DETECTION OF ANTIBIOTICS SENSITIVITY FOR AEROBIC AND ANAEROBIC MICROBIOTA ISOLATED FROM PATIENTS WITH CONJUNCTIVITIS.

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ABSTRACT

Conjunctivitis is one of the most common ocular infections, with bacterial infections accounting for 45-65% of all conjunctivitis cases. We conducted a study to determine the microorganism causing conjunctivitis and their antimicrobial susceptibility patterns in Al-Nahrian eye specialty center at Ramadi, west of Iraq. The patients, whose ages ranged (1 - 70) year, suffered from conjunctivitis. The cases of conjunctivitis were registered from September 2020 to February 2021. Preliminary diagnosis included 160 samples. The results demonstrated were bacterial infection, (45.6%) in total, gram positive (28.12%) and was Gram negative (17.5%). (9.4%) samples were fungal infections. After microscopic diagnosis, biochemical test, and VITEK 2 system as complementary diagnostic step, the study confirmed that the highest rate of infections resulted from Staphylococcus a spp. Negative swab refers to no growth, allergy resulting from chemical materials, foreign object, particle of dust, metal shard or viral growth. The rates were Staphylococcus aureus (21.59%), Pseudomonas aeruginosa (19.31%), Staphylococcus epidermidis (18.18%), Escherichia coli (12.5%) and Staphylococcus Schleiferi (11.36%) out of the total bacteria swabs. The ratios of sensitive and resistant isolates bacteria were different. Erthromycin and Amoxicillin were effective on all isoltes. Most of the isolates exhibited 100% resistance towards Ampicillin, Oxacilline, Methicillin and Pencillin G.

Keywords: microbiota conjunctivitis, antibiotic resistance, Staphylococcus spp.

I. INTRODUCTION

Conjunctivitis is a common disease of the eye characterized by pain, conjunctival hyperemia and discharge; common etiological agents being viruses, bacteria, allergens or chemical irritants. Pathogenic factors for this case are bacteria like Staphylococcus aureus. Viruses like, Adeno viruses and Herpes viruses. These infections do not cause pus secretions. Allergy or a foreign object can result in conjunctivitis. (1). Allergic conjunctivitis is the most frequent cause of conjunctivitis, affecting 15 to 40% of the population, and is observed most commonly in spring and summer. Bacterial conjunctivitis rates are highest from December to April. (2)

There are two types of conjunctivitis: acute and chronic. Symptoms of acute conjunctivitis include hyperemia, irritation, tearing, mucopurulent exudate, and mattering of the lids. It may begin as a unilateral condition (3). The most common bacteria species that cause infections are Staphylococcus aureus, Pseudomonas aeruginosa. Staphylococcus epidermidis, Escherichia coli. (4) and Staphylococcus Schleiferi. Staphylococcus aureus is the most common isolated bacteria. (5). Staphylococcus aureus (including drug-resistant strains such as MRSA) are found on the skin and mucous membranes, and humans are the major storage for these organisms. Lysozyme enzyme and antibiotics have no effect on microorganisms. Fungus and viruses can cause the same infections. Viral conjunctivitis is the most common cause of conjunctivitis. This infection is more common in adults than in children. The fungus in study was candida albicans.

II. MATERIALS AND METHODS

The study was conducted at Al-Nahrian conjunctivitis specialty center in Ramadi west of Iraq, from September 2020 to February 2021. During this period, patients with conjunctivitis were included in the study.
Patients who had already received antimicrobial treatment were excluded. Written informed consent was taken from the patients' guardians prior to inclusion in the study. Ethical clearance was taken from institutional ethical committee. Detailed history was taken from patients regarding such as name, age, sex, chronic diseases, previous prescriptions. For each patient, one sterile cotton swab moistened with sterile saline physiological was swept along the inferior palpebral conjunctiva, avoiding contamination from the skin and lid.

The swab was used to prepare the smearing. It was cultured on to brain-heart agar as transport media until the samples arrive at laboratory. The samples aerobically and anaerobically were cultured on MacConkey’s agar, blood agar and chocolate agar. Anaerobic growth is done by jar tank (figure 1). All the culture plates were incubated at 37°C and observed after 24 hours and 48 hrs of incubation. As a second step, a sample colony was placed on a clean glass slide, which was stained by Gram’s staining and scanned under oil immersion lens. Identification of bacterial pathogens was based on colony morphology, staining characteristics and biochemical properties following standard laboratory protocol (figure 2).

