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Full Length Research Paper

Impact of multi drugs resistant bacteria on the pathogenesis of chronic suppurative otitis media

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One hundred twenty patients with chronic suppurative otitis media (CSOM) in Basrah, 65 (54.2%) males and 55 (45.8%) females, with male: females ratio (1.2:1) and 60 individual without otological problems as control group were included in this study, which done during the period between March 2009 and January 2010. This includes the collection of aural swab samples, culturing of samples, identification of causative agent's species and antibiotic sensitivity. Gram's negative bacteria were the commonest microorganism comprises (60%). *Pseudomonas aeruginosa* was common causative agent (19.04%), followed by *Staphylococcus aureus* (16.7%) and *Klebsiella* spp. (14.3%). Mixed infection was found in high percent (74%), in which *P. aeruginosa* and other microorganisms were more common. The antibiotic sensitivity pattern showed that *P. aeruginosa* was sensitive to Ciprofloxacin, amoxicillin +clavulanic acid and gentamicin, while other is appeared resistant, *S. aureus* was sensitive to ciprofloxacin, amoxicillin+clavulanic acid, erthomycin, cephalexine and it is resistant to penicillin and ampicillin, *klebsiella* species were sensitive to ciprofloxacin, amoxicillin +clavulanic acid, gentamicin, while resistant to tetracycline.

Key words: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella* spp., chronic suppurative otitis media, aural swab, antibiotic sensitivity, gram s negative bacteria, otological.

INTRODUCTION

Otitis media is inflammation of the middle ear. This is commonly caused by the build up of fluid behind the ear drum, as a result of a blockage to the Eustachian tube. Otitis media is more common in children, as their Eustachian tube is shorter and more horizontal than adults and is made up of more flaccid cartilage, which can impair its opening (Bluestone and KLien, 2001). Otitis media can cause a mild to moderate hearing loss, due to the fluid interfering with the transmission of sound through to the inner ear. It can often affect the tympanic membrane causing it to retract or become inflamed. The fluid can cause the tympanic membrane to bulge and become inflamed and occasionally the tympanic membrane will perforate. There are three common types of otitis media, acute purulent otitis media, otitis media with effusion and chronic suppurative otitis media (Berman, 1997).

CSOM, for the purposes of this document, defined as a chronic inflammation of the middle ear and mastoid cavity, which presents with recurrent ear discharges or otorrhoea through a tympanic perforation (Howard, 2007). The disease usually begins in childhood as a spontaneous tympanic perforation due to an acute infection of the middle ear, known as acute otitis media which presents with a rapid onset of signs and symptoms, such as pain, fever, irritability; a red bulging ear drum and middle ear effusion (Jahn, 1991).

In CSOM the bacteria may be aerobic (e.g. *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Proteus mirabilis, Klebsiella* species) or anaerobic (e.g. *Bacteroides, Peptostreptococcus, Proprionibacterium*) (Saunders et al., 2009; Brook, 1996). The present studies aimed to identify the bacterial pathogens associated with CSOM, study the antibiotic susceptibility pattern of antibiotic against bacterial pathogen, and determined the

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Table 1. Bacterial types isolated from healthy person (control group).

Microorganisms	No. of isolates	(%)
Klebsiella spp.	2*	4
S. Spp.	3	6
E. coli spp.	2	4
Bacillus spp.	8	16
S. epidemedis.	20	40
Corynebacterium spp	15	30
No growth	10	16.66
Total	60	100%

* P<0.01

Table 2. Bacterial type isolated from patients with CSOM.

Caustive agents	No. of isolates	(%)
P. aeruginosa.	40*	19.41
S.aureus.	35	16.99
Klebsiella spp.	30	14.56
B.catarrhalis.	20	9.70
Proteus spp.	20	9.70
H.influenzae.	20	9.70
Streptococcus spp.	15	7.28
E.coli spp.	10	4.85
Corynebacterium spp.	08	3.88
Bacillus spp.	08	3.88
Total No. of isolates	206	100

** x^2=49.8 p < 0.01

mode of bacterial isolation and multi drugs resistant bacteria.

MATERIALS AND METHODS

Patients

A total of 120 patients with CSOM were included in this study, the diagnosis of CSOM was carried out according to clinical examination by otoscopic and tuning fork examination, and audiological investigation (pure tone audiometry and tympanometry under supervision of specialists of ENT. Microbiological investigation includes (culture, identification of causative agents and antibiotic sensitivity. The study was carried out in Basrah General Hospital, out patients E.N.T. clinic, during the period from March 2009 - January 2010.

