Effect of some antibiotics on aerobic pathogenic bacteria causing otitis media and urinary tract infection in Al-Manathera city in Iraq: A comparative in vitro study

Ahmed Abduljabbar Jaloob* and Fadaa Abdul-Ameer Gafil*

الخلاصة

أجريت الدراسة الحالية للفترة بين نيسان 2011 و ايار 2011في مستشفى المذ اذرة العام في مدينة المذ اذرة في العراق.وفق الدراسة للمقارنة بين فعالية البنسلينالجنتامايسين السبروفلوكساسين الريفادينالترايميثوبريمالسيفوتاكسيم ،ايتروفيورانتوين على البكتريا الهوائية المسببة لالتهاب الأذن الوسطى والتهاب المجاري البولية أوضحت نتائجالدراسة إن من بين 130 عينة مأخوذة من المرضى غير الراقدين (65مسحة أذن من المرضى المصابين بالتهاب الأذن الوسطى و 65عينة إدرارالمرضى المصابين بالتهاب المجاري البولية) كانت هناك 60 عينة بكتيرية معزولة من المرضى المصابين بالتهاب الأذن الوسطى (36 ذكور 60% و24إناث 40%)الإضافة إلىعينات لم يظهر بها نمو بكتيري كانت بكتريا المسبب البكتيري الرئيسي التهاب الإذن الوسطى (25عزلة *Pseudomonas aeruginosa* 41.666%). ما من بعينة 65إدرار من المرضى المصابين بالتهاب المجاري البولية كانت هناك 65عزلة بكتيرية (24ذكور 36.923% و 41إناث 63.077%)كانت بكتريا المسبب الرئيسي لالتهاب المجاري البولية (27عزلة 41.53%) .كان *K. pneumoniae* المضاد الحياتي *Nitrofurantoin* الأكثر تجاه كل العزلات البكتيرية المعزولة من المرضى المصابين بالتهاب الإذن الوسطى والتهاب المجاري البولية في حين كانت المضادات الحياتية Ampicillin و Pencillin الأقل فعالية تجاه معظم العزلات البكتيرية المعزولة.

Abstract

Background: Otitis Media (OM) is an inflammation of the middle ear drum and the inner ear, including a duct known as the eustachian tube. Otitis media is very common in children. Over 60 percent of the cases of otitis media are caused by bacteria. Urinary tract infection(UTI) refer to the presence of microbial pathogens within the urinary tract. A urinary tract infection is a condition where one or more structures in the urinary tract becomes infected after bacteria overcome its strong natural defenses. In spite of these defenses, urinary tract infections are the most common of all infections and can occur at any time in the life of an individual. Almost 95% of cases of UTIs are caused by bacteria that typically multiply at the opening of the urethra and travel up to the bladder.

*Kufa University College of Science -Department of Biology Kufa university-College of Medicine

Escherichia coli, K. pneumoniae and *Proteus mirabilis* are the bacteria most commonly cause UTI, followed by other gram-negative and gram-positive bacteria. A number of studies have reported on the prevalence of otitis media and urinary tract infection. However, majority of these studies have focused on clinical diagnosis with little or no report on the bacteriological etiology of otitis media and urinary tract infection so this study aimed to isolate, characterize and identify the bacteriological etiologic agents of otitis media and urinary tract and to select the best antibiotic for the treatment of such infections.

Objective: The study aimed to identify the aerobic pathogenic bacteria cause otitis media and urinary tract infection and to compare between pencillins, gentamicin, ciprofloxacin, refampicin, cefotaxim, trimethoprime, and nitrofurantoin effects on this bacteria.

