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Biological Characteristics and Inhibition by Both Natural Agents and Antibiotics of *Streptococcus Pyogenes*

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Abstract: In this review, the characteristics, classification, pathogenesis and diagnosis of *Streptococcus* pyogenes (*S. pyogenes*) infections are discussed. In addition, a list of antibiotics and natural agents necessary to inhibit *S. pyogenes* are listed herein. *S. pyogenes* is a group A beta-hemolytic *Streptococcus*. It is a facultative anaerobe, non-motile, non-sporing, spherical or ovoidal cocci. Many virulence factors are produced by *S. pyogenes* and are responsible for its infections such as streptolysin O, streptolysin S, *streptococcal* pyogenic exotoxin A and exotoxin C, streptokinase, hyaluronidase and C5a peptidase. Pathogenic causes of *S. pyogenes* are pharyngitis, impetigo, sepsis, erysipelas, Cellulities, puerperal fever, scarlet fever, rheumatic fever, glomerulonephritis and toxic shock syndrome. Serology, culturing and microscopic examination of *S. pyogenes* are the most important ways of diagnosis. The recent ways to inhibit *S. pyogenes* by natural agents (honey, essential oils, plant extracts) are also discussed.

Key words: *Streptococcus* pyogenes, classification, characterization, virulence, natural agents, diagnosi

INTRODUCTION

S. pyogenes is a member of Genus: Streptococcus within family Streptococcaceae; species of *Streptococcus* are classified based on their hemolytic properties (Brooks *et al*., 2004). It is a Gram-ve cocci and an blood agar colonies are usually compact, small and surrounded by a 2-3 mm zone of beta hemolysis; it form capsule which is composed of hyaluronic acid that covers pilli and mediated adherence of the organism to mucosal surface and M proteins of its cell surface are unique to each strain (Bae *et al*., 2007). Classification and characterization of strain belonging to *S. pyogenes* were described by Lancefield scheme using serological properties (Mora *et al.,* 2005). Recently 16 S rRNA cataloging analysis is used for *S. pyogenes* identification (Steer *et al.,* 2009).

Several virulence factors of *S. pyogenes* including streptolysin O, streptolysin S, streptococcal pyogenic exotoxin A and exotoxin C, streptokinase, Hyaluronidase, Streptodornase and C5a peptides are the main reasons of pyogenic infections (Kenneth and Lawrence, 2010). There are 517,000 deaths globally each year due to severe pyogenic infections. About 20% of them are a school-aged children (Bassen, 2009). Culturing of clinical samples, serology, microscopic examination are the effective ways of diagnosis of *S. pyogenes* infections (Chessbrugh, 2006). Traditional use of honey is treatment of *S. pyogenes* infections is discussed herein as well as the active components of honey. Essential oils and natural plant extracts are recently used for treatment of *S. pyogenes* infections (Enan *et al*., 2015a). In addition many recently published papers described the inhibition of *S. pyogenes in vitro* (Enan and Amri, 2006; Abdel *et al*., 2014; Enan *et al*., 2015b). The inhibitory activity was due to lactic acid and bacteriocins produced by probiotic cultures (Enan *et al*., 2012; Enan *et al*., 2013; Enan *et al*., 2014).

Nomenclature systematic position and classification of *S. pyogenes*: The name derives from the Greek word"streptos," meaning "twisted chain "due to the fact that the bacterium resembles astring of small pearls when viewed under the microscope (Commons *et al*., 2008). It belongs to kingdom: bacteria, Phylum: Firmicutes, Class: bacilli, Order: Lactobacillales, Family: Streptococcaceae, Genus: Streptococcus (Brooks *et al*., 2004).

Species of *streptococcus* are classified based on their hemolytic properties. Alpha hemolytic species cause oxidization of iron in hemoglobin molecules within red blood cells, giving it a greenish color on blood agar. Beta hemolytic species cause complete rupture of red blood cells. On blood agar this appears as wide clear zones of blood cells surrounding bacterial colonies (Kenneth and

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Lawence, 2010). Gamma-hemolytic species cause no hemolysis. The important members of this group are Streptococcus faecalis (Enterococcus) and Streptococcus faecum (Enterococcus) (Ruoff and Bisno, 2009).

Beta-hemolytic Streptococci are further characterized via Lancefield serotyping which describes specific carbohydrates present on the bacterial cell wall. There are 20 described serotypes, named Lancefield groups A to V (excluding I and J) (Facklam, 2002).

Genotyping classifications of Streptococci have been divided into six Groups on the basis of their 16S rDNA sequences: anginosus, bovis, Mitis, mutans, pyogenes and Salivarius. The 16 S groups have been confirmed by whole genome sequencing of Streptococcus pyogenes that belong to the pyogenes groups (Kawamura *et al*., 1995).

In 1928, Rebecca Lancefield published a method for serotyping of S.pyogenes that is based on its M protein. Lancefield serotyping describes specific carbohydrates present on the bacterial cell wall. There are 20 described serotypes, named Lancefield groups A to V (excluding I to J) (Bisno *et al.,* 2003).

Latter in 1946, Lancefield described the serologic classification of S.pyogenes isolates based on their surface variable trypsin-resistant antigen (T antigen). Four of the 20 T antigens have been revealed to be pili which are used by bacteria to attach to the host cells. Over 100 M serotypes and approximately 20 T serotypes are known (Mora *et al.,* 2005).

The traditional Lancefield classification system which is based on serotyping, has been replaced by emm typing which has been used to characterize and measure the genetic diversity among isolates of S.pyogenes. Determination of the emm type is mandatory for epidemiological investigations of Group A Streptococci (GAS) infections (Steer *et al*., 2009).

Characteristics of *S. pyogenes*

Morphology of *S. pyogenes*:

S. pyogenes is Group A Beta-hemolytic Streptococcus. It is a facultative anaerobe, Gram-positive extracellular bacterium (Cunningham, 2008). It is non-motile, non-sporing, spherical or ovoidal cocci that are less than 2 ìm in length and that form chains and large colonies greater than 0.5 mm in size (Kenneth and Lawrence, 2010).

Cultural characters of *S. pyogenes*: On blood agar plates colonies are usually compact, small and surrounded by a 2-3 mm zone of beta hemolysis (complete lysis of red cells surrounding the colony (that is easily seen and sharply demarcated. This type of reaction has long been used to classify the Streptococci (Ferretti *et al*., 2001). Crystal violet blood agar is useful selective media for isolation of *S. pyogenes* as crystal violet will inhibit the growth of S. aureus (Chessbrugh, 2006). In microscopic as 0.5-1.0 ìm spherical or ovoid cells in chains of short to medium length (Bassen, 2009).

Cell structure: The cell wall of group A Streptococci is built upon a peptidoglycan matrix that provides rigidity. Within this matrix lies the group-specific antigen which is composed of rhamnose and N-acetyl glucosamine. The cell surface is responsible for its virulence and anti-phagocytic factors. The outermost layer of the cell is the capsule which is composed of hyaluronic acid that covers pilli and mediated adherence of the organism to mucosal surface. Protein F is exposed on the streptococcal surface and it binds fibronectin and another cell surface protein which binds the Fc portion of antibodies. The latter aids molecular mimicry because it could leads to covering of antibody molecules on the streptococcal surface. This would provides some sort of "invisibility" to the host immune system and interference with the complement activation at the bacterial surface in tandem with M protein (Ferretti *et al*., 2001). The M protein is an important antigen and it is associated with virulent streptococci. This protein is found in fimbriae and binds fibrinogen from serum and blocks the binding of complement to the underlying peptidoglycan. This process inhibits phagocytosis. Some M protein types are rheumatogenic or. M proteins are unique to each strain and identification can be used clinically to confir m the strain causing an infection (Bae *et al*., 2007).

Pathogenesis and virulence factors of *S. pyogenes*: S.pyogenes has several virulence factors that enable it to attach to host tissues, envade the immune response and spread by penetrating host tissue layers. The capsule protects the bacterium from phagocytosis by neutrophils. In addition, the capsule and several factors embedded in the cell wall, including M protein, lipoteichoic acid and protein F facilitate attachment to various host cells. M proteins also inhibits opsonization by the alternative complement pathway by binding to host complement regulators .The M proteins found on some serotypes is able to prevent opsonization by binding to fibrinogen. However, the M protein is also the weakest point in this pathogen's defense, as antibodies produced by the immune system against M protein target which help bacteria for engulfment by phagocytes (Bisno *et al*., 2003).

The virulence factors of *S. pyogenes* are as follows: Streptolysin O which is an exotoxin that is one of the

bases of the organism's beta-hemolytic property. Group A *streptococcus* spp. encode a pore-forming protein streptolysin O that functions as a conduct to inject the toxin *S. pyogenes* (Bricker *et al*., 2002).

