

Isolation & Identification of aerobic bacteria Causing Infection eyes of newborn babies in in Al-Diwaniya city and its sensitivity to some antibiotics

عزل وتشخيص البكتريا الهوائية المسببة لالتهاب العيون عند الاطفال حديثي الولادة في مدينة الديوانية وحساسيتها لبعض انواع المضادات الحيوية

Aamal Ghazi Mahdi
College of Science
Al – Qadysia University

ABSTRACT

(175) samples were collected from newborn children infected by Ophthalmia neonatorum contacting the Educational Hospital for children and & Delivery in Al Diwaniya city , (149) isolated of bacteria was isolated , the dominance to the gram positive bacteria, it was isolated (134) isolate with percentage(%89.93) included *Staphylococcus aureus* with percentage(%54.36) and *Streptococcus pyogenes* with percentage (% 35.57), while the gram negative bacteria with percentage(%10.06) at (15) isolate included : *Pseudomonas aeruginosa* (% 6.71), *Neisseria gonorrhoeae* (%1.34) , *Proteus spp* (% 1.34) , *Moraxella lacunata* (% 0.67) .As for the effect of antibiotics on bacteria it was found the most powerfull antibiotic on *Staphylococcus aureus* was Cefotaxim with percentage (%81.50) then Erythromycin with percentage (% 61.59) as has been Cefotaxim the most powerfull antibiotic on *Streptococcus pyogenes* with percentage (% 83.45) then Cephalexin with percentage(%70.27) , while the lowest effective antibiotic on *Staphylococcus aureus* and *Streptococcus pyogenes* was Amikacin with percentage(% 2.57) and(%1.20) respectively.

الخلاصة

تم جمع (175) عينة من الاطفال حديثي الولادة المصابين بالتهاب العيون والمراجعين الى مستشفى الولادة والاطفال التعليمي في مدينة الديوانية , تم عزل (149) عذلة بكتيرية , كانت السيادة فيها للبكتريا الموجبة لصبغة غرام اذ عزلت (134) عذلة وبنسبة (%89.93) وشملت المكورات العنقودية الذهبية *Staphylococcus aureus* بنسبة (% 54.36) والمسبقيات القححية *Streptococcus pyogenes* بنسبة (% 35.57) بينما شكلت البكتريا السالبة لصبغة غرام نسبة (% 10.06) وبواقع (15) عذلة وضمت الانواع التالية :

Pseudomonas aeruginosa (% 6.71) , *Neisseria gonorrhoeae* (% 1.34) , *Proteus spp* (% 1.34) , *Moraxella lacunata* (% 0.67) .

كما اظهرت النتائج ان اكثر المضادات الحيوية تاتيها على بكتريا *Staphylococcus aureus* كان مضاد Cefotaxim وبنسبة (% 81.50) يليه مضاد Erythromycin بنسبة (% 61.59) , كما وقد كان مضاد Cefotaxim اكثر المضادات الحيوية تاتيها على بكتريا *Streptococcus pyogenes* بنسبة (% 83.45) يليه مضاد Cephalexin بنسبة (% 70.27) بينما كان مضاد الحياة Amikacin اقل المضادات الحيوية تاتيها على كل من بكتريا *Staphylococcus aureus* و *Streptococcus pyogenes* اذ اثر بنسبة (% 2.57) و (% 1.20) على التوالي .

INTRODUCTION

The eye is one of the five senses of the importance of life from birth until the end of his life, the attention given to eye health and cleanliness is very essential being exposed to outside influences and internal influences such as: dust, high temperature, bacteria , fungi, viruses and other which lead to several diseases, some cases lead to blindness .

