A Study on the Bacteria and Fungi causing upper Respiratory Tract Infections(URTIs) in Children visiting Benghazi pediatric Hospital

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DEDICATION

By the name of Allah the most beneficent.

I dedicate this thesis to my mother, my brothers, my sisters, my husband and my childrens without their loves and encouragements this thesis would not be materialized.

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Abbreviation or symbols	Meaning
URTI	Upper Respiratory Tract Infection
RTI	Respiratory tract infection
LRTI	Lower respiratory tract infection
GAS	Group A *Streptococcus*
ABRS	Acute bacterial rhinosinusitis
CT	Computed tomography
MHC	Major Histocompatibility Complex
ABS	Acute bacterial sinusitis
FRS	Fungal rhinosinusitis
GABHS	Group A beta-hemolytic *Streptococcus*
EDTA	Ethylenediamine-tetracetic acid
TMPD	tetramythyl –p– phenylenalanine dihydrochloride
API	Analytical Profile Index
PFGE	Pulsed-Field Gel Electrophoresis

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ABSTRACT

A total of 300 specimens of swabs were collected from patients who had upper respiratory tract infections for this study during a period of (August, 2016 to June, 2017). The specimens were taken from outpatient department (OPD) of the Children's Hospital in Benghazi, Libya, from different areas of upper respiratory tract which were nose, tonsils and pharynx, aged between one year to 13 years. The data collected were analyzed with respect to age, sex and areas of upper respiratory tract. The study showed that all patients tested had a single microbial infection. Males constituted 172 patients (57%) while females were 128 (42.6 %) of infected subjects. The results of the bacterial growth showed that 297 samples of isolates (99%) were positive of growth while there was no growth of pathogens in three patients or (1%) of samples.

The isolates were examined morphologically, by biochemical tests, API and BBL Streptocard. The infectious bacteria that appeared were: *Staphylococcus aureus* (31%), *Streptococcus pyogenes* (28.3%), *Streptococcus pneumoniae* (21.3%), *Klebsiella pneunoniae* (8%), *Pseudomonas aeruginosa* (6%), *Escherichia coli* (two isolates) and *Bacillus cereus* (two isolates), while *Staphylococcus epidermidis* (one isolate). The infectious fungi were *Aspergillus niger* (2.3%) and *Candida albicans* (0.3%).

Effective factors were studied regarding the spread of nose infection, tonsillitis and pharyngitis which were age, sex and area of infection. The high level of infections were in the first group (7-10 years), with 105 or (35%). The infections were distributed on the four seasons. The seasonal infections were in their highest level in winter with the rate of 108 or (36%).

The isolates were tested for antibiotic sensitivity using nine antibiotics. All isolates showed a high resistance against ampicillin whereas, most of the isolates were sensitive to ciprofloxacin and gentamicin.

CHAPTER 1

INTRODUCTION

1. Background :

Broad diagnosis of respiratory tract infection (RTI) includes the two principal sub-diagnoses of lower respiratory tract infection (LRTI) and upper respiratory tract infection (URTI), although it is often difficult to distinguish between them (Mansoor, 2009).

Upper respiratory tract infection (URTI) has been recognized as one of the most common medical problems in the daily lives of people worldwide. A strong confirmation for the prevention of URTI is rather inadequate, and thus, the patients take preventive measures on the basis of their own experience or preferences. However, an URTI is referred to as a viral infection causing inflammation and infection in the nose and throat. URTIs are contagious which remain for few hours to 2-3 days of exposure. Also, the symptoms have been knowm to last from 7-10 days, but reports have shown that the symptoms may last even longer. URTI has been regarded as a nonspecific term that is used to describe acute infections involving the nose, paranasal sinuses, pharynx, larynx, trachea, and bronchi. Although, there have been a range of related conditions that may have similar or overlapping clinical presentations within each category of illness, and hence, judgment is required in determining the affected respiratory mucosal part. Various signs and symptoms of URTIs have been reporets which include stuffy and runny nose, sneezing, coughing, sore throat, fever, vomiting, irritability, loss of appetite, and watery eyes. However, URTI infections have been suggested to be mild and self limiting, but they have been reported to lead to life threatening complications. Further, the cause of URTIs have been attributed to viral, but studies have also suggested the cause to be bacterial (Rohilla *et al*., 2013). URTI is inflammation of the nasal, paranasal and pharyngeal mucosa. It usually diffuses in all tissue especially in infants and small children due to immaturity of local anatomical barriers (Alsaeed *et al*., 2017). URTIs are defined as acute febrile illnesses presenting with cough, coryza, sore throat, or hoarseness, which are very common in the community and are one of the major reasons for visiting primary care physicians, particularly during the winter season (Khan and Khan, 2015). Various signs and symptoms of URTIs have been reported which include stuffy and runny nose, sneezing, coughing, sore throat, fever, vomiting,

irritability, loss of appetite, and watery eyes (Cooper *et al*., 2001). The inflammatory process always occurs with the same features: inflammation and edema of the mucosa, vascular congestion, hypersecretion of mucus and alteration of transport and mucus ciliary clearance (Mucia *et al*., 2015). The clinical manifestations of URTIs are variable depending on the causative organisms. URTIs symptoms can be relatively mild. They may begin with sore throat, dry cough, and runny nose. The cough may then become more severe, and can be associated with sputum. The mouth and throat may become swollen and red (Mansoor, 2009). Both viral and bacterial pathogens are considered to play an important role in the etiology of URTIs. Fungi, other micro-organisms, and chemicals (such as powder or oil that accidentally penetrate into the lungs) could also function as causative agents for URTIs (Khan and Khan, 2015). Numbers of causative factors have been found to be involved in the occurrence of rhinosinusitis, which include immunological deficiency, seasonal and altitude variation, severe common cold condition, allergies, unusual changes in anatomy of nasal septum, and smoke (Kennedy and Borish, 2013). Factors like cold, allergics, toxic fumes, accumulation of chemicals, and flu have been suggested to result in pharyngitis. Also, a number of viruses and bacteria have been noted to be involved in the origin and development of infectious pharyngitis (Rohilla *et al*., 2013). URTIs are highly prevalent, especially in children between the ages of two and four years (Mansoor, 2009). Children younger than five years may have between five and eight episodes a year (Paulo and Jose, 2003). Children less than six months old are relatively protected against community-based respiratory infections. The frequency of URTIs increases and becomes high during the second year of a child's life, and may increase again during child-bearing years. On the other hand, the frequency of respiratory infections decreases with increasing age of children (Mansoor, 2009).

Pathogenesis of rhinosinusitis involves three key elements: narrow sinus ostia, dysfunction of the ciliary apparatus, and viscous sinus secretions. The narrow caliber of the sinus ostia sets the stage for obstruction to occur. Factors that predispose the ostia to obstruction include those that result in mucosal swelling and those that cause direct mechanical obstruction. Of these multiple causes the viral upper respiratory infection (URI) and allergic inflammation are the most frequent and most important (Drettner, 1980). When obstruction of sinus ostium occurs, there is transient increase in pressure within the sinus cavity. Oxygen is depleted in this close space, the pressure in the sinus becomes negative relative to atmospheric pressure. This negative pressure may allow

the introduction of nasal bacteria into sinuses during sniffing or nose blowing (Mustafa *et al*., 2015). When obstruction of the sinus ostium occurs, secretion of mucous by mucosa continues, resulting in accumulation of fluid in the sinus (Gwaltney *et al*., 2000). Tonsil infections may be contagious, and can spread from person to person by contact with the mouth, throat, or mucous of someone who is infected. Generally, tonsillitis symptoms include a sore throat, fever, swollen glands in the neck, and trouble swallowing (Eisenberg, 2012). URTIs have been characterized as acute febrile illnesses presenting with cough, coryza, sore throat, and hoarseness, which forms the prime reason to get affected by URTI (Meydani *et al*., 2004). However, it has been suggested that the vast majority of URTIs cases have been benign, and thus, the exact etiology of URTIs has not been understood completely. The transmission of organisms causing URITs has been known to occur by aerosol, droplet, and direct hand-to-hand contact with infected secretions. In addition, subsequent passage to the nares and eyes also forms the basic procedure of acquiring infections, and hence, it has been suggested that the transmission occurs more commonly in crowded conditions (Aagaard and Gonzales, 2004).

CHAPTER 2

LITERATURE REVIEW

2.1 Background:

URTI occurs commonly in both children and adults and is a major cause of mild morbidity. URTIs have a high cost to society, being responsible for missed work and unnecessary medical care. Occasionally, they have serious sequelae (Cotton *et al*., 2004). Pharyngitis, the inflammation of pharynx or throat at back side, can be divided into two types, i.e., acute and chronic. The pharyngitis can be classified into viral pharyngitis and bacterial pharyngitis according to their cause, that has been known to occur at an age of 4-8 years (McGinn *et al*., 2003). Common symptoms of pharyngitis include rheumatic fever, red-sore throat, yellow coloured secretion from nose, hypertrophy of tonsils, coughing, conjunctivitis, severe pain, enlargement of lymphs, headache, malaise, and difficulty in swallowing (Rohilla *et al*., 2013).

Rhinosinusitis is defined as a symptomatic inflammatory condition of mucosa of the nasal cavity and paranasal sinuses, the fluids within these sinuses, and/or the underlying bone (Lanza and Kennedy, 1997). It is termed chronic when the duration of symptoms exceeds 12 weeks as shown in figure (1). (Beatrice *et al*., 2016). The sinuses have been classified into following subunits namely maxillary sinuses, frontal sinuses, ethmoid sinuses, sphenoid sinuses, anal sinuses, and dural venous sinuses (Piccirillo, 2004). In sinusitis, nasal endoscopy has been commonly referred for diagnostic purposes. In addition, sinusitis can be further classified into acute sinusitis and chronic sinusitis, based on the duration of occurrence and termination of symptoms (Sande and Gwaltney, 2004). The sinus infection may cause inflammation of Pharynx, Larynx, Bronchi and Gastric symptoms via constant descending infection. These conditions fail to respond to treatment unless the primary infection is recognized and treated early. Maxillary sinusitis is usually diagnosed on clinical and radiological grounds (Varalakshmi *et al*., 2016). Tonsilitis, another common type of URTIs, can be defined as the state of inflammed condition of palatine tonsils, pharyngeal tonsils, tubal tonsils, and lingual tonsil (Meydani *et al*., 2004). Tonsillitis is inflammation of tonsils, a common clinical condition caused by either bacteria or viral infection. It affects significant percentage of

population more so in children. The condition can occur occasionally or recur frequently. Acute tonsillitis is characterized by visible white streaks of pus on tonsils and the surface of the tonsils may become bright red colour, as shown in figure (2) (Vijayashree *et al*., 2014). Pharyngitis is one of the most common conditions encountered by the family physician (Ebell *et al*., 2000). The optimal approach for differentiating the various causes of pharyngitis requires a problem focused history, a physical examination, and appropriate laboratory testing (Vincent *et al*., 2004). Identifying the cause of pharyngitis, especially group A beta-hemolytic *Streptococcus* (GABHS), is important to prevent potential life-threatening complications (Singer, 2001).

2.2 Diagnosis

The diagnosis of the upper respiratory tract infections in most cases rests only upon recognition of the symptoms and physical examination. The classification of those diseases is built upon the clinical manifestations, as already mentioned. Some of these diseases can be treated at home. If the symptoms are severe, have unexpected prolonged duration, or in some other circumstances like in immunecompromised persons, or during epidemics, medical attention is necessary. The aim is to recognize or detect the causative agent and, thus, enable efficient therapy. In some instances, visualization and imaging techniques help in the management of these patients. The armamentarium of investigations to reach the final diagnosis is huge (Peros and Tekavec, 2014). The most common presentation is a persistent (and nonimproved) nasal discharge or cough (or both) lasting more than 10 days (Wald *et al*., 1991). Pharyngitis can present with sudden onset of sore throat, fever, headache, tender anterior cervical lymphadenopathy or lymphadenitis, and occasionally, abdominal pain, nausea, vomiting, fatigue, or rash as shown in figure (3). When GABHS is the etiologic agent, fevers are often > 38.5°C (101.3 °F), tonsillar exudates are common, and patients may experience fevers, chills, and myalgias (Hayes and Williamson, 2001). On examination, the typical findings of acute pharyngitis may include an erythematous and swollen pharynx, tonsillar hypertrophy and inflammation (with or without tonsillar exudates), fever, edematous uvula, petechial rash along the palate, and tender anterior cervical lymphadenopathy. Occasionally, a scarlatiniform rash may be present, often seen in association with a GABHS infection. The etiologic diagnosis is based on laboratory tests. Throat culture is the gold standard for diagnosing pharyngitis caused by *Streptococcus* and it has a

sensitivity ranging between 90% and 95%. Samples should be obtained by vigorously swabbing the tonsils and the posterior pharynx (Low, 2012). If the result is negative, most patients should not be administered antibiotic therapy (Anjos *et al*., 2014). However, when the result is positive, it does not eliminate the possibility of chronic colonization (Wessels, 2011). When an orbital or intracranial complication of ABS is suspected on clinical grounds, imaging studies are essential to confirm the diagnosis and to determine the need for surgical intervention. Computed tomography provides the best definition of bony structures and is most likely to show subperiosteal abscess and osteitis, particularly when orbital complications are suspected (Gregory and Wald, 2011).