Control group

A total of 60 individuals without otological problems, 30 males and 30 females in various age group, they were regarded as a control group.

Sampling

Two groups were included in this study: Group (1) 120 aural swabs were taken from infected ear of CSOM patients. Group (2) 60 aural swab were taken from a control group. Swabs were taken under sterile condition and transfer immediately to the laboratory by brain heart broth for aerobic bacteria, thioglycollate broth for anaerobic bacteria, and cultured on suitable media at 37˚C for 24 - 48 h. Primary isolation on (Blood agar, chocolate agar, nutrient agar), then on selective media identification and biochemical characterization were carried out according to standard routine techniques (Fingole and Baron, 2002). Note: All media are sterilized by autoclave (121˚C under 15 lbs pressure for 15 min). Antibiotics disc include:

1- Penicillin G 10 mg (Bioanalyse).
2- Erythromycin 15 mg (Bioanalyse).
3- Tetracycline 30 mg (Bioanalyse).
4- Ciproflaxin 5 mg (Bioanalyse).
5- Gentamicine 10 mg (Bioanalyse).
6- Ampicillin 10 mg (Bioanalyse).
7- Augminten 20 mg (Bioanalyse).
8- Trimethoprim 25 mg (Bioanalyse).
9- Streptomycin 10 mg (Bioanalyse).
10- Lincomycin 2 mg (Bioanalyse).

Statistical analysis

In order to determine the statistical significance among different variables, SPSS program (statistical program for social sciences) ver.11, was used for this purpose. The following statistical testes were performed: Chi-square (x^2) test and the difference between two proportions by T-test were used to assess the significance of difference between groups. P-value less than 0.05 was considered as statistically significant (S), p-value < 0.01 as highly significant and (HS), p-value < 0.001 as extremely significant (ES). p-value more than 0.05 was considered as statistically not significant (NS).

RESULTS

Table 1 show results of isolated bacterial from (60) healthy persons. The following bacteria were isolated, *Staphylococcus epidermidis* 20 isolates (40%), followed by *Corynebacterium* species 15 isolate (30%). Other types distributed according to species in Tables 3 - 8. Ten samples gave negative result for bacteria culture (16.66%).

Pathogenic bacteria isolated from patients with CSOM

The occurrence of various bacterial isolate among CSOM patients shown in Table 2 presents that *P. aeruginosa* was more frequently isolates 40 (19.41%), while *S. aureus* followed by *Pseudomonas* 35 (16.99%), *Klebsiella* 30 (14.56%) *Branhamella catarrhalis* 20 (9.70%), *Proteus* 20(9.70%), *Heamophilus influenzae* 20(9.70%), *Streptococcal* spp. 15(7.28%), *E. coli* 10(4.85%), *Corynebacterium* 8 (3.88), and *Bacillus* 8 (3.88).

Table 3. Relationship between caustive agents and hearing loss.

Causative agent	No. of isolates with Hearing Loss (%)				
	No. of isolates	Not applicable	CHL	MXHL	SNHL
P. aeruginosa	40(20)	6(15)	16(40)	10(25)	8(20)
S . aureus	35(17.5)	4(11.4)	15(42.8)	9(25.7)	7(20)
Klebsiela spp	30(15)	3(10)	12(40)	8(26.6)	7(23.3)
B. catarrhalis	20(10)	3(15)	6(30)	6(30)	5(25)
Proteus spp	18(9)	2(11.1)	9(50)	4(22.2)	3(16.6)
H. influenzae	16(8)	4(25)	6(37.5)	3 (18.7)	3(18.7)
Streptococcal spp	15(7.5)	2(13.3)	6(40)	4(26.6)	3(20)
E. coli spp	10(5)	3(30)	4(40)	2(20)	1(10)
Corynebacterium spp	8(4)	1(12)	3(37)	2(25)	2(25)
Bacillus spp	8(4)	2(25)	3(37)	2(25)	1(12)

CHL: Conductive hearing loss, SNHL: Senserineural hearing loss and MXHL: Mixed hearing loss.

Table 4. Standard antibiotic susceptibility test according to diameters of inhibition zone supplied by bioanalysis company.

