A Study of otitis media among children in Benghazi's children hospital

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Dedication

I would like to dedicate my project to my father, to my Mother, to my husband for his endless love and support, to sweet hearts my children to my brothers and sisters and to all who encouraged me to finish my research Project.

Acknowledgement

First of all, I give my thanks to my **Allah**, who gave me power, inspiration and helped me through my life to complete this research. I would like to thank my supervisor prof. **Saleh H. Baiu**, for his great support before and during my research and it was a great pleasure for me.

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Author

[Tahani Ali Elgadi]

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LIST OF ABBREVIATIONS

Abbreviation	Meaning
AOM	**Acute otitis media**
COM	**Chronic otitis media**
DALYs	**Daily Adjusted Life Years**
ET	**Eustachian tube**
GBD	**Global Burden of Disease**
IL-8	**Interleukin-8**
OME	**Otitis media with effusion**
TM	**Tympanic membrane**
URTI	**Upper respiratory tract infection**
WHO	**World Health Organization**

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Abstract

Otitis media has a worldwide prevalence . Though it is more common in children, otitis media can result in severe complications like hearing impairment, death and severe disability due to central nervous system.

This study was a survey to determine the microorganisms (bacteria and fungi) responsible for middle ear infection among 300 patients who had ear discharge and were visiting the children hospital in Benghazi city, during a period of 10 months (August 2016 to May 2017). The patients were aged between one month to twelve years. The highest incidence of infection (55.3%) was observed in the children aged between <1-2years. The infections were higher during the winter and Autumn months.

A total of 293 positive cultures representing 7 bacteria species were identified and their susceptibility to various antibiotics was tested *Pseudomonas aeruginosa* was the predominant (18.7%) pathogen isolated, followed by *Staphylococcus aureus* (13.3%), *Proteus mirabilis* (10%), *Streptococcus peneumoniae* (7.3%), *Klebsiella penumoniae* (6%), *Escherichia coli* (5.7%), and *Streptococcus pyogenes* (5%).

Fungi were isolated from (10.3%) of patients; *Candida albicans* was the predominant (6.7%) fungus isolated. Followed by *Aspergillus niger* (3.3%), and *Aspergillus fumigatus* with (0.7%).

Most of the isolates of Otitis media infection showed sensitivity to the antibiotics tested. Generally Gram-positive bacteria isolate were more sensitive to tested antibiotics. The Gram-negative bacteria (especially *Pseudomonas aeruginosa*) showed the highest resistance to the antibiotics.

Conclude that bacteria were more responsible for otitis media infections than fungi and the ciprofloxacin was the most effective drug against bacteria causing otitis media.

1-INTRODUTION

1.1 Background information:

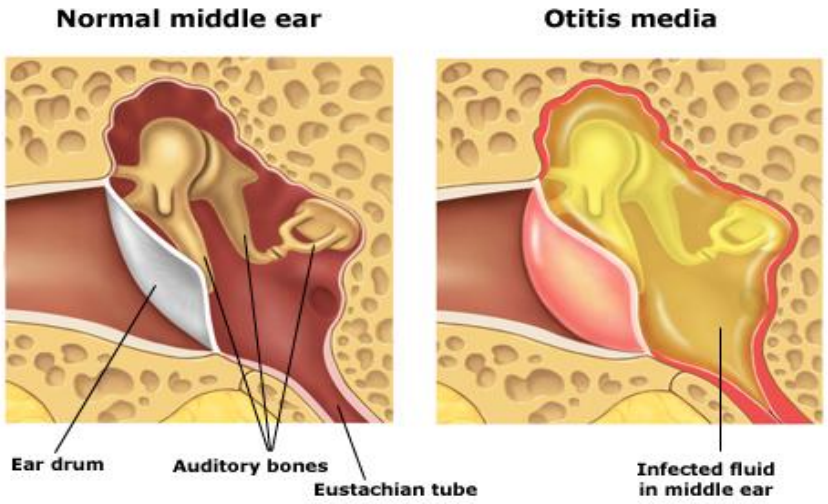
The ear is responsible for hearing and also maintaining balance, It is divided into the inner, middle and outer ear with the middle and outer regions being most susceptible to injury and infections (Richard and Roberts, 1996).

The ear can have different types of diseases with otitis media being the most common (Weiner and Collison, 2003). The inflammatory disease of the mucosal lining of the middle ear is called otitis media (OM) and refers to a group of complex infections and inflammatory diseases (Brook and Saantonsa, 1995).

OM occurs in the area between the ear drum and the inner ear, including aduct known as the eustachian tube (ET) mastoid anturm and mastoid air cells (Lieberthal *et al*., 2013), Figure (1).

OM affects all age groups but it is more common in children (Bluestone, 2005). Two out of three children will have at least one episode of OM before their third birthday. The children with the average toddlers have two to three episodes a year, and this is always accompanied by a viral upper respiratory infection (URTI), mostly common cold caused by influenza virus (Lieberthal *et al*., 2013).

OM can be caused by infections, allergy, anatomic or functional deviations of the middle ear or ET (Smith *et al*., 1996).



http://hpathy.com/homeopathypapers/homeopathy-for-themanagement-of-otitismedia

Figure (1): (a) Normal Middle Ear (b) Otitis Media

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Children below the age of seven years are much more susceptible to OM since the ET which drains the middle ear space to the posterior pharynx is shorter, more compliant, and more horizontally places than the adult ET. The result is an accumulation of middle ear secretions and creation of a negative pressure environment with a potential for aspiration of contaminated nasopharyngeal secretions to the middle ear space (Weiner and Collison, 2003). Maturational changes of skull enlargement and elongation produce a more vertical and functional ET in most adults (Poole, 1995). Figure (2).

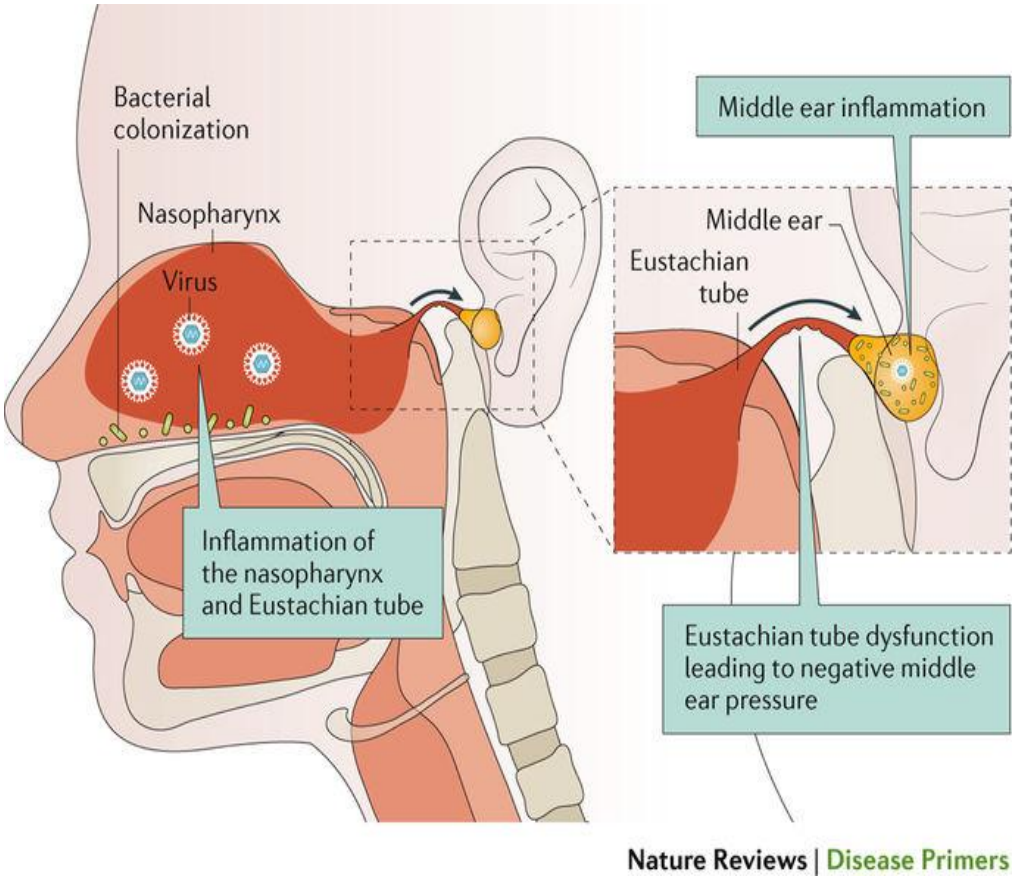

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Figure (2): Anatomical View of Otitis Media Ear

Breast feeding for the first twelve months of life is associated with a reduction in the number and duration of all OM infections (Miller, 1979) and (Bethesda, *et al*., 2002).

The OM can be acute (acute otitis media (AOM), chronic (chronic supportive otitis media (CSOM), or otitis media with effusion (OME). AOM is characterized by the rapid onset of signs of inflammation, specifically bulging and possible perforation of the tympanic membrane, fullness and erythema, as well as symptoms associated with inflammation such as fever, hearing impairment and a purulent discharge (otorrhea) through a perforation of the tympanic membrane (or tympanotomy tube) (Richard and Roberts, 1996; Bennett *et al*., 1998).

Symptoms are non-specific and may include irritability, restlessness, bouts of screaming, anorexia, vomiting, fever and occasionally, convulsions (Shimanura , *et al*., 1990). Pulling and rubbing of the ears with restlessness may be indirect evidence of acute otitis media. The clinical feature of AOM in older children may also be non-

specific. It is advisable therefore, to exclude AOM before any child with fever is labeled as having pyrexia of indeterminate origin.

AOM may progress to (CSOM) which defined as chronic supportive inflammation of mucus periosteal lining of middle ear cleft with tympanic membrane perforation (GBD, 2013). It causes conductive and sensorineual hearing loss and adverse effects on child development (Teele *et al*., 1990).

CSOM is of two types, mucosal or tube-tympanic type and cholestoma or atticoantral type (Brook and Burke, 1992). Tube tympanic type affective mainly mesotympanum , hypo-tympanum. (ET) and mastoid air cells whereas atticoantral is a dangerous one because of associated complications and may be life threatening at times infection can spread from middle ear to vital structures as mastoid, facial nerve, labyrinth, lateral sinus, meninges and brain leading to mastoid abscess (Poole, 1995).

facial nerve paralysis, deafness lateral sinus thrombosis, meningitis and brain abscess of all these complications, hearing loss associated with chronic ear discharge (Miller, 1979). The symptoms of CSOM this manifests as persistent purulent ear discharge for more than six weeks (Mawson and Pollack, 1988). It most often occurs in children with tympanic membrane perforation. The principal symptoms are hearing impairment and aural discharge. In the tub tympanic disease, the discharge tends to be profuse and is frequently muciod rather than frankly purulent (Roberts, 1980). In the atticoantral disease, the discharge is generally scanty, foul smelling and tends to be more chronic. The development of headache, vertigo or facial palsy is evidence of complication (Weiner and Collison , 2003; Roberts, 1980).

(OME) is typically not associated with symptoms (Brook, 1987). Occasionally a feeling of fullness is described. It is defined as the presence of non-infectious fluid in the middle ear for more than three months. OME usually presents with a symptomatic middle ear effusion that may be associated with a “plugged” ear feeling. The presence of an effusion is associated with either a mild or moderate conductive hearing impairment.

Diagnosis of the otitis media:

An auroscope / otoscope with a fresh bulb and a good power source, as well as a view of the tympanic membrane that is not obstructed by cerumen, are essential to making the diagnosis of OM. The appearance of the eardrum in AOM progresses from injection of the vessels along the handle of the malleus and around the periphery, to reddening with bulging of the eardrum and finally to perforation and discharge (Brook and Burke, 1992).

The discharge may be serous, serosanguineous or mucopurulent (Roberts, 1980). Crying or attempts to remove ceriman can cause erythema of the eardrum; therefore erythema of the tympanic membrane alone should not be the sole basis for the diagnosis of AOM (Barobby and Zadik, 1987). In OM with effusion, otoscopic findings include visualization of air-fluid levels and clear or amber middle ear fluid. It can also be associated with negative middle ear pressure which is suggested by prominence of the lateral process and shortening of the long arm of the malleus with a more horizontal orientation (Mawson and Pollack, 1988).

In cases of CSOM, visualization of the tympanic membrane will reveal a perforation. If the perforation is wide enough, the condition of the middle ear mucosa can be assessed (Ilechukwu, *et al*., 2014). In addition, polyps and discharge crusts may also be evident (Roberts, 1980). It may be necessary in children to examine the ears under general anesthesia in order to make a proper assessment (Roberts, 1980).

Pneumatic otoscopy / auroscopy : A pneumatic otoscope with a rubber suction bulb and tube is used to assess mobility of the tympanic membrane. Pneumatic otoscopy is necessary in all cases of suspect ion of AOM to avoid over diagnosing “red ears” (Mawson and Pollack, 1988).

In such cases, there is decreased mobility of the tympanic membrane if fluid accumulates in the middle ear (Glezen, 2000). Tympanometry can be used to identify an effusion but not inflammation (Mawson and Pollack, 1988). Because of the compliance of the cartilaginous canal of the infants, tympanometry is usually reserved for children over the age of 6 months (Mawson and Pollack, 1988).

The diagnosis of otitis media as it is typical symptoms overlap with other conditions, such as a cute external otitis, clinical history alone is not sufficient to predict whether AOM is present; it has to be complemented by visualization of the tympanic membrane. AOM in children with moderate to severe bulging to the tympanic membrane or new onset of otorrhea (drainage) is not due to external otitis. Also, the diagnosis may be made in children who have mild bulging of the ear intense erythema (redness) of the ear drum.

To confirm the diagnosis, middle-ear effusion and inflammation of the ear drum have to be identified; signs of these are fullness, bulging, cloudiness and redness of the ear drum (Sattout and Jenner, 2008). It is important to attempt to differentiate between (AOM) and (OME), as antibiotics are not recommended for OME (Sattout and Jenner, 2008). It has been suggested that bulging of the tympanic membrane is the best sign to differentiate AOM from OME (Coleman and Moore, 2008).

Viral otitis may result in blisters on the external side of the tympanic membrane, which is called bullous meningitis (myring being Latin for "ear drum") (Thompson *et al*. 2013). Sometimes even examination of the ear drum may not be able to confirm the diagnosis, especially if the canal is small (Qureishi, *et al*., 2014). If wax in the ear canal obscures a clear view of the ear drum it should be removed using a blunt cerumen curette ora wire loop (Benningerz, 2008). Also, an upset young child's crying can cause the ear drum to look inflamed due to distension of the small blood vessels on it, mimicking the redness associated with OM (Benninger, 2008).

1.2 A etiology:

The common cause of all forms of otitis media is dysfunction of the ET (Thanaviratananich *et al*., 2013). This is usually due to inflammation of the mucous membranes in the nasopharynx. which can be caused by a viral URTI, strep throat, or possibly by allergies (Thanaviratananich *et al*., 2013). Because of the dysfunction of the middle ear is trapped and parts of it are slowly absorbed by the surrounding tissue, a negative pressure in the middle ear can occur (John Donaldson, 2013).

Eventually the negative middle ear pressure can reach a point where fluid from the surrounding tissues is sucked in to the middle ear's cavity (tympanic cavity), causing a middle-ear effusion. This is seen as a progression from a type A tympanogram to a type C to a type B tympanogram (John Donaldson, 2013).

By reflux or aspiration of unwanted secretions from the nasopharynx into the normally sterile middle-ear space, the fluid may then become infected usually with bacteria (Shaikh, Nader, 2010). The virus that caused the initial URTI can itself be identified as the pathogen causing the infection (Thanaviratananich *et al*., 2013). Childhood AOM and (OME) can both cause long term changes of the tympanic membrane (Kozyrskyj *et al*., 2010). These changes may reduce the elastic properties of the TM, making it more susceptible to chronic perforation or retraction (Macfadyen *et al*., 2006).

Genetic and racial factors have been associated as risk factors for the development of OM. The incidence of OM varies in different populations, and in the developed world is highest among the Eskimos, Native Americans, New zeal and Maoris and Australian Aborigines (Hannley, 2000). It is significantly more common in cold and dump areas like among the Inuit (Eskimos) (Browning, 1988).

Environmental factors have also been associated as risk factors, the prevalence is higher in lower socioeconomic groups (Macfadyen and Acuin, 2005). Factors significant for draining ears were general health scores, maternal smoking and day care attendance (Macfadyen and Acuin, 2005).Other factors associated with OM are ET dysfunction which is more in patients with OM than normal individuals (Esposito *et al*., 1992). Craniofacial abnormalities are associated with increased risk of OM (Sheahan *et al*., 2002)

1.3 Epidemiology:

The reasons for the young-age preference includes: poorly developed immune defense, shorter and more horizontal Eustachian tube, well-endowed with lymphoid follicles and endenoids in the nasopharynx (Mawson and Pollack, 1988; Brook and Burke, 1992). By the age of 3 years, 80% of children have had at least one episode of

AOM, and nearly 50% have had 3 or more episodes (Glezen, 2000) (Thanaviratananich, *et al*., 2013). Incidence declines after 6 years of age (Mawson and Pollack, 1988; Glezen, 2000).

Predisposing factors for acute otitis media: they are protean, with controversies still surrounding most of them and include:

1. **Parental smoking and exposure to wood smoke:** parental smoking has been found to be a major risk factor for OM (Mawson and Pollack, 1988; Gracia *et al*., 1997). It was noted that children of mothers who smoked 20 or more cigarettes per day were at significantly increased risk of having AOM (Berman et al., 2001). This high lights the effect of close, prolonged contact between children and smokers on OM.
2. **Upper respiratory tract infections (rhinitis, nasopharyngitis):** Rhinitis and nasopharyngitis usually set the stage for infection of the middle ear by allowing spread of pathogenic organisms from the nasopharynx into the middle ear via the ET. The presence of viral infection has been shown to increase bacterial adhesion in the nasopharyngeal tissue (Brook and Burke, 1992; Sassen *et al*., 1997).
3. **Daycare attendance:** A significant increase in the number of children attending daycare centers has been linked to increased prevalence of OM in developing countries (Uhari *et al*., 1995; Kvaerner *et al*., 1996).
4. **Familial tendency:** These include allergic rhinitis, asthma, and cow's milk allergy, parental atop, history of parental otitis media (Stenstrome and Ingvarsson, 1997). The common postulated pathogenesis is that they cause nasopharyngeal lymphoid and adenoidal hypertrophy which mechanically block the ET leading to ET dysfunction and otitis media ultimately (Brook and Burke, 1992).
5. **Short duration of breast feeding and bottle feeding:** breast feeding is known to reduce the incidence of acute respiratory infection. It also prevents colonization with otitis pathogens though selective IgA antibody; and decrease the amount of contaminated secretions aspirated into middle ear space (Mawson and Pollack, 1988).
6. **Use of pacifiers and presence of digit sucking:** In ameta-analysis of the risk factors for AOM in children (Gracia *et al*., 1997) noted that the use of a pacifier increased the risk of AOM.

