American Journal of Scientific Research
ISSN 2301-2005 Issue 77 October, 2012, pp.130-141

http://www.eurojournals.com/ajsr.htm

RAPD-PCR Typing to Confirm the Role of Nasopharyngeal *Staphylococcus Epidermidis* as a Source of Conjunctivitis in the Same Patient

Munaff J. Abd Al-Abbas
Biology Department, College of Science
University of Basrah, Basrah, Iraq
E-mail: munaffjawdat@yahoo.com

Ahmed M. Al-Samak
Ophthalmology Department, College of Medicine
University of Basrah, Basrah, Iraq

Yasin Y. Al-Luaibi
Biology Department, College of Science
University of Basrah, Basrah, Iraq

Hisham F. Mohammed
Biology Department, College of Science
University of Basrah, Basrah, Iraq

Abstract

The frequency of bacterial infection associated to 24 conjunctivital patients and their nasopharynx was studied using API STAPH system. Relatedness and identity between *S.epidermidis* strains were determined by converting RAPD-PCR data into UPGMA to construct the dendrogram. *Staphylococcus epidermidis* was the most common axenic (single and pure) isolates 19 (79.1%), followed by 4 Gram positive rods (16.6%) and only one Gram negative rod (4.1%). Out of 8 patients, two (25%) have identity between strains of conjunctivitis (2 and 3) and their nasopharynx (2b and 3a, respectively). Fortuitously, a strain from nasopharynx (2a) was identical to strain from conjunctivitis (3), but from different patients. Three conjunctivital strains (5, 7 and 8) from different patients were identical. Closely relatedness between conjunctivital and nasopharyngeal strains (6 and 6a, respectively) from the same patient, and a closely relatedness strains (4 and 1a, respectively) from different patients, were reported. However, Chloramphenicol and cephalexin were an effective (95% sensitivity, for each) first line treatment for most cases of conjunctivitis. the study confirms the role of *S. epidermidis* as a common causative pathogen to conjunctivitis, and the species has the ability for transmission from nasopharynx to eye of the same individual via the nasolacrimal duct.

Keywords: *S.epidermidis*, nasopharynx, conjunctivitis, RAPD, PCR

Introduction

The conjunctiva is a thin, translucent, relatively elastic tissue layer with both bulbar and palpebral proteins, the bulbar protein of conjunctiva lines the outer aspect of globe, while palpebral protein covers the inside of eyelids, underneath the conjunctiva lie the episclera, therefore, conjunctivitis refers to any inflammatory condition of the membrane that lines the eyelids and covers the exposed surface of the sclera (Morrow and Abbot, 1998).

Conjunctivitis is a generic term due to various infections agents "bacteria, viruses or fungi" and noninfectious cases "allergic, chemical and mechanical" (Tarabishy and Jeng, 2008). Worldwide, there are an estimated of 5 million cases of infectious conjunctivitis per year (Wilhelmus, 2005). In the developed world, acute red eyes account for 1-4% of all general practitioner (GP) consultations, and about 50-75% of all cases are most frequently diagnosed as acute bacterial conjunctivitis (Sheikh and Hurwitz, 2001; Everitt and Little, 2002; Rietveld et al, 2005). In accordance with this, a study by Rose et al (2005) including clinical diagnosis of conjunctivitis yielded bacterial pathogens in 67% of the patients, viruses alone in 3% and both bacteria and viruses in 10%.

Bacterial conjunctivitis is usually divided according to its course and severity into hyperacute, acute and chronic forms, since *Neisseria gonorrhoeae* is the most frequent cause of hyperacute bacterial conjunctivitis and *Moraxella lacunata* is the species most common found in chronic angular blepharoconjunctivitis (Rubenstein, 1999; Mannis and Plotnik, 2005). Acute bacterial conjunctivitis is most frequently caused by *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Haemophilus influenza, Streptococcus pneumonia, Streptococcus viridans* and Gram negative intestinal bacteria (Seibel and Ruprecht, 1983; Weiss et al, 1993; Block et al, 2000; Wald et al, 2001; Normann et al, 2002; Buznach et al, 2005; Rose et al, 2005; Tarabishy et al, 2006). *Staphylococcus aureus* and *Staphylococcus epidermidis* are still the most common isolates among the known and opportunistic pathogen in conjunctival infection (Rubab et al, 2006; Sherwal and Verma, 2008).

Factors predisposing for conjunctival infection include ectropium and entropium, injured conjunctival epithelium following trauma, dry-eye disease or previous infection (Hovding, 2004; Mannis and Plotnik, 2005). Immunodeficiency syndrome and systematic immunosuppression also predispose for acute bacterial conjunctivitis (Friedlaender et al, 1980; Sharma et al, 2004). In vaginally delivered newborns, the birth canal of the mother (Isenberg et al, 1988; Normann, 2005), but this has been contracted by Krohn et al (1993), who suggested that bacteria causing acute conjunctivitis more commonly originated from the infants' care providers or from the infants' nasopharynx. Since, about 20% of people normally harbor staphylococci continually in the nasal passages and other 60% harbor it intermittently, in both cases, the bacteria may be a reservoir for recurrent ocular infection (Kluytmans et al, 1997). However, genotyping techniques have been used extensively to differentiate epidemiologically significant strains (Lainson et al, 2002). Random amplified polymorphic DNA (RAPD) analysis has been applied for the distinction of strains belonging to the same species (Welsh and McClelland, 1990). This method has been widely used in a variety of bacteria (Lam et al, 1995; Charlton et al, 1999; Dziva et al, 2001; Devi et al, 2012). It is a fast, sensitive for the epidemiological studies and PCR based method of genetic typing depending on genomic polymorphisms (Huber et al, 2002; Olorunfemi et al, 2005).

According to the acute conjunctival diagnosis oftenly the bacteria and the antibiotic resistance are a growing global problem (Rose et al, 2005). The studies should reinforce the need for antibiotic sensitivity of conjunctival bacteria.