Antimicrobial agent	Symbol	Conc. mcg	Zone diameter (mm).	
			Sensitive	Resistant
Ciprofloxacin.	(cip)	10	20 or less	29 or more
Amoxicillin + clavulanic acid.	(AMC)	20	19 or less	20 or more
Gentamicin	(CN)	10	10 or less	15 or more
Vancomycin	(VA)	30	9 or less	12 or more
Lincomycin.	(L)	2	9 or less	15 or more
Cephalexin	(CL)	30	14 or less	18 or more
Penicillin	(p)	10	11 or less.	22 or more
Erythromycin	(E)	15	13 or less.	18 or more
Ampicillin	(AM)	10	(11-21) or less.	(14 - 30) or more
Tetracycline	(T)	30		19 or more
Streptomycin	(s)	10	14 or less.	15 or more
Trimethoprim+ sulphamethoxazole	(SXT)	1.25	11 or less.	16 or more

Bacterial pathogens and hearing impairment

The occurance of various caustive agents isolates among CSOM patients in three types of hearing loss (CHL, SNHL, MXHL) are shown in Table 3. *P. aeruginosa* was more frequently isolated in senserineural and profound hearing loss (25 - 26.2%), while in conductive and mixed hearing loss (16.7 - 20.4%) *S. aureus* isolates, appeared more frequently among CSOM patients with conductive and mixed hearing loss (20.4 - 25%) than in senserineura and profound hearing loss (12.5 - 15%) *Klebsiella* species and other organisms isolated in various percentages from these three types of hearing loss.

Antibiotic sensitivity of P. aeruginosa

Table 5 show that the frequency of Ciprofloxacin, Amoxicillin + clavulanic acid (Augmentin) and Gentamicin are statistically significantly higher than other types of antibiotics. $P < 0.01$ in percentages of sensitivity between (50 - 75%) ($p < 0.01$), while 88% of *P. aeruginosa* isolates was resist by trimethoprim, 85% to Streptomycin, and 80% to Vancomycin, while other pattern of resistance were between 25 - 78% of various antibiotics $p < 0.01$.

Antibiotic sensitivity of S. aureus

Table 6 shows that in each drugs group, the frequency sensitivity of Ciprofloaxacin, Augmenten, Cephalexin and Penicillin (57 - 80%) were statistically significantly higher sensitive than other antibiotic. $P < 0.01$, while 83% of *S. aureus* isolates was resist to trimethoprim, 83% to Streptomycin, and 83% to Vancomycin, while other pattern of resistance were between 20 - 77% of various

Table 5. Antibiotic susceptibility pattern of *Pseudomonas aeruginosa*.

Drugs type	No. of isolates	Sensitive (%)	Resistant (%)
Ciprofloxacin	40	30*(75)	10 (25)
Augmentin	40	21(52.5)	19(47.5)
Gentamicin	40	20(50)	20(50)
Vancomycin	40	8(20)	32(80)
Lincomycin	40	9(22.5)	31(77.5)
Cephalexin	40	11(27.5)	29(72.5)
Penicillin	40	10(25)	30(75)
Erythromycin	40	12(30)	28(70)
Ampicillin	40	14(35)	26(65)
Tetracycline	40	13(32.5)	27(67.5)
Streptomycin	40	6(15)	34(85)
Trimethoprim	40	5(12.5)	35(87.5)

$X^2 = 25$ $p < 0.01$.

Table 6. Antibiotic susceptibility pattern of *S. aureus*.

Drugs type	No. of isolates	Sensitive (%)	Resistant (%)
Ciprofloxacin	35	20*(57.15)	15(42.85)
Augmentin	35	20(57.15)	15(42.85)
Gentamicin	35	15(42.85)	20(57.15)
Vancomycin	35	6(17.14)	29(82.86)
lincomycin	35	8(22.85)	27(77.15)
Cephalexin	35	20(57.15)	15(42.85)
Penicillin	35	12(34.28)	23(65.72)
Erythromycin	35	28(80)	07(20)
Ampicillin	35	10(28.57)	25(71.43)
Tetracycline	35	10(28.57)	25(71.43)
Streptomycin	35	6(17.14)	29(82.86)
Trimethoprim	35	6(17.14)	29(82.86)

$X^2 = 6.9$ $p < 0.01$.

Table 7. Antibiotic susceptibility pattern of *Klebsiella spp*.

