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Original Research Article

Bacterial Infections among Students of Department Clinical Laboratories /College of Applied Medical Sciences/University of Karbala

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Abstract: This study conducted at the College of Applied Medical Sciences /University of Kerbala focused on isolating and diagnosing bacteria from urine and tonsil samples collected from 50 students. The participants consisted of 25 females and 25 males, with ages ranging from 18 to 32 years. The samples were obtained within the university premises between December 20th, 2022, and March 25th, 2023. Standard culture media, including MacConkey agar, Blood agar, and chocolate agar, were utilized for direct inoculation of the samples. The cultures were incubated at 37 C for 24-48 hrs. Additionally, Muller Hinton agar was used for the Antimicrobial Susceptibility Test (Kirby-Bauer Disc Technique). Manual biochemical tests, such as Gram stain, Oxidase, Catalase, Urease, Coagulase, Citrate, and Triple Sugar Iron (TSI), were carried out for bacterial identification. The bacteria isolates were identified according biochemical tests into, namely *Escherichia coli (4), Streptococcus pyogenes (4), Staphylococcus aureus (13), Klebsiella pneumoniae (3), Haemophilus influenzae (7), Staphylococcus saprophyticus (4), Acinetobacter baumannii (1), Neisseria gonorrhoeae (1), Streptococcus agalactiae (1), Enterococcus faecalis (1), and Staphylococcus epidermidis (1)*.

Keywords: Tonsilliti, Bacterial Infections, Students, Clinical Laboratories.

INTRODUCTION

Tonsillitis, is an inflammation of the tonsils, usually caused by an infection by viruses or bacteria. Tonsils are lumps of tissue on both sides of the back of the throat that help the immune system protect the body from infections. Inflamed tonsils get red and swollen and can cause a sore throat, and may be covered with a yellow or whitish coating or spots. A urinary tract infection (UTI) is a bacterial infection that can impact different parts of the urinary system, such as the bladder, urethra, or kidneys. There are three main clinical syndromes associated with UTI: lower UTI (frequency dysuria syndrome), upper UTI (acute bacterial pyelonephritis), and asymptomatic bacteriuria. The aim of study: This study aimed to investigate and identify bacterial infections with tonsillitis and urinary tract infections among students at Department of Clinical Laboratories/College of Applied Medical Sciences/University of Kerbala.

History of Tonsillitis

Ancient Times: The earliest references to tonsillitis can be traced back to ancient Egyptian medical texts, dating back to around 1550 BCE. and prescribed various herbal remedies for their treatment. Middle Ages: During the middle Ages, medical knowledge and understanding of tonsillitis were limited .19th Century: In the 19th century, medical understanding of tonsillitis improved. Physicians began to differentiate between acute and chronic tonsillitis. French physician Guillaume Dupuytren made significant contributions by describing the anatomy and function of the tonsils.20th Century: In the early 20th century, the role of bacteria in tonsillitis became better understood. Streptococcus pyogenes, commonly known as group A streptococcus, was identified as a primary cause of tonsillitis.(Lilja *et al*., 1998)

History of Urinary Tract Infections

The existence of urinary tract infections dates back to ancient times, with the first documented description found in the Ebers Papyrus, dating back to around 1550 BC.(Al-Achi, 2008) The Egyptians described it as the bladder emitting heat.(Wilson & Miles, 1975) However, effective treatment for UTIs only became possible with the development and accessibility of antibiotics in the 1930s. Prior to that, remedies such as herbal treatments, bloodletting, and rest were recommended for managing UTIs.(Al-Achi, 2008).

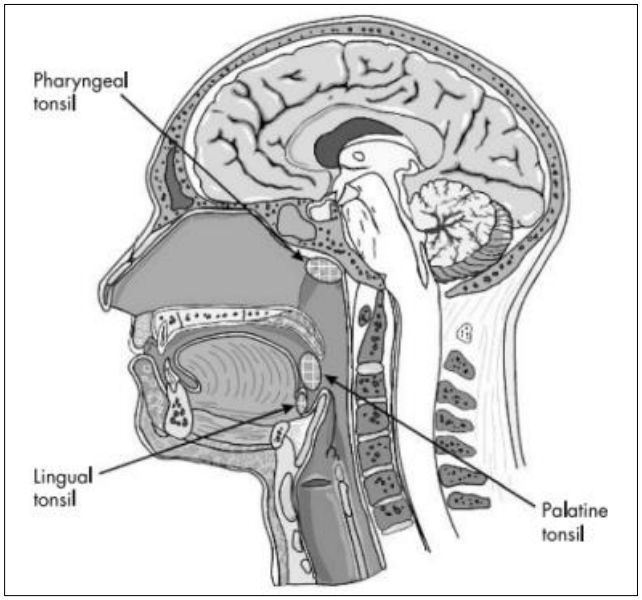


Figure 1: Type of tonsils (Syrjänen, 2004)

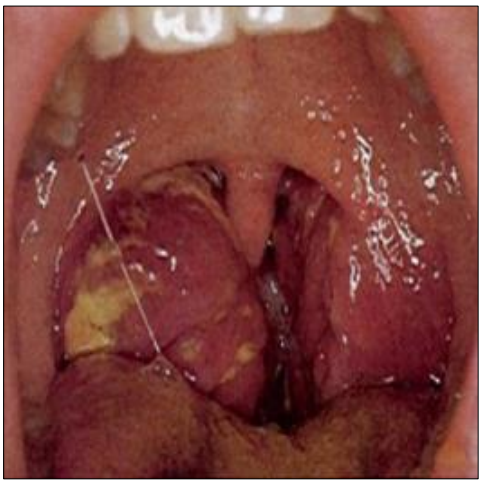


Figure 2: Acute tonsillitis of palatine tonsils (ELAINE C. JONG, MD DENNIS L. STEVENS, PhD, 2012)

Tonsillitis

Tonsil, small mass of lymphatic tissue located in the wall of the pharynx at the rear of the throat of humans and other mammals. In humans, the term is used to designate any of four sets of tonsils (two palatine tonsils, pharyngeal tonsil and lingual tonsils and two tubal tonsils) most commonly the palatine tonsils. (Syrjänen, 2004). The main function of tonsils is fighting infection. Your tonsils contain a lot of white blood cells, which help kill germs. As your tonsils are in the back of your throat, they can “catch” germs that enter your body through your nose or mouth. They help filter out germs that enter through your nose or mouth to protect the rest of your body from infection.(Arambula *et al*., 2021). Tonsillitis is inflammation of the pharyngeal tonsils (termed "adenoid"). The inflammation usually extends to the adenoid and the lingual tonsils.(Alasmari *et al*., 2017)Acute tonsillitis refers to any inflammatory process involving the tonsillar tissues of the oropharynx triggered by one of the several types of bacteria or viruses, and peritonsillar abscesses can also occur, pathogens are bacterial, with *Streptococcus pyogenes* contributing heavily to the incidence of this disease process(ELAINE C. JONG, MD DENNIS L. STEVENS, PhD, 2012) (figure 2). Chronic tonsillitis is a tenacious infection of the tonsils which may result in tonsil stones, observed in most cases of chronic tonsillitis, with alpha- and beta-hemolytic streptococcal species, *H influenza*, *S aureus*, and *Bacteroides* species having been recognized. When a person contracts tonsillitis on multiple

occasions each year, recurrent tonsillitis develops. Tonsils that are repeatedly inflamed are symptoms of both chronic and recurrent tonsillitis, both of which can have a negative impact on a patient's quality of life. Tonsillitis affects children frequently, while it is uncommon to see cases before the age of two. In cases of recurrent pharyngitis, a polymicrobial flora with both aerobic and anaerobic bacteria has been detected in core tonsillar cultures (Alasmari *et al*., 2017).