The present study was performed to determine the predominant bacteria causing conjunctivitis, and to confirm if the nasopharyngeal bacteria is responsible for a conjunctival infection by comparing the precise identical strains from both sources using RAPD analysis. The sensitivity of conjunctival bacteria to various antibiotics was also evaluated.

Materials and Methods

Sample Collection

From each case of 24 outpatients with conjunctivitis ,in Basrah city/Iraq in 2010, two samples were obtained as indicated, (i) conjunctival specimen: Before taking ocular specimens, any purulent exudates, if present, were first removed from the eye using a sterile cotton swab. A sterile needle was then used to scrap materials from the palpebral conjunctival epithelium (Yip et al, 2007). (ii) Nasopharyngeal specimen: A sterile cotton swab was inserted through the nares towards posterior nasopharynx until there was a reflection from the patient. These specimens were collected from volunteers' patients (between 23 to 74 in age) by the physician having the agreement from the ministry of health.

Isolation of Bacteria

All specimens were immediately inoculated into Brain Heart Infusion Broth (HIMEDIA) and incubated at 37^{O}C for 24h. Each tube growth was streaked onto Blood Agar and Chocolate Agar (ALPHA, for each) plates. All colonies were Gram stained, Gram positive cocci were tested for catalase, coagulase production with tube method. Staphylococcal isolates were then identified for their species using API STAPH test (bioMerieus S.A.) a biochemical identification kit.

DNA Extraction

Five ml of Tryptic Soy Broth (ALPHA) was inoculated with tested bacteria and incubated at 37^{O}C for 18h. (Japoni et al, 2004). The grown bacteria was rewashed three times by Phosphate Buffer Saline (Oxoid). DNA purification kit (Promega) was used, with some modification, by adding 3µl of proteinase K and 3µl of lysozyme (Promega, for each) together to lyse the bacterial cell wall. For checking DNA, the samples were loaded in 0.8% agarose gel of 1× TBE (54g Tris-base, 27.5g Boric acid, 20ml of 0.5M EDTA, 1.0 L distilled water, PH=8, then 100ml of the solution was diluted in 400ml of distilled water) containing 1µl ethidium bromide and electrophoresed at 60V for 30 min. Products were viewed under ultraviolet light system (VilberLourmata).

RAPD-PCR

RAPD-PCR protocol and primers were according to Olorunfemi et al (2005). PCR primers (5'-TCGCCAGCCA-3') and (5'-GACACGGACC-3') tested in the present study were purchased from Promega Co. and each of 10 nucleotides long. These primers were chosen as a result of their ability to amplify (together) a large number of bands (approximately 11) to reduce the probability of any mistake in the relatedness among strains during bands comparison, and also for their ability to amplify the DNA from all isolates. Amplifications were performed by thermocycler apparatus (Thermo Co.) in 20µl reaction mixture consisting of 5µl genomic DNA (1U) and 5µl of PCR PreMix (BIONEER) covered by Korea (1U Taq DNA polymerase, 250µM of each dATP, dCTP, dGTP, and dTTP, 10mM Tris-HCl, 30mM KCl and 1.5mM MgCl2), the two primers were used together in a reaction as 1.5µl (100pmol) for each followed by 7µl of free water. The reaction mixture was overlaid with 25µl of mineral oil to prevent evaporation. The cyclic program was (i) 1 cycle of 94^{O}C for 3 min.,(ii) 45 cycles of 94^{O}C for 1 min. (denaturation), 36^{O}C for 1 min. (annealing) and 72^{O}C for 2 min. (extension), and (iii) a final extension at 72^{O}C for 7 min. The reaction products were resolved by electrophoresis in a 2% agarose gel at 60V for 1.5h. prepared in 1×TBE as described above (DNA extraction). A 2 kb ladder (Promega) was inoculated as molecular size marker. Gel was viewed under ultraviolet light, and the banding patterns were photographed by digital camera (Sony).

Data Analysis

The identical, closely related and unrelated strains were detected. Since, the RAPD bands of each individual strain were calculated for their base pair (bp) based on the ladder's bands. According to Olorunfemi et al (2005), the data of the RAPD patterns of all strains, were transformed to the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm program creating and modifying by Garcia-Vallve and Puigbo (1999; 2009). Within, RAPD patterns of individual strains were compared based on the index of similarity between samples (Chansiripornchai et al, 2000), providing a mathematical model by calculating a similarity matrix, transforms similarity coefficients into distance matrix (Distance Matrix value "0.000" indicating identical strains) and makes a clustering to construct a dendrogram from a set of variables, to study genetic variation especially with difficult or closely related RAPD patterns. Computational analysis of this type allows for direct comparisons without the need to count bands, which is specially important after loss of resolution resulting from manuscript duplication via photocopying (Dautle et al, 2002).

Antibiotic Sensitivity

Antibiotic sensitivity for conjunctivitis bacterial infections was tested with disc diffusion (Bioanalyse) method of tetracycline (30µg), clindamycin (2µg), amoxicillin (15µg), rifampin (5µg), penicillin G (10µg), ampicillin (10µg), oxacillin "methicillin" (1µg), gentamycin (10µg), cephalexin (30µg), chloramphenicol (30µg), cloxacillin (1µg) and vancomycin (30µg) by spreading 0.1 of 1.5ml Brain Heart Infusion Broth with bacteria (18h) onto Muller Hinton Agar. Each isolate was tested for growth with all antibiotic discs according to NCCLS (2000).

