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УДК 577.1:54.057

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STUDY ON THE SENSITIVITY OF MICROORGANISMS TO ANTIBIOTICS OF VARIOUS CLASSES

This study collected throat swab samples from patients with chronic tonsillitis and successfully isolated four strains of bacteria, including both Gram-positive and Gram-negative bacteria, using broth culture medium. Subsequently, the Kirby-Bauer disk diffusion method was employed to assess the sensitivity and resistance of these strains to four commonly used antibiotics. The findings not only clarified the antibiotic sensitivity profiles of the different strains but also provided scientific evidence and guidance for the selection of antibiotics in the clinical treatment of chronic tonsillitis. This research contributes to optimizing treatment strategies and reducing the misuse of antibiotics.

Keywords: Chronic tonsillitis; Antibiotic resistance; Sensitivity; Kirby-Bauer method; Throat swab.

For citation: Wang Hui & Qin Xin Rui & Xu Siyan & Gu Minmin & Siyamak Shahab & Ye Meng. Study on the Sensitivity of Microorganisms to Antibiotics of Various Classes. Sophia. 2025;1:54–65. English.

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ИССЛЕДОВАНИЕ ЧУВСТВИТЕЛЬНОСТИ МИКРООРГАНИЗМОВ К АНТИБИОТИКАМ РАЗЛИЧНЫХ КЛАССОВ

В данном исследовании были собраны образцы мазков из зева у пациентов с хроническим тонзиллитом и успешно выделены четыре штамма бактерий, включая как грамположительные, так и грамотрицательные бактерии, с использованием питательного бульона. Впоследствии метод диско-диффузии (метод Кирби-Бауэра) был применен для оценки чувствительности и устойчивости этих штаммов к четырем commonly используемым антибиотикам. Результаты не только прояснили профили чувствительности к антибиотикам различных штаммов, но и предоставили научные доказательства и рекомендации для выбора антибиотиков при клиническом лечении хронического тонзиллита. Данное исследование способствует оптимизации стратегий лечения и снижению неправильного использования антибиотиков.

Ключевые слова: хронический тонзиллит; устойчивость к антибиотикам; чувствительность; метод Кирби-Бауэра; мазок из зева.

Образец цитирования: Ван Хуэй. Исследование чувствительности микроорганизмов к антибиотикам различных классов / Ван Хуэй, Цинь Синьжуй, Сюй Сыянь, Гу Минминь, Сиямак Шахаб, Е Мэн // София: электрон. науч.-просветит. журн. – 2025. – № 1. – С. 54–65.

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1. Introduction

In today's world of widespread antibiotic use, microbial resistance is very common. Therefore, for successful antibiotic therapy, antibiotic resistance of pathogenic microorganisms should be determined before prescribing and attempts should be made to overcome microbial resistance [1].

Various laboratory diagnostic methods can be used to identify microorganisms. The classic laboratory tests are culture and microscopic examination, both of which are constantly being improved.

Antibiotic resistance is a phenomenon in which strains of infectious agents become resistant to the action of one or more antimicrobial drugs [2], i.e., microbial cultures become less sensitive to the action of antimicrobial substances.

Microorganisms that develop resistance to one antibiotic also develop resistance to other antibiotics with a mechanism of action similar to that of the first antibiotic. This phenomenon is known as cross-resistance [3].

The aim of this laboratory study was to investigate the resistance and susceptibility to antimicrobial agents in swab isolate cultures from volunteers with chronic tonsillitis.

2. Materials and methods

2.1. Materials

Microorganisms extracted from the nasopharyngeal mucosa of 7 volunteers diagnosed with chronic tonsillitis with the aim of further studying their resistance to antibiotics - tobramycin, metronidazole, cefoperazone, ampicillin/sulbactam. show in *Figure 1*.