7. **Overcrowding:** Overcrowding predisposes to easy spread of droplet infections, including OM (Brook and Burke, 1992). However, in analyzing over cowing; other determinants such as room size and adequacy of ventilation have to be considered (Brook and Burke, 1992).
8. **Measles, pertussis, tuberculosis and immunosuppression:** OM is a known complication of pertussis, measles, diphtheria and tuberculosis (Browning, 1988; Ibeziako, 1999).Similarly, children with congenital or acquired immunodeficiency may have defects of phagocyte function or hum oral systems (Brook and Burke, 1992).
9. Infections of the respiratory tract, including OM, are associated with defects of chemo - taxis, phagocytosis.
10. **Cleft palate, Down syndrome and other craniofacial defects:** Patients with anomalies such as cleft plat and children with Down syndrome have higher incidence of ET dysfunction and chronic otitis media with effusion (Miller, 1979).

Acute otitis media is very common in childhood. It is the most common condition for which medical care is provided in children under five years of age in the US (Glasziou *et al*., 2004). Acute otitis media affects 11% of people each year (709 million cases) in the world with half occurring in those below five years (Monastal *et al*., 2010). Chronic supportive otitis media affects about 5% or 31 million of these cases with 22.6% of cases occurring annually under the age of five years. Otitis media resulted in 2,400 deaths in 2013 down from 4,900 deaths in 1990 (GBD, 2013).

Global burden of illness from COM is estimated to involve about 63-330 million individuals with draining ears, 60% of whom (39-200) million suffer from significant hearing impairment. It account for 28,000 deaths and disease burden of over 2 million disability adjusted life years (DALYS). Over 90% of the burden is borne by developing countries in south-east Asia, Western pacific regions and Africa. COM is uncommon in the Americans, Europe, the Middle East and Australia (Teele *et al*., 1990). In Britain, 0.9% of children and 0.5% of adults have COM (Jose, 2004).

however, some studies estimate the yearly incidence of COM to be 39 cases per 100,000 in children and adolescents aged 15 years and younger (Sierra et al., 2011).

1.4 Microbiology of Otitis Media:

The most common cause of OM is bacterial infection of the middle ear (Sierra *et al*.; Qureishi *at al*., 2011) . AOM is predominantly caused by *S. pneumoniae , H. influenzae* and *M. catarrhalis* (Sattar *et al*., 2012). However, *Ps. aeruginosa* and *Staph. aureus* are the most common aerobic microbial isolates in patients with COM, followed by *Prot. vulgaris* and *K. pneumoniae* (Sattar *et al*., 2012).

Studies on microbiologic diagnoses of COM differ in regard to patient age, geography and presence of complications such as cholesteatoma and these inconsistencies likely impact some of the variation in reported pathogens. A portion of the variability observed may be related to differences in sampling and processing methods (Roland, 2002; Vartiainen and Vartiainen, 1996). Bacteria are infrequently found on the skin of the external ear canal, but proliferate in the presence of trauma, inflammation, lacerations or high humidity. These bacteria may then gain entry to the middle ear through a chronic perforation (Kenna, 1990).

Pseudomonas is the most commonly isolated organism in COM (Thanaviratananich *et al*., 2013). *P. aeruginosa* can thrive well in the ear environment and is difficult to eradicate (Pollack, 1988). It has been proposed that *P. aeruginosa* evades the host defiance mechanism by taking advantage of a shall of surrounding damaged epithelium that causes decreased blood circulation to the area (Pollack, 1988). *P. aeruginosa* damages the tissues, interferes with normal body defenses and inactivates antibiotics by various enzymes and toxins (Donelli and Vuotto, 2014). COM can also be characterized by co-infections with more than one type of bacterial and viral pathogen (Vartiainen, 1996; Bakaletz, 2010).

Fungi have also been identified in cultures from patients with COM (Ibekwe *et al*., 1997). Fungi, particularly *Aspergillus* and *Candida* species, although rare are reported as pathogens as well (Acuin, 2007). However, the presence of fungi can be due to the treatment with antibiotic ear drops, which causes suppression of bacterial flora and the subsequent emergence of fungal flora (Saunders *et al*., 2011). This probably increased the incidence of fungal super-infection, and even the less virulent fungi become more opportunistic. Furthermore, there has been much disparity on the rate of isolation of fungi of CSOM patients (Prakash *et al*., 2013). This variation can be attributed to the

climatic conditions, as the most and humid environment favours the prevalence of fungal infections of the ear (Asish *et al*., 2014 and Juyal *et al*., 2014).

Interleukin-8 plays a role in the development of chronicity of OM and has also been related bacterial growth. Up regulation of these pro-inflammatory cytokines can cause tissue damage as well as transition from acute to chronic otitis media (Si *et al*., 2014)

1.5 Pathophysiology:

OM is considered a multifactorial disease resulting from a complex series of interactions between environmental, bacterial, host and genetic risk factors (Rye *et al*., 2012). It is important to identify the genes that contribute to CSOM susceptibility, which will provide insights into the biological complexity of this disease and ultimately contribute to improve the methods of prevention and treatment (Allen *et al*., 2014). Immune mechanisms such as the TLR4/MyD 88 pathway are particularly important in eliciting protective immune responses against bacteria (Allen *et al*., 2014). On the other hand, transforming growth factor-B pathway helps in balancing the adverse outcome of an exaggerated pro-inflammatory responses bacteria (Hernandez *et al*., 2008).

The roles of these pathways have been extensively studied in AOM; no studies are available in relation to CSOM (Harkness and Topham, 1998) and (Leichtle *et al*., 2011). Chronic otitis media is initiated by episode of a cute infection. The pathophysiology begins with initiation and subsequent inflammation if the middle ear mucosa. Ongoing inflammation eventually leads to mucosal ulceration and consequent break down of the epithelial lining. The cycle of inflammation and ulceration continues to late chronic phase with well-established intractable mucoperiosteal disease. The recurrent episodes of otorrhoea and mucosal changes are characterized by ontogenesis, bony erosions and otitis that include the temporal bone and ossicles. This is followed by acicular chain destruction and or alkalosis which together with the tympanic membrane perforation contribute to hearing loss (Seibert and Dannner, 2006).

Perforations of the TM are classified as central when the annulus is preserved and marginal when a portion of the annulus or the entire annulus is involved.

Marginal perforations are more frequently associated with cholsteatoma (Oktay, 2005). Etiologic agents for otitis media include viruses and bacteria. The notable viruses include respiratory syncytial virus, rhinovirus, adenovirus, Para influenza and corona virus while predominant bacteria that causes OM are *Ps. aeruginosa, Staph. aureus, s.*

pyogenes, Moraxella catarrhalis, and non-type able *H. influenza.* The primary site of pathology in OM is the ET connects the tympanic cavity with the nasopharynx and: is the primary defense mechanism of the middle ear, permits equilibration of middle ear pressure with atmospheric pressure, protect the middle ear from reflux of nasopharyngeal secretions; and drain secretions from the middle ear into the nasopharynx. Unlike adult, child's eustachian tube is more horizontally aligned. This allows for easier spread of infection from the nasopharynx to the middle ear (Oni *et al*., 2001). OM runs through the following stages: tubal occlusion, pre-suppuration, suppuration and resolution or complication (Onietal ., 2001).

Bacterial biofilms have gained attention in the pathogenesis of CSOM. Biofilms are resistant to antibiotics and other antimicrobial compounds (Stewart and Costerton, 2001). Therefore, they are difficult to eradicate and hence could lead to recurrent infections (Abdelshafy *et al*., 2015). In addition biofilms attach firmly to damaged tissue, such as exposed osteitic bone and ulcerated middle ear mucosa, or to ontological implants such as tympanostomy tubes, further aggravating the problem of eradication biofilms have been demonstrated in the middle ear of CSOM patients; their precise role in the pathophysiology of the disease is yet to be determined (Lampikoski *et al*., 2012).

Furthermore, the molecular mechanisms leading to biofilm formation in the middle ear during CSOM are also poorly understood (Lampikoski *et al*., 2012).

Cytokinase have also been implicated in the pathogenesis of OM. Most studies addressing the role of cytokines are in relation to AOM, and there are very limited studies available demonstrating the role of cytokines in the pathogenesis of CSOM. High levels inflammatory cytokines such as IL-8 have been demonstrated in the middle ear effusion of CSOM patients (Elmorsy *et al*., 2010).

1.6 Management:

Oral and topical pain are effective to treat the pain caused by OM. Oral agents include ibuprofen, paracetamol (acetaminophen), and opiates (Coleman and Moore, 2008). Topical agents shown to be effective include antipyrine and benzocaine ear drops (Sattout and Jenner, 2008).

Decongestants and antihistamines, either nasal or oral, are not recommended due to the lack of benefit and concerns regarding side effects (Coleman and Moore, 2008).

Half of cases of ear pain in children resolves without treatment in three days and 90% resolves in seven or eight days (Thompson *et al*., 2013). Treatment of CSOM otitis media can be medical intervention and /or surgery including rehabilitation through use of hearing aids (Sharma *et al*., 2004). The goals of treatment of CSOM are to stop otorrhoea, heal the TM, eradicate current infection, and prevent complication and recurrence. Medical treatment includes aural toilet, use of topical or systematic antibiotics, topical steroids and topical antiseptics (Saranya *et al*., 2015).

Although there are no randomized controlled trials (RCTs) evaluating its use, most experts agree that aural toilet is a key component of treatment of CSOM (Orji and Dike, 2015). Aural toilet should be used as supplement to antibiotic therapy. These can be administered as either topical or systemic agents. Topical antibiotics are the first line treatment of uncomplicated otorrhea (Macfadyen and Acuin, 2007).

Various topical agents have compared over the years: antibiotics, steroids, anti-fungals and antiseptics. Topical antibiotics with steroids are better than dry mopping alone (Haugsten and Lorentzen, 1980). Topical antibiotics are more effective than oral or intramuscular antibiotics (Mawson and Pollack, 1988). Systematic antibiotics should be considered in patients at risk for complicated or invasive ear infections, or in those who receive several coarses of empiric topical therapy and are at risk of developing resistant organisms (Wang, *et al*., 2014).

Surgical intervention is the treatment of choice to effect closure of perforation since spontaneous healing of chronic TM perforations is uncommon and medical interventions are not effective in promoting closure (Adoga *et al*., 2010). Surgery is indicated for patients who develop complications of CSOM to remove infected tissue in the middle ear or mastoid and to repair ear damage that results in hearing loss (Kumar and Seth, 2011).

Irreversible disease such as cholesteatoma, polypoid disease, and infected bone must be removed in order to create a dry, safe ear that is free of infection (Laissi *et al*., 2002). Preservation of anatomic contour is also important to preserve the acoustic characteristics of the ear, when possible, though some patients with intractable disease will require more aggressive approach such as mastoidectomy (Leibovitz, 2013).

Reconstruction of the sound transmission mechanism is important through use of ossicular prosthesis to replace damaged ossicles (Mansoor *et al*., 2009). Restoration of tympanic and mastoid aeration is required for both maintenance of adisease. Free state and for maximal auditory function (Mahmaud, 2015).

Treatment of acute otitis media controversial and contineously changing likely due to the increasing prevalence of resistant organisms (Barobby and Zadik, 1987; Browing, 1990). Accelerated patterns of bacterial resistance therefore, mandate an evidence-based approach to managing OM. There is wide variation in the use of antibiotics among doctors worldwide (Browing, 1990). The recommended treatment duration for uncomplicated AOM is 5-7 days (Obiakor, 2000). Amoxicillin has been the first line antibiotic for treating otitis media, even with a high prevalence of drug-resistant *S. pneumoniae,* because resistance to ß-lactam antibiotics, such as amoxicillin, develops as a stepwise process (Osazuwa *et al*., 2011). Amoxicillin-clavulanate combination is an appropriate choice as a second line antibiotic if a child is not responding to treatment after 72 hours on amoxicillin (Mawson and Pollack, 1988). The addition of clavulanate to amoxicillin will broaden the coverage while retaining efficacy against *S. pneumoniae* (Rajat *et al*., 2013). Other appropriate choices include erythromycin combined with a sulphonamide (Mawson and Pollack, 1988; Brook and Burke, 1992).

If the patient is allergic to the penicillins, the combination of oral erythromycin and sulfonamides is an alternative (Saini *et al*., 2005). Combined trimethoprim-sulfamethoxazole can also be given to penicillin- sensitive individuals (Tiedt *et al*., 2013). A child that remains symptomatomatic for more than three days while a second-line agent requires tympanocentesis to identify the causative pathogen (Roland *et al*., 2005). If a highly resistant pneumococcus is found or tympanocentesis is not feasible, clindamycin or intramuscular ceftriaxone appears to be the best third line agent (Mawson and Pollack, 1988).

Recent studies have shown that short courses (2-3 days of antibiotic) at conventional or high doses equally effective in terms of resolution of symptoms and signs (Bain, 1990). The role of antihistamine / decongestant in treating AOM is controversial (Barobby and Zadik, 1987). Other supportive therapy, such as analgesics, antipyretics and local heat, are helpful (Jose, 2004). In patients with unusually severe

earache, myringotomy may be re-evaluated approximately two weeks after the institution of treatment, for some otoscopic evidence of resolution, such as a decrease in inflammation and return of mobility of the tympanic membrane (Grevers *et al*., 2012). Periodic follow-up is indicated for patients who have had recurrent episodes (Egbe *et al*., 2010). Recurrent OM chemo-prophylaxis or tympanostomy tubes are often recommended for recurrent acute otitis media (Mawson and Pollack, 1988; Alho, et al., 1996).

Immunization with the polyvalent pneumococcal vaccine may be effective is preventing recurrent AOM when given to children above 2 years (Stenstrome and Ingvarsson, 1997). Adenoidectomy is another surgical option for preventing recurrent AOM but the benefit is short-lived, with significant morbidity and cost implication (Maw, 1987). The main reason to treat otitis media with effusion is to avoid the adverse effect of prolonged conductive hearing impaiment on language development and academic functioning (Mawson and Pollack, 1988). The management option include observation, antibiotics alone, and combination of antibiotic and corticost steroid therapy (David, 2002). It is recommended that ventilating tubes should be placed especially if condition is bilateral, associated with considerable subjective hearing loss (Mawson and Pollack, 1988; Maw, 1987).

Complications of otitis media develop if infection spreads from the middle ear cleft to structures from which this mucosa lined space is usually separated by bone. The complications are generally classified into two main groups:

A. Intratemporal (whith in the confines of the temporal bone): hearing impairment. It is more pronounsed and prolonged in chronic than acute supurative OM (Weiss, 1996). Acute mastoiditis: refers to the inflammation of the mucosal lining of antrum and the bony walls of the mucosal lining of antrum and the bony walls of the mastoid air cell system (Dhingra, 2004). It follows ASOM, the determining factors being high virulence of organisms or lowered resistance in the patient (Paradise, 2004). Petrositis results when the infection spreads from the middle ear and mastoid to the petrous part of temporal bone (Amusa *et al*., 2005). Diagnosis can be confirmed by x-ray and computarized axial tomography of the temporal bone (Brook and Burke, 1992; Alho, *et al*., 1996).

Labyrinthitis results if the infection progresses to involve the labyrinth (Brook and Burke, 1992). Facial paralysis can occur as a complication of both acute and chronic OM (Donelli and Vuotto, 2014). Facial nerve function fully recovers if AOM is controlled with systemic antibiotics (Bransko and Marko, 2011).

B. Intracranial complications of OM collection of pus between the bone and dura may occur both in acute and chronic infection of the middle ear giving rise to extra dural abscess. Pus can also collect between the dura and arachnoid leading to subdural abscess. Inflammation of the leptomenings (pia and arachnoid) and of the cerebro-spinal fluid (CSF) can result in meningitis (Glezen, 2000). Cerebral abscess is another serious complication of AOM in children. It is often associated with extradural abscess (Karim et al., 1981). Cerebellar abscess is a direct extension through the trautmain's triangle or by retrograde thrombophlebitis (Ogbogu *et al*., 2013). Generally, brain abscess is often associated with other complications, such as exradural abscess, pier-sinus abscess, meningitis, sinu thrombosis and labyrinthitis (Amusa *et al*., 2005). Thus, the clinical picture may overlapping. Lateral sinus thrombophlebitis (sigmoid sinus thrombosis) as an inflammation of the inner wall of the lateral venous sinus with formation of a thrombus and occurs as a complication of acute coalescent mastoiditis, masked mastoiditis or chronic suppuratized of middle ear and cholesteatoma (Amusa *et al*., 2005). Otitis hydrocephalus is characterized by raised intracranial pressure with normal cerebrospinal fluid findings (Brook and Burke, 1992; Maw, 1987). It is pathogenesis is that thought to result from thrombosis from the lateral sinus extending to the superior sagittal sinus (Oni *et al*., 2002).

Aims of Research Project

- To study the prevalence of the otitis media microbial (bacterial and fungi) infections among children visiting the pediatric hospital of Benghazi.
- .To make statistics on prevalence of the infection and regarding age.
- And to find out the antibiotics most sensitive to the identified bacterial isolates.

2-REVIEW 0F LITERATURE

Many studies have been conducted on microbiology of OM and showed different results from region to region (Ahmad, 2013). Microbiological cultures in some studies showed many, frequently multiple organisms and these vary depending on climate, patient population, collection and processing techniques of specimens and prior use of antibiotics (Jolivet-Gougeon, and Bonnaure-Mallet, 2014). Traditional swab specimen collection method has been associated with introclcuing contaminants with normal skin flora like *Staph. epidermidis* (Luntz *et al*., 2013).