Results

From each of 24 patients with conjunctivitis, a material scraped by needle was taken from the conjunctival infection and the swab from nasopharynx (Table 1). All conjunctivitis showed bacterial infections (100%). *Staphylococcus epidermidis* appeared to be the predominant bacteria in conjunctivitis (79.1%). Since, it was isolated as an axenic culture in 19 of 24 samples, except two strains (clear visually) together from patient No. 17. The remaining patients were carried 4 Gram positive rods (16.6%) and only 1 Gram negative rod (4.1%) as an axenic cultures too. In nasopharyngeal cultures, 13 *S. epidermidis* (54.1%) ,with a notification there were two strains (clear visually) in patient No. 2, 5 Gram positive rods (20.8%), 4 *Staphylococcus aureus* (16.6%), 2 *Staphylococcus intermidius* (8.3%) and 1 Gram negative rod (4.1%). Notification, no diagnosis could be performed for isolate 4a as this strain was lost during subcultivation. For strains detection, RAPD reactions (Figure 1 and 2) were performed with only individuals (1 to 8) having the same bacterial species (*S.epidermidis*) in both conjunctivitis and nasopharynx. The dendrogram (Figure 3) showed two of 8 patients (25%) having conjunctivital strains (2 and 3) identical to nasopharyngeal strains (2b and 3a), respectively, with Distance Matrix of 0.000 for each (Table 2). However, the nasopharyngeal strain (2a) was identical to conjunctivital strain (3) from other patient, with Distance Matrix of 0.000. On the other hand, three identical conjunctivital strains (5, 7 and 8) from different patients were observed, with Distance Matrix of 0.000. Some closely related relationships were distinguished between conjunctivital and nasopharyngeal strains (6 and 6a, respectively) from the same patient, with Distance Matrix of 0.138, and (4 and 1a, respectively) from different patients, with Distance Matrix of 0. 195. A closely related strains, with Distance Matrix of 0.120, were also observed between strains (1 and 3) from conjunctivitis of different patients. The remaining strains from different patients' sources were considered as unrelated.

Antibiotics against conjunctivital *S.epidermidis* showed high sensitivity to cephalexin and chloramphenicol (95%, for each) , while the sensitivity was decreased to rifampin, oxacillin, gentamycin, tetracycline, clindamycin and vancomycin (85, 80, 75, 75, 70, and 70%, respectively). In contrast, the lower sensitivity was to cloxacillin, ampicillin, penicillin G, and amoxicillin (35, 30, 30 and 15%, respectively). The four Gram positive rods isolates were sensitive to all antibiotics (100%) except to penicillin and cloxacillin (2 and 0%, respectively). The single isolate of Gram

negative rod was intermediate resistant to both cloxacillin and amoxicillin but sensitive to all other (Table 3).

Table 1: Distribution of conjunctivital and nasopharyngeal bacterial isolates in patients

Patient	Source of sample	No. of isolate	Bacteria	Patient	Source of sample	No. of isolate	Bacteria
1	conjunctivitis nasopharynx	1 1a 1b	*S.epidermidis* *S.epidermidis* *S.epidermidis*	13	conjunctivitis nasopharynx	13 13a	Gr+ve rod *S.epidermidis*
2	conjunctivitis nasopharynx	2 2a 2b	*S.epidermidis* *S.epidermidis* *S.epidermidis*	14	conjunctivitis nasopharynx	14 14a	*S.epidermidis* *S.epidermidis*
3	conjunctivitis nasopharynx	3 3a	*S.epidermidis* *S.epidermidis*	15	conjunctivitis nasopharynx	15 15a	*S.epidermidis* *S.epidermidis*
4	conjunctivitis nasopharynx	4 4a	*S.epidermidis* *S.epidermidis*	16	conjunctivitis nasopharynx	16 16a	*S.epidermidis* *S.epidermidis*
5	conjunctivitis nasopharynx	5 5a	*S.epidermidis* *S.epidermidis*	17	conjunctivitis nasopharynx	17a 17b 17	*S.epidermidis* *S.epidermidis* Gr+ve rod
6	conjunctivitis nasopharynx	6 6a	*S.epidermidis* *S.epidermidis*	18	conjunctivitis nasopharynx	18 18a	Gr+ve rod *S.epidermidis*
7	conjunctivitis nasopharynx	7 7a	*S.epidermidis* *S.epidermidis*	19	conjunctivitis nasopharynx	19 19a	Gr+ve rod *S.epidermidis*
8	conjunctivitis nasopharynx	8 8a	*S.epidermidis* *S.epidermidis*	20	conjunctivitis nasopharynx	20 20a	Gr+ve rod *S.epidermidis*
9	conjunctivitis nasopharynx	9 9a	*S.epidermidis* Gr+ve rod	21	conjunctivitis nasopharynx	21 21a	*S.epidermidis* *S.epidermidis*
10	conjunctivitis nasopharynx	10 10a	Gr+ve rod *S.epidermidis*	22	conjunctivitis nasopharynx	22 22a	*S.epidermidis* *S.epidermidis*
11	conjunctivitis nasopharynx	11 11a	*S.epidermidis* Gr+ve rod	23	conjunctivitis nasopharynx	23 23a	*S.epidermidis* Gr+ve rod
12	conjunctivitis nasopharynx	12 12a	*S.epidermidis* Gr+ve rod	24	conjunctivitis nasopharynx	24 24a	*S.epidermidis* Gr+ve rod

Figure 1: Agarose gel electrophoresis shows RAPD patterns of *Staphylococcus epidermidis* strains of conjunctivitis (Lanes: 1, 2, 3 and 4) and nasopharynx (Lanes: 1a, 2a, 2b and 3a) from four patients Lanes: Ls show 2kb ladders

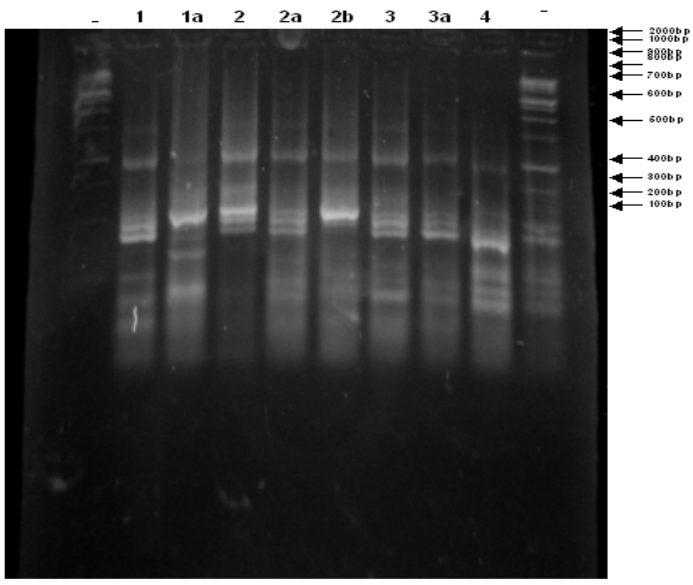

Figure 2: Agarose gel electrophoresis shows RAPD patterns of *Staphylococcus epidermidis* strains of conjunctivitis (Lanes: 5, 6, 7 and 8) and nasopharynx (Lanes: 5a, 6a, 7b and 8a) from four patients Lanes: Ls show 2kb ladders