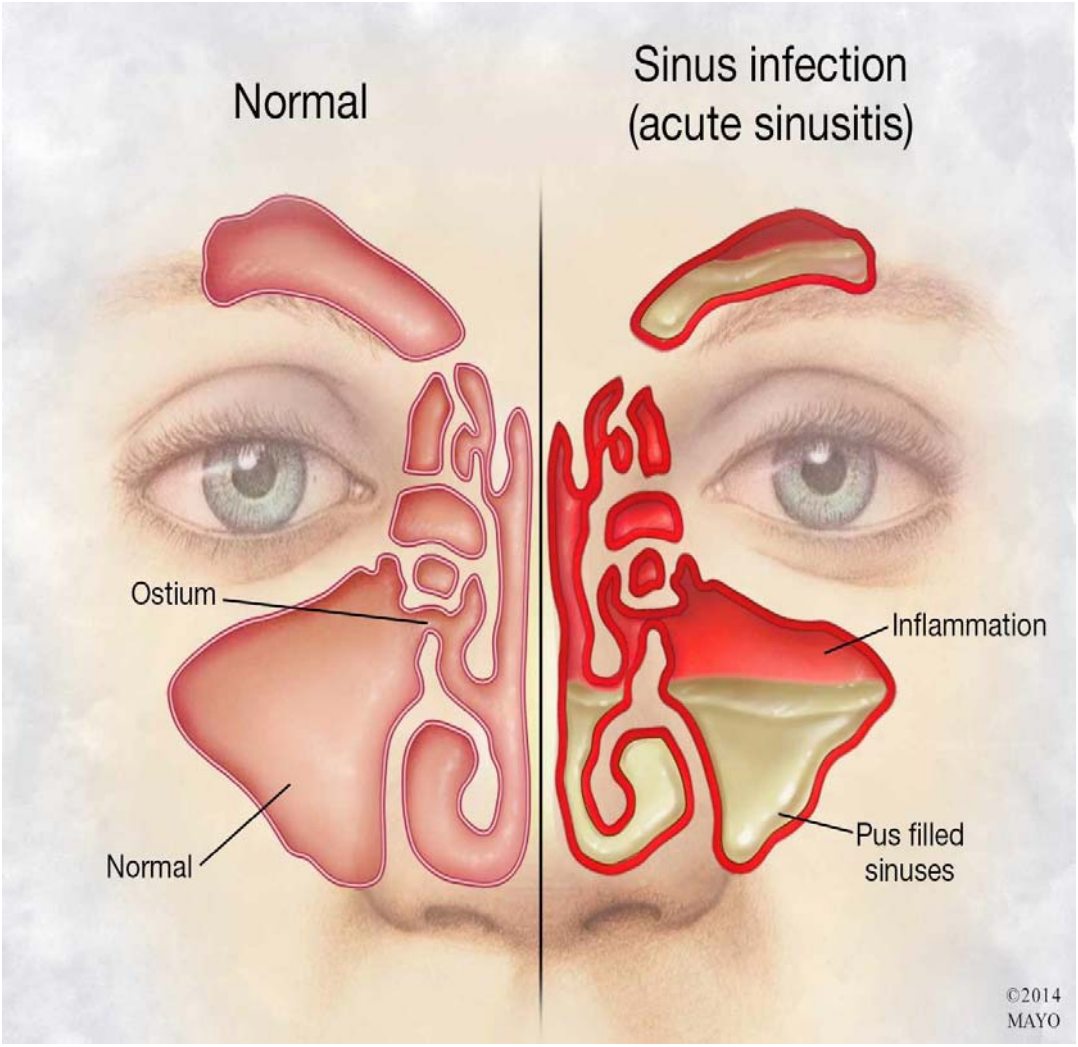

Figure (1): Normal sinus and sinusitis

https://newsnetwork.mayoclinic.org/discussion/mayo-clinic-q-a-chronic sinusitis-symptoms-often-resemble-a-cold-but-last-for-months/.

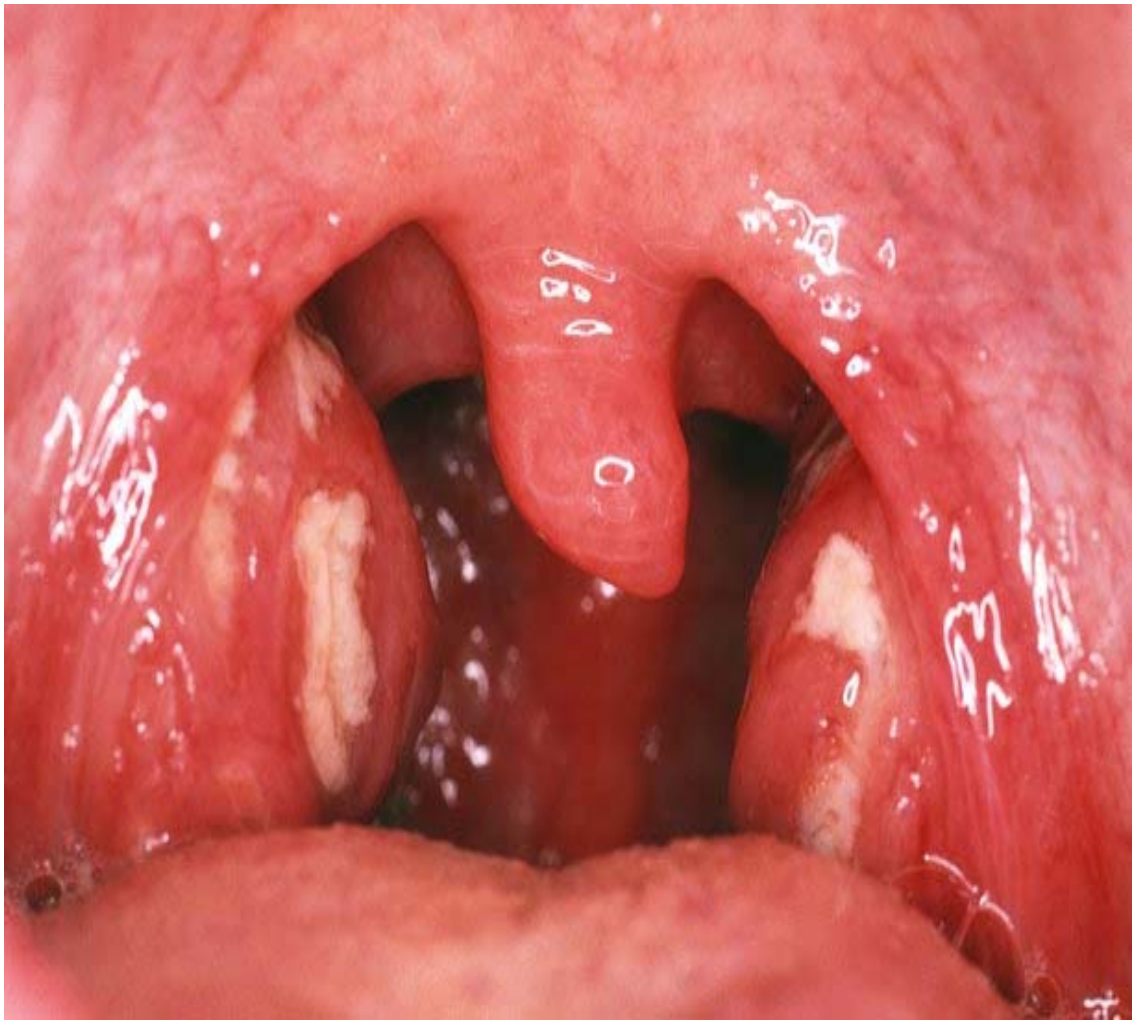

Figure (2): Tonsillitis

https://medikoe.com/article/tonsillitis-causes-and-treatment-4536.

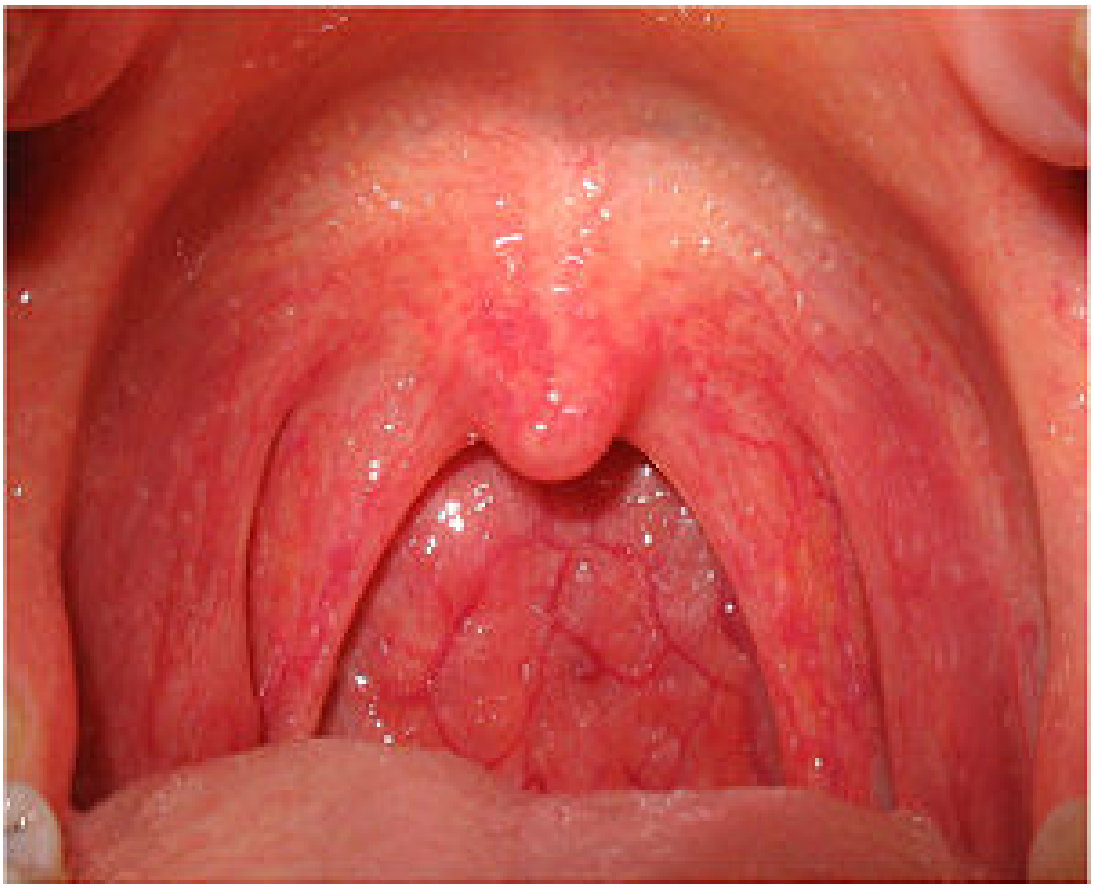

Figure (3): Pharyngitis

(https://en.wikipedia.org/wiki/Pharyngitis).

2.3 Seasonality of URTIs

Most URIs occur more frequently during the cold winter months, because of overcrowding. Adults develop an average of two to four colds annually (Khan and Khan, 2015). Epidemics and mini-epidemics are most common during cold months, with a peak incidence in late winter to early spring (Mansoor, 2009). The common factor amongst the wide range of seasonal diseases, from common cold to measles and pneumonia, is a correlation between the incidence of the disease and air temperature (Wells, 1944; Lieberman *et al*., 1999).