Aerobes and anaerobes play a pathogenic role in OM and they usually grow together in mixed cultures. Experiments show that when aerobes and anaerobes are inoculated together, they produce intense inflammation with production of pus (Yorgancilar *et al*., 2013) This reaction is attributed to the synergistic relationship between aerobes and anaerobes (Rajat *et al*., 2013). The production of beta–lactamase by anaerobes and some aerobes and their ability to pass on their protective role to other organisms increase their pathogenicity in the mixed state (Kumar and Seth, 2011).

In terms of polymicrobial versus monomicrobal cultures various researchers have shown different results (Acuin, 2007). In one study (Shrestha *et al*., 2011) in 204 cases of OM, monomicrobial growth was obtained in 118(57.48%) samples, 63(33.33%) samples yielded polmicrobial growth, where 18 (8.82%) showed no growth.

In a study conducted in a central district of Malawi in 2010 in 124 ears. results of the study showed that most ears (91%) incubated faecal bacteria (Alsaimary *et al*. 2010) . *P. mirabilis* (74%) and *enterococci* (60%) were the most frequently isolated microbes .Similar findings were seen in studies done in 2011 by Harnivender and Sonia in 2011.

In a study (Meera, 2012) it has been show that *Ps. aeruginosa* and *Staph. aureus* were are the most commonly isolated bacteria in several large case series. The ability of these organisms to form biofilm might contribute to their frequency in OM (Meera, 2012). Fungi particularly *Aspergillus* species and *Candida* species although rare were reported as pathogens as well (Meera, 2012) .

Although the majority of studies (Ahmed *et al*., 2010) on microbiology of OM showed that *Ps. aeruginosa* and *Staph. aureus* were commonest aerobic OM causing micro-organisms, a study in Nigeria that looked at retrospective chart review of 128 patients (Egbe *et al*., 2010) showed different microorganisms causing otitis media which were also different from those studies that found *Ps. mirabilis* as the most frequent bacteria causing otitis media.

The study by (Van Hasselt *et al*., 2013) Found out that most prevalent organisms causing OM were coliform bacteria with *Klebsiella* species as commonest followed by *E. coli.*

In a study by (Ogbogu *et al*., 2013), on 220 children with otitis media showed *Ps. aeruginosa* (33.33%) were the most prevalent microbial agent of otitis media followed by *Staph. aureus* (23.19%) while *Citrobacter* species and *Asperogillus* niger were the least prevalent with a prevalence of 0.48% each.

In a study by (Jik *et al*., 2015) out of the 182 samples of middle ear exudates exminted 154 specimens contained bacterial pathogens and 18 specimens were sterile. A total of 53 of the isolate wares confirmed as *Staph. aureus* and 34 of the isolates were confirmed *Ps. aeruginosa*. Children between age group 1-3 years were statistically significantly affected with total number of 59(38.31%) positive samples followed by age group 4-6 years with 47(30.52%), age group 7-9 with 30(19.48%) and age group 10-12 with 18(11.69%).

In a study by (Mahmoud, 2015) about bacteriological profile and resistance pattern of antibacterial agent of ear infection. a total of 96 study subjects were included in this study. Bacterial isolate identified were *Ps. aeruginosa* 64(75%), *Staph. aureus* 28(25%), *Proteus* species 8(7%). *Enterobacter* spesies 8(7%), and *Klebsiella* species 4(3.6%). The overall resistance profile of antibacterial agent, Ceftriaxone and Norfluxacilline showed high level of antibacterial effect on all identified bacterial species, where Gentamycin was moderately active.

In a study by (Jonathan *et al*., 2016) which was under taken to evaluate the antibacterial sensitivity of bacteria of otitis media to some antibiotics. A total of (54) samples were obtained from patients with OM coming into the national Ear care center

for the first time. The study revealed highest frequency of *Ps. aeruginosa* 60(71.43%) followed by *P. mirabillis* 14(16.67%), *K. pnuemoniae* 6(7.14%) and *E. coli* 4(4.76%). Antimicrobial ausceptibility test showed heighest frequency of resistance among all isolates to amoxicillin, tetracycline, contrimoxazole, nitrofurantoin and nalidixic acid .

However, gentamicin, ofloxacin, augmentin and tetra cycline were effective against *Ps. aeruginosa* but ineffective against other isolates.

In a study about the role of bacteria Bioilan and fungal infection in otitis media by (Abdelshafy *et al*., 2015). Showed most common isolated bacteria were *Ps. aeruginosa* (37.5%) followed by *Klebsiella spp*. (10%) and *Staph. aureus* (7.5%) and fungal culture gave results to *candida* (10%) and *asperigillus* (7.5%) mixed bacterial and fungal infection occurred in (11%) of patients.

In a study by (Saranya *et al*., 2015). was carried out to identify the common bacteria and fungi causing otitis media and to determine the antibiotic sensitivity of the bacterial isolates. The most commonly isolated bacteria were *Ps. aeruginosa* followed by *Staph. aureus, K. aerogenes, Prot. mirabilits* and *E. coli*. *Aspergillus* spp. were the only fungus isolated. Bacteria were sensitive to Ciprofloxacin, Amikacin, Amoxicillin, Clavulante and Ceftazidime.

In a study by (Ekpo *et al*., 2009). The number of occurrence of bacterial species out of the total isolates ranged from 1 and 15 with percentage frequency between 1.49 and 22.38 %. The following bacteria were isolate from otitis media samples : *Moraxella spp*., *Streptococcus spp*., *staphylococcus spp*., *Bacillus spp*., and *E. coli*, with a predominance of *Streptococcus spp*. The number of occurrence of fungal species in the samples ranged between 1 to 18 with percentage frequency between 2.04 and 20.4%. fungi isolated from otitis media samples were *Aspergillus spp*. , *Rhizopus spp*. , *Cephalosporium, penicillium spp*. , and *candida spp*. ,with *Aspergillus spp*., predominating. Forty nine patients (81.6%) had a single organism isolated from the middle ear culture. While the remaining 11(18.4%) patients had two or more organisms isolated . Infection was highest among the 1-10years and the lowest among aged 31 and above.

In a study by (Grevers *et al*., 2012). In 100 children with otitis media *H. influenzae* was identified in 21%, *S. pneumoniae* in 10%, *streptococcus* in 13% and *Moraxella* catarrhalis 1%. *H. Influenzae* was the most frequently identified pathogen in children from 12 months of age . *H. influenzae* and *S. pneumonia* were equally prevalent in children aged 3-11 months , but *S. pyogenes* was most frequently isolated in this age group.

Fungal infection of the middle ear are more common as fungi thrive well in moist ears (Tiedt *et al*. 2013). The most commonly found fungi in otitis media are *Candida* and *Aspergillus* species (Yorgancilar *et al*. 2013). A study of 204 clinically diagnosed patients with OM in India by (Saini *et al*., 2005) found fungal etiology in 25(12.25%), of which 7(29.17%) were Candida and 18(170.83%) Aspergillus species.

In a study done on patient with otitis media in Nigeria by (Musa *et al.*, 2015) Aspergillus species were isolated in 16(6.9%) and Candida species 6(2.6%).

A study done in Bosnia, *Aspergillus* species were isolated in 6(7.1%), *Candida* species in 8(9.4%) and Saprophytic flora 12(14.1%). The authors attributed this usually high prevalence of fungi to an excessive and uncontrolled use of antibiotics, There is also a possibility that in some cases unrecognized fungal inflammations of the auditory canal might be the cause of the infection (Bransko *et al*. 2011).

3.Material and Methods

3.1 Collection of Specimens:

Samples were collected from 300 children with signs and symptoms of the OM from patients visiting the children hospital in Benghazi-Libya, which included children between the ages of one months to twelve years. The specimens were collected during a period of 10 months (August 2016 to May 2017).

Swabs were taken from the auditory meatus, mainly in three suspected conditions, AOM, CSOM and OME. Swabs suitable for taking specimens of exudates from the ear consist of a sterile pledget of absorbent material, usually cotton wool swab (Citotest, China). The swab is a convenient sampling method. If possible, it should be 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 (Fischbach, 1996).

3.2 Culturing the Specimens

Swabs were cultured on two blood agar plates, one incubated aerobically at 37ºC for 24 hours and the other anaerobically, also at a third culture was on chocolate agar incubated in a carbon dioxide enriched atmosphere at 37ºC for 24-48 hours. Also, a forth culture was on MacConkey agar plate (Oxoid , England) incubated aerobically at 37ºC for 24 hours. Finally, if a fungal infection was suspected mycelium formation the specimen was cultured on sabouraud agar (Oxoid, England) and incubated aerobically at 35Cº-37ºC for 24 hours and then incubated at room temperature for 6 days.

3.2.1 Blood Agar

This plating medium supports the growth of most medically significant bacteria. It was used for primary plating and for subcultureing of colonies and is especially useful for detecting hemolytic activity of bacteria. With certain exceptions, human or animal blood is recommended for general use, mainly because colonies of ß-hemolytic streptococci show a characteristic clear zone on this blood medium (Collee *et al*., 1996).

3.2.2 Chocolate Agar

Chocolate agar is recommend as a primary plating medium swab culture that may contain fastidious organisms (Washington, 1985).

Because it supplies the special growth requirements (X and V factors) for *H. influenzae* when incubated in atmosphere with high CO2 concentration (Cheesbrough, 2000).

3.2.3 MacConkey Agar

MacConkey agar is a differential medium for the selection of Enterobacteriaceae (Koneman, *et al*., 1997). This medium used to isolate Gram- negative enteric bacilli from specimens containing mixture of bacterial species (Koneman, *et al*., 1997). This medium contains lactose as the only carbohydrate and neutral red as an indicator of acid production (Collee, *at al*., 1996). Lactose fermenting colonies are red, but colonies of non-fermenters are colorless (Washington, 1985).

3.2.4 Sabouraud Dextrose Agar

This medium was used for culturing , isolation, and identification of fungi (Washington,1985).

3.3 Identification of Bacteria and Fungi

3.3.1 Identification of Bacteria

3.3.1.1 Microscopic Examination of Bacterial pure Colonies.

After sub culturing , pure colonies were examined microscopically by making Gram staining to help identify pathogens in pure cultures by their Gram reaction and their morphology.

3.3.1.2 Biochemical tests

3.3.1.2.1 Catalase test

Catalase reagent acts as a catalyst in the breakdown of hydrogen peroxide to oxygen 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 methods: 2-3 ml of 3% hydrogen peroxide solution H_2O_2 (Oxoid, England) poured into a test tube (Reagenzglaser, Germany). A sterile wooden stick or a 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).

3.3.1.2.2 Coagulase test

Coagulase test was used to differentiate *Staph. aureus* which produces the enzyme coagulase from *Staph. epidermidis* and *Staph. saprophytic us* which do not produce coagulase. This enzyme causes plasma to clot by converting fibrinogen to fibrin (Cheesbrough, 2000). This test requires EDTA (ethylenediamine- tetra acitic 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(Sail Brand, China), and a colony of the tested microorganism from culture was emulsified in it. One drop of plasma (Oxoid, England) was added to the suspension and mixed gently for 10 seconds and the clumping was observed (Cheesbrough, 2000).

3.3.1.2.3 Oxidase test

It was used to identify *Pseudomonas* species from other members of the enterobacteriaceae. The solution used, was tetramythyl-p-phenylenalanine dihydrochloride (BDH chemicals 1td and merch company) in 2.5ml of sterile distilled water giving a final concentration of (1%) Two to Three drops of a freshly prepared oxidase reagent were placed in a petri dish (Oxoid, England) and one small piece of filter paper (Oxoid, England). The appearance of deep blue-purple color was observed in few seconds.

3.3.1.2.4 DNase test

DNase age (Oxoid, England) is a differential medium that tests the ability of an organism to produce an exoenzyme, called deoxyribonuclease or DNase, that

hudrolyzes DNA. DNase agar contains nutrients for the bacteria, DNA, and methyl green is action which binds to the negatively-charged DNA. it is purpose is 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 (Oxoid, England) to which DNA is added. The indicator methyl green produces a mint green medium.

The method:

1- Using a sterile loop, several colonies from an 18-24 hours culture is picked.
2- Incubate the test and control organism in each test area.
3- Incubate the plate at 35-37 Cº for 24 hours.
4- After incubation observe the color change in DNase with methyl green.
5- Result Interpretation:
    - Positive, development of clear halo around the colony.
    - Negative, no clear zone in the medium. Agar remains green due to no degradation of DNA.

3.3.1.2.5 Mannitol Salt Agar

Mannitol salt agar is a selective-differential medium for the isolation of staphylococci. The significant ingredients of this medium are the 7.5% sodium chloride (NaCl) mannitol, and the phenol red (PH indicator). The phenol red that gives ared appearance to the inoculated medium. In the presence of acid. The phenol red turns yellow color.

The method: A plate of mannitol salt agar is streaked with a pure colonies of the isolate, incubated at 37Cº for 2 hours. If the isolate is *Staph. aureus* (halophilic bacterium), it grows on the medium and ferments mannitol to acid products which turn the medium to a yellow color.

3.3.1.2.6 Streptocard Enzyme Latex Test (Oxoid , England):

This test is used for the identification of Streptococcal groups A, B, C, D, F, and G, Figure (3a and 3b). The procedure was carried out in according to the instruction of the manufacturer as follows:

Streptococcus extracted enzyme of the streptococcal groups supplied in a powder, was hydrated by adding 12mL of distilled water. Then 0.4mL of this solution was distributed in each 6 test tubes.

1. Five colonies of the test bacterium were emulsified in the enzyme containing test tubes, and incubated at 37C^{o} 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 spreaded over the entire area of the rings.
4. The slide was gently rotated, and the agglutination (positive reaction) was read in 1 minute (Agrawal, 1992)

Figure (3- a): Identification Kit of *Streptococcus* species

Figure (3- b): Identification test of *Streptococcus* species

3.3.1.2.7 API for Identification of Enterobacteracae

API 10 S system enables 10 tests to be recorded out quickly and easily for the biochemical identification of anaerobes. Other tests such as colonial and microscopic morphology, Gram-stain, ...etc. should be performed and the results to confirm or complete the identification table inserted at the end of package of the kit.

The method: The incubation box was prepared and 5ml of distilled water distributed into honeycombed wells of the tray to create a humid atmosphere. The strip was removed from package and placed in the incubation box. An ampule of API medium (NaCl) about 5ml. A wire loop was used to remove a single 18-24 hours old well isolated colony from a plate. Bacterial suspension was distributed into tube of the strip, and the incubation box closed and then incubated at 37ºC for 24 hours. After the incubation, some reactions required to added of reagents these were: TDA, IND. The strip was read by using a referring table

3.3.2 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).

3.3.2.1 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

3.3.2.1.1 Slide Culture

1cm of sabouraud dextrose agar (SDA) block is cut from the sterile plate and transferred to a sterile microscope slide (SAIL BRAND, CHINA) resting on a bent glass rod in a petri dish. Small fragments of the test colony are inculated using a mounted needle into the 4 sides of the block, which is then covered with a sterile coverslip (SAIL BRAND, CHINA). A few drops of water are added to the dish for not drying and then covered and incubated at 25-28ºC.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).

3.3.2.2 Identification of *Asperigillus sp.*

The identification of the organism depends on the colonial morphology and pigmentation and production of conidial heads (Raper and Fennel, 1965). *Asp. niger* colonies initially white, quickly becoming black, hyphae are hyaline and distinctly septet . Conidiophores are long and vesicle is usually not seen because it is covered with thick ball of spores. Aflaves spreading olive limo-green colonies with rough conidiophores and smooth conidia distinguishes this species (Sutton *et al*., 1998; Konemat *et al*., 1997).

3.3.2.3 Identification of *Candida albicans.*

C. albicans grows well on sabouraud agar, the colonies are white and having a distinctive yeast smell and the budding cells can be easily seen by direct microscopy in stained or unstained preparation.

C. albicans can be identified presumptively by a simple germ tube test.

3.3.2.3.1 Germ tube test

The simple identification method is the germ tube test. Very small quantities of a colony are used for inoculation of 0.5ml of mammalian serum (horse, human) in small

plastic tubes (Reagenzglaser, Germany). Then incubated for 3 hours at 37ºC. 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).

3.4 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 (drops, ointment) in different species, the patient and microorganism, simultaneously.

All bacterial isolates were tested for their sensitivity to antibiotics using the disk diffusion method of (Bauer *et al*., 1966), and using Nutrient agar (Oxoid, England) for Gram negative bacteria and blood agar for Gram positive bacteria. The following oxoid antibiotic discs (Oxoid , England) were used:

Amoxycillin Clavulanic acid (AMC), Fusidic Acid (FA), Chloramphenicol (C), Ciprofloxacin (CIP), Norfloxacin (NOR), Gentamycin (CN), Amikacin (AK), Amoxicillin (AX), and Ampicillin (AMP). Table (1).

3.5 Statistical analysis:

Frequency table and chart constructed for our date were analyzed statically using the Chi-Square test. We assumed results statistically significant when P-value is <0.05. the statistical analysis of the results were carried out according to the computer package (SPSS 8 Version).

Table(1): Antimicrobial susceptibility testing

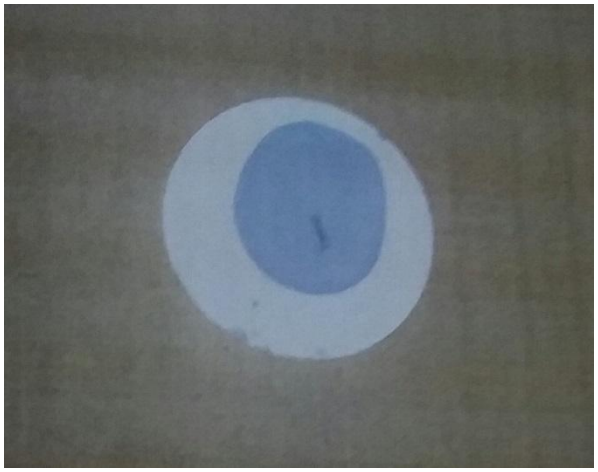
Antibiotic	Abbreviation	Concentration (µg)
Amoxycillin / clavulanic acid (Augmentin)	AMC	30
Fusidic Acid	FD	10
Chloramphenicol	C	10
Ciprofloxacin	CIP	5
Norfloxacin	NOR	15
Gentamycin	CN	10
Amikacin	AK	30
Amoxicillin	AML	30
Ampicillin	AMP	10

4.Results

The microbiological sample from 300 patients, at the children hospital in Benghazi were collect from out-patients visiting the clinic with signs and symptoms of the otitis media during a period of 10 months (August 2016 to May 2017). Two hundred and ninety three (97.7%) were positive for culture and seven (2.3%) had no bacterial or fungal infection.