Ophthalmia neonatorum defined as any inflammation of the eye conjunctiva with secretions from the eyes or one eye during the 28 days in the life of newborn, the cause is either *Neisseria gonorrhoeae* or *Clamidia trachomatis*, this is severe during the passage of child birth canal or after birth [1] . The symptoms and signs of inflammation of the eyes of newborn with presence of mucous pus usually caused by bacteria, the product of pus increase , conjunctival hyperemia and lid edema [2] . Microorganisms can invade the external surface of the eye or phrenic tissues or even

the bloody course ,the risk of eye damage because lens and vitreous non vascular system rich with protein the ideal of the proliferation of many pathogenic bacteria may provide the opportunity resulting from bacterial infection with pus a few millimeters in diameter but hinder vision and becomes a suspended eye necessary because of inflammation of the soles of the eye [3] and fearing from transmission of inflammation to the second eye by warmer face or fingers or anything else [4].Despite resistance of the outer surface of the eye of the bacterial infection through a variety of mechanisms , but the eye infection conjunctivitis occurs when the microbes have susceptibility to resist host defenses [5], host resistance as possible to weaken or damaged in some diseases such as tumor or in patients whose immune systems at risk always [6] , it can be classified bacterial infection of eye according to the duration of the disease to acute and chronic [7] the existence of the purulent secretions and purulent mucous diagnostic signs of inflammation of the eye conjunctivitis , therefore tend to stick eye lids after waking up from sleep , which depends on the degree of pus sign of the presence of bacterial causes, not viral causes inflammation of the eye [4] .

The most important causes of bacterial eye infections are :

Pseudomonas aeruginosa , *Neisseria gonorrhoea* , *Proteus spp* , *Haemophilus aegyptius* , *Moraxella spp* such *Moraxella lacunata* , *Moraxella liquefaciens* , *Moraxella catarrhalis* , *Staphylococcus aureus* ,*Streptococcus pyogenes* [8] . Most cases of acute bacterial conjunctivitis sharp eye is common and can affect all ages and both sexes [9] the cause of a number of gram positive cocci , in general, the incubation period of this inflammation less than 3 weeks [7] while in the case of inflammation of chronic bacterial conjunctivitis eye it is periods would be more than 4 weeks for this we can promise chronic it is usually different in terms of microbial causes from chronic bacterial conjunctivitis which was usually the result of continuous inoculation of bacteria associated to Blepharitis [10] , occurs because of *Staphylococcus aureus* and some gram negative bacilli *Moraxella lacunata* [4] and accompanied by a few discharge from eye [11] and that can happen is endemic also by pollution eye masturbation [12] .

The use of mandatory treatment in the case of Ophthalmia neonatorum common use erythromycin ointment with concentration (% 0.5) and Tetracycline ointment with concentration (% 1) and the use of silver nitrate in the form of droplets with concentration (% 1) , apart from the use of povidine – iodine solution with concentration (% 2.5) [14] and Benzyl –penicillin in the form of droplets [13] in any case, the best way to treat eye infections conjunctivitis is identification of microorganisms causing infections and the selection of specific antimicrobial treatment [15] taking into consideration patient age , environment , ocular finding [16] .The objective of this study as follows :

- 1 - Isolation and identification of the bacteria causing eye infection in newborn children
- 2 -Determine the effect of antibiotics on the prevailing bacteria isolated from eye infection in newborn children

METHODS & MATERIALS

1- Collection of Samples :(175) samples were collected from newborn children whome infected by Ophthalmia neonatorum contacting the Educational Hospital for children and & Delivery in Al Diwaniya city , was used sterile cotton swabs , the samples have been collected depending on the contents of [17] .

2- Direct examination of samples :

for the purposes of examining samples have been working directly smear from swabs and put the samples on sterile slides , after the fixation , samples were stained and read the result depending on the presence or absence of bacterial causes in infected eyes .

3- Isolation of bacteria : Blood agar ,MaCConkey agar ,Chocolate agar were used ,Culture media were streaking with 2 petridish for each, Placed in incubator with 37°c for 24h [18] .

4- Identification of bacterial isolates

Culture media were prepare for the purpose of purification, conservation, revitalization, identify qualities appearance and cultural recipes and making biochemical test of the bacterial isolates also

attended the dyes and solvents and reagents to complete biochemical test and studied following the completion of diagnosis :

A - Characteristics manifestation of the developing colonies:

Observed qualities manifestation of the colonies developing on culture media form color, the surface of the colony, strength, transparency, smell, lactose fermentation on Macconkey agar [18 ;19]

B-Microscopic characteristics of the developing colonies : Swabs were the work of the pure colonies segments of slides the colored character gram stain observed forms of cells ,arranged, responding to gram stain [18]