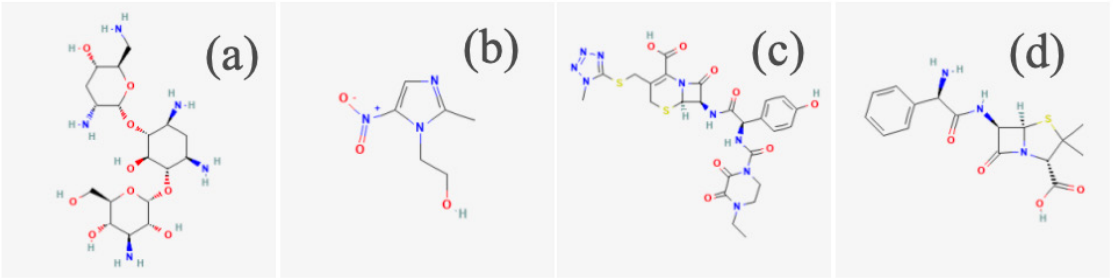

Fig. 1. The antibiotics used in the experiment:
a) Tobramycin; b) Metronidazole; c) Cefoperazone; d)Ampicilli.

2.2. Methods

Nasopharyngeal swabs were obtained from the nasopharynx of 7 volunteers diagnosed with chronic tonsillitis. The pharyngeal swabs were collected from the nasopharyngeal mucosal secretions. In preparation for the extraction of microflora from the pharyngeal swabs, it is important to

- Do not eat before analysis;
- Do not drink anything 2 hours before analysis;
- Do not brush your teeth or rinse your mouth before taking the material.

Subjects should not use mouthwashes or sprays containing antimicrobials or antibiotics before taking a throat swab. A wooden swab is used for collection, which is inserted into the mouth and gently pressed against the posterior pharyngeal wall and tonsillar surface .

It was then seeded on meantone saline agar. Meat peptone agar (MPA) is a nutrient rich medium. For preparation, we added 7.8 g of suspension to 200 ml of distilled water and boiled it for 2 minutes until the agar was completely melted, then filtered it through cotton filters and poured it into sterile bottles and autoclaved at 121 degrees for 15 minutes. The medium was then cooled to 45–50 degrees. The prepared agar is poured into cups [4], each layer being 5 mm thick. Leave the cups to solidify at room temperature. After the medium has solidified, dry it at 37 degrees for 40–60 minutes.

In disc diffusion method, we used Mueller-Hinton medium, which is specialized for antibiotic resistance and antibiotic susceptibility studies of microorganisms.

3. Results and discussion

3.1. Culture methods

We collected pharyngeal swabs [6] from the pharynx of patients with chronic tonsillitis and placed them on meat pine agar (MPA). We selected one (number 9) from all the pharyngeal swabs in *Figure 2*.

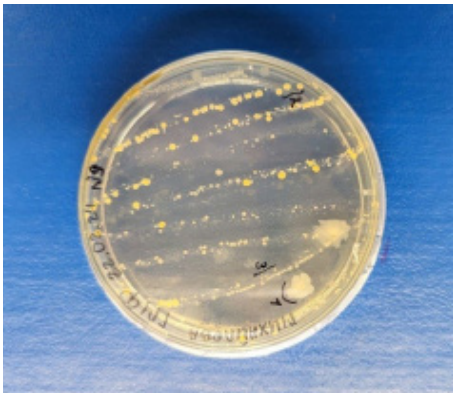

Fig. 2. Petri dish No. 9 with colonies a); b) and c) on MPA.

Petri dish No. 9 contained 3 different microorganisms, each of which was transplanted into a different petri dish in *Figure 3*.

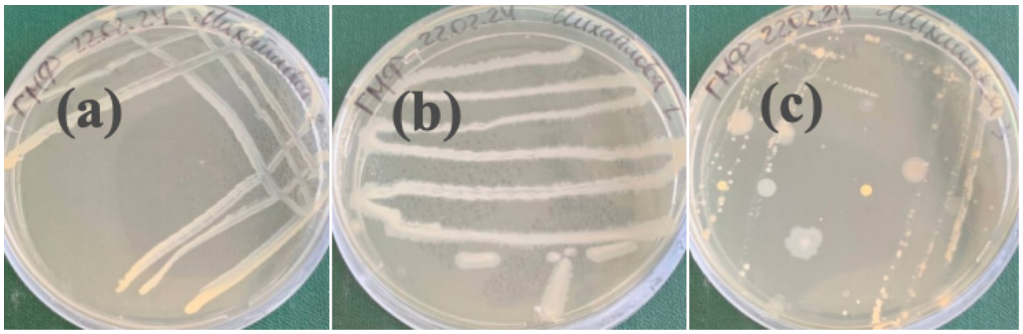

Fig. 3. Petri dish No. 9 with colonies a); b) and c) on MPA.