Surgical Anatomy of the Palatine Tonsil

The palatine tonsil is a lymphoid structure housed within the tonsillar fossa, which is bordered anteriorly and posteriorly by mucosal folds (commonly referred to as the anterior and posterior tonsil pillars) comprising the palatoglossus and palatopharyngeus muscles, respectively. The superior constrictor muscle forms the lateral border of the tonsillar fossa. Deep to this muscle is a layer of loose connective tissue and the buccopharyngeal fascia, which is the final boundary between the tonsil and the parapharyngeal space. The tonsils' medial free edge appears "pitted" due to the branching crypts, which average 10 to 30 per tonsil. Along the medial (luminal) surface of the tonsil, the crypts resemble tubular diverticula and have a fibrovascular core surrounded by lymphoid tissue. The epithelial surface is made up of non-keratinized stratified squamous epithelium. A non-uniform distribution of stratified squamous epithelium and reticulated crypt epithelium lines the crypts themselves..(fig 3) (Arambula *et al*., 2021) (Moller, 2000)

The tonsils are positioned laterally in the pharyngeal wall between the palatoglossal arch and palatopharyngeal arch (the anterior and posterior tonsillar pillars), which merge superiorly to become the soft palate. Figure A shows a sagittal section through the oropharynx. Figure B shows a transverse section through the tonsillar region from medial (palatoglossus muscle) to lateral (palatopharyngeus muscle). (Moller, 2000)

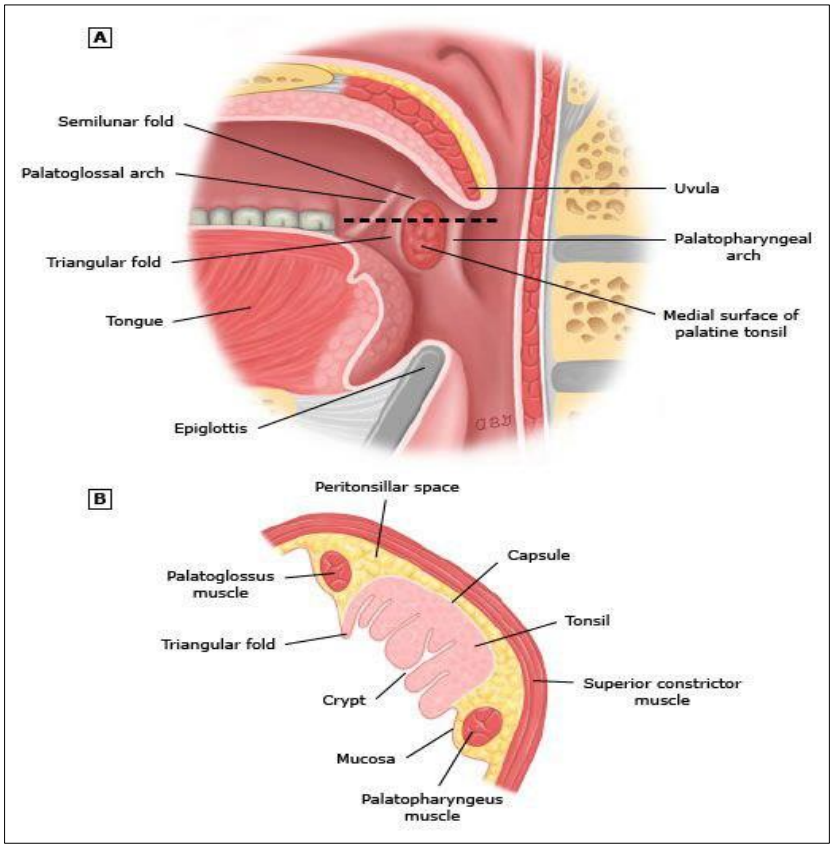


Figure 3: Anatomy of the palatine tonsil

Epidemiology of Tonsillitis

Numerous children so suffer from recurrent tonsillitis and sore throats that these illnesses become part of their life. For instance, according to one research, tonsillectomies are necessary in about 30% of cases of peritonsillar abscesses. Though it is more common in winter and early spring, the disease can occur at any time during the year. GABHS accounts for 5% to 15% of adults with pharyngitis and 15% to 30% of patients between the ages of five and fifteen. Viral etiologies are more common in patients under five. GABHS is rare in children under two years of age (Abu Bakar *et al*., 2018). Because there is a chance that group A beta-hemolytic streptococcus infection can progress to more severe complications like abscess, acute glomerulonephritis, rheumatic fever, and scarlet fever, it is critical to distinguish this bacterium from other bacterial or viral causes of pharyngitis and tonsillitis(Smith *et al*., 2023)

Signs and Symptoms

- Fever
- Sore throat and Foul breath
- Dysphagia (difficulty swallowing)
- Odynophagia (painful swallowing)
- Tender cervical lymph
- nodes (Alasmari *et al*., 2017)

Pathogenesis of Tonsillitis

Microorganisms that enter the oral and/or nasal cavities can be trapped and eliminated by the mucous blanket and the clearance system. However, certain microorganisms, including *S. pyogenes*, have the ability to breach the mucous film and attach to the epithelial lining. The precise molecular mechanisms involved in the interaction between microorganisms and the host at the mucosal membrane level are not yet fully understood. *S. pyogenes* can penetrate the mucous barrier, attach to epithelial cells, spread between cells, and potentially infiltrate the outermost layer of epithelial cells. These events trigger the production of cytokines and/or complement activation, leading to an inflammatory response in the tonsillar tissue.(Beachey & Courtney, 1987)(Gibbons, 1989)

2.4. Urinary Tract Infection (UTI)

UTI is a comprehensive phrase used to describe any infection that affects any segment of the urinary tract, which includes the urethra, bladder, kidneys, and ureters. The urinary tract is categorized into the upper (kidneys and ureters) and lower tract (bladder and urethra).(Orenstein & Wong, 1999)

Cystitis (Lower Urinary Tract Infection)

Cystitis, also known as a lower urinary tract infection, is characterized by a sudden onset of symptoms such as painful urination, frequent urination, and a strong urge to urinate. Some patients may also experience blood in their urine or a change in urine odor. Suprapubic discomfort and tenderness may also be present in some cases. In women, the absence of vaginal discharge, a personal or family history of UTI, recent sexual activity, and use of spermicide-based contraception are indicators of a higher likelihood of cystitis. Patients who have recently taken antibiotics or traveled to developing countries may be at risk of contracting an antimicrobial-resistant UTI. Other conditions that may present similarly to cystitis include urethritis (caused by *Chlamydia trachomatis, Neisseria gonorrhoeae, or herpes simplex virus*), vaginitis (caused by *Candida* or *Trichomonas species*), nephrolithiasis, irritant or atrophic vaginitis or urethritis, prostatism (in elderly men), and diabetes mellitus (especially if the main symptom is urinary frequency). *Escherichia coli* is the most common causative organism of uncomplicated cystitis, accounting for 80% to 90% of cases. *Staphylococcus saprophyticus* is responsible for 5% to 10% of cases, and the remaining cases are caused by non-*E. coli* gram-negative bacteria or, rarely, *enterococci*. Other bacteria such as gram-negative bacilli, gram-positive cocci (including *enterococci, Streptococcus species, Staphylococcus aureus*, and *coagulase-negativestaphylococci*) are also more frequently encountered in complicated cases.(Elaine c. Jong, md dennis l. Stevens, phd, 2012)

Pyelonephritis (Upper Urinary Tract Infection)

Pyelonephritis is a type of urinary tract infection that affects the upper urinary tract, including the kidneys. Symptoms can range from mild flank pain to severe urosepsis that requires intensive care. Typical features include fever, flank or back pain, nausea, and vomiting. Physical findings may include tenderness over the costovertebral angle, abdominal tenderness, tachycardia, and hypotension. The differential diagnosis includes other conditions such as diverticulitis, appendicitis, ectopic pregnancy, pelvic inflammatory disease, endocarditis, and nephrolithiasis. Acute prostatitis is a relatively uncommon condition that causes fever, perineal pain, dysuria, and extreme prostate tenderness, while chronic prostatitis is a more common condition that may be misdiagnosed as cystitis due to mild irritative voiding symptoms. The most common causative organism is *Escherichia coli*, which is also the most common pathogen in uncomplicated cystitis. *Staphylococcus saprophyticus* is less common in pyelonephritis, while non-E. coli gram-negative bacilli are more common. (Elaine c. Jong, md dennis l. Stevens, phd, 2012)

Pathogenesis of UTI

The presence or absence of future UTI is the outcome of a dynamic interaction between the host and the uropathogen. Symptomatic UTIs occur when uropathogens in the bladder or kidney promote the release of cytokines, resulting in an inflammatory response and symptoms. The large difference in UTI prevalence between men and women is thought to be due to a combination of factors, including: the greater distance between the anus (the usual source of uropathogens) and the urethral meatus; the drier environment surrounding the male urethra; the male urethra's greater length; and the antibacterial activity of prostatic fluid.(Hooton, 2000) The uropathogen initially adheres to the epithelial surface before colonizing and disseminating throughout the mucosa, causing tissue destruction. Pathogens can enter the urine bladder after the first colonization period, resulting in symptomatic or asymptomatic bacteriuria. Progression may

result in pyelonephritis and renal dysfunction. Bacterial resistance to the host's ordinarily effective defense mechanisms is caused by specific virulence factors found on the uropathogen's membrane.

