Egypt. Acad. J. Biolog. Sci., 12(1): 9-18 (2020)

Egyptian Academic Journal of Biological Sciences
G. Microbiology

ISSN: 2090-0872
www.eajbs.eg.net

Study of Aerobic Agents in Chronic Suppurative Otitis Media and their Sensitivity to Antibiotics in Ramadi City

Maysaa I. Al. Zubaidi[1], Shehab A. Lafi[2],and Raid M. Al-Ani[3]

1- Department of Microbiology, College of Medicine, University of Anbar,
2- Department of Microbiology, College of Medicine, HOD, University of Anbar.
3- Department of Surgery/ENT, College of Medicine, University of Anbar.

E.Mail: **maysaa_ahmed@yahoo.com** - **shehab_6555@ymail.com** - **raedalani2003@yahoo.com**

ARTICLE INFO

Article History
Received: 2/1/2020
Accepted:29/1/2020

***Keywords*:**
Otitis media; Suppurative; Sensitivity; Aerobic microorganisms; Chronic suppurative otitis media.

ABSTRACT

Objectives: Aims of this study were to evaluate the aerobic microorganisms in CSOM and antibiotics sensitivity to them.

Methods: The study was a cross-sectional, prospective study conducted during the period from 1st of November 2018 to 28th of February 2019 at Al-Ramadi Teaching Hospital and Microbiological Department of College of Medicine, University of Anbar. Patients with aural discharge ≥ 12 weeks, eardrum perforation and deafness were enrolled in the study. Samples of ear discharge were collected and cultured. The identification of bacterial species was performed with antimicrobial susceptibility testing according to EUCAST.

Results: Out of 63 patients, 29 (46.0%) were males and 34 (54.0%) females. Most of patients with unilateral disease 76.2% (n= 48) and bilateral 23.8% (n=15). Three of the bilateral cases presented with unilateral active aural discharge, so the total number of ear discharge specimens was 75. Out of 75 specimens, 68 (88.3%) showed aerobic microorganisms, while 9 (11.7%) showed negative growth. *Pseudomonas aeruginosa* is the most common isolate constituting (35, 45.4%), each *Staphylococcus aureus* and *Staphylococcus epidermidis* were isolated in (13, 16.8%), and the least microorganism isolated was *E. coli* (1, 1.3%). Impenem is the most effective (97.1%) antibiotic against the isolated microorganisms, while 72.4% isolates were sensitive to amikacin and 71.4 % to ciprofloxacin.

Conclusion: *Pseudomonas aeruginosa* is the most common organism found in this study. Since the price of ciprofloxacin is cheap and the study has proven to be sensitive in approximately 3/4 of microorganisms in CSOM, so it can be used as empirical treatment in this disease.

INTRODUCTION

CSOM is defined as chronic inflammation of the middle ear cleft, which presented with deafness and persistent or intermittent ear discharge through a perforated eardrum. Usually, the disease begins in childhood in the first 6 years of life with a peak at the age of 2 years.

The infection results either from acute suppurative otitis media (ASOM) with a spontaneous eardrum perforation or as a sequel of non-suppurative or secretory otitis media. The exact mechanism of chronicity has not been determined (Verhoeff, *et al.*, 2006). If CSOM disease is not treated properly, many 0extra and intracranial complications will be occurred (Gates, *et al.*, 2002).

The two most important causes of CSOM are Eustachian tube dysfunction and bacterial infection, so it is of utmost importance for the treatment of this disease is the selection of proper antibiotics. However, due to recent antibiotic abuse(Lee, *et al.*, 2010), there are changes in the common microorganisms causing CSOM in addition to their antibiotic sensitivity. The two most imported microorganisms found in CSOM are Staphylococcus and *Pseudomonas aeruginosa*; as revealed by many previous studies. However, due to the increasing resistance of these pathogens to the antibiotic results in difficulty in the treatment of them (Gates ,e*t al.,* 2002). If empirical treatment is used for patients with antibiotic-resistant microorganisms, like quinolone-resistant *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (MRSA), which were commonly found in infectious conditions, treatment failure or even disastrous complications is the end result (Karlowsky, *et al.,* 2013., Iwamoto, *et al.,* 2013). So culture and sensitivity is an essential step in the management of this common disease. To our best knowledge, this study is the first in Ramadi city in handling this topic. The objectives of the study are to determine the aerobic agent in patients with CSOM and their antibiotic sensitivity.