Figure (1): candle jar. Figure (2): Staphylococcus schleiferi on blood agar.

Figure (3) A: fungi on sabouraud’s Dextrose-agar agar.

Specimens were cultured on Candida chrom agar medium at 37°C for 24-48 hours (6). The vitek 2 compact system diagnosis confirmed the conventional biochemical test and microscopic diagnosis performed on the isolates. The diagnosed fungal conjunctiva infection was Candida albicans. It was diagnosed by forming the germ tube when incubated in 37°C 2-3 hours in 0.5 ml of human blood serum. Other types of Candida did not form it. Only C. albicans formed germ tube as (7) under microscopic exam. Candida albicans grew in sabouraud’s Dextrose-agar after 24 hours incubation in 37°C (figure 3) A. After being planted in chrom (figure 3) B.

Figure (3) B: Candida albicans on chrom agar.

Agar Candida culture media and incubated for 24-48 in 37°C, Candida albicans became green. This culture media was distinguishing one used with types of Candida. The diagnosis relied on the discoloring of the culture media (8). The biochemical tests, which were run on isolates, were Oxidase, Catalase, Simmon citrate, Coagulase, Indol, H2S and Urease.

Biochemical test

The isolates were diagnosed by Biochemical test to the selected colony:

Oxidase test

A colony was transported from the growing cultural media to the agar by sterilized wooden sticks to filtering papers wet with oxidase reagent. The paper turns purple after 30 seconds proves the test being positive (9).
**Catalase test**

The age of the cultural media must be 18-24 hours. A part of the colony was transported by a loop to a clean glass slide. A drop hydrogen peroxide 6%. The reagent was used to detect the bacteria ability to produce catalase enzyme that analyzes hydrogen peroxide H2O2. The test was positive if there were air bubbles (10).

**Coagulase test**

The test contains drops of plasma separated from blood with colony of bacterial cultural media placed on slide. Clotting means the test was positive (11).

**Urease test**

Urease test was prepared by adding 24.01 gm of to 950 ml of purified distilled water without autoclaving. 2 gm of urease were added to 5 ml of purified distilled water. This mix was added to urea base agar using filter paper. Urea agar media was prepared, slant. The media was vaccinated in the culture using planning method. Tubes were incubated in 37 Cº temperature for 24 hours. Pink color indicates positive results (12).

**Indol test**

The test was performed by vaccination. A single colony was placed in Peptone Water. Tubes were incubated in 37 Cº temperature for 24 hours. Then, 5 drops of Kovac’s reagent were added. The appearance of deep cherry red color at the top layer was considered as a positive test (indole reacts with Kovac’s reagent to produce deep cherry red Rosindole dye) (13).

**Citrate utilization test**

The citrate media was prepared by the slant method. The media was vaccinated by the required bacterial culture for 24 hours. If the media color changes from green to blue, as a result to PH change, the result will be positive. One of the most important gram negative opportunistic pathogens and isolates were revealed to have the ability to catabolize citrate promote hydrolysis of urea and decarboxylation of lysine and were also associated with negative indole production (14).

Simmon citrate agar test was prepared by adding 24.28 gm to 1000 ml of distilled water. The mix was sterilized by autoclave at 15 minutes and 121 pressure. The discoloration means that the test is positive (15).

**Growth test on kliglar iron agar test**

Prepared kliglar media tubes were vaccinated by planned slant and butt stabbing methods. They were also incubated in 37 C ° temperatures for 24 hours. Then, the butt was examined for the presence of H2S and glucose fermentation. The yellow color indicates the glucose fermentation in the media. On the other hand, the black color in the butt indicates the bacteria being able to produce H2S. The test was positive. kliglar iron agar was yellow in slant. (16) as in table (1)

Antimicrobial susceptibility testing was performed for all the bacterial isolates, as per the procedures described by CLSI (Clinical & Laboratory Standards Institute).

The antibiotics, used in the study, were Vancomycin 30 µg, Penicillin G 10 µg, Chloramphenicol 30 µg, Erythromycin 15 µg, Ampicillin 10 µg, Clindamycin 10 µg Trimethoprim 10 µg, Tetracycline 30 µg, ciprofloxacin 10 µg, Cefotaxime 30 µg, Levofloxacin 5 µg, Gentamicin 10 µg, and Oxacillin 1 µg.