Drugs type	No of isolated	Sensitive (%)	Resistant (%)
Ciprofloxacin	30	20* (66.66)	10 (33.34)
Augmentin	30	21(70)	9(30)
Gentamicin	30	16(53.33)	14(46.67)
Vancomycin	30	06(20)	24(80)
lincomycin	30	08(26.66)	22(73.34)
Cephalexin	30	12(40)	18(60)
Penicillin	30	10(33.34)	20(66.66)
Erythromycin	30	09(30)	21(70)
Ampicillin	30	11(36.66)	19(63.34)
Tetracycline	30	10(33.34)	20(66.66)
Streptomycin	30	10(33.34)	20(66.66)
Trimethoprim	30	08(26.66)	22(73.33)

$X^2 = 25$ $p < 0.01$.

antibiotics $p < 0.01$.

Antibiotic sensitivity of Klebsiella spp.

Table 7 shows that in each drugs group, the frequency of sensitivity of Ciprofloxacin and Augmentin (67 - 70%) were statistically significantly higher than other type of antibiotic drugs. ($p < 0.01$), while 73% of *Klebsiella spp* isolates was resist to trimethoprim, 70% to Erythromycin, and 80% to Vancomycin, while other pattern of resistance were between 30 - 73% of various antibiotics $p < 0.01$.

Antibiotic sensitivity of B. catarrhalis

Table 8 shows that in each drugs group, Ciprofloxacin, Augmentin, Cephalexin, Ampicillin, Gentamicin were statistically significantly higher sensitivity (50 - 75%)

Table 8. Antibiotic susceptibility pattern of *Branhamella spp*.

Drugs type	No. of isolates	Sensitive (%)	Resistant (%)
Ciprofloxacin	20	15* (75)	05 (25)
Augmentin	20	15(75)	05(25)
Gentamicin	20	10(50)	10(50)
Vancomycin	20	06(30)	14(70)
lincomycin	20	08(40)	12(60)
Cephalexin	20	12(60)	08(40)
Penicillin	20	10(50)	10(50)
Erythromycin	20	09(45)	11(55)
Ampicillin	20	11(55)	09(45)
Tetracycline	20	10(50)	10(50)
Streptomycin	20	06(30)	14(70)
Trimethoprim	20	05(25)	15(75)

$X^2 = 25$ $p < 0.01$.

Table 9. Antibiotic susceptibility pattern of *Proteus spp*.

Drugs type	No. of isolates	Sensitive (%)	Resistant (%)
Ciprofloxacin	20	14*(70)	06(30)
Augmentin	20	12(60)	08(40)
Gentamicin	20	12(60)	08(40)
Vancomycin	20	10(50)	10(50)
lincomycin	20	10(50)	10(50)
Cephalexin	20	08(40)	12(60)
Penicillin	20	05(25)	15(75)
Erythromycin	20	08(40)	12(60)
Ampicillin	20	05(25)	15(75)
Tetracycline	20	10(50)	10(50)
Streptomycin	20	08(40)	12(60)
Trimethoprim	20	10(50)	10(50)

$X^2 = 25$ $p < 0.01$.

against *Branhamella* spp than other type of antibiotic ($p < 0.01$), while 75% of *Branhamella* spp isolates was resist to trimethoprim, 70% to Streptomycin, and 70% to Vancomycin, while other pattern of resistance were between 25 - 60 of various antibiotics $p<0.01$.

Antibiotic sensitivity of Proteus spp.

Table 9 shows that in each drugs group, the frequency of Ciprofloxacin, Augmentin, Gentamicin and Trimethoprim were statistically significantly higher effective against *Proteus spp* than other type of Antibiotics, (60 - 70%) sensitive ($p < 0.01$), while 75% of *Proteus* spp isolates was resist to Ampicillin, 70% Erythromycin, and 75% to Penicillin, while other pattern of resistance were between 30 - 60% of various antibiotics $p < 0.01$.

Table 10. Antibiotic susceptibility pattern of *Heamophilus spp*.

Drugs type	No. of isolates	Sensitive (%)	Resistant (%)
Ciprofloxacin	20	15* (75)	5 (25)
Augmentin	20	12(60)	8(40)
Gentamicin	20	12(60)	8(40)
Vancomycin	20	10(50)	10(50)
Lincomycin	20	10(50)	10(50)
Cephalexin	20	8(40)	12(60)
Penicillin	20	8(40)	12(60)
Erythromycin	20	10(50)	10(50)
Ampicillin	20	6(30)	14(70)
Tetracycline	20	6(30)	14(70)
Streptomycin	20	4(20)	16(80)
Trimethoprim	20	7(35)	13(65)

$X^2 = 25$ $p < 0.01$.

