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MOLECULAR STUDY OF MOST COMMON PATHOGENIC BACTERIA ISOLATED FROM CONJUNCTIVITIS PATIENTS IN BAGHDAD

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Abstract

Aim of the study: The current study was conducted to investigate the causes of bacterial eye infections which included inflammation of conjunctivitis, and detection of molecular study of most common pathogenic bacteriaat different age groups .

Method: study was conducted in Educational laboratories of Microbiology-Baghdad medical city,involve taken sample from Ibn al-Hatham teaching hospital,AlKindi specialized hospital and Baghdad medical city / ghazi al– Hariri teaching hospital during the period from December / 2016 to Jun / 2017.

Results: Culture investigation showed 194 positive bacterial growth of 200,103 isolate represent Gram positive,91 isolate Gram negative, and 6 no growth.Most common bacteria *were Staphylococcus aureus* showed high percentage [41(20.5%)], Whileother bacteria less than *Staphylococcus aureus.*

Conclusion: To the best of our knowledge this study was the first study in Iraq explained the molecular study of conjunctivitis patients. That concluded there 41cases infection with *Staphylococcus aureus*and showed all cases were methicillin resistance (MRSA), but not all carried gene mecA only [35(85.37%)], 13(31.71%) carried Panton-Valentine leukocidingene (PVL), and no one have VanA gene responsible of Vancomycin resistance..

Introduction

Vision is the most important special sense in human being. Normal vision is essential for normal physical, mental, psychological development and education [1].Eye infections can be caused by several agents such as bacterial, viral and fungal or parasites . In some cases allergic response can cause signs similar to infection, this infection can involve the eye itself and /or the tissue surrounding the eye.Serious infections of the eyes

that involved the deeper interior portions of the eye can result in loss of sight . Also the infection may be unilateral or bilateral, meaning involving one or both eyes, or if the infection began in one eye it may spread to the other eye . Some common eye infections are;Conjunctivitis, Blepharitis, Keratitis andUveitis [2, 3].

Conjunctivitis is defined as inflammation of the conjunctiva . It is characterized by irritation, itching, foreign body sensation, and dischargefrom the eyes . Bacterial conjunctivitis may be distinguished from other types of conjunctivitis in presence of a yellow-white mucopurulent discharge . It is categorized by the redness or swelling of the conjunctiva and the membrane that lines the eyelid and eye surface . This membrane is typically clear but when it is infected, it becomes red or pink and swells [4]. Then conjunctiva is subdivided into three parts depending on location; palpebral conjunctiva, bulbar conjunctiva and conjunctival fornix . The palpebral (tarsal) conjunctiva lines the inner eyelid, the bulbar conjunctiva covers the sclera and the fornix conjunctiva forms

ajunction between the tarsal and bulbar sections . But the conjunctival epithelium is formed of a non-keratinized layer of columnar and squamous stratified epithelial cells 2-5 cells thick, which is interspersed with goblet cells [5].The conjunctiva plays a number of important roles, including acting as a reservoir for the tears, contributing to the secretions that make up the tears, and providing a protective surface against microbial invasion . Also the conjunctiva consists of a layer of cuboidal epithelium located atop heavily vascularized loose connective tissue . The conjunctival epithelium is composed of (2 – 5) stratified layers of loosely packed epithelial cells . The conjunctiva is very active metabolically as evidenced by the large number of mitochondria present within conjunctival cells and the 17 high levels of glycolytic, Krebs cycle, and respiratory chain enzymes found by histochemical techniques . The conjunctiva also contains numerous goblet cells, whose main function is to produce mucus, which maintains the wetability of the ocular surface [6, 7]. Therefore the conjunctiva is the most immunologically active outer layer of the eye and forms a natural barrier against the invasion of exogenous substances, developing lymphoid hyperplasia after stimulation . The normal conjunctival flora contributes to the defense of the eye by preventing the growth of more pathogenic species . But anatomical barriers, mucus and antibacterial agent secretion, and the local humoral and cellular immune response form the mixed defense mechanism of the conjunctiva . Finally the development of the normal microbial flora of the conjunctiva starts at birth and the flora is present throughout life [8, 9]. The red eye is complex because it is a nonspecific sign. Redness of the eye is a sign of ocular inflammation, resulting from dilatation and/or rupture of blood vessels in the eye[10]. Numerous conditions may result in redness of the eye, including conjunctivitis, blepharitis, canaliculitis, dacryocystitis, episcleritis, scleritis, uveitis, iritis, keratitis, orbital cellulitis, corneal injury, foreign body, chemical burn, subconjunctival hemorrhage, dry eye syndrome, and acute angle-closure glaucoma. But in most cases, redness of the eye is caused by benign conditions, such as conjunctivitis .Therefore important thing to remember is that conjunctivitis may lead to blindness. A single episode of severe conjunctivitis can cause corneal scarring that could affect vision or lead to conjunctival changes that become a chronic degenerative problem [11, 12]. Bacterial conjunctivitis is probably the most common ophthalmic infection, often seen by primary care physicians [13, 14]. Then the bacterial conjunctivitis, a common infection of the ocular surface that affects individuals of all ages, typically presents with erythema, mucopurulent discharge, and chemosis . It is an extremely contagious disease caused by one or more bacterial species and affects both sexes, all ages, ethnicities and countries , with outbreaks commonly seen in schools, households, and child care facilities [15]. In 1878, Koch first noted that different diseases were caused by Gram-positive cocci depending on whether they formed pairs, chains or clusters]. [Pasteur cultivated them for the first time in 1880 [16]. In 1882 Ogston named the clustered micrococci "staphylococci," from the Greek staphyle, meaning bunch of grapes].[In 1884 Anton J. Rosenbach (1842-1923), a German surgeon, isolated two strains of staphylococci, which he named for the pigmented appearance of their colonies: *Staphylococcus aureus*, from the Latin aurum for gold, and *Staphylococcus albus*(now called *epidermidis*), from the Latin albus for white [17, 18]. The *staphylococcus aureus* is the most common etiologic agents in ocular infections, they are the predominate organisms recovered from conjunctivitis, keratitis and endophthalmitis [19, 20].