Biochemical tests, which were the catalase test, Api 10s, Mannital salt agar test Figure(4), the Oxidase test Figure (5a and b), and DNase test Fig (6), showed positive result with others, Those results were used as a complement to other characteristics of bacterial species (Gram staining , morphology, conditions of growth), for identifying those genera and specie

Figure(4): Mannitol Salt Agar Showing Fermentation by *Staph. Aureus*



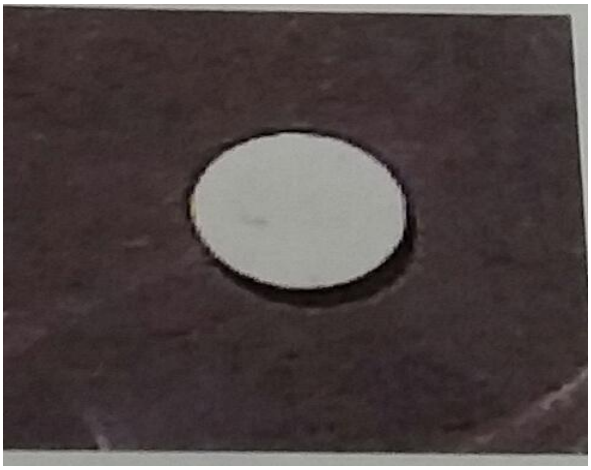


Figure (5-a): Oxidase positive test Figure (5-b): Oxidase negative test

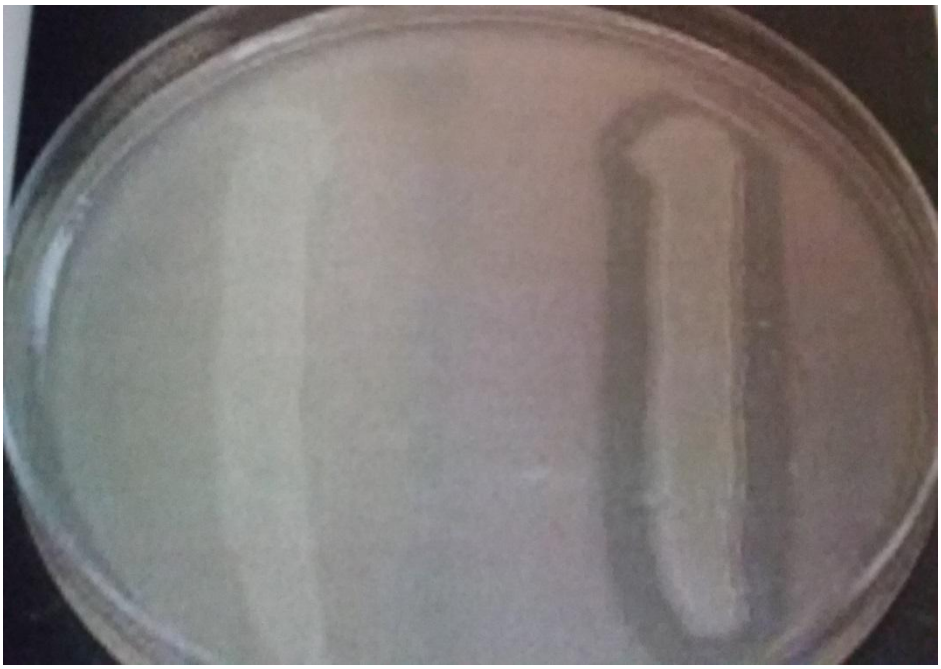


Figure (6): Positive DNase test

4.1. Age Distribution

Table (2) show the age distribution of otitis media infection .The age was between less than two years to twelve years. The age in years was divide into five group: (<1-2 years), (3 to 5), (6 to 8),(9 to 11), and (>11 years). The otitis media infections decreased with increase of the age of the patients.

Table (2): Groups of infected patients to age

Age (years)	Frequency	Percentage
< 1 - 2	166	55.3
3 - 5	51	17
6 - 8	46	15
9 - 11	26	8.7
> 11	11	3.7
Total	300	100

4.2. Relationship Between Age Sex and Infection

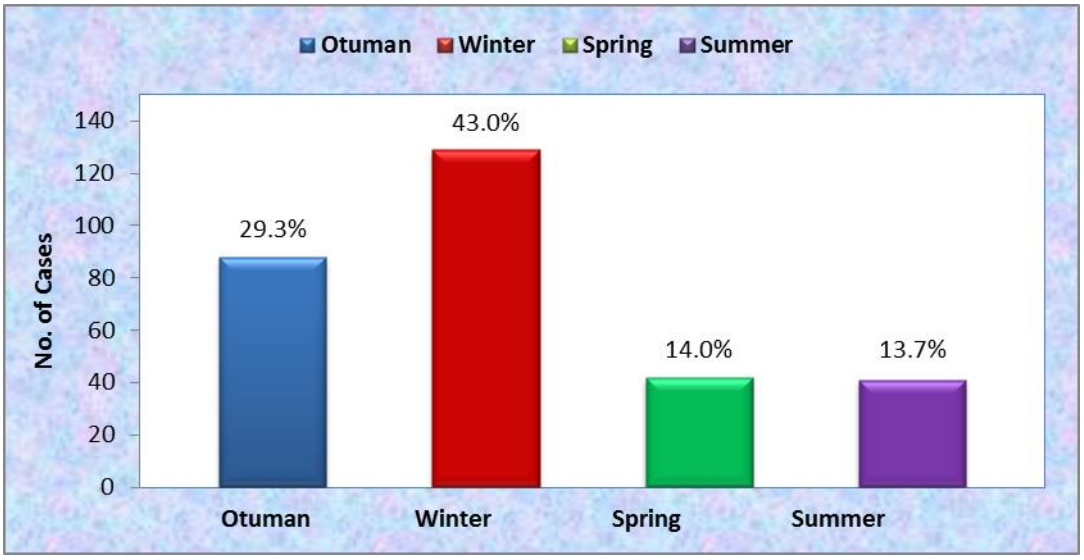
The age of the patients was between few months and 12 years (Table 3). The positive cases 179 males and 121 females .There was a difference between number of cases of males and females $P = 0.04$ ($P > 0.05$).

Table (3): Otitis media infection according to age and sex

Age (years)	Sex		Total
	Male	Female	
< 1 - 2	104 (34.7 %)	62 (20.7 %)	166 (55.3 %)
3 - 5	32 (10.7 %)	19 (6.3 %)	51 (17 %)
6 - 8	24 (8 %)	22 (7.3 %)	46 (15.3 %)
9 - 11	14 (4.7 %)	12 (4 %)	26 (8.7 %)
> 11	5 (7 %)	6 (2 %)	11 (3.7 %)
Total	179 (59.7 %)	121 (40.3 %)	300 (34.7 %)

4.3. Seasonal Distribution

Figure (7) and table (4) show the seasonal distribution of ear infection. The highest percentage was in winter 129 cases (43%), then the autumn where the number of cases was 88 (29.3%), followed by spring with 42 cases (14%) and the lowest percentage was in summer season with 41 (13.7%) cases.



Figure(7): Distribution of Otitis Media Patients Accotding to Year Seasons

4.4. Micro-organisms Distribution

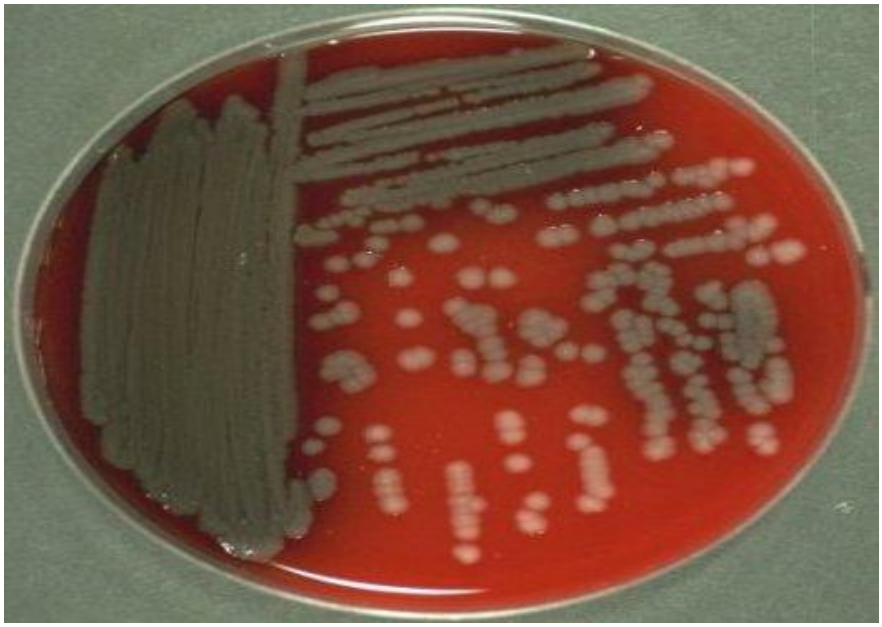
From the total 293 positive culture 198 (66%) were single bacterial infections, 31(10.4%) were fungal infections and 64(21.3%) were mixed infections (Two bacterial spp.), tables (5,6).The predominant microorganisms were *P. aeruginosa* (18.7%) as shown in figure (8), and *Staph. aureus* (13.3%) figure (9), then *prot. mirabilis* (10%) figure (10), *S. pneumoniae* (7.3%), *K. pneumoniae* (6%), *E.coli* (5.7%), and *S. pyogenes* (5%), two isolates of *A. fumigatus* about ((0.7%) figure (11), ten isolates of *A. niger* about (3.3%) figure (12), and isolates of *C. albicans* about (6%) figure (13) and table (7) describe distribution of the etiological agents, figure(14).

Table (4): Single and mixed infections of patients

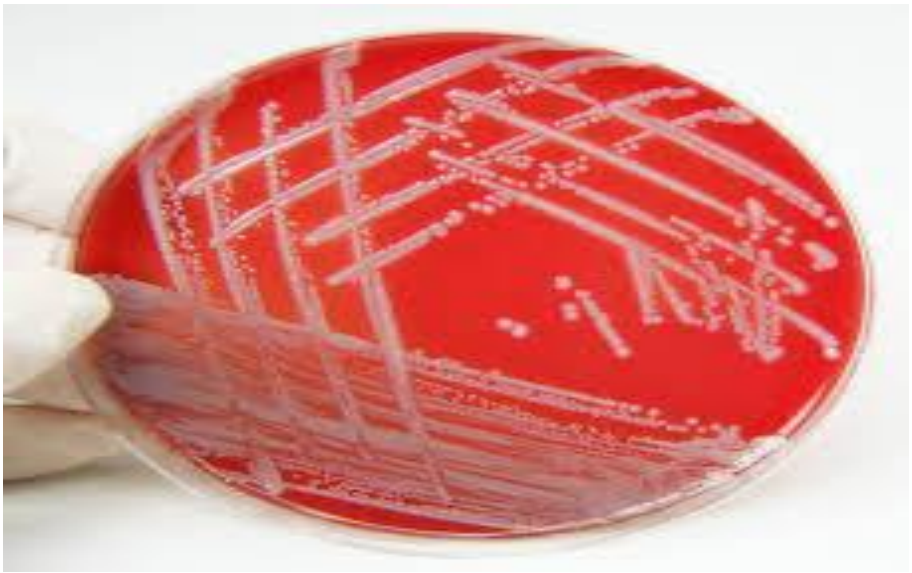
Infected cases	Single infection	Mixed infection	Total number of Infected cases
Number	229	64	293
Percentage	(76.3)	(21.3)	(7.6)

Table (5): Kinds of microbial agents causing the infection

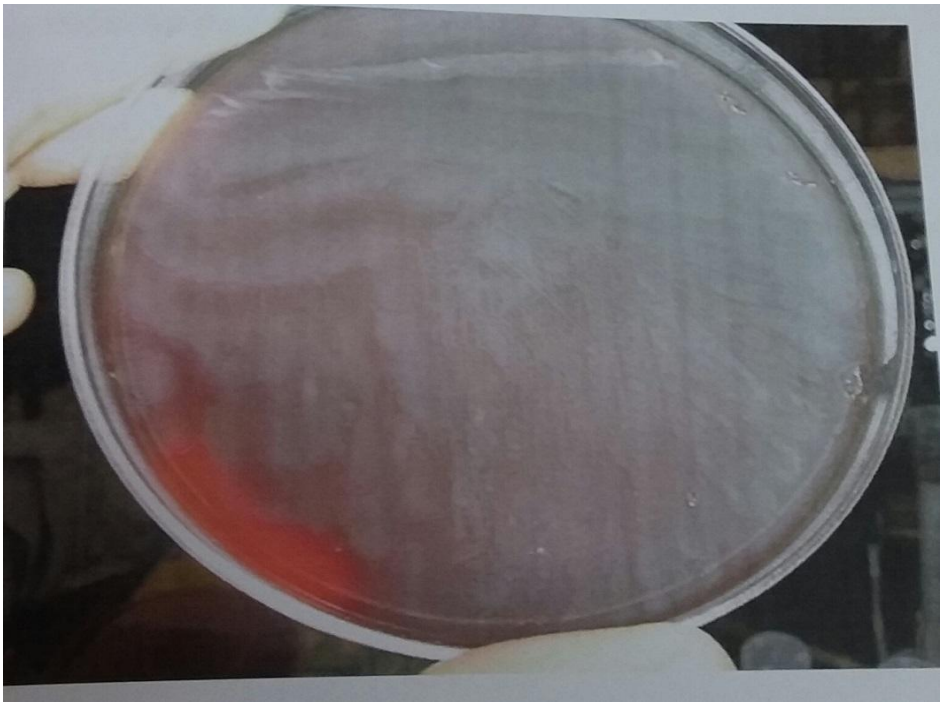
	Positive culture				
	Bacterial Infection	Fungal infection	Two bacterial spp	No growth	Total
Number	198	31	64	7	300
Percentage (%)	(66)	(10.4)	(21.3)	(2.3)	(100)



Figure(8): *Pseudomonas aeruginosa* on Blood Agar



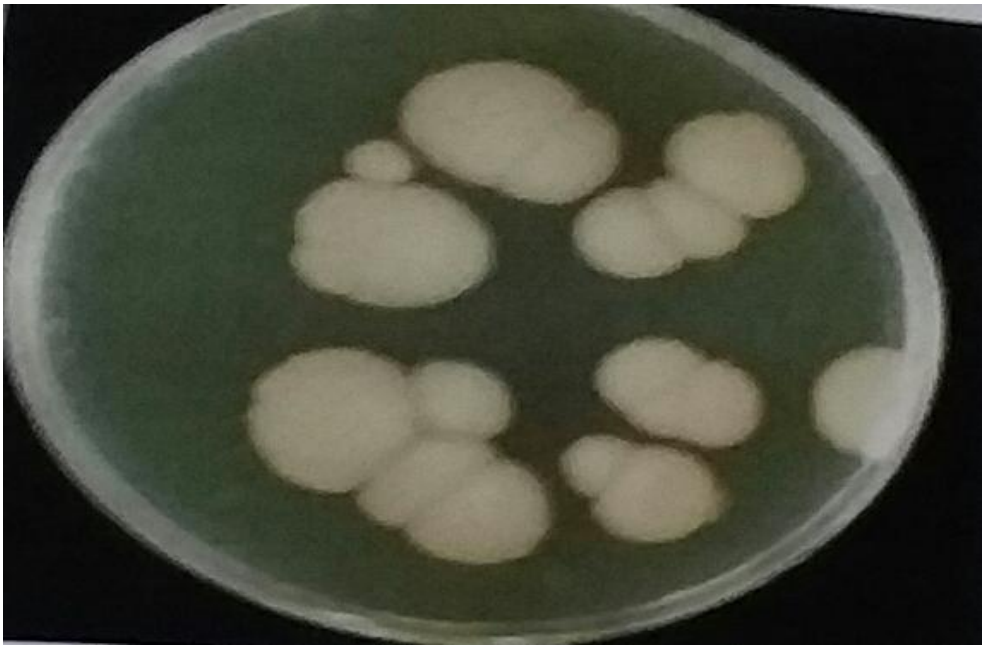
Figure(9): *Staphylococcus aureus* on Blood Agar



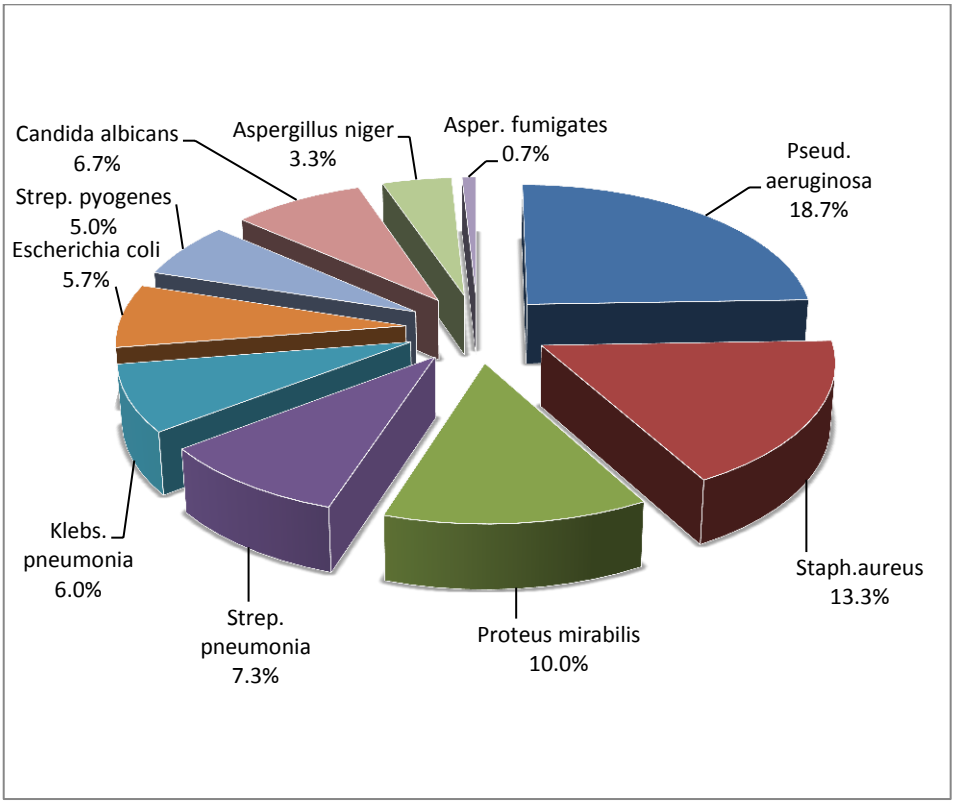
Figure(10): *Proteus mirabilis* on Blood Agar

Figure(11): *Asp. fumigatus* on Sabouraud Dextrose Agar

Figure(12): *Asp. niger* on Sabouraud Dextrose Agar



Figure(13): *Candida albicans* on Sabouraud Dextrose Agar



Figure(14): Percentage of micro-organisms which causes OM

4.5. Antimicrobial Sensitivity Test

The sensitivity test of the seven isolated species of bacteria was cerried out using 9 types of antibacterial drugs. The most effective antibiotic (76%) was Ciprofloxacin (5mg), Ampicillin (30mg) showed the lowest effect with (44%).