2.4 Microbiology

Bacterial causes are more important because of the non suppurative sequelae like rheumatic fever and rheumatic heart disease in group A haemolytic *Streptococcus* (GABHS) infection (Mustaq, 2011). The common bacteria isolated from patients having throat infections are *Staphylococcus aureus, Streptococcus pyogenes*, *Proteus spp*. *Klebsiella spp*., and *Pseudomonas aeruginosa*. The primary pathogen of oropharynx is *Streptococcus pyogenes* where *Staphylococcus aureus* is a secondary pathogen (Moirangthem and Gurung, 2013). The pathogens common to acute sinusitis, namely *Streptococcus pneumonia*e and *Staphylococcus aureus*, are also found in the chronic form. Anaerobes, enteric bacteria and *Pseudomonas aeruginosa* are often present. Anaerobes generally account for up to 25% of infections but different studies have yielded widely differing results (Sener *et al*., 1996). The frequency of each pathogen varies according to age, season, geographical area and also the immune status of the patient (Claassen, 2012). Patients with bacterial pharyngitis generally do not have rhinorrhea, cough, or conjunctivitis (Vincent *et al*., 2004). The incidence of bacterial pharyngitis is increased in temperate climates during winter and early spring (Zaoutis and Klein, 1998). There is often a history of streptococcal throat infection (strep throat) within the past year (Vincent *et al*., 2004). GABHS is the most common bacterial cause of pharyngitis (Bisno, 2001). GABHS infection symptoms of strep throat may include pharyngeal erythema and swelling, tonsillar exudate, edematous uvula, palatine petechiae, and anterior

cervical lymphadenopathy (Vincent *et al*., 2004). Patients with untreated streptococcal pharyngitis are infectious during the acute phase of the illness and for one additional week (Ebell *et al*., 2000). Other bacteria that can infect the tonsils and pharynx include *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Neisseria gonorrhoeae*. Fungi such as *Candida* species may cause sore throat in immunocompromised patients (Bartlett *et al*., 2015). Most forms of fungal sinusitis are found more commonly in males. The exact reason for this predisposition is not known (Chatterjee and Chakrabarti, 2009). They are commonly caused by members of the class *Zygomycetes* or by *Aspergillus* spp. This disease occurs more often in the immunocompromised patients, (Gowing and Hamlin, 1960) and associated with a mortality rate exceeding 50%. The disease is characterized by a time course of less than 4 weeks with predominant vascular invasion (Chatterjee and Chakrabarti, 2009). *Streptococcus pyogenes* (group A, Beta-hemolytic) is still the most important pathogen; *Streptococcus pyogenes* can present molecules on its cell wall, like M-protein identical to molecules found on cell membrane of human tissues in the skin, heart, joints, and brain. This mechanism of fooling the immune system is called (Molecular Mimicry). GAS produces at least 18 exotoxins able to act as Super-antigens that can avoid the MHC system processing immune response and induces exaggerated, nonspecific, harmful humoral response. All aseptic complications recognized till now following bacterial URTI are induced by GAS (Kirvan *et al*., 2006).

2.5 Management of URTIs

Upper respiratory tract infections are among the leading cause of acute morbidity and the most frequent cause of health service access worldwide (Hueston *et al*., 1999). URTI is the most common cause of antibiotic use in pediatrics in spite of all the argument about antibiotic close overuse and abuse. Many criteria and scores were developed to help clinicians find their way for the best use of antibiotics in treating URTI (Alsaeed *et al*., 2017). The management of URTIs depends on how soon the morbidity and mortality of the disease can be limited after the infection occurs (Mansoor, 2009). Antibiotics are prescribed more frequently in patients with URTIs (Teng *et al*., 2004 ; Chang and Thrasher, 2012).

Antibiotics have no role in the management of the common cold or any mild URTI. However, almost 75% of adults with URTIs are prescribed antibiotics by their physicians (Hall *et al*., 2011). Antibiotics misuse/overuse is an important public health issue that affects the community and the individual. Irrational reuse of antibiotics can lead to the development of antibacterial resistance, increasing the burden of chronic diseases, raising costs of health services, and the development of adverse effects (Alumran *et al*., 2011). The medical management of ABRS includes the use of antibiotics and adjuvants. The goals of therapy are to eliminate infection, decrease the severity and duration of symptoms, and prevent complications (Brook, 2013). This is because many of the predominant bacterial pathogens have developed resistance to commonly used antibiotics (Critchley *et al*., 2007). The emerging antimicrobial resistance among respiratory pathogens leads to the empiric overuse of broad-spectrum antibiotics, which generates selective pressure that promotes the emergence of greater antimicrobial resistance (Magee *et al*., 1999). Amoxicillin is a reasonable first line antibiotic choice for both adults and children, unless there is a high prevalence of B-lactamase producing strains. The higher dose (90 mg/kg/day) is recommended for children at higher risk of amoxicillin resistance, such as those who attend day care, were recently treated with antibiotics, or are under the age of 2 years. The addition of potassium clavulanate can also counter this antibiotic resistance. The most common side effects include abdominal cramping and diarrhea, which are quickly reversed upon discontinuation of the drug. Trimethoprim sulfamethoxazole is an alternative antibiotic in penicillin-allergic individuals; however, up to 20% of *Streptococcus pneumoniae* may be resistant to this alternative. In a meta-analysis of several randomized trials, folate inhibitors were found to be as effective as the newer, more costly antibiotics (Leung and Katial, 2008). Acute *Streptococcus pyogenes* pharyngitis is often a self-limiting disease. Fever usually resolves within 3-5 days and sore throat resolves in one week (Wessels, 2011). Antibiotics help to reduce the severity and duration of symptoms, limit disease spread, and prevent suppuration(e.g., peritonsillar or retropharyngeal abscess, cervical lymphadenitis, otitis media, and mastoiditis) and noninfectious complications such as acute rheumatic fever (Nakhoul and Hickner, 2013). There is some doubt whether post-streptococcal glomerulonephritis can be prevented by antibiotic treatment in streptococcal pharyngitis (Wessels, 2011). Antibiotics are less efficient in improving symptoms when started 2 days after disease initiation

(Nakhoul and Hickner, 2013). However, even when started 1-2 days after symptoms have begun, they are equally effective in preventing rheumatic fever (Barbosa and Müller, 2009). Narrow-spectrum penicillins are the first choice for treatment because of the rarity of documented resistance to penicillin by group A *Streptococcus* during pharyngitis treatment and because of their low cost (Chiappini *et al*., 2011). The recommended oral formulation is penicillin V. For complete agent eradication, it is important to emphasize that oral penicillin should be taken for 10 days, even if symptoms subside within a few days (Barbosa and Muller, 2009). It is difficult for patients to maintain treatment for ten days because of the drug's poor palatability, the need to take it several times a day, and the rapid symptom resolution. Because amoxicillin is reportedly equally effective and has a better palatability, it is a suitable option for children (Chiappini *et al*., 2011). In patients with penicillin allergy, cephalosporins can be an acceptable alternative, although a primary hypersensitivity to cephalosporins can occur (Chiappini *et al*., 2011). The macrolides are another option. *Streptococcus pyogenes* resistance to erythromycin has increased with increased macrolide use (Myers *et al*., 2009). Anti-inflammatory agents such as ibuprofen, ketoprofen, and diclofenac, or analgesic agents such as paracetamol can reduce severe symptoms and high fever (Pelucchi *et al*., 2013). Systemic corticosteroids should not be routinely prescribed in acute pharyngitis (Principi *et al*., 2013). The use of steroids in children displays a substantial enhancement in symptoms with slight side effects and without any effects on disease evolution. The best consequences were realized in verified streptococcal pharyngitis for dexamethasone (10 mg), as well as betamethasone (8 mg) and prednisolone (60 mg) with a perfect decrease in the pain and feeling of illness that associated with acute tonsillitis (Hayward *et al.*, 2012). Antiseptic mouthwashes with chlorhexidine or benzydamine show symptoms relief in children and adults (Cingi *et al*., 2011). Typical herbal gargles contain sage, thyme and chamomile, can lubricate and preserve the mucous membranes. However, several substances containing ethanol as an extraction solvent and are not approved for children <12 years old (Alotaibi, 2017).

A study by Varalakshmi and coworkers out of the 100 samples of paranasal sinusitis. Results of the study showed that most of 100 cases 96 (96%) were positive on culture of which 87 (90.6%) were positive for bacterial culture, 1(1.04%) pure

fungal and 8(8.33%) yielded mixed isolates. Bacterial isolate identified were *Staphylococcus areus*, *Streptococcus pneumoniae*, *Diphtheroids* and *Klebsiella pneumoniae* (Varalakshmi *et al*., 2016). the most commonly found bacteria in sinusitis was *Staphylococcus aureus* 43% of the patients followed by *Klebsiella spp*., (9%) and MRSA, (3%). Fungal organisms identified were *Aspergillus* and *Candida spp*. isolated from (9%) of the patients, which is very high compared to the other studies (Kamath *et al*., 2013). In 2018 Waheed and coworkers had showed that there were 523 consented patients with rhinosinusitis enrolled into the study. There were 51.6% males and 48.4% females. Microscopy culture and sensitivity revealed growths of *Streptococcus* (24.1%) and *Staphylococcus aureus* (18.5%) (Waheed *et al*., 2018). In a study by Udayasri and Radhakumari, (2016) on 125 patients with sinusitis showed that *Staphylococcus aureus* (43.92%*)* was the most common pathogen followed by Coagulase-negative *Staphylococci* (24.29), *Escherichia coli* (7.47%), *Klebsiella* species (11.21%), *Pseudomonas aeruginosa* (8.41%). Fungi isolated were 15 (12.29%) and *Aspergillus flavus* was the most common fungus.

Al Ahmary *et al*., (2012) showed that Fifty-two patients with tonsillitis showed that *Staphylococcus aureus* and *Group B Streptococcus*. Two Gram-negative bacteria, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were also isolated. No anaerobes were isolated Al Ahmary *et al*., (2012). Another study by Jayasimha *et al*. in (2013) on 50 children with tonsillitis showed that 42 *Staphylococcus* strains and 34 *Streptococcus pyogenes* were the most commonly isolated bacteria in tonsillitis. The other organisms isolated were *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and coagulase negative *Staphylococcus* (Jayasimha *et al*., 2013). Okoye and coworkers in (2016) found that out the most prevalent organisms causing tonsillitis were *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Escherichia coli*, *Staphylococcus* species, *Proteus* species, *Pseudomonas* species and *Klebsiella* species. *Streptococcus pyogenes* presented the highest number of the bacterial isolates recovered. The prevalence of bacterial tonsillitis, specifically *Streptococcus pyogenes*, is 8% in infants aged between 5 weeks to 11 months and 17% in children aged between 1-5 years (Okoye *et al*., 2016). Manandhar and coworkers studied the Bacteriological evaluation of tonsillar surface and tonsillar core micro flora in patients under going tonsillectomy, on forty-six patients with

tonsillitis showed *Staphylococcus aureus* was the commonest isolated organism in surface and core of tonsil followed by *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Klebseilla pneumoniae*, *Streptococcus viridians*. The maximum number of patients in children between 1-5 years (Manandhar *et al*., 2014). Vijayashree and his coworkers have reported about the bacterial tonsillitis caused mainly by β-haemolytic *Streptococcus*, extent by *Staphylococcus aureus* and several other bacteria. Sensitivity of isolated bacteria to different antibiotics and chemotherapeutic drugs indicated that Gram-positive bacteria were more susceptible to the antibiotics than Gram-negative bacteria. Majority of the isolates were susceptible to the antibiotics penicillin, erythromycin, amphicillin, gentamycin, chloramphenicol, ciprofloxacin, cephalexin, cefotaxime, cephotaxime and amikacin (Vijayashree *et al*., 2014).

