MICROBIAL STUDY ON CHRONIC SUPPURATIVE OTITIS MEDIA AND THEIR SENSITIVITY TO ANTIBIOTICS

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ABSTRACT

One hundred and thirty three ear swabs were collected from outpatients attending at Al- Batnan Medical Center/Tobruk,Libya during the period from January 2005 to September 2005, their ages ranged between 16-65 years and were from both sexes. Eleven different pathogens were isolated from patients and most of swabs (79.14%). indicated growth of single organism. Theresults of this study revealed that the Gram negative bacteria were more common (70.71%) .Pseudomonas aeruginosa was the most frequent pathogen with 51.42 %, followed by Staphylococcus aureus with 18.57%. The study also revealed that 20.86% of positive swabs showed the growth of mixed infection and Pseudomonas aeruginosa was the most frequent bacteria present in these swabs combined with other microorganism.

The antibiotic sensitivity pattern showed that most of Pseudomonas aeruginosa isolates were resistance to many antibiotic but were sensitive to Cefatoxim, Gentamycin and Tetracyclin with (97.3 %,95.4%,72.3%) respectively.

Key words: *Otitis Media, Antibiotics Sensitivity pattern, Pseudomonas aeruginosa*

INTRODUCTION

Chronic suppurative otitis media {CSOM} is an inflammation of part or all of the mucoperiosteal lining of the middle-ear which is slow and insidious in its course, tend to be persistent, and is very often destructive, sometimes with irreversible sequela (Mustafa *et al.,* 1994). Middle ear infection often develops as a complication of the common cold and include persistent and severe earache; temporary hearing loss: pressure in middle ear; bulging of the eardrum; nausea, vomiting, diarrhea and fever in young children; may lead to rupture of the eardrum, bloody discharge and then pus in the ear. Severe complication, including bone infection, permanent hearing loss, and meningitis (Gwendolyn *et al.,* 2004)

The bacterial flora found in chronic otitis media was studied on several occasions (Hassan, 1990). The microbiology of (CSOM) had been shown to be polymicrobial consisting of aerobic bacteria (Karma *et al.,* 1985). The bacteria isolated in their studies were mostly aerobic predominantly gram negative bacilli and staphylococci; these organisms are mostly likely secondary invaders that gain entrance to the middle ear and mastoid from the external auditory canal during the episode of acute otitis media and otorrhea (Hassan, 1990).

This study was designed to show the aetiogensis of chronic suppurative otitis media for consecutive outpatients in specific region, and to examine the antibiotic susceptibility patterns of bacteria isolated from these patients.

MATERIALS AND METHODS

The study was carried out on 133 consecutive out patients attending at Al-Batnan Medical Center (a big authorized center in eastern region of Libya) during the period between January 2005 to September, 2005, and were diagnosed with chronic suppoative otitis media. All specimens had been examined at the central laboratory and the following culture media were used for swab culturing:

Blood agar plates, Chocolate agar plates, and MacConkey's agar plates were used for detection and isolation of bacterial pathogens. The inoculated culture media were incubated aerobically at 37^{o}C for 24 hrs.

Sabouraud's agar was used to detect yeast and fungi; the inoculated plates were maintained at room temperature (26-28^{o}C) for 24 hrs.

Bacterial isolates were identified on the basis of their morphological and biochemical characteristics using conventional biochemical tests (Finegeol and Baron, 1990). The bacterial strains were identified according to the diagnostic article adopted by Finegeol and Baron (1994).

The susceptibility of bacterial isolates to a number of antibiotics was determined by using the disc diffusion method and interpreted according to (Barry, 1976; Daly *et al.,* 1999).

RESULTS

During the period from January 2005 - September 2005, 133 ear swabs were obtained from outpatients with ages between 16-65 years from both sexes. Out of the 133 swabs only 115 gave positive growth and the remaining18 exhibited negative growth.

Examination of the 115 positive specimens revealed the presence of 91 specimens showing growth of a single organism, 23 specimens showing growth of two organisms and only one specimen showed growth of more than two organisms (Table 1).

Table 2 shows the different types of microorganisms isolated from patients with CSOM and their frequency. It reveals that gram negative bacteria isolates gave the highest percentage (70.7%) of the total isolates, gram positive bacteria isolates represented (22.8%), *Aspergillus* species (3.5%) and Candida species (2.8%).

Bacterial and fungal isolates of different species and their frequency among patients with CSOM are presented in (table 3), eleven various microorganisms appeared as a causative agent of CSOM in this study and 140 isolates were obtained from the total 115 ear swabs.