C-Biochemical Tests : tests conducted a production of the enzyme Catalase , Oxidase , Coagulase Voges proskauer , Methyl red as stated in [20] Motility test , Urease , Indol , H₂S Production , Citrate utilization , Kligler agar Sugar fermentation as stated in [21] Haemolysis by the way [22] and growth on 10 °C ,45 °C according to [21]

5 - Sensitivity to antibiotics : from the use of nine types of antibiotics are:

Amikacin(AN) , Chloramphenicol (C) , Ampicillin (AM), Cefotaxime (CTX) , Amoxicillin (AMX) ,Tetracycline (TE),Cephalexin (KF), Cloxacillin (Clx) , Erythromycin (E) , determining sensitive and resistant bacteria to antibiotics by measuring the diameter of inhibition zone by Mm and then compared with the standard diameters that installed in the standard scales [23;24]

Results & Discussion

(149) isolated of bacteria was isolation of the total (175) sample of newborn children whome infected by Ophthalmia neonatorum , the purification and diagnosis of developing isolates was remained dependent on [19;21], identifying characteristics appearance and culture media, recipes cells under the microscope and results of biochemical tests as shown in table (1) the bacteria contain : *Staphylococcus aureus* and *Streptococcus pyogenes* as gram positive ,the gram negative contained *Pseudomonas aeruginosa* , *proteus spp*,*Neisseria gonorrhoea* , *Moraxella lacunata* as shown in table (2) .

Table (1) biochemical tests of gram positive bacteria isolated from samples and some attributes appearance and microscopic

Test \ Type of bacteria	<i>Staphylococcus aureus</i>	<i>Streptococcus pyogenes</i>
Coagulase	+	-
Catalase	+	+
Haemolysis	β	β
Oxidase	-	-
Mannitol fermentation	+	-
growth on 10 °C	+	-
growth on 45 °C	+	-
Qualities appearance	colonies of medium-to large-sized Milky yellow, smooth ,β -haemolysis	Colonies of small, white, transparent, granular appearance, β -haemolysis
Attributes microscopic	spherical form of the grapes , G +	spherical in the form of long chains , G +

Table (2) biochemical tests of gram negative bacteria isolated from samples

Type of bacteria \ Tests	oxidase	catalase	Indol	Methylred	Simmons citrate	Urease	Kligler agar	H2S production	Motility	Nitrate reduction	Glucose	Maltose	Lactose
<i>Pseudomonas aeruginosa</i>	+	+	-	-	+	V	K/K	-	+	+	-	-	-
<i>Neisseria gonorrhoea</i>	+	+	+	-	+	-	A/A	-	-	V	+	-	-
<i>Proteus spp</i>	-	+	-	+	+	+	K/A	+	+	+	+	-	-
<i>Moraxella lacunata</i>	+	+	+	-	-	V	K/A	-	-	V	-	-	-

Abbreviation

A : Acid , K : Alkaline , V : Variable , + : Positive , - : Negative
 K/A: Alkaline / Acid , A /A : Acid / Acid , K/K : Alkaline/ Alkaline

We shall note from the table (3) the arrangement bacteria isolated from samples of newborn children whome infected by Ophthalmia neonatorum

, it was a gram positive bacteria (% 89.93) at (134) isolates of the total isolates , while gram negative with percentage (% 10.06) at (15) isolates , while [25] isolated gram positive with percentage (% 41.90) and gram negative with percentage (% 85) , and is clear from the table the rule of *Staphylococcus aureus* on other type of isolated bacteria since been isolated (81) isolates with percentage (% 54.36) , the results were consistent with [8] isolate *Staphylococcus aureus* with percentage (% 59.71) and [26] with percentage (% 59.70) and [27] with percentage (% 53.06) , the cause may be in the presence of this type of bacteria in these high proportions in eye injuries in children to the pollution occurring in the delivery room or at home hence the birth of a child in the polluted atmosphere that lead to transition to him or because of natural presence on the skin and genital tract and once the contact between them with the newborn whose immunity is weak , have high potential to cause infection in eyes [28] these bacteria has a number of surface antigen and enzymes and toxins which enabled them to make infection [29] .As *Streptococcus pyogenes* came in second place as cause for of the eyes infection of newborn children , it was isolated (53) isolates with percentage (% 35.57) , had been isolated by [26]with percentage (% 40.3) and [30] with percentage (% 39.4) while [31] with percentage (% 0.55) , the reason is the proliferation of bacteria in eyes infection to possess quickly resistance to antibiotics and disinfectants used in hospitals [32] .