The study by Naveen *et al*., (2016) included 100 cases of acute pharyngitis in children between the age group of 5-15 years who attended pediatric outpatient department. Throat swabs were collected from all the 100 cases and subjected to rapid group A *Streptococcus* antigen detection, Gram stain, culture and antibiotic sensitivity testing. Seventeen (17) cases (21.25%) were due to *β hemolytic Streptococci*, (Naveen *et al*., 2016). Sridevi *et al*., (2016) out of the 100 Samples of Pharyngitis showed that *Streptococcus pyogenes* was the commonest isolate, followed by *Staphylococcus aureus* and *Candida albicans*. In 60% it was mixed infection. The susceptibility patterns varied depending on the drugs, but most of the organisms were susceptible to penicillin, erythromycin and vancomycin (Sridevi *et al*., 2016). In a study by Moirangthem and Gurung in (2013) a total of 55 throat swabs were collected from the patients with symptoms of pharyngitis, out of the 55 samples, culture was positive in 37 samples. Twenty one strains of *Staphylococcus aureus*, 13 strains of *Streptococcus pyogenes,* one strain of *Pseudomonas aeruginosa* and two strains of *Proteus spp*. were isolated by Moirangthem and Gurung in (2013). In Jimma town in (2015), results of a study showed that a total of 355 children with pharyngitis, *Streptococcus pyogenes* (GAS) was the most frequent bacteria causing pharyngitis (Tesfaw *et al*., 2015).

The aim of the Research Study

This study is amid :

(1) to found out the prevalence of the upper respiratory tract infection causing nose infection. pharyngitis and tonsillitis among children visiting the Benghazi Pediatric Hospital.

(2) to diagnose the infectious agents (bacteria and fungi).

(3) to study the antibiotic sensitivity of the isolated bacterial pathogens.

CHAPTER 3

MATERIALS AND METHODS

3.1 Collection of Specimens :

Samples were collected from 300 children with signs and symptoms of the upper respiratory tract infections from patients visiting the children hospital in Benghazi-Libya, which included children between the ages of one year to thirteen years. The specimens were collected during a period of 10 months (August 2016 to June 2017). The data collected were analyzed with respect to age, sex and site of collection. Swabs were taken from tonsillar surface, pharynx and from the infected nose. Swabs suitable for taking specimens of exudates from the tonsils, pharynx and nose consist of a sterile pledget of absorbent material, usually cotton (Wellkang Ltd, London). The swab is a convenient sampling method, it was well loaded with the exudates to be sampled and transmitted promptly to the laboratory for processing (Collee *et al*., 1996). Also for the prevention of specimen from drying out and to avoid the death of the microorganisms.

3.2 Culturing the Specimens :

Swabs were cultured on two blood agar plates, one incubated aerobically at 37°C for 24 hours and the other anaerobically, also a third culture was on chocolate agar incubated in a carbon dioxide enriched atmosphere at 37°C for 24-48 hours .Also, a fourth culture was on MacConkey agar plate incubated aerobically at 37°C for 24 hours. Finally, how either fungal infection was suspected, mycelium formation was cultured on sabouraud agar and incubated aerobically for 24 hours at 37°C and then incubated at room temperature for six days.

3.3 Subculturing of the Bacterial Cultures:

It is a standard method for preparing a pure culture for making a second level culture from a well-isolated colony. A tiny bit of cells is transferred onto a separate Sterile medium plate and incubated.

3.4 Identification of the Bacterial and Fungal isolates:

3.4.1 Identification of Bacteria

3.4.1.1 Microscopic Examination of Bacterial pure Colonies

After subculturing, pure colonies were examined microscopically by making Gram-staining to help identify the pathogens in pure cultures by their Gram reaction and their morphology on slide.

3.4.1.2 Biochemical tests :

3.4.1.2.1 Catalase test

Catalase reagent acts as a catalyst in the breakdown of hydrogen peroxide to hydrogen and water. Bubbles of hydrogen are released if the organism is a catalase producer (Cheesbrough, 2000). This test was done to differentiate between those bacteria that produce the catalase enzyme, such as *Staphylococci* and non-catalase producing bacteria such as *Streptococci* (Cheesbrough, 2000).

The method: 2-3ml of 3% hydrogen peroxide solution (H_2O_2) (Oxoid, England) poured into a test tube (Reagenzglaser, Germany). A sterile wooden stick or glass rod was used to take good growth of organism and immersed in the H_2O_2 solution. Positive result showed an active bubbling (Cheesbrough, 2000).

3.4.1.2.2 Coagulase test

Coagulase test was used to differentiate *Staphylococcus aureus* which produces the enzyme coagulase from *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* that do not produce coagulase. This enzyme causes plasma to clot by converting fibrinogen to fibrin (Cheesbrough, 2000). The test requires EDTA (ethylenediamine-tetracetic acid) (Oxoid,England) and anti-coagulated human plasma or Rabbit plasma. The plasma was allowed to warm before being used (Cheesbrough, 2000).

The method: A drop of physiological saline was placed on a slide, and a colony of the tested microorganism from culture was emulsified in it. One drop of plasma was added to the suspension and mixed gently for 10 seconds and the clumping was observed (Cheesbrough, 2000).

3.4.1.2.3 Oxidase test

It was used to identify *Pseudomonas* species from other members of the enterobacteriaceae. The solution used, was TMPD (BDH chemicals limited company, England) in 2.5 ml of sterile distilled water giving a final concentration of (1%).
The method: Two to three drops of a freshly oxidase reagent were placed in a petri dish (Wellkang Ltd, London) and small piece of filter paper (Oxoid, England). The appearance of deep blue-purple colour was observed in few seconds.

3.4.1.2.4 DNAase test

DNase agar (Oxoid, England) is a differential medium that tests the ability of an organism to produce an exoenzyme, called deoxyribonuclease or DNase, that hydrolyzed DNA. DNase agar contains nutrients for the bactreria, DNA, and methyl green is a cation which binds to the negatively-charged DNA, its purpose to see if the microbe can use DNA as a source of carbon and energy for growth. Use of DNA is accomplished by an enzyme called DNase, the medium used is DNase agar with methyl green. The medium is a nutrient agar to which DNA (Oxoid, England) is added. The indicator methyl green produces a mint green medium.

The method: Using a sterile loop, several colonies from an 18-24 hours culture is picked and incubated the plate at 37°C for 24 hours, then after Incubation observe the color change in DNase with methyl green, results interpretation:

- Positive: When DNA is hydrolysed, methyl green is released turning the medium colorless around the test organism.

- Negative: If there is no degradation of DNA, the medium remains green.

3.4.1.2.5 Mannitol Salt Agar

Mannitol salt agar (Oxoid, England) is a selective-differential medium for the isolation of *Staphylococcus aureus*. The significant ingredients of this medium are the

7.5% sodium chloride, mannitol, and the phenol red (PH indicator). The phenol red gives a red appearance to the uninoculated medium. In the presence of acid, the phenol red turns to yellow color.

The method: A plate of mannitol salt agar is streaked with a pure colony of the isolate, incubated at 37°C for 24 hours. If the isolate is *Staphylococcus aureus* (halophilic bacterium), it grows on the medium and ferments Mannitol to acid products which turn the medium to a yellow color.

3.4.1.2.6 Streptocard Enzyme Latex Test :

This test was used for the identification of Streptococcal groups A, B, C, D, E, F and G (Oxoid, England). The procedure was carried out in according to the instruction of the manufacturer as the following:

Streptococcus extracted enzyme of the streptococcal groups supplied in a powder (Oxoid, England), was hydrated by adding 12ml of distilled water, Then four ml of this solution was distributed in each of six test tubes.

1. Five colonies of the test bacterium were emulsified in the enzyme containing test tubes, and incubated at 37°C for 10 minutes.

2. One drop from each emulsified tested bacteria was dispensed into a circular ring on the slide.

3.One drop of latex was added to each of the six rings, then the mixture was spread over the entire area of the rings.

4. The slide was gently rotated, and the agglutination (positive reaction) was read after 1 minute.

3.4.1.2.7 API 20 for Identification of Enterobacteriaceae

API 20 system enables 20 tests to be quickly and easily read for the biochemical identification of anaerobes. Other tests such as colonial and microscopic morphology, Gram stain, should be peformed and the results to confirm or complete the identification table inserted at the end of the package of the kit (Biomerieux, France).

3.4.2 Identification of Fungi

The identification of fungi is based on their colonial morphology, pigmentation and microscopic appearances, shape of fungal spores, and production of conidial heads (Raper and Fennel, 1965).

3.4.2.1 Microscopic Examination of Fungi

The best examination of filamentous fungi is the slide culture of pure colonies and for *Candida spp* the germ tube test.

3.4.2.1.1 Slide Culture

One cm of sabouraud dextrose agar block was cut from the sterile plate and transferred to a sterile microscope slide (Oxoid, England) resting on a bent glass rod in a petri dish. Small fragment of the test colony was inoculated using a mounted needle into the four sides of the block, which is then covered with a sterile coverslip. A few drops of water are added to the dish for not drying and then covered and incubated at 25°C. After 7-14 days the agar block is discarded and the cover slip is placed in lactophenol cotton blue (Oxoid, England) on a fresh glass slide (Stokes *et al*., 1993); (Baiu, 2003).

3.4.2.2 Identification of *Aspergillus sp*

The identification of the organism depnds on the colonial morphology and pigmentation and producing of conidial heads (Varalakshmi *et al*., 2016). *Aspergillus niger* colonies initially white, quickly becoming black, hyphae are hyaline and distinctly septate. Conidiphores are long and vesicle is usually not seen because it is covered with thick ball of spores (Sutton *et al*., 1998).

3.4.2.3 Identification of *Candida albicans*

Candida albicans grows well on sabouraud agar, the colonies are white and having a distinctive yeast smell and the pudding cells can be easily seen by direct microscopy in stained or unstained preparation. *Candida albicans* can be identified presumptively by a simple germ tube test.

3.4.2.3.1 Germ tube test:

The simple identification method is the germ tube test. Very small quantities of a colony are used for inoculation of 0.5 ml of mammalian serum (horse, human) in small plastic tubes (Reagenzglaser, Germany). Then incubated for three hours at 37°C. At this stage a small loopful of serum is examined microscopically for the presence of small germ tubes originating from yeast cells (Stokes *et al*., 1993).

3.5 Antimicrobial Susceptibility Testing

The selection of appropriate antimicrobial therapy is the only decision made in medical practice that requires consideration of the pharmacology of a drug in different species, the patient and microorganism, simultaneously.

All bacterial isolates were tested for their sensitivity to antibiotics using disk diffusion method (Eldeeb and Khashan, 2006), and using Mueller-Hinton agar (Oxoid, England) for Gram negative bacteria and blood agar for gram positive bacteria. The following oxoid antibiotic discs were used: Amoxycillin clavulanic acid, fucidic acid, chloramphenicol, ciprofloxacin, norfloxacin, gentamycin, ampicillin, amoxicillin, amikacin as shown in table (1).

Table (1): Drugs used in the antimicrobial susceptibility testing

SL.NO	Antibiotic	Symbol	Concentration
1	Augmentin	AMC	30 µg
2	Fucidic Acid	FA	10 µg
3	Chloramphenicol	C	10 µg
4	Ciprofloxacin	CIP	5 µg
5	Norfloxacin	NOR	15 µg
6	Gentamycin	CN	10 µg
7	Ampicillin	AM	30 µg
8	Amoxicillin	AX	30 µg
9	Amikacin	AK	10 µg

CHAPTER 4

RESULTS

A total of 300 specimens of swabs were collected from patients who had upper respiratory tract infections for this study during a period of 10 months (August 2016 to June 2017). The specimens were taken from outpatient department (OPD) of the Children's Hospital in Benghazi, Libya, from different areas of upper respiratory tract which were nose, tonsils and pharynx, aged between one year to 13 years.

The data collected were analyzed with respect to age, sex and areas of upper respiratory tract. The study showed that all patients tested had a single microbial infection.

The isolates were examined morphologically, by biochemical tests which were the catalase test, Oxidase test, coagulase test, Mannitol salt agar test, DNase test, API 20 and Streptocard Enzyme Latex Test, showed positive results with other tests. Those results were used as a complement to other characteristics of bacterial species (Gram staining reaction, morphology, conditions growth), for identifying those genera and species). Examination of filamentous fungi is the slide culture of pure colonies and for *Candida spp* the germ tube test.

In this study three hundred samples from patients who suffered upper respiratory tracts infections (URTIs). Namely, 126 patients who suffered tonsillitis, 85 patients who had nose infections (sinusitis) and 89 patients with pharyngitis.

Some influencing factors on prevalence of inflammation on the upper respiratory tract were studied like age, gender, and the distributions of infection over the seasons of the year during study period.

The relation of some factors with infection percentage:

4.1 Age Distribution

The table (2) shows the prevalence of infection in different group ages with the difference of distribution of proportions as the results showed that the highest percentage of infection was among the age group of (7-10 years) with 105 patients

followed by age group (4-7 years) with 102 patients, then the age group (10-13 years) with 70 patients and the lowest age groups was (4-7 years). The upper respiratory tract infections increase of the age of the patients.