The most common microorganism that appeared with high percentage in both ,single infection and mixed infection, was *Pseudomonas aeruginosa* which had the frequency of 51.42%, followed by *Staphylococcus aureus* with 18.57%, *Proteus* species with (8.57%), and *Klebsiella* species with 7.85%. These results indicated that gram negative bacteria

were more common than gram-positive bacteria in causing CSOM. Yeast and fungus were also isolated, *Candida* and *Aspergillus* species detected in 2.8% and 3.5% respectively.

Table 4 reveals that gram negative bacteria were more common than gram positive bacteria in both single and mixed infections and appeared with a frequency of 75.6%, compared with 24.4% for gram positive bacteria.

Table 5 shows the coexistence of the most predominant bacteria *Pseudomonas aeruginosa*, *Proteus* species and *Staphylococcus aureus* with each other and with other pathogens in the mixed infection. *Pseudomonas aeruginosa*, coexisted more with *Proteus* species, than with *Staphylococcus aureus*. Twelve patterns of mixed infection were obtained.

The invitro susceptibility of the isolated microorganisms to 14 different antibiotics is presented in Figurs (1 - 9). All the isolated strains were significantly resistant to amoxicillin except *Staphylococcus aureus* and *Streptococcus pyogens* which were sensitive to this antibiotic with 100% each. All of the gram negative isolates were resistant to bacterim except E.coli and to Carpenicillin except *Klebsiella* and *Enterobacter* and majority of the isolates showed resistant to Ampicillin, Penicillin and Erythromycin. However, all isolated strains were sensitive to Chloroamphenicol except *Pseudomonas aeruginosa* and *Proteus* which were resistant to it with 66.6% and 58.3% respectively. 72 isolates of *Pseudomonas aeruginosa* were evaluated for sensitivity toward 10 antibiotics, the results showed that *Pseudmonas. aeruginosa* isolates were sensitive to Cefatoxim, Gentamycine and Tetracycline (97.3%, 95.4%, 72.3%) respectively, and it was resistant to the rest of antibiotics at different level.The study also indicated that *Pseudomonas aeruginosa* isolates showed multi resistance pattern Table 6.

DISCUSSION

Chronic Suppurative Otitis, which arises from a complex series of inflammatory events in the middle ear, develops from a chronic bacterial infection (Teel, 1983). Infection is usually polymicrobial and Secondary in nature, derived from the external auditory canal or commensal flora of nasopharynx (Gwendolyn *et al.,* 2004, Papastavros *et al.,* 1986). The result of this study showed that 13.5% of the specimens gave negative growth, this may refers that some patients were taking antibiotics and that suppresses the growth of organisms, also bacteria in their L-forms may or may not grow in culture and may or may not express pathogenicity at a particular time. Also the failure of employing proper anaerobic techniques may account for the relatively high rate of negative culture of middle ear effusion. This result is in agreement with that of (Mustafa *et al.,* 1994).

The aerobic organisms recovered in this study are similar to those found by others (Jero and Karma, 1997) where *Pseudomonas* and *Staphylococcus aureus* were the most commonly isolated bacteria followed by Proteus spp. This finding is in agreement with many others. (Mustafa *et al.,* 1994: Hirsch, 1992). However, Mustafa and his co-workers found the same frequency except for *Klebsiella pneumoniae,* and *Proteus*. spp which were in a higher percentages (39%, 18%) respectively.

This finding (for *Pseudomonas aeruginosa*) is due to many reasons, firstly *Pseudomonas aeroginosa* are the most secondary invaders when the resistance of middle ear is lowered (Lindman, 1988). Secondly the high incidence of *Pseudomonas aeruginosa* indicates more general antibiotics resistance than in the case of gram-positive bacteria (Chandler1968). And thirdly, the presence of exuberant granulation tissue reaction which formed in otitis infections makes difficult to eradicate such organisms like *Pseudomonas aeruginosa* (Zakouk .and Mahgoub, 1980). *Staphylococcus aureus* accepted as the primary invader of acute otitis media, has been frequently observed in chronic suppurative otitis media (Hassan, 1990) This high frequency may be due to firstly *Staphylococcus aureus* is inherent a nature of developing resistance strains (Lindman, 1988; Bluestone, 1983; Ashoor *et al.,* 1996). However, Kalantar *et al.,* 2006 found that *Staphylococci spp.,* coagulase negative *Staphylococcus aureuse* and *Streptococcus pneumonia* were the prominent isolates.