Antibiotic sensitivity of H. influenzae

Table 10 shows that in each drugs group, the frequency of Ciprofloxacin, Augmentin, Gentamicin, Vancomycin and Lincomycin (50 - 75%) were statistically significantly higher sensitive drugs against *H. influenzae* than other ($p < 0.01$), while 80% of *H. influenzae* isolates was resist to Streptomycin, 70% Tetracycline, and 70% to Ampicillin, while other pattern of resistance were between 25 - 65% of various antibiotics $p < 0.01$.

Antibiotic sensitivity of Streptococcus spp.

Table 11 shows that in each drugs group the frequency of Ciprofloxacin, Augmentin, Penicillin, Erythromycin and tetracycline were statistically significant higher sensitive (67 - 80%) than other type of Antibiotics ($p < 0.01$), while 60%, of *Streptococcus* spp isolates was resist to Trimethoprim, 53.33% to Streptomycin and 46% to Ampicillin, while other pattern of resistance were between 20 - 40% of various antibiotics $p < 0.01$.

Antibiotic sensitivity of E. coli

Table 12 shows that in each drugs group, the frequency of Ciprofloxacin, Augmentin, Gentamicin, Lincomycin and Cephalexin were statistically significantly higher sensitive drugs (60 - 80%) against *E. coli* than other type of drugs ($p < 0.01$), while 80%, of *E. coli* spp isolates was resist to Streptomycin, 60% to Trimethoprim and 60% to Ampicillin, Erythromycin and penicillin, while other pattern of resistance were between 20 - 40% of various

Table 11. Antibiotic susceptibility pattern of *Streptococcus spp*.

Drugs type	No. of isolates	Sensitive (%)	Resistant (%)
Ciprofloxacin	15	12* (80.00)	3(20.00)
Augmentin	15	10(66.66)	5(33.34)
Gentamicin	15	10(66.66)	5(33.34)
Vancomycin	15	9(60.00)	6(40.00)
Lincomycin	15	9(60.00)	6(40.00)
Cephalexin	15	10(66.66)	5(33.34)
Penicillin	15	10(66.66)	5(33.34)
Erythromycin	15	10(66.66)	5(33.34)
Ampicillin	15	8(53.33)	7(46.67)
Tetracyclin	15	9(60.00)	6(40.00)
Streptomycin	15	7(46.67)	8(53.33)
Trimethoprim	15	6(40.00)	9(60.00)

$X^2 = 10.8$ $p < 0.01$.

Table 12. Antibiotic susceptibility pattern of *E. coli*.

Drugs type	No. of isolates	Sensitive (%)	Resistant (%)
Ciprofloxacin	10	8* (80)	2(20)
Augmentin	10	8(80)	2(20)
Gentamicin	10	8(80)	2(20)
Vancomycin	10	6(60)	4(40)
Lincomycin	10	6(60)	4(40)
Cephalexin	10	6(60)	4(40)
Penicillin	10	4(40)	6(60)
Erythromycin	10	4(40)	6(60)
Ampicillin	10	4(40)	6(60)
Tetracycline	10	4(40)	6(60)
Streptomycin	10	2(20)	8(80)
Trimethoprim	10	4(40)	6(60)

$X^2 = 25$ $p < 0.01$.

Table 13. Antibiotic susceptibility pattern of *Corynebacterium spp.*

Drugs type	No. of isolates	Sensitive (%)	Resistant (%)
Ciprofloxacin	8	6* (75.0)	2(25.0)
Augmentin	8	6(75.0)	2(25.0)
Gentamicin	8	4(50.0)	4(50.0)
Vancomycin	8	3(37.5)	5(62.5)
Lincomycin	8	3(37.5)	5(62.5)
Cephalexin	8	4(50.0)	4(50.0)
Penicillin	8	6(75.0)	2(25.0)
Erythromycin	8	6(75.0)	2(25.0)
Ampicillin	8	6(75.0)	2(25.0)
Tetracycline	8	4(50.0)	4(50.0)
Streptomycin	8	5(62.5)	3(37.5)
Trimethoprim	8	5(62.5)	3(37.5)

$X^2 = 45.4$ $p < 0.01$.

antibiotics $p < 0.01$.

Antibiotic sensitivity of Corynebacterium spp.