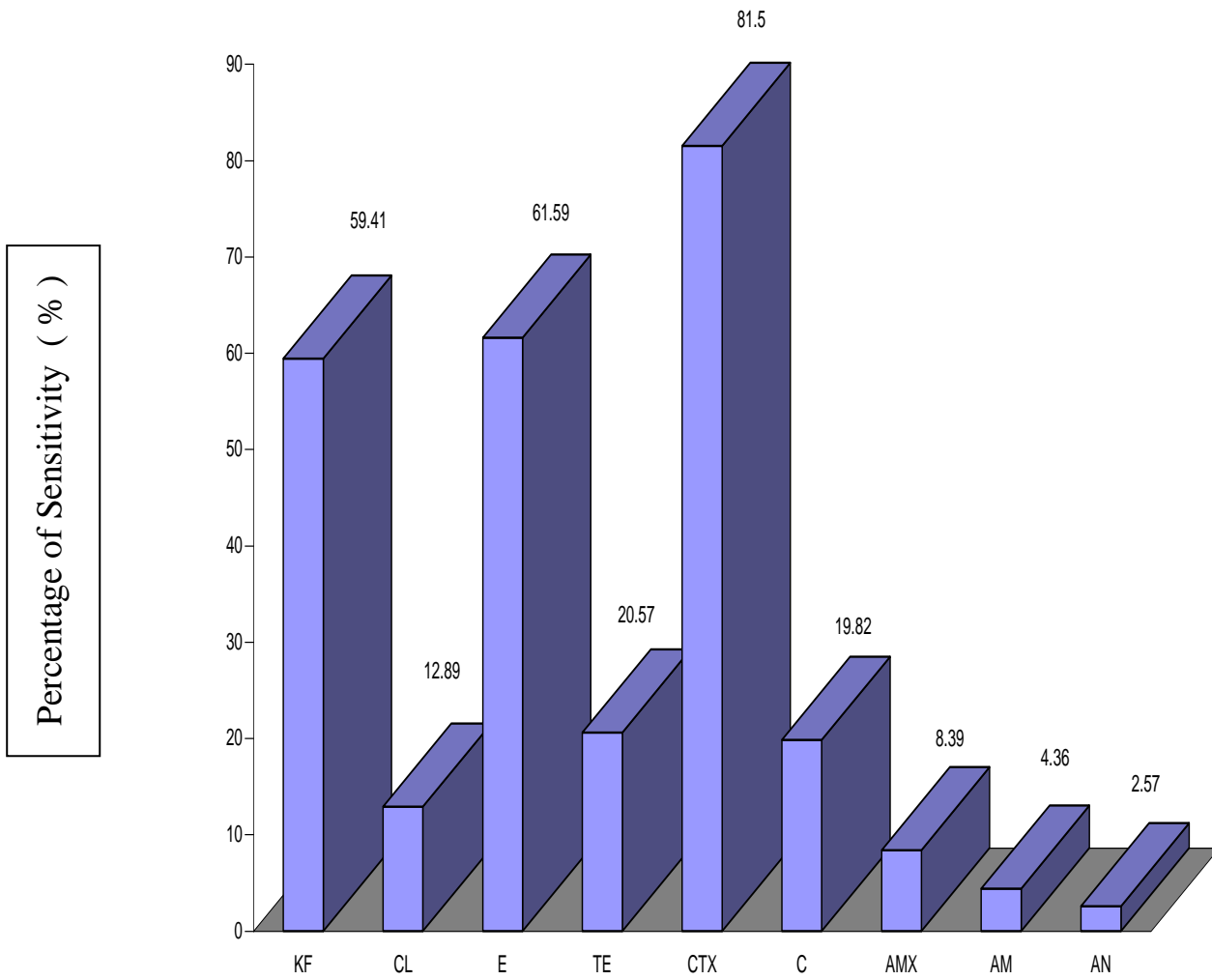
Pseudomonas aeruginosa came in third place , it was isolated (10) isolates with percentage (% 6.71) , had been isolated by [26]with percentage (% 7.69) , and explain the possible isolation of *Pseudomonas aeruginosa* to resistance to antibiotics and disinfectants used in hospitals [30] as well as the possession dyes that have important role in the process of colononization of the bacteria in the host as it works to give these bacteria force competition with the rest of other bacterial species and allow them the opportunity to sovereignty [33] it is a sign of endemic infection to the eye [28] as these bacteria known susceptibility to growth in various temperature making the process of proliferation and infection of children easy and quick sections within the hospital [34] [35] it also has the ability to stick to surfaces external environment by polar flagella [36] , this could be transmitted to another group of patients not infected by these bacteria causing serious injuries difficult to control [37] . It also has been isolated *Neisseria gonorrhoea* with percentage (% 1.34) , had been isolated by [26]with percentage (% 2.19) , according to the [38] (% 10) of the newborn children infected by *Neisseria gonorrhoea* which gained from the mother during the birth of the child by infected mothers previously infected with gonorrhoea , also stressed on [4] that these bacteria infection is acquired from mother during the passage of the child in the birth canal