After diagnosis, samples were cultured on Muller –Hinton agarusong sterile cotton swabs. The plates were left to dry for 5 minutes. After that, antibiotics discs were placed using sterile tweezers. The plates were incubated in 37 Cº for 24-48 hours. The bacteria sensitivity was confirmed by the emergence of a zone. The measure of the zone was compared with CLSI (17).

**III. RESULTS AND DISCUSSION**

The patients, whose ages ranged (1 - 70) year, suffered from conjunctivitis. The cases of conjunctivitis were registered from September 2020 to February 2021. Preliminary diagnosis included 160 samples. Negative swabs were 72 in number, 45 %. They mean that there was no growth, allergy resulting from chemical materials,
foreign object, particle of dust, metal shard or viral growth. The results demonstrated that were bacterial infection, (45.6%) in total, gram positive (28.12%) and was Gram negative (17.5%). (9.4%) samples were fungal infections. The study confirmed that the highest rate of infections resulted from *Staphylococcus aureus* (21.59)%, *Pseudomonas aeruginosa* (19.31)%, *Staphylococcus epidermidis* (18.18)%, and *Escherichia coli* (12.5)% and *Staphylococcus Schleiferi* (11.36)%. Fungal infections were 15 swabs, (17.04)%.

Patients with conjunctivitis were found to have a higher isolation rate of *Staphylococcus aureus*. Gram-negative bacilli, particularly *Pseudomonas* species were found in the single episode microbial keratitis group and conjunctiva (18). Contact lens can be the cause of conjunctivitis (19). *Pseudomonas aeruginosa* is the most significant conjunctivitis pathogen is a leading cause of bacterial keratitis in contact lens wearers (20).

The high ratio is attributed to the contagious diseases can be easily transmitted. The infection results from the direct contact with contaminated hands to the conjunctivitis, being exposed to contaminated air, the outbreak of pathogens, respiratory system secretions that lead to infections, some types of bacteria colonize the conjunctiva, some bacteria come from the nasopharynx due to the blockage of tear tract or nose and exchange personal objects with infected people (21).

Results showed that the infection rate in females was 59.82% and 40.18% for males. (Figure 4)

![Figure 4](image-url)

Figure (4) shows percentages of infections among age groups

![Figure 5](image-url)

Figure (5) shows percentages of infections among age groups
Group 1 = 1 ≤ 14 years old.
Group 2 = >14 ≤ 28 years old.
Group 3 = >28 ≤42 years old.
Group 4 = >42 ≤56 years old.
Group 5 = >56 ≤70 years old.

The high rates of infection cases among females can be attributed to using cosmetics like liners and mascaras. The cosmetic materials can be sources of ocular infections especially conjunctivitis. These materials, in cases of misuse, can transmit infection to other healthy people. Close contact between mothers and infants can cause the infection too.

The study demonstrated that ages (14-27) year are the highest rates of infections, 30.3%; (42-55) year 26.7 % as shown by (figure 5). The reason of the high rate of conjunctivitis in ages (14-27) year is due to activities like swimming, football, etc., make the conjunctivitis as easy target for infection and contamination. Conjunctivitis can be caused by bacteria, viruses, fungi, or typical organisms. It usually occurs in the pediatric population. Making an accurate diagnosis of these infections is further complications (22).

*Staphylococcus aureus* is one of the major and most common causes of conjunctivitis infections after surgeries. Visual impairment normally follows the infection because of the virulence of cell wall of *Staphylococcus aureus*. The virulence contributes to immunity motivation. For instance, Lipoteichoicacid and peptidoglycan (23).

*Staphylococcus aureus* mainly exists in the conjunctivitis. It is deemed the major cause of conjunctiva and it enters the upper respiratory system from the nose. Then, it moves from the nose to the conjunctiva via tear ducts resulting in conjunctivitis (24).