MATERIALS AND METHODS

The study was a prospective cross-sectional study carried out at Al-Ramadi Teaching Hospital and the Department of Microbiology in the College of Medicine, University of Anbar. Patients with active ear discharge of more than 12 weeks, conductive deafness and eardrum perforation, attending the Ear, Nose, and Throat (ENT) clinic were enrolled in the present study. Patients on antibiotics (local or systemic) in the last three days from the presentation, patients with acute suppurative otitis media and the patient or parent or caregiver who does not give his or her consent to participate in the research study were excluded from the study. The study was approved by the ethical committee of the University of Anbar.

Detailed history regarding the age, gender, residence, occupation, and religion, duration of the disease, laterality and aural symptoms (discharge, deafness, pain, itching, tinnitus, and vertigo) were taken from every patient. For the purpose of the study, the age at the onset (age at the presentation – duration of disease = age at the onset), was used as a reference for the age of the patients.

The otoscopic examination was done in all subjects. The type of perforation whether central, marginal and attic were registered. Swabs were collected from the discharging ears of patients with CSOM by inserting a sterile swab deep in the canal. The collected samples were placed in transport media (Amies media) and then transported to the Microbiology Laboratory in the College of Medicine – the University of Anbar for microscopy, culture and sensitivity tests. All specimens collected were subjected to the Gram stain. Then each smear was inoculated on blood agar and MacConkey agar plates. The plates of Blood and MacConkey incubated in for 18-24 hours, after that the plates were read and isolates identified according to standard methods using Viteck system (USA). Antibiotic sensitivity testing was performed following Kerby –Bauer technique using the bacterial suspension, which was set to 0.5 McFarland turbidity equal to 1.5×10^{8} CFU/ml. A sterile cotton swab was dipped into the suspension and squeezed against the side of the bottle, and then used to inoculate a Mueller Hinton agar before the application of single antibiotic discs and subsequently

incubated at 37°C aerobically for 18-24 hour. Zone diameters of inhibition around each disc were measured using a calibrated ruler and interpreted according to Clinical and Laboratory Standard Institute (CLSI). Data were analyzed using SPSS (Statistical Package for the Social Sciences) version 22. The results for frequencies put in figures or tables. A chi-square test was used for comparison of the variables. P value < 0.05 was considered significant.

RESULTS

Age and Sex Incidence:

In the current study, the age of patients at the time of presentation was between 1-70 years (mean 28.95, SD 20.32). While the age of our patients at the onset of the disease was ranged from 1-50 years (mean 16.21, SD 16.8). There were (n=29, 46.0%) males and (n=34, 54.0%) females. The high percentage of patients was within the age group more than 18 (63.5%), while the rate of patients in age group ≤ 18 years was (36.5%). On the other hand, the age of onset showed a high prevalence in age group ≤ 18 46 (73.0%) while in age group> 18 years 17 (27.0%) Table 1. Table 1 showed 36 (57.1 %) of patients were from the rural while the remaining 27 (42.9%) from urban areas. Most of our patients 50 (79.8%) with no history of otological trauma, 38 (60.3%) with duration ≤ 10, 49 (77.8%) were immunocompetent, and 54 (85.7%) with tubotympanic disease Table 2. There is no statistical significance P value> 0.05 for all studied factors in relation to the 2 most common isolates Tables 1 and 2.

Site of Infection:

Seventy-five ear swabs were collected from 63 patients, 48 from unilateral active and 27 swabs were collected from 15 (12 with bilateral and 3 with unilateral active disease) bilateral CSOM. The incidence of infections on the right side was (39, 52.0%) and the incidence on the left side was (36, 48.0%) (Table -3). The results showed that all bilateral infections were caused by a single species of bacteria.

Bacteriological Profile of CSOM Infections:

The results of the culture showed that (66, 88%) swabs gave positive bacterial culture, while (9, 12%) swabs reported no growth. Out of 66 positive swabs, 64 gave single isolate on culture, while 2 swabs gave 2 bacterial species, therefore the total number of the isolates was 68.There were (42, 62%), Gram-negative and (26, 38%) Gram-positive aerobic microorganisms Figure 1. Among the 6 isolated microorganisms, *P. aeruginosa* was the most frequent bacterial isolated (n=35 51.5%), and the least one was Proteus spp (n=1 1.5%) Figure2 .The result of this study showed that *Pseudomonas aeruginosa* was the most frequent bacterial species isolated from CSOM with a rate (51.5%), followed by *Staphylococcus aureus (20.6%)* and *Staphylococcus epidermidis* (17.6%). On the other hand, the results showed a low infection rate with *Klebsiella pneumonia* (5.9%), *E.coli* (2.9%), *Proteus* spp(1.5%) (Table-2 &Fig. 2).

Residence:

Among 63 patients from the different residences of Al-Ramadi city, patients were divided into two groups according to their housing in rural (57.1%) and in urban (42.9%), (Table-1).