The microbial species obtained by culturing in Petri dishes were made into smears and subsequently examined under a microscope *Figure 4*.

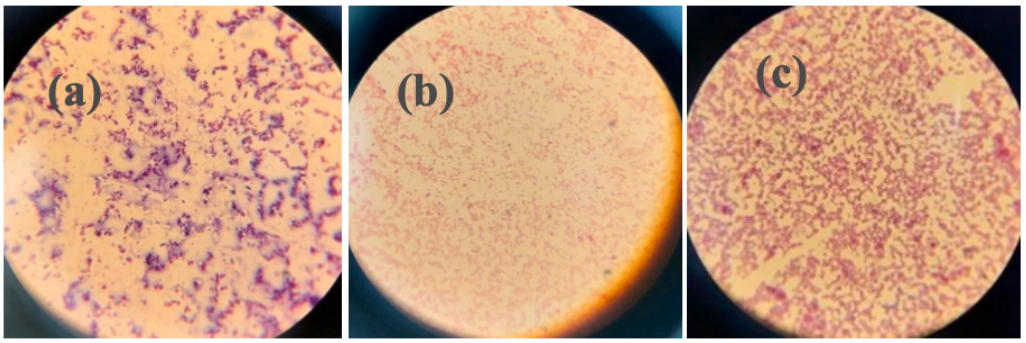

Fig. 4. Swab: a); b) and c) from Petri dish No. 9.

It was found that the following microorganisms were found in Petri dish No. 9:

1) *figure 4a* contained predominantly single gram-positive bacilli, presumed to be Corynebacterium rods;
2) *figure 4b* small gram negative bacilli, presumed to be Haemophilus influenzae;
3) *figure 4c* gram-positive cocci – Staphylococcus aureus and Staphylococcus aureus.

3.2. Morphological and vectorial characterization of microorganisms

The morphology and Gram properties of the microorganisms isolated from each colony were studied.

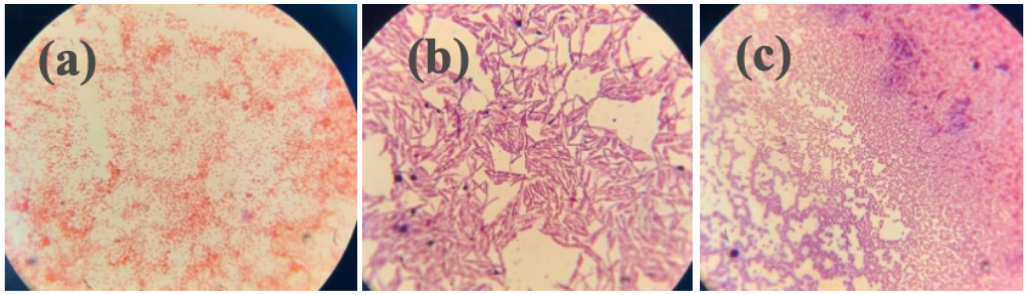

Fig 5. a) Gram-negative bacilli; b) Gram-positive bacilli; c) Gram-positive cocci.

As a result, the following microorganisms were detected in 7 patients with chronic tonsillitis (*Table 1*):

Table 1

Microorganisms Isolated

Microorganisms	***Number***
G+ Coccidioides	7
G+ Bacillus	5
G- Bacillus	4
G+ Spore Bacilli	3

Note: G+ Gram-positive microorganisms, G- Gram-negative microorganisms.

The results of the study are shown in *Figure 6*.

