TURKISH REPUBLIC OF NORTH CYPRUS
NEAR EAST UNIVERSITY
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ASYMPTOMATIC GROUP A BETA HEMOLYTIC STREPTOCOCCI PHARYNGEAL CARRIAGE IN NORTHERN CYPRUS

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MASTER THESIS

DEPARTMENT OF CLINICAL AND

MEDICAL MICROBIOLOGY

SUPERVISOR

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2020-NICOSIA

ACKNOWLEDGEMENTS

I am grateful and pleased for every individual that supported me in this thesis. First of all, I would like to thank to my supervisor Dr.Esref Celik for all her support, motivation and valuable guidance.

I am grateful for my family and my friends that supported me in this long hard working period. I am thankful for my dad and for my mum that always trusted me. Special thanks to my dear friends Ayse and Deniz that motivated and encorouged me all the time for this hard work.

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SYMBOL AND ABBREVIATIONS

APSGN	Acute Post Streptococcal Glomerulonephritis
ARF	Acute Rheumatic Fever
ASO	Anti Streptolysin O
DNA	Deoxyribonucleic acid
Fn	Fibronectin
GAS	Group A Streptococci
NAAT	Nucleic Acid Amplification Test
PCR	Polymerase Chain Reaction
RHD	Rheumatic Heart Disease
RNA	Ribonucleic Acid
SBA	Sheep Blood Agar
SLO	Streptolysin O
SLS	Streptolysin S
Spe	Streptococcal Pyrogenic Exotoxins
S.pyogenes	*Streptococcus pyogenes*
SXT	Sulfamethoxazole/trimethoprim

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ABSTRACT

Kumsal, R. Asymptomatic Group A Beta- Hemolytic Streptocci Pharyngeal Carriage in Northern Cyprus, Near East University Institute of Health Sciences, M.Sc. Thesis in Medical Microbiology and Clinical Microbiology Programme, Nicosia, 2019.

Group A beta-hemolytic streptococci (GAS) is an important bacteria that causes mild to serious life threatening infections. This includes pharyngitis and more serious complications as necrotiziing fasciitis and septicimia. Improper treatment and diagnosis of pharygitiscaused by GAS lead to immune-related complications of rheumatic fever, rheumatic heart disease and glomerulonephritis. GAS associated infections are global health problem. GAS mainly colonizes nasophayngeal mucosa and skin of individuals and may remain as asymptomatic. The transmission of GAS is airborne through the respiratory secretions or through skin contact of symptomatic or asymptomatic individuals.Asymptomatic carriers are important reservoir for transmission of GAS. The aim of this study was to identifyasymptomatic adult pharyngeal carriers of GAS in Northern Cyprus to prevent outbreaks and serious infections. A total of 307 participants were randomly selected from five districts of North Cyprus and pharyngeal specimens were collected. Throat culture and rapid strep A antigen detection tests were performed.This followed by gram staining and catalase tests of suspected colonies.Catalase negative and gram positive cocci were selected and subcultured. Subcultures were further analyzed by bacitracin sensitivity, SXT resistancy, PYR test and Lancefield latex agglutination test.Among 307 participants, the prevelance of GAS was found as 4.9 %. Participants with presence of cardiovascular or rheumatic disease were found to have higher risk of GAS carriage.As a result, recommendation of routine screening programmes should be carried out especially for patients with underlying serious conditions and in healthcare settings.

Key words:GroupA beta- hemolytic streptococci,*S.pyogenes*,asymptomatic carriage, GAS, North Cyprus

ÖZET

Kumsal, R. Kuzey Kıbrıstaki Asemptomatik Kişilerde Grup A Beta-Hemolitik Streptokok Taşıyıcılığı., Yakın Doğu Üniversitesi Sağlık Bilimleri Enstitüsü, Tıbbi Mikrobiyoloji ve Klinik Mikrobiyoloji Programı, Yüksek Lisans Tezi, Lefkoşa, 2019

A Grubu beta-hemolitik streptokoklar (AGBHS'lar), sadece insanlarda olmaküzere zaman zaman çocuklar arasında ciddi salgınlara neden olan gram pozitif zincir şeklinde üreyen koklardır. Farenjit ve nekrotizan fasiit, septisimia gibiciddi enfeksiyonlara neden olabilir. Grup A beta-hemolitik streptokokların, geçtanı ve yetersiz tedavisi, romatizmal ateş, romatizmal kalp hastalığı veglomerülonefrit gibi komplikasyonlarına yol açabilir. AGBHS'laresas olarak nazofarengeal mukozayı ve bireylerin cildin kolonize eder ve asemptomatik olarak kalabilir. Asemptomatik taşıyıcılar AGBHS bulaştırıcılığı icin önemli bir etkendir. Taşıyıcıların tespiti vegerekirse tedavisi, hastalığın önlenmesinde önemli bir adımdır. Özellikle taşıyıcıların kalabalık yerlerde çalışması bu riski daha da artırmaktadır. Bu çalışmanın amacı salgınları ve ciddi enfeksiyonları önlemek için Kuzey Kıbrıs'ta A grubu beta- hemolitik streptokokların asemptomatik taşıyıcılarını taramaktı. Kuzey Kıbrıs'ın beş ilçesinden, toplam 307 katılımcı seçilip, örnekler toplandı. Boğaz kültürü ve hızlı strep A antijen saptama testleri yapıldı. Şüpheli kolonilere, gram boyama ve katalaz testleri yapıldı. Katalaz negatif ve grampozitif koklar seçilip, tekrar ekim yapildi. Basitrasin duyarlılığı, SXT direnci,PYR ve Lancefield lateks aglütinasyon testi ile analiz edildi.307 katılımcıarasında GAS prevelansı %4.9 olarak bulundu. Kardiyovasküler veyaromatizmal hastalığı olan katılımcıların GAS taşıyıcılığı riski daha yüksek bulunmuştur. Sonuç olarak, özellikle altta yatan ciddi rahatsızlıkları olan bireyler için ve sağlık alanlarında asemptomatik taşıyıcılık için rutin tarama programları yapılmalıdır.

Anahtar Kelimeler: A-Grubu Beta-Hemolitik Streptokok, *S.pyogenes*, asemptomatik taşıyıcılık, AGBHS, Kuzey Kıbrıs

1. Introduction

Group A beta-hemolytic streptococci (GAS), *(S. Pyogenes)* is an important member of human pathogens. GAS is gram positive bacteria that can colonize skin and oropharynx. Its ability of causing self limiting toserious life threatening complications make it special importance for research. Mortalities from GAS related infections is anually about 517,000 which is a critically important rate and 1.78 million new cases of GAS infection are reported each year (Carapetis, J. R. *et al.* ,2005)GASis known mainly for causing pharyngitis but can cause more serious conditions of necrotiziing faciitis, toxic shock syndrome,septicimia andpostsequele complications as rheumatic fever, rheumatic heart disease and glomuneronephritis.GAS could cause outbreaks especially in healthcare settings which could lead to mortalities. The host of GAS is only humans so only humans could transmit the bacteria. The transmission of GAS is through respiratory droplets from orophayrnx of colonized individuals or from the skin of infected individuals. GAS could remain asymptomatic in humans, resulting asymptomatic carriers. Asymptomatic carriers could also transmit the bacteria and are important reservoir for transmission. The identification of carriers is crucial in order to preventoutbreaks and also for prevention of serious infections.

Our reseach took place in North Cyprus. In the 5 districts of North Cyprus; Nicosia, Famagusta, Kyrenia, Trigomo, Lefke, total of 307 volunteers were selected randomly and analyzed for the presence of GAS pharyngeal carriage. The aim of this research is to identify the asymptomatic pharyngeal carriers of GAS, in order prevent outbreaks and serious complications.

