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Screening And Characterization Of Ocular Infections Associated Bacterial Pathogens And Their Susceptibility Against Commonly Used Antibiotics

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Abstract

Eye infections are wide spread and are associated with different bacterial pathogens. In this study, bacterial isolates were obtained from mild, moderate and severe eye infections samples. Samples were collected from LRBT (Layton Rehmat Benevolent Trust) and spread on agar plates. Morphological, biochemical and characterization of bacterial isolates was done. The bacteria identified from mild eye infections were *Staphylococcus aureus* and *Haemophilus influenza* I. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were isolated from moderate ocula infections while from severe eye infections, *Treponema primitia* was isolated. The antibiotic sensitivity of these bacteria was also studied. All bacterial isolates showed resistance against Ampicillin. Bacterial isolates MS2B showed maximum sensitivity against Penicillin which was 11.5mm and bacterial isolates MS2A, MO1A and MO2 showed minimum sensitivity against Oxytetracycline which was 2.25mm. Optimum growth temperature observed was 37°C and pH 7. In current study, pathogenic bacteria isolated form eye infections showed resistance to commonly used antibiotics. It is because of misuse of antibiotics and self-medication resulted in resistance in pathogens against antibiotics. There is need to explore alternative ways to treat infections.

Introduction

In the today's era eye infections are spreading commonly in the global world. Eye infections pose a serious threat to vision and can also serve as a gateway for pathogens to spread beyond the orbit, potentially leading to systemic complications such as meningitis or even death. These infections may arise from viral, bacterial or fungal agents and can involve any part of the eye, ranging from the eyelids and cornea to the retina [1]. Ocular infections generally develop when harmful microorganisms invade the eyeball or its surrounding tissues. Ocular infections caused by opportunistic

pathogens are becoming more common due to the prevalent use of systemic immunosuppressive agents. The rising number of patients with human immunodeficiency virus (HIV) infection and those undergoing organ transplantation with immunosuppressive therapy further contributes to this trend. Additionally, the increased use of contact lenses provides another route for opportunistic pathogens to cause eye infections. Severe conditions such as endophthalmitis, particularly after cataract extraction and intraocular lens implantation, are frequently associated with these pathogens [2].

Although many eye infections are mild, certain cases demand immediate medical attention[3]. Other notable eye infections include stye, keratitis, cellulitis, herpes simplex virus, corneal ulcer and herpes zoster (shingles). The eye infection conjunctivitis is caused by pathogenic bacteria. Conjunctivitis is an infection or red lining on the white part of an eye and the bottom of the eye lid (conjunctiva) that can be due to disease, sensitivity, or actual physical providers like infra-red or ultra violet light. Conjunctivitis is the swelling of the conjunctiva, a lean, sensitive tissue layer that protects the eye itself and libraries the eye lid. Conjunctivitis is an extremely widespread eye problem because the conjunctiva is constantly exposed to bacteria, microorganisms and ecological vendors that can cause infections or allergies[4]. Conjunctivitis can be moderate or mild on how long the situation continues, the degree of symptom, and the type of patient or bacteria engaged. It can also impact one or both sight and, if due to disease, can be very easily passed on to others during close physical contact, particularly among kids in a childcare middle. Other brands for conjunctivitis consist of pinkish eye and red eye [5,6].