The 89 isolates of *Ps. aeruginosa* showed a high sensitivity to gentamicin (100%) and ciprofloxacin (95%), and low sensitivity to amoxycillin (30.4%), table(8), figure (15).

The highest rate of resistance of *Staph. aureus* isolates was against ampicillin (87%), and the highest percentage of sensitivity was to fusidic acid (100%), ciprofloxacin (98.4%) and gentamycin (96.7%) figure (16) table (9).

Strep. pyogenes strains were resistance to ampicillin (55%),and high percentage of sensitivity was to fusidic acid, chloraphenicol and gentamicin (100%) each Table (14) Fig(21).

Strep. Pneumoniae strains showed a high percentage of sensitivity to amoxycillin, fusidic acid, ciprofloxacin, and amoxicillin (100%) each, as shown figure (18) and table (11).

Proteus mirabilis was resistance to norfloxacin (55.5%) and amoxycillin (24%) and high percentage of sensitivity to ciprofloxacin and amikacin both (100%) each figure(17) and table(10).

E.coli strains were resistant to amoxycillin (54%), and very sensitivity to chloramphenicol and amikacin as shown in figure (20) and table (13)

Klebsiella pneumoniae isolates were resistant to amoxycillin (65%) and ampicillin (76%), and was (100%) sensitive to amikacin figure (19) and table (12).

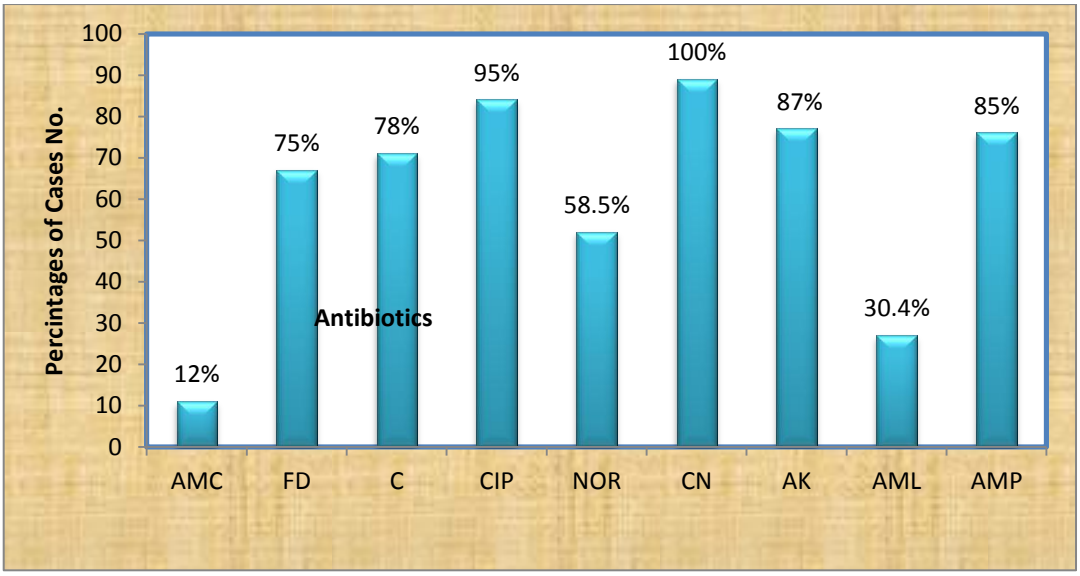


Figure (15): Percentage of Sensitivity of *Pseud. aeruginosa* Strains

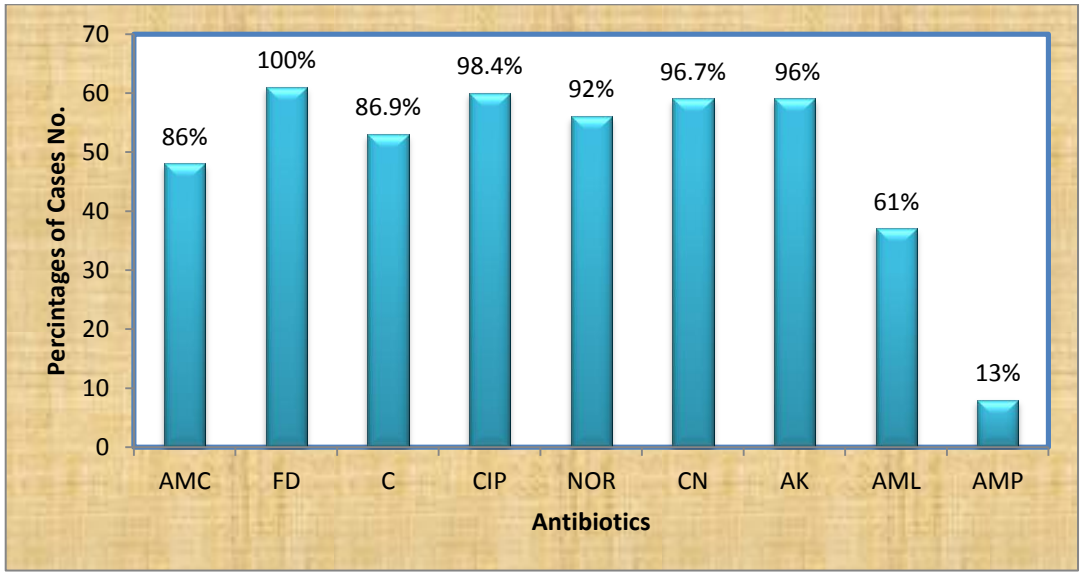
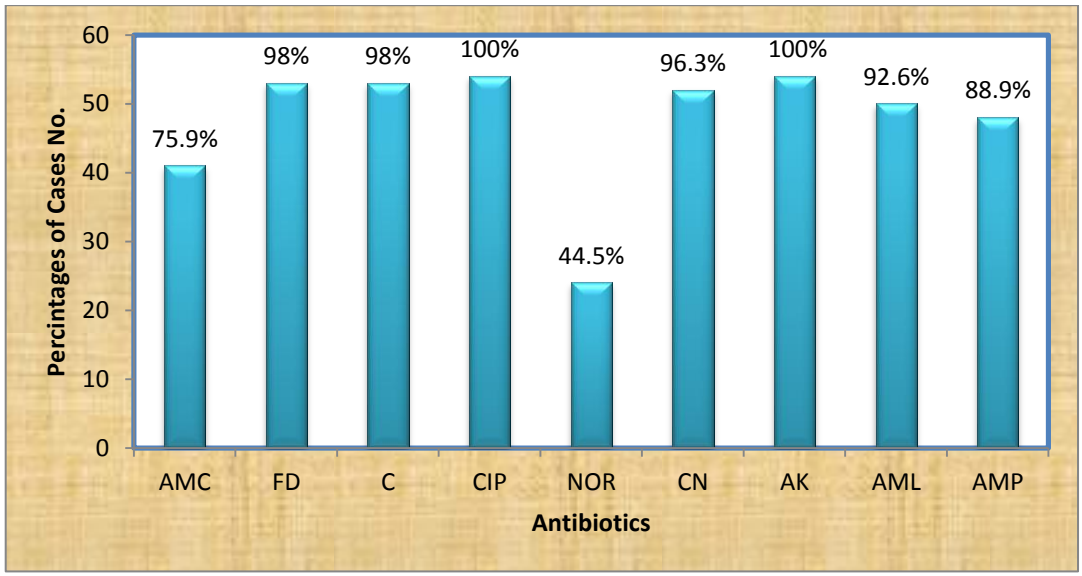


Figure (16): Percentage of Sensitivity of *Staph. aureus* Strains



Figure(17): Percentage of Sensitivity of *Proteus mirabilis* Strains

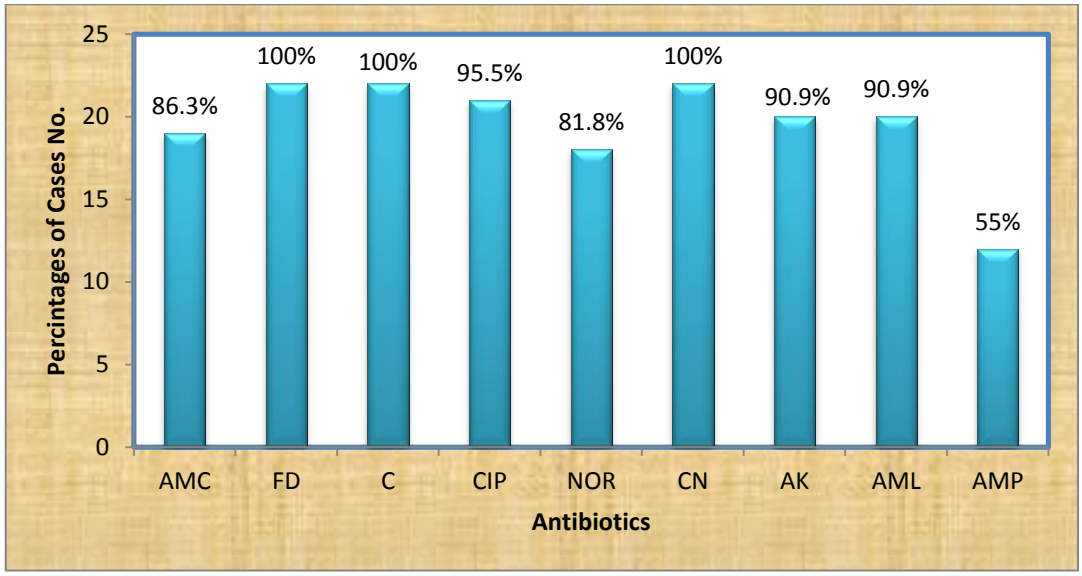
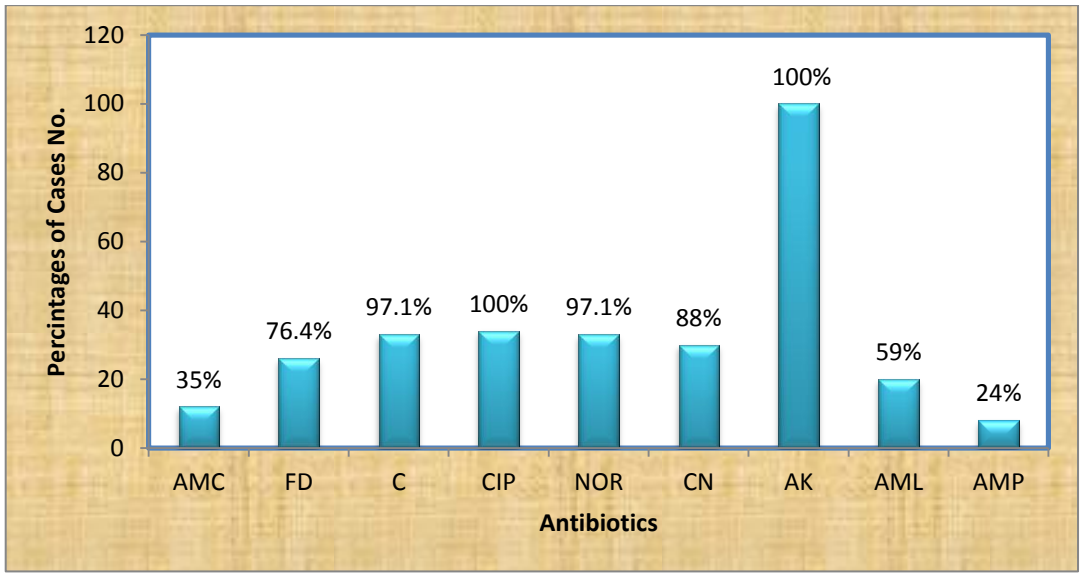


Figure (18): Percentage of sensitivity of *Streptococcus pneumoniae*



Figure(19): Percentage of Sensitivity of *Klebs. pneumoniae* Strains

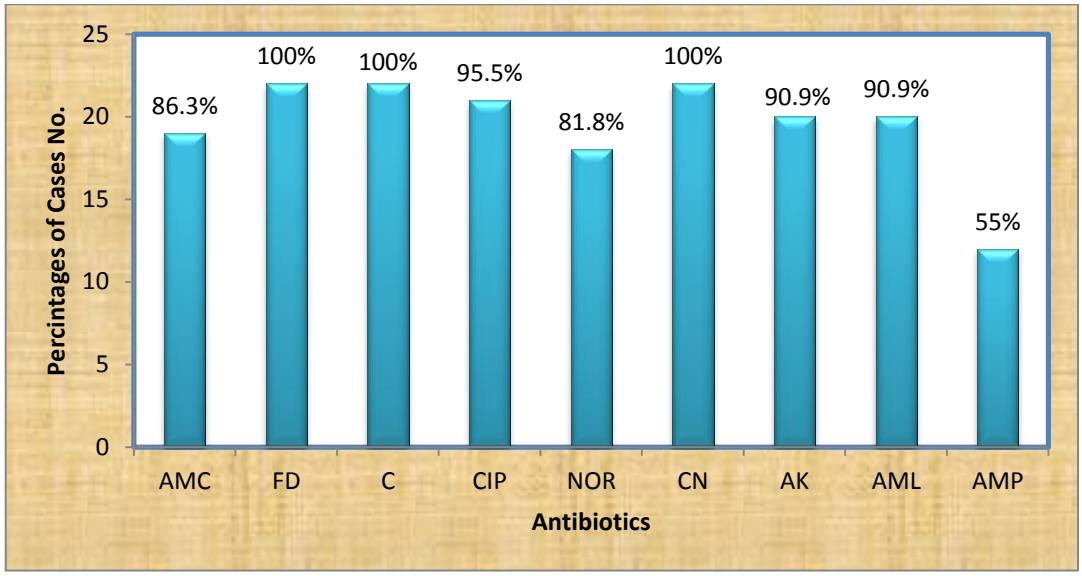
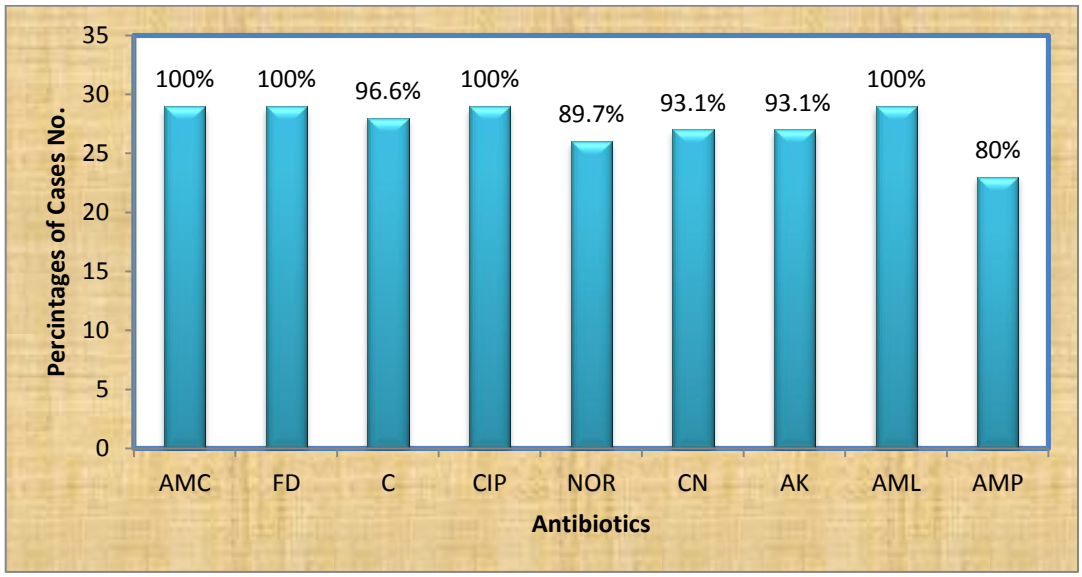


Figure (20): Percentage of Sensitivity of *E. coli* Strains



Figure(21): Percentage of Sensitivity of *Strep. pyogenes* Strains

Table(6): Antimicrobial sensitivity test for 89 strains of *Pseudomonas aeruginosa*

Antimicrobial Agents	Number of resistant isolates	%	Number of Sensitive isolates	%
Amoxicillin / Clavulanic acid (Augmentin)	78	88	11	12
Fucidic Acid	22	25	67	75
Chloramphenicol	18	20	71	78
Ciprofoxacin	5	5	84	95
Norfloxacin	37	41.5	52	58.5
Gentamicin	0	0	89	100
Amikacin	12	13	77	87
Amoxicillin	62	67.6	27	30.4
Ampicillin	14	15	76	85

Table(7): Antimicrobial sensitivity test for 61strains of *Staphylococcus aureus*

Antimicrobial Agents	Number of resistant isolates	%	Number of Sensitive isolates	%
Amoxicillin / Clavulanic acid (Augmentin)	8	14	48	86
Fusidic Acid	0	0	61	100
Chloramphenicol	8	13.1	53	86.9
Ciprofoxacin	1	1.6	60	98.4
Norfloxacin	5	8	56	92
Gentamicin	2	4	59	96.7
Amikacin	2	4	59	96
Amoxicillin	24	39	37	61
Ampicillin	53	87	8	13