Material and Methods

Total of (200) clinical samples were collected from patients (specific - bacterial conjunctivitis according to opinion of physician and confirmed by cultures) at Ibn

al-Hatham teaching hospital, AlKindi Specialized Hospitaland Baghdad Medical City / Ghazi al– Hariri Teaching Hospital during the period from December / 2016 to Jun / 2017 .Eye swabs were collected from patients with infected bacterial conjunctivitis by sterile swabs with transport media. The sterile swab was rubbed on the inflamed area and cultured onto blood agar, MacConkey agar and chocolatemedia.These were chosen as a selective media for differential of bacteria isolate. All collected samples were inoculated on Brain Heart infusion for primary identification. They were incubated aerobically at 35˚C for overnight. Positive growths were then subjected to the following procedure:Gram's staining for differentiation between gram positive and negative bacteria[21].All gram negative bacteria were identified by API-20 E System; the procedure was done as recommended by the manufacture's instruction (BioMérieux, France). And gram positive were identified by some biochemical test (manual) to differentiation them and the most common bacteria (*staph. Aureus*) were identified by Vitek2 compact.VITEK-2 Compact which represents an advanced colorimetric technology for bacterial identification and antibiotic susceptibility. At last Detection of some gene to most common bacteria according antibiotic resistance and sensitivewere PVL, vanA and mecA genes by PCR Assay.

Results

To the best of our knowledge this study was the first study in Iraq explained the molecular study of conjunctivitis patients. Out of 200 specimens of the present study, were divided according the gender, city, residency and culture results. This data presented in Table(1) represents the distribution of patients according to gender. Show that males had a tendency to getconjunctivitis more than females were (55.5%) of the patients were males and (44.5%) females with non-significant difference between the two groups (P>0.05).City of patients were Rasafa (77%) and Karakh (23%) with highly significant (P<0.01),Residencyof patientswere urban (92%) and rural (7.5%) with highly significant (P<0.01).And culture results were positive (97%) and negative (3%) with highly significant (P<0.01).Data illustrated in Table(2) showed the distribution of patients according to the type of bacteria (strains). The most common bacteria were *staph. Aureus* (20.5%).other bacterial percentage were *Haemophilusinfluenzae (14.5)*,*Streptococcus pneumoniae* (14%), *Pseudomonas aeruginosa* (11%),*Staphylococcus epidermidis* (7%),*Moraxella catarrhalis* (7%),*Streptococcus viridance*(6.5%), *Acinetobacter species*(6.5%), *Klebsiella pneumoniae* (4.5%),*E. coil* (2%), and (3%) No growth.

In this study, all S. aureus isolates (41 isolates) were screened againstseventeen anti - S. aureus antibiotics. The resistance results were determinedby using VITIKE2 system as presented in Tables (3, 3A, and3B). Comparison of percentage of resistance among tested S. aureus to individual antibiotics indicated the presence of diversity degrees of resistance to most of antibiotics tested. An isolate was characterized as resistant if the MIC was greater than the breakpoint MIC defined by CLSI, (2015) while it will be susceptible if it is less than the break point. S. aureus showed variable degrees of resistance after comparing the results with that in CLSI (2015). Antibiotic sensitivity test for Staphylococcus Aureus growth, documented that the best antibiotics with elevated percentage (%) of sensitivity were [Tigecycline 100%] followed by [Linezolid 95.12%], [Clindamycin 78.05%] & [Levofloxacin &Moxifloxacin 75.61%], while the others noted with low sensitivity& [Benzylpenicillin&Oxacillin 100%] resistance.

