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Microbiological study and antimicrobial susceptibility pattern of ear infections in patients with Chronic Suppurative Otitis Media (CSOM) in Basrah Province

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Abstract:

The ear infections are highly popular disease in the world. Chronic suppurative otitis media (CSOM) is one of the prevalent hearing problems, this infection of the middle ear may extend to cranium that can cause many serious complications if not treated duly. It is famed for its return enduring infection.

The causative agents of bacteria or fungi may be cause the CSOM infection. Therefore, our study is mainly aimed to identify the bacterial isolates which causing of CSOM and detect some isolates of fungi which implicated in this ear infection with performed of the antimicrobial susceptibility test.

The study was executed on people whose attending the outpatients clinic of ENT in AL-Mwanee General Hospital during the period from November, 2016 to May, 2017 in Basrah governorate. Ear swab samples were collected and processed from eighty five (85) patients of CSOM by the following of standard bacteriological procedures mainly, for isolation and identification the bacterial pathogens, in addition, common diagnosis methods was used to detect of some fungal isolates.

The positive microbial growth cultures were seen in 59 cases with frequent of (69.41 %) and 26 cases were negative in frequent of (30.58%). Their ages ranged from 1-60 years with high incidence of CSOM in age groups ≤ 10 and 31-50 years old in percent's of (38.82 % and 24.70 %) respectively. Polymicrobial samples from the total isolates (89) which identified in this study were (82) aerobic bacterial isolates in frequent (92.13 %) and 7 fungal isolates in frequent of (7.86 %).

The most predominant bacterial isolated which causing CSOM was *Pseudomonas aeruginosa* in ratio (34.83 %) followed by *Staphylococcus aureus*, *Streptococcus spp.*, *Klebsiella pneumonia*, *Escherichia coli*, *Proteus mirabilis* and *P.vulgaris* in proportions: 29.21% , 10.11% , 8.98% , 6.74% , 1.12% and 1.12% . While, the most common fungal detection which causing of CSOM was *Aspergillus spp.* and *Candida spp*. with frequently: 71.42% and 28.57%.

Susceptibility test was done for known the best antibiotic agents which can be used as a proper treatment to CSOM infection. In the present study the most effective antibiotics agents for most of bacterial isolates were Ciprofloxacin, Vancomycin, Amikacin, Augmentin, Amox-Clav and Gentamycin.

Key Words: Ear infection, Chronic suppurative Otitis Media, Pathogenic bacteria, Antibiotic susceptibility testing.

1- Introduction:

The ear infections are usually categorize into those which occur in the ear canal called otitis-externa and those which occur in the middle ear in small space behind the eardrum (Bojrab *et al*, 1996). Even though, the ear infection is a disease common in infants and young children but can occur at any age then, also affect the adults (Farhan *et al*, 2011).Otitis media is an inflammation of the middle ear. It's most commonly caused by the buildup of fluid behind the eardrum as a result of a blockage to the Eustachian tube. Otitis media is a common disease in people all over the world represents a major health problem. The prevalence of otitis media varies from place to place, also occurs with a high incidence and prevalence in both developed & developing countries (El-Gendy, 1998; Damoiseaux,

