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Erythromycin resistance in *Group A Beta-hemolytic streptococci*

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ABSTRACT

Aim: *Streptococcus pyogenes* (*Group A Beta-hemolytic streptococci*, GABHS) is one of the important bacterial pathogens in clinical microbiology. It often causes upper respiratory tract infections such as tonsillitis, pharyngitis, and laryngitis. It also leads to complications such as acute rheumatic fever and post-streptococcal glomerulonephritis. Early diagnosis and treatment of these bacterial infections will prevent suppurative and non-suppurative complications, the transmission of infection to other people, and chronic carriage. Today, the treatment of streptococcal infections relies entirely on chemotherapy. *Beta hemolytic group A streptococci* and generally other *beta-hemolytic streptococci* in groups B (GBBHS), C, and G are generally sensitive to many chemotherapeutics, especially Penicillin and Erythromycin. In patients with penicillin allergy, erythromycin, amoxicillin-clavulanate, or oral cephalosporins are used instead of penicillin. However, it has recently been understood that there are strains resistant to Erythromycin in GABHS and are increasing. In this study, the situation in our region of Erythromycin resistance, which is used as an alternative for people allergic to Penicillin in the treatment of streptococcal infections, was investigated.

Material and Method: In our study, throat swab samples were taken from 150 pharyngitis patients and 94 GABHS were obtained by applying the Bacitracin-SXT test with the culture method, and antibiotic susceptibility tests were performed on these 94 GABHS by Kirby-Bauer agar disc diffusion method.

Result: GABHS was found susceptible to Bacitracin and resistant to SXT. GBBHS is resistant to Bacitracin and SXT. other *beta-hemolytic streptococci* were resistant to Bacitracin and susceptible to SXT.

Conclusion: In this study, Erythromycin's resistance was found to be 19.1%. it is observed that Erythromycin resistance has increased over the years when compared to previous studies. Erythromycin should not be used empirically in treatment. An antibiotic susceptibility test should be performed and the antibiotic should be selected according to the results of the antibiogram test.

Keywords: Penicillin, erythromycin, *Streptococcus pyogenes*, tonsillitis, pharyngitis, laryngitis

INTRODUCTION

Streptococci can be found in the normal flora of humans, but also can be important bacterial pathogens. Specifically, GABHS is the most important causative agent for acute pharyngitis. They cause both suppurative and non-suppurative complications. Streptococci are gram-positive bacteria, which are round or oval and form pairs or rings. They form longer rings in mediums containing blood and serum. They are non-motile and they do not form spores. Most of them have a capsule that contains hyaluronic acid. Streptococci which have capsules are resistant to phagocytosis. They are facultative anaerobes and are catalase and oxidase negative (1-4). They can grow in simple media, but the amount and the rate of growth increase when substances like blood, serum, and glucose are added to the media. Their growth is maximum in media containing horse or sheep erythrocytes. Selective media for streptococci are known as; Trypticase soy, heart infusion, Todd-Hewitt infusion media, Columbia agar, Colistin-Nallidixic acid agar (CNA), Phenyiethyi alcohol agar (PEA), and chocolate agar (5). Not only the nutrients but also fermentable carbohydrates that can affect the hemolysis of *beta-hemolytic streptococci* are useful. They grow better in anaerobic or with a CO_2 concentration of 10% conditions, at 37°C in a period between 18-24 hours (1,5-7). Streptococci have a colony morphology of white-grey color, with a diameter of 1-2 µm on blood agar. M-protein-forming strains have a dull appearance. Strains that do not form or form fewer M proteins have bright colonies (8). Generally, streptococci colonies formed on the blood agar show different features. According to hemolysis properties, they form

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either small or large complete hemolysis zones (beta hemolysis), due to partial lysis of erythrocytes, a green hemolysis zone (alpha hemolysis) is formed, or there is no hemolysis at all (gamma hemolysis) (4).

Group A Beta Hemolytic Streptococci can cause diseases such as erysipelas, scarlet fever, tonsillopharyngitis, infective endocarditis, post-streptococcal diseases, acute joint rheumatism, and acute glomerulonephritis (4,9-11). It is most commonly seen in the age group 5-15 years. The probability is lower in people who are under 3 or above 5 (7,12). GABHS' are isolated from the normal population in 5-25% proportions (1,13,14). The transmission is by direct contact or via droplets. Contaminated food such as milk, eggs, and ice cream can cause food poisoning or pharyngitis epidemics. Transmission to belongings or clothes does not carry a significant risk (1,7,15-17).