Antimicrobial Sensitivity Test:

The results of this study designed to determine the antibiotics sensitivity of the two most common bacterial

species caused CSOM, the results of antibiotics sensitivity showed that the most effective antibiotic was Impenem and followed by Ciprofloxacin and Amikacin (Tables 3&4 and Fig.3).

Table 1: Predominant two organisms with demographic factors of patients with CSOM

	No	Percentage (%)	*P. aeruginosa*	Percentage (%)	Staph. *aureus*	Percentage (%)	P value
Sex							
Male	29	46.0%	17	48.6%	6	46.2%	0.739
Female	34	54.0%	18	52.4%	7	53.8%	
Age at presentation							
≤ 18	23	36.5 %	13	37.1%	6	46.2%	0.575
> 18	40	63.5 %	22	62.9%	7	53.8%	
Residence							
Rural	36	57.1 %	21	60.0%	9	69.2 %	0.222
Urban	27	42.9 %	14	40.0%	4	30.8 %	

Table 2: Predominant two organisms with clinical features of CSOM

	Frequency	Percentage (%)	*P. aeruginosa*	Percentage (%)	Staph. *aureus*	Percentage (%)	P value
Otological trauma							
Present	13	20.6%	7	20.0%	4	30.7%	0.195
Absent	50	79.4%	28	80.0%	9	69.3%	
Duration							
≤ 10	38	60.3%	23	65.7%	7	53.8 %	0.778
> 10	25	39.7%	12	34.3%	6	46.2%	
Immunity status							
Immunocompetent	49	77.8%	28	80.0%	11	84.7%	0.992
Immunocompromised	14	22.2%	7	20.0%	2	15.3%	
Classification							
Tubotympanic	54	85.7%	31	88.5%	9	69.2%	0.576
Attico-antral	9	14.3%	4	1.5%	4	30.8%	

Table 3: Predominant two organisms with side of infection with CSOM

Side	No.	Percentage (%)	*P. aeruginosa*	Percentage (%)	Staph. *aureus*	Percentage (%)	P value
Right	39	52.0%	19	54.2%	9	64.2%	0.332
Left	36	48.0%	16	45.8%	5	35.8%	

Table 4: Antibiotic susceptibility patterns of *Pseudomonas aeruginosa* (n = 35)

Antibiotics		Resistant	Intermediate	Susceptible
Symbol	Name			
P10	penicillinG	35(100%)	0	0
AK	Amikacin	10(28.6%)	0	25(72.4%)
CN	Gentamicin	13(37.1%)	2(5.7%)	20(57.2%)
AX	Amoxicillin	34(97.15%)	1 (2.85%)	0
CAZ	Ceftazidime	32(91.4%)	0	3 (8.6%)
CRO	Ceftriaxone	30 (85.7%)	0	5 (14.3%)
CFM	Cefixime	30 (85.7%)	0	5 (14.3%)
CIP	Ciprofloxacin	8 (22.9%)	2 (5.7%)	25 (71.4%)
AMC	Amoxicillin/clav	30 (85.7%)	2 (5.7%)	3 (8.6%)
ATM	Azteronam	8 (22.9%)	1 (2.8%)	26 (74.3%)
IMP	Impenem	1 (2.85%)	0	34 (97.1%)
MET	Metronidazole	34(97.15%)	0	1 (2.85%)

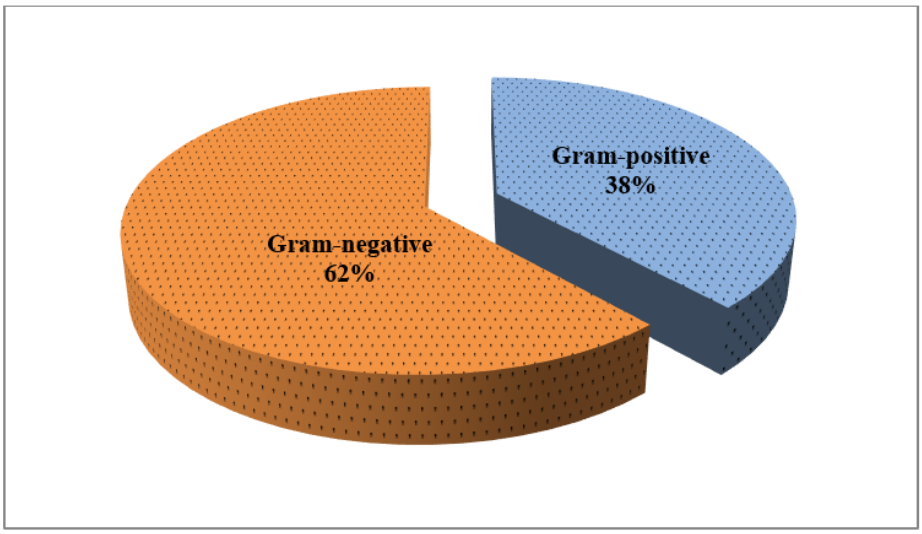

Fig. 1: Distribution of Gram-positive and Gram-negative from the total isolates.