2005; Sauver *et al*, 2006). The ear infection may be a cute, chronic or recurrent infections also, can be suppurative or non-suppurative as a cute suppurative otitis media. ASOM is a disease common in childhood and can be progress to chronic suppurative otitis media CSOM (Leach and Morris, 2006; Wariso and Ibe, 2006; Wasihum and Zemene, 2015). The chronic suppurative otitis is defined as infection of the middle ear cleft that lasting from two weeks to more than three months and is accompanied with otorrhea and perforation of the tympanic membrane mainly caused by bacteria (Rao and Reddy, 1994; Agrawal *et al*, 2013; Mesfin and Muluken, 2014). The infection in otitis media is mainly dependent on the route by which infection reaches to the middle ear and the chief route by which it occurs through the auditory tube (Healy and Teele, 1977; Daly, 1997). Also, the otitis media is related anatomically and pathologically with upper respiratory tract therefore the nasopharynx is a natural reservoir for several bacterial species (Fadon, 1997). CSOM is a destructive disease with sequential irreversible and can proceed to seriously intra or extra cranial complications (Poorey and Lyer, 2002). The causative organisms may be isolated in CSOM can be aerobic, anaerobic, mixed bacteria or fungi (Brook, 2003; Yeo *et al*, 2007; Tahira *et al*, 2009). Depending on the according reports of many studies, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Escherichia coli* are the common bacteria which isolated from the cases of ear infection (Abera and Kibret, 2011; Muluye *et al*, 2013; Seid *et al*, 2013). The entry of bacteria to the middle ear may gain through a chronic perforation (Acuin, 2004). The predisposing factors to the ear infection are higher in children than adults because, the anatomy of Eustachian tube in children easier permits access of the microorganisms through the nasopharynx (Anthony, 1997). The incidence of otitis media varies from place to place, in developing countries it is on the rise while it is declining in the developed world because of awareness (Adoga *et al*, 2010; Farhan *et al*, 2011).

The aim of this study mainly, is to detect the bacterial isolates and some other fungi in patients with otitis media infections also, apply their antibiotics susceptibility pattern against bacterial pathogens among patients who visited ENT Clinics in Basrah.

2- Materials and Methods:

The population under study was among people attending to the outpatients clinic of ENT in AL-Mwanee General Hospitals. A total of (85) patients with signs and symptoms of CSOM who were not on any antibiotics were included and investigated in this study, their ages ranged from 1 to 60 years, the investigation period extended from November, 2016 to March, 2017.

The specimens collected from patients with ear exudates by using three sterile cotton swabs under strict aseptic precautions and assist of aural speculum then processed immediately in the Lab. of microbiology. The first swab was used for the bacterial detection in microscopically and culturing on MacConkey agar, Blood and Chocolate agar which were then incubated for 24-72 hrs. at 37 C^0 in aerobic condition also, in CO2 incubator. Then, a Gram stain examination was made from the second swab of specimen with direct microscopy of sample in KOH for fungal detection. Also, the microbiological investigations were done which included: cultures, the identification of isolates after grown by using colonial morphology, characteristics of cultures and complete of the standard biochemical reactions (Cruickshank *et al*, 1975; Holt *et al.*, 1994). Furthermore, the antibiotic sensitivity testing of the diagnosed organisms was determined by Kirby Bauer Method on Muller Hinton agar. The result of plates were read after incubation overnight at 37 C^0 by measure of the inhibition zones around the discs of antibiotics as per Clinical Laboratory Standards Institute (CLSI, 2011). Finally, the third swab was used for mycology culture which included: inoculation on two slants of media on Saboroud Dextrose Agar (SDA) in 25 C^0 and 37 C^0 with the addition of chloramphenicol material. Later the examination and detection of slants were done.

3- Results:-

In the present study out of total 85 ear swabs samples were collected and processed from 85 patients suffering from CSOM. The microbial growth was seen in 59 (69.41 %) as positive culture while 26 (30.58 %) samples showed no growth.

Their ages ranged from 1 to 60 years. The peak incidence of CSOM in age groups ≤ 10 (38.82 %), 31-50 (24.70 %) followed by age groups 11-30 and 51-60 in frequents (21.17 %) and (15.29 %), respectively (Table 1).

Table 1: Distribution of CSOM patients according to age groups

Age groups (year)	No. of CSOM patients	Frequency (%)
≤ 10	33	38.82
11-30	18	21.17
31-50	21	24.70
51-60	13	15.29
Total	85	69.41

The total isolates 89 including; polymicrobial samples were detected and identified in which aerobic bacteria were 82 (92.13 %) and 7 (7.86 %) the fungal isolates Table (2).