The polymicrobial aerobic or aerobic / anaerobic nature of CSOM has been established by several studies (Mustafa *et al.,* 1994; Desai *et al.,* 1985; Karma et al., 1985; Vaishnav and Changani, 1981). The result of this study showed that out of 133 specimens, 91 gave single organism and 23 specimens gave two organisms, and only 1 specimen gave three organisms. Our result was in agreements with those results reported by (Mustafa *et al.,* 1994; Jero and Karma, 1997; Karma *et al.,* Desai *et al.,* 1985). However Vanshave and Changani (1986) who studied 100 cases with CSOM found that all infections were nonbacterial. The objective of treatment with antibiotics is to cure the infection and eradicate the aerobic as well as the anaerobic organisms. Because the most frequently cultured bacteria were gram negative, antimicrobial agents that are effective against these organisms should be selected (Chandler, 1968). Gentamycin is bactericidal to most gram negative and gram positive bacteria (Mansour *et al,* 2008, 2009). In our study Pseudomonas aeruginoas, *Staphylococcus aureus* and *E.coli* showed sensitivity to Gentamycin but Proteus spp Were resistant to it with 66.6%. The study also revealed that Chloramphenicol was effective against *Streptococcus spp, Klebsiella spp.* and *E.coli* .

CONCLUSION

The positive ear swabs showed the presence of single and mixed infections and gram negative bacteria were more common than gram positive bacteria. The most frequent pathogen was *Pseudomonas aeruginosa* and its susceptibility test to antibiotics showed that this bacteria was resistant to most antibiotics.

Table 1 : Number of positive swabs according to their growth in culture.

Type of growth		No-swabs	%
Mono growth		91	79.14
Mixed growth*	Di-	23	20
	Poly	1	0.86
Total		115	100

* Di- =two organisms, Poly=more than two organisms.

Table 2 : The frequency of groups of microorganisms isolated from ear swabs.

Microorganism	Single infection	Mixed infection	No. of isolates	%
Gram-positive bacteria	18	14	32	22.86
Gram-negative bacteria	68	31	99	70.71
Candida spp.	2	2	4	2.86
Aspergillus spp.	3	2	5	3.57
	91	49	140	100

Table 3 : Microorganism isolated and their frequency from patient with CSOM.

Microorganism isolates	Single infection	Di-infection	Poly infection	Total	%
Staphylococcus aureus	16	9	1	26	18.57
Streptococcus pyogenes	2	3	-	5	3.57
Streptococcus viridance		1	-	1	0.71
Pseudomonas aeruginosa	55	16	1	72	51.42
Proteus spp	4	7	1	12	8.57
Klebsiella. spp	6	5	-	11	7.85
E.coli	1	1	-	2	1.42
Enterobacter spp	1	-	-	1	0.71
Citrobacter spp	1	-	-	1	0.71
Candida spp	2	2	-	4	2.85
Aspergillus spp	3	2	-	5	3.57
Total	91	46	3	140	100

Table 4 : The frequency of gram positive and gram negative isolates in Single and mixed infections.

Gram stain	Single infection	Mixed infection	Total / %
Gram-positive bacteria	18	14	32 / 24.4
Gram-negative bacteria	68	31	99 / 75 .6
Total	86	45	131 / 100

Table 5 : Patterns and frequency distribution of mixed microorganisms with each other.

Microorganisms	Freq./ No. of isolates
Pseudomonas aeruginosa + Proteus spp	7 14
Pseudomonas aeruginosa + Staphylococcus aureus	5 10
Pseudomonas aeruginosa + E.coli	1 2
Pseudomonas aeruginosa + Klebsiella spp	1 2
Pseudomonas aeruginosa + Candida spp	1 2
Pseudomonas aeruginosa + Staphylococcus aureus + Proteus	1 3
Pseudomonas aeruginosa + Aspergillus spp	1 2
Staphylococcus aureus + Klebsiella spp	3 6
Streptococcus pyogenes + Klebsiella spp	1 2
Streptococcus pyogenes + Streptococcus viridance	1 2
Streptococcus pyogenes + Aspergillus spp	1 2
Staphylococcus aureus + Candida spp	1 2

Table 6 : The antibiotic resistance patterns of *Pseudomonas aeruginosa* strains occurring 3 times or more.