Streptolysin S which is a cardiotoxic exotoxin that is another beta-hemolytic component. Streptolysin S is not immunogenic. A potent cell poison affecting many types of cell including neutrophils, platelets and sub-cellular organelles (Brooks *et al*., 2004).

Streptococcal pyogenic exotoxin A and exotoxin C which are super antigens secreted by many strains of *S. pyogenes*. This pyrogenic exotoxin is responsible for the rash of scarlet fever and many of the symptoms of streptococcal toxic shock syndrome (Fraser and Proft, 2008).

Streptokinase which is enzymatically activates plasminogen, a proteolytic enzyme into plasmin which in turn digests fibrin and other proteins (Kenneth and Lawrence, 2010).

Hyaluronidase facilitates the spread of the bacteria through tissues by breaking down hyaluronic acid Streptodornase: four different forms of it are secreted by *S. pyogenes*. It also called DNase (Buchanan *et al*., 2006).

MATERIALS AND METHODS

The C5a peptidase cleaves a potent neutrophil chemotaxin called C5a which is produced by the complement system. C5a peptidase is necessary to minimize the influx of neutrophils early in infection as the bacteria are attempting to colonize the host's tissue (Kenneth and Lawrence, 2010).

Genetic regulation of pathogenesis: Spyogenes infects many different tissues including the skin, throat, muscle and blood. To cause these infections, Spyogenes produces various virulence factors and regulated the expression of their genes (Trevino *et al*., 2010).

Regulation of virulence gene expression in Group A Streptococci (GAS) has been studied extensively at the level of transcription and in all cases, "growth phase regulation (overrids all other types) control. Even when all repressors are absent and all activator are present, genes are expressed only in the appropriate phase of growth. It was found that mRNA decay plays a major role in growth phase regulation (Bugrysheva and Scott, 2010).

There are two classes of mRNA class I and II. Class I includes the operon which encodes the enzymes for synthesis of the hyaluronic acid capsule. Class II includes transcripts of genes which encode known or suggested virulence factors for GAS (sagA, encoding streptolysin S, sda encoding a DNAse and arc, encoding arginine deiminase which plays a role in pH homeostasis) (Barnes *et al*., 2007)

Diseases caused by *S. pyogenes*

Pyogenic local infections

Pharyngitis: Acute streptococcal pharyngitis is the most characterized by fever, enlarged tonsils, tonsillar exudate, sensitive cervical lymph nodes and malaise. If the patient is not treated, the organism is present for 1-4 weeks after symptoms have disappeared. Spread occurred is by direct contact with the mucosa or secretions (Kenneth and Lawrence, 2010).

Impetigo: Localized skin infection that is characterized by multiplication and lateral spread of S.pyogenes in deep layers of the skin. Infection takes place through minor trauma (e.g., insect bites). It usually affects children in the 2-5 year old range. Frist it forms a small vesicle enlarges, becomes pustular and late breaks to form a yellow crust (Bisno and Stevens, 2010).

Systemic infection

Bacteremia/sepsis: Infection of traumatic or surgical wounds with Streptococci results in bacteremia which rapidly can be fatal. *S. pyogenes* bacteremia can also follow skin infections such as Cellulities and rarely pharyngitis (Brooks *et al*., 2004).

Invasive diseases

Erysipelas: This type of infection is superficial cutaneous Cellulities that affects the skin and subcutaneous tissues and usually occurs on the face. It is characterized by a spreading area of erythema and edema with rapidly advancing, well demarcated edges, pain and systemic manifestations, including fever, chills, malaise and lymphadenopathy (Celestin *et al*., 2007).

Cellulities: The most common invasive disease account for 20-40% of GAS (Lamagni *et al*., 2008) which is characterized by redness and inflammation of skin with associated pain and swelling. It has acute onset and usually accompanied by generalized symptoms such as fever (Bernard, 2008).

Puerperal infection: Infection of the endometrium at or near delivary is a life threating of GAS. It is now relatively rare but in the 19th century the clinical findings of child bed fever (Kenneth and Lawrence, 2010).

Other infections: Septicemia, otitis media, mastitis, sepsis, myositis, osteomyelitis, septic arthritis, meningitis, endocarditis, pericarditis and neonatal infections are all less common infections due to *S. pyogenes* (Aziz *et al*., 2010).

Toxigenic diseases

Scarlet fever: Infections due to certain strains of S.pyogenes can be associated with the release of bacterial

toxins. Throat infections associated with release of certain toxins lead to scarlet fever (pink-red rash and fever). The buccal mucosa, temples and cheeks are deep red, except for a very characteristic pale area around the mouth and nose. The hard and soft plates are affected by punctuate hemorrhages and the tongue gets covered in a yellow-white secretion. On the second day of illness, a diffuse red rash appears, spreading from the upper chest to the trunk and extremities (Kenneth and Lawrence, 2010).

Streptococcal toxic Shock Syndrome (STSS): Streptococcal toxic shock syndrome may begin at the site of any GAS infection even at the site of seemingly minor trauma. The systemic illness starts with vague myalgia, chills and severe pain at the infected site, most commonly in the skin and soft tissues and leads to necrotizing myonecrosis. It continues with nausea, vomiting and diarrhoa followed by hypotension, shock and organ failure. The laboratory findings are lymphocytosis. Impaired renal function (azotemia) and in over half the cases bacteremia. Some patients are in irreversible shock by the time they reach a medical facility. Many survivors have been left as multiple amputees as result of metastatic spread of streptococci (Kenneth and Lawrence, 2010).

Necrotizing fasciitis: Streptococcus pyogenes invasion and multiplication in the fascia can lead to necrotizing fasciitis, often given the popular name "flesh eating bacteria "this disease rarely occurs in children. Infection may arise after a surgical procedure, intra muscular injections, intra venous infusions and minor insect bites (Mora *et al*., 2005).

Compartment syndrome is often a present and predisposing condition as in Fournier's gangrene. This severe infection leads to necrosis of subcutaneous tissue and the fascia surrounding it. Some symptoms include fever with sunburn rash, shock and light headache, difficulty in breathing and cellulites (Olsen *et al*., 2009). Necrotizing fasciitis is a life-threatening condition requiring surgical removal. Rabid and progressive infection of subcutaneous tissue, massive systematic inflammation, hemorrhagic bullae, crepitus and tissue destruction) are some of the more serious complication involving S.pyogene infections (Cole *et al*., 2011).

e- Post streptococcal diseases:

Rheumatic fever: *S. pyogenes* can also cause a disease in the form of post infectious"non pyogenic" (not associated with local bacterial multiplication and pus formation) syndromes. These autoimmune-mediated complications follow a small percentage of infections and include rheumatic fever (joint inflammation, carditis and neurological complications). A condition secondary to an acute streptococcal infection appear several weeks following the initial streptococcal infection. Rheumatic fever is characterized by inflammation of the joints and/or heart following an episode of streptococcal pharyngitis. Rheumatic fever is acute and progressive and damages the heart, especially its valves (Kenneth and Lawrence, 2010).

Glomerulonephritis: It is manifested in the form of post streptococcal disease. Acute post infectious glomerulo nephritis which is characterized by inflammation, hematuria, fever, edema, hypertension, urinary sediment abnormalities and severe kidney pain (Brooks *et al*., 2004).

Epidemiology of *S. pyogenes*: Different clinical manifestations of this bacterium are more common in different parts of the world. *S. pyogenes* is responsible for a wide range of both invasive and non-invasive infections. It causes diseases ranging from mild superficial infections such as impetigo to life-threatening systemic diseases including toxic shock and necrotizing fasciitis (Kenneth and Lawrence, 2010).

Infections typically begin in the throat or skin. There are at least 517,000 deaths globally each year due to severe S.pyogenes infections. Necrotizing fasciitis kills about 30% of patients and Streptococcal Toxic Shock Syndrome (STSS) has mortality rate of 30-70% (Starr and Engleberg, 2006). This disease still remain a major public health concern both in developed and developing countries (Commons *et al*., 2008).There is no commercial vaccine to prevent GAS infection (Henningham *et al*., 2012).

Worldwide, *S. pyogenes* causes over 18 million cases of severe diseases resulting in over a half million annual deaths. There are 616 million cases of pharyngitis caused by S.pyogenes world-wide each year (Carapetis *et al*., 2005). Streptococcal pharyngitis is predominant in temperate areas and peaks in late winter and early spring. About 15-20% of school-aged children has *S. pyogenes* in its carrier form in their throats and are more at risk of having the disease. Impetigo is more common with children in warm humid climates. Crowding and poor hygiene increase the chance of an outbreak of GAS infection (Bessen, 2009).