Table 13 shows that in each drugs group, the frequency of Ciprofloxacin, Cephalexin, Erythromycin, Ampicillin and Penicillin were statistically significantly higher sensitive drugs (75%) against Corynebacterium spp ($p < 0.01$), while 63%, of *Corynebacterium* spp isolates was resist to Lincomycin, 63% to Vancomycin and 50% to Gentamicin and Cephalexin, while other pattern of resistance were between 25 - 38% of various antibiotics $p < 0.01$.

Antibiotic sensitivity of Bacillus spp.

Table 14 shows that in each drugs group, the frequency of Ciprofloxacin, Erythromycin, Ampicillin, and Trimethoprim were statistically significantly higher sensitive drugs (75%) against *Bacillus spp* than other type of drugs ($p < 0.01$), while 50%, of *Bacillus* spp isolates was resist to Lincomycin, 50% to Vancomycin and 50% to Cephalexin, Penicillin and Streptomycin, while other pattern of resistance were 25 - 37.5% of various antibiotics $p < 0.01$.

Types of infection according to number of causative agent

Table 15 shows that the frequency of double causative agents (55 isolates, 45.83%) was statistically significantly higher than single causative agent (38 isolates, 31.66%), three causative agents (18 isolates, 15%) and more than three (9 isolates, 7.5%). There was no difference between male and female in the frequency of various types of mode of isolates.

Table 14. Antibiotic susceptibility pattern of *Bacillus spp.*

Drugs type	No. of isolates	Sensitive (%)	Resistant (%)
Ciprofloxacin	8	6 *(75)	2(25)
Augmentin	8	4(50)	4(50)
Gentamicin	8	6(75)	2(25)
Vancomycin	8	4(50)	4(50)
Lincomycin	8	4(50)	4(50)
Cephalexin	8	4(50)	4(50)
Penicillin	8	4(50)	4(50)
Erythromycin	8	6(75)	2(25)
Ampicillin	8	6(75)	2(25)
Tetracycline	8	5(62.5)	3(37.5)
Streptomycin	8	4(50)	4(50)
Trimethoprim	8	6(75)	2(25)

X^2=1.26 P>0.05.

Table 15. Modes of isolation of the bacterial pathogens among patients with CSOM.

Modes of isolated	Male	Female	Total
	No. of patients (%)		
Single causative agent	18*(15.00)	20 (16.66)	38(31.66)
Double causative agent	30(25.00)	25 (20.83)	55(45.83)
Three causative agent	12 (10.00)	6 (05.00)	18(15.00)
More than three	5(04.16)	4 (03.33)	9(07.50)
Total	65(54.16)	55(45.83)	120(100)

*p < 0.01.

Bacterial agents and antibiotics

Table 16 shows that in each isolates group the frequency of susceptibility to antibiotic. *P. aeruginosa* was statistically significantly higher resistance than other bacterial isolates (10.19%) followed by *S. aureus* (8.73%), *Klebsiella* (7.76%), *B. catarrhalis*, *Proteus spp*, *H. influenza* (6.97%), *Streptococcal* spp. (4.85%), *Corynebacterium* (0.9%) and *Bacillus* spp. (0.9%) p < 0.01.

DISCUSSION

Chronic suppurative otitis media was develops from a chronic bacterial infection. However, the bacteria that caused the initial episode of acute otitis media with perforation are usually not those isolated from the chronic discharge when there is a chronic infection in the middle ear and mastoid infection usually polymicrobial and secondary in nature, derived from the external auditory canal or commensal flora of nasopharynx (Bluestone and KLien, 2001). The infection causes a build up of fluid in the middle ear. The pressure exerted by this fluid can build up to the point where the ear drum perforated. The fluid build up and ear drum perforation inhibit the transmission or conduction of sound through the ear (Howard, 2007).

Our result goes with the study which was done by Guo (1994); Engel (1998), that show most patients with CSOM infected by more than one pathogenic bacteria leading to hearing loss, about 40 patients, (33.4%) of patients with CSOM suffered from bilateral hearing loss, while (80 patients, 66.6) of patients with CSOM have unilateral hearing. Guo et al. (1994) studied found the effect of endotoxic damage to the strial vascularis and concluded that lipopolysaccharide induced by strial ototoxicity produced ion imbalance, causing changes in endolymph composition and energy failure in the middle and inner ears organ explaining the pathogenesis of hearing loss in CSOM.

Engel et al. (1998) studied the passage of streptolysin-O and albumin through the round window membrane and proposed that the passage of macromolecule, such as protease, from a purulent middle ear effusion may be facilitated by pore forming toxins, resulting in middle and inner ear organs damage and hearing loss. Karma et al. (1978) have used gram stain not only to confirm the presence of cultured bacteria but to detected and identify

Table 16. Relationship between causative agents and antibiotics (resistance patterns).