Table(8): Antimicrobial sensitivity test for 54 strains of *Proteus mirabilis*

Antimicrobial Agents	Number of resistant isolates	%	Number of Sensitive isolates	%
Amoxicillin / Clavulanic acid (Augmentin)	13	24.1	41	75.9
Fusidic Acid	1	2	53	98
Chloramphenicol	1	2	53	98
Ciprofoxacin	0	0	54	100
Norfloxacin	30	55.5	24	44.5
Gentamicin	2	3.7	52	96.3
Amikacin	0	0	54	100
Amoxicillin	4	7.4	50	92.6
Ampicillin	6	11.1	48	88.9

Table(9): Antimicrobial sensitivity test for 29 strains of *Streptococcus pneumoniae*

Antimicrobial Agents	Number of resistant isolates	%	Number of Sensitive isolates	%
Amoxicillin / Clavulanic acid (Augmentin)	0	0	29	100
Fusidic Acid	0	0	29	100
Chloramphenicol	1	3.4	28	96.6
Ciprofoxacin	0	0	29	100
Norfloxacin	3	10.3	26	89.7
Gentamicin	2	6.9	27	93.1
Amikacin	2	6.9	27	93.1
Amoxicillin	0	0	29	100
Ampicillin	6	20	23	80

Table(10): Antimicrobial sensitivity test for 34 strains of *Klebsiella pneumoniae*

Antimicrobial Agents	Number of resistant isolates	%	Number of Sensitive isolates	%
Amoxicillin / Clavulanic acid (Augmentin)	22	65	12	35
Fusidic Acid	8	23.6	26	76.4
Chloramphenicol	1	2.9	33	97.1
Ciprofoxacin	0	0	34	100
Norfloxacin	1	29	33	97.1
Gentamicin	4	12	30	88
Amikacin	0	0	100	100
Amoxicillin	24	41	20	59
Ampicillin	26	76	8	24

Table(11): Antimicrobial sensitivity test for 37 strains of *E . coli*

Antimicrobial Agents	Number of resistant isolates	%	Number of Sensitive isolates	%
Amoxicillin / Clavulanic acid (Augmentin)	20	54	17	46
Fusidic Acid	12	30.5	25	69
Chloramphenicol	0	0	37	100
Ciprofoxacin	1	2.7	36	97.3
Norfloxacin	1	2.7	36	97.3
Gentamicin	16	42	21	58
Amikacin	0	0	37	100
Amoxicillin	3	8.2	34	91.8
Ampicillin	15	41	22	59

Table(12): Antimicrobial sensitivity test for 22 strains of Streptococcus pyogenes

Antimicrobial Agents	Number of resistant isolates	%	Number of Sensitive isolates	%
Amoxicillin / Clavulanic acid (Augmentin)	3	13.7	19	86.3
Fusidic Acid	0	0	22	100
Chloramphenicol	0	0	22	100
Ciprofoxacin	1	4.5	21	95.5
Norfloxacin	4	18.8	18	81.8
Gentamicin	0	0	22	100
Amikacin	2	9.1	20	90.9
Amoxicillin	2	9.1	20	90.9
Ampicillin	10	45	12	55

Table (13): Mixed isolates

Name mixed isolates	Frequency	Percent
Pseudomonas aeruginosa + Proteus mirabilis	15	5
Streptococcus pyogenes + Klebsiella pneumoniae	7	2.3
Staphylococcus aureus + Escherichia coli	6	2
Staphylococcus aureus + Pseudomonas aeruginosa	12	4
Proteus mirabilis + Escherichia coli	8	2.7
Streptococcus pneumoniae + Escherichia coli	6	2
Pseudomonas aeruginosa + Klebsiella pneumoniae	5	1.7
Streptococcus pneumoniae + Proteus mirabilis	1	0.3
Staphylococcus aureus + Klebsiella pneumoniae	3	1

5.Discussion

The number of the out-patients who attended the clinic at the Children Hospital during the period August, 2016 to May, 2017 (ten months study) was 300 patients, all with otitis media and discharge. An overall prevalence of (97%) of culture–positive otitis media was observed in this study. This prevalence was higher than previous studies in other countries (Giebink, 1989; Maharjan *et al.*, 2006).

It has been reported that the prevalence of otitis media is higher in developing countries when compared with advanced countries inaccessibility to health care facility, local customs and beliefs, harmful traditional practices and poor treatment of acute cases by the first contact health personnel have been suggested as possible reason for the difference in prevalence (Lasisi and Ajuwon, 2001; lasisi *et al*., 2008).

In this study (3%) of the taken swabs did not give any positive growth .In a similar study in Tamilnadu, also 4% of samples were without growth (Nwabaisi and Olige, 2002), and 7% in a study in India (Prakash *et al*., 2013).

The bacteria in the middle ear exudates failed to grow because: (1) they were strictly(Milton and Edson, 1989).

(2) The presence of viral etiological agents when the infection was caused by respiratory syncytial virus, influenza virus or adenovirus (Henderson *et al*., 1982; Riding *et al*., 1978), or the patient was taking an antibacterial drug and did not tell physician.

In our study, otitis media was more common in males compared to females. It was found 40.3% of females and 59.7% of males with OM infection. This result correlates with the studies reported by (Iqbal *et al*. 2011; Nwasbisi *et al*., 2002 and Kumar *et al*., 2011). This may be due to difference some tradition, cotton swabs were used by in transferring of pathogens from the external skin to the middle ear. Other studies have challenged this with equal distribution (Katzen meyer *et al*., 1999).

A seasonal variation played an important role regarding ear infection we found 43% of the infected cases during the winter months. These results were in agreement with

other workers (Fleming *et al*., 1992; Katzenmeyer *et al*., 1999; Meyer and Lawson, 1986; Gates, 1998). Particularly OM was more in winter time because it was correlated with the upper respiratory tract infection (Gates, 1998; Hall and Colman, 1987; Katzenmeyer *et al*., 1999; Meyer and Lawson, 1986).Also during the autumn months, the cases of OM infections were in increase. This is not in agreement with (Pelton and Klein, 1988).

The group with the highest OM infections were the people aged between less than one year (<1 year) and 2 years old. After that, there was a correlation between age and OM infection; that is the increase of age resulted in less infection. Our results were in agreement with previous studies (Johnson and Yu, 1997; Mims *et al*., 1998 ; Gates, 1998; Gorbach *et al*., 1997; Mandell *et al*., 1990 and Katzenmeyer *et al*., 1999).

A study on chronic suppurative Otitis media (CSOM) in benghazi (libya) showed that 53% of age group of newborn to 15 years had CSOM (Rao *et al*., 1992). The great frequency of OM in infant and children because their ET are shorter and more flaccied eartilage which causes impaired opening of the tube (Blue stone and Klein, 2001; Lanphear *et al*., 2014). And this tube permitting direct access from the nasopharynx to the middler ear (Meyers and Lawson, 1986).

This condition is extremely common in infant and small children, partly because related to forced, improper positioning of infants during breast feeding and bottle feeding (Mims *et al*., 1998). Also it is because of the poorly developed immunity with recurrent upper respiratory tract infection (Gates, 1998; Maran, 1988; Veenhoven *et al*., 2004; Gross *et al*., 1992).

A total of 326 bacterial isolates were isolated from 300 OM patients. The individuals who had bacterial isolates, 66% had single bacterial infection while 21.3% had mixed bacteria- bacteria infection. This is comparable with the rates of previous studies conducted in Ethiopia (Abera and Kibret, 2011) and Nigeria (Osazuwa *et al*., 2011), and other workers (Chonmaitree *et al*., 1986; Giebink, 1989; Jero and Karma, 1997).

Regarding the etiological agents that were isolated and identified. We found that *Ps. aeruginosa* was contributing the highest percentage with 18.7% followed by *Staph.*

aureus which accounted for 13.3% of the total isolates. Similar results have been observed by other authors who reported that *Ps. aeruginosa* and *Staph. aureus* were the most common organisms isolated from OM patients (Bardonis *et al*., 2003; Arshad *et al*., 2004; Abdelraouf *et al*., 2014). our results supported the previous studies (Oyeleka, 2009; Oni *et al*., 2002) but it is inconsistent with other studies conducted in different area which showed that *S.aureus* is the most predominant isolate (Ekpo *et al*. 2009; Akinjogunta, 2011).

This may due to the fact that bacterial colonization of OM increases as temperatures raises, which in turn increases the isolation rate of bacteria. As a result difference in isolation rate might be occuring related to the effect of climate and geographical variation. The frequency of *Ps. aeruginosa* and *Staph. aureus* in the present work may due to the ubiquitous nature of *P. aeruginosa* and the availability of *S. aureus* as normal flora of the nares ,mouth and some other non-sterile sites (Ekpo *et al*., 2009). Additionally, the virulent nature and rapid colonization properties of these two Organisms also contributes to their high rate of recovery (Sweeny *et al*., 1982).

Enteric bacteria *(Prot. mirabilis, E.coil* and *K. pneumoniae*) were representing 5-10% of all ear discharge specimens. Karim et al.,1981 found a higher percentage (6-9%). It can be surmised that these organisms also become opportunistic pathogens in the ear when the resistance is lowered (Rao *et al*., 1992).

S. pneumoniae strains made up 7% of all isolates, this is similar to the study by Karim et al.who found 6% in the year 1981 and was different from 17% by Roland *et al*., in 2009. This difference might be due to the high number of simples (1309) taken by Roland *et al. S. Pyogenes* found in our study as 5%, and this is approximately similar to a study in Benghazi, conducted by Karim *et al*., 1981. who reported 4% of *Strep. Pyogenes*, and a Norwegian study (7%) reported by Haugsten and Lorentzen in 1980. Japanese workers found a higher percentage (14%) (Sugita *et al*., 1982).

The fungal spp. Caused ear infection in our survey were *Asp. niger, A. fumigatus*, and as well as the yeast *candida albicans*. Our results were in agreement of (Grigorious and Font in 1970; Pahwa *et al.,* in 1983 and Southgate et al in 1997). Howerer Rao *et al*., in 1992 isolated more *Candida spp*. than *Aspergillus spp.*

Ps. aeruginosa strains were highly resistant to Augmentin (Amoxycillin / Clavulanic acid), Amoxycillin and Norfloxcin. However they were sensitive to Gentamicin (100%), Ciprofloxacin (95%), Amikacin (87%) and Ampicillin (85%). A study by Mansoor *et al*., 2009. showed that *Pseudomonas* was sensitive to Amikacin, gentamicin and Ciprofloxacin whereas the study of Jane *et al*., 2004. showed high resistance to Ciprofloxacin.

Staph. aureus was the second organism causing OM infection. Its strains were highly sensitive to Ciprofloxacin (98%), Amikacin (96%) and Norfloxacin (92%); however, resistant to Ampicillin (91%) and Amoxycillin (86%). In the study by kumar *et al*., 2011. it was found that *Staph. aureus* was sensitive to Amikacin, Ciprofloxacin and Linezolid and resistant to Ampicillin and Cefotaxime. Aonther study by Johnson and Yu in 1997 showed that most *Staph. aureus* isolates were resistant to Penicillin, Ampicillin, and Amoxycillin. This is most probably because of different strains of the genus *Stapylococcus* acquired different mechanisms of resistance to different antibiolics . At the beginnning of the antibiotic era, virtually all *staph. aureus* were susceptible to penicillin. Currently, all hospital isolates and and most community isolates are producing B-Lactamases and are resistant to penicillin , Ampicillin and Amoxycillin (Johnson and Yu,1997).

K. pneumoniae isolates were sensitive to ciprofloxacin, Amikacin and resistant to Amoxycillin and Norfloxacin. *Prot. mirabilis* was sensitive to ciprofiaxacin and resistant to Norfloxacin. *Klesiella spp*. and *Proteus spp*. were sensitive to ciprofloxacin in the study of Alsaimary *et al*., 2010. Most known strains of Proteus produce B-lactamase which cleave the B-lactam of pencillin and its derivates making them without effect (Balows and Duerden, 1998).

The isolates of *S. pneumoniae* were sensitive to all antibotics tested. Our results were in agreement with John-son and Yu in 1997. Brooks *et al*. 1998, reported that 5 to 10 percent of pneumococci isolated in the USA were resistant to penicillin.

Most strains of *Escherichia coli* were sensitive to all antibiotics tested expect Amoxycillin and Ampicillin. These results were very similar with this of Iqbal *et al*., 2011. This resistance to these penicillin derivaives might be because of the acquisition by these strins of plasmide coding for B-lactamase.

Streptococcus pyogenes isolates were sensitive to most antibiotics tested however, these strains were less sensitive to Ampicillin and Norfloxacin. This result is similar to results seen by Arshad *et al*., 2004; Sharma *et al*., 2004; Abdelraouf *et al*., 2014.

6-Conclusion

Through this study on the etiological agent causing Otitis media infections in patients of Benghazi area, the following can be concluded:

1- The highest middle ear infections were in the patients between below <1 to 2 years.

2- Otitis media infections were found to be more active in autumn and winter months.

3- Bacteria were more responsible for Otitis media infections than fungi.

4- Major bacterial infections agent were *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and fungal infections were *Candida spp.* and *Aspergillus spp*.

5- Different bacterial isolates were sensitive to different antibiotics but, generally the Ciprofloxacin was the most effective drug against bacteria causing otitis media.

7.References

Abdelraouf, A., Elmanama, N., Abu Tayyem, E. and Nassrallah, S. A. (2014). The bacterial etiology of Otitis media and their antibiogram among children in Gaze strip, palestine. Egyption J of Ear, Nose, Throat and Allied Sciences, 15: pp87-91.

Abdelshafy, I. A., Haleem, A. A., Khalil, Y. A. and Ghazal, A. A. (2015). Microbiology of Chronic Suppurative Otitis media, study of the Role of Biofilm and Fungal infection. J Otolarynol ENT Research. 3(1): pp51.

Abera, B. and Kibret, M. (2011). Bacteriology and Antimicrobial Susceptibility of otitis media at Dessie Regional Health Research Laboratory. Ethiopia.J. Health Dev. 52(2): pp161-167.

Acuin JM. (2007). Chronic otitis media : Adisease waiting for solutions. Common Ear Hearing H . 4(6): 17-19.

Adoga, A. S., Malu, D., Badung, Bp., Obiesie IV, (2010). Swab and Aspiration Collection methods and antibiograms in chronic otitis media at Jos. University Teaching Hospital. Which is superior? Ann Afr Med. Vol 9. Issue 4:230-234.

Agrawal, S., M. Husein, D. Macrae. (1992). Complications of Otitis Media: An Evolving State. The Journal of Otolarynology; 34: p33-38.

Ahmad, S. (2013). Antibiotics in Chronic Suppurative Otitis Media: A bacteriologic study. Egyptian Journal of Ear, Nose, Throat and Allied Sciences. 14: pp191-194.

Ahmed, M., Ihsan, E., and Jassim, M., (2010). Prevalence and patterns of chronic suppurative otitis media and hearing impairment in Basrah city. Jornal of medicine and medical sciences. 1(4): pp129-133.

Akinjogunla, O. J. (2011). Aetiologic agents of acute otitis media :prevalence, Antibiotic Susceptibility, B. Lactamase (BI) and extended spectrum B-Lactamase production J.Microbiology Biotechuol food Sci, 12: pp333-53.

Alho, O. P., Laara, E. and Oja, H. (1996). What is the natural history of recurrent Acute otitis media in infanty?. The Jornal of Family Practice. 43: pp258-264.

Allen, E. K., Manichaikul, A. and Sale, M. M. (2014). Genetic contributors to Otitis media : agnodtic discovery approaches. Curr Allergy Asthma Rep. 14: p411.

Alsaimary, B. L., Alabbasi, A., and Najim, J., (2010). Antiboitic susceptibility of bacterial pathogens associated with otitis media. Jornal of Bacteriology Research. 2(4): pp41-50.

Amusa, Y. B., Ijadunola, I. K. T. And Onayade, O. O. (2005). Epidermiology of otitis media in Local Tropical African Population, West African Journal of Medicine. 24: pp227-230.

Arshad, M., Khan, N. U., Ali, N. and Afridi, N. M. (2004). Sensitivity and spectrum of bacterial isolate in infection otitis externa J coll physicians surg pak,14: pp146-149.

Asish, J., Amar, M., Vinay, H., Sreekantha, Avinash, S. S. And Amareshar, M. (2013). To study the bacteriological and mycological profile of chronic suppurative otitis media patients and their antibiotic sensitivity pattern. Int. J. Pharma. Bio. Sci 4: pp186-199.

Bain, J. (1990). Childhood Otalgia: Acute otitis media. Justification for antibiotic use in general practice. British Medical Journal. 300: pp1006-1007. http://dx.doi.org/101136/bmj.300.673.1006.

Balows, A. and Duerden, B.(1998). Microbiology and Microbial Infection Vol2: systematic bacteriology 9th ed. Arnold, London, UK.

Bardanis, J., Batzakakis, D. and Mamataas, S. (2003). Types and causes of Otorihea Auris Nasus Larynx. 30: pp253- 257.

Barobby, G. W. And Zadik, P. (1987). Bacteriology of otitis media in Gana. Tropical Doctor. 17: pp 91-92.

Bauer, A. K., Kirby, W. M., Sheiris, J. C., Turck, M. (1966). Antibiotic susceptibility testing by astandardized single disk method . American Journal of climical pathology. 45: pp 493-496.

Bennett, K. E. and Haggard, M. P. (1998). Accumulation of factors influencing children's middle ear disease: risk factor modeling on alarge population cohort. Journal of epidemiology and community health. 52: pp 786-793.

Benninger, Michael S. (2008). “Acute bacterial rhinosinusitis and otitis media: changes in pathogenicity following widespread use of pneumococcal conjugate vaccine”. Otolaryngology- head 138(3): 274-278. doi: 10.1016/ j.otohns. ISSN 0194-5998. PMID18312870.

Berman, S., Johnson, C., Chan, K., Kelley, P. (2001). Ear, Nose and Throat. In: Hay, W. W., Hayward, A. R., Levin, M. J., Sondheimer, J. M., Eds., Current pediatric diagnosis and treatment, Mc Grow-Hill Companies Inc., NewYork, 400-410.

Bethesda, E. H., Author. National Institute on Deafiness and other communication Disorders. Otitis media NIH Publication. (2002). No 974216.