This study went along with (25) including *Staphylococcus* spp., *Pseudomonas* spp., other Gram-negative and fungal species like *Candida albicans*. These results went along with (26) where *Staphylococcus aureus, and Pseudomonas aeruginosa* and fungi such as *Candida albicans* are conjunctivitis bacteria. After microbiological diagnosis, biochemical test, and VITEK 2 system as complementary diagnostic step *Staphylococcus aureus* was positive to Coagulase test but *Pseudomonas aeruginosa, Staphylococcus epidermidis Staphylococcus Schleifeir and Escherichia coli* were negative. *Pseudomonas aeruginosa* was positive to Oxidase test but *Staphylococcus aureus, Staphylococcus epidermidis Staphylococcus Schleifeir and Escherichia coli* were negative. *Escherichia coli*

![Kligler Simmon Indol Urease Citrate ( A )](image)

*Escherichia coli* were negative to Indole test. The others were negative. *Escherichia coli* were negative to catalase test. The others were positive. To urease test, *Staphylococcus aureus, Staphylococcus epidermidis and Pseudomonas aeruginosa* were positive. *Escherichia coli* and *Staphylococcus Schleifeir* were negative. (27) said that *Staphylococcus schleiferi* tests negative. (28) identified *E. coli* isolates as lactose-fermenting, citrate- and urease-negative. (Figure 6) A shows the negative and .(Figure 6)B positive tests.
Figure (6). A shows the negative and B positive tests.

The study went along with (29) who stated that the common conjunctivitis pathogens are Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans. For detecting mixed Candida spp. It had Virulence factors like morphogenesis, penetration, hyphae, adhesion, biofilm formation and hydrolic enzymes (30). Positive bacteria are prevalent because they have multi virulence factors. The reason behind the prevalence of Gram positive bacteria, compared to the Gram negative, is that Gram positive bacteria naturally coexist in human skin (31). Gram positive bacteria contain a peptidoglycan layer thicker than the Gram negative; in addition to Techoiccid acid and some protein components that colonize the host’s tissues and inhibit Phagocytosis and Tissue necrosis (32).

Figure (7): Staphylococcus aureus resistance to Oxacilline

The most important factor is the abuse of antibiotics; some antibiotics may block the microorganism growth or be resistant to antibiotics. This misuse has increased the spread microorganisms such as bacteria and fungi. All Staphylococcus aureus isolates resisted the following antibiotics: Erthromycin 60%, Ampicillin (100%), Oxacilline and Pencillin G (100%). The resistance ratio was (100%) (figure 7).

These results went along with (33). The resistance rates were as follows: complete resistance (100%) toward Methicillin and Oxacilline. High resistances were to Ampicillin (91%), Piperacilllin (85.7%) and penicillin G (77%). Moderate resistance was to Tetracycline (67%), Cefotaxime (58.57%) and Clindamycin (34.28%). While low resistance was towards Gentamicin (18.57%), Levofoxacin. This went along with (34) who stated that There were a significant number of bacteria present which were resistant to ampicillin, and cefotaxime. These results agreed with (35) who stated great extent of resistance to ampicillin (100%), penicillin G (80.8%), tetracycline (77.8%) and oxacilline (66.7%).

Staphylococcus spp resistance to β- lactam antibiotic is ascribed to the ability to produce β- lactamase which breaks the antibiotic β- lactam ring. The β- lactam ring interferes in the synthesis of peptidoglycan which is one of the components of the cell wall to inhibit the construction of bacterial cell wall.

Pseudomonas Aeruginosa isolates showed high resistance to most of the antibiotics(36). It was 100% resistant towards Trimethoprime, Clindamycin and Tertracycline. The isolates were( 100%) resistant and close to (37) who said that this resistance comes from the antibiotic failure to reach the inhibiting concentration inside the cell because of the encrypted processes in the plasmids. These processes either reduce, absorb or easily move antibiotic away from the cell (38).

The results revealed that isolates resistances were (100%) to Penicillin G and (100 %) to Ampicillin. These results went along with (39) and (40) who stated that the resistance of pseudomonas aeruginosa to Amoxicillin, Ampicillin and Penicillin G was (100 %) due to having wide spectrum β lactamasas enzymes. Being on-plasmids enzymes makes it easy to move among the species of these bacteria (41).

These bacteria were (80%) resistant to Chloramphenicol, as (42) explained that These bacteria resisted Chloramphenicol. This resistance is ascribed to having many enzymes that release and block this antibiotic like...
Chloramphenicol acetyl transferase enzyme. This enzyme prevents the multi peptide from elongation, forming peptidoglycan layer and obstructs the antibiotic permeability related to antibiotic flow pumping activity.

The study results showed that all \textit{E. coli} were sensitive to most of the antibiotics. The isolates were (100%) sensitive towards Chloramphenicol Erthromycin, Tertracycline and Cefotaxime. They were (75%) sensitive towards Penicillin G, and Clindamycin. They were (80%) sensitive towards Trimethoprime.