Materials and methods: The study was carried out on 130 out patients (65 patients 36 male and 24 female infected with otitis media and 65 patients 24 male and 41 female infected with urinary tract infection). The specimens were collected with sterile swab sticks (for patient infected with O.M) and collected with sterile container (for patient infected with UTI) which were properly labeled for each patient. Specimens were inoculated onto Blood, Chocolate , MacConkey and Manitol salt agar plates. All plates were incubated immediately for 24 hours aerobically with the exception of Chocolate agar that was incubated in a candle jar. Emergent colonies were identified according to standard bacteriological methods

Results: From the 130 specimens from out patients (65 ear swab from patients infected with otitis media and 65 urine specimens from patients infected with urinary tract infection) , there were 60 bacterial isolates from patients infected with otitis media (36 male 60% and 24 female 40%) with 5 specimens no growth. *Pseudomonas aeruginosa* was the most prevalent bacteriological etiologic agent of otitis media (25 isolates 41.666%).While from the 65 urine specimens from patients infected with urinary tract infection , there were 65 bacterial isolates(24 male 36.923% and 41 female 63.077%) *K. pneumoniae* was the most prevalent etiologic agent of urinary tract infection (27 isolates 41.53%).

Conclusions: Nitrofurantoin antibiotic was active against all of the bacterial isolates from patients infected with otitis media and from patients infected with urinary tract infection. While Ampicillin and Pencillin were not active against most of bacterial isolates.

Recommendations: Otitis media and UTI characterized by the presence of many drug resistant cases due to different pathogenic bacteria involved in their etiology so further clinical studies are required to select the most appropriate and effective antibiotic in the treatment of such cases.

Introduction

Otitis Media (OM) is highly prevalent worldwide (Favour *et al*,2011). Its inflammation of the middle ear drum and the inner ear, including a duct known as the eustachian tube (Arroll,2005). Otitis media is very common in children(Li et al,2001). Children below the age of fifteen years are much more susceptible to otitis media (Weiner and Collison,2003).Over 60 percent of the cases of otitis media are caused by bacteria, Occasionally, otitis media may be caused by fungi, viruses, *Mycoplasma pneumoniae* and *Chlamydia* (Favour *et al*,2011).

Among the most common infectious diseases, urinary tract infections (UTIs) are encountered diseases by clinicians in developing countries with an estimated annual global incidence of at least 250 million, (UTIs) refer to the presence of microbial pathogens within the urinary tract. (Beyene and Tsegaye , 2011). A urinary tract infection (UTI) is a condition where one or more structures in the urinary tract becomes infected after bacteria overcome its strong natural defenses. In spite of these defenses, urinary tract infections are the most common of all infections and can occur at any time in the life of an individual. Almost 95% of cases of UTIs are caused by bacteria that typically multiply at the opening of the urethra and travel up to the bladder (Abu Setta and Doma ,2008).Urinary tract infections occur commonly both in outpatients and inpatients, *Escherichia coli, K. pneumoniae* and *Proteus mirabilis* are the bacteria most commonly isolated from infected urine specimens, followed by other gram-negative and gram-positive bacteria, they found that each of them was sensitive to most of antibiotics but with different percentages. (Giammancong and Sarina ,1994).

Otitis media and respiratory tract infections are the common infections diagnosed in pediatric emergency and outpatient settings, and occasionally occur in association with urinary tract infection; however, the significance of combined infections in the pathogenesis of urinary tract infection remains uncertain. Otitis media and upper respiratory tract infection occurs in 13 to 30% of patients with urinary tract infection (Balat,2009).

A number of studies have reported on the prevalence of otitis media and urinary tract infection (Minja and Machemba ,1996; Ong *et al*,2008;Aich *et*

al,2009). However, majority of these studies have focused on clinical diagnosis with little or no report on the bacteriological etiology of otitis media and urinary tract infection . Against this background, this study aimed to isolate, characterize and identify the bacteriological etiologic agents of otitis media and urinary tract infection in Al-Manathera city , Iraq. And then choose the best type of treatment (antibiotic) for these bacteria.

Patients and methods

The study was carried out on 130 out patients (65 patients 36 male and 24 female infected with otitis media and 65 patients 24 male and 41 female infected with urinary tract infection) during the period between March,2011 to April ,2011 in Al-Manathera general hospital in Al-Manathera city, informed concent was taken from all the patients

Collection of specimens:-

The specimens were collected with sterile swab sticks (for patient infected with O.M) and collected with sterile container (for patient infected with UTI) which were properly labeled for each patient. The specimens were taken to the medical microbiology laboratory, Al-Manathera general hospital for analysis, immediately after collection.