Table 2: Category of isolates

Organisms	No. and percentage (%) of isolates (89)
Bacteria	82 (92.13)
Fungal	7 (7.86)

The most common bacteria causing CSOM was *Pseudomonas aeruginosa* in 31 (34.83 %) of samples followed by *Staphylococcus aureus* 26 (29.21 %), *Streptococcus.spp* 9 (10.11 %), *Klebsiella pneumonia* 8 (8.98 %), *Escherichia coli* 6 (6.74 %), *Proteus mirabilis* 1 (1.12 %) and *P.vulgaris* 1 (1.12 %). While, the most common fungus which causing CSOM was *Aspergillus spp*. 5 (71.42 %) followed by *Candida spp*. 2(28.57%) Table (3).

Table 3: List of various Microorganisms (Bacteria, Fungi) isolated from cultures

Bacterial isolates	No. of isolates (82)	Relative frequency (%)
Pseudomonas aeruginosa	31	34.83
Staphylococcus aureus	26	29.21
Streptococcus.spp	9	10.11
Klebsiella pneumonia	8	8.98
Escherichia coli	6	6.74
Proteus mirabilis	1	1.12
P.vulgaris	1	1.12
Fungal isolates	**No. of isolates (7)**	**Relative frequency (%)**
Aspergillus spp.	5	71.42
Candida spp.	2	28.57

The results of sensitivity testing are described in Tables 4, 5, 6 and 7. Among the 31 isolates of *Pseudomonas aeruginosa* it was sensitive to Ciprofloxacin (83.87 %), Levofloxacin (77.41 %), Gentamicin (70.96 %), Amox-Clav (54.83 %), Augmentin (51.61 %) and it resistant to Trimethoprim (87.09 %), Ampicillin (83.87 %), Vancomycin (80.64 %), Cefotaxime (74.19 %), Streptomycin (67.74 %), Erythromycin (64.51 %) and Tetracyclin (58.06 %) as shown in Table (4). While, from the total number 26 of *Staphylococcus aureus* isolates the results showed that, S.aureus was sensitive to Vancomycin (100 %), Ciprofloxacin (80.76 %), Amox-Clav (73.07 %), Augmentin (65.38 %), Amikacin (61.53 %), Gentamicin and Amoxicillin in (57.69 %) but it was resistant to Ampicillin (88.46 %), Cefotaxime (73.07 %), Erythromycin (69.23 %) and Penicillin (65.38 %) as found in Table (5).

Table 4: Antibiotics susceptibility pattern in *Pseudomonas aeruginosa*

Type of Drugs used	Total No. of isolates 31	
	Sensitive (%)	Resistant (%)
Cefotaxime	8 (25.80)	23 (74.19)
Gentamicin	22 (70.96)	9 (29.03)
Ciprofloxacin	26 (83.87)	5 (16.12)
Augmentin	16 (51.61)	15 (48.38)
Levofloxacin	24 (77.41)	7 (22.58)
Vancomycin	6 (19.35)	25 (80.64)
Erythromycin	11 (35.48)	20 (64.51)
Setreptomycin	10 (32.25)	21 (67.74)
Tetracyclin	13 (41.93)	18 (58.06)
Trimethoprim	4 (12.90)	27 (87.09)
Ampicillin	5 (16.12)	26 (83.87)
Amox-Clav	17 (54.83)	14 (45.16)

Table 5: Antibiotics susceptibility pattern in *Staphylococcus aureus*

Type of Drugs used	Total No. of isolates 31	
	Sensitive (%)	Resistant (%)
Ciprofloxacin	21 (80.76)	5 (19.23)
Amox-Clav	19 (73.07)	7 (26.92)
Ampicillin	3 (11.53)	23 (88.46)
Gentamicin	15 (57.69)	11 (42.30)
Vancomycin	26 (100)	0 (00.00)
Amikacin	16 (61.53)	10 (38.46)
Cefotaxime	7 (26.92)	19 (73.07)
Penicillin	9 (34.61)	17 (65.38)
Erythromycin	8 (30.76)	18 (69.23)
Amoxicillin	15 (57.69)	11 (42.30)
Augmentin	17 (65.38)	9 (34.61)