2. General Information

2.1.Discovery of Group A Beta-Hemolytic Streptococci

Primary identification of streptococcal infection was described by Theodor Billroth, from wound infections and erypsipelas. Isolation of streptococci wasdoneby Louis Pasteur in 1874. Puerperal fever was major cause of mortalities for women at that time. Streptococcus was isolated from blood and uterus of infected women. *Streptoccocus pyogenes* name was given by Friedrich Julius Rosenbach. Strepto meaning chain, coccus meaning berry in Latin and pyogenes came from pyo (pus) and genes (forming), which means pus forming (Ferretti, et al, 2016).

2.2 Classification Group A Beta-Hemolytic Streptococci

In 1903 bloodagar plates were introduced by Hugo Schottmuller. This was a major importance for differentiation of streptococci. In 1919, Brown classification was introduced. This resulted in classification of streptococci according to hemolytic characteristics observed on bloodagar. Alpha, beta and gamma hemolysis are the names given to 3 hemoytic patterns. In alpha hemolysis green coloured zones and in beta hemolysis complete discoloration of clear patterns are observed. However, in gamma hemolysis no patterns of hemolysis appears. It is known as non hemolytic pattern (Patterson MJ,1996). Brown classification by its own is not enough for identification. Lancefield introduced serologic classification according to differences in surface antigens of different streptococci. Names starting from A to X are given to groups. The name of GroupA beta hemolyticstreptococci (*S.pyogenes)* came from presence of group A carbohydrate, N-acetylglucosamine. Griffifth demonstrated slide agglutination by T-antigen determination. Further on, with the presence of M-protein resulted in subdivision of *S.pyogenes* into various antigenic types. M protein is the major virulance factor of *S.pyogenes* which was identified by Lancefield.

2.3.Structure and Virulence Factors of Group A Beta-Hemolytic Streptococci

GAS*(S.pyogenes)*are gram positivecocciarranged in chains (Figure 2.1). It is important to note that the only resorvoir for GASis humans. The antigenic structure of *S.pyogenes* is widely researched. The composition of cell wall is similar to other gram positive bacteria as having thick peptidoglycan cell layer and importantly having specificantigens related to groups. GAS has specific group carbohydrate of N-acetylglucosamine,which is used in classification and differentiation from other groups.This is responsible for the name of group A streptococci.Virulence of GAS depends on various abilities of bacteria. This includes toxin, enzyme production, inhibition of host immune attacts by prevention of phagocytosis and opsonization and adhesion to host cells. One of the important component of cell wall is M-protein which is the major virulance factor of GAS.M-like protein, F-protein and lipoteichoic acid are other crucial cell wall components and these are all adhesins. Also, hyaluronic acid capsule is present in some strains of GAS. Hyaluronic acid capsule is important characteristic as it is antigenically similar to connective tissues in humans. This makes these strains to survive from phagocytic attacts and cause more serious infections including rheumatic fever. GAS has different mechanisms for protection from host immune system.

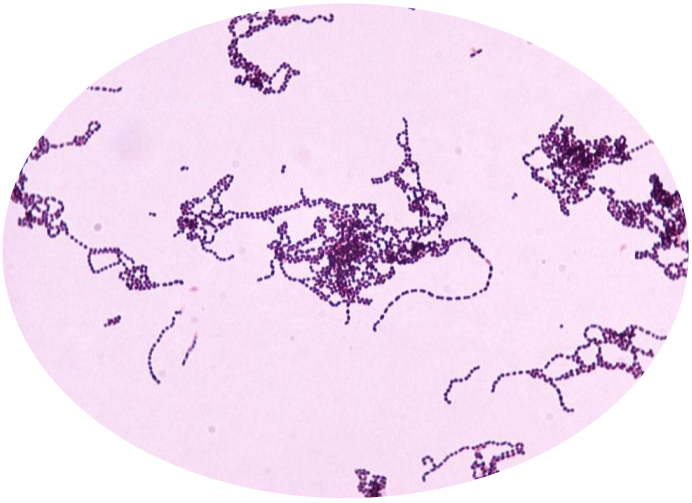

Figure 2.1.Microscopy of GAS (Buensalido,2019)

2.3.1.Toxins and Enzymes of Group A Beta-Hemolytic Streptococci

Different serotypes of *S.pyogenes*(GAS) produces various types of toxins and enzymes.These are streptococcal pyrogenic exotoxins (Spe), streptolysin S, streptolysin O, streptokinases and DNases.

Streptococcal pyrogenic exotoxins (Spe) are toxins that is exclusively specific to streptococcal lysogenic strains. These are also known as erythrogenic toxins. There are four antigenically different types and these are Spe A, Spe B, Spe C and SpeF. These are all function as superantigens. These toxins are related to many conditions such as scarlet fever, toxic shock syndrome, necrotiziing fasciitis.

Hemolysins are responsible for the hemolytic activities. There are 2 types of hemolysins associated with *S.pyogenes*. Streptolysin S and streptolysin O. Streptolysin S (SLS) is nonimmunogenic and oxygen stable exotoxin. Cytotoxic action of SLS effects specific types of eukaryotic cells especially platelets, leukocytes and erthyrocytes causing them to lyse. SLS is responsible for the beta hemolysis seen on blood agar. Streptolysin O (SLO) is type of exotoxin. SLO is oxygen labile and immunogenic. SLO affects host cells by causing pores in their membranes and leading to apoptosis. Host cells includes epithelial cells and white blood cells such as neutrophils. SLO is also present in other pathogenic bacteria such as*Clostridium perfringes, Streptococcus pneumonia, Bacillus cereus.* Antibodies against SLO are formed readily and are known as anti-streptolysin O (ASO). ASO is good indicator for recent *S.pyogenes* infection. For cutaneous types of infections ASO do not produced because cholesterol on skin inhibits SLO.

Streptokinase A and B are enzymes produced by *S.pyogenes* which are the spreading factors. These two breaks down plasminogen into fibrin and fibrinogen and accelerate the spread of *S.pyogenes*. Anti-streptokinase antibodies are crucial diagnostic marker for infection.

DNases areenzymes that are function as spreading factors. There are 4 clasesses of DNases, A, B, C, and D. These enzymes degrade DNA and liquify pus which accelerates the spread of bacteria. Anti-DNase B antibodies are good indicator for streptococcal cutaneous infections.

Hyaluronidase and C5a peptidase are other enzymes. Hyaluronidase is also spreading factor in connective tissues that cleaves hyaluronic acid. C5a peptidasebreaks down C5a chemotaxin in complement system and prevents neutrophils attacking the bacteria.

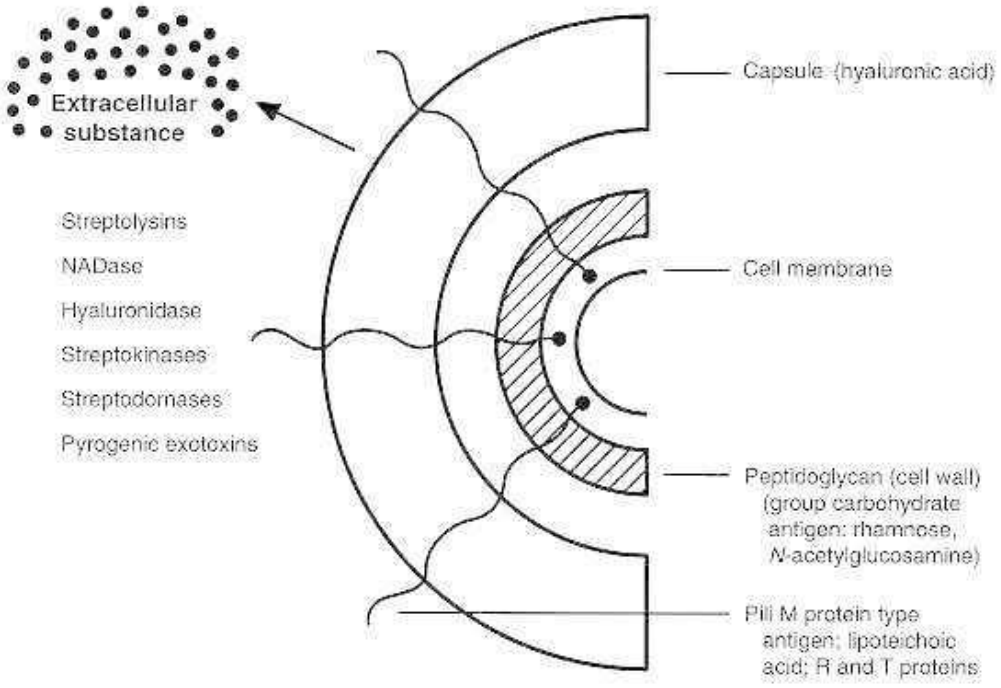

Figure 2.2. Structure of cell wall and extracellular substances (toxins) of S.pyogenes(GAS) (Patterson, 1996)

