and gram-negative bacteria against different antibiotics used. Appropriate statistical tools included ANOVA, t test were used.

In our study, 74 cases were found to have bacterial growth and 46 cases were not having bacterial growth. The bacterial growth cases were considered for the sensitivity and resistance studies.

Gender wise categorization

A total of 64 males and 56 females were enrolled. Out of which, the samples of 43 males and 31 females showed bacterial growth.

Age wise categorization

Our study included patients of 1-90 years of age, among which 9 patients were age in age group of 1-20, 20 patients in 21-40, 26 patients in 41-60 age groups and 19 patients in the age group of above 60 years.

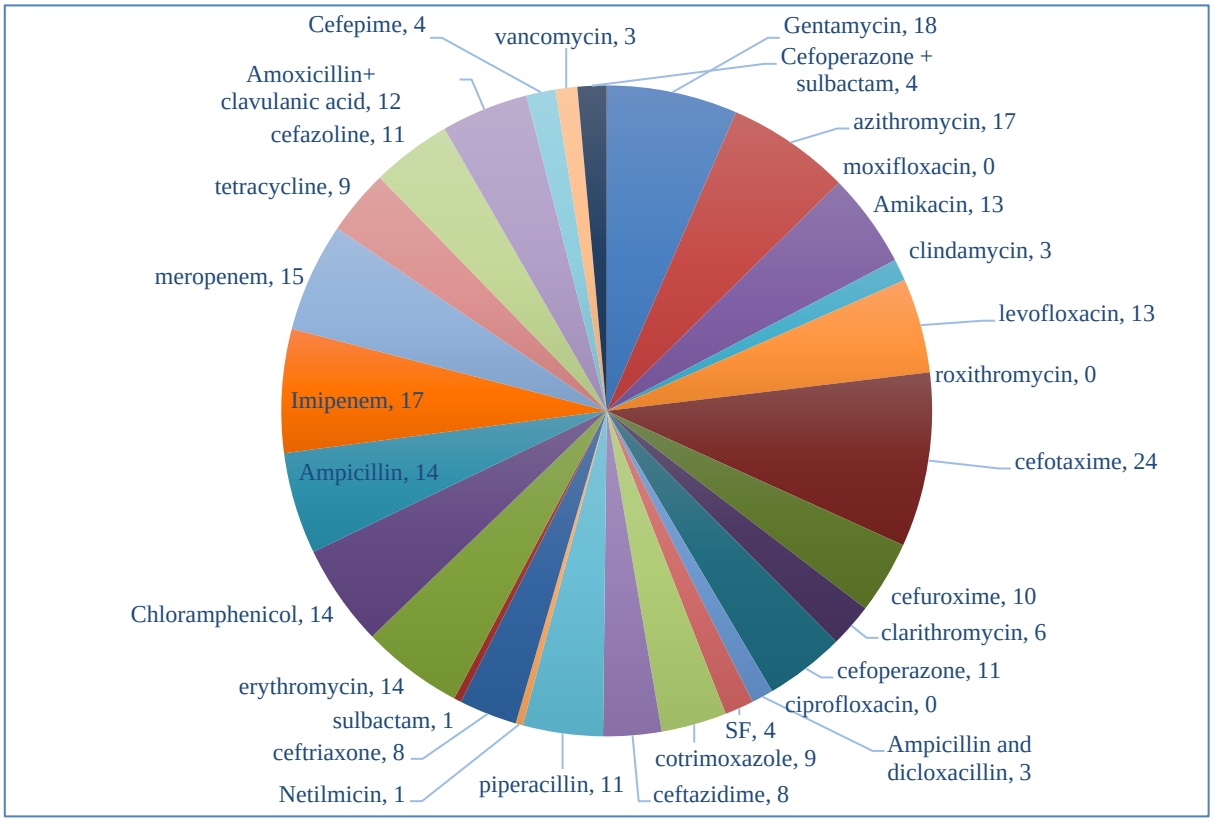

Figure 1: Sensitivity of antibiotic drugs to gram-positive bacteria.