Finally, the antibiotic sensitivity tests to Gram negative bacteria other than *P.aeruginosa* were described in Table (6). Among the 16 isolates of results it were sensitive to Amox-Clav and Amikacin (93.75 %), Ciprofloxacin (68.75 %),Cefotaxime (62.5 %) also,

sensitive to Chloramphinicol, Gentamycin and Cefazolin in frequent (56.25 %). Furthermore, resistant to Ampicillin (100 %), Tetracycline (81.25 %), Erythromycin (68.75 %) and Augmentin (62.5 %). While, from the total isolates 9 of Streptococcus.spp the antibacterial sensitivity tests were sensitive to Ciprofloxacin and Augmentin in frequent (88.88 %), Gentamicin (77.77 %) and Clindamycin (66.66 %) as well, it resistant to Chloramphenicol (99.99 %), Erythromycin and Amox-Clav in frequent (77.77 %), Amoxicillin (66.66 %) and Tetracycline (55.55 %) as shown in Table (7).

Table 6: The number and percentage of antibiotics susceptibility pattern in Gram negative bacteria other than *P. aeruginosa*

Type of Drugs used	Total No. of isolates 16	
	Sensitive (%)	Resistant (%)
Chloramphenicol	9 (56.25)	7 (43.75)
Ciprofloxacin	11 (68.75)	5 (31.25)
Amox-Clav	15 (93.75)	1 (6.25)
Cefotaxime	10 (62.5)	6 (37.5)
Gentamicin	9 (56.25)	7 (43.75)
Cefazolin	9 (56.25)	7 (43.75)
Erythromycin	5 (31.25)	11 (68.75)
Ampicillin	0 (00.00)	16 (100)
Augmentin	6 (37.5)	10 (62.5)
Tetracycline	3 (18.75)	13 (81.25)
Amikacin	15 (93.75)	1 (6.25)

Table 7: Antibiotics susceptibility pattern in *Streptococcus spp.*

Type of Drugs used	Total No. of isolates 9	
	Sensitive (%)	Resistant (%)
Augmentin	8 (88.88)	1 (11.11)
Amoxicillin	3 (33.33)	6 (66.66)
Tetracycline	4 (44.44)	5 (55.55)
Chloramphenicol	0 (00.00)	9 (99.99)
Erythromycin	2 (22.22)	7 (77.77)
Gentamicin	7 (77.77)	2 (22.22)
Ciprofloxacin	8 (88.88)	1 (11.11)
Clindamycin	6 (66.66)	3 (33.33)
Amox-Clav	2 (22.22)	7 (77.77)

4- Discussion

The most disease in frequently for patients to visit clinicians with take antibiotics is ear infection and the serious health care concern worldwide, it is the otitis media. Because of, not only the distress it the patients also their families but, it also because the imposes of the substantial economic burden on the care health system. The incidence of the otitis media in previously publications which reported its depend on the race the socioeconomic factors (Shenoi, 1988 ; Rao and Reddy, 1994 ; El-Gendy, 1998 ; Ologe and Nwawolo, 2002 ; Leach and Morris, 2006 ; Egbe *et al.*, 2010 ; Grevers, 2010).

CSOM is one of the most common ear diseases, it's a chronic infection of the middle ear may lead to deafness that's for causing of intracranial dreadful complications and irreversible local destruction of the middle ear structures. Also, this disease is notorious and more reported in lower socioeconomic status groups as well as in rural populations. If the CSOM disease untreated or poorly treated that's can lead to many complications as meningitis, mastoiditis and brain abscess (Anifasi and Tumushime- Buturo, 1989; Rao and Reddy, 1994; Fairbanks, 1996; Poorey and Lyer, 2002; Loy *et al.*, 2002; Wariso and Ibe, 2006; Taneja and Taneja, 2009).

In our study among 85 discharging ear swabs were collected from 85 patients with CSOM. Microbial growth was found in 59 (69.41 %) as a positive culture, this is in accordance with Deb and Ray, 2012 ; Prayaga *et al.*, 2013 but differ from Khanna *et al.*, 2000; Poorey and Lyer, 2002; Agrawal *et al.*, 2013 while 26 (30.58 %) samples showed no growth as negative cultures which is near similar to the prior studies of Chakraborty *et al.*, 2005 and Fatema *et al.*, 1998 those found higher percentage of negatively culture samples whereas not accordance with Vijaya *et al.*, 1998; Prakash *et al.*,2013 and Dayasena *et al.*, 2011 study. These negative cultures of results may be the reason of the modification in bacterial flora in ears affected by the previous empirical antibiotic therapy.