Table (2): Age distribution of patients

S.NO.	Age (years)	Frequency patients	Percent (%)
1	1-4	23	7.7
2	4-7	102	34
3	7-10	105	35
4	10-13	70	23.3

4.2 Gender distribution of patients

Table (3) and figure (4) shows prevalence of infection in among males by (57.3%) more than that of females by (42.7%). The distribution was more in male patients compared to female patients.

Table (3): Gender distribution of patients

Gender	Frequency patients	Percent (%)
Male	172	57.3%
Female	128	42.7%

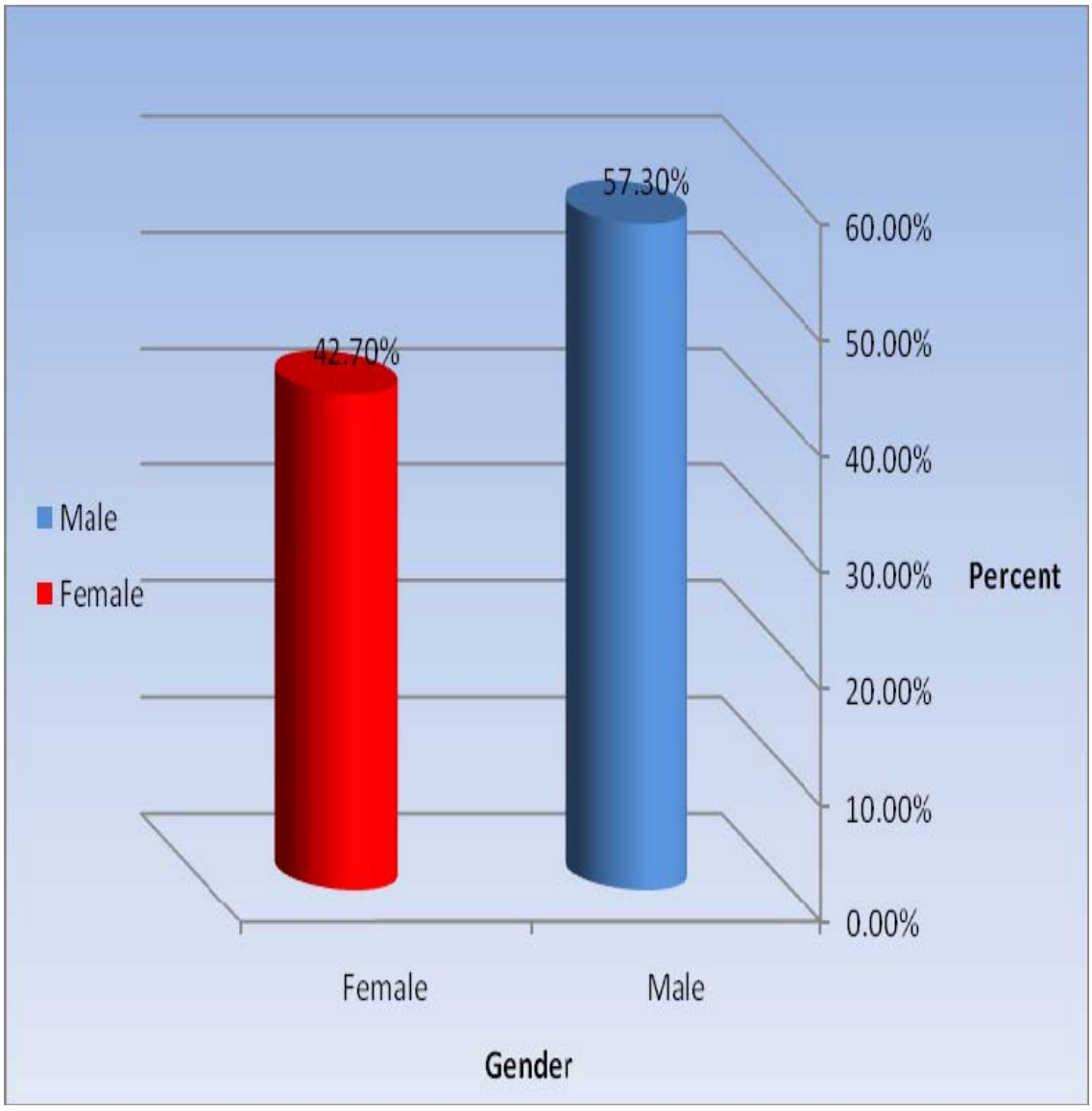

Figure (4): Distribution of patients according to gender

4.3 Seasonal Prevalence of the URTI Pathogens

Table (4) and figure (5) show the seasonal distribution of the upper respiratory tract in the period of august 2016 to june 2017 was studied, which the results showed the highest percentage of infection from winter by 36% and in second place spring by 28%, while fall by 25% and the lowest percentage of infection was in summer by 11%.

Table (4): Seasonal prevelance of URTIs pathogens

S.NO.	Season	Frequency	Percent (%)
1	Autumn	76	25.3
2	Winter	108	36
3	Spring	84	28
4	Summer	32	10.7

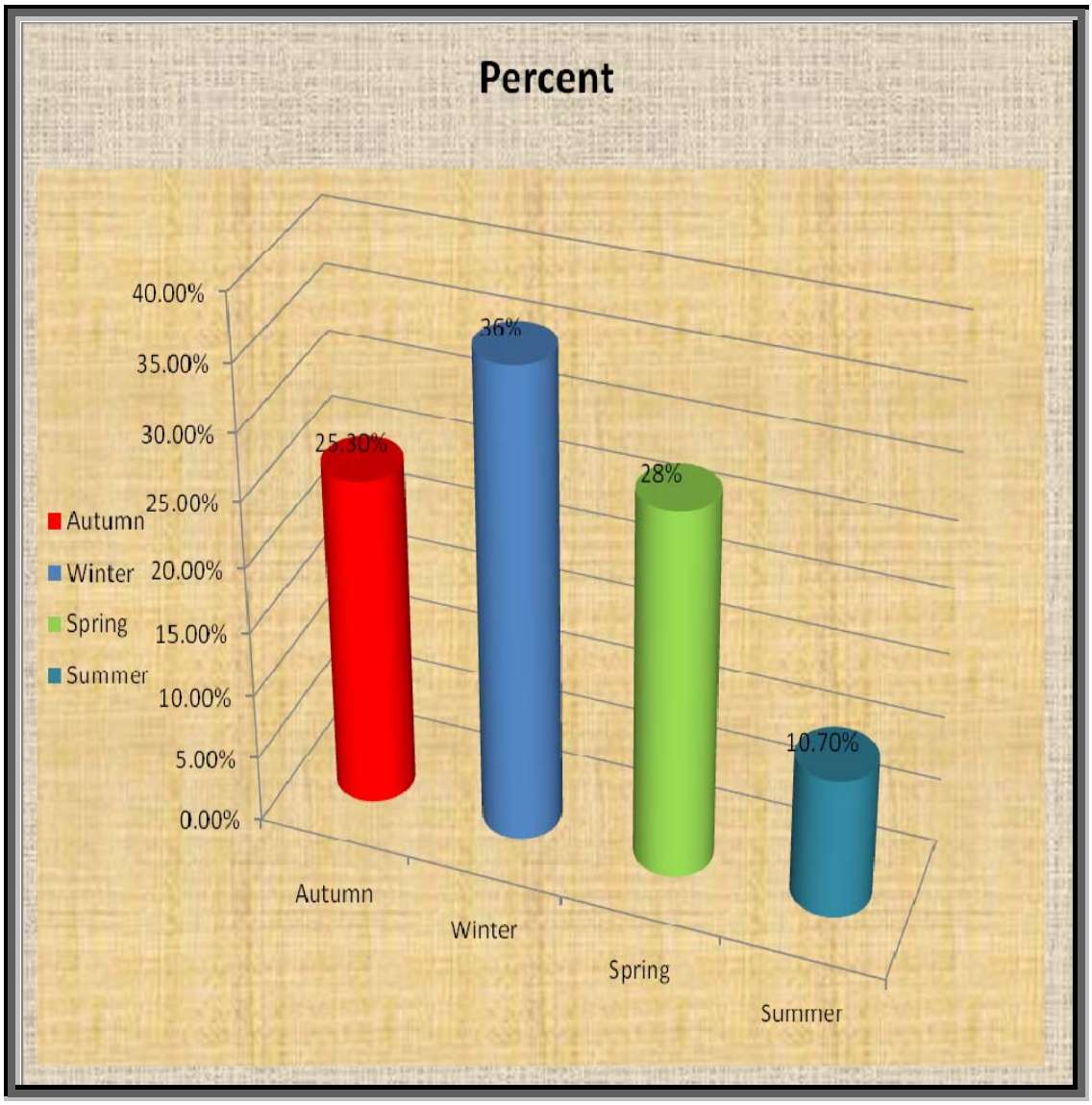

Figure (5): Seasonal prevalence of URTIs pathogens

4.4 Type of Infection

In this study we studied three hundred samples from patients who suffered upper respiratory tracts infections (URTIs). Distribution of the patients according to Type of infection showed that who suffering from tonsillitis were 42% (126 patients), nose infection 28.3% (85 patients) and pharyngitis 29.6% (89 patients), is shown in table (5) and figure (6).

Table (5): Type of infection distribution

SL.NO	Frequency	Percent (%)
Tonsillitis	**126**	**42**
Nose infection	**85**	**28.3**
Pharyngitis	**89**	**29.6**

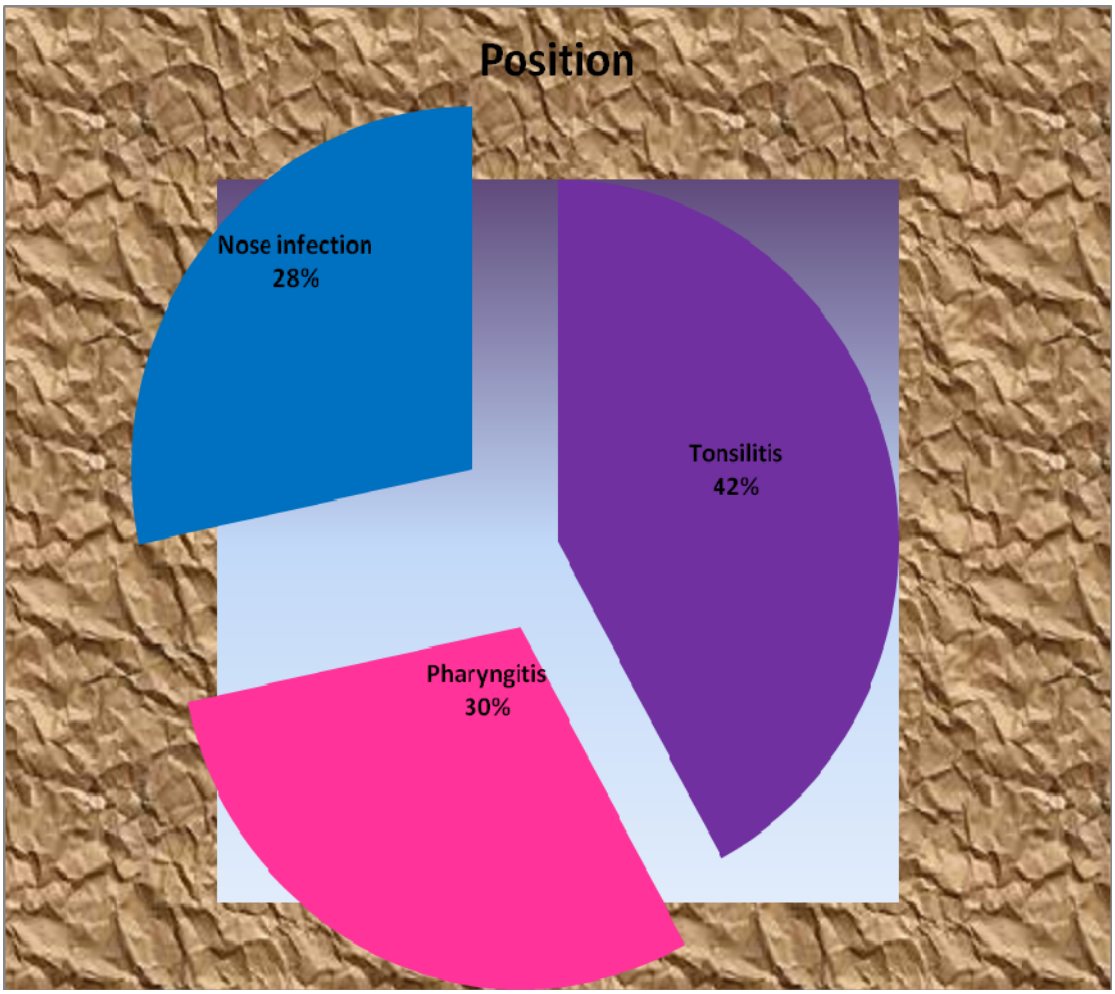

Figure (6):Type of infection distribution

4.5 Distribution of Patients according to their Age and Type of Infection

Table (6) shows that inflammation of the nose is more common in the age group of (4-7 years) by 32%. Pharyngitis infection is more in the (4-7 years) by 36% and lowest percentage in the age group (1-4 years), while tonsillitis is more common in the age group of (7 to 10 years) by 39% and lowest in (1 to 4 years) by 11%.