Bacterial virulence factors influence whether an organism invades the urinary tract and the severity of infection acquired. *Uropathogenic E. coli* (UPEC) can infect the urinary system by expressing particular virulence factors that allow adhesion and colonization of the lower urinary tract (Schlager *et al*., 2002) (Yamamoto *et al*., 1997)(figure 4) (Mulvey, 2002).The microorganism's adhesion is based on three crucial environmental factors: first, the bacteria's own adhesive properties, second, the receptive features of the urothelium, and finally, the fluid that exists between both surfaces (Schaeffer *et al*., 1981).

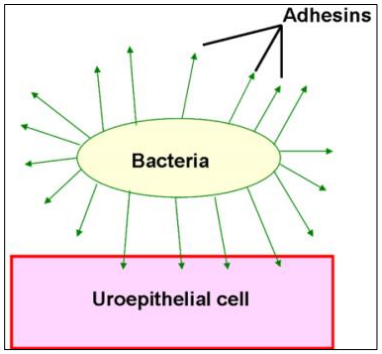


Figure 4: Adhesions on the uropathogen are responsible for attachment of the bacteria to the uroepithelial cell membrane of the host. (Mulvey, 2002)

Host Response to Pathogenic Adherence

After the uropathogen clings to the mucosal surface, the host activates a variety of defense responses. Within hours of the initial infection, epithelial cells exfoliate, and infected urothelial cells are lost (Mysorekar *et al*., 2002). Type 1 piliated bacteriae that trigger cell death mediate secretion and excretion of infected urothelial cells (Mulvey *et al.*, 1998). The epithelium covering the surface of the bladder is dormant in healthy patients because the umbrella cell layer is regenerated every few months. In the murine cystitis model, however, these typically repressed proliferation and differentiation pathways are rapidly triggered after the infective process. Within 24 hours of exfoliation, these proliferation cascades have the capacity to cause efficient regeneration of an umbrella cell layer (Fig. 5). Another study in mice found that exfoliation of urothelial cells reduces *uropathogenic E. coli* cluster formation(Anderson, Martin, *et al*., 2004). Notably, mice who underwent modest exfoliation in response to the uropathogen were more prone to produce biofilms that moved deeper into the skin.

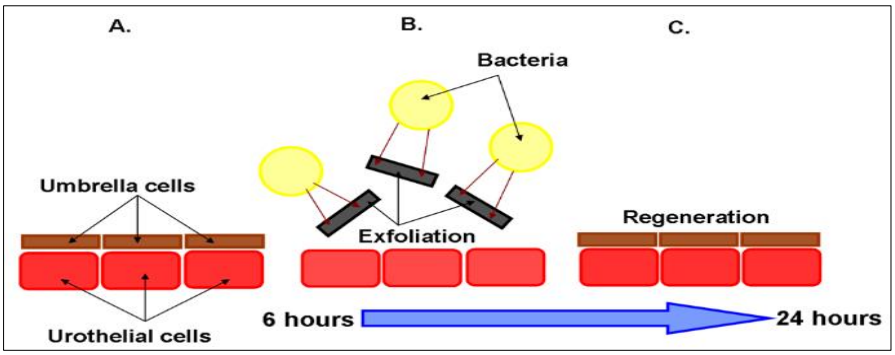


Figure 5: In healthy patients the umbrella cell layer lining the lumen of the urinary bladder is renewed every few months (A). However, epithelial cells exfoliate within 6 hours after infection with an invasive uropathogen (B). This excretory process allows for effective excretion of infected urothelial cells. Within 24 hours of exfoliation proliferation cascades induce effective regeneration of a new umbrella cell layer. (Mysorekar *et al*., 2002)

The innate immune response of the host is principally in charge of giving resistance to the invading uropathogen. As the uropathogen invades, numerous cell types such as neutrophils, macrophages, eosinophils, and natural killer cells get activated. Furthermore, polymorphonuclear leukocytes produce nitric oxide by boosting the transcription of nitric oxide synthase, a process that is harmful to the invading pathogen(Poljakovic & Persson, 2003) (Poljakovic *et al*., 2001). It is vital to note that neutrophils play an important role during the early inflammatory response stage as they travel towards the infected location. Pathogen-associated molecular pattern receptors (PAMPs) and Toll-like receptors (TLRs) mediate the migratory process (Anderson, Martin, *et al*., 2004). TLRs activate signaling pathways that induce immunological and

inflammatory responses to eliminate infections after recognizing lipopolysaccharides (LPS), peptidoglycans (PG), and other bacterial components (Anderson, Dodson, *et al*., 2004). TLR4 and its co-receptors (CD14 and MD2) detect Gram-negative bacterial LPS and trigger an innate immune response (Haraoka *et al*., 1999). TLR11 is also secreted from the kidney and activated to prevent infection from spreading to the renal parenchyma (Zhang *et al.*, 2004). Notably, more recent research has shown that uropathogens can suppress NFB and so reduce the host's inflammatory response. This strategy allows the uropathogen to infiltrate deeper tissues (Klumpp *et al*., 2001). After 7-10 days, the adaptive immune response is triggered, with B and T cells recognizing specific uropathogens with high affinity antibodies.

Escherichia Coli

Escherichia coli, a type of bacteria commonly found in the human gut, was first isolated by the German pediatrician Theodor Escherich (1857-1911) in 1885 from the feces of infants.(Feng *et al*., 2002). *Escherichia coli*, commonly referred to as *E. coli*, is a gram-negative, non-sporulating, rod-shaped, facultative anaerobic, and coliform bacterium belonging to the genus Escherichia. It is commonly found in the environment, as well as in the lower gut of warm-blooded animals, including humans, and can also be found in certain foods. (Campbell *et al*., 2002). Although the majority of *E. coli* strains are harmless, certain serotypes can cause various illnesses when ingested through contaminated food or water. Some of these illnesses include diarrhea, urinary tract infections (UTIs), respiratory infections, anemia, and kidney infections. (Ingerson-Mahar & Reid, 2011). However, certain strains of E. coli have evolved to become pathogenic by acquiring virulence factors through the use of plasmids, transposons, bacteriophages, and/or pathogenicity islands. (Kaper *et al*., 2004).

There Are Several Different Categories of *E. coli* Strains That Cause Disease

- *Enterohemorrhagic E. coli (EHEC)* strains, such as E. coli 0157:H7, cause hemorrhagic colitis and produce toxins similar to those found in Shigella dysenteriae.
- *Enteroinvasive E. coli (EIEC)* strains, on the other hand, invade intestinal epithelial cells and biochemically resemble Shigella.
- *Enteropathogenic E. coli (EPEC)* strains adhere to intestinal mucosa and produce a characteristic lesion in the gastrointestinal tract, but do not produce enterotoxins and are not invasive.
- *Enterotoxigenic E. coli (ETEC*) strains colonize the small intestine without invading and produce either or both heat-labile and/or heat-stable enterotoxins.
- *Enteroaggregative E. coli* adhere to tissue-culture-based assays in a characteristic "stacked brick" pattern, unlike EPEC strains which show localized adherence. (Nataro & Kaper, 1998)

Klebsiella Spp

Klebsiella is one of the most important members of *Klebsiella* genus in *Enterobacteriaceae* family, which is responsible for pneumonia (Puspanadan *et al*., 2012). The main Klebsiella species caused pneumonia disease is *K. pneumoniae* followed but to lesser degree by *K. oxytoca*. *Klebsiella spp* are facultative, anaerobic, non-motile, lactose-fermenting, and Gram-negative rods (0.3-1 μm in width and 0.6-6 μm in length) arranged singly, in pairs or in short chains that possess a prominent polysaccharide capsule (CPS), which gives the colonies their appearance on agar plates. Klebsiella colonies appear large, mucoid, and red with diffusing red pigment on MacConkey agar indicating fermentation of glucose and acid production (Siri *et al*., 2011).