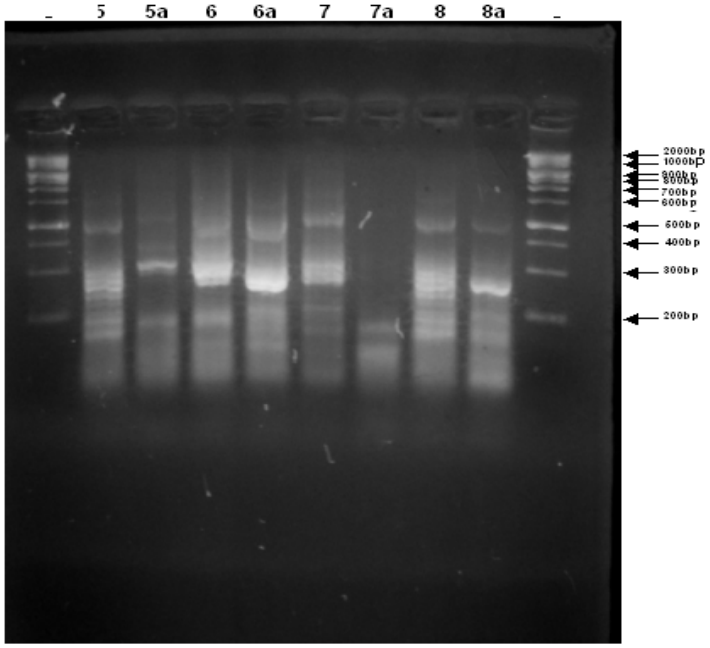

Figure 3: Dendrogram of *Staphylococcus epidermidis* strains from conjunctivitis (1, 2 , 3, 4, 5, 6, 7 and 8) and nasopharynx (1a, 2a, 2b, 3a, 5a, 6a, 7a and 8a) constructed by a set of variables (base pair of RAPD bands) using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm. Bootstrap values after 100 repetitions are indicated

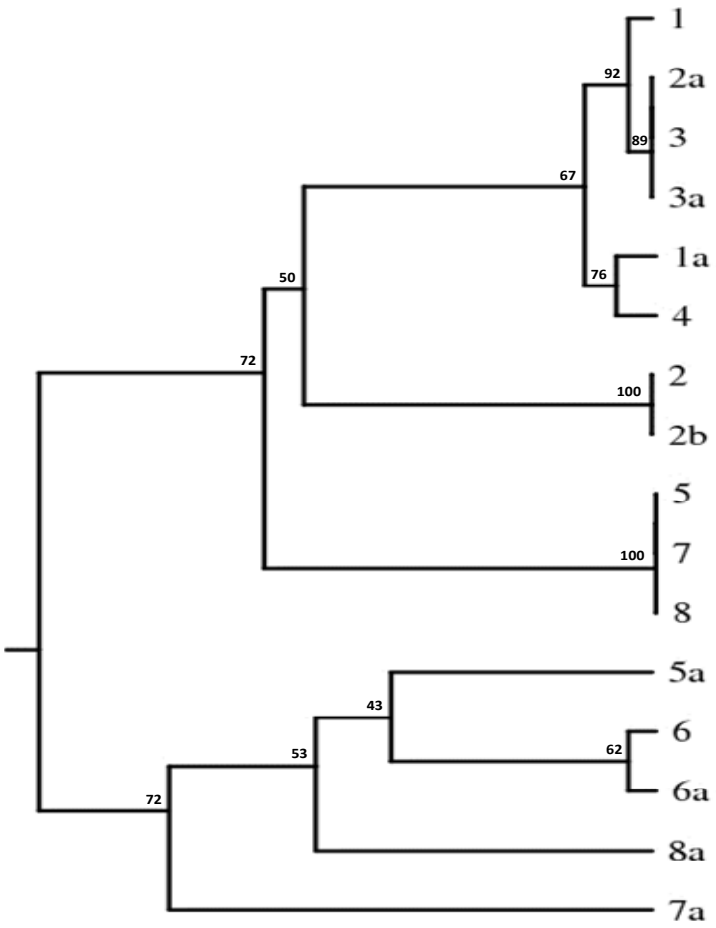

Table 2: Distance Matrix between conjunctivital and nasopharyngeal *Staphylococcus epidermidis* strains

Strains	1	1a	2	2a	2b	3	3a	4	5	5a	6	6a	7	7a	8	8a
1	0	0.293	1.751	0.120	1.751	**0.120**	0.120	0.404	2.135	3.043	2.749	2.751	2.135	3.686	2.135	2.344
1a		0	1.780	0.303	1.780	0.303	0.303	**0.195**	2.184	3.061	2.722	2.720	2.184	3.661	2.184	2.330
2			0	1.757	**0.000**	1.757	1.757	1.786	1.793	3.379	3.143	3.143	1.793	3.910	1.793	2.795
2a				0	1.757	**0.000**	**0.000**	0.396	2.165	3.112	2.773	2.778	2.165	3.743	2.165	2.344
2b					0	1.757	1.757	1.786	1.793	3.379	3.143	3.143	1.793	3.910	1.793	2.795
3						0	**0.000**	0.396	2.165	3.112	2.773	2.778	2.165	3.743	2.165	2.344
3a							0	0.396	2.165	3.112	2.773	2.778	2.165	3.743	2.165	2.344
4								0	2.150	2.976	2.598	2.595	2.150	3.563	2.150	2.187
5									0	2.746	2.646	2.634	**0.000**	3.271	**0.000**	2.486
5a										0	1.358	1.300	2.746	1.814	2.746	1.978
6											0	**0.138**	2.646	2.399	2.646	1.419
6a												0	2.634	2.302	2.634	1.425
7													0	3.271	**0.000**	2.486
7a														0	3.271	2.807
8															0	2.486
8a																0