Treatment of specimens:-

Specimens were inoculated onto Blood, Chocolate , MacConkey and Manitol salt agar plates. All plates were incubated immediately for 24 hours aerobically with the exception of Chocolate agar that was incubated in a candle jar. Emergent colonies were identified according to standard bacteriological methods McFadden (2000). Disc susceptibility test was performed according to National Committee for Clinical Laboratory Standards (NCCLS)(1993).

Statistical analysis:- Statistical analysis was made using (graph pad prism version 4) computer software using chi square (X2) test. P value less than the 0.05 level of significance was considered statistically significant.

Results

From the 130 out patients (65 ear swab from patients infected with otitis media and 65 urine specimens from patients infected with urinary tract infection , there were 60 bacterial isolates from patients infected with otitis media (36 male 60% and 24 female 40%) with 5 specimens no growth. *Pseudomonas aeruginosa* was the most prevalent bacteriological etiologic agent of otitis media (25 isolates 41.666%) while the least was *E.coli* (2

isolates 3.333%)(Table 1, Figure 1,2). Patients that were 1-10 years (11 isolates 30.55%) and 11-20 years (11 isolates 30.55%) had significantly higher prevalence (P<0.05) of otitis media in male compared with others ages groups. While patients that were 1-10 years(15 isolates 62.5%) had significantly higher prevalence (P<0.05) of otitis media in female compared with others ages groups(Table 2, Figure 3). The antibiotic susceptibility profile of the bacteria isolates revealed moderate level resistance. Cefotaxim and Nitrofurantoin were active against all of the bacterial isolate with inhibition percentage 100%. While Ampicillin and Pencillin were low active against all of the bacterial isolate with inhibition percentage 6.4% , 10.5% respectively (Table 3, Figure 4).

Table 1:- Numbers and percentage of bacteria isolated from patients infected with otitis media.

Ear swab				
Type of bacteria	Male isolates	Female isolates	Total isolates	100%
P.aeruginosa	15	10	25	41.666
S.aureus	9	4	13	21.668
S. epidermidis	4	4	8	13.333
E.coli	2	0	2	3.333
P.mirabilis	5	4	9	15
P. Vulgaris	1	2	3	5
No growth	3	2	5	7.7
Total	39	26	65	100%

Table 2:- Numbers and percentage of bacteria isolated from patients infected with otitis media according to sex and ages groups.

Ages groups (years)	Male 60% *		Female 40%	
	Total isolates	100%	Total isolated	100%
1-10	11	30.55	15	62.5
11-20	11	30.55	8	33.3
21-30	9	25	1	4.2
Above 30	5	13.9	0	0
Total	36+3 no growth=39	100%	24+2 no growth=26	100%

Table 3:- Sensitive pattern of bacterial isolate from patients infected with otitis media.

Antibiotic	Total numbers (N) and percentage (%) of bacterial isolates that sensitive to antibiotics.						
	P.aeruginosa N=25	*S.aureus N=13*	*S.epidermidis N=8*	*E.col N=2*	*P.mirabilis N=9*	*P. Vulgaris N=3*	Average *100%*
Gentamicin	12(48)%	7(53.8)%	4(50)%	2(100)%	4(44.4)%	2(66.6)%	60.4%
Amoxicillin	10(40)%	11(84.6)%	7(87.5)%	0%	5(55.5)%	2(66.6)%	55.7%
Augmentin	23(92)%	13(100)%	8(100)%	1(50)%	7(77.7)%	2(66.6)%	81.0%
Cirpofloxacin	15(60)%	8(61.5)%	4(50)%	2(100)%	9(100)%	3(100)%	78.5%
Rifampin	10(40)%	13(100)%	8(100)%	2(100)%	5(55.5)%	2(66.6)%	77.0%
Cefotaxim	25(100)%	13(100)%	8(100)%	2(100)%	9(100)%	3(100)%	100%
Trimetheprim	9(36)%	10(76.9)%	6(75)%	0%	8(88.8)%	2(66.6)%	57.2%
Ampicillin	0%	2(15)%	1(12.5)%	0%	1(11.1)%	0%	6.4%
Pencillin	0%	5(38.4)%	2(25)%	0%	0%	0%	10.5%
Nitrofurantoin	25(100)%	13(100)%	8(100)%	2(100)%	9(100)%	3(100)%	100%
Amikacin	25(100)%	12(92.3)%	6(75)%	2(100)%	9(100)%	3(100)%	94.5%