In the present research the increased prevalence of CSOM were in groups of age ≤ 10, 31-50 and 11-30 with frequent (38.82 %), (24.70 %), (21.17 %) respectively which is in agreement with the prior literature of Loy *et al.*, 2002 ; Brook, 2003 ; Healy and Teele, 1977 ; Shyamala and Reddy, 2012 ; Oni *et al.*, 2002 due to, these age of groups exposed to several predisposing factors for occurrence of otitis media any way, the more incidence of CSOM was in the first and second decade of life with accounted in 59 % of the cases during this study in addition to the ratio of age group 31-50 years old.

In our study, the totally 89 isolates were isolated and identified which consisting of polymicrobial samples in frequent (92.1 %) of 82

isolates were aerobic bacteria and fungal isolates in frequently 7 (7.8 %). This is in accordance with Sunilkumar and Roopa, 2015. On the other hand, in our research the results showed that, *Pseudomonas aeruginosa* was the most predominant aerobic isolate in causing of CSOM with percentage (34.83 %) followed by *Staphylococcus aureus* in frequent (29.21 %) this is in agreement with other reports (Arshad *et al.*, 2004; Goyal *et al.*, 2009; Malkappa, 2012; Muluye *et al.*, 2013 and Abdelraouf *et al.*, 2014).

Also, in this study among the other gram negative pathogens which isolated next to *Pseudomonas aeruginosa* were including: *Klebsiella pneumonia* (8.98 %), *Escherichia coli* (6.74 %), *Proteus mirabilis* (1.12 %) and *P.vulgaris* (1.12 %). While, *Streptococcus spp*. in frequent (10.11 %) next to *Staphylococcus aureus* as gram positive pathogens.Wheres, the secondary invader was the fungal infections of the middle ear. The most common fungus causing of COSM in current study was *Aspergillus spp*. in isolation rate (5.61 %) followed by *Candida spp*. in frequent (2.24 %), these results are consort with observations of (Sunilkumar and Roopa, 2015; Kumar and Seth, 2011. Commonly, the wet pus in the middle ear may expedite the growth of the fungi. Diagnosis of the causative microorganisms is essential for suitable management the cases of CSOM disease.

In the current study, the antibiotic susceptibility test was performed for all the isolated microorganisms, most of the microbial isolates which identified in this study were found to be susceptible to Ciprofloxacin. This is in accordance with study of (Prayaga *et al.*, 2013). But, nearly 80 % of the microbial isolates showed resistance to Ampicillin drug, this was agreement with a report of (Seid *et al.*, 2013).

In the present study, the *Pseudomonas aeruginosa* was highly resistance (87.09 %, 83.87 % and 80.64 %) to Trimethoprim, Ampicillin and Vancomycin respectively.

Muluye *et al.*, 2013 study revealed, the resistance levels of *P.aeruginosa* to Ampicillin and Amoxicillin / Clavulanic acid were 86-91.8 % and 60-67 % , this result was somewhat in accordance with the our findings. However, the P.aeruginosa showed high levels of sensitivity (83.87 %, 77.41 %, and 70.96 %) to Ciprofloxacin, Levofloxacin and Gentamicin respectively. This was consisting with the results of other studies (Abera and Kibret, 2011; Wasihun and Zemene, 2015).