which leads to inflammation of the eye conjunctivitis by these bacteria .As has been isolated *Proteus spp* with percentage (% 1.34) which is causing widespread infections in the eye [30], the air play an important role in the transfer of these bacteria to hospital delivery rooms especially when opening the doors and windows which facilitates transfer it to the baby[26]. Also isolated *Moraxella lacunata* with percentage (% 0.67) this is bacteria as an indicator of the epidemic eye infection [28]and cause Blepharitis with a few eye secretions [11] .It is worth noting that there are(26) samples with percentage(% 4.85) did not show bacterial growth was due to the possibility the presence of other such as viral causes or clamyia [39]or one type of yeasts [4] .

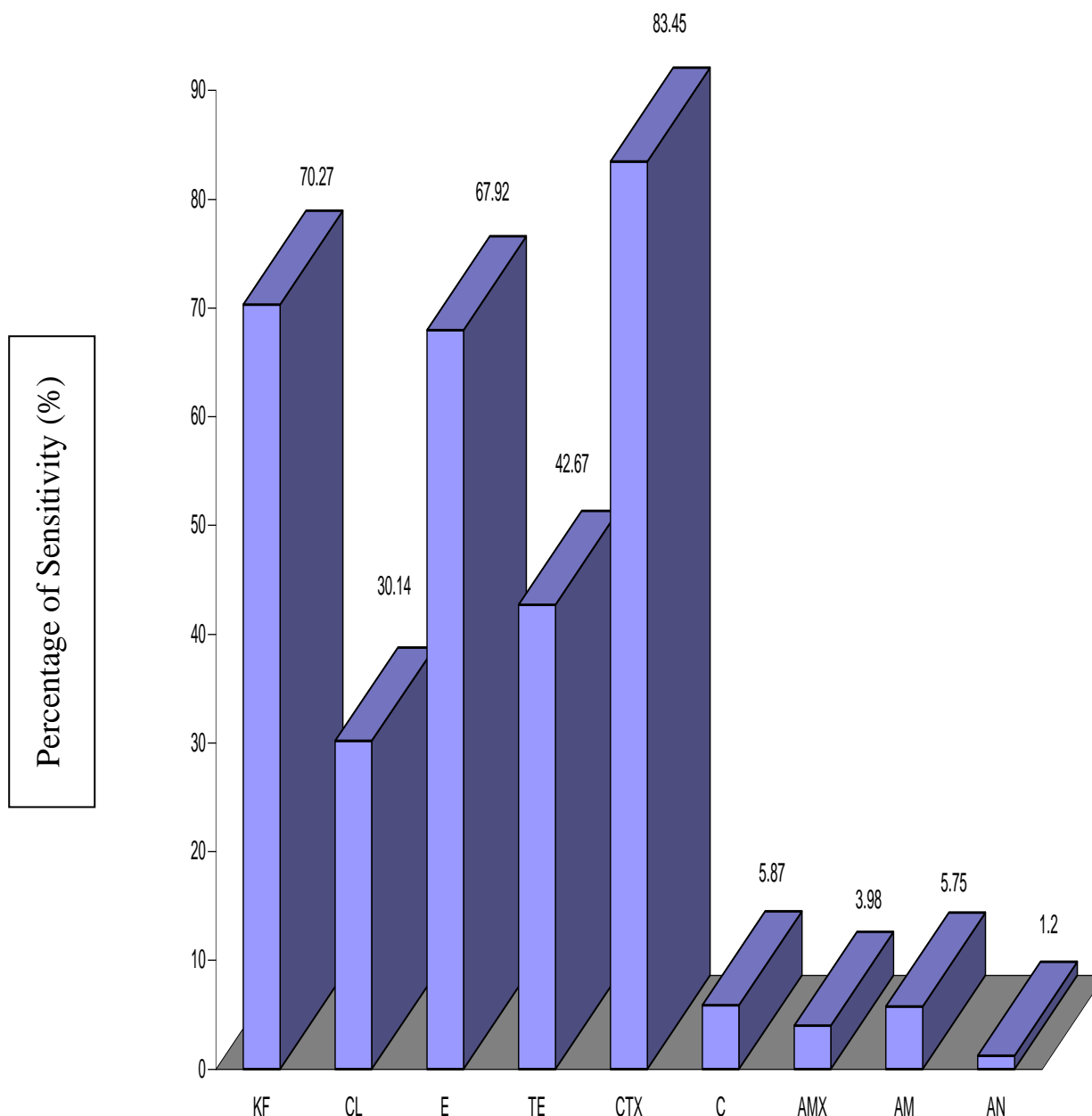
Table (3) types and numbers of bacteria isolated from samples of children with eyes infection