On the other hand from the 65 urine specimens from patients infected with urinary tract infection , there were 65 bacterial isolates(24 male 36.923% and 41 female 63.077%) . *Klebsiella pneumoniae* was the most prevalent etiologic agent of urinary tract infection (27 isolates 41.53%) while the least was *P.aeruginosa* (2 isolates 3.11%) (table 4 figure 1,2). Patients that were above 30 years old (12 isolates 50%) had significantly higher prevalence ($P<0.05$) of urinary tract infection in male compared with others ages groups. While patients that were 21-30 years olds (16 isolates 39.1%) had significantly higher prevalence ($P<0.05$) of urinary tract infection in female compared with others ages groups (Table 5, figure 3). The antibiotic susceptibility profile of the bacteria isolates revealed moderate level resistance. Amikacin and Nitrofurantoin were active against all of the bacterial isolate with inhibition percentage 100%. While Ampicillin and Pencillin were low active against all of the bacterial isolate with inhibition percentage 11.1% , 5.5% respectively (Table 6, Figure 4).

Table 4:- Numbers and percentage of bacteria isolated from patients infected with urinary tract infection.

Urine specimens				
Types of bacteria	Male isolates	Female isolates	Total isolates	100%
S.aureus	2	1	3	4.61
P.mirabilis	2	4	6	9.23
P. Vulgaris	3	5	8	12.30
K. pneumoniae	10	17	27	41.53
E.coli	6	10	16	24.61
P.aeruginosa	1	1	2	3.11
Mix growth	0	3	3	4.61
Total	24	41	65	100%

Table 5:- Numbers and percentage of bacteria isolated from patients infected with urinary tract infection according to sex and ages groups.

Age groups (years)	Male (36.923%)		Female (63.077%)*	
	Total isolates	100%	Total isolated	100%
1-10	4	16.6	4	9.7
11-20	2	8.4	13	31.7
21-30	6	25	16	39.1
Above 30	12	50	8	19.5
Total	24	100%	41	100%

Table 6:- Sensitive pattern of bacterial isolates from patient infected with urinary tract infection.

Antibiotic	Total numbers (N) and percentage (%) of bacterial isolates that sensitive to antibiotics.						
	P.aeruginosa N=2	*S.aureus N=3*	*K.pneumoniae N=27*	*E.coli N=16*	*P.mirabilis N=6*	*P. Vulgaris N=8*	*Average 100%*
Gentamicin	2(100)%	3(100)%	20(74)%	16(100)	3(50)%	3(37.5)%	76.9%
Amoxicillin	2(100)%	2(66.6)%	0%	0%	2(33.3)%	3(37.5)%	39.5%
Augmentin	1(50)%	3(100)%	14(51.8)%	12(75)%	4(66.6)%	7(87.5)%	71.8%
Cirpofloxacin	2(100)%	2(66.6)%	25(92.5)%	12(75)%	5(83.3)%	7(87.5)%	84.1%
Rifampin	1(50)%	2(66.6)%	10(37)%	16(100)	4(66.6)%	6(75)%	65.8%
Cefotaxim	2(100)%	3(100)%	7(25.9)%	0%	6(100)%	8(100)%	70.9%
Trimetheprim	1(50)%	2(66.6)%	0%	14(87.5)	4(66.6)%	4(50)%	53.4%
Ampicillin	0%	2(66.6)%	0%	0%	0%	0%	11.1%
Pencillin	0%	1(33.3)%	0%	0%	0%	0%	5.5%
Nitrofurantoin	2(100)%	3(100)%	27(100)%	16(100)%	6(100)%	8(100)%	100%
Amikacin	2(100)%	3(10%	27(100)%	16(100)	6(100)%	8(100%	100%