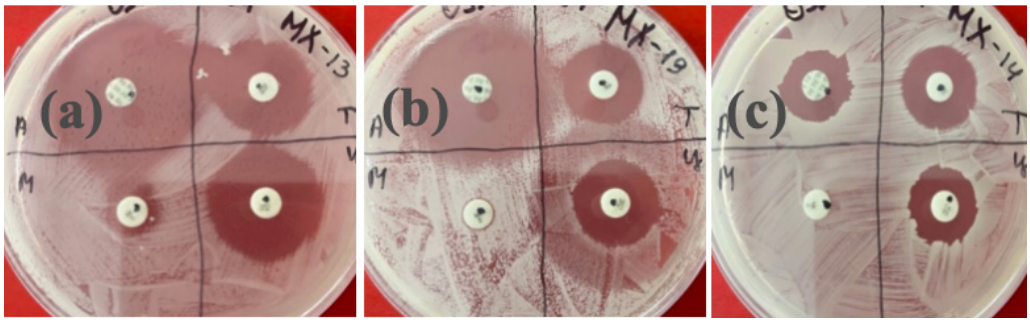

Fig. 6. Results of the paper diffusion method
for detecting changes in microbial growth inhibitory diameter
(A – ampicillin/sulbactam; T – tobramycin; M – metronidazole; C – cefoperazone)

3.3. Antibiotic susceptibility and resistance assessment of microorganisms

The results of the Antibacterial preparation (ABP) susceptibility assessment were interpreted by placing thetest cultures into one of three categories:

- Sensitive – strains are inhibited at the concentrations and recommended doses of ABP produced in human organs and tissues.

- Moderately susceptible – cultures are considered to be inhibited at concentrations at the maximum administered dose but within the recommended dose range.
- Resistant (drug-resistant) – microorganisms whose growth cannot be inhibited even with the maximum allowable dose of drug [5].

Sensitivity and resistance results were evaluated based on the data presented in *Table 2*.

Table 2

Criteria for interpretation of microbial susceptibility/resistance results for the following antibiotics

Designation	***Drug content in round tablets, μg***	***Diameter of growth inhibition, mm***		
		R	***R/S***	***S***
Ampicillin/ sulbactam (A)	10/10	<14	14 – 15	>16
Metronidazole(M)	5	0	0	>15
Tobramycin (T)	10	<16	16 – 18	>19
Cefoperazone (C)	75	<15	16 – 20	>21

Note: S – Sensitive; R – Resistant, including 0 – complete absence of microbial growth inhibition zones; R/S – moderately resistant

Table 3

Diameter of Microbial Growth Inhibition Zones

Samples	***Diameter of growth inhibition, mm***				***Type of microorganism***
	A	***T***	***M***	***Ц***	
1	12	27	0	14	Gram-negative small bacilli
2	37	19	0	22	Gram-positive cocci
3	45	28	10	27	Gram-positive bacilli with spores
12	36	22	0	20	Gram-positive cocci
4	23	21	8	21	Gram-positive bacilli
5	15	19	0	13	Gram-negative small bacilli
6	30	26	0	15	Gram-positive cocci
7	40	22	0	31	Gram-negative small bacilli
8	29	26	11	18	Gram-negative bacilli
9	21	24	17	12	Gram-negative bacilli
10	31	17	0	15	Gram-positive cocci
11	46	31	24	28	Gram-positive cocci
13	32	21	11	24	Gram-positive bacilli with spores
14	17	18	0	16	Gram-positive cocci
15	32	21	0	13	Gram-positive bacilli
16	28	17	0	13	Gram-positive bacilli
17	11	16	0	12	Gram-positive cocci

18	34	23	9	12	Gram-positive bacilli with spores
19	34	18	0	18	Gram-positive bacilli

Note: A – ampicillin; T – tobramycin; M – metronidazole; C – cefoperazone

Antibiotic resistance and antibiotic susceptibility results obtained from nasopharyngeal cultures of patients with chronic tonsillitis, depending on the type of infection, are presented in *Tables 4*, *5*, *6* and *7*.

Table 4

Antibiotic Resistance Data for Gram-Positive Coccidioides Infections to Selected ABP

Sample	***A***		***T***		***M***		***C***	
	Diameter, mm	***R, S***	***Diameter, mm***	***R, S***	***Diameter, mm***	***R, S***	***Diameter, mm***	***R, S***
2	37	S	19	S	0	R	22	S
12	36	S	22	S	0	R	20	R/S
6	30	S	26	S	0	R	15	R
10	31	S	17	R/S	0	R	15	R
11	46	S	31	S	10	R	28	S
14	17	R/S	18	R/S	0	R	16	R/S
17	11	R	16	R/S	0	R	12	R

Note: R – resistant, S – sensitive, R/S – moderately sensitive;