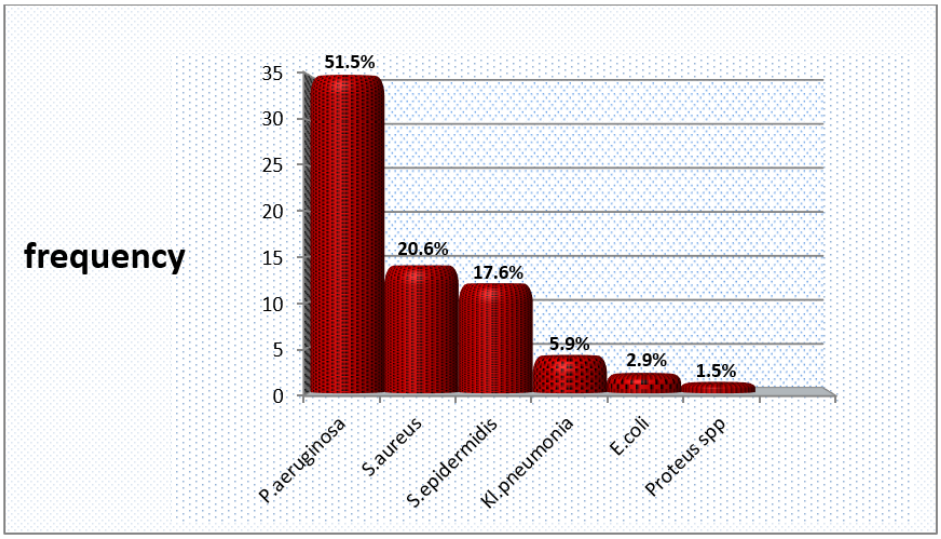

Fig. 2: Frequency of bacterial species isolated from 63 patients with chronic supportive otitis media.

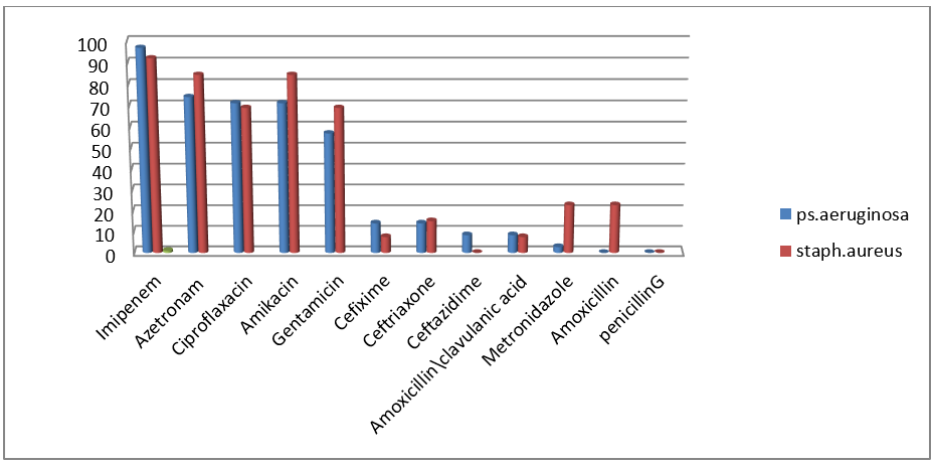

Fig.3. The sensitivity rates of the 12 antibiotics against the two common agents.

DISCUSSION

In the past, CSOM is defined as an inflammation of the middle ear and mastoid cavities, it is characterized by persistent or intermittent ear discharge through a perforated eardrum for at least 6 weeks. However, it is not necessary a chronic inflammation of the middle ear cleft associated with eardrum perforation, so nowadays the term chronic otitis media (COM) is used to cover all types of chronic inflammation of the middle ear whether there is a tympanic perforation or not (Kim *et al.*, 2015). Here in the present study, we took specimens from active COM with perforated tympanic membranes for 3 months or more and sent them for culture and sensitivity. The condition and its complications are one of the common problems seen by an otologist and even general practitioners (Brook, 2008). CSOM results from various microorganisms, including aerobic and anaerobic bacteria, and even fungi. Ciprofloxacin is usually used as an empirical treatment to attack the most causative agent (*Pseudomonas aeruginosa*) while waiting for culture and sensitivity result (Browning & Gatehouse 1992).Culture and sensitivity are the golden slandered technique in achieving the best medical therapy for such conditions.