Highly significant difference (P<0.01), for antibiotics except Gentamicin was

non-significantdifference (P=0.061, P>0.05).
The data in table (3A) presented the Intermediate resistance of antibiotic the percentage of Teicoplanin were(24.39%),while Vancomycin (2.44%) with highly sign.[P=0.00(P<0.01)]. Data illustrated in Table(3B) showed all cases were (MRSA) Cefoxitin Screen resistance (100%), and all cases were sensitive for Inducible Clindamycin(100%) with highlysign.(P=0.00, P<0.01). These high results of resistance may be due to the transmission mode of S. aureus and MRSA through hands, which maybecome contaminated by contact with colonized or infected individuals or through contact with colonized or infected body sites of other persons. Other factors contributing to transmission include close skin to- skincontact, crowded conditions, and poor hygiene. This agree with many study[22]
our study agree with some antibiotic in paper of Jeaz,[23, 24]. The results of Alsamarai revealed that all bacterial isolates showed high resistance (100%), to Methicillin,Ceftazidime and Oxacillin. The resistance to Ceftriaxone in the present study was 97.68%, while it was 93.02% to Cefotaxime. And the study agree in most antibiotic works in paper[25].Our study indicated a high resistant rate of MRSA to the tested antibiotics, Unfortunately this finding may lead to increased risk of MRSA infections as a result of indiscriminate using of multiple broad spectrum antibiotic[26], This can be attributed to the fact that, antibiotics may have revolutionized the treatment of commonbacterial infections[27]. Our study disagree with[28].After the sensitivity test was performed, it was found that most or all of the bacteria were resistant to methicillin. We worked with the genes that match or confirm this speech as*PVL, MecA* and *VanAgenes*Amplification of genes by PCR technique was done for the genomic DNA of 41 isolates of *Staphylococcus Aureus* to detect *PVL, MecA* and *VanA*genes. Results showed that from 41 isolates [13(31.71%)] Carry *PVL* gene, with[P=0.028 Sign. (P<0.05)], This result agreed with other studies in which PVL values were found in African countries such as Copeverde island (35%),Mali (23.9%) & in aremote population of Gabonese babongo pygmies (55.9%) of the MSSA isolated from patient , and in India such as Karnataka (62.8%) , Mumbai (64%) PVL positive MRSA[29-32], Subarna explain the high percentage of PVLproducing MRSA as well as MSSA strains is interesting and can be due to the carriage of large parts of SCC mec including the luk-F and luk-S genes toother resistant strains of S.aureus by various ways of recombination and also be due to misuse of antibiotics in thecountry causing selective pressure for development of resistant strains along with the virulence factor i.e. PVL. While disagreed with other studies in which PVL gene was less than(5%) in France, 4.9% in UK, 8.1% in Saudi Arabia,9.1% in Philippine, 14.3% in Bangladesh[33, 34] During large study period (5year) by [35]Istanbul, Turkey , a total of 102 MRSA isolates were collected from different source were isolated from skin and soft tissue infections (54.9%), respiratory tract infections (17.6%), bloodstream infections (16.7%) and(10.8%) from other sites of infection (i.e., urinary tract, intra-abdominal, osteoarticular and ocular infections) . Of the 102 isolates, only seven (6.9%) were positive for PVL genes, which disagreement with our results (31.71%)of isolates carry PVL gene.
35(85.37%) with highly sign.[P=0.00(P<0.01)] carry MecA gene These results were in agreement with some study,[36] who referred that 21 cases with methicillin resistance, 17(81%) occurred in 2005and the increase is due to community-acquired methicillin-resistant S.aureus ,in[37, 38] MRSA has emerged in patients withoutestablished risk factors, that MRSA infections by (15%) were community-associated and (85%) were health care-associated., while VanA gene did not appear in any isolate 41(100%),this agreed with[39].Table (4).

Conclusion

The current study concluded there that S. aureus that the most common pathogen causes of eye infections (conjunctivitis),Conjunctivitis was the most common infection among children was less than < 20 years, isolates were present in high percent among males 22 (53.7%) than in females 19 (46.3 %) patient with conjunctivitis. The antibiotics susceptibility obtained by VITEK-2 system methods revealed that S. aureus isolates showed multidrug resistance belong to Cefoxitin Screen resistance, Benzylpenicillin&Oxacillin 100%, whereas the most isolates were sensitivity Tigecycline 100% followed by Linezolid 95.12%, Clindamycin 78.05% &Levofloxacin &Moxifloxacin 75.61%], while the others noted with low sensitivity. PCR was a reliable technique and sensitive enough for the detection of S. aureus virulence gene such as vancomycin resistant Van A gene, PVL gene and MecA gene.