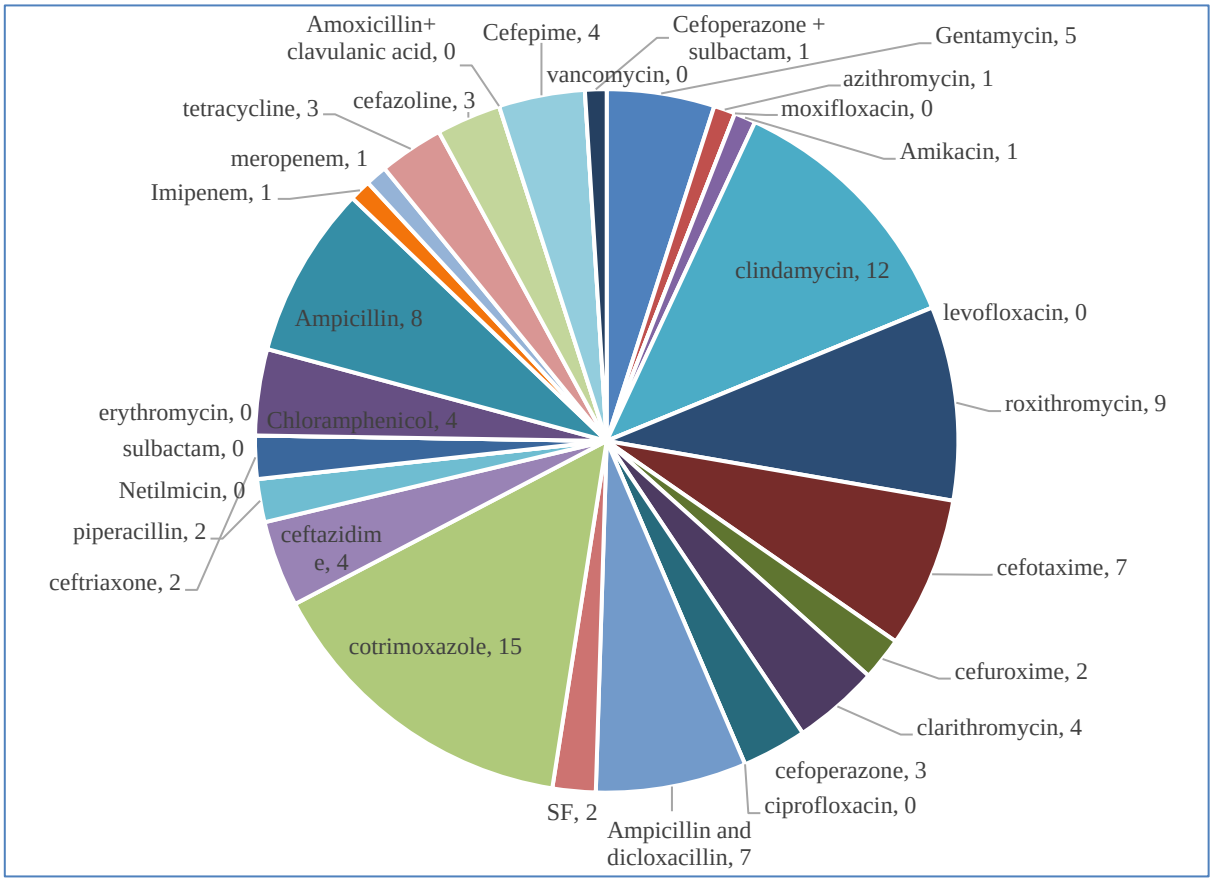

Figure 2: Resistance of antibiotic drugs to gram-positive bacteria.