Epidemiologic studies showed that the resurgence of severe invasive GAS infection was not limited to sporadic cases; rather, it represented a global spread, ushering in a new pandemic, similar to that reported in the earlier part of the 20th century. An important feature of this latest pandemic is its association with a distinct epidemiologic shift in GAS serotypes (Aziz and Kotb, 2008).

There are 115.6 million cases of rheumatic heart disease yearly and at least 18.1 million cases of invasive infections, predominantly in older populations. Post-streptococcal glomerulonephritis is seasonal and is

more common in children (Cunningham, 2008). Annually, there are 400,000 deaths and hundreds of thousands of children died due to rheumatic fever and rheumatic heart diseases (Arafa *et al*., 2008). One of the most serious manifestations of cute rheumatic fever is carditis which is evident in 30-45% of individuals with rheumatic fever (Hafez *et al*., 2013).

Laboratory diagnosis of infections caused by *S. pyogenes*:

Accurate diagnosis followed by appropriate therapy of *S. pyogenes* infections prevents the disease, its transmission and its sequelae (Capoor *et al*., 2006).

Specimens: It differs depending on disease manifestations, pharyngeal secretion, blood, cerebrospinal fluid, joint aspirate, leading edge aspirate of cellulities, skin biopsy specimen, epiglottic secretions, broncho-alveolar lavage fluid, thoraco-centesis fluid and abscess fluid may be sources for locating the organism. In cases of suspected necrotizing fasciitis , a frozen section biopsy obtained in the operation room may be great value in confirming the diagnosis and may aid in defining haw much surgical debridement of devitalized tissue is necessary (Schroeder, 2003)

In surgical site infection: samples are obtained using sterile swabs from deep seated wounds. Endotracheal aspirate are collected from patients having chest infections, infection in intensive care unit by a sterile catheter. A portion of catheter containing significant amount of the aspirate is aseptically cut in a sterile test tube. Catheterized patients: sterile containers are used to collect urine samples aseptically. Non catheterized patients: they are asked to avoid and mid-stream catch urine is collected in a sterile container. Blood samples about 5 ml blood from septicemic patients under aseptic technique in a sterile syringe and used for inoculating blood culture bottle (Chessbrough, 2006).

In case of ear, nose and throat samples: ear-by a sterile swab is introduced carefully to avoid touching the external auditory canal (avoid touching the drum). Nasal samples: by sterile swab is introduced by rotating action to collect nasal secretions. Throat: by a sterile swab is used to touch the inflamed tonsils or pharyngeal wall. The swabs are introduced gently while using tongue depressor in a good lightning and care is taken to avoid touching the palate or buccal mucosa (Kotloff and Beneden, 2008).

Pus from abscesses: A sterile swab is used to collect pus from the floor and the wall of abscesses after their evacuation. Samples of Cerebrospinal Fluid (CSF) are aspirated by lumbar puncture using spinal needles under complete aseptic technique. CSF is collected in a sterile syringe or sterile test tube. Conjunctival samples: Sterile swab in a parallel position to the eye and rolled it in the lower fornix. Sputum samples: sterile containers are used to collect sputum samples and skin swab is used to collect exudate from skin infection sites (Chessbrough, 2006).

RESULTS AND DISCUSSION

Specimen transport

Swabs: Immediate inoculation of specimens onto blood agar plates is optimal. If the time between specimen collection and plating is greater than a few hours a transport medium (e.g., Stuart's medium or the silica-gel) is recommended. It is important to note that high temperature and high humidity can adversely affect viability of GAS if plating is delayed (Kotloff and Beneden, 2008).

Fluids and tissue sample: Aspiration into a syringe is the standard method of sampling body fluid collected from a normally sterile site and abscess fluid. Biopsy material may also be obtained for the diagnosis and treatment of necrotizing fasciitis. These fluid and tissue specimens should be immediately transported to the laboratory in a sterile container. Tissue specimens must be kept moist by adding a few drops of sterile, non-bacteriostatic saline to the container. Fluid or pus may also be sent on swabs and transported to the microbiology lab (Kotloff and Beneden, 2008).

Microscopic examination: Gram stain of specimen shows Gram-positive cocci in chains. It is non-motile, non-sporing spherical or ovoidal cocci that are<2 im in length and that form chains and large colonies greater than 0.5 mm in size. In broth medium, it forms long chains of cocci (Kenneth and Lawrence, 2010).Because *S. pyogenes* do not normally colonize the skin surface, the finding of Gram-positive cocci in pairs and chains in association with leukocytes is important (Murray *et al*., 2009). Fluorescence microscopy by using a combination of two fluorochromes that stains live bacteria green and dead bacteria red (Braga *et al*., 2003).

Culture media: S.pyogenes grow readily on 5% sheep blood agar, chocolate agar, columbia agar with colistin and nalidixic acid, phenylethyl alcohol agar, crystal violet blood agar but they can be isolated more easily using selective media that inhibit the growth of normal pharyngeal flora e.g., 5% sheep blood agar containing trimethoprim-sulfamethoxazole (1.25 mcg/ml^{-1} TMP and 23.75 mcg\ml^{-1}) and enrichment broth e.g., serum blood or blood broth (Kotloff and Beneden, 2008). To accurate haemolysis colonies should be inoculated on 5% agar

sheep blood agar by stabbing the inoculatory loop into the agar several times. As visualization of beta-hemolysis is enhanced by anaerobic conditions, plates should be colourless shiny with mucoid appearance and produce complete heamolysis (Chessbrough, 2006).

Throat culture: because pharyngitis and tonsillitis may result from various infections etiologies other than Spyogenes infection, the diagnosis should be confirmed. Throat culture remains the criterion standard diagnostic test for streptococcal pharyngitis. If performed correctly, culture of a single throat swab on a blood agar plate yields a sensitivity of 90-95% for the detection of *S. pyogenes* in the pharynx. Although some throat culture results are false-positive (e.g., they do not reflect acute infection but rather symptomatic carriage), all patients with positive culture results are treated with antibiotics (Shulman *et al*., 2012).

Blood culture: For infections associated with Bacteremia or septicemia e.g., bacterial endocarditis and puerperal sepsis. It is more valuable in diagnosis than the uterine swab which is often contaminated with normal flora (Chessbrough, 2006).

Criteria used for *S. pyogenes* identification

Catalase test: This test is used to differentiate those bacteria that produce catalase such as staphylococci, from non-catalase producing bacteria such as streptococcci. Total 2-3 ml hydrogen peroxide 3% is poured into a test tube and using a sterile wooden stick or glass rode to remove several colonies of the tested organism and immersed in hydrogen peroxide. If active bubbling immediately appear so it is catalase positive test; if no bubbles appear so it is catalase negative test. Care should be taken to avoid touching the blood agar medium to avoid false positive reaction. Positive catalase control (Staphylococcus species) and negative catalase control S.pneumoniae ATCC4619 should be use (Chessbrugh, 2006).

Pyrrolidonyl test (PYR): This detects pyrrolidonyl peptidase enzyme activity. *S. pyogenes* is PYR positive. This test can be rabidly and simply performed using PYR impreginated strips (Chessbrugh, 2006).

Bacitracin disc sensitivity: Bacitracin disc is applied to sheep blood agar plate streaked with the tested organism. After an overnight incubation at 35°C in CO_2 incubator, zones of inhibition>14 mm is indicative of sensitivity of the tested strain and the organism is identified as *Streptococcus* pyogenes (Kenneth and Lawrence, 2010).

Rapid antigen detection test: When the diagnosis of streptococcal pharyngitis seems particularly likely based on examination findings or when social factors necessitate an immediate decision about antibiotic therapy, the use of rapid antigen detection tests capable within minutes of identifying *S. pyogenes* directly from the throat swab is a reasonable option in most practice settings. Most kit use antibodies for the detection of group A carbohydrate antigen. Tests can be completed in a matter of minutes (Chessbrugh, 2006).

The currently available rapid streptococcal tests have a sensitivity of 70-90% compared with standard throat cultures. In contrast to their relatively low sensitivity, the specificity of these rapid tests has consistently been 90-100%.Therefore, if a rabid streptococcal test result is positive, a culture is not necessary and appropriate antibiotic therapy can be immediately initiated. However, when a negative rapid test result is encountered a standard throat culture should always be obtained (Casey and Pichichero, 2005).