Endophthalmitis is described as an infection from the bacteria that produces within the eye. Endophthalmitis is considered a serious side-effect following cataract surgery treatment but can also happen after an infiltrating injury or injury to the eye. Endophthalmitis can cause pain, swelling, redlining and serious puffiness within the eye [7,8]. The condition is handled strongly with medications, which are treated directly by injecting into the eye. If not handled efficiently, surgery treatment is sometimes required. Prolonged eye damage and vision loss can happen if neglected. Bacterial endophthalmitis is an inflamed reaction of the intraocular liquids or tissues due to bacterial creatures. The vitreous acts as a superb channel for bacterial growth, and, in the past, animal vitreous was used as a culture channel. Bacteria, as foreign things, provoke an inflamed response. The stream of inflamed products happens resulting in a rise in the blood-ocular hurdle malfunction and a rise in inflamed cell recruiting. The damage to the eye happens from the malfunction of the inflamed tissues releasing the digestive support minerals as well as the possible poisons produced by the bacteria [9]. Devastation happens at all tissue levels that are in contact with the inflamed tissues and poisons. Another type of eye infection is corneal ulcer is an open painful area on the cornea, the evident structure in the front side of the eye. The cornea lies in front of the iris, the colored portion of the eye. Corneal ulcers typically present as grayish to whitish area on the otherwise transparent corneal surface [10,11]. In some cases, the ulcers may be too small to detect without adequate magnification and illumination.

The present work was carried out on isolated pathogenic bacteria from eye infections.

The aim of work was:

To isolate bacteria from mild to severe eye infections

To identify and characterize these bacterial isolates on the basis of

Morphological,

Biochemical and

To evaluate the optimal growth conditions of bacterial isolates

To determine the antibiotic resistance of bacterial isolates

Materials and Methods

Sample Collection:

Samples of eye infections ranging from mild to severe cases were obtained from LRBT (Layton Rehmat Benevolent Trust) using sterilized culture sticks (Figure 3). These samples were transported to the laboratory, where they were spread on agar plates [12,13].

Culture Media Used:

The primary growth medium, nutrient agar, was made by dissolving 1.6 g of nutrient agar in 1000 ml of distilled water and then sterilizing it in an autoclave at 121 °C for 15 minutes

Isolation of Culture:

The autoclaved medium was then poured into the petri plates which were allowed to solidify at room temperature. After solidification, the samples were spread onto the agar plates and incubated overnight at 37 °C. Bacterial colonies appeared which were then picked and re-incubated overnight at 37 °C (Figure 4).

Glycerol Stock Preparation:

Glycerol stocks were prepared for the purpose of preservation of bacterial cultures and LB broth or nutrient broth was prepared and autoclaved and then incubated at 37ºC for overnight. For preservation, 0.5 ml of culture broth was mixed with 500 µl of sterile glycerol and stored at –20 °C

Morphological and Biochemical Characterization of Bacterial Strains:

The isolated bacteria were characterized by morphological and biochemical tests. Morphological tests include different types of motility and staining test [14,15].

Gram's Staining:

Gram’s staining is used characterize and discriminate Gram’s negative bacteria from Gram’s positive bacteria.

Motility Test:

The gel like medium for motility test was prepared. The culture was inoculated by stabbing and incubated for 12-24 hours. The growth along line of stab was observed which reveals positive result.

Biochemical Tests:

Various biochemical tests were performed to identify the bacterial cultures, including: MacConkey test, hydrolysis of gelatin, Indole test, Starch test, oxidase test, carbohydrate fermentation test, TSI test, urease test MRVP test, citrate test, H_2S test, and catalase and nitrate reduction test.

Catalase Test:

The catalase test was performed to evaluate the ability of microorganisms to degrade hydrogen peroxide through the enzyme catalase. The appearance of gas bubbles indicated a positive result.

Urease Test:

The urease test is used to identify bacteria that use the urease enzyme to break down urea. The purple color was indicated which showed positive test.

Gelatin Hydrolysis Test:
The purpose of this test is to check the gelatinase enzyme's hydrolytic activity. Tubes remained frozen in which no hydrolysis occurred.

Carbohydrate Fermentation Test:
This test is performed to check some microorganisms which can ferment some carbohydrates e.g. sucrose and fructose with the production of an acid or gas.

Litmus Milk Test:
This test is used to distinguish between some microbes that enzymatically convert certain milk substrates into metabolic end products. The change in color of the broth was detected.

Triple Sugar Iron Test:
This test is performed to characterize various groups' even genera of enterobacteriaceae. The yellow slant and butt suggested sucrose fermentation, the red slant and butt revealed glucose fermentation. Additionally, no fermentation was indicated by the red slant and butt or no change.