Bluestone, C. (2005). Eustachian tube: structure, function, role in otitis media. Hamilton, london: BC Decker. pp1-219. ISBN 978155009066.

Bluestone, C. D. and Klein, J. O. (2001). Microbiology. In: Bluestone CD, Klein Jo, editors. Otitis media in Infants and children. 3rd ed. Philadelhia: P A W 13. Saunders; 21: pp 79-101.

Bransko, K., and Marko, B., (2011). Microbiology of chronic otitis media. Medici ski Glasnik. 8(2).

Brook, I. (1987). The role of anaerobic bacteria in otitis media: Microbiology, pathogensis, and implications on therapy. Amj Otolaryngol. pp 8-109.

Brook, I. and Burke, P. (1992). The management of acute, serous and COM: the role of anaerobic bacteria. J. Hosp. Infect. 22(75).

Brook, I. and Saantonsa, G. (1995). Microbiology of chronic suppurative otitis media in children is surabaya, indonesia, Int. J. Paditr otorhnolarygol. (3): pp 23-28.

Brooks, G. F., Butel. J. S., Morse, S. A. (1998). Medical Microbiology 31st ed Applection anf Lang Stamford Education . USA.

Browing, G. G. (1988). Medical management of active chronic otitis media: a controlled study. J Laryngol Otitis. pp102-491.

Browing, G. G. (1990). Childhood Otalgia: Acute Otitis media. Antibiotics Not Necessary in Most Cases. British Medical Journal. 300: pp 673-1005.

Cheesbrough, M. (2000). Laboratory practice in tropical countries part2. Cambridge University Prees UK.

Chonmaitree, T., Howie, U. M., Truant, A. L. (1986). Presence of Respiratory viruses in middle Ear Fluids and Nasel wash specimens from cilfren with acute Otitis Media. Pediatrics.J. 77: pp698-702.

Coleman, C. and Moore, M. (2008). Coleman, C. ed. "decongestants and antihistamines for a cute otitis media in chidren". Cochrane Database Syst. Rev. (3): CD 001727. Doi: 10.1002/14651858. CD 001727. Pub4. PMID 18646076.

Collee, J.G., Fraser, A. G., Marmion, B. P., and Simmon, S. A. (1996). Practical Medical Microbiology. 14th ed Cherdill stone. NewYork.

David, S. H. (2002). Pearl Operative antibioticts in chronic otitis madia. Ear, Nose and throat Journal.

Dhingra, P. L. (2004). Disorders of Middle Ear. In: Dhingra, P. L., Diseases of Ear, Nose and Throat, 3rd Edition, Elsevier, NewDelhi, pp 80-112.

Donelli, G., and vuotto, C. (2014). Biofilm- based infections in long-term care facilities. Future Microbial 9, pp175-188.

Egbe, C., Mordi, R., Omoreaie, R., and Enabulele, O., (2010). Prevalence of otitis media in Okada community, Edo State, Nigeria, Maced. J. Med.. 3(3): pp 299-302.

Ekpo, M., Akinjogunla, O. and Idiong, D. (2009). Microorganisms associated with a cute otitis media diagnosed in Uyo City. Nigeria. Scientific Research and Essay. 4(6): pp 560-564.

Elmorsy, S., Shabana, Y. K., Raouf, A. A., Naggar, M. E., Bedir, T., Taher, S. and Fath-Aallah, M. (2010). The role of IL-8 in defferent types of otitis media and bacteriological correlation. Jint Adr Otol. 6: pp269-273.

Esposito, S., Noviello, S. and Montanaro, C. (1992). Topical Ciprofloxacin vs. Intramuscular gentamycin for COM-Archives of Otolaryngol Head and Neck Surg. 118: 842-844.

Fishbach, F. (1996). A Manual of Laboratory and Dignostic test. 5th ed, J.B. Lippincott company. Philadelphia. USA.

Fleming, D., Norbury, C. and Crombie, D. (1992). Annual and Seasonal Variation in the Incidence of common Diseases . Occasional paper 53. London: Royal colleage of general practitioners.

Gates, R. H. (1998). Infection Diseases Secret . Hanley and Belfus. INC . philadelphia. USA.

GBD (2013). Mortality and causes of death, collaborators (17 December 2014). "Global regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the global burden of disease study 2013". Lancet. 385(9963): 117-71.dio:10.1016/S0140-6736(14)61682-2. PMC4340604. PMID 255 30442.

Giebink, G. S. (1989). Infection of the middle and inner ear, springervelag New York Bertin. Heideberg- London.

Glasziou, P. P., Dei Mar, C. B., Sanders, S. L. and Hayem, M. (2004). "Antibiotics for a cute otitis media in chidren" The Cochrane database of systematic reviews (1): CD 000219. dio: 10-1002/ 14651858. CD000219.PMID 14973951.

Glezen, W. P. (2000). Prevention of acute otitis media by prophylaxes and treatment of influenza virus infection. Vaccine. 19(1): pp 56-58.

Gorbach, S. L., Bartlett, J. G. and Blacklow, N. R. (1992). Infection Diseases W.B Saunders company. philadelphia .USA.

Gracia, V. C., Galve, R. F., Penascal, P. E., Rubio, S. F. and Olmedillas, A. M. J. (1997). Acute Otitis Media in the first year of life and it is relationship with various Risk factors. Anales de Pediatria. 47: pp 473-477.

Grevers, G., Wiedemann, S., Bohn, J. C., Blasius, R. W., and Marano, V. (2012). Identification and characterization off the bacterial etiology of clinically problematic acute ototis media after tympancentesis or spontaneous otorrhea German children BMC Ifection Disease. 12: pp312.

Grigoriou, D. and Font, N. (1970). Les Otomycoses. Dermatologica J, 141: pp 138-142.

Gross, S., Blasiss, M. S. and Herrod, H. G. (1992). Role of immunogloulin Subclassed and specific Antibody Determination in the Evaluation of Recurrent Infection in children.J. pediatr. 12(4):pp 516-522.

Hall, I. S. and Colman, B. H. (1987). Diseases of the Nose Throat and Ear 13th ed . Churchill Living Stone, New York. USA.

Hannley, M. T. (2000). Use of ototopical antibiotics in treating 3 common ear disease. Otolaryngol Head and Neck Surg. 122-934.

Harkness, P. and Topham, J. (1998). Classification of Otitis media. Laryngoscope 108: pp1539-1543.

Harnivender, K., and Sonia, S., (2011). Bacterial and fungal study of 100 cases of chronic suppurative otitis media. Journal of clinical and diagnosis research (suppl-1). 5(6): pp 1224-1227.

Haugsten, P. and Lorentzen, P. (1980). The bacterial etiology of acute suppurative otitis media. J Laryngol; 94:169.

Henderson, F. W., Collier, A. M. and Sanyal, M. (1982). Alongitudinal study of Respiratory Viruses and Bacteria in the etiology of acute otitis media with Effusion. N. Engl. J. M., 306:1377.

Hernandez, M., Leichtle, A., Pak, K., Ebmeyer, J., Euteneuer, S., Obonyo, M., Guiney., D. G., Webster, N. J., Broide, D. H. and other authors (2008). Myeloid differentiation primary response gene 88 is required for the resolution of otitis media. J. Infect. Dis 198: pp1862-1869.

Ibekwe, A. O., Alshareef, Z. and Benayam, A. (1997). Anaerobes and fungi in chronic suppurative otitis media. Ann. Otol Rhinol Laryngol 106: pp 649-652.

Ibeziako, N. S. (1999). Common Bacterial Infections. In: Azubuike, J. C. and NK anginieme, K. E. O., Eds., paediatrics and childhealth in a tropical region. African Educational Services, Owerri, 410-425.

Ilechukwu, G. C., Ubesie, A. C., Ojinnaka, Emechebe, G. O. Iloh, K. K. (2014). Otitis media in children, Nigeria open journal of pediatriics4: pp 47-53.

Iqbal, K., Khan, M. and Satti, L. (2011). Microbrology of chronic supporative otitis media Experience at Dera Ismail Khan, Gomal Journal of Medical Sciences 9(2): pp189-193.

Jane, C. H., Park, S. Y. (2004). Emergence of ciprofloxacin resistant pseudomonas in chronic Suppurative otitis media Clin Otolaryngol. 29: pp321-323.

Jero, J., Karma. P. (1997). Bacteriological finding and persistance of middle ear effusion in otitis media with effusion Acuta. Otolaryngol. 529: pp22-26.

Jik, A. W, Ogundeji, E. B, Maxwell, I. K, Ogundeji, A, O, Samaila, J. M., Sunday, C. D., Gullek, J. S., Baso, L. A., Agbaje, A. O. and Onuoha, M. (2015). OSR Journal of Dental and Medical Sciencess. 14(12): pp61-67.

John Donaldson. "Acute otitis media" Med scape. Retrieved 17 march 2013.

Johnson, J. and Yu, V. (1997). Infections Diseases and Antimicrobial Therapy of the Ears, Nose and Throat. W. B. Saunders company. Philadelphia.USA.

Jolivet-Gougeon, A. and Bonnaure-Mallet, M. (2014). Biofilms as a mechanism of bacterial resistance. Drug Discov. Today Technol. 11: pp49-56.

Jonathan, M., Mustapha, A., Moi I. M., Lawal, G., and Abimbola, O. (2016). Antibacterial Susceptibility spectrum of same gram negative bacteria from suspected otitis media patients Vol. 10(3): pp1280-1285.

Jose, A. (2004). Chronic otitis media: Burden of illness and management. Child and Adollescent Health and Development Prevention of Blindness and Deafness. World Health Organization (WHO). Geneva, Switzerland.

Juyal, D., Negi, V., Sharma, M., Adekhandi, S., Prakash, R. and Sharma, N. (2014). Significance of fungal flora in chronic suppurative otitis media. Ann Trop Med public Health 7: 120-123.

Karim, Q. N., Hason, D. N. and Abdulla, E. M. (1981). Ear infection among children in Benghazi. An Analysis of 177 cases. Gary Med. J. Jan: pp 85-88.

Katzen meyer, K., Deskin, R., and Quinn, F. (1999). Otitis media. Grand Rounds presentation UTMB .Dept, of Otolaryngology.

Kenna, M., (1990). Incidence and prevalence of complications of otitis media. ANN Otol Rhinol Laryngol., 99 (7) (Suppl.149): pp38-39.

Kline, M. W. (1999). Otitis Media. In: McMillan, J. A., DeAngelis, C. D., Feigin, D. R., Warshaw, J. B., Eds. Oski's pediatrics. Principles and practice. Lippincot Williams and wilkins, philadelphia, pp1302-1304.

Koneman, E. W., Allen, S. D., Janda, W. M., Schreckenberger, P. C. and Winn, W. C. (1997). Color Atlas Text Book of Diagnostic Microbiology. 5th ed., Lippincott, New York. USA.

Kozyrskyj, A., Klassen, T. P., Moffat, M. and Harvey, K. (2010). "Short course antibiotics for acute otitis media". The Cochrane database of Systematic reviews. (9): CD001095. dio: 10.1002/14651858. CD001095 pub2. PMID 20824827.

Kumar, H., and Seth, S. (2011). Bacterial and fungal study of 100 cases of Chronic Otitis Media. J Clin Diag Res. 5: pp1224-1227.

Kvaerner, K. J., Nafasted, P., Hagen, J. A., Mair, I. W. and Jaakkola, J. J. (1996). Early Acute Otitis Media and siblings Attendance at Nersery. Archives of Disease in childhood. 75: 338-341. htt://dx.dio.org/ 10.1136/adc. 75.4.338.

Laissi, A. O. Nwaogu, O. G. B., Grandawaa, H. I. and Isa, A. (2002). A 15 years review of otoloic surgery in Ibadan, Nigeria:problems and Prospects .Nig J. Surg. Soc .Res. 4(1-2): pp 45-49.

Lampikoski, H., Aarnisalo, A., Jero, J. and Kinnari, T. (2012). Mastoid biofilm in chronic otitis media. Otol Neurotol. 33: pp785-788.

Lanphear, B. P., Byrd, R. S., Auinger, P. and Hall, C. B. (2014). Increasing prevalence of recurrend. Otitis media among children in United states. Pediatrics 1997; E1-7.

Lasisi, A. O. (2008). Otolaryngological practice in developing countives a profile of met and unmet needs. East Central Afr. J.surg. 13(2): pp101-104.

Lasisi, A. O. and Ajuwon, J. H. (2001). Belifs and perceptions of ear , nose and throat-related condition among residents of a traditional community in Ibadan, Nigeria. Afr.J.med. Sci. 31(1): pp 49-52.

Leibovitz, E. (2008). Complicated otitis media and implication vaccine. 26: pp16-19.

Leichtle, A., Lai, Y., Wollenberg, B., Wasserman, S. I. and Ryan, A. F. (2011). Innate signaling in otitis media: pathogenesis and recovery. Curr. Allergy Asthma Rep 11: pp78-84.

Lieberthal, A., Carroll, A., Chonmatree, T., Ganiats, T., Hoberman, A., Jackson, M., Joffe, M., Miller, D., Rosenfeld, R., Sevilla, X., Schwartz, R., Thomas, P., Tunkel, D. (March 2013). The diagnosis and management of acute otitis media. Pediatrics. 131(3): pp64-99. dio: 10.1542/peds. 2012-3488. PMID 23439909.

Luntz, M., Yehudai, N., Haifler, M., Sigal, G. and Most, T. (2013). Risk factors for sensorneural hearing loss in chronic otitis media. Acta Otolaryngol 133: pp1173-1180.

Macfadyen, C. A. and Acuin, J. M. (2005). Topical antibiotics with steroids for chronically discharging ears with underlying tympanic membrane perforation. Cochrane Database Syst. Rev. CD 004618.

Macfadyen, C. A. and Acuin, J. M. (2005). Topical antibiotics with steroids for chronically discharging ears with underlying tympanic membrane perforation. Cochrane Database Syst Rev. CD004618.

Macfadyen, C. A., Acuin, J. M. and Gamble, C. (2006). “Systematic antibiotics versus topical treatments for chronically discharging ears with underlying eardrum perforations. “The

cochrane database of systematic review (1): CD 005608. dio:10.1002/1465858. CD 005608PMID 16437533.

Maharjan, M., Bhandari, S., Singh I. and Mishra, S. C. (2006). Prevalence of ototis me”’’.njdia in G.R.(1991). Bacteremia with otitis media pediatrics 87: pp28-33.

Mahmoad, M. A. (2015). Bacteriological profile and durg resistance patterns otitis media patients. International Jourrual of Adanced Biological Research. 5(3): pp277-280.

Mandell, G. L., Douglas, R. G. and Bennett, J. E. (1990). Principles and Practice of infection disease Wiley, New York. USA.

Mansoor, T., Musani, M., Khalid, G. and Kamal, M. (2009). *Pseudomonas aeruginosa* in chronic suppurative otitis media: sensitivity spectrum against various antibiotics in Karachi. J Ayub Med Coll AbboHabad: 21(2): pp120-123.

Maran, A. G. (1988). Logan Turners Disease of the Nose Turners and Ear 9th ed. Butter worth-Heinemann Oxford. UK.

Maw, A. R. (1987). Is Your Grommet Really Necessary? Archives of disease in childhood. 62: pp 656-658.

Mawson, S. and Pollack, M. (1988). Special role of Pseudomonas aeruginosa in chronic otitis media. Ann Otol Rhinol Laryngol Head and Neck Surg. 97(suppl 130): pp10-13.

Meera HPC. (2012). "Correlating the severity of conductive heating loss with size and site of pars tensa TM perforations using video. Autoscopy. MMED Thesis

Meyers, R. and Lawson, W. (1986). I : Infectious disease and medical microbiloogy. Braude et al.,eds. 2 ed . W, B. Saunders company. Philadelphia . USA.

Miller, M. L. (1979). Epidemiology of otitis media: problem and reseach focus for geographers. Social Science Medicine. (13): pp233-236.

Milton, D. U. and Edson, R. R. (1989). Bacteriology if chronic otitis media affecting children living Rio de Janeiro. Ear nose and throatt. 68: pp448.

Mims, C., Playfair, J., Roit, I., Wakelin, D. and Wiliams, R.(1998). Medical Microbiology. Masby International. London.UK.

Monastal, L., Ronfani, I., Marchetti, F., Montico, M., Vrecchi-Brunetti, L., Bavcar, A., Grasso, D., Barbiero, C. and Tamburlini, G. (2010). 'Burden of disease caused by otitis media: systematic review and global estimates". PLOSONE. (4): e36226.dio:10.137/ journal. pone. 0036226. PMC 33403 47. PMID22558393.

Musa, T. S., Bemu, A. N., Grema U.S., and Kirfi, A. M., (2015). Pattern of otitis externa in kaduna Nigeria. Pan Afr. Med. J. 21(1): 165.

Nwabuisi, C., Olige, F. E. (2002). pathogenic agents of chronic suppurative otitis media in Ilorin, Nigeria. East African Medical Journal. 79(4): pp202-205.

Obiakor, M. N. (2000). Diseases of the Ear. Nose and Throat. Ochumba press ltd., Enugu, pp55-86.

Ogbogu, P. I., Eghafona, N. O. and Ogbogu, M. I. (2013). Microbiology of Otitis media among children attending a tertiary hosital in Benin city, Nigeeria. 5(7): pp280-84.

Oktay, M. F. (2005). Tympanic membrance changes in central tympanic membrance perforations. AmJ Otolaryngol. 26: p393.

Oni, A. A., Bakare, R. A., Nwaorgu, O. G. B., Ogunkunle, M. O. and Toki, R. A. (2001). Bacterial Agents of Discharging Ears and Antimicrobial Sensitivity patterns in children in Ibadan, Nigeria. West Africa Journal of Medicine. 20: pp131-135.

Oni, A. A., Nwaorgo, O, G., Bakare, R. A., Ogunkunle, M. O. and Taki, R. A. (2002). Discharaging ear in adult in Ibadan, Nigeria causative pattern. Afr. J. clin. Exp. Microbiol.; 3: pp1-5.

Orji, F. T. And Dike, B. O. (2015). Observations on the current bacteriological profile of chronic suppurative otitis media in south eastern Nigeria. Ann Med Health Scires. 5: pp124-128.