Table 3: Antibiotic sensitivity of conjunctivital bacterial isolates

Isolates	**no.**	**Antibiotic sensitivity no. (%)**											
		TE 30 µg	**DA 2 µg**	**AM 15 µg**	**RA 5 µg**	**P 10 µg**	**APX 10 µg**	**OX 1 µg**	**CN 10 µg**	**CL 30 µg**	**C 30 µg**	**CX 1 µg**	**VA 30 µg**
S.epidermidis	20	15 (75)	14 70	3 15	17 85	6 30	6 30	16 80	15 75	19 95	7 95	14 35	70
Gr+ve rods	4	4 **(100)**	4 **(100)**	4 **(100)**	4 **(100)**	2 **(50)**	4 **(100)**	4 **(100)**	4 **(100)**	4 **(100)**	4 **(100)**	0 **(0)**	4 **(100)**
Gr-ve rod	1	S	S	I	S	S	S	S	S	S	S	I	S

S: sensitive, I: intermediate, TE: tetracycline, DA: clindamycin, AM: amoxicillin, RA: rifampin, P: penicillin G, APX: ampicillin, OX: oxacillin (methicillin), CN: gentamycin, CL: cephalexin, C: chloramphenicol, CX: cloxacillin, VA: vancomycin

Discussion

A total of 24 conjunctivital patients, *Staphylococcus epidermidis* was the most isolated bacteria (79.1%), followed by Gram positive rods (16.6%), while only one Gram negative rod (Table 1). Since, the predominant organisms isolated from ocular infections were coagulase negative staphylococci, *Corynebacteria* and *Klebsiella* (Haas et al, 2005; Mannis and Plotnik, 2005). All studies are needed to diagnose the causative pathogen precisely. Since, the infection of eye leads to conjunctivitis which is responsible for increased incidence of morbidity and blindness worldwide (Chirambo et al, 1986; Juarez-Verdayes et al, 2006). Although, *S.epidermidis* is frequently present on the healthy conjunctiva, but more traditionally pathogenic organisms, such as *Staphylococcus aureus*, streptococci, *Haemophilus* species, moraxellae and Gram negative coliform rods are also occasionally isolated from non-inflamed eyes (Cagle and Abshire, 1981; Seibel and Ruprecht, 1983; Olafsen et al, 1986; Weiss et al, 1993; Thiel and Schumacher, 1994; Mannis and Plotnik, 2005). However, A primary pathogen will regularly cause infection, an opportunistic pathogen causes infection in immunocompromized individuals, while normally occurring microorganisms may act as incidental pathogen, replicating and causing disease when host defense mechanisms have been impaired (Wilhelmus, 2005). On the other meaning, any microorganism can cause infection (Sherwal and Verma, 2008). Furthermore, *S.epidermidis* can lead to chronic blepharitis, keratitis and conjunctivitis (Baum, 1978; Pinna et al, 1999; Wieser and Busse, 2000), suggesting that opportunistic infection with *S.epidermidis* is reflecting the status of the host. However, in the present study, *S.epidermidis* was isolated from all the conjunctivital infections as an axenic cultures revealing it is the only causative pathogen of conjunctivitis in those patients. On the other hand, *S.epidermidis* was isolated from the nasopharynx of the same patients in 54.1%. Although, *S.epidermidis* is present in the nares of almost all healthy individuals (Hu et al, 1995). But, it may harbor pathogenicity (Ueta et al, 2007).

Eight patients have *S.epidermidis* in their conjunctivital and nasopharyngeal samples, and in order to explain the genetic relationships of the strains level between these two sources. Since, it is of importance in epidemiology and ecology to be able to identify bacterial species and strains accurately. However, closely related isolates are difficult to identity and differentiate using the biochemical methods (Olorunfemi et al, 2005). Moreover, some coagulase–negative species can be distinguished only by a limited number of stable biochemical test, but the precise assignment of a staphylococcal strain to a species is difficult to obtain (Wieser and Busse, 2000). Therefore, the objective of the present study is to carry out a genetic differences to different strains of *S.epidermidis* using random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) as shown in Figure (1 and 2). This RAPD procedure works with anonymous genomic markers, requires only small amounts of DNA and when compared with the biochemical methods, is simpler, cheaper and less labor intensive than other DNA marker methodologies (Olorunfemi et al, 2005). Nevertheless, the use of different and more than one RAPD primers may improve differentiation power of RAPD process (Ozbey et al, 2004). Two patients of 8 (25%) have the same strains of *S.epidermidis* in conjunctivitis and their nasopharynx , with Distance Matrix of 0.000 for each (Figure 3 and Table 2). This result confirms that the nasopharynx could be the source of conjunctivital *S.epidermidis*, which agrees with Rubab et al (2006); Yip et al (2007) suggesting that the bacteria ,in general, were also of interest to evaluate the risk of remote site like sinuses and nasopharyngeal colonization causing ocular infection. Similarly to Hovding (2008), the same strain of *Haemophilus influenza* was always found in both the eye and nasopharynx. Although, contact with contaminated fingers, eyelid margins and adjacent skin were believed to be a common cause of acute infective conjunctivitis, and more rarely from genitals or via the blood stream (Sherwal and Verma, 2008). But, the present study excluded the probability that *S.epidermidis* were transmitted between the two sources by external way. Because, from the two patients' history under study, (i) the care was taken to avoid contact with the eyelid margins during conjunctivital scraping (ii) patients were washes their faces with soap many times daily (iii) no one of them has been used the hand to scrub his eye or nose (iv) and one of them has a cold symptom with acute internal inflammation causing obstructed lacrimal duct during sampling. Interestingly, this study suggested that the rout of *S.epidermidis* for transmission from nasopharynx to conjunctiva is via internal way, particularly via the nasolacrimal duct. Since, the obstructed nasolacrimal duct and abnormal lacrimal fluid were predisposes for bacterial infection (Hovding, 2004; Mannis and Plotnik, 2005). Patient No.2 showed strain (2a) from nasopharynx was identical to conjunctivital strain (3) from patient No.3. Based on this fact, it is possible for microorganisms to spread to multiple areas from a single source (Dautle et al, 2002).