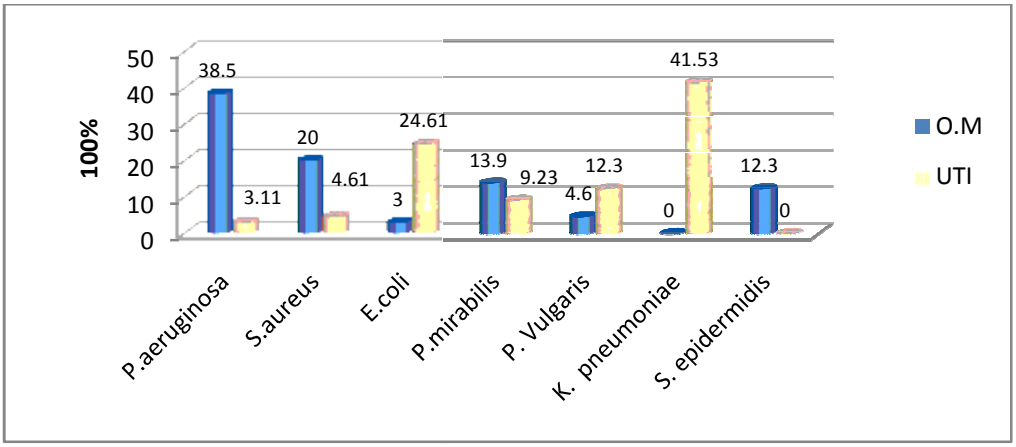

Figure 1:-Comparison between bacteria isolated from patients infected with otitis media and from patients infected urinary tract infection according to percentage.

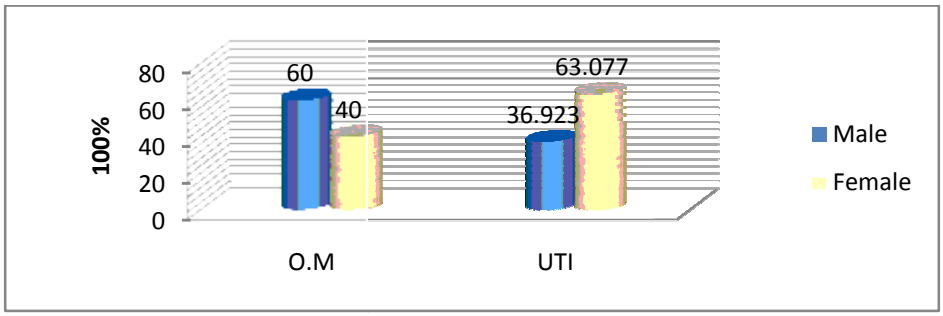

Figure 2:-Comparison between bacteria isolated from patients infected with otitis media and from patients infected urinary tract infection according to sex.

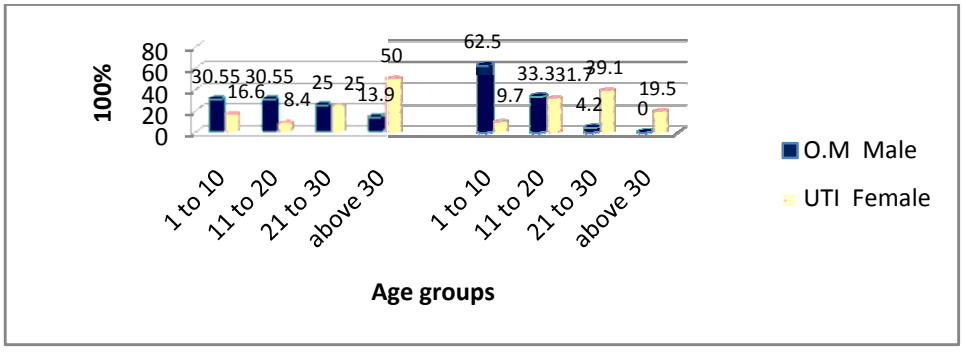

Figure 3:-Comparison between bacteria isolated from patients infected with otitis media and from patients infected urinary tract infection according to ages groups.