2.3.2. Importance of M protein

M protein was identified by Rebecca Lancefield. M protein is an important and primary virulance factor for GAS. Vaccine researchers on GAS mainly focuses on the M protein as primary target. Still, there is no successful vaccination against GAS because of its complex structure, especially the ability of antigenic diversity. It has crucial functions in GAS virulance. Antiphagocytic property, resistancy to opsonization, inhibition of complement cascade, makes GAS more powerful and resistant in human system. M protein is encoded by *emm* gene. There are more than 200 *emm* types (Gherardi, 2018 ; Mcmillan, et al, 2013). In the world according to

various studies *emm* type distribution varies by countries economic status and geographical position (Walker, 2014).Each *emm* type causes different disease (Table 2.1.). The structure of M protein is composed of two polypeptide chains coiled in alpha helical coil. C-terminal region (carboxy terminus)of the M protein is the conserved part which all group A streptocci have in common. N-terminal region which is amino terminus, is the variable part of M protein and the reason of antigenic differences.

Further on M protein is divided into class I and class II proteins. Class I includes proteins that share exposed antigens where as in Class II proteins absence of exposed antigens. In acute rheumatic fever outbreaks association with class I M proteins was perceived (Bessen, et al, 1989).

GAS has different mechanisms for inhibitionof phagocytosisand opsonization. M protein plays an important role in this mechanisms. It prevents the complement C3b binding, also complement cascade could be inhibited by M proteins by binding of Fc part of antibodies. M protein has another critical role in adhesion of GAS to host cell.

M proteins cross react with cardiac myosin which causes rheumatic heart disease. Specific M types causes serious complications such as necotizing fasciiis, sepsis and rheumatic sequelae.

Clinical presentation	Associated M types
Pharyngitis	M1, M3, M5, M6, M12, M14, M17, M19, M24
Acute rheumatic fever (ARF)	M1, M3, M5, M6, M11, M12, M14, M17, M18, M19, M24, M27, M29, M30, M32, M41
Epidemic ARF	M5, M18
Geographically widespread epidemics	M1
Fatality	M1, M3, M12, M28
Necrotizing fasciitis	M1, M3, M28
Streptococcal toxic shock syndrome (STSS)	M1, M3
Impetigo	M33, M41, M42, M52, M53, M70
Puerperal sepsis	M28
Acute glomerulonephritis	M1, M4, M12, M49, M55, M57, M60
Meningitis	M1, M12

Table 2.1. Different M serotypes and clinical presentations (Metzgar, D. and Zampolli, A. 2011)

2.4.Transmission and Pathogenesis of Group A Beta-Hemolytic Streptococci

GAS(S.pyogenes) mainly colonizes nasophayngeal mucosa and skin of individuals and may remain as asymptomatic. This results in the occurance of asymptomatic carriers. The colonization of GAS on skin, causes transmission from person to person through contact and the colonization of GAS in pharynx causes transmission via respiratory droplets.

Primarily, infection of GASbegins with the adhesion to epithelial cells by fibronectin (Fn) binding proteins. Epithelial cells includes skin, nasal or oral cavities. Extremely virulent strains of GASexpresess one or more than one Fn binding proteins. At the end of primary invasion, GAS could invade various organs as its capibility of evading hosts immune cells. The pathogenecity of the GAS depends on the M serotype (Table 2.1.).

2.5. Disease of Group A Beta-Hemolytic Streptococci

GAShas ability to cause wide range infections which can range from benign to severe. Mailny GAS colonizes skin and pharynx. Addition to this, rectum and vagina could be colonized. GAScauses, supporativeandnon- supporative infections. Supporative infections include pharngitis, impetigo, scarlet fever, cellulitis and more seriously necrotizing fasciitis, toxic shock syndrome and sepsis. Non-supporative infections which are post-streptococcal squele include rheumatic fever and acute glomerulonephritis (Figure 2.3).

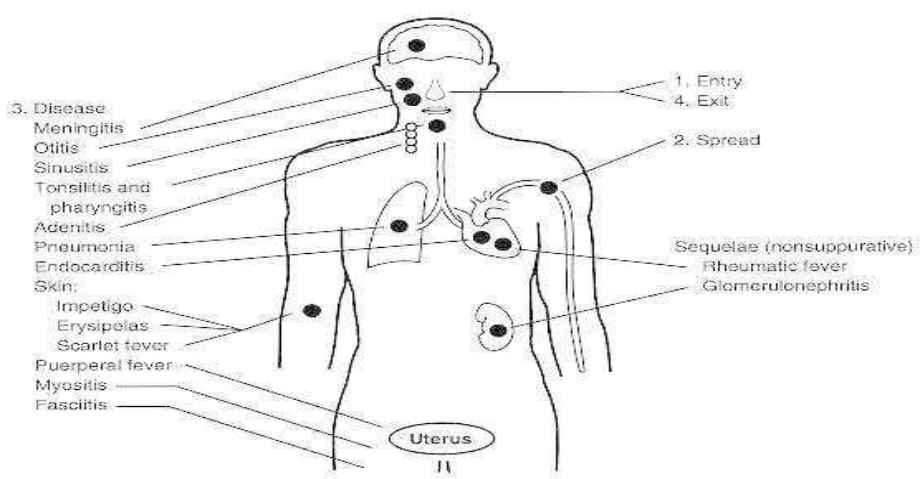

Figure 2.3. Summary of GAS disease (Patterson, 1996)

2.5.1. Pharyngitis and Scarlet Fever

GASis well known for causing pharyngitis which is inflammation of posteior pharynx. Mainly in winter, also in spring period the incidince of pharyngitis rises. Each year 616 million new cases of streptococcal pharyngitis occur worldwide (Carapetis, 2005).Individuals at all ages are susceptible for transmitting the bacteria, especially crowded environments such as schools, millitaries etc. Proper diagnosis and treatment of symptomatic pharyngitis caused by GAS is important for prevention of post sequele complications such as rheumatic fever, rheumatic heart disease and acute glomuneronephritis. There is a strong correlation between pharyngitis and acute rheumatic fever (ARF). ARF causes damage to heart valves and may result to heart failure and mortality.Serotypes1, 3, 5, 6, 14, 18, 24 of M protein are found to have relation with pharyngitis and ARF. According to studies it has been found that in developed countries, the incidence of symptomatic pharyngitis among school aged children is 15% and in adults is 4-10%. In less developed countries the incidince of pharyngitisrises approximately4 to 10 fold(Carapetis, 2005; Bennett, 2019) .

Scalet fever is a condition that appears after steptococcal pharyngitis. Strains infected with lysogenic bacteriophage can cause scarlet fever. Pyrogenic exotoxin

production is stimulated by the bacteriophage. This causes erythematous rash, starting primarily from upper chest and disseminate throughout whole body. Other complications of scarlet fever includes strawberry tongue, pastia lines, circumolar pallor, desquamation. In the preantibiotic era, scarlet fever had caused mortalities especially in children. After discovery of antibiotics and improvements in living conditions, scarlet fever incidences had been decreased. However, in the recent years the incidences of scarlet fever had increased in countries such as Hong Kong, China and UK (Efstratiou, 2016). Mainly scarlet fever outbreaks occurs in spring. The main reason of the new incidences was not clearly understand. In the UK, scarlet fever cases had been increased, especially in 2016 data, 19,206 cases had been identified(Lamagni, 2019).