A close relation was found between conjunctivital and nasopharyngeal strains from the same patient (6 and 6a, respectively) or from different patients (4 and 1a, respectively). However, it was a hypothesis that the frequent occurrence of mutants might be responsible for a level of variation among the strains (Olorunfemi et al, 2005), producing closely relation relatedness. Particularly, when the bacteria changed their environmental site (Schwebke, 2005; Abd Al-Abbas, 2012), either inside the same patient or between different patients.

Three identical strains (5, 7, and 8) of conjunctivitis were identified from three different patients. Since, the potential transfer of microorganisms from patient to patient is either via direct contact or exposure to a common source of pathogen (Dautle et al, 2002). Such as implicating the use and sharing of mascara as a possible cause of conjunctivitis (Schwartz et al, 1989). The remaining strains of *S.epidermidis* were genetically different, as a result of multiple sources of bacterial contamination existance (Chansiripornchai et al, 2000).

Sensitivity of *S.epidermidis* was checked against antibiotic using standard sensitivity discs. It is reassuring to confirm that chloramphenicol and cephalexin (95%, for each) are still an effective and economical first line treatment for most cases of conjunctivitis, which coincides with Rubab et al (2006). Additionally, rifampin and methicillin (85 and 80%, respectively) have the second high percentage. Since, in outpatients who were found to have conjunctivitis caused by *Staphylococcus* species, the rate of methicillin resistance was lower than inpatients (Tarabishy et al, 2006). The next

level of the effective was with gentamycin and tetracycline (75%, for each), which accepted with Rubab et al (2006) result. A total of 70% of conjunctivital *S.epidermidis* were sensitive to vancomycin, but there is no vancomycin using in Basrah city. Therefore, the 30% resistance could be due to the plasmid transferring (Abd Al-Abbas, 2012). Moreover, antimicrobial resistance is not a phenomenon restricted to a specific class of antimicrobials because of cross-resistance due to overlapping targets of different antimicrobials or co-selection related to genetic linkage between resistance genes (Simonsen et al, 2003). However, *S.epidermidis* isolates showed low sensitive to antibiotics including amoxicillin, penicillin, ampicillin and cloxacillin. Since, resistant organisms are most commonly found in the intestine, but organisms living freely on the external skin of conjunctiva can also become resistant due to routine exposure to antibiotics secreted in sweat or to salted lacrimal fluid (Salyers et al, 2002; Abd Al-Abbas et al, 2012).

Conclusions

Based on the present study, *S.epidermidis* is the predominant causative pathogen to conjunctivitis. The DNA fingerprinting to detected the genetic differences among *S.epidermidis* strains showed that the nasopharynx could be the source of bacterial conjunctivitis via the nasolacrimal duct of the same patient. Simultaneously, the same strain could be responsible to conjunctivitis for different patients. However, Chloramphenicol and cephalexin are the best for conjunctival treatment.

References

[1] Abd Al-Abbas, M. J., 2012. "Antimicrobial susceptibility of *Enterococcus faecalis* and a noval *Planomicrobium* isolate of bacteria", *Int. J. Med. Med. Scien*. 4(2): 19-27.

[2] Abd Al-Abbas, M. J., Al-Hadithi, H. T. and Al-Badran, A. I., 2012. "MLST of *S.aureus* isolates identified by 16S rRNA gene sequencing", Lap Lambert Acade. Publish. Gmbh & Co. KG. Germany.

[3] Baum, J. L., 1978. "Current concepts in ophthalmology", *Ocular infections. N. Engl. J. Med*. 299: 28-31.

[4] Block, S. L., Hedrick, J., Tyler, R., Smith, A., Findlay, R., Keegan, E. and Stroman, D.W., 2000. "Increasing bacterial resistance in pediatric acute conjunctivitis (1997-1998)", *Antimicrob. Agents. Chemother*. 44: 1650-1654.

[5] Buznach, N., Dagan, R. and Greenberg, D., 2005. "Clinical and bacterial characteristics of acute bacterial conjunctivitis in children in the antibiotic resistance era", *Pediatr. Intact. Dis. J*. 24:823-828.

[6] Cagle, C. D. and Abshire, R. L., 1981. "Quantitative ocular bacteriology", *Invest. Ophthalmol*.. Vis. Sci. 20: 751-757.

[7] Chansiripornchai, N., Ramasoota, P., Bangtrakulnonth, A., Sasipreeyajany, J. and Svenson, S. B., 2000. "Application of randomly amplified polymorphic DNA (RAPD) analysis for typing avian Salmonella enteric subs", enteric. FEMS. *Immunol. Med. Microbiol*. 29: 221-225.

[8] Charlton, B. R., Biokford, A. A., Walker, R. L. and Yamamoto, R., 1999. "Complementary randomly amplified polymorphic DNA (RAPD) analysis patterns and primer set to differentiate *Mycoplasma gallisepticam* strains", *J. Vet. Diagn. Invest*. 11: 158-161.

[9] Chirambo, M. C., Tielsch, J. M., West, K. P. and Katz, J., 1986. "Blindness, and visual impairment in Southern Malawi", *Bull. WHO*. 64: 567-572.

[10] Dautle, M. P., Ulrich, R. L. and Hughes, T. A., 2002. "Typing and subtyping of 83 Clinical isolates purified from surgically implanted silicone feeding tubes by random amplified polymorphic DNA amplification", *J. Clin. Microbiol*. 40 (2): 414-421.