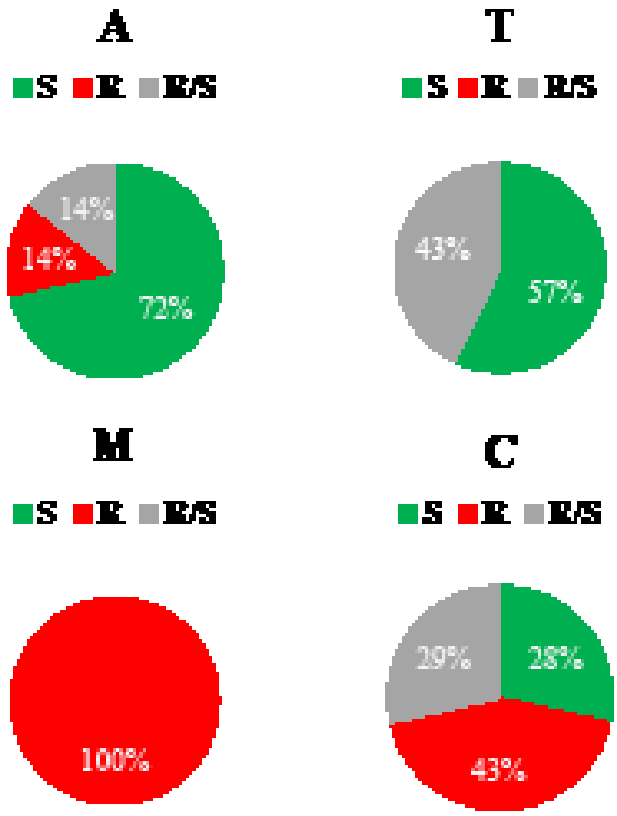

Fig. 7. Susceptibility of Coccidioides immitis to selected ABPs (in %).

- Thus, for ampicillin/sulbactam, Coccidioides microorganisms showed high susceptibility (mean diameter – 35) in 5 samples and resistance in 2 samples. The diameters of the no-growth zones were 11 mm and 17 mm, respectively. This may be due to individual characteristics of microorganisms belonging to different individuals.
- Seven samples were resistant to metronidazole. The diameter of the no-growth zone ranged from 0 to 10 mm (mean diameter 1.5 mm).
- Four microorganisms were susceptible to tobramycin and three samples were moderately susceptible to tobramycin. The zone of no growth ranged from 17 to 31 mm in diameter (mean diameter 24 mm).
- Two samples were susceptible to cefoperazone, two were susceptible to cefoperazone, and three were resistant to cefoperazone. The diameter of the no-growth zone ranged from 12 to 28 millimeters (mean diameter 20 millimeters).

Table 5

Antibiotic Resistance Data for Gram-Positive Bacteria to Selected ABP

Sample	*A*		*T*		*M*		*C*	
	Diameter, mm	*R, S*	*Diameter, mm*	*R, S*	*Diameter, mm*	*R, S*	*Diameter, mm*	*R, S*
4	45	S	28	S	10	R	27	S
7	40	S	22	S	0	R	31	S
15	32	S	21	S	0	R	13	R
16	28	S	17	R/S	0	R	13	R
19	38	S	18	R/S	0	R	18	R/S

Note: R – resistant, S – sensitive, R/S – moderately sensitive;

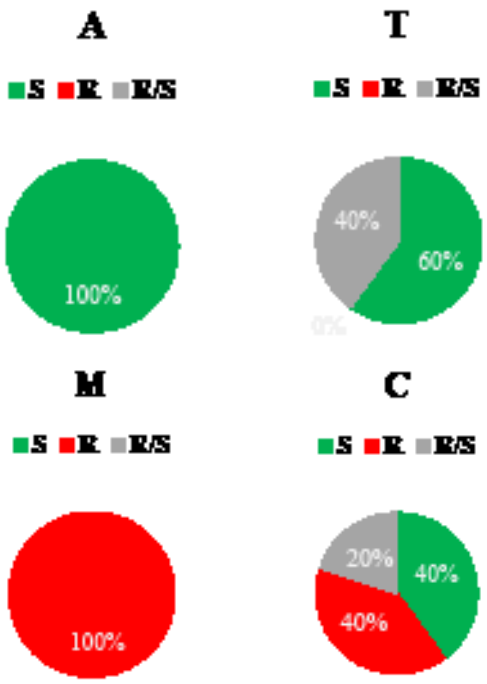

Fig. 8. Susceptibility of Gram-positive bacilli to selected ABPs (in %).