Methyl Red-Voges Proskauer Test:
This test is helpful to distinguish between non-spore forming, Gram negative rods and few other species of Bacillus. MR-VP broth medium was used. Red color gives a positive result.

Citrate Test:
The basis of this test is the microbes' capacity to use ammonia as their only sources of nitrogen and citrate as the only source of carbon. The growth on medium was a positive result.

Hydrogen Sulphide Test:
For this test, Kliger iron medium was an important medium used to determine the ability of some bacteria which can produce hydrogen sulphide. After incubation blackening occurs which showed positive result.

Starch Test:
To check the starch hydrolyzing ability of bacterial strains starch test is used in which starch agar media. The Clear zone around the growth showed the positive result.

Nitrate Reduction Test:
Red color was a sign of positive result.

Blood Agar Test:
To test that the bacterial strains are pathogenic or not this test was performed. To prepare media, 2.8g of nutrient agar was mixed in 100ml of sterilized water and then autoclaved. Five milliliters of non-coagulated blood were added to this medium when temperature had decreased to 50°C. After that, the mixture was poured into petri plates, streaked and then the plates were incubated for 24 hours at 37°C. The growth shows a positive result.

MacConkey Agar Test:
The medium was made by dissolving five grams MacConkey agar in distilled water which was taken up to 100ml. After that media was autoclaved and then poured into

plates. Then streaking was done with bacterial cultures and incubated at 37°C for overnight. The pink colored colonies were observed which showed positive result.

Oxidase Test:
This test is performed to detect the presence of specific oxidase enzymes in bacteria that facilitate the transfer of electrons between bacterial electron donors and the redox dye tetramethyl-p-phenylenediamine. The dye develops a deep blue color due to oxidase activity. Using a sterilized loop, the bacterial colonies were picked and streaked onto strips impregnated with a aminodimethylaniline 1% solution. The appearance of a blue color indicated a positive reaction within 10–30 seconds.

Indole Test:
This test indicates the ability of microorganisms to decompose amino acid tryptophan to indole which accumulates in medium. Aqueous layer upon standing and a reddening of alcohol layer within few minutes indicated the positive result.

Antibiotic Resistance:
Seven agar plates were prepared, and the bacterial strains were evenly spread on them. Four different antibiotic sensitivity discs— Penicillin (Pn), Ampicillin (Am), Oxtetracycline (T) and the same Doxycycline (Do) were placed at separate regions on each plate. The plates were then incubated for 24 hours at 37°C. Clear zones around the discs indicated bacterial sensitivity to the respective antibiotics, while areas showing growth represented resistance. The diameter of the zones of inhibition was measured in millimeters using a scale (Figures 5,6).

Determination of Optimum Growth Conditions:
Two main factors i-e, pH and temperature were considered in determining the optimum growth conditions for each bacterial culture.

Determination of Optimum Temperature:
10ml of LB broth was poured into each of seven test tubes, one for each bacterial strain ranging from mild to severe, and autoclaved. After sterilization, each tube was inoculated with 20 µl of a fresh culture of the respective bacterial strain. The seven sets of test tubes were incubated at three different temperatures—25°C (room temperature), 37°C (incubator), and 4°C—for 12–14 hours. After overnight incubation, the optical density (O.D.) was measured at 600 nm. A graph was plotted to determine the optimum temperature for each strain, with temperature on the X-axis and optical density on the Y-axis.

Determination of Optimum pH:
10ml of LB broth was prepared in seven different test tubes, one for each bacterial isolate. The pH of the medium was adjusted to 5.5, 6.6, 7.0, and 8.0, and then tubes were autoclaved. After sterilization, each tube was inoculated with 20 µl of the respective bacterial culture and incubated the culture overnight at 37°C. After overnight incubation, the optical density (O.D.) was measured at 600 nm. A graph was plotted to determine the optimum pH for each isolate, with optical density on the Y-axis and pH on the X-axis.