Type of bacteria	No. of isolates	Percentage %	No. of isolates	Percentage %
<i>Staphylococcus aureus</i>	81	54.36	134	89.93
<i>Streptococcus pyogenes</i>	53	35.57		
<i>Pseudomonas aeruginosa</i>	10	6.71	15	10.06
<i>Neisseria gonorrhoea</i>	2	1.34		
<i>Proteus spp</i>	2	1.34		
<i>Moraxella lacunata</i>	1	0.67		
Total	149	100		

Shown in Figure (1) the sensitivity of *Staphylococcus aureus* isolates to antibiotics used showed the highest sensitivity to Cefotaxime with percentage (%81.50) followed by Erythromycin with percentage (% 61.59) while the proportion was less sensitive to Amikacin with percentage(% 2.57) , results were similar to [26] as the proportion of the sensitivity of *Staphylococcus aureus* isolated (% 77.1) to Cefotaxime ,(% 54.7) to Erythromycin and (% 3.2) to Amikacin .As shown in figure (2) the highest sensitivity record *Streptococcus pyogenes* was to Cefotaxime with percentage (% 83.45) followed by Cephalexin with percentage (% 70 .27) while the proportion was less sensitive to Amikacin with percentage(%1.20), results were similar to the [26] as the proportion of the sensitivity of *Streptococcus pyogenes* isolated (% 80 .6) to Cefotaxime and Cephalexin with percentage (%72.5)while fully showed resistance to Amikacin with percentage (% 100) , it is possible interpretation of these results and in accordance with the contents of [40] which indicated that the Cefotaxime use the newly in those years and the use has dropped in a certain period then re-use making a bacteria quick response to it , it is possible explanation for the high resistance to Amikacin on the basis of vulnerability resistance bacteria to the formation of an enzyme is to modify the antibiotic and thus lose its effectiveness [41] or the result of the loss of outer membrane proteins thereby reducing the permeability of the antibiotic within cell [42] [43]



Form (1) the sensitivity of *Staphylococcus aureus* for some types of antibiotics laboratory



Form (2) the sensitivity of *Streptococcus pyogenes* for some types of antibiotics laboratory

References

- 1 – Moni , J.K and vidya , R. M. (1997) . Conjunctivitis infection in human , Review manual . Surv . Ophthalmama . 20 :12- 15 .
- 2 – Dawson , C. R . (1982) . Follicular conjunctivitis in ophthalmology . Vol .4 , Ed . Thomas Duane , chapter 7 .
- 3 - Al-Dewagi , H. S . (1987) . Book in the studios of Pathology. 11 edition, Part 3 , translation of selected faculty members in the Iraqi universities .
- 4 - Gorback , J.;Bartlett , R.and Blaklow , A. (1998) . Infection disease , 2 nd . ed ., America . 177 -178
- 5 - Mannis , M.J. (1990) . Bacterial conjunctivitis . In :Tasman W , Taeger , E.A , (eds) . Duanes clinical ophthalmology .Vol.4 Philadelphia : JB Lippincott , 1 – 7 .
- 6 - Gerald , I.,Man,D.;Johne , E. and Bennett , R .(1995) . Principles and practice of infectious disease , part I .