One of these is pastorex strep test which is a rapid agglutination test for grouping of streptococci according to Lancefield classification. The test involves use of latex suspensions specific for group A. Pastorex strep uses a simple enzymatic extraction. The antigen contained in the extract is identified using latex particles coated with group specific homologous antibodies. In the presence of the homologous antigen, the latex particles agglutinate. In the absence of antigen, they remain in homogenous suspension (Chessbrugh, 2006).

Anti-Streptolysin O antibody tests (ASO): Elevated streptococcal antibody titers in the setting of hypo complementemic nephritis are essentially diagnostic of post streptococcal glomerulonephritis. With rare exceptions, neither post treatment throat cultures in a symptomatic patients nor routine cultures in asymptomatic family contacts are necessary.

Rapid simple to perform latex agglutination and other carrier particle tests are widely available to screen for and measure semi quantitative raised levels of ASO antibody in serum. Most tests have a detection limit of 200 IU/ml^{-1}. It is important in investigation of post streptococcal disease especially rheumatic fever (Chessbrugh, 2006).

Deoxyribnuclease B antibody (DNase B): The rise in DNase B antibody usually occurs later than the rise in ASO antibody. Measurement of DNase B antibody is of value when investigating acute glomerulonephritis following streptococcal skin infection rather than streptococcal sore throat. This is because is not usually raised following ASO antibody titer streptococcal skin infection whereas arise in titer of anti-DNase B (Chessbrugh, 2006).

Other laboratory investigations in streptococcal infections: Other additional laboratory tests [e.g., Complete Blood Count (CBC), White Blood Cell (WBC) count, erythrocyte sedimentation rate and C-reactive protein] may also be useful, depending on the manifestation of disease under consideration.

Other tests, depending on disease syndrome can be very diverse in nature. For example, a histopathologic analysis of skin biopsy specimens which may need to be analyzed intra operatively is warranted in cases of suspected necrotizing fasciitis. Calculation of creatinine clearance may be valuable in assessing the extent of renal dysfunction for nephritis (Teglund *et al*., 2005).

C-Reactive Protein (CRP): Creactive proteins is a substance produced by the liver in response to inflammation. A reading of<1 mg/L^{-1} indicates a low risk of cardiovascular disease. A reading between 1 and 2.9 ml/L^{-1} denotes intermediate risk. A readin> 3ml/L^{-1} means you are at high risk for cardiovascular disease. A reading above 10ml/$^{-1}$ may indicate a need for further testing to determine the cause of severe inflammation in your body.

The Erythrocyte Sedimentation Rate (ESR): The Erythrocyte Sedimentation Rate (ESR), is a measure of the settling of red blood cells in a tube of blood during one hour, the rate is an incubation of inflammation and increases in many disease. Normal values for the westergren method are:

- Women under 50:under20mm/hr^{-1} women over50: under 30mm hr^{-1}
- Men under 50: under 15 mm hr^{-1}, men over 50:under20 mm hr^{-1}
- Newborns: under 2 mm hr^{-1}, children before puberty: 3-13 mm hr^{-1} (Saadeh *et al*., 1998)

Complete Blood Count (CBC), differential and platelet count: Elevated leukocyte count suggests deep-seated or systemic infection. Decreased platelet counts suggest Bacteremia, the toxic shock syndrome or gas gangrene (Eron, 2009). An elevation in the total WBC count (WBC>11.000/ml^{-1}) is called leukocyte which is most commonly identifies infection, tissue inflammation or tissue necrosis associated with disorders such as acute myocardial infarction, burns, gangrene, leukemia, radiation (Oloughlin *et al*., 2007).

Natural products used in treatment of *S. pyogenes*

Honey: Honey is a sweet and viscous fluid produced by honeybees (and some other species) and derived from the nectar of flowers. It has a similar composition of granulated sugar (50% fructose and 44% glucose) and approximately the same relative sweetness, 97% of the sweetness is sucrose (Riddle, 2001). Honey is a natural product that has been widely used for its therapeutic effects. It has been reported to contain about 200 substances. Honey is composed primarily of fructose and glucose but also contains fructo-oligosaccharides and many amino acids, vitamins, minerals and enzymes (Chow, 2002).

Honey has had a valued place in traditional medicine for centuries (Zumla and Lulat, 1989). However, it has a limited use in modern medicine due to lack of scientific support (Ali *et al*., 1991). For a long time, it has been observed that honey can be used to overcome liver, cardiovascular and gastrointestinal problems (El-Arab *et al*., 2006). Ancient Egyptians, Assyrians, Chinese, Greeks and Romans employed honey for wounds and diseases of the intestine (Al-Jabri, 2005).

Naturally darker honey has greater antioxidant properties. acetic, butanoic, formic, citric, succinic, lactic, malic, pyroglutamic, gluconic acids and a number of aromatic acids are found in Honey. Bee's honey is free of Cholesterol (Anupama *et al*., 2003).

Honey has been found to bestow a number of health and nutrition benefits. It may be used alone or in combination with other substances and is administered both orally and topically. Some of which have been mentioned below:

Cut and wounds: apply bee's honey on cuts and wounds. Eyes: honey is very good for eyes and eyesight. Appetite: honey is good for improving appetite, especially in children. Burns: apply fresh bee's honey directly. Cardiac Tonic: honey contains flavonoids; antioxidants which help reduce the risk of heart disease. Blood sugar regulation: even though honey contains simple sugars, it is NOT the same as white sugar or artificial sweeteners. Its exact combination of fructose and glucose actually helps the body regulate blood sugar levels. Some honeys have a low hypoglycemic index, so they don't jolt your blood sugar. Arthritis: place a spoonful of honey with two tablespoons of warm water, one teaspoon cinnamon, Make the dough and rub the affected area, the pain will disappear within minutes. Drinking a glass of warm water with two tablespoons of honey and one teaspoon cinnamon, morning and evening to ease the pain of arthritis and cure. Bladder infections and Dysuria: mix two tablespoons of cinnamon, one teaspoon of honey in a cup of warm water and drink it. This drink eliminates the bacteria in the bladder. Eyesight: 10 ml^{-1} of honey mixed with 10 ml^{-1} of carrot juice and consumed regularly will improve eyesight. Cold and cough: mix 10 ml of honey

with equal quantity of ginger juice and consume twice a day. Obesity: keep garlic immersed in bee's honey for one year. Then, use 1 clove of garlic daily before breakfast. This is used as a home remedy. One glass of warm water taken with 10 ml^{-1} of honey and 5 ml^{-1} of lemon juice in early morning reduces fat and purifies blood. Skin Disorders: applying honey and cinnamon powder in equal parts on the affected parts of skin helps to cure eczema, ringworm and many other types of skin infections. Bad Breath: in the morning, gargle with one teaspoon of honey and cinnamon powder mixed in hot water, so breath stays fresh throughout the day. It maintains dental hygiene and cleanliness of the smell of the mouth. It also acts as a good carrier for other medicines. Honey increases the beneficial qualities of the medicines it carries. It is for this reason that honey is used as a base for a lot of medicines. Honey has a laxative effect on the digestion system of an individual and helps provide relief from constipation. Cholesterol: two tablespoons of honey, cinnamon and 3 tablespoons water in 16 oz tea, can reduce cholesterol by 10% in only 2 h. If they dealt with treatment 3 times daily for a week may heal completely. Eating a tablespoon of honey taken before food cholesterol protects against infection. The Immune System: the regular use of honey per day with cinnamon strengthens the immune system and protects the body from bacteria and viruses. Honey contains many vitamins and iron. The daily use of honey strengthens the white blood corpuscles to fight bacteria and viral diseases.

Honey has been used since ancient times for the treatment of some diseases and for the healing of wounds, however, its antibacterial activity was first reported by scientists in 1892. Recently, numerous studies have been published on the antimicrobial activities of honey showing its biological activities (Packer *et al*., 2012; Maddocks *et al*., 2012). It is used as antimicrobial agent against antibiotic-resistant bacteria (Kwakman and Zaat, 2010; Jenkins *et al*., 2011). Antibiotic susceptible and resistant isolates of Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecium, Escherichia coli, Streptococcus pyogenes, Pseudomonas aeruginosa, Enterobacter cloacae and Klebsiella oxytoca were killed within 24 h by 10%-40% (v/v) honey (Kwakman *et al*., 2008). Honey has been used to treat adult and neonatal post-operative infection (Ergul and Ergul, 2009; Mat Lazim *et al*., 2013). Burns, necrotizing fasciitis (Tahmaz *et al*., 2006). Infected and non-healing wounds and ulcers (Schumacher, 2004). Pilonidal sinus (Thomas *et al*., 2011), venous ulcers and diabetic foot ulcers (Jull *et al*., 2013). When ingested, honey also promotes healing and shows antibacterial action by decreasing prostaglandin levels, elevating nitric oxide levels and exerting probiotic effects (Kamaratos *et al*., 2014).