\textit{Staphylococcus epidermidis} highly resisted penicillin G (100%) and (75%) ampicillin (43) who stated \textit{Staphylococcus epidermidis} highly resisted penicillin G.

The isolates of \textit{Staphylococcus schleiferi} showed (15 %) resistance to Oxacilin and (65%) Vancomicin (44) who confirmed that \textit{Staphylococcus schleiferi} is resistant to cefotoxine and Oxacilin. It is also resistant to β lactam and methicillin and sensitive to erythromycin (45) and (46).

<table>
<thead>
<tr>
<th>Test type</th>
<th>\textit{Staphylococcus aureus}</th>
<th>\textit{Pseudomonas aeruginosa}</th>
<th>\textit{Staphylococcus epidermidis}</th>
<th>\textit{Staphylococcus schleiferi}</th>
<th>\textit{Escherichia coli}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram stain</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Oxidase</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Simmon citrate</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urease</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Indol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>H2S</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coagulase</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Growth on kligle r</td>
<td>Acid/acid</td>
<td>Alkaline / Alkaline</td>
<td>Acid/acid</td>
<td>Acid/acid</td>
<td>Alkaline / Acids</td>
</tr>
</tbody>
</table>

Table (2) percentages of isolates resistance and sensitivity to antibiotics\textsuperscript{44}.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Staph. Aureus</th>
<th>Staph. epidermis</th>
<th>P.aeruginosa</th>
<th>E.coli</th>
<th>Staph. Schleiferi</th>
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</thead>
<tbody>
<tr>
<td>Pencillin G</td>
<td>100%R</td>
<td>100%R</td>
<td>100%R</td>
<td>75%S</td>
<td>20%R</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>100%R</td>
<td>75%R</td>
<td>100%R</td>
<td>62%S</td>
<td>80%R</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>60%R</td>
<td>65%S</td>
<td>90%R</td>
<td>100%S</td>
<td>70%S</td>
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<tr>
<td>Clindamycin</td>
<td>80%S</td>
<td>70%S</td>
<td>100%R</td>
<td>75%S</td>
<td>70%S</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>75%R</td>
<td>70%R</td>
<td>80%R</td>
<td>100%S</td>
<td>70%R</td>
</tr>
<tr>
<td>Vancomicin</td>
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<td>20%S</td>
<td>0</td>
<td>0</td>
<td>65%R</td>
</tr>
<tr>
<td>Levofoxacin</td>
<td>80%S</td>
<td>70%S</td>
<td>70%S</td>
<td>60%S</td>
<td>80%S</td>
</tr>
<tr>
<td>Tetracycline</td>
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<td>100%S</td>
<td>100%R</td>
<td>100%S</td>
<td>25%R</td>
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<td>Ceprofloxacin</td>
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<td>15 %R</td>
<td>55%S</td>
<td>80%S</td>
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</tr>
<tr>
<td>Cefotaxime</td>
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<td>30%R</td>
<td>25%R</td>
<td>100%S</td>
<td>20%R</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>15 %R</td>
<td>60%S</td>
<td>100%R</td>
<td>20%R</td>
<td>40%R</td>
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<tr>
<td>Gentamicin</td>
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<td>50%R</td>
<td>80%S</td>
<td>80%S</td>
<td>70%S</td>
</tr>
<tr>
<td>Oxacilin</td>
<td>100% R</td>
<td>80%R</td>
<td>0</td>
<td>0</td>
<td>15%R</td>
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</table>

IV. CONCLUSION

The present study reached the following conclusions:

1. Conjunctivitis was the most spread and frequent eye infection.
2. Most of the cases of Conjunctivitis were viral and allergic because of being exposed to chemical materials or foreign bodies.
3. \textit{Staphylococcus spp.} was prevalent in Conjunctivitis. \textit{Staphylococcus aureus} comes first.
4. \textit{P. aeruginosa} exhibited resistance to multi antibiotics in study.
5. The most effective antibiotic was Tetracycline. *P. aeruginosa* isolates exhibited resistance to this antibiotic.

6. *P. aeruginosa* isolates had virulence factors. For instance, hemolysin, protease and urease contrasting results.

7. *Candida albicans* caused all of the fungal infection cases.

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