- In all samples, Gram-positive bacilli were highly susceptible to ampicillin/sulbactam. The diameter of the no-growth zone ranged from 28 mm to 45 mm (mean diameter 37 mm).
- The bacilli in all samples were resistant to metronidazole. The diameter of the no-growth zone ranged from 0 to 10 mm, with a mean diameter of 2 mm. The mean diameter was 2 mm.
- Gram-positive bacilli in three samples were susceptible to tobramycin. Two samples were moderately susceptible to the antibiotic. The diameter of the no-growth zone ranged from 17 mm to 28 mm. The mean diameter was 21 mm.
- Two microorganisms were susceptible to cefoperazone, bacilli showed resistance in two samples, and one sample showed intermediate susceptibility because there was no zone of inhibited bacterial growth. The diameter of the no-growth zone ranged from 13 mm to 31 mm (mean diameter 20 mm). This may be due to individual characteristics of microorganisms belonging to certain individuals.

Table 6

Antibiotic Resistance Data Gram-negative bacilli resistance data for selected ABP

Sample	*A*		*T*		*M*		*C*	
	Diameter, mm	*R, S*	*Diameter, mm*	*R, S*	*Diameter, mm*	*R, S*	*Diameter, mm*	*R, S*
1	12	R	27	S	0	R	14	R
5	15	R/S	19	S	0	R	13	R
8	29	S	26	S	11	R	18	R/S
9	21	S	24	S	17	S	12	R

Note: R – resistant, S – sensitive, R/S – moderately sensitive;

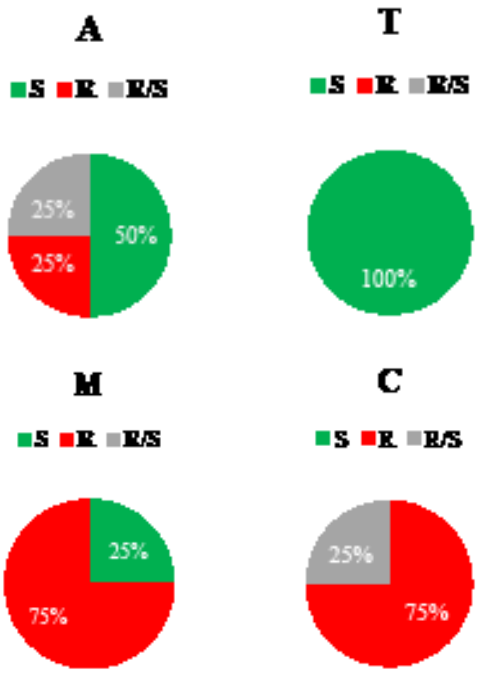

Fig 9. Susceptibility of Gram-negative bacilli to selected ABP (in %).

- Gram-negative bacilli showed high susceptibility to ampicillin/sulbactam in two samples, resistance in one sample, and moderate susceptibility in one sample. The diameter of the no-growth zone ranged from 12 mm to 29 mm (mean diameter 19 mm).
- Metronidazole showed susceptibility in 1 sample of gram-negative bacilli and resistance in 3 samples because of the absence of a zone of inhibited bacterial growth. The diameter of the no-growth zone ranged from 0 mm to 17 mm. The mean diameter was 7 mm.
- Gram-negative bacilli were susceptible to tobramycin in all samples. The diameter of the no-growth zone ranged from 19 mm to 27 mm. The mean diameter was 24 mm.
- Three microorganisms were resistant to cefoperazone, and bacilli in one sample showed intermediate susceptibility. The diameter of the no-growth zone ranged from 12 mm to 18 mm (mean diameter 14 mm).