Epidemology

In nature, *Klebsiella spp* are common in the environment, where they can be found in surface water, sewage, soil, and on plants, and the mucosal surfaces of mammals like humans, horses, or swine. *K.peumoniae* is found in the normal flora of the intestines but usually in low numbers compared with E. coli. They may also colonize the mouth and skin(Ullmann, 1998)

K. Pneumoniae

Established in normal mouth, skin, and intestines flora and feces of about 5% of people. It triggers tiny bacterial pneumonias. It may cause substantial hemorrhagic necrotizing lung consolidation. Occasionally, it induces urinary tract infection and focal lesion bacteremia in compromised patients. virulence factors of *K.pneumoniae* ; The pathogens of Klebsiella infections have been searched for some bacterial factors that share these bacteria's pathogenesis which inculde Capsular Polysaccharides, Lipopolysaccharides, Siderophores, Adhesins and Biofilm Formation by *K. pneumoniae*(Jasim, 2020).When these bacteria get into other areas of the body, they can cause infection. These infections could include:

- Urinary tract infections;
- Pneumonia;
- Bloodstream infections (also called sepsis)
- Wound or surgical site infections; and
- meningitis(Pennsylvania Department of Health, 2017)

Streptococcus Pyogenes

Streptococcus pyogenes is a facultative, Gram-positive bacteria that grows in chains. Pyogenic, viridans, lactic, and *enterococci* were the four basic divisions of *streptococci* that were used to classify them before the invention of molecular typing techniques. *Group A streptococcus* (GAS), among other species connected to illnesses in humans and animals, was present in the pyogenic division(Reglinski & Sriskandan, 2015). The pyogenic division included the beta-hemolytic strains with defined group antigens (A, B, C, E, F, and G). This division of the Streptococci is not appreciably different from that of today's identification systems based on sergrouping. The sort of hemolysis that is formed on blood agar plates serves as the basis for preliminary identification of hemolysis. Instead of using human erythrocytes, which typically contain anti-streptococcal antibodies, agar plates containing sheep erythrocytes are employed. Hemolysis, Alpha-hemolysis, and Gamma-hemolysis are the three different forms of hemolytic patterns. Usually, a brown or green colorization surrounds these streptococcal colonies(Bryant & Stevens, 2021).

Cell Wall Associated Components

- **C-carbohydrate:** The serogroup is described by this structure. It is a 2:1 branching polymer of L-rhamnose and N-acetylglucosamine found in *group a streptococci*. The latter is the antigenic determinant connected to the peptidoglycan by bridges made of phosphate.
- **Capsular Polysaccharides:** The antigenic specificity of the capsular polysaccha rides is used to classify S. pneumoniae into 84 types and to type the *group B Streptococci*
- **M protein:** M protein is one of the classical virulence determinants of *S.pyogenes*, appearing as hair-like fibrils at the bacterial surface, sequencing of the gene encoding M-protein is providing a rapid definitive way of comparing M-typeable and M-non-typeable strains(Pancholi *et al*., 2010).

Epidemiology

GAS have a narrow host range, identified almost exclusively in humans and only rarely in other species. GAS is highly communicable and can cause disease in individuals of all ages who do not have type-specific immunity. Diseases related to *S. pyogenes* infections have been indicated ranging from noninvasive symptoms (pharyngitis and impetigo) to severe scarlet fever, necrotizing fasciitis and toxic shock syndrome resulting in high morbidity and mortality(Ferretti *et al*., 2016).

MATERIALS AND METHODS

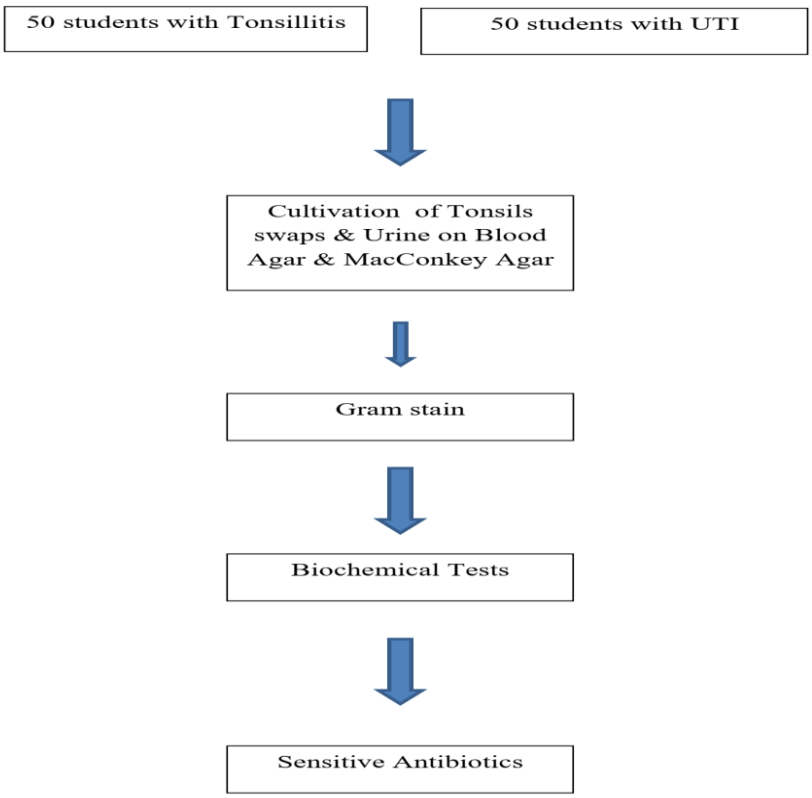


Figure 6: Study Plan

Table 1: Equipment

Equipment	Company	Origin
Autoclave	Labtech	Korea
Incubator	MMM Medcenter	Germany
Refrigerator	arcelik	Turkey
Water Stills	Gesellschaft für Labortechnik	Germany
Vortex	Human	Germany
Heater	Heidolph	Germany
Microscope	Human	Germany
Electronic balance	Kern & Sohn	Germany

Table 2: Materials

Materials	Company	Origin
Blood agar	Hi Media	India
MacConkey agar	Hi Media	India
EMB	Hi Media	India
Urease	Hi Media	India
Simmon citrate agar	OXIOID LTD	England
Kovac's reagent's	Alpha Chemika	India
Muller Hintun agar	Biolab Zrt	Hungary
Hydrogen peroxide	Alpha Chemika	India
Gram stain	Alpha Chemika ……	India

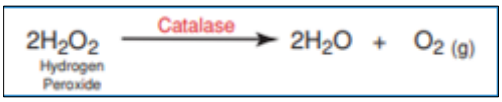
Sample Collection

The current study included of isolation and diagnosis of bacteria from urine and tonsil samples collected randomly from 50 (25 males and 25 females) students of the College of Applied medical Sciences/University of Kerbala during the period from December 20, 2022 to March 25, 2023, The ages of the students ranged from 18 to 32 years old. These patients were separated by residence, age, sex, place of infections. After completion of the questionnaire for each student (see page 41), urine samples (approximately 10 mL) were collected into sterile, disposable containers with instructions for students to swab the area and collect a 'midstream' sample and tonsil samples were collected using a cotton swab. We made sure that there would be no more than 30 minutes between obtaining the sample and implanting it.

Biochemical Tests

Catalase Test

Catalase tests are used to identify the organisms that produce the catalase enzyme. They can be used to differentiate the members of the *Micrococcaceae* from those of the *Streptococcaceae*, and variations in this test can also be used to identify *Mycobacterium species*(Leboffe & Pierce, 2021). Catalase is an enzyme that catalyses the breakdown of H2O2 into water and oxygen (**Error! Reference source not found.**) (Torok *et al*., 2009).



Equation 1: Catalase Mediated Conversion of H_2O_2 [42]

A catalase-positive culture produces oxygen gas bubbles immediately when hydrogen peroxide is added to it (**Error! Reference source not found.**. The organism is catalase-negative if there are no bubbles visible.

Note: Media containing blood may produce a false-positive result. (Torok e*t al*., 2009)

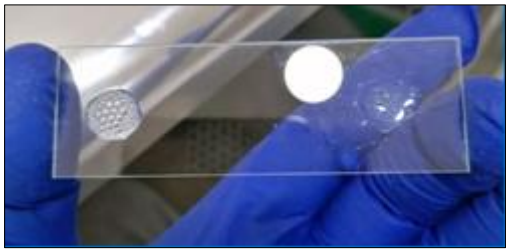


Figure 7: Slide catalase test results. (Left) The positive reaction was produced by Staphylococcus aureus; (Right) the negative reaction was produced by Streptococcus pyogenes

Coagulase Test

Coagulase is an enzyme that converts fibrinogen into fibrin. This test is used to distinguish between staphylococci. Staphylococcus aureus is an opportunistic pathogen that is resistant to both the natural immune response and antimicrobial agents. Its resistance can be related in part to the creation of a coagulase enzyme. Coagulase collaborates with other plasma components to construct protective fibrin barriers around individual bacterial cells or groups of cells, protecting them from phagocytosis and other sorts of attack. (Leboffe & Pierce, 2021)

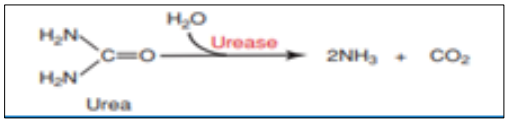
Procedure:

A colony is emulsified in a tube containing plasma and incubated at 37°C for 4h. A visible clot indicates a positive result. If negative at 4h, the tube should be reincubated overnight. (Torok *et al*., 2009)

Urease Test

The Urease Test is used to distinguish organisms based on their capacity to hydrolyze urea with the enzyme urease. Urinary tract infections of the genus *Proteus* can be recognized from other enteric bacteria by their fast urease activity. Urea is an outcome of the decarboxylation in certain amino acids. Bacteria that have the enzyme urease can hydrolyze it to ammonia and carbon dioxide.(Leboffe & Pierce, 2021)

Urea hydrolysis (**Error! Reference source not found.**) to ammonia by urease positive organisms will overcome t he buffer in the medium, turning it from orange to pink. (FIG 8) During the incubation period, the agar must be inspected everyday.