Table (6): Distribution of patients according to their age and type of infection

Type infection / Age group	1- 4y	4- 7y	7-10 y	10-13 y
Nose infection	7 (8.2%)	27 (31.8%)	26 (30.6%)	25 (29.4%)
Pharyngitis	5 (5.6%)	32 (36%)	30 (33.7%)	22 (24.7%)
Tonsillitis	11 (8.7%)	43 (34.1%)	49 (38.9%)	23 (18.3%)

4.6 Distribution of Patients according to their Gendr and Type of infection

Table (7) and figure (7) shows the distribution of the patients according to their sex and the type of infection. This results reveal that the infection of all types were higher among males than females. This findings are as following: Nose infection among males was 58% while infected females were 41%. In the case of pharyngitis 63% were male and just 37% were females. As for tonsillitis 53% was male and 48% female.

Table (7): Distribution of patients according to their gender and type of infection

Type infection	Gender	Male	Female
Nose infection		**50 (58.8%)**	**35 (41.2%)**
Pharyngitis		**56 (62.9%)**	**33 (37.1%)**
Tonsillitis		**66 (52.4%)**	**60 (47.6%)**

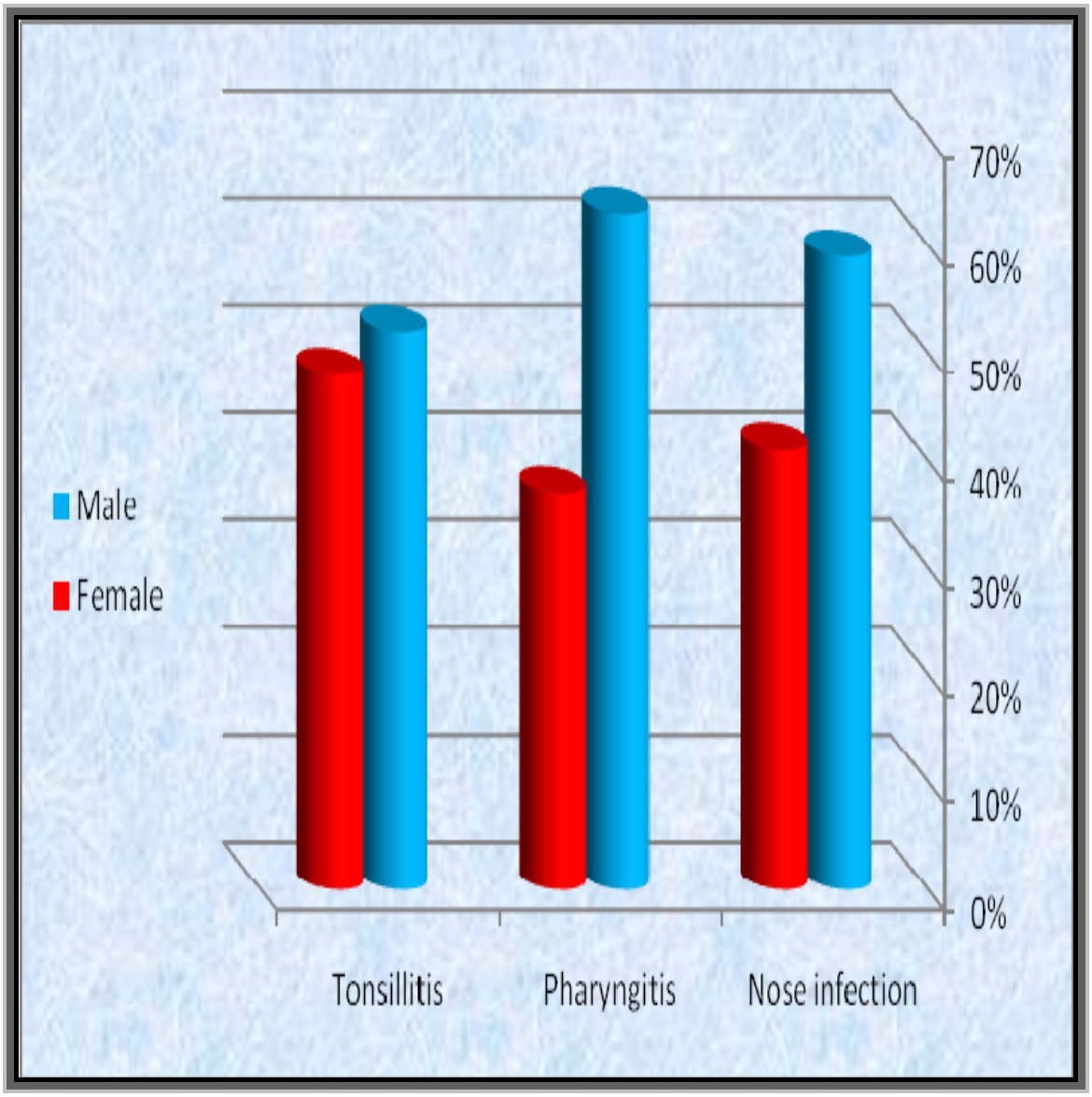

Figure (7): Type of infection and gender distribution

4.7 Distribution of patients based on the impact of the Seasons and the type of infection:

The table (8) shows that inflammation of the nose is more common to be infected by in winter by 37.6% and less to be infected by in summer by 8.2%, also pharyngitis is highly to be infected by in winter by 32.6% and less in summer by 15.7%, while tonsillitis the highest percentage to be infected by in winter by 37%, and the lowest percentage in summer by 8.7%. The spread of infection was in the winter and its disappearance in the summer. In this study that the total infected cases were during the spring and autumn, and a little in the summer (8.2%).

Table (8): Distribution of URTI types according to seasonal infection

Infection type	Autumn	Winter	Spring	Summer	Total
Nose infection	23 (27.1%)	32 (37.6%)	23 (27.1%)	7 (8.2%)	85 (28.3%)
Pharyngitis	25 (28.1%)	29 (32.6%)	21 (23.6%)	14 (15.7%)	89 (29.7%)
Tonsillitis	28 (22.2%)	47 (37.3%)	40 (31.7%)	11 (8.7%)	126 (42%)

4.8 Micro-organisms Distribution according to the Type of Infection

Table (9) shows the prevalence of microorganisms isolated from the patients according to the type of infection. The results of the microbiall growth showed that 289 samples of bacterial isolates (96.3 %), and 8 samples (2.7%) of fungal isolates were positive of growth, while there was no growth of pathogens in 3 patients or (1%) of samples.

Table (9): Kind of microbial agents causing the infection

SL.NO.	Frequency	Percent (%)
Bacterial infection	**289**	**96.3**
Fungal infection	**8**	**2.7**
No growth	**3**	**1**

Micro-organisms Distribution according to the Type of Infection. Of the isolated strains, *Staphylococcus aureus* was the most frequent in sinusitis followed by *Streptococcus pyogenes* which was the most common in pharyngitis and the most microorganisms in tonsillitis were *Streptococcus pneumoniae* and *Streptococcus pyogenes*. Data in the table show that 297 different strains were isolated. *Staphylococcus aureus* was the most prevalent organism (31%), followed by *Streptococcus pyogenes* (28.3%), *Streptococcus pneumoniae* (21.3%), *Klebsiella pneumoniae* (8%), *Pseudomonas aeruginosa* (6%), *Aspergillus niger* (2.3), *Candida albicans* (0.3%), *Escherichia coli* (0.6%), *Bacillus cereus* (0.6%) and *Staphylococcus epidermidis* (0.3%) as shown in table (10).

Table (10): Micro-organisms distribution according to type of infection

Type of infection / Type of pathogen	Total (URTIs)		Sinusitis		pharyngitis		Tonsillitis	
	F*	%	F	%	F	%	F	%
No growth	3	1	0	0	3	3.4	0	0
1- *Staphylococcus aureus*	93	31	80	94.1	10	11.2	3	2.3
2- *Streptococcus pyogenes*	85	28.3	1	1.2	56	62.9	28	22.2
3- *Streptococcus pneumoniae*	64	21.3	1	1.2	8	9	55	43.6
4- *Klebsiella pneumoniae*	24	8	1	1.2	2	2.2	21	16.6
5- *Pseudomonas aeruginosa*	18	6	1	1.2	0	0	17	13.4
6- *Escherichia coli*	2	0.6	0	0	0	0	2	1.6
7- *Bacillus cereus*	2	0.6	0	0	2	1.6	0	0
8-*Staphylococcus epidermidis*	1	0.3	0	0	1	1.1	0	0
9- *Candida albicans*	1	0.3	0	0	1	1.1	0	0
10- *Aspergillus niger*	7	2.3	1	1.2	6	6.7	0	0

* means frequency

4.9 Antimicrobial susceptibility patterns:

Antimicrobial susceptibility patterns for the isolated strains were studied using Bauer and Kirby method. The sensitivity test of the eight isolated species of bacteria was carried out using nine types of antimicrobial drugs. Tables (11 to 18) show the susceptibility patterns of the isolated microorganisms against different antimicrobial agents. Both ciprofloxacin and gentamicin showed the best activity, followed by chloramphenicol, norfloxacin and amikacin, while the low activity were by ampicillin and amoxicillin.

The results of the study showed all *Staphylococcus aureus* strains isolated were resistant to ampicillin and high sensitivity (100%)to fucidic acid, ciprofloxacin (98.9%), chloramphenicol (97.8%) and gentamycin (96.7%) as illustrated at the table 11.

Chloramphenicol, ciprofloxacin, gentamycin, amoxicillin and fucidic acid had a high effect (100%) on *Streptococcus pyogenes* and amikacin had a moderate effect on this bacterium (63.5%). (Table 12) .

Streptococcus pneumoniae had few resistance to amikacin (42.1%) and higher sensitivity of ciprofloxacin (100%), fucidic acid (100%) and amoxillin clavulanic acid (100%), as illustrated at the table 13.

Ciprofloxacin (100%), gentamycin (91.6%) and amikacin (87.5%) had a high effect on *Klebsiella pneumoniae*, whereas high resistant to amoxillin and ampiciilin had a low effect(100%) on this bacteria (table 14), How ever *Pseudomonas aeruginosa* had high sensitivity of gentamycin (94.4%) and ciprofloxacin (88.8%), and resistant to ampicillin (100%). (Table 15).

Escherichia coli had high sensitivity to ciprofloxacin (100%), gentamycin (100%), amikacin (100%) and norfloxacin (100%). And resistant to ampicillin, a amoxillin clavulanic acid, fucidic acid and amoxillin (100%). (Table 16).

Staphylococcus epidermidis and *Bacillus cereus* showed ahigh sensitivity (100%) to all antimicrobial agents, (table 17 and table 18).