Bacterial isolate Type	No. of Isolates	Susceptibility to drugs								Total	
		(1) drug		(2) drugs		(3) drugs		More than (3) drugs			
		R	S	R	S	R	S	R	S	R	S
Ps.aeruginosa	40	1	2	**2**	3	**8**	6	**1**0	8	21(10.19)	19(9.22)
Staph. aureus	35	2	3	2	4	**6**	**5**	**8**	5	18(8.73)	17(8.25)
Klebsiela	30	1	**3**	**2**	**3**	5	4	**8**	4	16(7.76)	14(6.79)
Br.catarrhalis	20	**2**	1	**3**	**1**	4	2	**5**	**2**	14(6.97)	6(2.91)
Proteus spp	20	2	1	**3**	1	4	2	5	2	14(6.97)	6(2.91)
H.influenza	20	2	1	3	1	4	2	5	2	14(6.97)	6(2.91)
Strept..spp	15	2	1	2	1	3	2	3	1	10(4.85)	5(2.42)
E.coli spp	10	2	1	1	2	1	1	2	0	6(2.91)	4(1.94)
Corynebacterium spp	8	**1**	2	0	**1**	**1**	**1**	0	**2**	2(0.9)	6(2.91)
Bacillus spp	8	**1**	2	0	2	1	**1**	0	1	2(0.9)	6(2.91)

them as well, gram stain smear were obtain from 108 ear swab; in 98 (91%) of them bacteria were found, seven of the 108 ear swab (6%) were devoid of bacteria both in culture and in the gram stain. Papastavros et al. (1986) indicated that this practice considerable error, because non viable bacteria can be as equally incriminated as the main pathogens present, furthermore, if the patients is under antimicrobial treatment. In our study, we found that, the different type of bacterial flora in the external canal were founded, *S. epidermidis* is the most common (20 isolates, 40%), followed by *Corynebacterium* species about (15 isolates, 30%), while other type have various percentages of isolation. Our result is agreed with (Pelton et al., 1980; Brook et al., 1996), while, it is against the result is of (Saunders et al., 2009). Pelton et al., (1980); Brook et al. (1981) showed that the predominant microflora were *S. epidermidis, diphtheroid, and S. aureus.*

In the present study, the number of *P. aeruginosa* isolates was (40 isolates, 19.41%). our result agreement with studies done by (Aslam et al., 2004); (Verhoeff, 2006) that *Pseudomonas* most common agents in patients with CSOM, and not approved with (Saunders et al., 2009) found *S. epidermidis* most common causative agents. Aslam et al. (2004) showed that *P. aeruginosa* is the most common isolates from infected mastoid cavity and chronic otitis media and the most common aerobic bacteria isolated from chronic suppurative otitis media. Verhoeff et al. (2006), stated that *P. aeruginosa* was the most prevalent bacteriological agent in chronic otitis media, followed by *S. aureus*. Saunders et al. (2009) stated that *S. epidermidis* species was the most prevalence bacteriological agent in chronic otitis media.

In this study we found that *S. aureus* (35 isolates, 16.99%) followed *P. aeruginosa* in their incidence, our result agree with study done by (Aslam et al., 2006), while against the study done by (Saunders et al., 2009). Saunders et al. (2009) found that *S. epidermidis* (6%) was the most common bacteria isolated from patients with suppurative otitis media, followed by methicillin resistant *S. aureus* (3%) and *P. aeruginosa* (1%). In our study, we found that *Klebsiella* species isolated from patients suffering from chronic suppurative otitis media was (30 isolates, 14.56%). our patients infected by Enterobacteriaecea such as *Klebsiella* species, most of them are among children and infants group, because the Eustachian tube in children are shorter and wider than adult. Bluestone et al. (1974) showed that young children have shorter, straighter and more compliant Eustachian tube than adult; this permits a reflex from nasopharynx to the middle ear with the consequence of bacterial contamination. Brook and Yocum (1989) found that Klebsiella species (6.2%) isolate from patients with CSOM, while Ostfeld and Rubinstein (1980) stated that (20%) of Klebsiella species presented in young infant with acute otitis media, but rarely appear in the middle ear effusion of older children with otitis media.