Equation 2: Urea hydrolysis produces ammonia, which raises the pH in the medium and turns the pH indicator pink (Leboffe & Pierce, 2021).

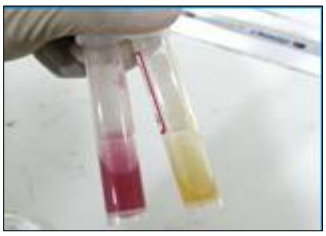
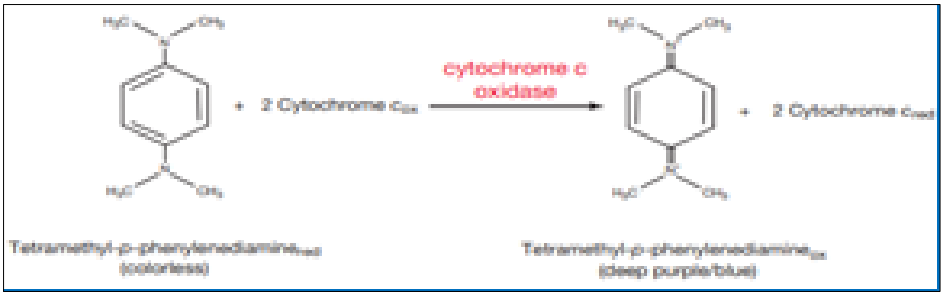


Figure 8: Urease Agar Test Results Urease agar tubes after a 24 hour incubation. Proteus spp. (urease-positive) is on the left. E.coli (urease-negative) is on the right

4.2.4. Oxidase Test

This test reveals whether an organism has the cytochrome oxidase enzyme and is used to differentiate *Pseudomonas, Neisseria, Moraxella, Campylobacter, and Pasteurella spp*. (oxidase-positive).(Torok *et al*., 2009)

It is able to do so because cytochrome c oxidase has the unique capacity to catalyze the reduction of cytochrome c by a chromogenic reducing chemical called tetramethyl-p-phenylenediamine. Chromogenic reducing agents are compounds that change color when they are oxidized (**Error! Reference source not found.**).(Leboffe & Pierce, 2021)



Equation 3: Chemistry of the Oxidase Reaction

Oxidase findings are obtained using the tetramethyl variant of the oxidase reagent (N,N,N,N tetramethyl-1,4-phenylenediamine, 1% aqueous solution).

Procedure:

This solution is placed on filter paper, and a portion of the colonial growth is rubbed onto the reagent with a platinum loop, cotton swab, or wooden applicator stick. Within 10 seconds, a dark purple color will appear with fresh cultures (**Error! Reference source not found.** 9).

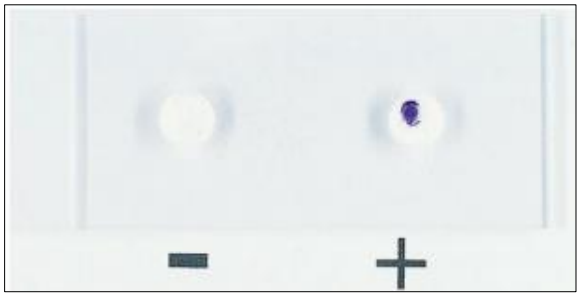
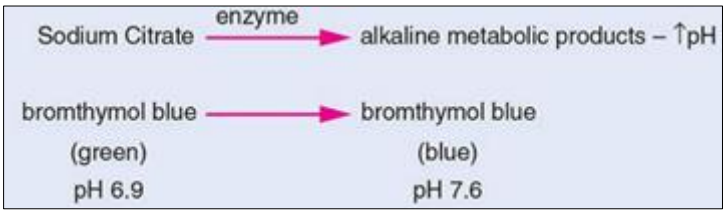


Figure 9: Oxidase Test

This test can easily be performed directly by applying Kovac's reagent (1% tetramethyl-p-phenylenediamine dihydrochloride) onto the colonies or indirectly by rubbing the colonies onto filter paper moistened with the reagent. If colonies rubbed onto filter paper moistened with the reagent remain colorless, the organism is oxidase negative (left). However, if the colonies turn dark blue or purple within 10 to 30 s, the test is positive (right).

Citrate Utilization Test

The Citrate Utilization Test is used to determine the ability of an organism to use citrate as its sole source of carb.Some microbes utilize sodium citrate as the sole carbon source. As a result, the medium employed to test citrate consumption cannot contain proteins or carbs as carbon sources. The medium contains sodium citrate as the sole carbon source and ammonium phosphate as the sole nitrogen source. Bacteria that employ citrate will excrete ammonia after consuming the nitrogen from the ammonium phosphate, resulting in the alkalinization of the medium. The indicator is bromthymol blue. This feature aids in the identification of numerous *Enterobacterales* members.(De la Maza *et al*., 2013). Citrate Simmons Agar is a defined medium that consists of sodium citrate as the sole carbon source and ammonium phosphate as the sole nitrogen source. As an indicator, bromthymol blue dye is used, which is green at pH 6.9 and blue at pH 7.6 (**Error! Reference source not found.**). Bacteria that grow in the medium and use the citrate convert the ammonium p hosphate to ammonia (NH3) and ammonium hydroxide (NH4OH), both of which alkalinize the agar. As the pH rises, the medium turns from green to blue (**Error! Reference source not found.** 10).(Leboffe & Pierce, 2021)



Equation 4: Citrate Test Principle (Leboffe & Pierce, 2021).

Figure 10: Citrate Test Resultss immons citrate agar inoculated Citrobacter diversus (-ve) on the left, Bacillus cereus (+ve) on the right

Procedure

A well-isolated colony is picked from the surface of a primarily isolation medium and inoculated as a single streak on the slant surface of the citrate agar tube. The tube is incubated at 35°C for 24–48 hours.

Triple Sugar Iron (TSI) Agar

Triple sugar iron (TSI) agar is a tubed differential media used to determine carbohydrate fermentation and H2S production. Gas produced by carbohydrate metabolism can also be detected. Bacteria can metabolize carbohydrates either aerobically (with oxygen) or fementatively (without oxygen).(Lehman, 2005) Is largely used to distinguish members of the *Enterobacteriaceae* from other Gram-negative rods such as *Pseudomonas*. TSIA is a nutrient-dense media developed to separate bacteria based on glucose fermentation, lactose fermentation, sucrose fermentation, and sulfur reduction. The pH indication is phenol red, while the hydrogen sulfide indicator is iron in ferrous sulfate(Leboffe & Pierce, 2021).

The oxidase enzyme shown is not involved directly in the indicator reaction as shown. Rather, it removes electrons from cytochrome c, making it available to react with the phenylenediamine reagent. (Leboffe & Pierce, 2021).

The reaction of two iron salts, ferrous sulfate and ferric ammonium citrate, with the H2 S results in the formation of a black ferrous sulfide precipitate. In the TSI tube, half of the length of the agar is at a slant and thus aerobic due to oxygen exposure, while the butt is protected from air and thus anaerobic (**Error! Reference source not found.**11). P roduction of the gases CO2 and H2 is also detected by observing cracks or bubble. If only glucose is fermented, the bottom (butt) portion of the slant will be yellow due to acid production during anaerobic glucose fermentation; however, the top (slant) portion will be alkaline (pink) due to oxidative degradation of the peptones during aerobic conditions(alkaline/acid [Alk/A]).(De la Maza *et al*., 2013)