[11] Devi, B., Dowarah, P., Unni, B. G., Wanns, S. B., Singha, I. M. and Samanta, R., 2012. "Characterizations of selected alpha-amylase producing bacterial strains isolated from soil

samples of the alluvial plains of Southern Brahmaputra corridor of Assam", *Afric. J. Microbiol*. Res. 6 (25): 5335- 5343.

[12] Dziva, F.., Christensen, H., Olsen, J. E. and Mohan, K., 2001. "Random amplification of polymorphic DNA and phenotypic typing of Zimbabwean isolates of *Pasteurella multocida"*, *Vet. Microbiol*. 82:361-372.

[13] Everitt, H. and Little, P., 2002. "How do GPs diagnose and manage acute infective conjunctivitis ?. A GP servey", *Fam. Pract*. 19: 568-660.

[14] Friedlaender, M. H., Masi, R.J., Osumoto, M, Smolin, G. and Ammann, A. J., 1980. "Ocular microbial flora in immunodeficient patients", *Arch. Ophthalmol*. 98: 289-294.

[15] Garcia-Vallve, S. and Puigbo, P., 1999-2009. "DendoUPGMA: A dendrogram construction utility", Universal Rovira i Virgili (URV). Tarragona. Spain.

[16] Haas, J., Larson, E., Ross, B., See, B. and Saiman, L., 2005. "Epidemiology and diagnosis of hospital queried conjunctivitis among neonatal intensive care unit patients", *Infect. Dis. J*. 24: 586-589.

[17] Hovding, G., 2004. "Akutt bakteriell konjunktivitt (Acute bacterial conjunctivitis)", Tidsskr. Nor. Loegeforen. 124: 1518-1520.

[18] Hovding, G., 2008. "Acute bacterial conjunctivitis", *Acta. Ophthalmol*. 86 (1): 5-17.

[19] Hu, L., Umeda, A. and Amako, K., 1995. "Typing of *Staphylococcus epidermidis* colonizing in human nares by pulsed-field gel electrophoresis", *Microbiol. Immunol*. 39: 315-319.

[20] Huber, B. S., Allred, D. V., Carman, J. C., Frame, D. D., Whiting, D. G., Cryan, J. R., Olson, T. R., Jackson, P. J., Hill, K., Laker, M. T. and Robison, R. A., 2002. "Random amplified polymorphic DNA and amplified fragment length polymorphism analyses of *Pasteurella multocida* isolates from fatal lowl cholera infection", *J. Clin. Microbiol*. 40: 2163-2168.

[21] Isenberg, S. J., Apt, L., Yoshimori, R., McCarty, J. W. and Alvarez, R. N., 1988. "Source of the conjunctivital bacterial flora at birth and implications for ophthalmia neonatorum prophylaxis", *Am. J. Ophthalmol*. 106: 458-462.

[22] Japoni, A., Alborzi, A., Rasouli, M. and Pourabbas, B., 2004. "Modified DNA extaction for rapid PCR detection of methicillin resistant staphylococci", *Biomed. J*. 8 (3): 161-165.

[23] Juarez-Verdayes, M, A., Reyes-Lopes, M. A., Cancino-Diaz, M. E., 2006. "Isolation, vancomycin resistance and biofilm production of Staphylococcus epidermidis from patients with conjunctivitis, corneal ulcers, and endophthalmitis", *Rev. Latin. Microbiol*. 48 (3-4): 238-246.

[24] Kluytmans, J., Van Belkum, A. and Verbrugh, H., 1997. "Nasal carriage of *Staphylococcus aureus* : epidemiology, underlyning mechanisms, and associated risks", *Clin. Microbiol. Rev*. 10: 505-520.

[25] Krohn, M. A., Hillier, S. L., Bell, T. A., Kronmol, R. A. and Grayston, J. T. (1993). The bacterial etiology of conjunctivitis in early infeny. *Am. J. Epidemiol*. 138: 326-332.

[26] Lainson, F. A., Aitchison, K. D., Donachie, W. and Thomson, J. R., 2002. "Typing of Pasteurella multocida isolated from pigs with and without porcina dermatitis and nephropathy syndrome", *J. Clin. Microbiol*. 40: 588-593.

[27] Lam, K. M., Yamamoto, R. and DaMassa, A. J., 1995. "DNA diversity among isolates of Campylobacter jejuni detected by PCR-based RAPD fingerprinting", *Vet. Mjcrobiol*. 45: 269-274.

[28] Mannis, M. J. and Plotnik, R. D., 2005. "Bacterial conjunctivitis", In: Tasman, M. and Jaeger, F. A. (eds). Duane's clinical ophthalmology. 4: 1-11. Philadelphia: Lippincott, Williams and Wilkins.

[29] Morrow, G.L. and Abbott, R.L., 1998." Conunctivitis", *Am. Fan. Physician*. 57 (4): 735-746.