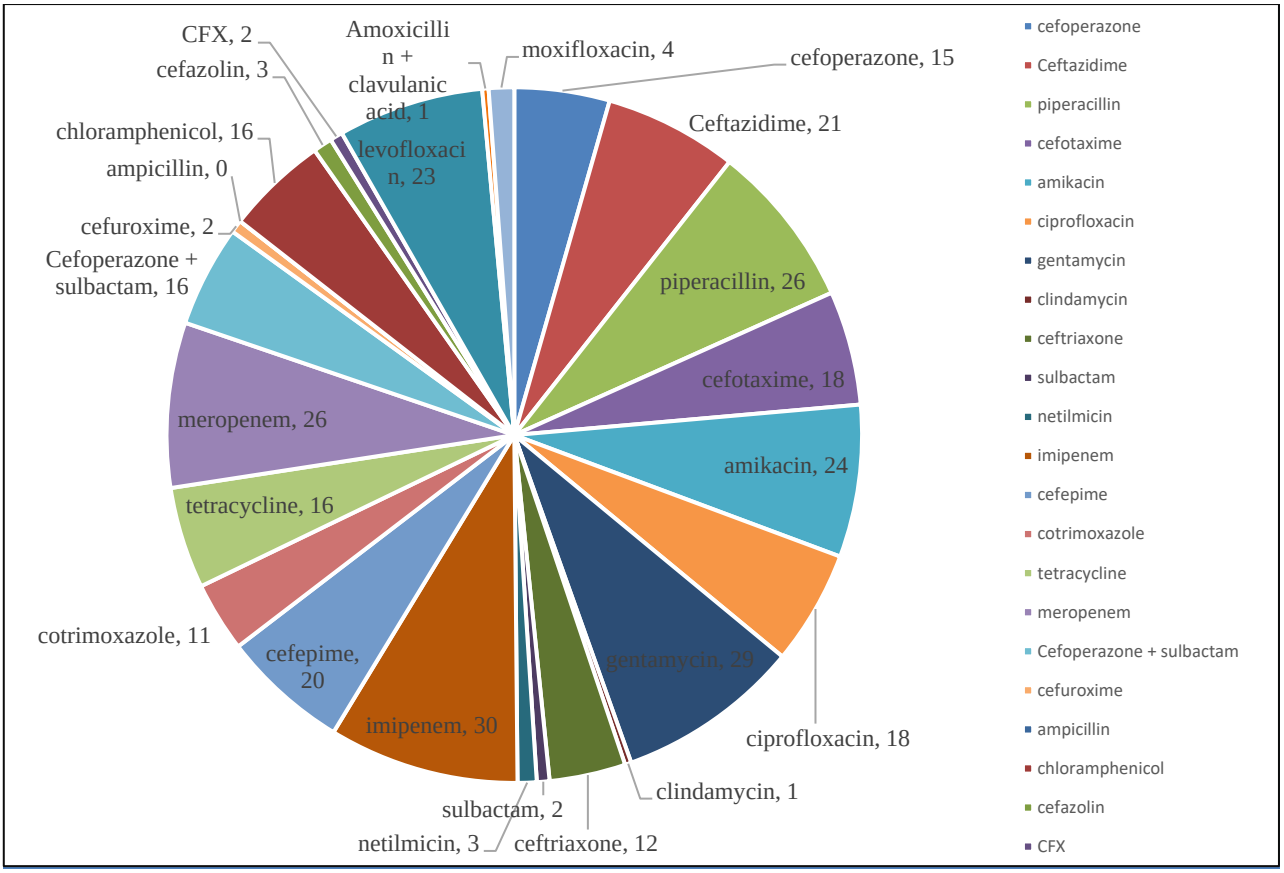

Figure 3: Sensitivity of antibiotic drugs to gram-negative bacteria.