- 7 - Antonions , S . and Tabbara , K.F. (1986) . Bacterial conjunctivitis in infections eye . Eds : Tabbara , K.F and Hyndiuk , R.A;Little Brown and CO .Boston &Toronto :413– 420
- 8 - Stuart , D. B. (1999) . A study about bacterial conjunctivitis .AMJ . J . Ophthalmol . 102 : 210 – 212 .
- 9 - Wan,W.;Farkas , G and May , W. (1986) . The clinical characteristics and course of adult gonococcal conjunctivitis.J. Am. Ophthal ., 102 (1): 575 – 83
- 10 - Syed , M. A. and Hyndiuk , R. A. (1992) .Infectious conjunctivitis .Infect . Dis . Clin . North Am ., 6 :789 – 805 .
- 11 -Seal , D. V . Barrett , S.P . and McGill , J . I . (1982) . Aetiology and treatment of acute bacterial infection of the external eye . J.Ophth.,66:357
- 12 -Schwartz , B;Harrison , L.H. and Motter , J.S. (1988) . Investigation of an outbreak of *Moraxella* conjunctivitis at a Navajo boarding school Am . J. Ophth . 107 : 341 – 7 .
- 13 - Chen , J.Y. (1992) .Prophylaxis of ophthalmia neonatorum :comparison of silver nitrate , Tetracycline , Erythromycine and no prophylaxis . J pediater infect . Dis (11):1026 .
- 14 -Hedberg , K;Ristinen , T.L;Soler, J.T;White , KE; Hedberg,CW;Osterholm , M. and Macdonald , KL. (1990) . Outbreak of erythromycine –resistant *Staphylococcus* conjunctivitis in a newborn nursery .Pediatr.infect .dis.Med . 9:268-73 .
- 15 - American optometric association . (1995) . Panel on care of the patient with conjunctivitis 243 N .Lindberg Blvd .,St.Louis , MO 63/41-7881.
- 16 -Janus, S.D. (1989) .Antiinflammatory drugs in clinical ocular pharmacology .2nd .ed .Boston:Bartlett J.D, Janus, S.D,eds.Butterworths , 163-97 .
- 17 - Graig , V. and Lechmann , S.(1998) . Practicle microbiology Churchill livingstone . London . 88 .
- 18 - Collee , J . G . ; Fraser , A . G . ; Marmion , B . P . and simmons , A . S . (1996) . practical medical microbiology . 14th . ed . churchill Livirgstone
- 19 - Betty ,A.F;Saham,D.F and Weissfeld,A.(1998).Diagnostic Microbiology . 10thed .Mmosby
- 20 - Baron , E.J. and Fingold , S.M. (1990) , Baily and Scotts diagnostic microbiology . (8 thed) .C.V.Mosby ,U.S.A .25 : 101 – 105 .
- 21 - Cowan ,S. T. (1985) . Cowan & Stells manual for identification of medical bacteria . 2 nd ed .Cambridge . Univ . Press .,U.K. 1982
- 22 - Holt , J . G . ; krieg , N . R . ; sneath , p . H . A . ; staley , J . and williams , S . T . (1994) . Bergey , S manual of Determinative Bacteriology . 9th ed . williams and wilkins , Baltimore , U . S . A .
- 23 - Bauer, A . W; Kirby, W. M.; Sherris , J . C. and Turk , M . (1966) . Antibiotic susceptibility testing by standarized single disk method . Am . J . clin . Pathol . (45): 493 .
- 24 -National Comite of Clinical Clinical Laboratory Standards.(NCCLS). (1991) .Performance standerd antibiotic susceptibility testing . NCCLS . Villanova , pa.
- 25 – Hayath. K, Esaki . S . (2008) . High isolation rate of *Staphylococcus aureus* from surgical site infections in an Indian hospital. Journal of Antimicrobial Chemotherapy, (2) : 2 – 5
- 26 - Al-Oqaili ,N .(2004) .Study of microbial eye infection of newborn babies in Diwaniya city .Msc thesis . College of education . Al-Qadisiya University , pp 44 – 54 .
- 27 - Al-Saimary (2007) . A study of antibacterial activity of plant extracts on bacterial pathogens isolated from Eye infections (conjunctivitis) . *The Internet Journal of Microbiology*. Volume 4 Number 1
- 28 - Landman, D. (2001) . Management of infections due to resistance *Staphylococcus aureus* Am . Infect . 30 :225 – 240 .
- 29 - Jarvis, W.R. (1996) .Selected aspects of the socioeconomic impact of nosocomial infection morbidity , mortility , Cost and prevention , infect . control Hosp . Epidemiol . 17 (8) :552-557 .
- 30 -Sleigh , DJ . and Timbury , MC . (1998) . Notes on medical bacteriology , 5 ed . Singapore . 116 – 120 .