2.5.2.Pyoderma and Erysipelas

Pyoderma (impetigo) is superficial infection of skin which is extremely contagious and mainly seen in children. The infected areas are mainly face, legs and arms.The transmission is due to close contact withGASinfected individuals or fomites. Crowded enviroments such as school and inadequate hygienic conditions increases the spread. Improper treatment could cause severe complications such as glomerulonephritis. Glomerulonephritis development after impetigo can be seen in %5 of patients(Stevens, 2016).

Erysipelas are infection of dermis with involment of lymphatics. Streptococci was primarily identified by Theodor Billroth from erysipelas. The transmission of erysipelas is due to trauma of skin or the individual can infect theirselves with carrying GASin nasal passages or skin. Prognosis of erysipelas is succesful if diagnosed and treated early. Erysipelas mainly effects children and older adults.

2.5.3. Cellulitis, Necrotiziing Fasciitis, Streptococcal Toxic Shock Syndrome

Cellulitis is inflammation of deeper subcutaneous tissues and skin. Erythema, swelling, pain on the affected area are seen in cellulitis. The transmission of cellulitis is due to trauma of skin such as burns, insect bites, wounds etc.

Necrotiziing fasciitis is GAS infection that spreads rapidly infecting fascia and subcutaneus tissues, andcauses necrosis. Streptococcal gangrene, flesh eating diseaseare other names given to necrotiziing fasciitis. Early diagnosis of necrotiziing fasciitis is important.

Streptococcal toxic shock syndrome is a serious infection of GAS that causes multiple organ failure. Pyrogenic exotoxin is responsible for streptococcal toxic shock syndrome.

2.5.4.Acute Rheumatic Fever and Rheumatic Heart Disease

Rheumatic fever is important post sequeale complication of untreated or improper treatment of GAS pharyngitis which causes rheumatic heart disease (RHD). Rheumatic fever still remains a global problem in low and middle income countries (Bennett, J et al 2019).Rheumatic fever causes manifestations of arthritis, carditis, chorea, erythema marginatum and subcutaneous nodules. Autoimmunity and molecular mimicry is the key reason for the complications leading crossreaction of antibodies with host tissues and GAS proteins. This damages the host tissues. This is also the case in RHD, which causes 275,000 mortality, 9 million disability cases in the world, predominantly in developing countries. In the world there are 33.4 million patients with RHD(Mayosi, 2017).M protein have similar α-coiled structure with heart cardiac protein myosin, and this leads to crossreaction that damages the heart tissue(Zuhlke, 2017).

2.5.5. Acute Post Streptococcal Glomerulonephritis

APSGN is a postsequele complication after the pharyngitis or impetigo that are caused by the infection with nephritogenic strains of GAS. M serotypes that are the reason of nephritis are 1, 2, 4, 12, 18, 25, 49, 55, 57, and 60. APSGN is a serious condition of the kidney inflammation that can lead to kidney failure. Annually 470,000 new cases of APSGN and 5,000 mortalities occur worldwide due to APSGN(Carapetis, 2005).

2.6. Asymptomatic Carriage of Group A Beta-HemolyticStreptococci

Asymptomatic carrier state is defined as the posterior pharyngial existence of *S.pyogenes* (GAS) without causing any indications. The throat culture is positive in asymptomatic carriers. However, the antibodies against GAS do not produced and remain negative. These antibodies are anti-deoxyribonuclease B, antistreptolysin-O and anti-hyaluronidase. GAS carriage is still diffucult to understand. It is critical to distinguish carriers from truely infected individuals, in order to prevent unnecessary usage of antibiotics. Importantly, asymptomatic carriers still have a risk of transmitting GAS. The risk is low but still present.

There are different theories for GAS throat carriage. Primary theory is that production of β-lactamase by the normal pharyngial flora bacteria. This β-lactamase producing bacteria inhibits the working of penicillins and causes carries for GAS. *Moraxella, Staphhylococcus aureus, Bacteroides* sp. and *Haemophius,* had been found to have a relation with carriage and also causes unsuccesful treatment for GAS. Second theory supports that GAS species have the capability for inhibition of antibiotics by getting internalized into epithelial cells. This hypothesis supported by invivo studies of GAS. 70% of carriers had been found to have GASintracellulary in epithelial cells. This was proved by tonsillarbiopsies (Osterlund, et al, 1997).Third theory is the capability of GAS for biofilm production (Ogawa, et al, 2011). So all of these shows that GAS has ability ofavoiding antibiotic treatments, which could lead to pharyngeal carriage.

The crucial question to ask is that when the carriage of GAS becomes important. First thing to consider is, the risk of carrier developing nonsuppurative complications. This complications, include acute rheumatic fever, acute glomerulo nephritis and rheumatic heart disease. In studies, it had been shown that there is a low risk of carriers to develop nonsuppurative complications. Secondly, the risk of carries to transmit the GAS to others shoud be considered. Carriers have low risk of trasmitting the GAS while people who have active acute infection has the highest risk. Carriers have a risk of transmission of GAS when infected with another virus which causes respiratory symptoms. In the cause of invasive disease, the state of carriers is not obvious. It had been found in a study that 27% of individuals

colonized with GAS, who had close contact with patients having invasive infections (Weiss,et al, 1999).

In the clinical settings, the most confusion of carriers cause a problem in the incorrect diagnosis of acute pharyngitis. This is due to a carriers become infected with a virus and showing the symptoms of sore throat. The rapid antigen tests and the throat culture would give a positive results that causes unneccesary prescription of antibiotics. This is a crucial problem as can result in antibiotic resistancy.

Important considerations in treatment arises when GAS carrier is identified. The first one if the treatment eradicates the carriage and secondly does the eradication provides any benefit to the patient or to the population. There are contraversials about treatment of carriers. It is crucial to note that low risk of transmission and for the occurence post sequele complications, the treatment of carriers would provide little or no benefits. However, there are some exceptional situations where treatment of carriers could be an option.These situations includes, outbreaks in communities of GAS invasive disease, pharyngitis, rheumatic fever, glomerulonephritis , individuals with personal or family history of rheumatic fever, recurrent GAS pharyngitis in family and in tonsillectomy because of carriage(Shulman, et al, 2012)

Carriers could acquire new strains of *emm* and this could increase the rheumatic fever risk. So it is important to test carriers for new *emm* types, in the cases of symptomatic phayngitis.

2.7 Antibiotics and Group A Beta-Hemolytic Streptococci

GAS is susceptible for penicillin and is still the best treatment of choice for GAS associated infections. The susceptibility to penicillin had not been changed for 50 years. However, there are some concerns in some GAS strains that penicillin therapy may fail to succeed. This could be due to the GAS ability to escape from penicillin by internalizing into epitheil cells, biofilm formation or presence of beta-lactamase bacteria that protect GAS. These are only some theories that not been proven (Walker, 2014) . Patients allergic to penicillin are treated with other types of antibiotics. These involves macrolides, tetracyclines and fluoroquinolones. In these

alernatives of antibiotics, there are some resistancy mechanisms and this is an important concern.

2.8.Laboratory Diagnosis of of Group A Beta-HemolyticStreptococci

2.8.1. Culture

Culture is the golden standard of diagnosis for GAS(Shulman, et al., 2012). Culture has sensitivity of 90-95% for detection of GAS(Bisno,2002). Blood enriched agar and especially %5 sheep blood agar is the preferred media for inoculation. It is important to obtain the specimens correctly from patients. Specimens should be carefully obtained from tonsils and posterior wall of pharynx. Improper collection could lead to false negative results. Antibiotic usage during or short period before culture test also results in false negativity. Beta hemolysis detection on blood agar is an important step for identification of GAS. Incubation of the plates for 24 hours at 37^{o}C is optimal conditions for *S.pyogenes* growth. Importantly, suspected negative plates should be incubated 24 hours more in order to get accurate results. Colony morphology of GASis white smooth colonies which have beta hemolytic clear zones (Figure 2.4.).