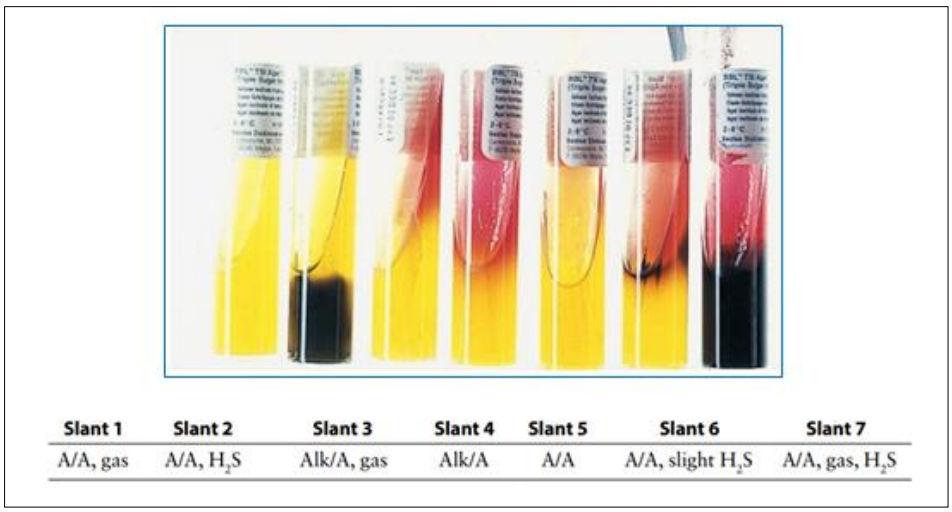


Figure 11: TSI Agar Slants [(De la Maza *et al*., 2013)

Antimicrobial Susceptibility Test

A systematic procedure called antimicrobial susceptibility testing is used to assess how well antibiotics and other chemotherapeutic drugs work against pathogenic germs. It is frequently a crucial tool for prescribing the right treatments(Leboffe & Pierce, 2021). Antibiotic sensitivity is a term used to indicate how amenable bacteria are to antibiotics. An antibiotic's ability to treat a bacterial infection in vivo can be determined using an antimicrobial susceptibility test (AST). Determining the etiological agent and its pertinent antibiotic sensitivity will result in the ideal antibiotic treatment(White, 2014). Kirby-Bauer Disc Technique: The agar diffusion method and the disk diffusion method are other names for this procedure. All aspects of the Kirby-Bauer procedure are standardized to ensure reliable results. Therefore, care must be taken to adhere to these standards. Mueller-Hinton agar, which has a pH between 7.2 and 7.4, is poured to a depth of 4 mm in either 150 mm or 100 mm Petri dishes.(Leboffe & Pierce, 2021).

Kirby-Bauer disc technique procedure:

1. Prepare a pure culture of the bacteria using McFarland standard (0.5) and antibiotic discs.(fig 11)
2. Spread the bacterial suspension on a Mueller-Hinton agar plate.
3. Place antibiotic discs on the agar surface, ensuring spacing.
4. Incubate the plate for 18-24 hours.
5. Measure the zone of inhibition around each disc.

6. Determine susceptibility (clear zone) or resistance (no zone).
7. Record and report the results for each antibiotic.

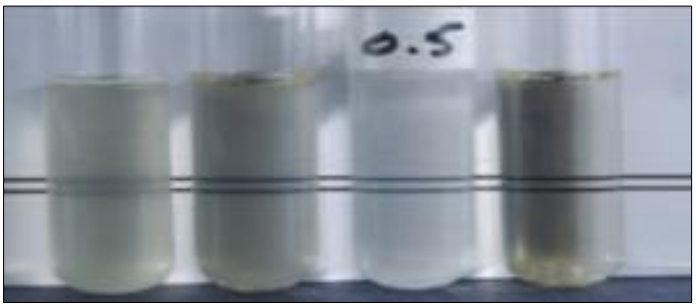


Figure 12: 0.5 McFarland turbidity standard (Leboffe & Pierce, 2021).

Preparation McFarland standard (0.5):

The original McFarland standards were created through the combination of predetermined quantities of barium chloride and sulfuric acid. When these two compounds are mixed, they react to form a precipitate of barium sulfate, resulting in turbidity within the solution.(fig 12) To prepare a 0.5 McFarland standard, 0.05 mL of 1.175% barium chloride dehydrate ($BaCl_2 \cdot 2H_2O$) is mixed with 9.95 mL of 1% sulfuric acid (H_2SO_4).(Wikler, 2006)

RESULTS AND DISCUSSION

The clinical symptoms of students from whom samples were obtained differed according to gender and age. The symptoms of students who had a positive transplant result were as follows, (as in the table 3):

- **Dysuria:** Usually happens due to bladder muscle contraction and peristalsis of the urethra, which ends up causing the urine to come in contact with the inflamedmucosal lining, which in turn stimulates pain receptors and causes one to feel pain and/orburning. (Bremnor & Sadovsky, 2002)
- **Pelvic Pain:** The bacteria responsible for a urinary tract infection (UTI) have the ability to infiltrate the inner lining of your urinary tract. Consequently, this invasion can trigger inflammation and irritation of the pelvic and cause pain. (Lane & Takhar, 2011)
- **Hypertension:** The most common cause of upper airway obstruction is often attributed to the enlargement of the tonsils. When there is a persistent and severe obstruction, it can result in the development of obstructive sleep apnea syndrome (OSA). OSA is characterized by disrupted sleep patterns, excessive snoring, and behavioral abnormalities. Additionally, OSA can also contribute to the development of hypertension. (Görür *et al*., 2001)
- **Fever:** Pyelonephritis refers to a genuine infection that affects the kidneys, the organs responsible for urine production. This condition can give rise to symptoms such as fever. (Orenstein & Wong, 1999)
- **Shortness of Breath:** Infectious mononucleosis has the potential to cause significant enlargement of the tonsils and swelling of the pharynx, which can result in upper airway obstruction. This obstruction may lead to symptoms such as shortness of breath or breathlessness.(Philpott-Howard, 1988)
- **Swollen Tonsils:** A bacterial infection affecting the tonsils can result in swollen tonsils 6(12%).

According to the questionnaire with the questions directed to the students obtained from them the symptoms of students suffering from urinary tract infection were burning urination 16 (26%), pelvic pain 5 (10%), dysuria 6(12%), some of them had a high temperature 11(22%) and headache10(20%). Those who were suffering from burning urination that the reason behind this is that bacteria cause inflammation in the lining of the bladder and urethra, as well as inflammation of the prostate in men.

Table 3: Demographic Distribution of Students

		No. (%)
Age	18-22 years	31(62%)
	23-32 years	19(38%)
Sex	male	25(50%)
	female	25(50%)
Clinical symptoms	headache	10(20%)
	Dizziness	4(8%)
	Swollen tonsils	6(12%)
	Fever	11(22%)
	Chills	9(18%)
	dysuria	6(12%)
	shortness of breath	11(22%)

	burning urination	13(26%)
	cough	9(18%)
	Pelvic pain	5(10%)
	Asthma	2(4%)
	Hypertension	1(2%)
Tonsillitis		23(46%)
UTI		19(38%)

Women experience UTIs significantly more frequently than males do, for a variety of reasons. Microbes can more easily ascend to the bladder in women because the urethra is shorter in them than in men and straight rather than curved as in men. Antibacterial characteristics of prostate secretions further safeguard the man.

Tonsillitis in male 8 (32%), in female 15(60%), while healthy student in male 17(68%) and female10(40%). Urinary tract infection in male 6(24%), in female 13(52%), while healthy student in male 19(76%) and female 12(48%). Total ratio of tonsillitis for both 23(46%). Total ratio of urinary tract infection is 19(38%). (Table 4, fig 15) Total distribution of positive cultures (UTI and Tonsillitis) according to Gender shows infections among males 14(28%), female 28(56%).(Table 4, fig 14)

While in other study in Karbala shows the percentage of infections among males (32.66%), (67.33%) females, and the maximum UTI infections in patients in age groups (1-60) were (54%).(Ali Al-Saadi, 2010) And other study about tonsillitis in Sudan shows the percentage of infections among Males accounted for 52 (67.5%) while females accounted for 25 (32.4%) of the patients. The majority 22 (28.6%) of patients were between the age of 11-20 years. (Alrayah, 2023)

Table 4: Distribution of bacterial infection among students

		Tonsillitis	**Healthy**	**Uti**	**Healthy**	**Total.I**
Sex	Male	8(32%)	17(68%)	6(24%)	19(76%)	14(28%)
	Female	15(60%)	10(40%)	13(52%)	12(48%)	28(56%)
Total		23(46%)	27(54%)	19(38%)	31(62%)	42(42%)