Pattern of multi resistant	No of isolates
Pen	5
Pen+ Amox	3
Pen + Amp + Amox	23
Pen + Amp + chlor	6
Pen + Amp + Amox + chlor	10
Pen + Amp + Amox + tetra	3
Pen + Amp + chlor + tetra	3
Pen + Amp + chlor + carb	3
Amp + tetra + chlor + carb	3

Pen : Penicillin **Amox:** Amoxycillin **Amp :** Ampicillin
Chlor : Chlorramphenicol **Tetra :** Tetracycline **Carb :** Carbenicilln

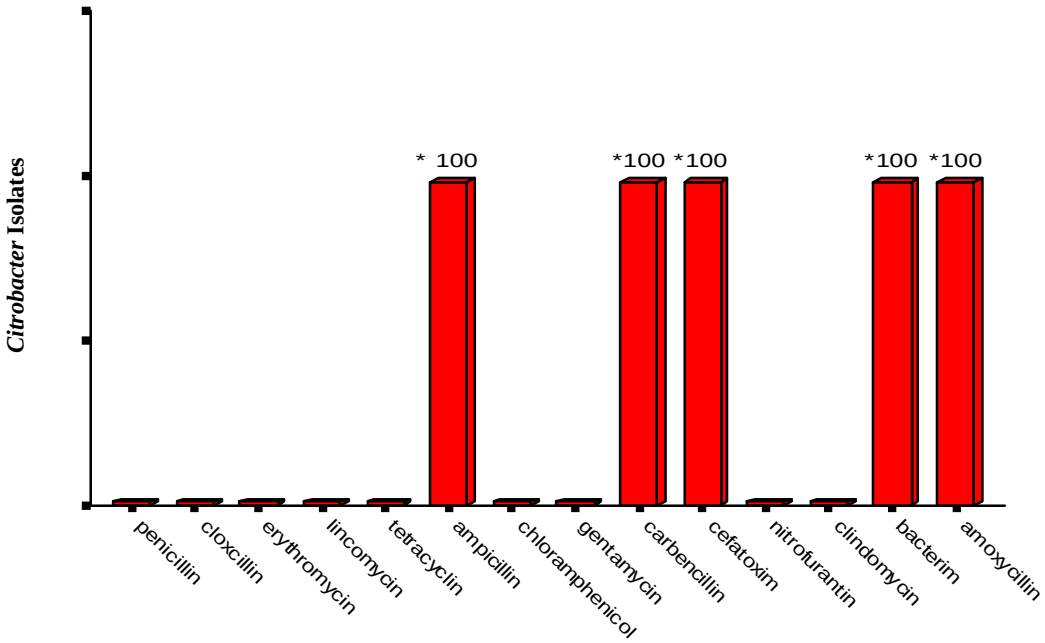

Figure 1 : The percentage resistance of Citrobacter to antibiotics CHI Square (with df =13) =0.00000404 * significant at P=0.005.

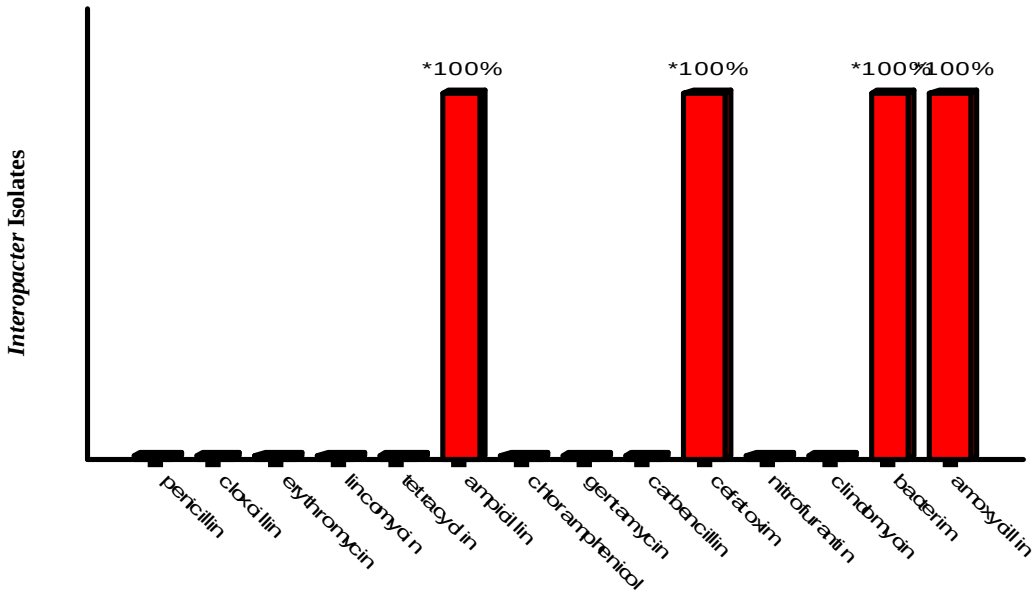

Figure 2 : The percentage resistance of *Enterobacter* to antibiotics CHI Square (with df =13)=0.0000121 * Significant at P=0.005.