Table (11): Antimicrobial sensitivity test of *Staphylococcus aureus*

S.NO.	Antibiotic Type	Resistant	Percent (%)	Sensitivity*	Percent (%)
1	AMC	18	19.35	75	80.6
2	FA	0	0	93	100
3	C	2	2.1	91	97.8
4	CIP	1	1	92	98.9
5	NOR	27	29	66	70.9
6	CN	3	3.2	90	96.7
7	AM	93	100	0	0
8	AX	25	26.8	68	73.1
9	AK	9	9.67	84	90.3

*Sensitivity was counted when the diameter of the inhibition zone was more than 14 mm or higher

Table (12) : Antimicrobial sensitivity test of *Streptococcus pyogenes*

SL.NO.	Antibiotic Type	Resistant	Percent (%)	Sensitivity	Percent (%)
1	AMC	17	20	68	80
2	FA	0	0	85	100
3	C	0	0	85	100
4	CIP	0	0	85	100
5	NOR	7	8.2	78	91.7
6	CN	0	0	85	100
7	AM	5	5.8	80	94.1
8	AX	0	0	85	100
9	AK	31	36.4	54	63.5

Table (13): Antimicrobial sensitivity test of *Streptococcus pneumoniae*

SL.NO.	Antibiotic Type	Resistant	Percent (%)	Sensitivity	Percent (%)
1	AMC	0	0	64	100
2	FA	0	0	64	100
3	C	5	7.8	59	92.1
4	CIP	0	0	64	100
5	NOR	12	18.7	52	81.2
6	CN	6	9.3	58	90.6
7	AM	10	15.6	54	84.3
8	AX	12	18.7	52	81.2
9	AK	37	57.8	27	42.1

Table (14): Antimicrobial sensitivity test of *Klebsiella pneumoniae*

SL.NO.	Antibiotic Type	Resistant	Percent (%)	Sensitivity	Percent (%)
1	AMC	10	41.6	14	58.3
2	FA	8	33.5	16	66.6
3	C	18	75	6	25
4	CIP	0	0	24	100
5	NOR	8	33.3	16	66.6
6	CN	2	8.3	22	91.6
7	AM	24	100	0	0
8	AX	24	100	0	0
9	AK	3	12.5	21	87.5

Table (15): Antimicrobial sensitivity test of *Pseudomonas aeruginosa*

S.NO.	Antibiotic Type	Resistant	Percent (%)	Sensitivity	Percent (%)
1	AMC	8	44.4	10	55.5
2	FA	10	55.5	8	44.4
3	C	14	77.7	4	22.2
4	CIP	2	11.1	16	88.8
5	NOR	3	16.6	15	83.3
6	CN	1	5.5	17	94.4
7	AM	18	100	0	0
8	AX	14	77.2	4	22.2
9	AK	8	44.4	10	55.5

Table (16): Antimicrobial sensitivity test of *Escherichia coli*

S.NO.	Antibiotic Type	Resistant	Percent (%)	Sensitivity	Percent (%)
1	AMC	2	100	0	0
2	FA	2	100	0	0
3	C	0	0	2	100
4	CIP	0	0	2	100
5	NOR	0	0	2	100
6	CN	0	0	2	100
7	AM	2	100	0	0
8	AX	2	100	0	0
9	AK	0	0	2	100

Table (17): Antimicrobial sensitivity test of *Bacillus cereus*

S.NO.	Antibiotic Type	Resistant	Percent (%)	Sensitivity	Percent (%)
1	AMC	0	0	2	100
2	FA	0	0	2	100
3	C	0	0	2	100
4	CIP	0	0	2	100
5	NOR	0	0	2	100
6	CN	0	0	2	100
7	AM	0	0	2	100
8	AX	0	0	2	100
9	AK	0	0	2	100

Table (18): Antimicrobial sensivity test of *Staphylococcus epidermidis*

S.NO.	Antibiotic Type	Resistant	Percent (%)	Sensitivity	Percent (%)
1	AMC	0	0	1	100
2	FA	0	0	1	100
3	C	0	0	1	100
4	CIP	0	0	1	100
5	NOR	0	0	1	100
6	CN	0	0	1	100
7	AM	0	0	1	100
8	AX	0	0	1	100
9	AK	0	0	1	100

CHAPTER 5

DISSCUTION

In this project we studied three hundred samples from patients who suffered upper respiratory tracts infections (URTIs). Namely, 126 patients who suffered tonsillitis, 85 patients who had nose infections (sinusitis) and 89 patients with pharyngitis. During the taking of the samples, we found out that male patients were more than female patients (172 males and 128 females). These findings were in aggrement with other researchers. These were He *et al*., (2014); Falagas *et al*., (2007); Asghar *et al*., (2017); Eldeeb and Khashan, (2006). Upper respiratory tract infections are the most common group of illnesses in young children. In the preschool children, the recorded incidence approximated one medical consultation per annum, although obviously many other episodes are treated without medical referral, and their frequency has been shown to be related to seasonal factors, family size, age and school attendances, especially in developing countries (Masavkar and Naikwadi, 2016). In the present study, maximum cases were observed in the age group (7-10 years) with 105 patients followed by age groups (4-7 years) with 102 patients which were in agreement with Stover and Litwin, (2014). School children are quite susceptible to the disease because tonsillitis can be transmitted directly from person to person as well as via formites (Okoye *et al*., 2016). The reasons for such high incidence in school children may be due to low immunity in the children, cross infection because of overcrowded class rooms and poor ventilation of the class rooms (Vijayashree *et al*., 2014). The results showed that 36% of the infected cases were during the winter season; this was in agreement with Van Der Gaag and Van Droffelaar, (2012); Tamerius *et al*., (2013); Shek and Lee, (2003).

Rhinosinusitis is a widely prevalent disease affecting more than 14% of adults and children (Poole, 1999). There were differences in the nose infection cases regarding sex. Hence, we found that male patients were more than female patients suffering from nose infection. These results were in agreement with (Kamath *et al*., 2013) and (Waheed *et al*., 2018). Probably because a number of patients admitted were more than female patients (Vijayashree *et al*., 2014). The seasonal variation played an important role

regarding nose infection and the seasonal or cyclic variation incidence is a notable feature of many nose infections. In this study we found a high increase in the infected cases during the winter months. Gregory and wald, (2011) found that the peak prevalence of complications of acute bacterial sinusitis (ABS) occurs in the winter months. The seasonal exposure to cold air causes an increase in the incidence of URTI due to cooling of the nasal airway (Eccles, 2002).

Regarding the etiological agents that were isolated and identified in the present study, 85 clinical isolates were isolated from patients suffering from nose infection. *Staphylococcus aureus* was contributing to the highest percentage followed by *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae, Pseudomonas aeruginosa*, and finally by the fungus *Aspergillus niger*. In most of studies investigating the microbiology of chronic sinustis, it has been found that *Staphylococcus aureus* was the most common organism isolated from sinusitis as reported by Montgomery *et al*., (1990); Zaki, (2000); Eldeeb and Khashan, (2006); Kamath *et al*.,(2013); Busaba *et al*., (2004); Doyle and Woodham, (1991). These results were similar to the six mentioned studies in the kind of the pathogens and also in having *Staphylococcus aureus* the most common organism isolated. With *Aspergillus niger* with one isolate or (1.2%), similar results have been observed by Kamath *et al*., (2013). The pathogens *Streptococcus pyogenes, Streptococcus pneumoniae, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were isolated as one single organism. Also similar results were found by Udayasri and Radhakumari, (2016); Eldeeb and Khashan, (2006); Santos *et al*., (2017); Attia (1992); Kamath *et al*., (2013); Busaba *et al*., (2004) and Doyle and Woodham, (1991).

Acute pharyngitis is the most common upper respiratory tract infection responsible for significant morbidity in the childhood (Naveen *et al*., 2016). In this study we found that male patients with pharyngitis were more than female. These results were in agreement with other researchers who were Agarwal *et al*., (1981); Bach *et al*., (1996); Naveen *et al*.,(2016). These results showed that most of patients with pharyngitis belonged to 4-7 years of age. These results were in agreement with Naveen *et al*., (2016). In the present study, pharyngitis had the highest percentage in winter followed by Autumn and the spring. These results were in agreement with (Vincent *et al*., 2004). Peak seasons for sore throat included late winter and early spring (Gerber, 1998). The incidence of bacterial pharyngitis is increased in temperate climates during winter and

early spring (Zaoutis and Klein, 1998). Streptococcal pharyngitis in temperate climates, is highest in winter and early spring (Wessels, 2011).

Streptococcus pyogenes was the most common organism isolated followed by *Staphylococcus aureus*, similar results have been observed by others Agarwal *et al*., (1981); Naveen *et al*., (2016) and Sridevi *et al*., (2016). These findings were quite with observations of (Sobhan *et al*., 2001) who also found most prevalent organism in throat swab cultures was *Streptococcus pyogenes*. The high prevalence of *Streptococcus pyogenes*, among patients of pharyngitis was also reported by other studies carried out by Roos *et al*.,(1985); Fluckiger *et al*.,(1998); Zwart *et al*., (2001); Eldeeb and Khashan, (2006) and Gupta *et al*., (1992). *Staphylococcus aureus* was the second isolated pathogen in pharyngitis. The present results were in agreement with Naveen *et al*., (2016); Chakrabarthi *et al*., (1997); Gulati and Prabhakar, (1981). *Streptococcus pneumoniae* isolates with pharyngitis in this study made (9%), our results were in agreement with Eldeeb and Khashan, (2006). *Bacillus cereus* was also isolated from pharyngitis patients but occurred at much lower rates with two isolates. *Staphylococcus epidermidis* was isolated as one isolate of a patient with pharyngitis. Bacteria of the genus *Staphylococcus* belong to the commensal flora of humans and other animals and may act as opportunist pathogen. *Staphylococcus epidermidis* is considered one of the most common species of this genus, and is frequently isolated from several inflamed tissues and organs, including the nasal cavity (Fornazari *et al*., 2012). The fungal species that caused nose infection in our study were *Aspergillus niger* and *Candida albicans*. This result correlates with the study reported by Sridevi *et al*., (2016).

Tonsillitis or throat infection is one of the most frequent health problems worldwide (Klug, 2009). In the present study of tonsillitis 126 specimens of them 66 were in males and 60 in females. Some other studies that were similar to our findings were found by Vijayashree *et al*., (2014); and Al Ahmary *et al*., (2012), but the present study is inconsistent with other studies in different percents that males were less than females (Shah *et al*., 2014; and Okoye *et al*., 2016). In this study the occurrence of tonsillitis with respect to population distribution was found to vary differently. Among the reported age groups, maximum tonsillitis cases were observed in the age group (7-10 years) followed by age group (4-7 years). That result correlates with the studies reported by Middleton *et al*., (1989); and Vijayashree *et al*., (2014). The seasonal effects were very important regarding tonsillitis, in our results 37% of the infected cases were

during the winter season. Tonsillitis is more common in younger people, especially in Autumn and Winter (Bartlett *et al*., 2015). and during spring the infection of tonsils was in increase. The upper respiratory tract infection occurrence in the season with highest prevalence (winter-spring) (Peros and Tekavec, 2014).

Streptococcus pyogenes strains were the second organisms causing tonsillitis which was found in our study 19.23% of all isolates. This is similar to a study by Eldeeb and Khashan, (2006); Badr, (1991); and El-Maraghy, (1985). *Staphylococcus aureus* found in present study in three cases, it is inconsistent with other studies conducted in different areas which showed that *Staphylococcus aureus* was the most predominant isolate (Eldeeb and Khashan, 2006 and Al Ahmary *et al*., 2012). *Klebsiella pneumoniae* (8%) and *Pseudomonas aeruginosa* (6%) were found in our study. Our results were in agreement to Vijayashree *et al*., (2014); Okoye *et al*., (2016); Baum *et al*., (2010); Jayasimha *et al*., (2013); Surrow *et al*., (1989); Brook *et al*., (1981) and Eldeeb and Khashan, (2006). The difference in the results may be attributed to that organisms responsible for chronic suppurative media which varied from one place to another depending on socioeconomic conditions, which are considered important predisposing etiological factors (Okafor, 1984).