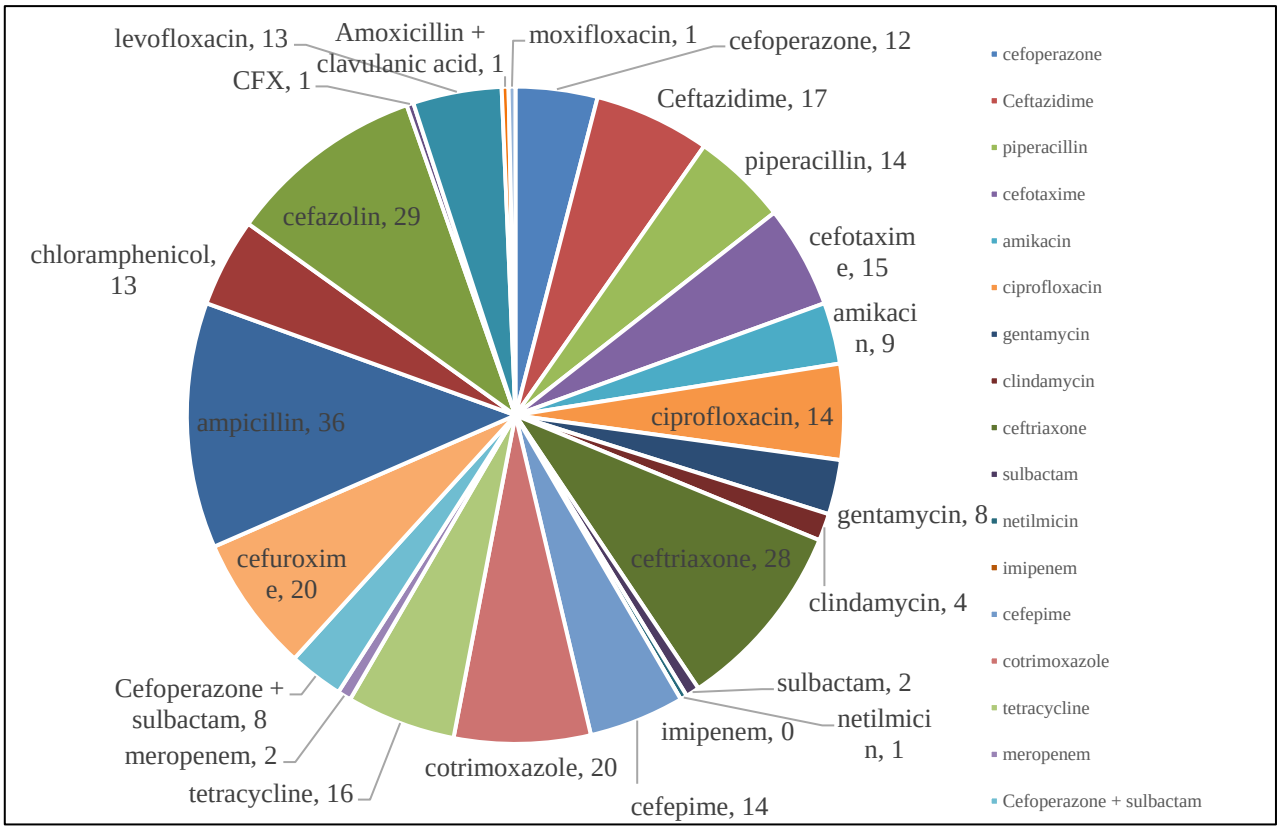

Figure 4: Resistance of antibiotic drugs to gram-negative bacteria.

Categorisation based on bacterial strians present in the culture

In our study two major gram-positive strains were found, that is, *Streptococcus pneumonia* (15) and *S. aureus* (17) and two gram-negative bacteria *K. sepsis* (23) and *P. aeruginosa* (19).

Growth of gram-positive and gram-negative bacteria

Table 1 was analysed by taking the common drugs tested against both gram-positive and negative bacteria and calculating the percentage of those common drugs sensitivity and resistance on a whole.

Figure 1 explains the final count of the sensitivity of drugs, analysed in the study period.

Figure 2 explains the final count of resistance of drugs, analysed in the study period.

Figure 3 explains the final count of the sensitivity of drugs analysed in the study period.