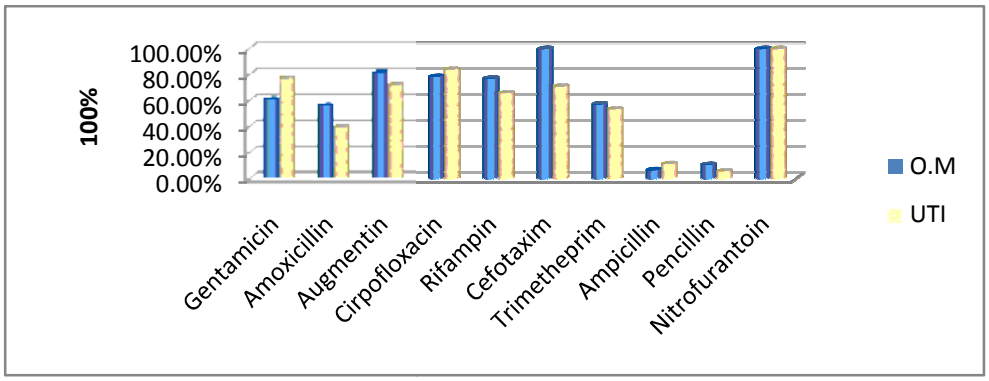

Figure 4:- Comparison between bacteria isolated from patients infected with otitis media and from patients infected urinary tract infection according to their sensitivity to antibiotics.

Discussion

Otitis media is a common diagnosis in pediatric emergency and outpatient settings, occasionally associated with urinary tract infection, a not uncommon systemic bacterial infection (Torrijos *et al.*,2007). Otitis media is frequently encountered in tropical and subtropical areas (Yehia *et al* ,1990). Diagnosis of this disease is often based solely on the clinical symptoms. It has been reported that children less than 10 years are more prone to otitis media due to shorter and more horizontal eustachian tube, lower immunity of children compared to adults and the fact that bacteria adhere better to epithelial cells of children that adults (Li *et al.*,2001; Weiner and Collison,2003; Egbe *et al.*,2010). In this study there was significant difference (p=□ 0.05) between male and female patients in the prevalence of otitis media (36 male 60% and 24 female 40%). This result is in agreement with (Parry and Roland,2002). Some studies have reported male gender to be a significant risk factor for acquisition of otitis media (Egbe *et al.*,2010; Favour *et al.*,2011).The most common pathogen isolated from the ear canal of patients with otitis media is bacteria (Parry and Roland,2002). *Pseudomonas aeruginosa* was the predominant isolate causing otitis media generally, this is in agreement with some studies (Nwabuisi and Ologe ,2002; Egbe *et al.*,2010). But differs from studies in others countries were *Staphylococcus aureus, Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* predominate (Bluestone *et al*,1992; Al-mohana *et al.*,2006). Otitis media may be caused by fungi, viruses, *Mycoplasma pneumoniae* and *Chlamydia* (Favour *et al.*,2011).

Urinary tract infections are among the most commonly observed infections in clinical practice, and more than 25% of all women experience

some form of UTI at least once during their lifetime.(Noor *et al.*,2004). The literature is unclear about the age urinary tract infection becomes less prevalent for male and female. Our data proved that females (39.1%) younger than 20 year of age are at higher risk for urinary tract infection than those in the first year of life. The incidence of UTI is higher among females, in this study there was significant difference (p=□ 0.05) between male and female patients in the prevalence of UTI (24 male 36.923% and 41 female 63.077%) in whom it commonly occurs in an anatomically normal urinary tract. Conversely, in males , UTI generally reveals a urinary tract lesion that must be identified by imaging and must be treated to suppress the cause of infection and prevent recurrence. UTI can be restricted to the bladder (essentially in females) with only superficial mucosal involvement, or it can involve a solid organ (the kidneys in both genders, the prostate in males)(Meyrier,2009).Or may be because of our small number of males with urinary tract infection. Some studies suggest that males and females younger than 15 year of age are most at risk for urinary tract infection, but males and females less than 5 year of age are not most at risk for urinary tract infection.(Kathy *et al.*,1998). Method of urine collection, and eligibility criteria, most have small sample sizes, and none have been true prevalence studies in which data are collected on all children.