Osazuwa, F., Osazuwa, E., Osime, C., Igharo, E. A., Imade, P. E., Lofor, P., Momoh, M., Omoregie, R. And Dirisu, J. (2011). Etiologic agents of otitis media in Benin City Nigeria. N.Am. J. Med. Sci. 3(2): pp95-98.

Oyeleke, S. B. (2009). Screening for bacteria agents responsible for otitis media and their antibiogram. African Journal of Microbiology resareh; 3(5): pp249-522.

Pahwa, V., K., Chamiyal, P. C., Suri, P. N. (1983). Mycological study of Otomycosis India J. of medical. Research.77: pp344-338.

Paradise, J. L. (2004). Otitis Media. In: Behrman, R. E., Kliegman, R. M., Jenson, H. B., Eds., Nelson Textbook of Pediatrics, 17th Edition, Saunders, philadelphia, pp 2138-2149.

Pelton, S. L. and Klein, J. O. (1988). The draining ear. Otitis media and Externa,infect. Dis. Clin. North Am. 2: pp117-129.

Pollack, M. (1988). Special role of Pseudomonos aeruginose in chronic suppurative otitis media. Arch Otolaryngol Head Neck Surg 97, pp10-13.

Poole, M. D. (19950. otitis media complication and treatment failures: Implication of Pneumococcal resistant peediator. Infec Dis. J. 14(4): pp23-26

.

Prakash, R., Juyal, D., Negi, V., Pal, S., Adekhandi, S., Sharma, M. and Sharma, N. (2013). Microbiology of chronic suppurative otitis media in a tertiary healty care setup of Utlarakhand state, India. North Am.J. Sci. 5(4) : pp282-287

Qureishi, A., Lee, Y., Belfield, K., Birchall, J. P., Daniel, M.(10 january 2014). "Update on otitis media prevention and treatment". Infection and drug resistance 7:15-24.dol:10.2147/IDR.S39637.

Rajat, P., Deepak, J., Vikran, N., et al. (2013). Microbiology of chronic otitis media in a Tertially care set up of Utterrakhand state. N Am J Med Sci. 5(4): pp8282-8285.

Rao, B. N., Kashbur, I. M. and Reddy, M. S. (1992). Chronic suppurative Otitis Media in Benghazi- A prospective study. Gary. Med. Journal, pp68-77.

Raper, K. B. and Fennel, D. J. (1965). The genus Aspergillus. Williams and Wilkins company, Baltimore, USA.

Richard, E. B. and Roberts, M. K. (1996). Otitis media and it is complications in nelson's texbook of peadiatrics. pp1814-1824.

Riding, K., Bluestone, C. D., Michaels, R. H., Cantekin, E. L., Doyle, W.J and Poziviak, C. S. (1978). Microbiology of chronic and recurrent otitis media with Effusion in young in fant .J. paediatr. 93:730.

Roberts, D. (1980). The etiology of bullous myringitis and the role of mucoplasm as in ear disease. Rev. Pediatrics. 65(4): pp761-766.

Roland, P. S. (2002). Chronic otitis madia : aclinical verview. Ear Nosa Throt J. 18:8.

Roland, P. S., Parry, D. A. and Stroman, D.W. (2005).Microbiology of acute otitis media with Tympanstmy tube. Otolaryngol Head Neck surg. Oct; 133(4): pp585-95.

Rye, M. S., Blackwell, J. M. And Jamieson, S. E. (2012). Genetic Susceptibility to Otitis media in childhood. Laryngoscope 122: pp665-675.

Saini, S., Gupta, N., and Sachideva, O. (2005). Bacteriological study of paediatric and adult chronic otitis media. Indian J. Pathol Microbiol. 48(23): pp413-416

Saranya, S. K., Vazhavandal, G., Vallab, G., Ismail, M. and Uma, A. (2015). Thirumalai Kolundu Subramaniam P. Bacteriological and Mycological Profile of Chronic Suppurative Otitis media in A Tertiary Teaching Hospital, Trichy, Tamilnadu. International Journal of Pharmaceuticed Science. 1(4): pp13-19.

Sassen, M. L., Brand, H. and Grote, J. J. (1997). Risk Factors for Otitis Media with Effusion in Children 0-2 years of age. American Jornal of Otolaryngology.18:324-330.htt://dx.doi.org/10.1093/clinids/22.6.179.

Sattar, A., Alamgir, A., Hussain, Z., Sarfaz, S., Nasir, J. and Badar-Alam (2012). Bacterial spectrum and thier sensitivity pattern in patients of chronic suppurative otitis media. J coll physicians Surg pak 22, pp128-129.

Sattout, A. and Jenner, R. (2008). Best evidence topic reports. Bet 1. The role of topical analgesia in acute otitis media. Emerg. Med. J. 25(2): pp 103-104. doi: 10.1136/ emj. 2007. 056648.PMID 18212148.

Saunders, J. Murray, M. and Alleman, A. (2011). Biofilms in chromic suppurative otitis media and cholesteatoma : scanning electron microcopy findings . Am J Otolaryngol. 32: pp32-37.

Seibert, J. W. and Danner, C. J. (2006). Eustachian tube funcyion and the middle ear. Am clin Otolaryngol North. 39: p1211.

Shaikh, Nader (2010).”Videos in clinical medicine. Diagnosing otitis media-otoscopy and cerumen removal”. NEJM362(20):e62. doi:10.1056 /NEJM vcmo 904397. PMID 20484393. Retrieved Feb 11, 2015.

Sharma, S., Rchan, H. S., Goyal, A., Jha, A. K., Upadhyaya, S. and Mishra, S. (2004). Bacteriological profile in Chronic Suppurative otitis media in Easter. Nepal. Trop. Dactor 34(2): pp102- 104.

Sheahan, P., Blanley, A. W. (2002). Sequelae of otitis media with effusion among children with cleft lip and/or cleft palate. Clinical otolaryngology. 27: pp494-500.

Shimanura, K., Shigemi, H., Kurono, Y. and Mogi, G. (1990). The role of bacterial and hence in otitis media with effusion. Arch Otolaryngol. Head Neck Surg. 116(10): pp1143-1146.

Shrestha, B. L., Amatya R. C. M., Shrestha, I. and Ghosh, I. (2011). Microbiological profile of chronic suppurative otitis media. Nepalese Journal of ENT Head and Neck Surgery. 2(2): pp6-7.

Si, Y., Zhang, Z. G., Chen, S. J., Zheng, Y. Q., Chen, Y. B., Liu, Y., Jiang, H., Feng, L. Q. And Huang, X. (2014). Attenuated TLRs in middle ear mucosa contributes to susceptibility of chronic suppurative otitis media. Hum Immunol 75: pp771-776.

Sierra, A., Lopez, P., Zapata, M. A., Vanegas, B., Castrejon, M. M., Deantonio, R., Hausdorff, W. P. and Colindres, R. E. (2011). Non-typeable *Haemophilus influenzae* and *Streptococcus pneumoniae* as primary causes of a cute Otitis media in colombian children: a prospective study. BMC Infect Dis. 11, 4.

Smith, A., Macharia, I., Mugwe, P., Hatcher, J. (1996) Randomized control trial of treatment of chronic otitis media in kenyau school children. Lancet. 348: pp1138-1133.

Southgate, L., Lockie, C., Heard, S. and Wood, M. (1997). Infection Oxford University. UK.

Stenstrom, C. and Ingvarsson, L. (1997). Otitis-Prone children and controls: A study of Possible predisposing Factors. 1Heredity, Family Background and perinatal period. A cuta Oto-Laryngologica, 117: pp87-93.

Stewart, P. S. and Costerton, J. W. (2001). Antibiotic resistance of bacteria inbiofilms. Lancet. 358: pp785-788.

Stokes, E. J., Ridgway, G. L. and Wren, M. W. (1993). Clinical Microbiology. 7th ed. Edwared Arnold, london. UK.

Sugita, R., Kawamura, S. and Fujimaki, Y. (1982). pract otol (Kyoto) 75:921.

Sutton, D. A., Fothergill, A. W. and Rinaldi, M. G. (1998). Significant Fungi Williams and Wilkins. USA.

Sweeny, G., picozzi, G. L., and Browning, G. (1982). A Guantitative study of aerabic and an aerobic Boderia in chronic suppurative otitis media. J. Infect. 5:47.

Teele, D. W., Klein, J. O., Chase, C. and Menyuk, P. (1990). The Greater Boston Otitis Media infancy and intellectual ability, School achievement, speech and languege at age 7 years. J. Infect. Dis. 162: pp 658-694

Thanaviratananich, S., Laopaiboon, M. and Vatanasapt, P. (2013). "Once or twice daily versus three times daily amoxicillin with or without clavulanate for the treatment of a cute otitis media". The cochrane database of systematic reviews 12: CD004975. Pub3. PMID 24338106.

Thompson, M., Vodicka, T. A. Blair, Ps., Buckley. DI., Heneghan, C., Hay, AD., TARGET Programme, Team (Dec 11, 2013). "Duration of symptoms of respiratory tract infections in children: systematic review". BMJ (clinical research ed.) 347:f7027.doi:10.1136/bmj.f7027. PMC3898587.

Thompson, M., Vodicka, T. A., Blair, P. S., Buckley, D. I., Heneghan, C., Hay, A. D. and Target Programme Team (2013). Duration of symptoms of respiratory tract infections in children: systematic review "BMJ" (Clinical researched)347:f 7027. doi:10. 1136/ bmj. F7027. PMC 3898587.

Tiedt, N. J., Butler, I. R., Atkins, M. D., Elliot E. et al. (2013). Pediatric chronic otitis media in the free state province; clinical and audiological features. The south african Med J. 103(7).

Uhari, M., Hietala, J. and Tuokko, H. (1995). Risk of Acute Otitis Media in Relation to the viraliology of infection in children. Clinical Infections Diseases. 20: 521-524. htt://dx.dio.org/10.1093/ clinids/ 20.3.521.

Van Hasselt, et al. Bacteriology of chronic otitis media amongst children in Nkhota District of Malawi ENT and Audiology News. (12) May/ June (2013).

Vartianien, E., Vartiainen, j. (1996). Effect of aerobic bacteriology on the clinical presentation and rteatment results of COM. J Laryngol Otol. 110:315.

Veenhoven, R., Rijkers, G., Schilder, A., Adelmeijer, J., Viterwoal, C. and Kuis, W. (2004). Immunoglobulin in otitis media-prone children pediatr. Res. 55(1): pp152-162.

Wang, J. C., Hamood, A. N., Saadeh, C., Cunningham, M. J., Yim, M. T. and Cordero, J. (2014). Strategies to prevent biofilm-based tympanostomy tube infections. Int J. Pediatr. Otorhinolaryngol. 78: pp1433-1438.

Washington, J. A. (1985). Laboratory Produres in Clinical Microbiology, seconded. Mayo foundation. New york. USA.

Weiner, R. and Collison, P. J. (2003). Middle ear pathogens in otitis prone children. South dakota J. Med. 56: pp103-107.

Yorgancilar, E., Yildirim, M., Gun, R., Bakir, S., Tekin, R., Gocmez, C., Meric, F. and Topcu, I. (2013). Complications of chronic suppurative otitis media: a retrospective review. Ear Arch Otorhinolaryngol. 270: pp69-76.

APPENDICES

(A) Data Collection Guide Questions for Parents

Date:.....

1. Age: .....
2. Gender: .....
3. History of otitis media:.....

a. Episodes reported.

b. Medical documentation.

(B) Culture Media

(1) Blood Agar: (Liofilchem – ITALY)

Sterile Blood (human blood)	50 ml
Blood Agar Base	42 g

Direction of use:

Amount of 42g of blood agar base in 950ml of distilled water and sterilized by autoclaving at 121ºC for 15 minutes. After cooling to about 50ºC, 50ml (5%) of sterile blood were add and well mixed, and poured into plates or tubes.

(2) Chocolate agar: (Liofilchem – ITALY)

Sterile Blood (human blood)	50 ml
Blood Agar Base	42g

Direction of use:

Amount of 42g of blood agar base were dissolved in 950ml of distilled water and sterilized by autoclaving at 121ºC for 15 minutes. After cooling to about 80ºC, 50ml of sterile blood were add and well mixed, and poured into plates or tubes.

(3) MacConkey agar: (Liofilchem, ITALY)

Formula	Gram per liter
Panncreatic digest of gelatin	17.0
Peptones (meat and casein)	3.0
Lactose monohydrate	10
Sodium chloride	5.0
Bile salts	1.5
Agar	15.0
Neutral red	0.03
Crystal violet	0.001

Direction of use:

Amount of 61.5g of the ready made medium were suspended in 1 liter of distilled water. The medium was completely dissolved by heating to the boiling and then sterilized by autoclaving at 121ºC for 15 minutes. The medium was cooled to about 50ºC and poured into petri dishes. The surface of the agar was dried before use.

(4) Sabouraud Dextrose Agar: (HIMEDIA- INDIA)

Formula	Gram per liter
Mycological peptone	10.00
Dextrose	40.00
Agar	15.00

Direction of use:

Amount of 65g of the ready medium were suspended in 1 liter of distilled water, sterilized at 121ºC for 15 minutes, and poured into plates or tubes.

(5) Nutrient agar: (Oxoid, England)

Formula	Gram per liter
Beef extract	3.0
Peptone	5.0
Sodium chloride	8.0
Agar	12.0

Direction of use:

Amount of 28g of the ready made medium were suspended in 1 liter of distilled water. The medium was completely dissolved by heating to the boiling and then sterilized by autoclaving at 121ºC for 15 minutes. The medium was cooled to about 50ºC and poured into plates or tubes.

(6) DNAse Agar: (Oxoid, England)

Formula	Gram per liter
Tryptose	20.0
Deoxyribonucleic acid	2.0
Agar	12.0
Sodium chloride	5.0

Direction of use:

Amount of 39g of the ready made medium were suspended in 1 liter of distilled water. The medium was completely dissolved by heating to the boiling and then sterilized by autoclaving at 121ºC for 15 minutes. The medium was cooled to about 50ºC and dispensed into sterile petri dishes.

(C) Reagents

(1) Oxidase reagent:

Tetra methyl-p-phenylenediumine dihydrochloride	0.1g
Distilled water	10ml

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

(2) Catalase reagent:

H2O2 hydrogen peroxide

Stock solution, 30% H2O2	10ml
Distilled water	90ml

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

(3) Coagulase reagent:

One-six dilution of the plasma (human) in saline (0.85%) NaCl. The plasma was stored in small portions at -20ºC and kept as a stock at 4ºC. Before it is use, it was brought to room temperature

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

Formula	
Latex reagent	0.1% sodium azide
Positive control reagent	0.1% sodium azaid
The extraction enzyme solution	0.01% thiomersal

(D) Stains

(1) Gram's stain

A. Crystal violet

Crystal violet	20g
Ammonium oxalate	9.0g
Ethanol absolute	95g
Disilled water	to 1.0 Liter

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

B. Lugol's iodine solution

Potassium iodide	20g
Iodine	10g
Distilled water	to 1.0 liter

The potassium iodide was weighed, and transferred to a clean brown bottle to hold 1 liter with distilled water.

C. Acetone- alcohol decolorizer

Aceton	500ml
Ethanol absolute	475ml
Distilled water	25ml

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

D. Neutral red

Neutral red	1.0g
Distilled water	1.0 liter

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

(2) Lactophenol blue stain

Phenol crystals	20g
Lactic acid	20ml
Glyserol	40ml
Distilled water	20ml
Methyl blue	0.075g

The components of the stain were dissolved and use

دراسة حول ألتهاب الأذن الوسطى بين الأطفال الزائرين لمستشفى الأطفال بنغازي

إعداد

تهاني علي القاضي

إشراف الأستاذ الدكتور

صالح حمد بعيو

الملخص

إلتهاب الأذن الوسطى هو مرض واسع الإنتشار، ويعتبر أكتر إنتشارا وشيوعا عند الأطفال، قد ينتج عن إلتهاب الأذن الوسطى العديد من التعقيدات التى قد تؤدي إلى إضعاف السمع، والموت وإتلاف وعجز الجهاز العصبي المركزي.

إن هذه الدراسة عبارة عن مسح لتحديد الكائنات المجهرية (البكتيريا والفطريات) المسؤول عن إلتهاب الأذن الوسطى مابين 300 مريض كان لديهم علامات وأعراض إلتهاب الأذن الوسطى وكانوا يترددون على مستشفى الأطفال فى مدينة بنغازى خلال فترة عشرة أشهر من (أغسطس 2016 الى مايو 2017). كانت تتروح أعمارهم ما بين شهر إلى 12 سنة. أعلى حدوث للإصابة (55.3%) وجد عند الأطفال الذين أعمارهم ماهم أصغر من السنة الى السنتين. أعلى الإصابات كانت فى فصلي الشتاء والخريف.

من إجمالي 293 مزرعه إيجابية 7 أنواع من البكتيريا تم تعريفها واختبار مدى حساسيتها لمضادات حيوية مختلفة. كانت *pseudomonas aerugions* الميكروب السائد حيث عزلت

بنسبة (18.7%) يليها بكتيريا *Staphylococcus aureus* بنسبة (13.3%) أما باقى الأنواع فهى *Proteus mirabilis* بنسبة (10%)، وبكتيريا *Streptococcus Pneumoniae* بنسبة (7.3%)، *Klebsiella pneumoniae* بنسبة (6%) وبكتيريا *Escherichia coli* بنسبة (5.7%) وأقل نسبة لبكتيريا *Streptococcus pyogenes* (5%).

عزلت الفطريات بنسبة حوالى (10.3%) من المرضى، كان فطر *Candida albicans* الفطر السائد حيث عزل بنسبة (6.7%), وفطر *Aspergillus niger* بنسبة (3.3%)، أما فطر *Asperaillus fumigatus* بنسبة (0.7%).

أغلب العزلات البكتيرية المسحية لالتهابات الأذن الوسطى أظهرت حساسية للمضادات الحيوية التى تم اختبارها عامة، البكتيريا الموجبة لصبغة جرام كانت اكتر حساسية لهذه المضادات الحيوية، البكتيريا السالبة لصبغة الجرام خاصة *Pseudomonas aeruginosa* أكبر درجة من المقاومة لهذا المضادات الحيوية .

دراسة حول التهاب الأذن الوسطى بين الأطفال الزائرين لمستشفى الأطفال بنغازي

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أ.د. صالح حمد بعيو

قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في

علم النبات.

جامعة بنغازي

كلية العلوم

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