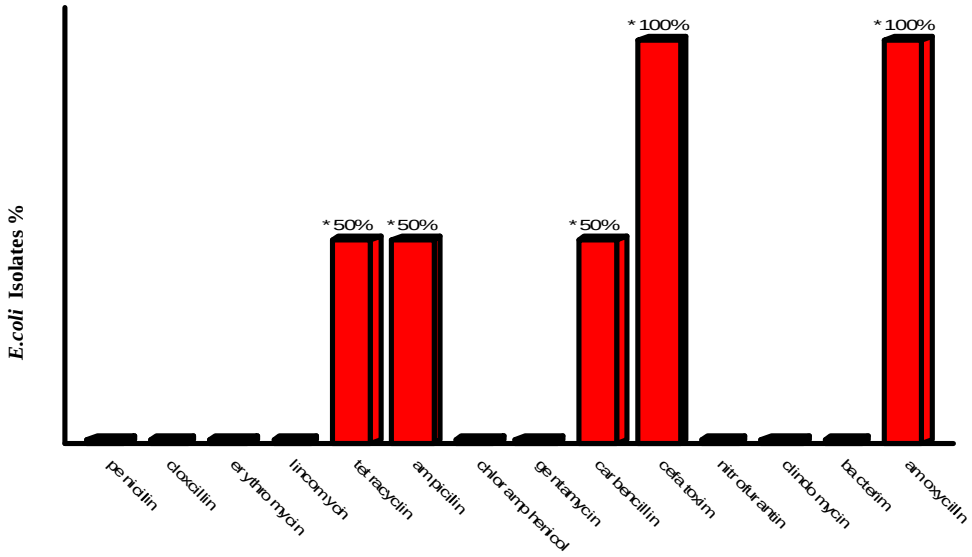

Figure 3 : The percentage resistance *of E.coli* to antibiotics CHI Square (with df = 13) = 0.000342 * Significant at P=0.005.

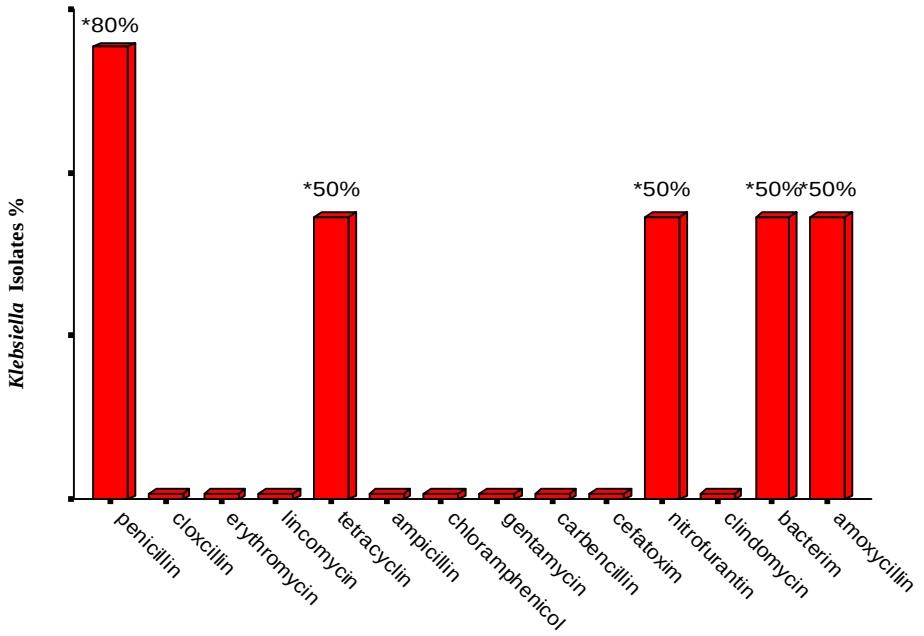

Figure 4 : The percentage resistance of *Klebsiella* to antibiotics CHI Square (with df =13)=0.0081 * Significant at P=0.005.