Figure 4 explains the final count of resistance of drugs analysed in the study period.

Table 1: Bacterial growth of gram-positive and gram-negative bacteria.

S. no.	Drug name	Sensitivity	Percentage (%)	Resistance	Percentage (%)
1	Cefoperazone	26	21.6	15	12.5
2	Ceftazidime	29	24	21	17.5
3	Piperacillin+tazobactam	37	30.8	16	13.33
4	Cefotaxime	42	35	22	18.33
5	Amikacin	37	30.8	10	8.33
6	Ciprofloxacin	18	15	14	11.6
7	Gentamycin	47	39.16	13	10.83
8	Clindamycin	4	3.33	16	13.33
9	Ceftriaxone	20	16.6	30	25
10	Sulbactam	03	2.5	2	1.66
11	Netilmicin	04	3.33	1	0.83
12	Imipenem	47	39.16	1	0.83
13	Cefepime	24	20	18	15
14	Cotrimoxazole	20	16.6	35	29.16
15	Tetracycline	25	20.83	19	15.83
16	Meropenem	41	34.16	03	2.5
17	Cefoperazone+sulbactam	31	25.83	12	10
18	Cefuroxime	12	10%	22	18.33
19	Ampicillin	14	11.66	44	36.66
20	Chloramphenicol	30	25%	17	14.16
21	Cefazolin	14	11.66	32	26.66
22	Levofloxacin	36	30%	13	10.83
23	Amoxycillin + clavulanic acid	13	10.83	1	0.83
24	Moxifloxacin	4	3.33	2	1.66
25	Azithromycin	17	14.16	1	0.83
26	Roxithromycin	0	0	9	7.5
27	Clarithromycin	6	5	4	30.33
28	Erythromycin	14	11.6	0	0
29	Vancomycin	3	2.5	0	0

Table 2: Sensitivity and resistance of antibiotic drugs and their percentages against the gram-positive bacteria.

S. no.	Drug name	Total	Sensitivity	Percentage	Resistance	Percentage (%)
1	Gentamycin	32	18	56.25	5	15.62
2	Azithromycin	32	17	53.12	1	3.12
3	Moxifloxacin	32	0	0	0	0
4	Amikacin	32	13	40.6	1	3.12
5	Clindamycin	32	3	9.37	12	37.5
6	Levofloxacin	32	13	40.6	0	0
7	Roxithromycin	32	0	0	9	28
8	Cefotaxime	32	24	75	7	21.8
9	Cefuroxime	32	10	31.25	2	6.25
10	Clarithromycin	32	6	18.75	4	12.5
11	Cefoperazone	32	11	34.3	3	9.35
12	Ciprofloxacin	32	0	0	0	0
13	Ampicillin and dicloxacillin	32	3	9.37	7	21.8
14	SF	32	4	12.5	2	6.25
15	Cotrimoxazole	32	9	28.12	15	46.8
16	Ceftazidime	32	8	25	4	12.5
17	Piperacillin	32	11	34.3	2	6.25
18	Netilmicin	32	1	3.12	0	0
19	Ceftriaxone	32	8	25	2	6.25
20	Sulbactam	32	1	3.12	0	0
21	Erythromycin	32	14	43.75	0	0
22	Chloramphenicol	32	14	43.75	4	12
23	Ampicillin	32	14	43.75	8	25
24	Imipenem	32	17	53.12	1	3.12
25	Meropenem	32	15	46.8	1	3.12
26	Tetracycline	32	9	29.12	3	9.37
27	Cefazoline	32	11	34.37	3	9.37
28	Amoxicillin+clavulanic acid	32	12	37.5	0	0
29	Cefepime	32	4	12.5	4	12.5
30	Vancomycin	32	3	9.37	0	0
31	Cefoperazone+sulbactam	32	4	12.5	1	3.12

Table 3: Paired t test (gram-positive bacteria).