Under the current situation there is an urgent need to discover alternative antimicrobial agents against the antibiotic and antimycotic resistant pathogens. Therefore, the present study was conducted to investigate the in-vitro antimicrobial effects of diluted (10%) and pure Egyptian cotton flower honey against highly pathogenic bacterial and mycotic isolates of animal origin which have high public health hazards and compare its activity with reference antibiotic and antimycotic drugs(Moez *et al*., 2010).

Essential oils: Essential oils, also called volatile odoriferous oil, are aromatic oily liquids extracted from different parts of plants, for example, leaves, peels, barks, flowers, buds, seeds and so on. They can be extracted from plant materials by several methods, steam distillation, expression and so on. Among all methods, for example, steam distillation method has been widely used, especially for commercial scale production (Cassel and Vargas, 2006; Di Leo Lira *et al*., 2009).

Essential oils have been widely used as food flavors. Essential oils found in many different plants, especially the aromatic plants vary in odor and flavor which are governed by the types and amount of constituents present in oils. Additionally, the amount of essential oil from different plants is different and this determines the price of essential oil (Burt, 2004).

Several compounds in essential oils have the structure mimicking the well-known plant phenols with antioxidant activity. Among the major compounds available in the oil, thymol and carvacrol were reported to possess the highest antioxidant activity .

The antimicrobial properties of essential oils derived from many plants have been empirically recognized for centuries, but scientifically confirmed only recently (Deans and Ritchie, 1987; Janssen *et al*., 1987). Practical uses of these activities have long been suggested in humans and animals but only in the last years has it been reported that some essential oils are capable of inhibiting food borne bacteria and extending the shelf-life of processed food (Conner and Beuchat, 1984; Kim *et al*., 1995; Smith-Palmer *et al*., 1998). Among chemical components in several essential oils, carvacrol has been shown to exert a distinct antimicrobial action (Veldhuizen *et al*., 2006). Carvacrol is the major component of essential oil from oregano (60%-74% carvacrol) and thyme (45% carvacrol) (Lagouri *et al*., 1993; Arrebola *et al*., 1994).

Essential oils have a broad spectrum of antimicrobial activity against most Gram-positive and Gram-negative bacteria (Friedman *et al*., 2002). Carvacrol disintegrates the outer membrane of Gram-negative bacteria, releasing

lipo-polysaccharides and increasing the permeability of the cytoplasmic membrane to ATP (Burt, 2004). For Gram-positive bacteria, it is able to interact with the membranes of bacteria and alter the permeability for cations like H+ and K+ (Veldhuizen *et al.,* 2006). In general, the higher antimicrobial activity of essential oils is observed on Gram-positive bacteria than Gram-negative bacteria (Kokoska *et al*., 2002; Okoh *et al.,* 2010).

Essential oils obtained from Cinnamomum verum, Cymbopogon citratus, Thymus vulgaris, Origanum compactum and Satureja montana are the most active oils tested against S.pyogenes, with inhibition zones average ranging from 48.0-35.0 mm. *S. pyogenes* is sensitive to Eugenia caryophyllus and Cymbopogon martinii var.motia (means inhibition diameters, resp., 18.3 and 15.3mm). Cinnamomum camphora, Mentha piperita, Thymus vulgaris CT thujanol ,Origanum majorana, Lavandula stoechas, Melaleuca cajuputi, Melaleuca alternifolia showed a moderate inhibitory activity against *S. pyogenes* (means inhibition diameters ranging from 13.0 to 9.0 mm).

Medicinal plants: Many medicinal plants have been recognized as valuable resources of natural antimicrobial compounds (Mahady, 2005) Medicinal plant extracts offer considerable potential for the development of new agents effective against infections currently difficult to treat (Iwu *et al*., 1999).

A wide range of phytochemicals present in plants are known to inhibit bacterial pathogens (Cowan, 1999; Medina *et al*., 2005; Romero *et al*., 2005). Successful determination of such biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Organic solvents such as ethanol, acetone and methanol are often used to extract bioactive compounds (Eloff, 1998). Ethanol, however is the most commonly used organic solvent by herbal medicine manufacturers because the finished products can be safely used internally by consumers of herbal extracts. Additionally, the bioactivity of plant extracts depends on the water and ethanol concentration used in the extraction process (Ganora, 2008).

Numerous plants and secondary metabolites isolated from plants have been reported to possess antimicrobial properties (Ali and Qaiser, 2009; Qadrie *et al*., 2009; Nisar *et al*., 2010; Khan *et al*., 2011). Many plants have been used because of their antimicrobial traits which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances for example, the phenolic compounds which are part of the essential oils (Jansen *et al*., 1987) as well as in tannin (Saxena *et al*., 1994).

Total of 315 extract fractions from 63 traditionally used Ethiopian plants were subjected to antibacterial screening against known strains of microorganisms. It was found that all the plants showed activity against one or more of the microorganisms (Desta, 1993). Organium vulgare L. had a variety of biological properties and its antibacterial activities had received a renewed interest for using in food conversation.

Aqueous and alcoholic extracts of Adiatum veneris inhibited the growth of *S. pyogenes*, B. subtilis, Corynebacterium ovis (Mahran *et al*., 1990). The leaves and pits extracts of Phoenix dactylifera L. have shown promising antibacterial activity against *S. pyogenes*. In an earlier study it was shown that P. dactylifera fruit extract neutralized the hemolytic activity of the streptococcal exotoxins, streptolysin O and 96% inhibition was obtained at a very low concentration (1:262144 DE dilutions). Water, ethanol, methanol, acetone, hexane and butanol of the Ocimum tenuiflorum and Acalypha hispida showed antimicrobial activity against hazardous bacteria at a higher dose (Elamparithi *et al*., 2014).

Antibiotics used for inhibition of *S. pyogenes* infections: Different types of antibiotics affect Streptococcus pyogenes. For examples, some of them act on bacterial cell wall synthesis and others act on 70 S bacterial ribosome, some other antibiotics such as penicillin are essentially nontoxic to the patient, unless hypersensitivity has developed.

Antibiotics that affect on cell wall: The B-lactam antibiotics include the penicillins, cephalosporins, carbapenems and monobactams. Their name derives from the presence of a B-Lactam ring in their structure, this ring is essential for antibacterial activity.

Penicillins: Penicillin G (phenoxy methyl penicillin) is active primarily against Streptococcus pyogenes, Penicillin G is the least toxic and least expensive of all the penicillins. Its modification as penicillin V confers acid stability, so it can be given orally. It inhibits the biosynthesis of cell wall mucopeptides. When adequate concentrations are reached and are most effective during the stage of active multiplication (Kenneth and Lawrence, 2010).

Amoxicillin: Amoxicillin is a drug of choice for GAS pharyngitis. It is a derivative of ampicillin and has a similar antibacterial spectrum. With a bacterial action comparable to penicillin, amoxicillin acts on susceptible bacteria during the multiplication stage by inhibiting cell-wall mucopeptide biosynthesis. It has superior bioavailability and stability to gastric acid and has a broader spectrum of activity than penicillin (Steer *et al*., 2009).

Piperacillin: It is an extended-spectrum beta-lactam of the ureidopenicillin class. The chemical structure of piperacillin and other ureidopenicillins incorporates a polar side chain that enhances penetration into Gram-(-) bacteria and reduces susceptibility to cleavage by Gram-(-) beta lactamase enzymes (Tan and File, 1995).

Piperacillin is not absorbed orally and must therefore be given by intravenous or intramuscular injection. It has been shown that the bactericidal actions of the drug do not increase with concentrations of piperacillin higher than 4-6x MIC which means that the drug is concentration-independent in terms of its actions. Piperacillin has instead shown to offer higher bactericidal activity when its concentration remains above the MIC for longer periods of time (50% time>MIC showing the highest activity). This higher activity (present in continuous dosing) has not been directly linked to clinical outcomes but however, does show promise of lowering possibility of resistance and decreasing mortality (Lau *et al*., 2006).

Imipenem: Imipenem is a broad-spectrum â-lactam antibiotic. It was the first carbapenem antibiotic selected for development more than two decades ago because it was a highly potent, broad-spectrum antimicrobial agent with a good safety profile (Birnbaum *et al*., 1985).

Cephalosporins: The cephalosporins are classified by generation first, second, third and fourth. The first-generation cephalosporins. The first- generation cephalosporins cefazolin and cephalexin have a spectrum of activity against Gram-positive organisms that resembles that of the penicillinase-resistant penicillins. These agents continue to have therapeutic value because of their high activity against Gram-positive organisms and because a broader spectrum may be unnecessary (Murray *et al*., 2009).