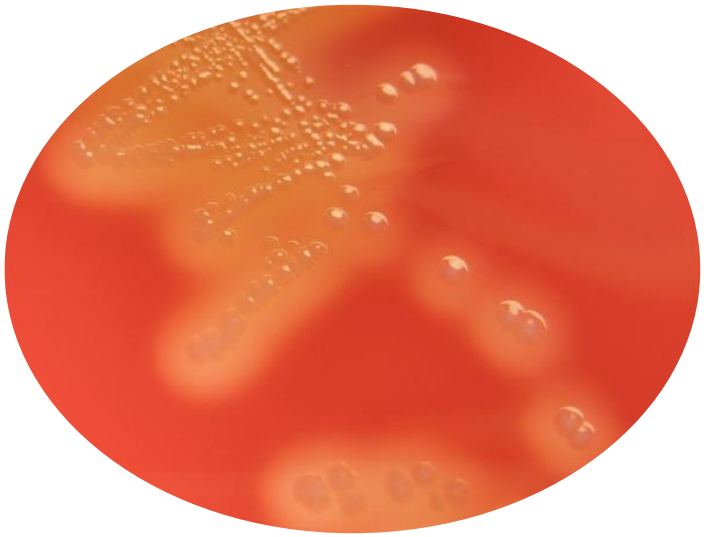

Figure 2.4. Colony Morphology of GAS (Spellerberg, Brandth, 2016)

2.8.2. Rapid Strep A Antigen Detection Tests

Rapid Strep A testsare widely used in clinical settings. The main working principle is that it detects directly the specific group-A carbohydrate from the throat swab and provide results in minutes. This provides rapid treatment of pharyngitis and reduces the unnecessary prescription of antibiotics. However, it is important to consider doing both rapid strep A test and culture together. Culture is still the golden tool for diagnosis. Rapid strep A tests have high specificity but the sensitivity changes from 58% to 96% (Uhl et al,2003)(Pritt et al, 2016). The sensitivity of the test is based on the disease severity. False positive results from rapid strep a test is relatively uncommon so treatment can be decided with the positive result (Bisno2002). However, all the negative rapid strep A test especially from children should be confirmed with culture as there is a risk of false negativity (Shulman, et al., 2012). Also children are susceptable for streptococcal pharyngitis more than adults and developing of nonsuppurotive complications such as rheumatic fever. So accurate diagnosis is crucial.

2.8.3. Catalase Test

Catalase test is used for conformation of colonies for streptococci. It is used for differentiation of staphylococci colonies from streptococci. GAS is catalase negative.Catalase test is performed by adding %3 H_2O_2 to bacterial colony transformed into clean slide and mixed. Positive results give appeareance of bubbles on slide and negative results no bubbles are observed. This is due to the catalase enzyme activity.

2.8.4. Bacitrasin Susceptibility and Sulfamethoxazole/ TrimethoprimResistancy

Bacitrasin sensitivity is important for differention of *S.pyogenes*(GAS) from other non- group A beta hemoltyic streptococci such as *S. iniae* and *S. porcinus*. However, there some types of group C and G streptococcus that are known to be sensitive to bacitracin. In order to increase the accuracy of the GAS test, bacitracin and SXT discs should be performed together. GAS(*S.pyogenes)* is sensitive to bacitracin and is resistant to SXT, while group C and G streptococcus are sensitive to SXT. In order to perform bacitrasin and SXT test, subculture should be obtained

from the strain on %5 sheep blood agar (SBA). Bacitrasin and SXT discs should be placed on SBA inoculated with pure strain and incubated at 37^{o}C for 24 hours.

2.8.5. PYR Test

PYRtestis pyrrolidonyl aminopeptidase test that is used in order to differentiate *S.pyogenes* from other types of beta hemolytic streptococci. PYR is a colorimetric test. It works by clarifing the presence of enzyme pyrolidonyl aminopeptidase. *S.pyogenes* is PYR positive.

2.8.6. Lancefield Antigen Determination

Identification of antigens on surface of streptococci by Rebecca Lancefield was a great discovery. Commercial kits are available which results of identification of Lancefield antigens. Substrates are supplied by commercial kits that allow to extract antigen and this followed by agglutination of specific antibodies for Lancefield antigens A, B, C, F, and G. However by its own is not enough for determination of *S.pyogenes* as there are several types of streptococci such as *Streptococcus anginosus* that have the group A antigen (Facklam, 2002). Further analysis should be carried for confirmation of *S.pyogenes* as PYR test and bacitracin susceptibility .

2.8.7. Nucleic Acid Tests

Nucleic acid probe and nucleic acid amplification tests are available for the identification of *S.pyogenes* (GAS). The primary nucleic acid probe assay that developed was GEN-probe. This works by the princible of nucleic acid hybridization that identifies rRNA of GAS from patients pharyngeal specimens by nucleic acid probe. The sensitivity of nuclear probe is 89%.

There are several nucleic acid amplification tests (NAAT) for the identification of GAS. The sensitivity and specificity of NAAT are relatively similar to culture. NAAT for the identification of GAS uses polymerase chain reaction (PCR) assay, that is developed by Roche company and named as Light Cycler Strep-A assay. It had been found in some researchers that by the single test of Light Cycler Strep-A, provides highly accurate results and sensitivity as high as

culture(Uhl2003)(Luo2019. The advantage of this test is that it is sensitive than rapid antigen detection tests and shorter time for analysis compared to culture. However, the main disadvantage of this test is the cost (Pritt2016).

2.8.8. Serological Tests for Antibody Detection of GAS

Serologicaltests are important for the diagnosis of postsequaele complications of GAS, such as rheumatic fever and glomerulonephritis. Patients develop antibodies againts the group A streptococcal enzymes. Anti-streptolysin O (ASO) and anti-DNase-B antibodies are the mostly used antibodies for the diagnosis of postsequaele infections.

Streptolysin O is oxygen labile and immunogenic exotoxin. Antibodies against streptolysin O (ASO) starts to rise at the week 1 from primary exposure to GAS and become the highest in the weeks of 3 to 6 (Spellerberg et al, 2016). ASO levels rise in the cases of streptococcal phayngitis and it is important that for the streptococcal cutaneous infections such as pyoderma, not enough immune response occurs against streptolysin O. This is due to the cholesterol in the skin inhibits the streptolysin O.

There are 4 types of DNases produced by the S.pyogenes, A, B, C and D. DNase B has the strongest host immunologic response and antibodies starts to develop following 2 weeks from the primary exposure to bacteria. In both streptococcal phayngitis and streptococcal pyoderma cases anti-DNase B antibodies develop and can be detected.

2.8.9. Molecular Typing of GAS (*S.pyogenes*)

Subtyping of GAS is only used in research laboratories and in the cases of outbreaks, in order to understand the strains of the bacteria. There are different typing systems for GAS focused on T and M surface proteins. The golden standard in the molecular typing is *emm*typing which analyses the primary virulance factor M which is encoded by *emm* gene. The *emm* typing is simple and practical with high signifance rate (Spellberg, 2016) .

3.MATERIAL AND METHOD

3.1.Type of Study

This study is a prospective study and was conducted from April 2019 until December 2019. This study was approved by the Institution Ethics Evaluation Board YDU/2019/67-768 on March 28, 2019. Total of 307 healthy adult participants were participated in this study. In this period, samples had been collected randomly from healthy 307 adult participants with the sign of clarified consent form (Appendix 2). The samples had been collected from Nicosia, Famagusta, Kyrenia, Trigomo, Omorfo and Lefke districts.