In our work, we found that *B. catarrhalis* was (20 isolates, 9.7%). Faden (1994) found that, *Moraxella catarrhalis or B. catarrhalis* were common organisms, *Diplococcus* are considered as part of the normal flora of human upper respiratory tract, classified as causative agents to middle ear infection; it had constituted approximately 10% of all isolates. Hanan (2000) showed that *M. catarrhalis* secreted lactamases (cephalosporinases) may protect these bacteria and other type from antimicrobial agents to which the second target pathogen ordinarily might be susceptible, which can be differentiated from the other *Neisseriae* spp by its lack of carbohydrate fermentation and by its DNase production. In our study, we found *proteus* species isolated (20 isolates 9.7%). Iseh and Adegbite (2004) found that proteus species (12.8) isolated from 41 patients with acute suppurative otitis media. Vaishnav and changani (1981), found that *Proteus* species with highest incidence (44%) of isolates from 100 cases with CSOM.

In our result, we found that *H. influenzae* was (20 isolates 9.7%), while *S. pneumonia* (15 isolates, 7.28%). Yamanaka et al. (2008) showed that *H. influenzae* and *Streptococcus pneumonia* are the most prevalent organisms responsible for acute otitis media. However, most studies from different parts of Africa suggest various bacterial pathogens as accusatives agents. Hence, *S. aureus* and *S. pyogenes* appear to be the most dominant causative organisms among Africans Hussain et al. (1991). Bluestone and Klein (2001) found that *S. pneumonia* and *H. influenzae* are the most common bacteria species causing middle ear infection in acute otitis media. Some European studies found *H. influenzae* to be the most common organism followed by *S. pneumonia* and *B. catarrhalis* (Gray and Canter, 1997). In our result, we found that, the frequency of *E. coli* was (10 isolates, 4.85%) isolated from patients with chronic suppurative otitis media. *E. coli* belong to enterobacteriaceae, pathogenic causative agent in acute suppurative otitis media in children and infant (Bluestone, 1990). Iseh (2004) found *E. coli* in patients with acute suppurative otitis media second causative agent, Ear swab was cultured in only 41 patients (36%). *S. aureus* (46.2%) was the commonest bacteria cultured followed by *E. coli* (23.1%). In our result, we found that *Corynebacterium* and *Bacillus* species were (8 isolates 3.88%), for each presents in external canal and middle ear cleft as apportuenstic normal flora in individual without otological problems. (Brook and Schwartz, 1981) showed that *Corynebacterium* species was predominant in external canal and middle ear cleft, while (Kurono et al., 1988) isolated 12 different bacterial species, in which *Bacillus subtilis* from middle ear cleft and external canal.

The organisms that cause otitis media become more resistant to antibiotic, for example, according to recent studies, between (30 - 60%) of S. pneumoniae bacteria is now partially resistant to the antibiotic such as penicillin and amoxicillin. Antibiotic lose their effectiveness in children who have been continuous treated with them in a short period of time. Ciprofloxacin and Augmentine (amoxicillin-clavulante) is more abundant bactericidal agent for many gram positive and gram negative bacteria in AOM, CSOM. Gehaanno (1997); Winter (1994) (90 - 95%) of cases of Acute otitis media (AOM) with otorrhoea occur in children aged (1 - 12) years, and typically (2 - 6) episodes of AOM. Ciprofloxacin is an effective and safe therapy for AOM and chronic suppurative otitis media (CSOM) (Force et al., 1995). The efficacy and safety of a combination of topical dexamethasone 0.1% and ciprofloxacin 0.3% in children with (AOM), otorrhoea resolved more rapidly with combination preparation than with ciprofloxacin alone and produce significantly greater clinical responses early after completion of seven days course of treatment (Zipfel, 1999).

In our study we noted that, Ciprofloxacin, (Amoxicillin+ clavulanic acid), Augmentin, Gentamicin were abroad spectrum antibiotic (70 - 80%) sensitive to different species of gram negative and gram positive bacteria in CSOM. Topical treatment is better than systemic therapy; this is probably because a higher local concentration of antibiotic is achieved. Macfadyen et al. (2006) the antibiotic should have activity against gram negative bacteria, especially *Pseudomonas*, and gram positive bacteria, especially *S. aureus*. The amino glycosides and the fluoroquinolones, both of them meet these criteria but the former may be ototoxic, failures of the antibiotic are usually due to failure to penetration of the debris rather than bacterial resistance. Marais et al. (1998). Amino-glycosides are contraindicated; there is evidence that they may cause hearing loss (Bance et al., 2005).

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