Today, the treatment of streptococcal infections is completely based on an antimicrobial basis. Generally, streptococci are susceptible to penicillin and erythromycin (18). For this reason, when streptococci are isolated, the treatment can be started without the need for antimicrobial resistance tests. However, nowadays, it is found that there are resistant origins of *Group A Beta-hemolytic streptococci*, and the resistance is on increase (19). The first choice of treatment is penicillin (20). GABHS' are susceptible to penicillin (14). Starting penicillin treatment within 9 days after the onset of symptoms prevents the formation of acute joint rheumatism (1,6,8,21). Erythromycin is preferred in patients with penicillin allergy. In addition, ampicillin, amoxicillin, cefaclor, and cefadroxil can also be used (18). However, it should be kept in mind that patients who have a penicillin allergy have a 10% probability of having a cephalosporin allergy too (22). It is found that there is a 6-38% probability of failure in oral or penicillin treatment of GABHS (23). Erythromycin used in this study is an antibiotic from the macrolide group. This group of antibiotics is called macrolides due to the presence of a macrocyclic lactone ring, with one or two deoxyribose radicals connected to it. They are also referred to as the name of the first member of the group (erythromycin group antibiotics). Erythromycin is the best and most commonly used alternative for penicillin as primary indications are limited. In this study, we aim to investigate the resistance of Group A Beta Hemolytic Streptococci to erythromycin.

MATERIAL AND METHOD

This article is a thesis work. It was made and completed with the approval of the institution before 2020. Ethical approval was not obtained because there was no ethics committee at that time. All procedures were performed adhered to the ethical rules and principles of the Helsinki Declaration. In this study, 150 throat swab samples were examined. Sore throat, fever, headache, nausea, vomiting, and other symptoms and signs were investigated. Throat cultures of 150 patients with a diagnosis of acute tonsillopharyngitis were taken by using a sterile swab, in accordance with the technique. In our study, throat swab samples were taken from two tonsil surfaces with the help of a tongue depressor and cotton swabs. Swabs were dipped in sterile broth tubes to prevent the material from drying out and immediately inoculated on 5% defibred sheep blood agar and incubated for 24 hours in an oven at 37°C. At the end of incubation on sheep blood agar, colonies showing beta hemolysis were found to be streptococci by Gram stain and negative catalase reaction. Later, to obtain pure culture, its passages were made and incubated at 37°C for 24 hours. Bacitracin susceptibility test was performed to distinguish whether *beta-hemolytic streptococci* with single colony passage were from group A or not. For this purpose, Bacitracin discs (oxoid) each containing 0.04 units of Bacitracin were used. After overnight incubation at 35-37°C, it was analyzed by the Kirby-Bauer disk diffusion method. A few colonies of streptococci showing beta-hemolysis on Kirby-Baner Disc Diffusion Method 5% sheep bloody agar was taken and inoculated into 1-2 ml broth. It was incubated at 37°C for 2-5 hours. When turbidity was equal to the MC Farland 0.5 standard, with the help of a cotton-tipped eraser, before planting, sowing was done on 5% sheep blood agar surface, which was dried in an oven with the lids ajar and upside down, to spread all over in the form of zigzag lines intersecting each other. The plates were left to dry at room temperature for 5-16 minutes. While the discs (penicillin-G, erythromycin, SXT, bacitracin) were arranged on the plate oxoid firm discs were used. The diameters of the zones of inhibition were measured from the lower face of the plate. Zone sizes measured in millimeters were evaluated according to **Table 2** and the results were reported as sensitive, less sensitive, and resistant. The active substance amounts of Bacitracin, trimethoprim-sulfamethoxazole, Erythromycin, and Penicillin discs used in this test are shown in **Table 1**.

Table 1. Amount of substance per antibiotic disc

Antibiotic name	Amount of active matter on the drive
Bacitracin	0.04 units
Penicillin G	10 units
Erythromycin	15 micrograms
Trimetoprimt+ Sulfamethoxazole	1.25+23.75 micrograms

Table 2. Antibiotic resistance and susceptibility status according to disc diffusion diameter

Antibiotic Name	Diameter of growth prevention area (mm)		
	Resistant	Medium susceptible	Susceptible
Penicillin-G	≤11	12-21	≥22
Erythromycin	≤13	14-17	≥18
Trimetoprimt+ Sulfamethoxazole	≤10	11-15	≥16
Bacitracin	≤8	9-12	≥13

RESULTS

In our study, a total of 150 patients of different ages and genders who were isolated beta-hemolytic streptococcus from throat cultures were evaluated. Of the *beta-hemolytic streptococci*, 94 were identified as Group A (62.7%) and 56 (37.3%) as Non-*Group A Beta-hemolytic streptococci* by Bacitracin test (**Table 3**).

Table 3. Distinctive properties of streptococci

Streptococci	Hemolysis	Susceptibility		Results	
		Bacitracin	SXT	Number	%
Group A S. pvogenes	Beta	+	-	94	62.7
Group B S. agalactiae	Beta	-	-	7	4.7
Other Beta Hemolytic Streptococcus	Beta	-	+	49	32.6

SXT: Trimetoprim sulfometaksazol

According to the characteristics seen in **Table 3**, 94 (62.7%) of them were GABHS among a total of 150 *Beta-hemolytic streptococci* (BHS). GABHS was found susceptible to Bacitracin and resistant to SXT. Among all BHSs, 7 (4.7%) were Group B and resistant to Bacitracin and SXT. 49 (32.6%) of them were evaluated as other *beta-hemolytic streptococci*. They were resistant to Bacitracin and susceptible to SXT. Erythromycin and Penicillin susceptibility was detected by disk diffusion method from 94 BHS strains which were identified as Group A by the Bacitracin-SXT test. The results are shown in **Table 4**.