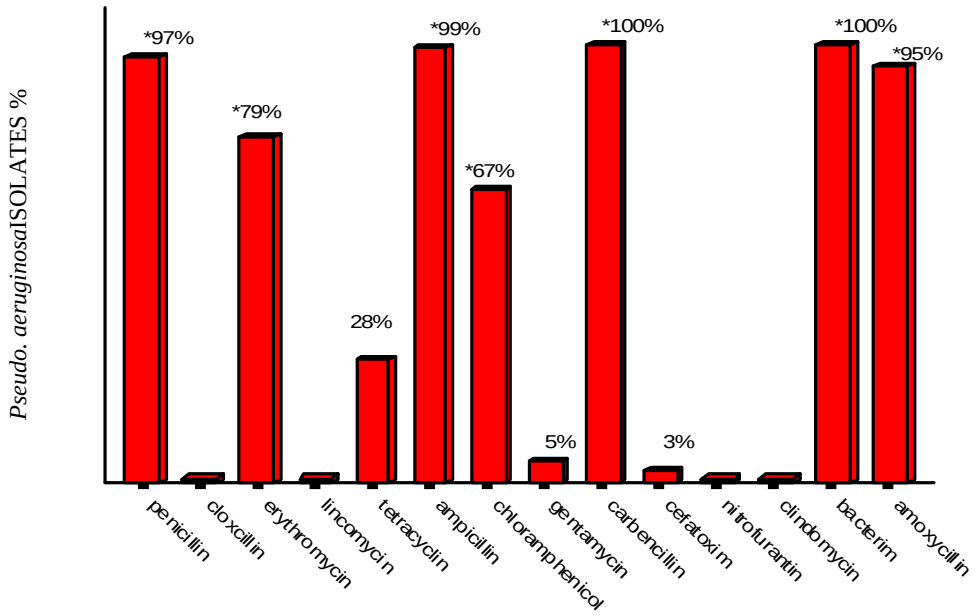

Figure 5 : The percentage resistance of *Pseudomonas aeruginosa* to antibiotics CHI Square(with df =13)=0.000017 * Significant at P=0.005

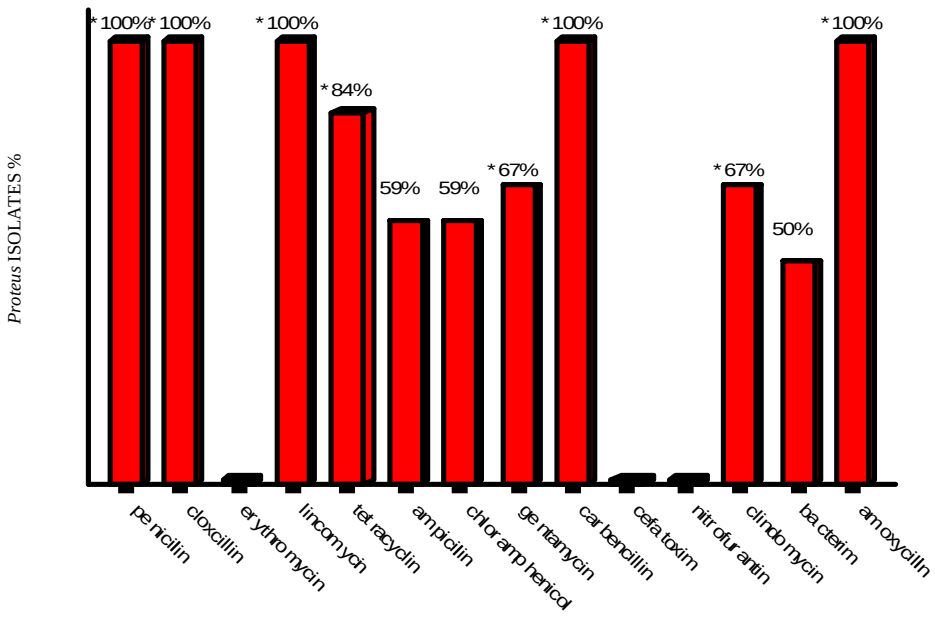

Figure 6 : The percentage resistance of *Proteus* isolates to antibiotics CHI Square (with df =13)=0.00026 * Significant at P=0.005