On the other hand the present results demonstrated that most isolates isolated from patients infected with otitis media and urinary tract infection offered high degree of resistance against the commonly used antibiotics (Table 3,6). These resistant organisms can then pass on their resistance genes to their offspring by replication or to related bacteria through conjugation and mechanisms of resistance such as plasmid mediated or reduced outer membrane permeability could be involved in the resistance to β-lactams (Noor *et al.*,2004). The production of extended spectrum β- lactamase among *E. coli* ,*P.aeruginosa* and *K. pneumoniae* also contributed significantly to the resistance of these isolates (Jenks *et al*., 1995). Resistance to aminoglycosides and Cirpofloxacin in gram-negative bacilli is often mediated by β-lactamases which are unaffected by exposure of the bacterium to the potential drugs (Shafran, 1990). The resistance pattern to Ampicillin, Pencillin Amoxicillin , and gentamicin has been the most common according to the present findings.(Sahm *et al.,* 2001). Analysis of bacterial collections from the pre-antibiotic era indicates that although plasmids were present in some of the strains but did not harbor antibiotic resistance genes (Hughes and Datta, 1983). It seems, the development of antibiotic resistance among bacteria occurred after the

introduction of antibiotics into clinical use. Epidemiological studies have suggested that antibiotic resistance genes emerge in microbial populations within 5 years of the therapeutic introduction of an antibiotic (Chakrabarty *et al*., 19ue to 90). Further, the antibiotic resistance genes (found in human and animal isolates) could have originated in the industrial microbes that are used for the production of antibiotics (Webb and Davis, 1993).

Conclusions and recommendations

Nitrrofurantoin is more effective in the treatment of otitis media and UTI than penicillin and ampicillin.

Otitis media and UTI are diseases characterized by the presence of drug resistant cases due to different types of pathogenic bacteria involved in their etiology so we are in need for further studies to select the most appropriate and effective antibiotic for the treatment of such infections.

References

1- Abu Setta, A.M. and Doma ,A. M.(2008). Isolation of microorganisms associated with urinary tract infection in diabetic patients in Libya and their antibotic susceptibility pattern. The Egyptian Journal of Hospital Medicine. Vol., 31: 211– 221.

2- Aich, M.L.; Biswas, A.C.; Ahmed, M.; Joarder, M.A.; Datta, P.G. and Alauddin, M.(2009). Prevalence of Otitis media with effusion among school going children in Bangladesh. Bangladesh J Otorhinolaryngol. 15: 31 – 34.

3- Al- Mohana, A.; Al-Yasiri, I. K. and Al- Toriahi ,T. A.(2006).The study Of B-lactamase producing bacteria from patients infected with otitis media.J.M.

4- Arroll, B.(2005). Antibiotics for upper respiratory tract infections. "An Overview of Cochrane Reviews'' Med 3: 255 – 261.

5- Balat,Y.(2009). Infectious Diseases Concomitant with Urinary Tract Infections in Children . J. of Medical Sciences 9. 65–68.

6- Beyene ,G. and Tsegaye,W.(2011). Bacterial pathogens in urinary tract infection and antibiotic susceptibility pattern in jimma university hospital. Ethiop J Health Sci. Vol. 21, No. 2.

7- Bluestone, C.D.; Stephenson, J.S. and Martin, L.M.(1992). Ten-year review of Otitis media pathogens. Paediatr infect Dis J.11: 57 – 11.

8- Chakrabarty, A.M.; Dastidar, S.G.; Ganguli, M. and Chattopadhyay. (1990). DNA as contaminants in antibiotics and its capacity to transform bacteria to drug resistance. *Indian J. Exp. Biol*. **28**: 58-62.