3.2. Materials

- Transport Medium Swabs
- Sterile Loops
- %5 Sheep Blood Agar
- Streptococcus pyogenes Group A Rapid AntigenTest
- Streptococcal Grouping Latex Kit
- PYR Test
- Gram Staining Kit
- Hydrogen Peroxide (H_2O_2)
- Bacitracin Antibiogram Disks
- Sulfamethoxazole/ Trimethoprim Antibiogram Disks
- Microscope Slides

3.3. Collection of Specimens

Specimens had been collected from the posterior pharynx and tonsils of 307 participants using sterile transport medium swabs. The collected specimens had been transferred immediately to microbiology laboratory.

3.4. Questionerre

Participants had been asked kindly to complete a questionerre (Appendix 1), in order to understand the risk of GAS carriage. First of all, for all participants personal data were reported. Personal data information had reported name, surname, age, residental area, occupational area of the participants .In the questionerre questions about cigarette, alcohol usage,presence of cardiovascular and rheumatic disease, unprescribed usage of antibiotics were enquired.

3.5.Investigation of Specimens

Collected specimens were inoculated into %5 defibrinated sheep blood agar and incubated for 24-48 hours at 37^{o}C and rapid strept A antigen test was performed. After 24 hours, the plates were examined. Colonies with beta hemolysis were suspected as GAS (Figure 3.1.) and further analyzed. This follows catalase test and gram staining was performed for the suspected coloniesof GAS. GAS are catalase negative and morphology is gram positive cocci arranged in chains. Catalase test negative colonies with the confirmation of gram staining were subcultured into %5 sheep blood agar for 24 hours at 37^{o}C . For the subculture, bacitrasin and SXT discs were placed (Figure 3.2.). Bacitracin sensitive and STX resistant colonies were further analyzed with PYR test. PYR test positive colonies were tested with Lancefield latex test and Group A beta-hemolytic streptococci were identified.

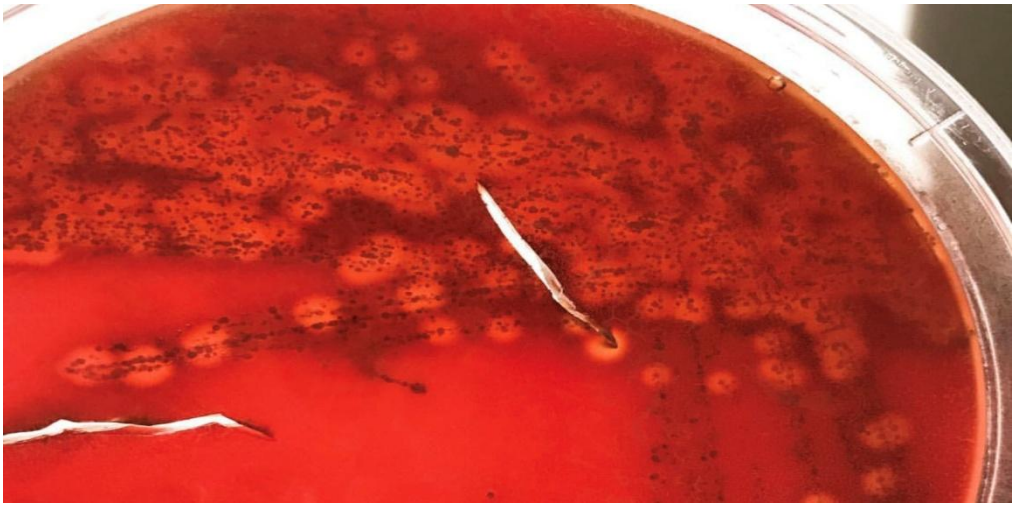

Figure 3.1. Primary culture for suspected GAS with beta hemolysis

Figure 3.2. Subculture of GAS with beta hemolytic colonies and bacitracin/SXTdiscs

4. RESULTS

Total of 307 individuals were participated in this study. Participants were selected and tested randomlyfor GAS pharyngeal carriage. This study was conducted from April 2019 until December 2019 in North Cyprus. According to our data, %4.9 of GAS carriers were identified (Table 4.1).

Table 4.1.Rate of GAS carriers identified in total of 307 participants

GAS PharyngealCarriage		Frequency (n)	Percent (%)
	NEGATIVE	292	95,1
	POSITIVE	15	4,9
	Total	307	100,0

In this study, 205 (66,8 %) female and 102 (33,2 %) male participants were attended.Age groups among 307 participants were as follows; age group 18-29 , (n: 63, 20.5 %) , age group 30-39 (n:50, 16.3 %), age group 40-49 (n:52, 16.9 %), age group 50-59 (n: 69, 22.5 %) and age group >60 (n:73, 23.8 %) (Table 4.3).There was no statistical significance among GAS carriage with age (p>0.05, t-test)(Table 4.2.).

Table 4.2. GAS carriage rate distribution within age

	GAS CARRIAGE	N	t	p
AGE	NEGATIVE	292	0.25	0.79 ns
	POSITIVE	15		

Table 4.3.Distribution of participants according to age groups of study and GAS carriage within age groups

Age Range		Frequency (n)	Percent (%)	GAS Frequency (n)	GAS Percent (%)
	18-29	63	20,5	4	6,3
	30-39	50	16,3	3	6,0
	40-49	52	16,9	2	3,8
	50-59	69	22,5	3	4,3
	>60	73	23,8	3	4,1

The distributions of participants according to districts were Nicosia (n: 155, 50.5%), Famagusta and Trigomo (n: 54, 17,6 %), Kyrenia (n:47, 15.3%), Lefke and Omorfo (n:51, 16.6 %) (Table 4.4). According to statistical analyses of Chi square test, the distrubition of GAS carriage with districts were not statistically significant (p>0.05).

Table 4.4. Distribution of participants among districts

Districts		Frequency (n)	Percent(%)	
	NICOSIA	155	50,5	p >0.05
	FAMAGUSTA+ TRIKOMO	54	17,6	
	KYRENIA	47	15,3	
	LEFKE+OMORFO	51	16,6	

The nationality of participants were Cypriots (n:268, 87,3 %) and other nationalities (n:39, 12,7 %). The GAS carriage among Cypriots was 3.6% and other nationalities was 1.3% . Total carriage was observed as 4.9 %. Statistical analysis of Chi square test had showed, the distribution among Nationality with GAS carriage was not significant ($p>0.05$) However, ODDs (OD: 2,67) ratio had showed that there was a higher risk of GAS carriage between other nationality and Cypriots (Table 4.5.).

Table 4.5. Description of GAS pharyngial carriage among nationalities

Nationality	**Frequency (n)**	**Percent (%)**	**GAS Frequency**	**GAS Percent**	**P OR**
Cypriot	268	87,3	11	3,6	p>0.05
Other Nationality	39	12,7	4	1,3	OR=2.67

The occupational area of participants were distributed as healthcare workers (n: 79, 25,7 %) and other areas (n:228, 74,3 %). There was no statistical significance observed by the use of Chi square test, between the occupational areas and GAS carriage of the participants ($p>0.05$) (Table 4.6). However, within the age group of 18-29,there was a statistical significance observed ($p<0.05$) between the occupational areas and GAS carriage. Fisher exact chi square test was used and p value was found as 0.04 as statistically significant result (Table 4.7.).

Table 4.6.Description of GAS pharyngial carriage among occupational areas

Occupational Area	**Frequency (n)**	**Percent (%)**	**GAS Frequency**	**GAS Percent**	**P OR**
Healthcare	79	25,7	7	2,3	p>0.05
Other	228	74,3	8	2,6	

Table 4.7. Descriptionof GAS pharyngial carriage among occupational areaswithin the age group 18-29

AGE RANGE 18-29					
Occupational Area	**Frequency (n)**	**Percent (%)**	**GAS Frequency**	**GAS Percent**	**P OR**
Healthcare	16	4,8	3	4,8	p<0.05
Other	47	1,6	1	1,6	

Pharyngeal carriage of GAS with presence of romatoid and cardiovascular disease, was found statistically significant (p<0.05)(Chi square test) . Participants with presence of romatoid disease was found to have 6 times higher risk (OR=6.386) and for presence of cardiovascular disease 4 times (OR=4.044) of GAS pharyngeal carriage (Table 4.8) than the participants who did not have any romatoid or cardiovascular disease.