[30] NCCLS, national committee for clinical laboratory standard, 2000. "Performance standard for antimicrobial susceptibility testing: Supplemental tables", M100. S12. Wayne, PA, USA.
[31] Normann, E. K., 2005. "Conjunctivitis in children", *Lancet*, 366: 6-7.
[32] Normann, E. K., Bakken, O., Peltola, J., Andreasson, B., Bubl, S., Sigg, P. and Nielsen, K., 2002. "Treatment of acute neonatal bacterial conjunctivitis: a comparison of fusidic acid to chloramphenicol eye drops", *Acta. Ophthalmol. Scand*. 80: 183-187.
[33] Olafsen, L. D., Storvold, G. and Melby, K., 1986. "A microbiological study of conjunctivitis with emphasis on Chlamydia trachomatis in Northern Norway", *Acta. Ophthalmol*. 64: 463-470.
[34] Olorunfemi, O. B., Onasanya, A. A. and Adetuyi, F. C., 2005. "Genetic variation and relationship in *Staphylococcus aureus* isolates from human and food samples using random amplified polymorphic DNAs", *Afri. J. Biotechnol*. 4 (7): 611-614.
[35] Ozbey, G., Kilic, A., Ertas, H. B. and Muz, A., 2004. "Random amplified polyphormic DNA (RAPD) analysis of *Pasteurella multocida* and *Manheimia haemolytica* strains isolated from cattle, sheep and goats", *Vet. Med*. 49 (3): 65-69.
[36] Pinna, A.., Zanetti, S., Sotgiu, M., Sechi, L. A., Fadda, G. and Carta, F., 1999. "Identification and antibiotic susceptibility of coagulase negative staphylococci isolated in corneal/external infections", *Br. J. Ophthalmol*. 83: 771-773.
[37] Rietveld, R. P., Ter-Riet, G., Bindels, P. J. E., Bink, D., Sloos, J. H. and Van Weert, H. C. P.M., 2005. "The treatment of acute infections conjunctivitis with fusidic acid: a randomized controlled trial", *Br. J. Gene. Pract*. 55: 924-930.
[38] Rose, P. W., Harnden, A., Brueggemann, A. B., Perera, R., Sheich, A., Crook, D. and Mant, D., 2005. "Chloramphenicol treatment for acute infective conjunctivitis children in primary care: a randomized double-blind placebo-controlled study", *Lancet*. 366: 37-43.
[39] Rubab, S., Awan, H. and Khan, W. A., 2006. "Comparison of indigenous microbial flora of the eye to that found in conjunctival and corneal infections in a hospital based study", *Pak. J. Ophthalmol*. 22 (2): 97-107.
[40] Rubenstein, J. B., 1999. "Disorders of the conjunctiva and limbus", In: Yanoff, M. and Duker J. S. (eds). Ophthalmology. St. Louis, 12-18.
[41] Salyers, A. A. and Whitt, D. D., 2002. "Bacterial pathogenesis, A molecular approach", (2 eds). Washington, D.C. USA.
[42] Schwartz, B., Harrison, L. H., Motter, J. S., Motter, R. N., Hightower, A. W. and Broome, C. V., 1989. "Investigation of outbreak of *Moraxella* conjunctivitis at a Anaraio boarding school", *Am. J. Ophthalmol*. 107: 341-347.
[43] Schwebke, J. R., 2005. "Abnormal vaginal flora as a biological risk factor for acquisition of HIV infection and sexually Transmitted disease", *J. I. D*. 192: 1372-1380.
[44] Seibel, W. and Ruprecht, K. W., 1983. "Bacreriologische Befunde in Bindehnautabs trichen (Bacteriological finding in conjunctivital smears)", *Klin. MbI. Augenhe*. 183: 60-62.
[45] Sharma, T., Grewal, J., Gupta, S. and Murray, P. I., 2004. "Ophthalmic manifestations of acute leucemias: the ophthalmologist's role", *Eye*. 18: 663-672.
[46] Sheikh, A. and Hurwitz, B., 2001. "Topical antibiotics for acute bacterial conjunctivitis: a systematic review", *Br. J. Gene. Prac*. 51: 473-477.
[47] Sherwal, B. L. and Verma, A. K., 2008. "Epidemiology of ocular infction due to bacteria and fungus-A Prospective stiudy", *J. Med. Educa. Res*. 10(3): 127-131.
[48] Simonsen, G. S., Smabrekke, L., Monnet, D. L., Sorensen, T, L., Moller, J. K. and Kristinsson, K. G., 2003. "Prevalence to resistance to penicillin, gentamycin and vancomycin. In: *Enterococcus faecalis* and Enterococcus faecium isolates from clinical specimens and use of antimicrobials five nordic hospitals", *J. Antimicrob. Chemother*. 51:323-331.
[49] Tarabishy, A. B. and Jeng, B. H., 2008. "Bacterial conjunctivitis: A review for internists", *Clevel. Clin. Medic*. 75 (7): 507-512.

[50] Tarabishy, A. B., Hall, G. S., Procop, G. W. and Jeng, B. H., 2006. "Bacterial culture isolates from hospitalized pediatric patients with conjunctivitis", *Am. J. Ophthalmol*. 142: 678-680.

[51] Thiel, H. J. and Schumacher, U., 1994. "Uber die standortflora der menschlichen bindehaut. Untersuchungen von 135 personen unterschiedlichen Alters (Normal flora of the human conjunctiva: a study of 135 persons of various ages)", *Klin. Monatbl. Augenheilk*. 205: 348-357.

[52] Ueta, M., Iida, T., Sakamoto, M., Sotozono, C., Takahashi, J., Kojima, K., Okada, K., Chen, X., Kinoshita, S. and Honda, T., 2007. "Polyclonality of *Staphylococcus epidermidis* residing on the health ocular surface", *J. Med. Microbiol*. 56: 77-82.

[53] Wald, E. R., Greenberg, D. and Hoberman, A., 2001. "Short term oral cefixine therapy for treatment of bacterial conjunctivitis". *Pediatr. Infect. Dis. J*. 21: 1039-1042.

[54] Weiss, A., Brinser, J. H. and Nazar-Stewart, V., 1993. "Acute conjunctivitis in childhood", *J. Pediatr*. 122: 10-14.

[55] Welsh, J. and McClelland, M., 1990. "Fingerprinting genomes using PCR with arbitrary primers", *Nucl. Ac. Res*. 18: 7213-7218.

[56] Wieser, M. and Busse, H., 2000. "Rapid identification of *Staphylococcus epidermidis*", *Inter. J. Syst. Evol. Microbiol*. 50: 1087-1093.

[57] Wilhelmus, K. R.,2005. "Epidemiology of ocular infections", In: Tosman, M. and Jaeger, E. A. (eds). Duane's clinical ophthoalmology. 2: 1-64. Town: Lippincott, Williams and Wilkins.

[58] Yip, T. P. P., Chan, W. H., Yip, K. I, Que, T. L., Lee, M. M., Kwong, N. S. and Ho, C, K., 2007. "Incidence of neonatal chlamydial conjunctivitis and its association with nasopharyngeal colonization in a Hong Kong hospital, assessed by polymerase chain reaction", *Hong Kong. Med. J*. 13: 22-26.