9- Egbe, C.A.; Mordi, R,; Omoregie, R, and Enabulele, O.(2010).Prevalence of Otitis media in Okada Community, Edo State, Nigeria. Macedonian J. Med. Sci. 3(3): 299 – 302.
10- Favour, O. ; Bmls, A. ; Aimlta, l. ; Emmanuel, O. and Clement, O.(2011). Etiologic agents of otitis media in Benin city, Nigeria .North American Journal of Medical Sciences , Volume 3. No. 2.
11- Giammanco,G. and Sarina, P.(1994). Rapid identification of micro-organisms from urinary tract infections by *p-g* l ucuronidase, p henylalanine deaminase, cytochrome oxidase and indole tests on isolation media J. Med. Microbio1.-Vol. 41, 389-392.
12- Hughes, V.M. and Datta, N. (1983). Conjugative plasmids in bacteria of the "Pre antibiotic" era. *Nature* (London). **302**: 725-726.
13- Jenks, P.J., Hu, Y.M., Danel, F., Mehtar, S. and Livermore, D.M. (1995). Plasmid mediated production of class I (AmpC) β-lactamase by two *Klebsiella pneumoniae*. *J. Antimicrob. Chemotherp.* 3**5**: 235-236.
14- Kathy, N.; Shaw, M.; Gorelick, ; L. and McGowan, N.(1998). Prevalence of Urinary Tract Infection in Febrile Young Children in the Emergency Department .J. Pedia.
15- Li, W.C.; Chiu, N.C.; HSU, C.H. and Lee, K.S.(2001). Pathogens in the middle ear effusion of children with persistent otitis media: Implications of drug resistance and complications. J Microbiol Immunol Infect. 34: 190 – 194.
16- McFadden, J. F. (2000). Biochemical tests for identification of medical bacteria (3rd ed.), Lippincott Williams and Wilkins. USA.
17- Meyrier, A.(2009).Urinary tract infection. (4th ed.), Wilkins. USA.
18- Minja, B.M. and Machemba, A.(1996) Prevalence of Otitis media, hearing impairment and cerumen impaction among school children in rural and urban Dar es Salam, Tanzania. Int J Pediatr Otorhinolaryngol. 37: 29 – 34.
19- National Committee for Clinical Laboratory Standards. (1993). Approved Standard M2 – A5 for antibacterial disc susceptibility test. Villanova.
20- Noor, N.; Ajaz ,M. and Rasool,S.(2004).Urinary tract infection associated with multi drug resistance bacilli. Pakistan Journal of Pharmaceutical Sciences Vol. 17, No., pp.115-123
21- Nwabuisi, C. and Ologe, F.E.(2002). Pathogenic agents of chronic suppurative otitis media in Ilorin, Nigeria. East Afr Med J. 79(4): 202 – 205.
22- Ong, D.S.Y.; Kuyvenhoven, M.M.; Dijk, L. and Van.T.J.M.(2008). Antibiotics for respiratory, ear and urinary tract disorders and consistency among GPs. Journal of Antimicrobial Chemotherapy. 62(3), 587-592.

23- Parry, D. and Roland, D.(2002). Middle Ear Chronic Suppurative otitis media. Medical treatment. pp. 12 – 15.
24- Sahm, D.F.; Thornsberry, C.; Mayfield, D.C.; Jones, M.E. and Karlowsky, J.A. (2001). Multidrugresistant urinary tract isolates of *Escherichia coli*: prevalence and patient demographics in the United States in 2000. *Antimicrob Agents Chemother*. **45**: 1402-1406.
25- Shafran, S.D. (1990). The basis of antibiotic resistance in bacteria. *J. Otolaryngol*. **19**: 158-168.
26- Torrijos, E.; Abdul, J.; Khan, M.D. and Mary, B.(2007). Urinary tract infections associated with otitis media in infants and children . Brooklyn, New York .
27- Webb, V. and Davis, J. (1993). Antibiotic preparations contain DNA: a source of drug resistance genes. *Antimicrob. Agents Chemother*. **37**: 2379-2384.
28- Weiner, R. and Collison, P.J.(2003). Middle Ear pathogens in Otitis prone children, South Dakota J Med. 56: 103 – 107.
29- Yehia, M.M.; Habib, H.M. and Shehab, N.M.(1990). Ear infections. J. Laryngol.. Otol 104: 387 – 389.