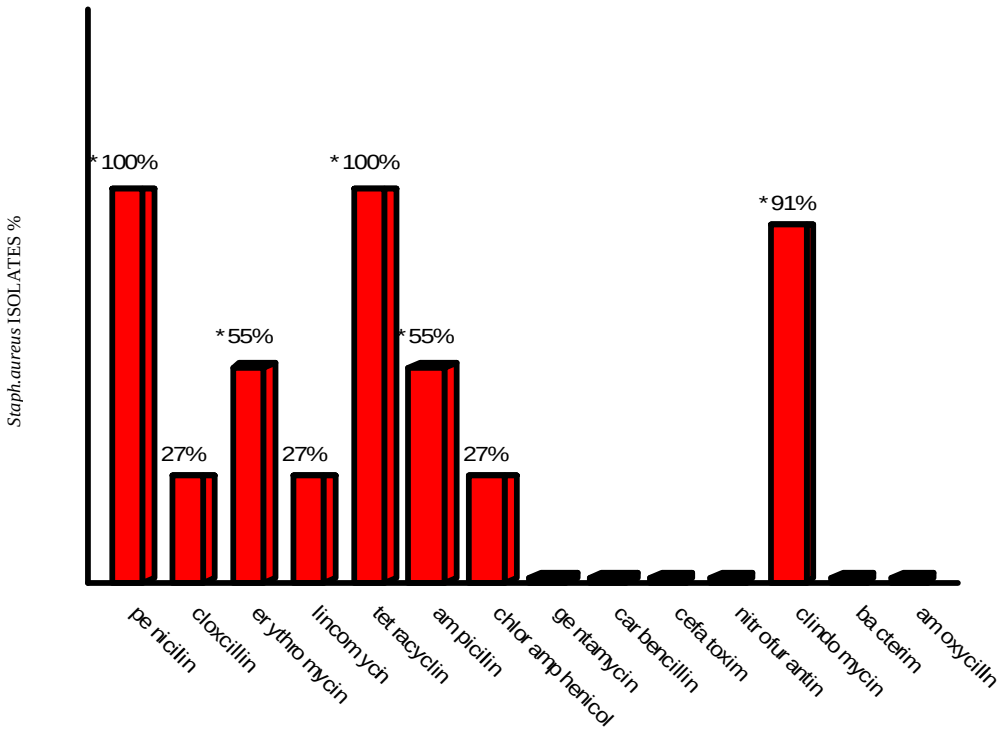

Figure 7 : The percentage resistance of *Staphylococcus aureus* to antibiotics CHI Square (with df =13)=0.0002 * Significant at P=0.005.

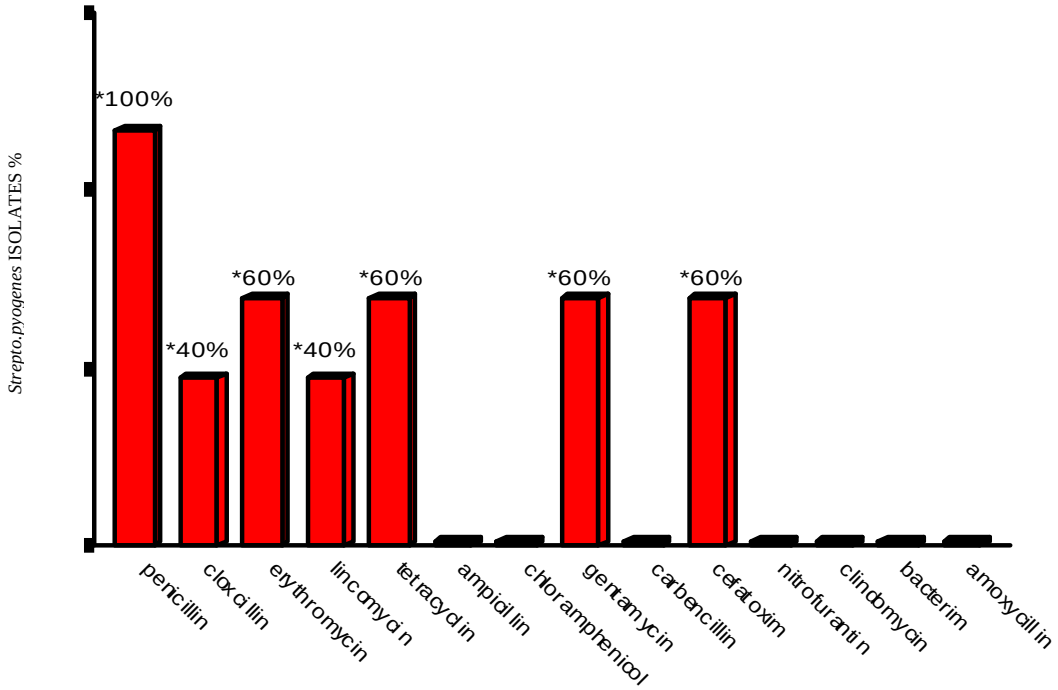

Figure 8: The percentage resistance of *Streptococcus pyogenes* to antibiotics CHI Square(withdf=13)=0.001 * Significant 0.005.