- 31 – Al – Sherwal (2008) . Epidemiology of Ocular Infection Due to Bacteria and Fungus – A Prospective Study. Medical College New Delhi & HIM S, Dehradun, Uttaranchal, India (10) No.3 : 1 – 7
- 32 - Arque , M.; Nieves , B.; Ruiz , O. and Dager , M. (2000) . Characterization of plasmids which mediated resistance to multiple- Antibiotic in gram negative bacteria on nosocomial origin . *Enferm , Infect . microbial Clin* .15 (6) : 299 – 305 .
- 33 - Green , W.; Slack , G. and Peuther , J.F. (1997) . Medical immunity laboratory diagnosis and control 13 th .ed . Churchill livingstone .
- 34 - Jawetz , E.; Melnick , J.L. and Adelberg's , E.A.; Brook , G.F; Butel .J.S. and Morse , S.A . (2001) . Medical Microbiology. 2nd . ed . Appleton and Lange. Norwalk , connectical .
- 35 - Rello , J.; Rue , M. and Jubert , P . (1997) . Survival in patients with Nosocomial wound infection : impact of severity of illness and the etiologic agents . *Crit .Med* . 25 : 1862 – 7 .
- 36 - Tredget , E.; Shankowk , H. and Dalang , K . (1992) . Epidemiology of infections with *Pseudomonas aeruginosa* and other organisms in burn patients . *Clin. Infect . Dis* . 13 : 119 – 121.
- 37 - Marty , N.; Pasquier , G.; Dournes , J . I ; Chemin , K.; Chavoght , F.; Gunard , N.; Chabanon , G. pipy , B. and Montrozier , H. (1998) . Effect of characterized *Pseudomonas aeruginosa* exopoly saccharides on adherence to human tracheal cells , *J. Med . Microbiol* , 97 : 129 – 134 .
- 38 - Nelson , P. (1996) . Disorder of conjunctivitis in text book of pediatrics , 15 nd .ed ., India . 410 – 413 .
- 39 - Monney , BR.; Green , BJ .and Hashisaki , pA. (1994) . Non gonococcal ophthalmia associated with erythromycine ointment prophylaxis of gonococcal ophthalmia neotorum . *Infect . Control* . 5 : 138 – 40 .
- 40 - Segreti , J.; Levin , S. and Marshel , A. (1996) . B acteriological and clinical application of anew extended spectrum poreteral cephalosporin . *AM. J. Med* . , 100 : 455 – 515 .
- 41 - Saeed , R.S. (1993) . *Proteus* and other microorganisms causing urinary tract infection in children . Msc thesis , college of medicine , university of Baghdad .
- 42 - Mingeot , L.J.; Glupezynski , Y. and Tulken , R.M. (1999) . Aminoglycosides Activity and resistance . *Antimicrobiol – agents chemother* 43 (4) : 727 – 737 .
- 43 - Murray , B.E. (1997) Antibiotics resistance advance in internal medicine 22 . 2 33 – 367 .