Manual biochemical tests for bacterial isolates were used to identify the species to bacterial species. Gram-Positive and gram-negative were utilized for bacterial identification according gram stain and Oxidase,Catalase, Urease, citrate and TSI. Table (6). Gram-Positive bacteria that causes Tonsillitis ((*S.pyogenes 4* (9.5%), *S.aureus (9)*21.4%, *E.faecalis (1)*2.3%)) and UTI ((*S.saprophyticus(4)*9.5%, *S.aureus (4) 9.5%, S.agalactiae (1)* 2.3%, *S.epidermidis (1)* 2.3%, *candida albicans* (2) 4.7%)), total: 26(62%). (Table 5, fig 16). While gram-negative bacteria that causes Tonsillitis ((*K.pneumoniae (2)* 4.7%, *H.influenzae (7*)16.6 %)) and UTI ((*E.coli (4) 9.5%, N.gonorrhoeae (1)* 2.3%, *A.baumanni (1)* 2.3%, *K.pneumoniae*(1) 2.3%)). Total :16(32%). (Table 5, fig 16). In other study the distribution of the different isolates are shown: *E.coli* 48 (39.02%), Proteus mirabilis 20 (16.26%), P.euroginosa 10(8.13%) *Enterobacter* 8 (6.50%), *Klebsiella* 1(0.81%), *Staphylococcus aureus* 31(25.20%) ,*Streptococcus* 5(4.06%).(Ali Al-Saadi, 2010)

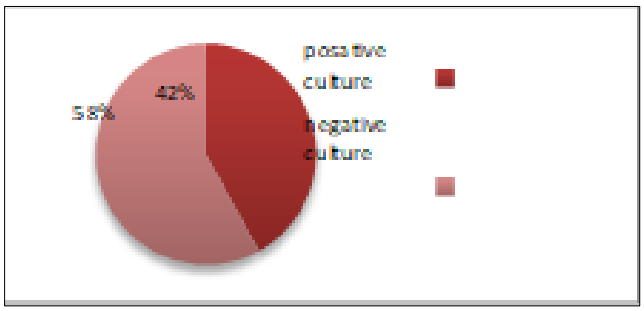


Figure 13: Distribution of bacterial growth among cultured samples

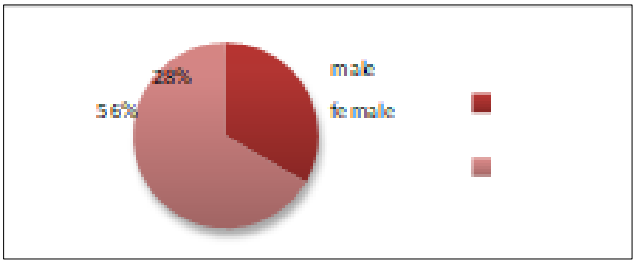


Figure 14: Distribution of positive cultures according to Gender

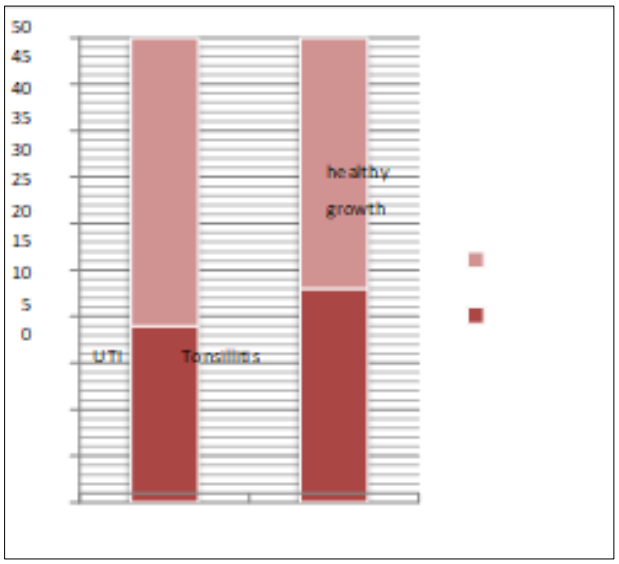


Figure 15: Distribution of bacterial isolates according to Type of infection and total sample

Table 5: Bacterial isolates

	Gram Positive (No.)%	Gram Negative (No.)%	Total
Tonsillitis	*S.pyogenes (4)*9.5% *S.aureus (9)*21.4% *E.faecalis (1)*2.3%	*K.pneumoniae (2)* 4.7% *H.influenzae (7)*16.6 %	*(23)*54.7%
UTI	*S.saprophyticus (4)*9.5% *S.aureus (4) 9.5%* *S.agalactiae (1)* 2.3% *S.epidermidis (1)* 2.3% *candida albicans* (2)4.7%	*E.coli (4) 9.5%* *N.gonorrhoeae (1)* 2.3% *A.baumanni (1)* 2.3% *K.pneumoniae* (1)2.3%	*(19)*45.2%

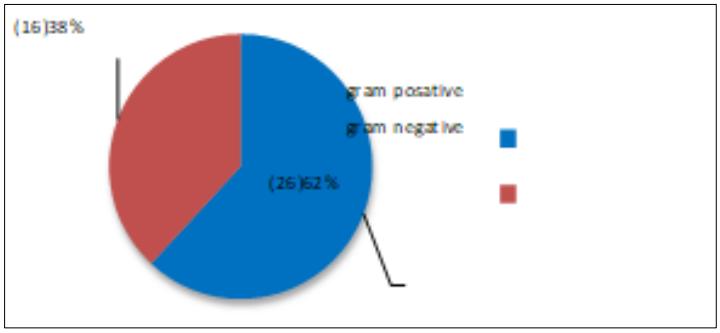


Figure 16: Number and percentage of bacterial isolates according to gram stain investigation

Table 6: Biochemical Tests of Bacterial Isolates

Bacterial Isolates	No.	Gram	Catalase	Oxidase	Urease	Citrate	Tsi
E.coli	4	-	+	-	-	-	A/A G
S.pyogenes	4	+	-	-	-	+	A/A G
S.aureus	13	+	+	-	+	+	A/A
K.pneumoniae	3	-	+	-	+	+	A/A G
H.influenzae	7	-	+	+	+	-	Alk/A
S.saprophyticus	4	+	+	-	+	+	A/A ,G,H2S
A.baumanni	1	-	+	-	-	+	Alk/A
N.gonorrhoeae	1	-	+	+	-	-	Alk/A
S.agalactiae	1	+	-	-	-	-	A/A
E.faecalis	1	+	-	+	-	-	A/A
S.epidermidis	1	+	+	-	+	-	A/A ,G,H2S+

TSI: Triple sugar iron, A: Acid, Alk: Alkaline, G: Gas, +: positive result, - : negative result

The interpretation of the results of carbohydrate fermentation tests can provide valuable information about the metabolic capabilities of microorganisms. Here are some common interpretations of the results: Table (6)

- Alkaline/acid (red slant/yellow butt) reaction: This indicates that the microorganism can ferment glucose, but not lactose or sucrose. For example, Neisseria gonorrhoeae exhibits this pattern of fermentation.
- Acid/acid (yellow slant/yellow butt) reaction: This indicates that the microorganism can ferment glucose, lactose, and/or sucrose. For example, Staphylococcus aureus exhibits this pattern of fermentation.
- Alkaline/alkaline (red slant, red butt) reaction: This indicates that the microorganism is unable to ferment any of the carbohydrates present in the medium.
- Blackening of the medium: This occurs in the presence of H2S produced by the microorganism. For example, Staphylococcus saprophyticus can produce H2S, which leads to blackening of the medium.
- Gas production: The formation of bubbles or cracks in the agar indicates the production of gas (CO2 and H2) by the microorganism. For example,
- Staphylococcus epidermidis can produce gas during fermentation.

Results and Interpretation of AST

The concentration drops as a function of the square of the distance of diffusion when the chemical diffuses from the filter paper into the agar. The antibiotic is diluted to the point where it no longer prevents bacteria growth at a specific distance from each disk. The existence of growth-inhibition zones indicates the efficacy of a certain antibiotic. These zones of inhibition (ZOIs) are seen as distinct regions The size of this zone is dependent on a number of factors, including the sensitivity of the microbe to the antibiotic, the rate of diffusion of the antibiotic through the agar, and the depth of the agar (Philpott-Howard & Williams, 1982).

Result, some drugs kill the organism and are said to be bactericidal. Other drugs are bacteriostatic; they stop growth but don't kill the microbe.

If susceptible to the antibiotic, the test organism will be unable to grow in the area immediately surrounding the disk, displaying a zone of inhibition (see figure below 17). Microorganisms that are resistant to an antibiotic will not show a zone of inhibition (growing right up to the disk itself) or display a relatively small zone (Leboffe & Pierce, 2021).