In a prospective study by Browning *et al.*, of 2000 British patients, they found that the prevalence of healed COM was 11.9%, inactive 2.6%, and active 1.5%. For those with active and inactive COM (4.1%), 3.1% with unilateral and 1% with bilateral disease. The study also evaluated the relationship between the prevalence of COM and age, gender and socioeconomic class. Subjects in the age group 40-80 years were double to have COM than those age group 18-40 years. There was no statistical difference regarding the prevalence of COM and gender. The study also showed that the lower socioeconomic class with manual workers was twice as likely to have COM than those non-manual workers (Browning & Gatehouse 1992). CSOM is a disease of childhood. In clinical practice, we saw a lot of cases of CSOM with durations of many years. In this study, we took the age at onset of the disease (age at the presentation - duration of disease = age at onset) as a reference to the age of the patient. The study showed that 47 (73%) of our patients in the age group ≤ 18 years. In addition 13 from 63 patients with a history of traumatic eardrum perforation, 11 (84.6%) in age group >18 years. These findings support the fact that CSOM is more prevalent in children and adolescents. The present study showed that the females were slightly more than males. Which was similar to the finding by previous 2 studies (Deb & Ray 2012., Kim *et al.*, 2015). The prevalence of CSOM is much more common in developing and underdeveloped than in developed countries. Overcrowding, passive smoking, malnutrition, lower socio-economic class, and low personal hygiene increases the incidence of CSOM. This study showed no statistical significance among those from the urban or rural area, this might be attributed that the study is a clinical-based, not a population-based. Many microorganisms are detected on culture, single or multiple, the results of these depend on the weather, type of population and whether antibiotics have been used for years or newly used. Previous studies showed different isolates in different types of the population(Sweeny *et al.*, 1982). The results of the current study showed that Gram-negative bacteria (62%) were more predominant than Gram-positive bacteria (38%). This result was similar to the previous study(Khan *et* a*l.*, 2016). While the previous 2 studies (Ahmad 2013., Kim *et al.*, 2015) have reported that Gram-positive were more prevalent than gram-negative microorganisms. The present study has reported that *Pseudomonas ae**ruginosa*** is the commonest agents isolated from specimens of active CSOM in the Iraqi population, followed by S. aureus. This finding is in agreement with previous studies (Das *et al.*, 2006), while it is in disagreement with others

(Brooks *et al.*, 2013). High rate isolation of *Pseudomonas aeruginosa* in this study was might be attributed to *Pseudomonas aeruginosa* is inherently resistant to various antimicrobial agents, mainly due to the synergy between multi-drug efflux system or a type1 AmpC β-lactamase, low outer membrane permeability, its antibacterial products i.e., pyocyanin and bacteriocin, and minimum nutritional requirements. In addition to above-mentioned reasons, *Pseudomonas aeruginosa* uses its pili to attach to the necrotic or diseased epithelium of middle ear (Elsayed *et al.*, 2003., Kim *et al.*, 2016), as a result of this attachment, *Pseudomonas aeruginosa* produces enzymes e.g. proteases, lipopolysaccharides, etc. to protect itself from normal body defense mechanism required for attacking infections. In this study, we examined the relationship between various factors (age, gender, residence, duration, side, history of TTMP, classification of the disease and immunological status of the patients) with the two most common pathogens, but there was no statistically significant result (P value > 0.05).