Table 4.8.Description of GAS carriage with romatoid and cardiovascular disease

	Frequency (n)	**Percent (%)**	**GAS Frequency**	**GAS Percent**	**P OR**
Romatoid Disease	14	4.6	3	1	p<0.05 OR 6,38
Cardiovascular Disease	20	6,5	3	1	p<0.05 OR 4,04

The distribution of antibiotic usage without doctor presciription and GAS pharyngeal carriage wasnot statistically significant (p>0.05) (Chi square test) (Table 4.9). The participants that use antibiotics without doctor prescription was 27,7 %.

Table 4.9.Description of GAS carriage with antibiotic usage with or without doctor prescription

	Frequency (n)	**Percent (%)**	**GAS Frequency**	**GAS Percent**	**P OR**
Antibiotic usage with dr prescription	222	72,3	4	1,3	p>0.05
Antibiotic usage without dr prescription	85	27,7	11	3,6	

5.DISCUSSION

GAS is responsible for diverse range of disease and outbreaks. GAS remains as a global health problem. Annually, 517,000 mortalies were reported as a result of GAS associated diseases (Carapetis, JR, et al. 2005). Identification of GAS asymptomatic carriers is crucial in order to prevent the diseases and outbreaks.

In this study, we had examined the asymptomatic carriage of GAS among 307 adult individuals, living in Northern Cyprus. This study mainly focused on the Cypriot nationality. The participants were selected mainly from Cypriots (87.3%). The carriage rate of GAS among Cypriot nationality was found as 3.6 % (Table 4.5). The overall carriage rate was found 4.9 % (Table 4.1). To our knowledge, this is the first study that has conducted in North Cyprus for the identification of asymptomatic GAS carriers. In North Cyprus, only one study was conducted that had investigated the rate of GAS among only in pharmacy students. The carriage rate was found as 4.6% among total of 140 students from Iran, Syria, Iraq and Nigeria (Haddah, S et al. 2019) . The carriage rate had showed similarity with this study.

In the literature, the studies about GAS carriage in adults is limited. In meta analysis studies, the prevelance rateof GAS carriage among adults was compared with OECD and non-OECD studies. In OECD studies, the prevelance of GAS among adults was found as 2 % and in non-OECD studies as 4.6%(Oliver, J. *et al.* , 2018). This study had showed similarity with the prevelance rate of GAS with non-OECD studies(Oliver, J. *et al.* , 2018).

The carriage rate among total of 187 young adult students within the age range of 18-27 was investigated in one study carried out in USA. The samples from 87 participants were collected in late winter/early spring and 100 samples in late autumn. In the late winter/early spring prevelance of GAS was found as 11.5% and in autumn as 8%>The overall asymptomatic carriage rate was found as 9.6 % (Levy, RM et al, 2015) .In our study the asymptomatic carriage within the age group of 18-29 was found as 6.3 % (Table 4.3).

The other study was conducted in Poland where 205 participants within age range of 18-44 were selected and examined in 12 month period from March 2013 to February 2014. The carriage rate was found as 1.5 % in this age group (Bura, M. et al 2016).

In Turkey, total of 134 participants were selected in three different groups for GAS asymptomatic carriage. The groups were as follows, the first group was the age group of 10-17 from social services, second group was the age group of 20-25 from police students and the third group was the age group of 60-90 from nursing home. The distribution of GAS, in order of age groups was 27%, 26% and 16.2 %. The start date of the study was as January 2005, however end date was not reported (Nese Demirturk, et al, 2007). The carriage rates among groups were found higher than this study and from the literature.

According to these different studies, the differences could be seen in the carriage of GAS. This can be due to the demographic differences, the period of the study, climate and the methods used.

In this study, the risk of carriage of GAS in the participants with conditions of rheumatic and cardiovasculardisease had been found higher and significant ($p<0.05$). This is important. The asymptomatic carriers with underlying conditions of rheumatic and cardiovascular disease should consult doctor in the case of sore throat, in order to take early precauticions.

In the occupational status of the participants within the age group of 18-29 and GAS phayngeal carriage, statistical significance was observed ($p<0.05$) (Table 4.7.). GAS could cause outbreaks in hospital settings.The eradication of GAScarriage in healthcare settings is considerable in presence of transmission to patients (Steer et al,2011) .

The questionerre answers from the participants attended to this study had touched upon an important point. 27.7% of the participants were using antibiotics without doctor prescription. There was not significant difference of GAS carriage and unprescribed usage of antibiotics according to this study. However, unnecessary usage of antibiotics results in antibiotic resistancy.

The methods that used in this study were highly significant. In the future, molecular analysis and emm typing can be carried out. *emm* typing is important in order to understand *emm* prevalance in North Cyprus.

In the literature, there were not many studies on asymptomatic carriage of GAS among adult population and this is a limitation in this study. Many studies had been investigate the GAS asymptomatic carriage in children.It is crucial and

significantly important to know the asymptomatic carriage rate among adults, in order to take precautions for outbreaks, prevention of serious complications and for epidemiologic studies.

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6.CONCLUSION

In this study, the asymptomatic group A beta-hemolytic streptococci carriage among 307 adult participants living in North Cyprus were investigated and as a result the carriage rate was found as 4.9%. According to our knowledge, this is the first study conducted in North Cyprus for the prevelance of GAS. It is important to have more studies especially in healthcare settings and schools. In North Cyprus more studies should conducted, in order to know the risk status pattern of GAS. GAS is a global health problem. Considering GAS as one of the pathogens that causes morbidity and mortality all over the world, it is a good reason for implementation of screening programmes.

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Appendix 1

ANKET

Kuzey Kıbrıstа Sağlıklı Kişilerde A-Grubu Beta Hemolitik Steptokok Taşıyıcılığı

1. İsim

   _____

2. Yaşınız

   _____

3. Köken
   - KKTC
   - Yabancı Uyruklu (Ne kadar süredir KKTC'de ikamet ediyor)

4. Yaşadığınız Bölge

   _____

5. Cinsiyet
   - E
   - K

6. Meslek

   _____

7. Bir yılda ne kadar sıklıkla tonsilit/faranjit/boğaz ağrısı şikayetiniz oluyor?
   - 2 ve daha az
   - 3 ve 4
   - 5 ve daha fazla

8. Boğazınız ağrıdığında doktora başvuruyormusunuz?
   - Evet
   - Hayır

9. Teşhis için boğaz kültürü yapıldımı ?
   - Evet
   - Hayır

10. Tedaviniz için hekim tarafından verilmiş antibiyotik kullandınızmı?
    - Evet
    - Hayır

11. Doktora gitmeden antibiyotik kullanıyormusunuz?
    - Evet
    - Hayır

12. Bademcik ameliyatı (tonsillektomi) geçirdinizmi?
    - Evet
    - Hayır

13. Sigara içiyormusunuz?
    - Hayır
    - Günde 5 ve daha az
    - Günde 10
    - Günde 20 ve daha fazla

14. Alkol kullanıyormusunuz?
    - Evet (ne sıklıkta)
    - Hayır

15. Gece uyku probleminiz var mı, horlama/ uyku apnesi/ burun tıkanıklığı?
    - Evet
    - Hayır

16. Geçirdiğiniz herhangi bir romatizmal hastalık var mı?
    - Evet
    - Hayır

17. Geçirdiğiniz herhangi bir kalp rahatsızlığı var mı?
    - Evet
    - Hayır

Appendix 2

ARAŞTIRMA AMAÇLI ÇALIŞMA İÇİN AYDINLATILMIŞ ONAM FORMU

Rüyam Kumsal'la yeni bir araştırma yapmaktayız. Araştırmanın ismi "Asymptomatic Group A- Beta hemolytic streptococci pharyngeal carriage in Northern Cyprus. (Kuzey Kıbrıstaki asymptomatik kişilerde Grup A beta hemolitik streptokok taşıyıcılığı) dir.