Table 4. Antibiogram results of 94 strains identified as GABHS

Antibiotic name	Diameter of growth prevention area (mm)		
	Susceptible	Medium susceptible	Resistant
Penicillin-G	94 (100%)	-	-
Erythromycin	67 (71.3%)	9 (9.6%)	18 (19.1%)
Trimethoprim+ Sulfamethoxazole	-	-	94 (100%)
Bacitracin	94 (100%)	-	-

As can be seen in **Table 4**, in the antibiotic susceptibility trial to 94 GABHS strains, 67 (71.3%) were susceptible, 9 (9.6%) were moderately susceptible, and 18 (19.1%) were resistant to Erythromycin. In the susceptibility experiment with Penicillin-G, 94 of them (100%) were found to be susceptible, and no moderately susceptible and resistant strains were found.

DISCUSSION

GABHSs are known to be one of the most common factors causing pharyngitis among all age groups. Causes of non-suppurative infections that have an adverse impact on personal and community health like acute rheumatism and post-streptococcal glomerulonephritis should be considered as occurring only after GABHS infections which makes it clear how important *beta-hemolytic streptococci* are in terms of diagnosis, treatment, and patient follow-up.

Today, in Group A streptococcal pharyngitis cases, Erythromycin is used in cases of allergy to Penicillin without doing antibiograms. However, it has recently been reported that there are refractory origins against Erythromycin in GABHS (11,24-30). In our study, throat swab samples were taken from 150 pharyngitis patients and 94 GABHS were obtained by applying the Bacitracin-SXT test with the culture method, and antibiotic susceptibility tests were performed on these 94 GABHS by Kirby-Bauer agar disc diffusion method. As mentioned in the findings section, 67 (71.3%) of the 94 strains were sensitive to Erythromycin, 9 (69.6%) were moderately sensitive, and 18 (19.1%) were found to be resistant. While all 94 strains (100%) were sensitive to Penicillin, no moderately sensitive and resistant strains were found. In various studies conducted in our country and published between 2000-2006, erythromycin resistance in *S. pyogenes* was generally reported to be below 10%, but between 0% and 23% (31,32,36). Again in 2021, in a study conducted in our country, Altun et al. (33) found that BHS isolates (23.5%) were resistant to erythromycin. In our study, if the moderately sensitive group is added, the resistance ratio was found to be towering at 28.7%.

Erythromycin was first used in Gram (+) coccal infections in 1952. Especially for patients who are sensitive to Penicillin, Erythromycin has been used as an alternative in the treatment of streptococcal infections. Penicillin-G is still valid in the treatment of *S. pyogenes* infections and Erythromycin is a safe and useful drug (34). Erythromycin resistance of *S. pyogenes* was first reported 30 years ago in Birmingham (Australia) hospital. Erythromycin-resistant strains of GABHS were reported in the UK, USA, and Canada in 1968. More than 50% of the *S. pyogenes* strains isolated in Japan in the late 1970s were resistant to Erythromycin. In the US, this resistance was reported as 5% (25). The prevalence of *S. pyogenes* infections in Western Australia has been investigated and resistance to Erythromycin has increased from 1% in 1985 to 9.1% in 1986 and 17.6% in 1987 (24). A 1988 study by Philips and his colleagues, showed that Erythromycin resistance increased with the number of months. Total resistance was reported as 22.8% in 413 cases (35). In a study conducted by Seppala et al. (26). in Finland, they stated that the sensitivity of GABHS to Erythromycin varies according to different regions and years. For instance, from January to December 1990, the resistant strain increased from 7% to 20%. In the same study, resistance differences were also found between

regions and they stated this was between 2% and 29%. The same researchers cannot explain the reason for the difference in resistance between time and regions

As can be seen from the studies conducted, Erythromycin resistance varies according to countries, years, months, and regions, and the reason for these differences in the resistance stays unknown. However, in-vitro studies suggest that resistance may be with plasmids or bacteriophages (25).

In this study, Erythromycin's resistance was found to be 19.1%. Among many studies conducted to date, Erythromycin resistance (0%-23%) has been found in GABHS and it is observed that Erythromycin resistance has increased over the years from all these studies.

CONCLUSION

Erythromycin should not be used empirically in treatment. To have a good response from the treatment we apply to the patient, first of all, an antibiotic susceptibility test should be performed and the antibiotic should be selected according to the results of the antibiogram test. The war between microorganisms and antibiotics continues, and resistance rates to antibiotics are increasing day by day. Studies should be continued at different times to assess erythromycin resistance over the years.

ETHICAL DECLARATIONS

Ethics Committee Approval: This article is a thesis work. It was made and completed with the approval of the institution before 2020. Ethical approval was not obtained because there was no ethics committee at that time.

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