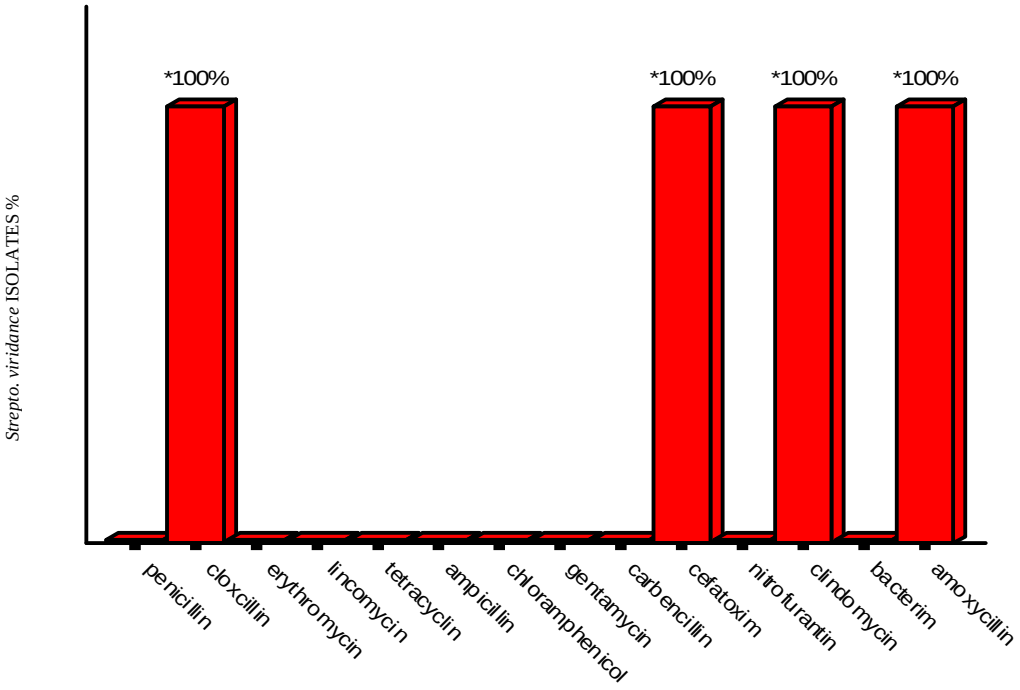

Figure 9 : The percentage resistance of *Streptococcus viridance* to antibiotics CHI Square (with df =13)=0.0001* Significant at P=0.005.

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Received on 23 / 2 / 2010

الملخص العربى

دراسة ميكروبية على إلتهابات الأذن الوسطى وحساسيتها للمضادات الحيوية

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أخذت ١٣٣ مسـحـة أذن من المرضى الخـارجـيين المراجـعين لمركـز البطنان الطبى / طبـرق من الفـتـرة يناير ٢٠٠٥ - أيلول ٢٠٠٥ تراوحت أعـمارهم من ١٦ - ٦٥ ومن الجنسين حيث تم عزل ١١ كائن مجـهـــرى ممـرض، وقـد بينت نتـائج زرع المسـحـات أن النمو المفرد كـان أكثر سيادة حيث بلغـت نسـبته 79.44% وكانت البكتريـا السـالبة لصبغـة جـرام هـى الأكـــثر شـــيوعاً حيث بلـــغـــت (70.71%)، كانت بكتريا *Pseudomonas aerugenosa* الأكثر تردداً (51.42%) تلتها بكتريا *Staphylococcus aureus* حيث بلغت 18.57% كذلك بينت هذه الدراسة أن 20% من المسحات الموجبة الـزرع أظهـرت النمو المختلط وكانت بكتريا *Pseudomonas aeruginosa* هى الأكـثر تـرددا فى هـذه المسـحات مشـتـركة مع غـيرها من العـزلات، كذلك تمـت دراســـة مـقـاومـــة العـزلات البكتيـرية لأربـعـــة عشر مضـاداً حيوياً وبينت الدراســة أن بكتريا *Pseudomonas aeruginosa* كانت مقاومـة لمعظم المضادات الحيوية لكنها كانت حساسة لكل من *Tetracyclin, Gentamycin, Cefatoxim* وبنسب 72.3, 95.4, 97.3 على التوالى.

JOESE 5

MICROBIAL STUDY ON CHRONIC SUPPURATIVE OTITIS MEDIA AND THEIR SENSITIVITY TO ANTIBIOTICS

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Reprint

from

Journal of Environmental Sciences, 2010; Vol. 39, No. 4

http://www.mans.edu.eg/facscim/english/MJI **ISSN 1110-192X**