Paired t test	
P value	0.0001
Significantly different (p<0.05)?	Yes
One or two-tailed p value?	Two-tailed
t, df	t=4.385, df=30
Number of pairs	31

Table 4: Sensitivity and resistance of antibiotic drugs and their percentages against the gram-negative bacteria.

Sr. No.	Drug Name	Total	Sensitivity	Percentage (%)	Resistance	Percentage (%)
1	Cefoperazone	42	15	35.71	12	28.5
2	Ceftazidime	42	21	50	17	40.47
3	Piperacillin/tazobactam	42	26	61.9	14	33.3
4	Cefotaxime	42	18	42.8	15	35.71
5	Amikacin	42	24	57.1	9	21.42
6	Ciprofloxacin	42	18	42.8	14	33.3
7	Gentamycin	42	29	69	8	19.04

Continued.

Sr. No.	Drug Name	Total	Sensitivity	Percentage (%)	Resistance	Percentage (%)
8	Clindamycin	42	1	2.38	4	9.52
9	Ceftriaxone	42	12	28.5	28	66.6
10	Sulbactam	42	2	4.76	2	476
11	Netilmicin	42	3	7.14	1	2.38
12	Imipenem	42	30	71.4	0	0
13	Cefepime	42	20	47.6	14	33.3
14	Cotrimoxazole	42	11	26.1	20	47.61
15	Tetracycline	42	16	38	16	38
16	Meropenem	42	26	61.9	2	4.76
17	Cefoperazone+sulbactam	42	16	38	8	19.04
18	Cefuroxime	42	2	4.76	20	47.6
19	Ampicillin	42	0	0	36	85.71
20	Chloramphenicol	42	16	38	13	30.95
21	Cefazolin	42	3	7.14	29	69.04
22	Cefixime trihydrate	42	2	4.76	1	2.38
23	Levofloxacin	42	23	54.7	13	30.95
24	Amoxicillin+clavulanic Acid	42	1	2.38	1	2.38
25	Moxifloxacin	42	4	9.52	1	2.38

Table 5: Paired t test (gram-negative bacteria).

Paired t test	
P value	0.5802
Significantly different (p<0.05)?	No
One or two-tailed p value?	Two-tailed
t, df	t=0.5607, df=24

Table 6: Antibiotic susceptability pattern of both gram-positive and negative bacteria.

Antibiotic susceptability		
Gram-positive bacteria	*S. pneumonia*	*S. aureaus*
Cotrimoxazole	13	06
Clindamycin	06	06
Cefepime	02	00
Gram-negative bacteria	*K. spesis*	*P. aeruginosa*
Ampicillin	13	12
Cefazolin	08	10
Ceftriaxone	07	11

DISCUSSION

According to the inclusion and exclusion criteria, a total of 120 cases were collected, out of which 46 cases showed NBG and 74 showed bacterial growth. 42 cases were gram-negative bacterial strain and 32 cases were a gram-positive bacterial strain. Gender wise classification of the patients revealed that number of male patients (43) were more compared to the female patients (31) with bacterial growth.

According to age wise categorisation of patients enrolled in the study, the highest number of bacterial growth (26) was found in the age group of 41-60 years (35%) and least number (9) was found in the age group of 1-20 years (12%).

Patients of viral and fungal aetiology were eliminated. The sensitivity and resistance of antibiotic drugs were noted after doing the culture sensitivity test accurately. The culture and sensitivity test presence of gram-positive species including *S. pneumonia* (15) and *S. aureus* (17) and gram-negative bacteria species including *K. sepsis* (23) and *P. aeruginosa* (19)

A list of 25 antibiotic drugs in gram-negative and 31 drugs in gram-positive strain, their sensitivity and resistance were taken and noted, respectively.