Staphylococcus aureus strains were highly resistant to ampicillin (100%), and sensitive to fucidic acid (100%), ciprofloxacin (99%), chloramphenicol (98%) gentamicin (96.7%) and amikacin (90.3%). A study by Okoye *et al*., (2016) showed that *Staphylococcus aureus* was (100%) sensitivite to gentamicin. Eldeeb and Khashan, (2006) reported that amikacin and gentamicin were the most active antibiotics showing 100% activity followed by ciprofloxacin (98.98%). Zaki, (2000) reported 100% activity for ciprofloxacin, amikacin and gentamicin. Results of the mentioned studies agree with those of the present in having quinolones the most active antibiotics against *Staphylococcus aureus.* Martin, (2001) recommended the use of ciprofloxacin as an empiric therapy of presumed *Staphylococcus aureus* infections. *Streptococcus pyogenes* strains were highly sensitive to most antibiotics tested however; ciprofloxacin, chloramphenicol, gentamycin, amoxillin and fucidic acid had the greatest activity (100%) against *Streptococcus pyogenes* followed by ampicillin (94.1%), norfloxacin (91.7%). Baquero *et al*., (1999) reported that the susceptibility of *Streptococcus pyogenes* was (90%) for ciprofloxacin. *Streptococcus pneumoniae*: ciprofloxacin, fucidic acid and amoxicillin/ clavulanic acid (augmentin) showed the greatest activities

(100%) against *Streptococcus pneumoniae* strains followed by chloramphenicol and gentamicin. Hawan, (2000) reported 94.74% activity for ciprofloxacin. *Klebsiella pneumoniae*: ciprofloxacin showed (100%) activity against *Klebsiella pneumoniae* isolates. Gentamycin (91.6%) and amikacin (87.5%) also showed high activities. High resistance to amoxicillin and ampicillin. El-Daly *et al*., (1990) reported (100%) activity for gentamicin, Kamal, (1999) found that amikacin and ciprofloxacin were the most potent antimicrobials against *Klebsiella* spp. *Pseudomonas aeruginosa*: the following susceptibility pattern gentamicin (94.4%), ciprofloxacin (88.8%) and norfloxacin (83.3%) showed the greatest activity, followed by amikacin (55.5%), and amoxicillin/clavulanicacid(augmentin)(55.5%),amoxicillin(22.2%)and chloramphenicol (22.2%). Wilkie *et al*., (1992) reported that new quinolones such as norfloxacin and ciprofloxacin had a broad spectrum of activity and were effective against Gram-positive and Gram-negative bacteria including *Pseudomonas aeruginosa*. Gebreel *et al*., (2000) reported in their study on *Pseudomonas aeruginosa* isolates that sensitivity to ciprofloxacin was (100%), and ampicillin 0%, respectively. These results are in agreement with those obtained in the present study. The low susceptibility of *Pseudomonas aeruginosa* to amoxicillin was also reported by Abdel-Salam *et al*., (2003).

Escherichia coli: ciprofloxacin, chloramphenicol, gentamicin, amikacin and norfloxacin showed the best activity (100%). *Escherichia coli* strains were resistant to other drugs. Oteo *et al*., (2002) investigated the susceptibility of *Escherichia coli*. The study reported (82.8%) activity for ciprofloxacin and (93.6%) for gentamicin. The present study showed similar activity for ciprofloxacin. *Bacillus cereus* and *Stapylococcus epidermidis* were sensitive to all antibiotics drugs. The sensitivity to all tested antimicrobials suggests that these strains have not been previously exposed to such drugs (Fornazari *et al*., 2012).

CONCLUSIONS

In this study, we found that:

1. The highest number of children with the upper respiratory infection are in the age group (7-10 years).

2. URTIs infections were found to be more active in winter and spring months.

3. Bacterial and fungal infections were found in the upper respiratory tract infections. Majority of bacteria were *Staphylococcus aureus* and *Streptococcus pyogenes*, while fungi were *Aspergillus niger* and *Candida albicans* that were less responsible for URTIs .

4. The bacterial isolates were sensitive to different antibiotics, but the ciprofloxacin and gentamicin had the highest effect on bacteria causing upper respiratory tract infections.

RECOMMENDATIONS

1. Health institutions must take preventive measures and to return to the medical examination to diagnose and prevent the spread of the infection. Prevention of contact with individuals who are ill or patients who are immunocompromised is beneficial, and to conduct such tests for students Schools, , to prevent a health and economic disaster.

2. Improved personal hygiene and health education of the masses on how to care for nose , throat and tonsils will greatly reduce these microbial infections.

3. Future studies on this subject are required, including most factors affecting infection in addition to age and gender.

4. Adequate rest for children accelerates recovery.

5. The study revealed that evaluation of the upper respiratory tract infections and antimicrobial susceptibility is still in need for more studies. This is due to the continuous development of newly resistant strains and the relatively little number of isolates in some species.

6. In future studies pulsed-field gel electrophoresis (PFGE) technology should be used.

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APPENDICES

A: Data Collection Guide Questions for parents

Date: ……

Age: ……

Gender: ……..

History of infection: ……

B : Culture Media

(1) Blood agar: (Liofilchem- Italy)

Typical formula (g/l)

Sterile Blood (human blood)……50 ml

Blood Agar Base ……..….42 g

Direction of use :

An amount of 42 g of blood agar base were dissolved in 950 ml of distilled water, heated to boiling and autoclaved at 121℃ for 15 minutes. After cooling to about 50°C, 50ml (5%) of sterile blood were added and well mixed, and poured into plates (Wellkang Ltd, London) or tubes (Reagenzglaser, Germany).

(2) Chocolate agar (Liofilchem– Italy)

Typical formula (g/l)

Blood Agar Base…….. ……. 42 g

Sterile Blood …….....50 ml

Direction of use:

An amount of 42 g of blood agar base were dissolved in 950 ml of distilled water, heated to boiling and autoclaved at 121℃ for 15 minutes. After cooling to about 80 °C, 50 ml of sterile blood were add and well mixed, and poured into plates or tubes .

(3) MacConkey agar: (Liofilchem – Italy)

Typical formula (g/l)

Pancreatic Digest of Gelatin.....17

Peptones(meat and casein).....3

Lactose monohydrate.....10

Bile Salts N˚3.....1.5

Sodium Chloride.....5

Neutral Red.....0.03

Crystal Violet.....0.001

Agar.....,,,,,.....15

Direction of use :

An amount of 51.5g of the ready made medium were suspended in 1 litre of distilled water. The medium was completely dissolved by heating to the boiling and then sterilized by autoclaving at 121°C for 15 minutes. The medium was cooled to about 50°C and poured into petri dishes.

(4) Sabouraud dextrose agar: (HIMEDIA-INDIA)

Typical formula (g/l)

Mycological peptone 10

Dextrose.....40

Agar.....15

Direction of use :

Sixty five grams of the ready-made medium were suspended in 1litre of distilled water, sterilized at 121°C for 15 minutes and poured into plates or tubes.

(5) DNase Agar (Oxoid ,England)

Typical formula	(g/l)
Tryptose	20
Desoxyribonucleic Acid	2
Sodium Chloride	5
Agar	12

Direction of use :

Thirty nine grams of the ready- made medium were suspended in one litre of distilled water. The medium was completely dissolved by heating to the boiling and then sterilized by autoclaving at121°C for 15 minutes. The medium was cooled to about 50°C and dispensed into sterile petri dishes.

(6) Muller-Hinton agar (Oxoid , England)

Typical formula	(g/l)
Beef Extract	2
Casein hydrolysate	17.5
Starch	1.5
Agar	17

Direction of use :

An amount of 38g of the ready-made medium were suspended in one liter of distilled water. The medium was completely dissolved by heating to the boiling and then sterilized by autoclaving at 121°C for 15 minutes. The medium was cooled to about 50°C and poured into plates or tubes.

C: Reagents

(1) Oxidase reagent: (BDH chemicals limited company,England)

Tetramethyl-p-phenylenediumine dihydrochloride .....0.1 g

Distilled water.....10 ml

The chemical was dissolved in the water and the reagent was used immediately.

(2) Catalase reagent : (Oxoid,England)

H_2O_2 hydrogen peroxide

Stock solution 30 % H_2O_2.....10 ml

Distilled water.....90 ml

Stable for 6 months at 4°C in brown bottle .Aliquots used at the bench should be discarded weekly .

(3) Coagulase reagent (Oxoid, England)

One -in-6 dilution of the plasma (human) in saline (0.85%) NaCl.

The plasma was stored in small portions at-20°C and kept as a stock at 4°C . Before its use, it was brought to room temperature .

(4) A latex agglutination test (Oxoid, England) for the identification of streptococcal groups:

Latex reagent……0.1% sodium azaid

Positive control reagent…….....0.1% sodium azaid

The extraction enzyme solution……0.01% thiomersal

D: Stains

(1) Gram's stain (Oxoid, England)

A. Crystal violet

Crystal violet ……...……. 20 g

Ammonium oxalate ……. 9 g

Ethanol absolute …….……. 95 g

Distilled water …….……... to 1 litre

The crystal violet was weighed on a piece of clean paper,and transferred to a clean brown bottle to hold 1 litre.The absolute ethanol was added and mixed until the dye was completely dissolved.The ammonium oxalate was weighed and dissolved in 200 ml of distilled water ,it was then added to the stain and to make a final 1 litre with distilled water,and mixed very well.

B. Lugol's Iodine Solution

Potassium iodide ……..……20 g

Iodine …….……..10 g

Distilled water…….…….1 litre

The potassium iodide was weighed, and transferred to a clean brown bottle to hold one litre with distilled water. The iodine was added and heated with mixing until iodine was completely dissolved.

C. Acetone-alcohol decolorizer

Acetone …….…..…….. 500 ml

Ethanol or methanol, absolute ……..……. 475 ml

Distilled water……..……... 25 ml

The distilled water mixing with the absolute ethanol. The solution was transferred to a clean bottle of 1 litre capacity. The acetone was added immediately to the alcohol solution, and mixed well.

D. Neutral red

Neutral red……..….1g

Distilled water……..1 litre

The neutral red was dissolved in the distilled water and used.

(2) Lactophenol blue stain (Oxoid, England)

Phenol crystal…….....……20 g

Lactic acid…….....20ml

Glyserol…….....……..40 ml

Distilled water……...…….……..…20ml

Methyl blue…….....…..0.075 g

The components of the stain were dissolved and used.

دراسة على البكتيريا والفطريات المسببة لإلتهابات الجهاز التنفسي العلوي في الاطفال الزائرين مستشفى الأطفال في بنغازي

اعداد

سالمة احمد العوامي

المشرف

أ.د صالح حمد بعيو

الملخص

تضمنت الدراسة 300 عينة من مرضى الجهاز التنفسي العلوي. خلال الفترة (أغسطس 2016 إلى يونيو2017). تم أخذها من قسم العيادات الخارجية (OPD) مستشفى الأطفال في بنغازي، ليبيا، من اجزاء مختلفة من الجهاز التنفسي العلوي تتضمن الانف والبلعوم واللوزتين. تتراوح أعمارهم ما بين(سنة واحدة الى 13 سنة). تم تحليل البيانات التي تم جمعها بناء علي العمروالجنس والجزء المصاب. حيث شكلت نسبة الذكور(57.3٪) 172 من الأطفال المصابين بالإلتهابات في حين كانت نسبة الإناث (42.6 ٪) 128 طفلة. أظهرت نتائج نمو الكائنات الممرضة أن 297 عينة من العزلات نمت (99٪)، بينما ثلاث عينات (1%) لم تظهر اي نمو. تم فحص العزلات مظهريا ومجهريا وبالاختبارات الكيموحيوية، وكذالك التشخيص بنظام API 10 و BBL Streptocard.

الممرضات البكتيرية التي ظهرت في الدراسة :

Staphylococcus aureus (31%)
Streptococcus pyogenes (28.3%)
Streptococcus pneumoniae (21.3%)
Klebsiella pneunoniae (8%)
Pseudomonas aeruginosa (6%)
Escherichia coli (0.6%)
Bacillus cereus (0.6%)
Staphylococcus epidermidis (0.3%)

ونوعان من الفطريات هي :

Aspergillus niger (2.3%)
Candida albicans (0.3%)

تمت دراسة بعض العوامل المؤثرة علي نسبة انتشار التهابات الجهاز التنفسي العلوي وهي العمر والجنس والجزء المصاب وتأثير فصول السنة خلال فترة الدراسة.

حيث ظهرت اعلى نسبة للإصابة في الفئة العمرية من 7 – 10 سنوات بنسبة 35%, اما العدوى الموسمية بلغت ذروتها في فصل الشتاء 108 بمعدل (36%).

تم اختبار حساسية العزلات البكتيرية باستخدام تسعة أنواع من المضادات حيوية وأظهرت جميع العزلات مقاومة عالية ضد الأمبيسلين Ampicillin في حين كانت معظم العزلات حساسة لسسيبروفلوكساسين Ciprofoloxacin وجينتاميسين Gentamicin.

دراسة على البكتيريا والفطريات المسببة لإلتهابات الجهاز التنفسي العلوي في الاطفال الزائرين مستشفى الأطفال في بنغازي

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قدمت هذه الرسالة استكمالا لمتطلبات الحصول علي درجة الماجستير في علم النبات.

جامعة بنغازي

كلية العلوم

ديسمبر 2019