Table 7

Antibiotic Resistance Data for Gram-Positive Bacillus to Selected ABP

Sample	*A*		*T*		*M*		*C*	
	Diameter, mm	*R, S*	*Diameter, mm*	*R, S*	*Diameter, mm*	*R, S*	*Diameter, mm*	*R, S*
3	27	S	30	S	25	S	39	S
13	32	S	21	S	11	R	24	S
18	34	S	23	S	9	R	12	R

Note: R – resistant, S – sensitive, R/S – moderately sensitive;

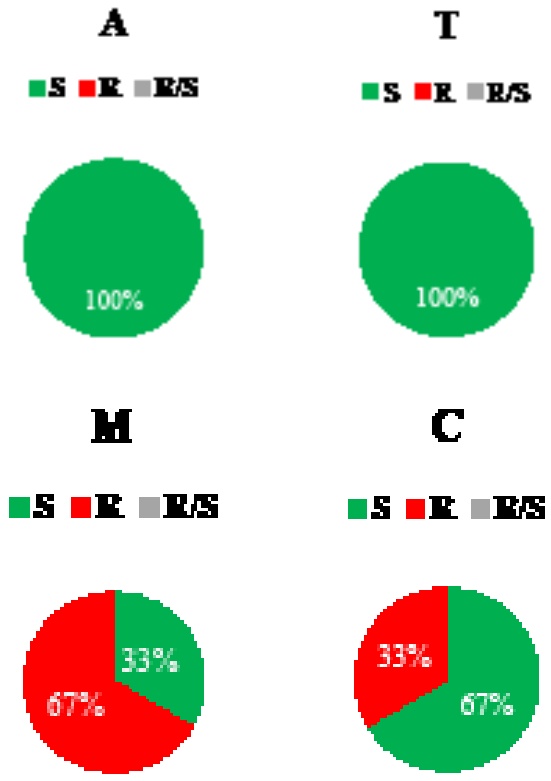

Fig. 10. Usceptibility of Gram-positive bacilli to selected ABPs (in %).

Spores are sensitive to three ABPs and resistant to one.

- Ampicillin/sulbactam: The diameter of the no-growth zone ranged from 27 to 34 mm. The mean diameter was 31 mm.
- Metronidazole is sensitive to one sample and resistant to two, with a no-growth zone ranging from 9 to 25 mm in diameter. The average diameter is 15 mm.
- Tobramycin: The diameter of the no-growth zone varies from 21 to 30 mm. The mean diameter was 25 mm.
- Cefoperazone: two samples were sensitive and one was resistant. The diameter of the no-growth zone ranged from 12 to 39 mm. The average diameter was 25 mm.

Antibiotic resistance is a phenomenon in which strains of infectious agents become resistant to the action of one or more antimicrobial drugs, and is defined as a decrease in the susceptibility of microbial cultures to the action of antimicrobial drugs.

4. Conclusion

Cultures isolated from the nasopharyngeal portion of volunteers suffering from chronic tonsillitis were characterized by high resistance and high susceptibility to various antimicrobial drugs (ampicillin/sulbactam, metronidazole, tobramycin, cefoperazone), which acted on different targets of the bacterial cell.

The following conclusions were drawn from the study:

1. The following microorganisms were detected in 7 patients with chronic tonsillitis:

- Gram-positive cocci infection in 7 samples;
- Gram-negative bacilli infection in 4 samples;
- Gram-positive bacilli infection in 5 samples;
- Gram-positive bacilli with spores in 3 samples.

2. By the paper diffusion method, we found that the same microorganisms belonging to different individuals had different diameters of growth retardation zones, which were related to the individual characteristics of different individuals.

3. Research findings:

- Among the antibiotics studied, ampicillin/sulbactam had the highest bacterial activity against gram positive cocci infections and gram positive and sporulating microorganisms but resistant strains were found in gram negative bacilli infections and the mean diameter of the no growth zone was the largest of all the microorganisms which was 32 mm.
- Metronidazole showed high resistance to sporulating microorganisms, Gram-positive bacteria, Gram-negative bacilli and Gram-positive cocci infections. The mean diameter of the no-growth zone was 10 mm.
- In most cases, the susceptibility of Gram-positive cocci, Gram-positive and Gram-negative bacilli to tobramycin was 57%, 85% and 80%, respectively. Sporulating microorganisms were sensitive to all samples. The mean diameter of the no-growth zone was 24 mm.
- Microorganisms were both sensitive and resistant to cefoperazone. For the most part, Gram-positive cocci were resistant and Gram-positive and Gram-negative bacilli showed sensitivity, resistance and intermediate sensitivity. The sporulating microorganisms were sensitive in all samples. The mean diameter of the no-growth zone was 20 mm.

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