Among gram-negative bacteria, the highest sensitivity was observed in imipenem (71.4%), followed by gentamycin (69.0%), piperacillin+tazobactam (61.9%) and meropenem (61.9%). The highest antibiotic resistance was seen in ampicillin (85.71%) which was similar to the study result of Ingale et al followed by cefazolin and ceftriaxone with 69.04% and 66.66%, respectively. Least sensitivity is observed in clindamycin, amoxicillin+clavulanic acid with 2.38% each, followed by sulbactam and cefuroxime with 4.76% each, netilmicin (7.14%). No sensitivity was found in ampicillin which has the greatest resistance. 0% resistance is seen in imipenem which has the highest sensitivity, followed by netilmicin, moxifloxacin, amoxicillin+clavulanic acid with resistance rate of 2.38% each and sulbactam, meropenem with 4.76% resistance and then clindamycin with 9.52%.

Among gram-positive bacteria the highest sensitivity was noted in cefotaxime (75%), followed by gentamycin with 56.25% and azithromycin and imipenem with 53.12% each. The highest resistance was seen in cotrimoxazole (46.8%), followed by clindamycin (37.5%) and roxithromycin (28.12%). The least sensitivity was seen in netilmicin, sulbactam, with 3.12% each, followed by ampicillin+dicloxacillin, clindamycin, vancomycin 9.3% each, cefepime and cefoperazone+sulbactam with 12.5% sensitivity. 0% sensitivity was seen in moxifloxacin, roxithromycin.

In one of the previous study conducted by Ingale et al noted that antibiotic resistance was commonly observed in generated antibiotics such as ampicillin, amoxicillin, clavulanic acid and amoxicillin. Cerebrospores antibiotics were less widely used and displayed resistance in about 77.7 percent of cases. Cotrimoxazole has shown resistant cases of about 40 percent. The less commonly used antibiotic was vancomycin. However, showed high sensitivity (100%) followed by linezolid (91.3%) and clindamycin (81.4%). Erythromycin showed 76% sensitivity while ciprofloxacin showed a low sensitivity of 35.5% followed by cephalosporins (22.2%).[17]

A prospective observational 3 month analysis was performed by Ingale et al.[17] Throat swabs were collected randomly from 50 patients attending the ENT OPD.

A patient's informed consent was obtained before starting the study. The antibiotics widely used displayed a higher level of resistance compared with the antibiotics less widely used. Ampicillin and amoxicillin, in both *Streptococci* and *Staphylococci*, showed poor sensitivity results.[18]

Paired t test of sensitive and resistant drugs to gram-positive bacteria showed a significant difference of $p=0.0001$ ($p<0.05$), that is the number of drugs resistant to the gram-positive bacteria is lesser than the number of drugs sensitive, while the same was not noted in gram-negative bacteria.[19,20]

Limitations

There were very few draw backs occurred in our research. They were patient complication, collection of samples and data, contamination of the samples after collection, irregular use of antibiotics by patients, improper data reporting by the patient, reduced hospital activity by patients. Majority of the samples showed no bacterial growth after exhibiting symptoms.

CONCLUSION

Sensitivity and resistance of each drug in both gram-positive and gram-negative bacteria were discussed and their percentage of sensitivity and resistance were known. The study results showed more patients in the age group of 41-60 years were infected. Various gram-positive species like *S. pneumonia* and *S. aureus* and gram-negative strains like *K. species* and *P. aeruginosa* were found in the study. Among gram-positive bacteria, the highest sensitivity was found in cefotaxime and highest resistance was found in cotrimoxazole. Among gram-negative bacteria the highest sensitivity was observed in imipenem and gentamycin and highest resistance was found in ampicillin. Statistical results revealed that the number of drugs resistance to gram-positive bacteria is less than the number of drug resistance. The number of drugs resistant to the gram-positive bacteria are lesser than the number of drugs sensitive, which showed significant difference ($p<0.05$), a significant difference of antibiotic drugs was not found in gram-negative bacteria. Our study findings help in appropriate and guarded use of the antibiotic drugs in acute tonsillitis, minimizing the exposure of individuals to antibiotic resistance by choosing an appropriate sensitive drug, therefore improving the quality of therapy.

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