Biofilm formation by such types of bacteria also complicated cases more and more leading to high resistant isolates protected by such biofilms. The same interpretations could be suitable to explain the high rate of staphylococcus isolation. *Staphylococcus aureus* possesses many virulence factors like Protein A, toxins and enzymes like Coagulase enzyme enable it to be in the high rank of isolation from patients with CSOM (Elsayed *et al.*, 2003 ., Brooks *et al.*, 2013). High resistance rate to antibiotics was due to arising of more virulent strains of *Staphylococcus aureus* which are penicillin and methicillin-resistant (MRSA) (Shaikh *et al.*, 2015). Antibiotics sensitivity test of 35 *P.aeruginosa* isolates revealed that all the isolates were resistant to penicillin (100%). This result agreed with (Ahmad ., 2013) who reported Penicillin with a rate (97.6%). Most of the isolates were resistant to cephalosporin antibiotics such as Ceftazidime, Cefixime, and Ceftriaxone with percentage (91.15%), (85.7%), (85.7%) respectively. These results were agreed with (Shaikh *et al.*, 2015) who reported resistance to Ceftazidime (91.4%), Cefixime (85.7%) and Ceftriaxone (85.7%). On the other hand, most of *P. aeruginosa* isolates were highly sensitive to Imipenem, which belonged to Carbapenemes antibiotic class with susceptibility rate (97.1%). This result agreed with (Sattar *et al.*, 2012) who reported (98%) sensitivity rate to Impenem. Ciprofloxacin belongs to Fluoroquinolone antibiotics showed good effectiveness towards most of *P. aeruginosa* isolates with sensitivity rate (71.4%). This result agreed with (Orji & Dis 2015) who reported a sensitivity rate (72%) and disagreed with (Ahmad 2013) who reported a sensitivity rate (98.9%). In this study, *P.aeruginosa* isolates showed different rates of sensitivity towards the aminoglycoside antibiotics including Amikacin (71.4%) and Gentamicin (57,2%). These results were agreed with (Juyal *et al.*, 2017) who reported sensitivity rates towards Amikacin (81.7%) and Gentamicin (29.3%) and disagreed with (Prasad *et al.*, 2017) *who reported* sensitivity rate to *Amikacin* (65.7%) and Gentamicin (45.7%).Also, Aztreonam showed good effectiveness against *P. aeruginosa* isolates with a sensitivity rate (74.3%). This result was agreed with) who reported a sensitivity rate to Aztreonam (71.3%). *Staphylococcus aureus* was the second more common bacterial species isolated from CSOM infections in this study. The results showed a high sensitivity rate to Impenem (92.3%).The most isolates were resistant to penicillin (100%) this result agreed with (Kim *et al.*, 2015) who reported that resistant rate to a penicillin(100%).and Amoxicillin with the resistant rate (69.2%), Cefotaxime (92.3%), Amoxicillin/clav(76.9%), this result agreed with (Katmanii & Anand 2015) who reported the resistant rate(100%) to Cefotaxime and closely rate with Amoxicillin, Co-Amoxiclav. The results showed sensitivity rate to Amikacin (84.6%), Ciprofloxacin(69.2 %)and Gentamicin(69.2%) this result agreed with [Prakash *et al.*, 2013] who reported sensitive

rate to Amikacin (89%) and close rates with Ciprofloxacin(77 %)and Gentamicin(83%). Other antibiotics appeared high resistant rate like Cefixime (92.3%), Amoxicillin/clav (76.9%), Metronidazole (76.9%) , Amoxicillin (69.2%) and Ceftriaxone (53.8%) this result disagreed with (Prakash *et al.*, 2013)who reported sensitivity rate to Ceftazidime (87%) ,Ceftriaxone(89%). From the above different antibiogram for studied isolates of ***pseudomonas aeruginosa*** and Staphylococcus, it was found that different response to different antibiotics, this can be ought to :

1-The fact that microbial undergo modulation through eras to keep virulence and resistance to antibiotics (Adoga *et al.*, 2011., Alshameri 2019) in addition to that.

2- Response of bacterial isolates to antimicrobial agents is different regarding the type of isolate and type of antimicrobial agent and the site of infection (Lutenbach *et al.,* 2011., Brooks *et al.,* 2013., Lafi *et al.,* 2018).

3- Abuse of antibiotics in a community leads to increasing resistance of bacteria to antibiotics due to arising of resistance factors like penicillinases of different spectrums (Bradford *et al.*, 2001., Makena *et al.*, 2016) . Thus continuous study and check up for the bacterial agents involved in infection and its antibiotic profile to defeat microbes with proper antibiotic treatment particularly with abuse of antibiotics communities. So regarding CSOM patients, culture and sensitivity must be done before treatment to select the most appropriate antibiotic as an optimal treatment, to avoid the patients from complications, to keep the continuity of hearing, and to prevent eardrum perforation. Although the complications of CSOM are low, however, they are mostly serious ones. In general, the complications divided into extra-cranial (e.g. eardrum perforation, tympanosclerosis, acute and chronic mastoiditis, sensorineural deafness etc….) and intracranial complications (e.g. extradural, subdural and intraparenchymal brain abscess, meningitis, and etc….). The goals of the Otolaryngologists in the management of CSOM are to eradicate the infection, making the ear dry, preventing its complications, and improving patients' hearing. Most of the cases of CSOM are treated medically which includes: meticulous cleaning of the ear to decrease the bacterial load by either suction or dry mopping, avoidance of water entry to the ear, and in some cases prescribing topical and systemic antibiotics depending on the knowledge of the most common causative microorganisms and on culture and sensitivity(Ahmad, 2013). Those cases in which they are resisting the medical management or presenting with complications are mostly treated by surgical intervention. General practitioners who are limited to empirical treatment treat often patients with CSOM and they are not sent them to the Otolaryngologist unless the use of empirical treatment is failing. The disease faces a major challenge in its treatment due to its recurrent nature form and the development of the microorganism resistance to antibiotics(Ahmad,2013). In addition to misuse or overuse of antibiotic in our country Iraq because of the lack of local guidelines for prescribing the antibiotics for CSOM and inactivation of the law towards the buying by hand from the patients or their relatives by the pharmacies, these 2 factors will enhance the resistance of the agents to the antibiotics. From the previously mentioned factors, it is of utmost importance to perform periodic surveillance of the microbiological profile and their antibiotic sensitivity in CSOM. The limitations of the study were: small sample size and not cultured the anaerobic bacteria and fungi as well as viruses, which are blamed in this infection because of the lack of suitable media, and increment in the cost of the study. In addition, we performed sensitivity to only 12 most local available antibiotics. We recommend further studies to cover other etiological agents for CSOM. In conclusion, most of the affected individuals in the present study were in the age group ≤ 18 years. *Pseudomonas aeruginosa* was the most commonly isolated microorganisms; it is a multifactorial resistant pathogen. Although Impenem is the most effective