Sizin de bu araştırmaya katılmanızı öneriyoruz. Bu araştırmaya katılıp katılmamakta serbestsiniz. Çalışmaya katılım gönüllülük esasına dayalıdır. Kararınızdan önce araştırma hakkında sizi bilgilendirmek istiyoruz. Bu bilgileri okuyup anladıktan sonra araştırmaya katılmak isterseniz formu imzalayınız.

Bu araştırmayı yapmak istememizin nedeni, boğazdaki (A-grubu beta hemolitik streptokok) beta bakterisinin varlığının araştırmasıdr. Yakın Doğu Üniversitesi Sağlık Bilimleri Fakültesi Anabilim Dalları'nın ortak katılımı ile gerçekleştirilecek bu çalışmaya katılımınız araştırmanın başarısı için önemlidir.

Eğer araştırmaya katılmayı kabul ederseniz boğazınızdan bir kültür örneği alınacaktır. Bu çalışmaya katılmanız için sizden herhangi bir ücret istenmeyecektir. Çalışmaya katıldığınız için size ek bir ödeme de yapılmayacaktır.

Sizinle ilgili tıbbi bilgiler gizli tutulacak, ancak çalışmanın kalitesini denetleyen görevliler, etik kurullar ya da resmi makamlarca gereği halinde incelenebilecektir.

Bu çalışmaya katılmayı reddedebilirsiniz. Bu araştırmaya katılmak tamamen isteğe bağlıdır ve reddettiğiniz takdirde size uygulanan tedavide herhangi bir değişiklik olmayacaktır. Yine çalışmanın herhangi bir aşamasında onayınızı çekmek hakkına da sahipsiniz.

Katılımcı
Adı, soyadı:
Adres:
Tel.
İmza

Görüşme tanığı
Adı, soyadı:
Adres:
Tel.
İmza:

Araştırmacı
Adı soyadı, unvanı:
Adres:
Tel.
İmza:

CURRICULUM VITAE

Name	Rüyam	**Surname**	Kumsal
Place of Birth	Nicosia	**Date of Birth**	13.10.1991
Nationality	Cypriot	**Tel**	05488668841
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Educational Level

	Name of the Institution where he/she was graduated	**Graduation Year**
Undergraduate	University of Nottingham (Bsc Biology)	2014
High School	Near East College	2009

Job Experience

Duty	**Institution**	**Duration**
Biologist	Özel Başkent Hastanesi	2015-Present
Intern	Dr.Burhan Nalbantoğlu State Hospital Emergency Laboratory	20.07.2012-03.08.2012
Intern	Dr.Burhan Nalbantoğlu State Hospital Genetics Laboratory	10.08.2012-31.08.2012

Foreign Languages	**Reading Comprehension**	**Speaking**	**Writing**
English	Proficient	Proficient	Proficient
Greek	Basic	Basic	Basic

Computer Knowledge	**Use Proficiency**
Office Programes	Very good
SPSS	Good

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YAKIN DOĞU ÜNİVERSİTESİ
BİLİMSEL ARAŞTIRMALAR ETİK KURULU

ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

Toplantı Tarihi :28.03.2019
Toplantı No : 2019/67
Proje No : 768

Yakın Doğu Üniversitesi Tıp Fakültesi öğretim üyelerinden Prof. Dr.Turgut İmir'in sorumlu araştırmacısı olduğu, YDU/2019/67-768 proje numaralı ve **"Asymptomatic Group A- Beta Hemolytic Streptococci Pharyngeal Carriage İn Northern Cyprus. (Kuzey Kıbrıstaki Asymptomatik Kişilerde Grup A Beta Hemolitik Streptokok Taşıyıcılığı)"** başlıklı proje önerisi kurulumuzca değerlendirilmiş olup, etik olarak uygun bulunmuştur.

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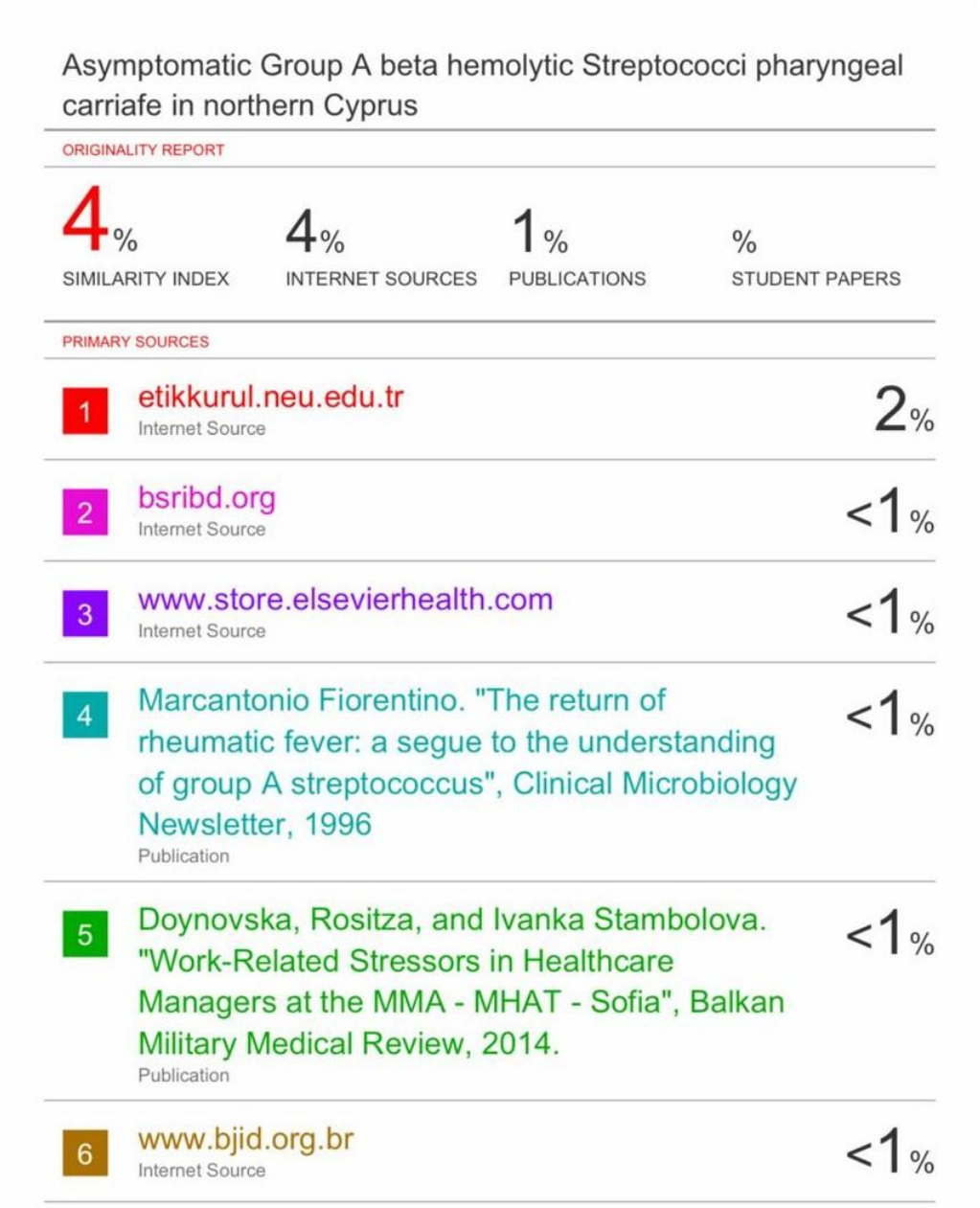

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