Cephalexin a first-generation cephalosporin, arrests bacterial growth by inhibiting bacterial cell wall synthesis. It has bactericidal activity against rabidly growing organisms. The drug's primary activity is against skin flora, cephalexin is used for skin infections and for prophylaxis in minor procedures. Oral cephalosporins are effective in the treatment of streptococcal phyrngitis (Steer *et al*., 2009).

Cefoxitin a second-generation cephalosporins which resistant to B-Lactamases and are also active against anaerobes.

Ceftazidime, Cefoperazon third-generation cephalosporins, they have an even wider spectrum, they potency, broad spectrum and low toxicity of the third-generation cephalosporins have made them the preferred agents in life-threatening infections in which the causative organism has not yet been isolated. Selection depends on the clinical circumstances. Cefepime a fourth-generation cephalosporins have enhanced ability to cross the outer membrane.

Inhibitors of protein synthesis

Antibiotic that binding to the 30 S ribosomal subunit: Tetracyclines (e.g. Tetracycline) which inhibit protein synthesis by binding to the 30 S ribosomal subunit at a point that blocks attachment of aminoacy-tRNA to the acceptor site on the mRNA ribosome complex (Forbes *et al*., 2007). Resistance to tetracycline is conferred by ribosome protection genes such as tet (M) or tet (O) and by efflux pumps encoded by the tet (K) or tet (L) genes, although these last genes are relatively rare (Rubio *et al*., 2012).

Antibiotic that binding to the 50 S ribosomal subunit

Clindamycin: Clindamycin is chemically unrelated to the macrolides but has a similar mode of action and spectrum .Clindamycin inhibits bacterial growth by blocking dissociation of peptidyle transfer ribonucleic acid (tRNA) from ribosomes, causing RNA-dependent protein synthesis to arrest Clindamycin is a perfectly adequate substitute for a macrolide in many situation.

Clindamycin is a lincosamide for the treatment of serious skin and soft-tissue staphylococcal infections. It is also effective against aerobic and anaerobic streptococci (except enterococci). Patients with invasive *S. pyogenes* infections (e.g. necrotizing fasiitis and sepsis) shoud be treated with IV penicillin in combination with clindomycin. Because, the pathophysiology of invasive *S. pyogenes* infection is largely toxin mediated, the use of a protein synthesis inhibitor (e.g., clinamycin) offers a theoretical advantage (Steer *et al*., 2009).

CONCLUSION

The nomenclature and systems of classification of strain belonging to S.pyogenes are given in this review. Cultural, morphological and biological characteristics including virulence factors of *S. pyogenes* are discussed and their role in identification is showed herein. All infections caused by *S. pyogenes* and their diagnosis are showed. Treatment of *S. pyogenes* infection by both classical antibiotics and recent natural agents are also included.

REFERENCES

Abdel, S.S., A.A.R. Mohammadi, S. Negm and G. Enan, 2014. Antibacterial activity of Lactobacillus delbreukii subspecies bulgaricus isolated from Zabady. Life Sci. J., 11: 284-270.

Al-Jabri, A.A., 2005. Honey, milk and antibiotics. Afr. J. Biotechnol., 4: 1580-1587.

Ali, A.T., M.N. Chowdhury and A.M.S. Humayyd, 1991. Inhibitory effect of natural honey on Helicobacter pylori. Trop. Gastroenterol., 12: 139-143.

Ali, H. and M. Qaiser, 2009. The ethnobotany of Chitral valley, Pakistan with particular reference to medicinal plants. Pak. J. Bot., 41: 2009-2041.

Anupama, D., K.K. Bhat and V.K. Sapna, 2003. Sensory and physico-chemical properties of commercial samples of honey. Food Re. Int., 36: 183-191.

Arafa, M.A., S.R. Zaher, E.A.A. Dowaty and D.E. Moneeb, 2008. Quality of life among parents of children with heart disease. Health Qual. Life Outcomes, 6: 1-7.

Arrebola, M.L., M.C. Navarro, J. Jimenez and F.A. Ocana, 1994. Yield and composition of the essential oil of *Thymus serpylloides* ssp. serpylloides. Phytochem., 36: 67-72.

Aziz, R.K. and M. Kotb, 2008. Rise and persistence of global M1T1 clone of streptococcus pyogenes. Emerge Infect. Dis., 14: 1511-1517.

Aziz, R.K., R. Kansal, B.J. Aronow, W.L. Taylor and S.L. Rowe *et al*., 2010. Microevolution of group a streptococci *in vivo*: Capturing regulatory networks engaged in sociomicrobiology, niche adaptation and hypervirulence. PloS. One, 5: 9798-9800.

Bae, S.Y., J.S. Kim, J.A. Kwon, S.Y. Yoon and C.S. Lim *et al*., 2007. Phenotypes and genotypes of macrolide-resistant *Streptococcus pyogenes* isolated in Seoul, Korea. J. Med. Microbiol., 56: 229-235.

Barnes, J., L.A. Anderson and J.D. Phillipson, 2007. Herbal Medicines. 3rd Edn., Pharmaceutical Press, London, UK., ISBN-13: 9780853696230, Pages: 710.

Bernard, P., 2008. Management of common bacterial infections of the skin. Curr. Opin. Infect. Dis., 21: 122-128.

Bessen, D.E., 2009. Population biology of the human restricted pathogen, *Streptococcus pyogenes*. Infect. Genet. Evol., 9: 581-593.

Birnbaum, J., F.M. Kahan, H. Kropp and J.S. MacDonald, 1985. Carbapenems, a new class of beta-lactam antibiotics. Discovery and development of imipenem/cilastatin. Am. J. Med., 78: 3-21.

Bisno, A.L. and D.L. Stevens, 2010. Streptococcus Pyogenes. In: Mandell GL, Douglas and Bennet Principles and Practice of Infectious Diseases, Mandell, G.L., R. Dolin and J.B. Bennet (Eds.). Churchil Living Stone, Philadelphia, Pennsylvania, pp: 2593-2610.

Bisno, A.L., M.O. Brito and C.M. Collins, 2003. Molecular basis of group a streptococcal virulence. Lancet Infect. Dis., 3: 191-200.

Braga, P.C., C. Bovio, M. Culici and D.M. Sasso, 2003. Flow cytometric assessment of susceptibilities of *Streptococcus pyogenes* to erythromycin and rokitamycin. Antimicrob. Agents Chemother., 47: 408-412.

Bricker, A.L., C. Cywes, C.D. Ashbaugh and M.R. Wessels, 2002. NAD+-glycohydrolase acts as an intracellular toxin to enhance the extracellular survival of group a streptococci. Mol. Microbiol., 44: 257-269.

Brooks, F., J.S. Butel and S.A. Morse, 2004. The Streptococci. In: Jawetz, Melnick and Adelberg's Medical Microbiology, Brooks, G.F., K.C. Carroll, J. Butel, S. Morse and T. Mietzner (Edn.,) 25th Edn., Lange Medical Brooks/McGraw-Hill, New York, pp: 195-209.

Buchanan, J.T., A.J. Simpson, R.K. Aziz, G.Y. Liu and S.A. Kristian *et al*., 2006. DNase expression allows the pathogen group A Streptococcus to escape killing in *Neutrophil extracellular* traps. Curr. Biol., 16: 396-400.

Bugrysheva, J.V. and J.R. Scott, 2010. The ribonucleases J1 and J2 are essential for growth and have independent roles in mRNA decay in *Streptococcus pyogenes*. Mol. Microbiol., 75: 731-743.

Burt, S., 2004. Essential oils: Their antibacterial properties and potential applications in foods-a review. Int. J. Food Microbiol., 94: 223-253.

Capoor, M.R., D. Nair, M. Deb, K. Batra and P. Aggarwal, 2006. Resistance to erythromycin and rising penicillin MIC in *Streptococcus pyogenes* in India. Japanese J. Infect. Dis., 59: 334-336.

Carapetis, J.R., A.C. Steer, E.K. Mulholland and M. Weber, 2005. The global burden of group A streptococcal diseases. Lancet Infect. Dis., 5: 685-694.

Casey, J.R. and M.E. Pichichero, 2005. Metaanalysis of short course antibiotic treatment for group a *Streptococcal tonsillopharyngitis*. Pediatr. Infect. Dis. J., 24: 909-917.

Cassel, E. and R.M.F. Vargas, 2006. Experiments and modeling of the *Cymbopogon winterianus* essential oil extraction by steam distillation. J. Mexican Chem. Soc., 50: 126-129.