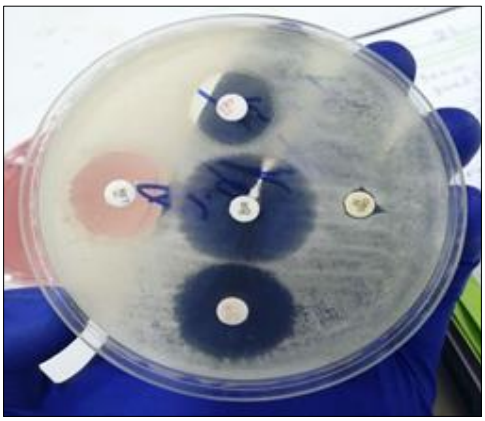


Figure 17: AST Result

Antimicrobial Susceptibility of Bacterial Isolates to (10) Drugs Was Studied

Bacterial isolates were susceptible for antimicrobial agents as (Table7):

S.aureuas (11,7,6,8,2) isolates were susceptibility for (CIP,NET, NOR, CLR, ERY) respectively, *E.coli* (3, 2,) isolates were susceptibility for (CIP, SXT, NOR,) respectively, *H.influenzae* (5, 4, 5) isolates were susceptibility for (CIP, SXT, LVX) respectively, isolates were susceptibility for *K.pneumoniae* (2) isolates were susceptibility for (CIP), *S.pyogenes* (4,1, 1, 3,3) isolates were susceptibility for (CIP,NET,LVX, CLR, ERY) respectively, *S.saprophyticus* (2, 1) isolates were susceptibility for (CIP,CLR) respectively, *A.baumanni* (1) isolates were susceptibility for (SXT), *N.gonorrhoeae* (1,1 ,1) isolates were susceptibility for (CIP,CRO,ERY) respectively, *S.agalactiae* (1,1) isolates were susceptibility for (CIP ,CRO) respectively, *E.faecalis* (1,1,1,1) isolates were susceptibility for (CIP , LVX, NOR,ERY) respectively, *S.epidermidis* (1 ,1 , 1) isolates were susceptibility for (NOR ,CLR , ERY) respectively.

Bacterial isolates were resistance for antimicrobial agents as (Table 7):

S.aureuas (3,2,2,5) isolates were resistance for (CIP, NET, CLR, PRY) respectively, *E.coli* (1 ,2 ,4 , 1, 1) isolates were resistance for (CIP, SXT, CRO, CLR, CAZ) respectively, *S.pyogenes* (1,3,1) isolates were resistance for (NET, CRO,ERY) respectively, *K.pneumoniae* (1,2,3,3, 2) isolates were resistance for (CIP, SXT,CRO,CLR, CAZ) respectively, *H.influenzae* (2, 3,2) isolates were resistance for (CIP, ERY, CAZ) respectively, *S.saprophyticus* (1, 3,3,2,2) isolates were resistance for (CIP , NOR,TEC, CLR,ERY respectively, Our study was indicated the isolates showed multi-drug resistance.

Table 7: In Vitro Antibiotic Sensitivity and Resistance pattern of isolated organisms

Bacteria (No.) Antibiotic		E.Coli (4)	S.Pyogenes (4)	S.Aureus (13)	K.Pneumoniae (3)	H.Influenzae (7)	S.Saprophyticus (4)	A.Baumanni (1)	N.Gonorrhoeae (1)	S.Agalactiae (1)	E.Faecalis (1)	S.Epidermidis (1)
CIP	S	3	4	11	2	5	2	0	1	1	1	0
	R	1	0	2	1	2	1	1	0	0	0	1
NET	S		1	7								
	R		1	2				1				
SXT	S	2				4		1				
	R	2			2							
LVX	S		1			5					1	
	R							1				
CRO	S	0							1	1		
	R	4	3		3			1				
NOR	S	2		6							1	1
	R						3					
TEC	S											
	R						3					1
CLR	S		3	8			1					1
	R	1		2	3		2			1		
ERY	S		3	2					1		1	1
	R		1	5		3	2					
CAZ	S											
	R	1			2	2				1		

CIP: Ciprofloxacin, NET: Netilmicin, CRO: Ceftriaxone, SXT: sulfamethoxazole, ERY: Erythromycin, FOX: Cefoxitin, LVX: Levofloxacin, CAZ: Ceftazidime, NOR: Norfloxacine, CLR: clarithromycin TEC: Teicoplanin, R: resistant, S: sensitive

In other studies conducted in the province of Karbala for patients with urinary tract infections as follows: Antimicrobial susceptibility of bacterial isolates to (14) drugs was studied .Fifteen isolates of *S.aureus* were susceptible to (AK), nine isolates of *E.coli* were susceptible to (AK) and (Cf) whereas (30),(26), (21) isolates of *E.coli* were resist to (C,CFX,CL,CN,CT,E) respectively. Bacterial isolates were resistance for antimicrobial agents : *S.aureuas* (16,16,18 ,19) isolates were resistance for (C, Cfx, E, P) respectively, *E.coli* (21,30,21,26, 27) isolates were resistance for (AMP,C,CEF,CFX,CL,CN,CT,E,P,T) (Ali Al-Saadi, 2010). And in another Antimicrobial Susceptibility of Tonsillar Diseases. The results of antimicrobial susceptibility of *S. aureus* isolates showed that 169 (91.48%) isolates were susceptible to all the selected antibiotics whereas 20 (10.87%) isolates were resistant to fusidic acid and only 1 (0.5%) isolate was resistant to both methicillin and fusidic acid. The antibiotic cotrimoxazole showed the highest rate of resistance against majority of the bacterial isolates including *Group A beta haemolytic streptococci* (GABHS) with 11 (2.37%) resistant and 3 (0.64%) susceptible; *Streptococcus pneumoniae* with 3 (0.64%) resistant; *Haemophilus influenzae* with 27 (5.81%) resistant and 59 (12.71%) susceptible, 30 (6.46%) isolates of *Klebsiella pneumoniae* resistant to ampicillin, and 1 (0.21%) isolate of each of *Acinetobacter baumannii*, and *Enterobacter* cloacae resistant to ampicillin (Alasil *et al*., 2013). The reason why bacteria become resistant to antibiotics is due to the types mechanisms of resistance:

a. Changes in the target site: The receptors that connect to antibiotics may undergo alterations in their structure, particularly in the regions where the antibiotics bind. These changes can occur in various enzymes and ribosomes. Notably, resistance associated with modifications in ribosomal targets is commonly observed with macrolide antibiotics. (Ayliffe, 1997), (Oppenheim, 1997)
b. Enzymatic inactivation of antibiotics: Many Gram-positive and Gram-negative bacteria produce enzymes that degrade antibiotics, leading to their inactivation. This enzymatic inactivation is a significant mechanism of resistance.(Yüce, 2001) (Bryant & Stevens, 2021)(Bassetti *et al*., 2013)

c. Reduction of membrane permeability: Resistance can occur through changes in the permeability of the inner and outer membranes of bacterial cells. This can result in a decreased uptake of drugs into the cell or rapid expulsion through active pump systems. (Yüce, 2001)(Bryant & Stevens, 2021)(Bassetti *et al*., 2013)(Nikaido, 1994)
d. Active efflux pump systems: Resistance can develop through active pump systems that actively expel antibiotics from the cell. This mechanism is commonly observed in the tetracycline group of antibiotics. Tetracyclines are actively pumped out of the cell, preventing their accumulation. Active pump systems, controlled by plasmids or chromosomal genes (Yüce, 2001),(Bryant & Stevens, 2021),(Nikaido, 1994)
e. Alternative metabolic pathways: Some bacteria can develop resistance by utilizing alternative metabolic pathways that bypass the need for the drug's target.(Yüce, 2001),(Jawetz *et al*., 1998)

The use of broad-spectrum antibiotics to treat mild infections, such as uncomplicated urinary tract infections, may lead to resistance in the treatment of more serious diseases. As a result, some specialists recommend saving broad-spectrum antibiotics for more serious diseases and instead using narrow-spectrum antibiotics(Charpentier & Courvalin, 1999).

CONCLUSION

In This Study

1. Urine and tonsil samples from 50(25 male and 25 female) students at the College of Applied medical Sciences/University of Kerbala were analyzed.
2. Multiple bacterial species were identified, including *E.coli* (4), *S.pyogenes* (4), *S.aureus* (12), *K.pneumoniae* (3), *H.influenzae* (7), *S.saprophyticus (4), A.baumannii (1), N.gonorrhoeae (1), S.agalactiae (1), E.faecalis (1), and S.epidermidis (1).*
3. The study provides insights into the prevalence and distribution of these bacterial species among university students, contributing to our understanding of urinary and tonsil infections in the region.

Recommendation

1. Check lab safety equipment at the start of the session.
2. Inspect personal protective equipment as you hand it out.
3. Ensure emergency switches are operable.
4. Provide the chemical safety data sheets (SDS) for each chemical used in that session.
5. Check vent positions and function.
6. Remove clutter from work and storage areas.
7. Eliminate any food or beverages from the area.
8. Update any relevant records.

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