In the current study, *Staphylococcus aureus* displayed low levels of resistance (0.00 % , 19.23 % and 26.92 %) to Vancomycin , Ciprofloxacin and Amox- Clav with a highest sensitivity levels to Vancomycin , Ciprofloxacin and Amox- Clav in frequently (100 % , 80.76 % and 73.07 %) respectively. This is in accordance with research of (Sunilkumar Biradar and Roopa, 2015). And was somewhat in agreement with the results of (Hailu *et al.*, 2016 ; Muleta *et al.*, 2004 ; Abera and Kibret, 2011; Muluye *et al.*, 2013 ; Wasihun and Zemen, 2015 ; Sunilkumar Biradar and Roopa, 2015) for Ciprofloxacin , Vancomycin and Amox- Clav. On the other hand, *S.aureus* isolates were resistance to Ampicillin, Cefotaxime, Erythromycin and Penicillin with levels of resistance (88.46 %, 73.07 %, 69.23 % and 65.38 %) respectively. Anyhow, our results were supported by other workers (Muluye *et al.*, 2013; Prayaga *et al.*, 2013; Seid *et al.*, 2013; Sunilkumar Biradar and Roopa, 2015 and Hailu *et al.*, 2016) observations. For irrational manner with increased use of wide-spectrum antimicrobial agents that lead to spread development of resistance in the microbial isolates, this becomes today, a major global that bluster the health. Also, the microorganisms usually keep changing in the resistance pattern to antibiotics (Wasihun and Zemen, 2015).

In the present study, the gram negative bacterial isolates showed high levels of susceptible with fairly proportion (93.75 %) to both Amox- Clav and Amikacin drugs while, moderate susceptible to Ciprofloxacin (68.75 %) . And, exhibited high levels of resistance (100 %, 81.25 %) to Ampicillin, Tetracycline in addition, moderate resistance (68.75 %) to erythromycin. Our results were consistent with (Sunilkumar Biradar and Roopa, 2015) and were somewhat in accordance with (Muluye *et al.*, 2013; Seid *et al.*, 2013; Wasihun and Zemen, 2015 and Hailu *et al.*, 2016). Furthermore, in this study, Ciprofloxacin, Augmentin and Gentamycin were found to be an effective antimicrobial agents to *Steptococcus spp*. with susceptible levels corresponding to (88.88 %, 88.88 %, 77.77 %) respectively. While, exhibited high levels of resistance to Chloramphenicol and Amox-Clav. Our findings were somewhat in agreements with the observations of (Ferede and Geyid, 2001; Mesfin and Muluken, 2014; Suman and Heba, 2014; Muluye *et al.*, 2013 and Hailu *et al.*, 2016).

Our results revealed that, the agent of Ciprofloxacin was shown to be the most effectiveness agent against all the bacterial isolates with highest sensitivity for most the commonly isolated microorganisms as that of study performed by (Afolabi

et al., 2012). Furthermore, in the present study this effectiveness of Ciprofloxacin agent followed by Vancomycin , Amikacin , Augmentin , Amox-Clav and Gentamicin . In contrast to previously studies of (Brook and Frazier, 1996 ; Ibekwe *et al.*, 1997 ; Oni et al., 2001 and Rebeca Mitchell & Noel Cranswick, 2008) whose recommended to Quinolone as second line treatment of microorganisms cause CSOM while the Penicillin can serve as the first line for drug treatment . Whereas, the others researchers revealed that, the Gentamicin agent represent the most sensitive.

In Conclusion: In our study, the chronic suppurative otitis media is still one of the most common infections in age group of ≤ 10 years old as a childhood problem and the commonest infectious bacterial agents which implicated in causing of CSOM were *Pseudomonas aeruginosa* followed by *Staphylococcus aureus* . While, the most common fungal infection which causing of CSOM was Aspergillus spp.

Ciprofloxacin , Vancomycin , Amikacin , Augmentin , Amox-Clav and Gentamicin were found to be most effectiveness antimicrobial agents against the most bacterial isolates including genera of gram negative and gram positive bacteria which isolated and detected in present study . While, its resistant to Trimethoprim, Ampicillin, Tetracycline, Chloramphenicol and Erythromycin. Most bacterial isolates were adapted to the common antibiotics and that leads to acquired resistance phenomena problem Therefore, a suitable antimicrobial drugs should be specified after proper and early diagnosis of the causative microorganisms with guided by the antimicrobial susceptibility testing of the isolates also, recommended to use of antibiotics in judicial manner with complete the full course of patients treatment and stopped the heavy used of antimicrobial agents in hospitals further to, practicing the good hand hygiene that ensures of effective the treatment to avoid the complications of CSOM disease.

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