Celestin, R., J. Brown, G. Kihiczak and R.A. Schwartz, 2007. Erysipelas: A common potentially dangerous infection. Acta Dermatovenerologica Alpina Pannonica Adriatica, 16: 123-127.

Chessbrugh, M., 2006. Microbiological Tests. In: District Laboratory Practice in Tropical Countries Part 2, Chessbrough, M. (Ed.). Cambridge University Press, New York, USA., pp: 45-143.

Chow, J., 2002. Probiotics and prebiotics: A brief overview. J. Renal Nutr., 12: 76-86.

Cole, J.N., T.C. Barnett, V. Nizet and M.J. Walker, 2011. Molecular in sight into invasive group a streptococcus disease. Nature Rev. Microbiol., 9: 724-736.

Commons, R., S. Rogers, T. Gooding, M. Danchin, J. Carapetis, R. Robins-Browne and N. Curtis, 2008. Superantigen genes in group A streptococcal isolates and their relationship with emm types. J. Med. Microbiol., 57: 1238-1246.

Conner, D.E. and L.R. Beuchat, 1984. Effects of essential oils from plants on growth of food spoilage yeasts. J. Food Sci., 49: 429-434.

Cunningham, M.W., 2008. Pathogenesis of group A streptococcal infections and their sequelae. Adv. Exper. Med. Biol., 609: 29-42.

Deans, S.G. and G.A. Ritchie, 1987. Antimicrobial properties of Plant essential oils. Int. J. Food Microbiol., 5: 165-180.

Desta, B., 1993. Ethiopian traditional herbal drugs part II antimicrobial activity of 63 medicinal plants. J. Ethnopharmacol., 39: 129-139.

Di leo Lira, P., D. Retta, E. Tkacik, J. Ringuelet, J.D. Coussio, C. van Baren and A.L. Bandoni, 2009. Essential oil and by-products of distillation of bay leaves (*Laurus nobilis* L.) from Argentina. Ind. Crops Prod., 30: 259-264.

El-Arab, A.M.E., S.M. Girgis, E.M. Hegazy and A.B. Abd El-Khalek, 2006. Effect of dietary honey on intestinal microflora and toxicity of mycotoxins in mice. BMC Complement Altern. Med., 6: 6-6.

Elamparithi, D., P. Mani and V. Moorthy, 2014. Antimicrobial activity and GC-MS analysis of *Ocimum tenuiflorum* and *Acalypha hispida* extract against *Streptococcus pyogenes*. Malaya J. Biosciences, 1: 259-266.

Eloff, J.N., 1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants? J. Ethnopharmacol., 60: 1-8.

Enan, G. and A.A. Al Amry, 2006. Novel plantaricin UG1 production by *Lactobacillus plantarum* UG1 in enriched whey permeate in batch fermentation processes. J. Food Agric. Environ., 4: 85-88.

Enan, G., A.A.I.A. Khair, S.S. Abdel and A.A.R. Mohammadi, 2015. Evalution of the use of *Enterococcus faeciva* NM_2 as a probiotic for inhibition of some urogential pathogens. J. Food Agric. Environ., 13: 7-7.

Enan, G., E.G. Didamony, E.H. Mohamed and A. Zakaria, 2014. Novel antibacterial activity of *Enterococcus faecium* NM_2 isolated from urine of healthy people. Asian J. Appl. Sci., 7: 66-78.

Enan, G., N. Awny, A.A.A. Zeid and M.A. Abdou, 2012. Incidence and virulence of Bacillus cereus isolated from Egyptian foods during four seasons. Afr. J. Microbiol. Res., 6: 4816-4824.

Enan, G., S. Abdel-Shafi, M.E.F. Abdel-Haliem and S. Negm, 2013. Characterization of probiotic Lactic acid bacteria to be used as starter and protective cultures for dairy fermentation. Int. J. Probiotics Prebioics, 8: 157-164.

Enan, G., T.I. Elsayed, D. Atef, M. Amer and A. Mahdy, 2015. Causal organisms, pathogenicity, laboratory diagnosis and treatment of candidiasis. Res. J. Appl. Sci., 10: 115-135.

Ergul, E. and S. Ergul, 2009. The effect of honey on the intestinal anastomotic wound healing in rats with obstructive jaundice. Med. J., 111: 265-270.

Eron, L.J., 2009. Cellulitis and soft-tissue infections. Ann. Internal Med., 150: 1-1.

Facklam, R., 2002. What happened to the streptococci: Overview of taxonomic and nomenclature changes. Clin. Microbiol. Rev., 15: 613-630.

Ferretti, J.J., W.M. McShan, D. Ajdic, D.J. Savic and G. Savic *et al*., 2001. Complete genome sequence of an M1 strain of *Streptococcus pyogenes*. Proc. National Acad. Sci., 98: 4658-4663.

Forbes, S.A., D.H. Sahm and W.A.S. Feld, 2007. Principles of Antimicrobial Action and Resistance. In: Baily and Scott Part I: Diagnostic Microbiology Betty, Forbes, A., F.D. Sahm and S.W.F. Alice (Eds.). Elsevier, Philadelphia, Pennsylvania, pp: 172-186.

Fraser, J.D. and T. Proft, 2008. The bacterial superantigen and superantigen-like proteins. Immunol. Rev., 225: 226-243.

Friedman, M., P.R. Henika and R.E. Mandrell, 2002. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella enterica*. J. Food Prot., 65: 1545-1560.

Ganora, L., 2008. Herbal Constituents: Foundations of Phytochemistry. Herbal Chem Press, Louisville, Kentucky, pp: 38-52.

Hafez, M., S. Yahia, W. Eldars, H. Eldegla and M. Matter *et al*., 2013. Prediction of residual valvular lesions in rheumatic heart disease: role of adhesion molecules. Pediatric Cardiol., 34: 583-590.

Iwu, M.M., A.R. Duncan and C.O. Okunji, 1999. New Antimicrobials of Plant Origin. In: Perspectives on New Crops and New Uses, Janick, J. (Ed.). ASHS Press, Alexandria, VA., USA., ISBN-13: 9780961502706, pp: 457-462.

Janssen, A.M., J.J. Scheffer and A.B. Svendsen, 1987. Antimicrobial activity of essential oils: A 1976-1986 literature review. Aspects of the test methods. Planta Med., 53: 395-398.

Jenkins, R., N. Burton and R. Cooper, 2011. Manuka honey inhibits cell division in methicillin-resistant *Staphylococcus aureus*. J. Antimicrob. Chemother., 66: 2536-2542.

Jull, A.B., N. Walker and S. Deshpande, 2013. Honey as a topical treatment for wounds. Cochrane Database Syst. Rev., Vol. 28,

Kamaratos, A.V., K.N. Tzirogiannis, S.A. Iraklianou, G.I. Panoutsopoulos and I.E. Kanellos *et al*., 2014. Manuka honey-impregnated dressings in the treatment of neuropathic diabetic foot ulcers. Int. Wound J., 11: 259-263.

Kawamura, Y., X.G. Hou, F. Sultana, H. Miura and T. Ezaki, 1995. Determination of 16S rRNA sequences of *Streptococcus mitis* and *Streptococcus gordonii* and phylogenetic relationships among members of the genus Streptococcus. Int. J. Syst. Evol. Microbiol., 45: 406-408.

Kenneth, J.R. and W.D. Lawrence, 2010. The Streptococci and Enterococci. In: Sherris Medical Microbiology, Kenneth, J.R., C.R. George, J.C. Sherrisch, N. Ahmed and W.D. Lawrence *et al.* (Eds.). McGraw-Hill, New York, USA., pp: 443-469.

Khan, M.A., H. Inayat, H. Khan, M. Saeed and I. Khan, 2011. Antimicrobial activities of the whole plant of *Cestrum nocturnum* against pathogenic microorganisms. Afr. J. Microbiol. Res., 5: 612-616.

Kim, J., M.R. Marshall and C.I. Wei, 1995. Antibacterial activity of some essential oil components against five foodborne pathogens. J. Agric. Food Chem., 43: 2839-2845.

Kokoska, L., Z. Polesny, V. Rada, A. Nepovim and T. Vanek, 2002. Screening of some Siberian medicinal plants for antimicrobial activity. J. Ethnopharmacol., 82: 51-53.

Kotloff, M. and V.C. Beneden, 2008. Standardization of epidemiologic protocols for surveillance of cute diseases caused by *Streptococcus pyogenes*. Pharyngitis impetigo Invasive Dis., 1: 9-28.

Kwakman, P.H. and S.A. Zaat, 2010. Antibacterial components of honey. IUBMB. Life, 64: 48-55.