(97.1%) antibiotic against isolated agents, it cannot use as empirical treatment of CSOM because of its high price. Instead. We recommended using ciprofloxacin (low cost, tolerable and effective in 71.4% in this study) as an empirical treatment as a local or systemic, for active CSOM while waiting for culture and sensitivity.

REFERENCES

AcuinJ. and G. G. Browning, "Clinical reviewChronic suppurative otitis mediaCommentary: Interpreting the evidence," Bmj, vol. 325, no. 7373, pp. 1159–1160, 2002.

Ahmad,S., "Antibiotics in chronic suppurative otitis media: A bacteriologic study," Egypt. J. Ear, Nose, Throat Allied Sci., vol. 14, no. 3, pp. 191–194, 2013.

Adoga,A.A, A. Bakari, O. A. Afolabi, A. M. Kodiya, and B. M. Ahmad, "Bacterial isolates in chronic suppurative otitis media: a changing pattern?," Niger. J. Med. J. Natl. Assoc. Resid. Dr. Niger., vol. 20, no. 1, pp. 96–98, 2011.

Alshameri,A., W. Xinghu, A. S. Dawood, C. Xin, and A.M. Chunjie Yan, "Characterization of Yemeni Natural Zeolite (Al-Ahyuq Area) and its Environment Applications: A Review," J. Ecol. Eng., vol. 20, no. 4, pp. 157–166, 2019.

Bradford, P.A.,"Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat," Clin. Microbiol. Rev., vol. 14, no. 4, pp. 933–951, 2001.

Brook, I."The role of anaerobic bacteria in chronic suppurative otitis media in children: implications for medical therapy," Anaerobe, vol. 14, no. 6, pp. 297–300, 2008.

Brooks,G.F., J. S. Butel, K. C. Carroll, T. A. Mietzner, and S. A. Morse, "Jawetz, Melnick, & Adelberg's medical microbiology, 13th ed. " 2013.

BrowningG.G. and S. Gatehouse, "The prevalence of middle ear disease in the adult British population," Clin. Otolaryngol. Allied Sci., vol. 17, no. 4, pp. 317–321, 1992.

Deb T.,and D. Ray, "A Study of the Bacteriological Profile of Chronic Suppurative Otitis Media in Agartala," Indian J. Otolaryngol. Head Neck Surg., vol. 64, no. 4, pp. 326–329, 2012.

Das,R.N., T. S. Chandrashekhar, H. S. Joshi, M. Gurung, N. Shrestha, and P. G. Shivananda, "Frequency and susceptibility profile of pathogens causing urinary tract infections at a tertiary care hospital in western Nepal," Singapore Med. J., vol. 47, no. 4, p. 281, 2006.

Elsayed,S., B. L. Chow, N. L. Hamilton, D. B. Gregson, J. D. D. Pitout, and D. L. Church, "Development and validation of a molecular beacon probe–based real-time polymerase chain reaction assay for rapid detection of methicillin resistance in Staphylococcus aureus," Arch. Pathol. Lab. Med., vol. 127, no. 7, pp. 845–849, 2003.

Gates *G. A.et al.*, "Recent advances in otitis media. 1. Definitions, terminology, and classification of otitis media.," Ann. Otol. Rhinol. Laryngol. Suppl., vol. 188, p. 8, 2002.

Iwamoto M., Yi Mu, Ruth Lynfield, Sandra **N**. Bulens, Joelle Nadle, Deborah Aragon, Susan Petit, Susan M. (2013).Trends in invasive methicillin-resistant Staphylococcus aureus infections," Pediatrics, vol. 132, no. 4, pp. e817–e824, DOI: https://doi.org/ 10.1542/ peds.2013-1112

Juyal, D., M. Sharma, V. Negi, R. Prakash, and N. Sharma, "Pseudomonas aeruginosa and its sensitivity spectrum in chronic suppurative otitis media: A study from Garhwal hills of Uttarakhand State, India," Indian J. Otol., vol. 23, no. 3, p. 180, 2017.