Discussion

This study shows that both Gram positive and Gram negative share in Eye infection. Were divided according the gender, city, residency and culture results.According to gender. Show that males had a tendency to getconjunctivitis more than females.,Residencyof patientsinurbanwere more than ruralagree with study[40].And culture results were positive (97%) and negative (3%) agree with[41].The most common bacteria were *staph. Aureus* (20.5%).Which agreed with[42, 43],

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Table (1): Studied parameters Distributions.

Studied parameters		Total N=200		Binomial (Z) Test (P-value)
		N	%	
Gender	Male	111	55.5%	P=0.137 Non sign. (P>0.05)
	Female	89	44.5%	
City	Rasafa	154	77%	P=0.00 Highly sign. (P<0.01)
	Karakh	46	23%	
Residency	Urban	185	92.5%	P=0.00 Highly sign. (P<0.01)
	Rural	15	7.5%	
Culture results	Positive	194	97%	P=0.00 Highly sign. (P<0.01)
	Negative	6	3%	

Table (2):Types of bacterial isolation distributions.

Types of bacterial isolation	N	%
Staphylococcus Aureus	41	20.5%
Haemophilusinfluenzae	29	14.5%
Streptococcus pneumoniae	28	14%
Pseudomonas aeruginosa	22	11%
Staphylococcus epidermidis	14	7%
Moraxella catarrhalis	14	7%
Streptococcus viridance	13	6.5%
Acinetobacter species	13	6.5%
Klebsiella pneumoniae	9	4.5%
Enterococcus. Species	7	3.5%
E. coil	4	2%
No growth	6	3%
Total	200	100%

Table (3):Antibiotic Sensitivity test for *Staphylococcus aureus.*

Antibiotic Sensitivity test (*Staphylococcus Aureus*)	Total N=41				Binomial (Z) Test (P-value)
	Sensitive		Resist		
	N	%	N	%	
Benzylpenicillin	0	0	41	100	P=0.00 Highly sign. (P<0.01)
Oxacillin	0	0	41	100	P=0.00 Highly sign. (P<0.01)
Gentamicin	14	34.15	27	65.85	P=0.061 Non sign. (P>0.05)
Tobramycin	8	19.51	33	80.49	P=0.00 Highly sign. (P<0.01)
Levofloxacin	31	75.61	10	24.39	P=0.001 Highly sign. (P<0.01)
Moxifloxacin	31	75.61	10	24.39	P=0.001 Highly sign. (P<0.01)
Erythromycin	7	17.07	34	82.93	P=0.00 Highly sign. (P<0.01)
Clindamycin	32	78.05	9	21.95	P=0.00 Highly sign. (P<0.01)
Linezolid	39	95.12	2	4.88	P=0.00 Highly sign. (P<0.01)
Tetracycline	3	7.32	38	92.68	P=0.00 Highly sign. (P<0.01)
Tigecycline	41	100.00	0	0.00	P=0.00 Highly sign. (P<0.01)
Fosfomycin	11	26.83	30	73.17	P=0.004 Highly sign. (P<0.01)
Trimethoprim	11	26.83	30	73.17	P=0.004 Highly sign. (P<0.01)

Table (3)A: Antibiotic Sensitivity test for *Staphylococcus aureus.*

Antibiotic Sensitivity test (*Staphylococcus Aurues*)	Total N=41				Binomial (Z) Test (P-value)
	Sensitive		Intermediate		
	N	%	N	%	
Teicoplanin	31	75.61	10	24.39	P=0.001 Highly sign. (P<0.01)
Vancomycin	40	97.56	1	2.44	P=0.00 Highly sign. (P<0.01)

Table (3)B: Antibiotic Sensitivity test for *Staphylococcus aureus*.

Antibiotic Sensitivity test (*Staphylococcus Aureus*)	Total N=41				Binomial (Z) Test (P-value)
	Positive		Negative		
	N	%	N	%	
Cefoxitin Screen (MRSA)	41	100	0	0	P=0.00 Highly sign. (P<0.01)
Inducible Clindamycin Resistance	0	0	41	100	P=0.00 Highly sign. (P<0.01)

Table (4): Genetics study distributions according to *Staphylococcus aureus*.

Genetics test (*Staphylococcus Aureus*)	Total N=41				Binomial (Z) Test (P-value)
	Positive		Negative		
	N	%	N	%	
Vancomycin resistance gene	0	0	41	100	P=0.00 Highly sign. (P<0.01)
Panton-Valentine leukocidin	13	31.71	28	68.29	P=0.028 Sign. (P<0.05)
Methicillin resistance mecA	35	85.37	6	14.63	P=0.00 Highly sign. (P<0.01)