Kwakman, P.H., V.D.J.P. Akker, A. Guclu, H. Aslami and J.M. Binnekade *et al*., 2008. Medical-grade honey kills antibiotic-resistant bacteria in vitro and eradicates skin colonization. Clin. Infect. Dis., 46: 1677-1682.

Lagouri, V., G. Blekas, M. Tsimidou, S. Kokkini and D. Boskou, 1993. Composition and antioxidant activity of essential oils from oregano plants grown wild in Greece. Zeitschrift Lebensmittel Untersuchung Forschung, 197: 20-23.

Lamagni, T.L., J. Darenberg, L.B. Harari, T. Siljander and A. Efstratiou *et al*., 2008. Epidemiology of severe Streptococcus pyogenes disease in Europe. J. Clin. Microbiol., 46: 2359-2367.

Lau, W.K., D. Mercer, K.M. Itani, D.P. Nicolau and J.L. Kuti *et al*., 2006. Randomized, open-label, comparative study of piperacillin-tazobactam administered by continuous infusion versus intermittent infusion for treatment of hospitalized patients with complicated intra-abdominal infection. Antimicrob. Agents Chemother., 50: 3556-3561.

Lazim, N.M., B. Abdullah and R. Salim, 2013. The effect of Tualang honey in enhancing post tonsillectomy healing process: An open labelled prospective clinical trial. Int. J. Pediatric Otorhinolaryngology, 77: 457-461.

Maddocks, S.E., M.S. Lopez, R.S. Rowlands and R.A. Cooper, 2012. Manuka honey inhibits the development of Streptococcus pyogenes biofilms and causes reduced expression of two fibronectin binding proteins. Microbiol., 158: 781-790.

Mahady, G.B., 2005. Medicinal plants for the prevention and treatment of bacterial infections. Curr. Pharm. Des., 11: 2405-2427.

Mahran, G.H., E.T.M. Alfy, K.F. Taha and E.M. Tantawy, 1990. Chemical composition and antimicrobial activity of the volatile oil and extracts of fronds of Adiantum capillus veneris L. Bull. Faculty Agric. Univ. Cairo, 41: 555-572.

Medina, A.L., M.E. Lucero, F.O. Holguin, R.E. Estell and J.J. Posakony *et al*., 2005. Composition and antimicrobial activity of Anemopsis californica leaf oil. J. Agric. Food Chem., 53: 8694-8698.

Moez, A.E.S., F.Y. Ahmed, A.A. Samy and A.R. Ali, 2010. Probiotic activity of acidophilus against major food-borne pathogens isolated from broiler carcasses. Nat. Sci., 8: 69-78.

Mora, M., G. Bensi, S. Capo, F. Falugi and C. Zingaretti *et al*., 2005. Group A Streptococcus produce pilus-like structures containing protective antigens and Lancefield T antigens. Proc. National Acad. Sci., 102: 15641-15646.

Murray, P.R., K.S. Rosenthal and M.A. Pfaller, 2009. Antimicrobial Agents in Medical Microbiology. Mosby Publisher, Maryland Heights, Missouri, pp: 199-208.

Nisar, M., M. Qayum, M.R. Shah, W.A. Kaleem, I. Ali and M. Zia-ul-Haq, 2010. Antimicrobial screening of impatiens bicolor royle. J. Bot., 42: 523-526.

Oloughlin, R.E., A. Roberson, P.R. Cieslak, R. Lynfield and K. Gershman *et al*., 2007. The epidemiology of invasive group A streptococcal infection and potential vaccine implications: United States, 2000-2004. Clin. Infec. Dis., 45: 853-862.

Okoh, O.O., A.P. Sadimenko and A.J. Afolayan, 2010. Comparative evaluation of the antibacterial activities of the essential oils of *Rosmarinus officinalis* L. obtained by hydrodistillation and solvent free microwave extraction methods. Food Chem., 120: 308-312.

Olsen, R.J., S.A. Shelburne and J.M. Musser, 2009. Molecular mechanisms underlying group A streptococcal pathogenesis. Cellular Microbiol., 11: 1-12.

Packer, J.M., J. Irish, B.R. Herbert, C. Hill and M. Padula *et al*., 2012. Specific non-peroxide antibacterial effect of manuka honey on the *Staphylococcus aureus* proteome. Int. J. Antimicrob. Agents, 40: 43-50.

Qadrie, Z.L., B. Jacob, R. Anandan, B. Rajkapoor and M.R. Ulla, 2009. Antibacterial activity of ethanolic extract of *Indoneesiella echioides* (l) Nees. evaluated by the filter paper disc method. Pak. J. Pharm. Sci., 22: 123-125.

Riddle, J.A., 2001. NOSB apiculture task force report draft organic apiculture standards addendum I. Definition Honey Honey Prod., 48: 1498-1502.

Romero, C.D., S.F. Chopin, G. Buck, E. Martinez, M. Garcia and L. Bixby, 2005. Antibacterial properties of common herbal remedies of the Southwest. J. Ethnopharmacol., 99: 253-257.

Rubio, L.V., S. Valdezate, D. Alvarez, P. Villalon and M.J. Medina *et al*., 2012. Molecular epidemiology, antimicrobial susceptibilities and resistance mechanisms of *Streptococcus pyogenes* isolates resistant to erythromycin and tetracycline in Spain (1994-2006). BMC. Microbiol., 12: 1-11.

Ruoff, K. and A.L. Bisno, 2009. Classification of Streptococci. In: Principles and Practice of Infectious Diseases, Mandell, D. and S. Bennett (Eds.). Churchill Lvingstone, Philadelphia, Pennsylvania, pp: 2591-2593.

Saadeh, C., 1998. The erythrocyte sedimentation rate: Old and new clinical applications. South. Med. J., 3: 220-225.

Saxena, G., A.R. McCutcheon, S. Farmer, G.H.N. Towers and R.E.W. Hancock, 1994. Antimicrobial constituents of Rhus glabra. J. Ethnopharmacol., 42: 95-99.

Schroeder, B.M., 2003. Diagnosis and management of group A streptococcal pharyngitis. Am. Family Physician, 67: 880-883.

Schumacher, H.H.A., 2004. Use of medical honey in patients with chronic venous leg ulcers after split-skin grafting. J. Wound Care, 13: 451-452.

Shulman, S.T., A.L. Bisno, H.W. Clegg, M.A. Gerber and E.L. Kaplan *et al*., 2012. Clinical practice guideline for the diagnosis and management of group A streptococcal pharyngitis: 2012 update by the Infectious Diseases Society of America. Clin. Infect. Dis., 55: 86-102.

Smith-Palmer, A., J. Stewart and L. Fyfe, 1998. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. Lett. Applied Microbiol., 26: 118-122.

Starr, C.R. and N.C. Engleberg, 2006. Role of hyaluronidase in subcutaneous spread and growth of group A streptococcus. Infect. Immunity, 74: 40-48.

Steer, A.C., I. Law, L. Matatolu, B.W. Beall and J.R. Carapetis, 2009. Global emm type distribution of group A streptococci: Systematic review and implications for vaccine development. Lancet Infect. Dis., 9: 611-616.

Tahmaz, L., F. Erdemir, Y. Kibar, A. Cosar and O. YalcyN, 2006. Fournier's gangrene: Report of thirty-three cases and a review of the literature. Int. J. Urology, 13: 960-967.

Tan, J.S., T.M. File, 1995. Antipseudomonal penicillins. Med. Clin. North Am., 79: 679-693.

Teglund, N.A., M.P. Muller, A. McGeer, B.S. Gan and V. Guru *et al*., 2005. Successful management of severe group A streptococcal soft tissue infections using an aggressive medical regimen including intravenous polyspecific immunoglobulin together with a conservative surgical approach. Scand. J. Infect. Dis., 37: 166-172.

Thomas, M., M. Hamdan, S. Hailes and M. Walker, 2011. Manuka honey as an effective treatment for chronic pilonidal sinus wounds. J. Wound Care, 20: 528-530.

Trevino, J., N. Perez and P. Sumby, 2010. The 4.5 S RNA component of the signal recognition particle is required for group A Streptococcus virulence. Microbiol., 156: 1342-1350.

Veldhuizen, E.J., J.L. Tjeerdsma van Bokhoven, C. Zweijtzer, S.A. Burt and H.P. Haagsman, 2006. Structural requirements for the antimicrobial activity of carvacrol . J. Agric. Food Chem., 54: 1874-1879.

Zumla, A. and A. Lulat, 1989. Honey-a remedy rediscovered. J. Royal Soc. Med., 82: 384-385.