Karlowsky J.A. Heather J. Adam, Marc Desjardins, Philippe R. S. Lagacé-Wiens, Daryl J. Hoban, George G. Zhanel,(2013).Changes in fluoroquinolone resistance over 5 years (CANWARD 2007–11) in

bacterial pathogens isolated in Canadian hospitals," J. Antimicrob. Chemother., vol. 68, no. suppl_1, pp. i39–i46.

Kim, S.H., M. G. Kim, S. S. Kim, S. H. Cha, and S. G. Yeo, "Change in detection rate of methicillin-resistant Staphylococcus aureus and Pseudomonas aeruginosa and their antibiotic sensitivities in patients with chronic suppurative otitis media," J. Int. Adv. Otol., vol. 11, no. 2, p. 151, 2015.

Khan,M., A. S. Akram, and S. Bin Faiz, "Isolation Of Microflora Involved In Chronic Suppurative Otitis Media And Finding Antibiotic Sensitivity In Kharian," Pakistan Armed Forces Med. J., vol. 66, no. 3, pp. 337–340, 2016.

Kattimani M.V., and S. Anand, "Aerobic bacteriology of chronic suppurative otitis media: our experience," J. Evol. Med. Dent. Sci., vol. 4, no. 67, pp. 11686–11694, 2015.

Lee, S.K., M. S. Lee, S. Y. Jung, J. Y. Byun, M. S. Park, and S. G. Yeo, "Antimicrobial resistance of Pseudomonas aeruginosa from otorrhea of chronic suppurative otitis media patients," Otolaryngol. Neck Surg., vol. 143, no. 4, pp. 500–505, 2010.

Lafi, S. A., M. Al-Shamarry, M. Ahmed, and W. Ahmed, Bacterial profile of Infected Traumatic Wound and the Antibiogram of predominant Bacterial Isolates Using Viteck Automated System in Ramadi Teaching Hospital, Iraq., vol. 10. 2018.

Lautenbach *E.*, Irving Nachamkin ., Baofeng Hu ., Neil O. Fishman. , Pam Tolomeo ., Priya Prasad., Warren B. Bilker and Theoklis E. Zaoutis "Surveillance cultures for detection of methicillin-resistant Staphylococcus aureus: diagnostic yield of anatomic sites and comparison of provider-and patient-collected samples," *Infect. Control Hosp. Epidemiol.*, vol. 30, no. 4, pp. 380–382, 2009.

Makena A., Duzgun A., Brem J., Mac Donough MA. And Rydzik AM. "Comparison of Verona integron-borne metallo-β-lactamase (VIM) variants reveals differences in stability and inhibition profiles," *Antimicrob. Agents Chemother.*, vol. 60, no. 3, pp. 1377–1384, 2016.

Orji F.T.,and B. O. Dike, "Observations on the current bacteriological profile of chronic suppurative otitis media in South eastern Nigeria," Ann. Med. Health Sci. Res., vol. 5, no. 2, pp. 124–128, 2015.

PrakashR., et al., "Microbiology of chronic suppurative otitis media in a tertiary care setup of Uttarakhand state, India," N. Am. J. Med. Sci., vol. 5, no. 4, p. 282, 2013.

Sweeney,G., G. L. Picozzi, and G. G. Browning, "A quantitative study of aerobic and anaerobic bacteria in chronic suppurative otitis media," J. Infect., vol. 5, no. 1, pp. 47–55, 1982.

Shaikh,S., J. Fatima, S. Shakil, S. M. D. Rizvi, and M. A. Kamal, "Prevalence of multidrug resistant and extended spectrum beta-lactamase producing Pseudomonas aeruginosa in a tertiary care hospital," Saudi J. Biol. Sci., vol. 22, no. 1, pp. 62–64, 2015.

Sattar,A., A. Alamgir, Z. Hussain, S. Sarfraz, and J. Nasir, "Bacterial spectrum and their sensiSattar, A., Alamgir, A., Hussain, Z., Sarfraz, S., & Nasir, J. (2012). Bacterial spectrum and their sensitivity pattern in patients of chronic suppurative otitis media. Journal of the College of Physicians and Surgeons--P," J. Coll. Physicians Surg. JCPSP, vol. 22, no. 2, pp. 128–129, 2012.

Verhoeff,M. E. L. van der Veen, M. M. Rovers, E. A. M. Sanders, and A. G. Schilder,M. "Chronic suppurative otitis media: a review," Int. J. Pediatr. Otorhinolaryngol., vol. 70, no. 1, pp. 1–12, 2006.