Results
In the present study, different bacteria involved in the eye diseases of humans were isolated from ocular infection samples. The bacteria that were isolated from ocular

infections were also identified as genus *Haemophilus* sp., *Staphylococcus* sp., *T. primitia*, and *Pseudomonas* sp. (Figures 7-10).

Bacterial Strain 1 (MS2A):

MS2A was isolated from mild eye infection samples. This bacterial strain was identified as *Staphylococcus aureus* (Table 1). It is gram-positive coccus bacterium. Every individual get infected caused by staphylococcus, ranging from minor to severe infection at least once in their lifetime. The strain tested positive for indole, litmus and carbohydrate fermentation tests. (Table 2). It was non-motile and did not form endospores. *Staphylococcus aureus* exhibited resistance to Penicillin and Ampicillin (Table 3) while showing the maximum sensitivity to Doxycycline, with zone of inhibition measuring 5.57mm (Table 3).

Bacterial Strain 2 (MS2B):

MS2B was isolated from mild eye infection samples. This bacterial strain was identified as *Haemophilus influenzae* (Table 1). It is gram negative rod shaped bacteria. It is a common disease causing bacteria. It gives positive result for oxidase, catalase and indole test (Table 2). The antibacterial resistance of strain MS2B was observed. *H. influenzae* showed maximum sensitivity against Doxycycline and zone of inhibition is 14.5mm (Table 3). This bacterium was also found to be sensitive against Oxytetracycline and Ampicillin with zone of inhibitions 7.25mm and 4mm respectively (Table 3). This bacterium showed resistance against Penicillin.

Bacterial Strain 3 (MO1A):

MO1A was isolated from moderate eye infection samples. This bacterial strain was identified as *Staphylococcus aureus* (Table 1). It showed resistance against Penicillin and Ampicillin (Table 3) and showed maximum sensitivity against Doxycycline with zone of inhibition 5.57mm (Table 3).

Bacterial Strain 4 (MO1B):

MO1B was isolated from moderate eye infection samples. This bacterial strain was identified as *Pseudomonas aeruginosa* (Table 1). It is gram negative rod shaped bacterium. It gives positive result for indole and litmus milk tests (Table 2). It shows motility. The antibacterial resistance of the strain MO1B was observed. *Pseudomonas aeruginosa* showed maximum sensitivity against Oxytetracycline with zone of inhibition 6.6mm (Table 3). This bacterium was found to be resistant against Penicillin and Ampicillin (Table 3).

Bacterial Strain5 (MO2):

MO2 was isolated from moderate eye infection samples. It was characterized as *Staphylococcus aureus* (Table 1). It showed maximum sensitivity against Doxycycline with zone of inhibition 6.0mm (Table 3). It also showed resistance against Ampicillin and Penicillin (Table 3).

Bacterial Strain 6 (SS1):

SS1 was isolated from severe eye infection samples. This bacterial strain was identified as *Treponema primitia* (Table 1). It is gram negative spirilla shaped bacterium. It is a common disease causing bacteria. It gives positive result for oxidase, litmus and indole test (Table 2). The antibacterial resistance of strain SS1 was observed. *T. primitia* showed maximum sensitivity against Penicillin with zone of inhibition 11.0mm (Table 3). This bacterium was also found to be sensitive against Oxytetracycline with zone of inhibitions 7.0mm respectively (Table 3).

Bacterial Strain 7 (SS2):
SS2 was isolated from severe eye infection sample. This bacterial strain was identified as *Treponema primitia* (Table 1). It is gram negative spirilla shaped bacterium. Its oxidase and indole tests are positive (Table 2). The antibacterial resistance of stain SS2 was observed. *T. primitia* showed resistance against Doxycycline and Ampicillin (Table 3) and also showed maximum sensitivity against Penicillin with zone of inhibition is 11.5mm (Table 3).

Optimum Growth conditions:
Optimum temperature for growth of all pathogens was observes as37°C (Figure 1). Optimum pH recorded for the bacterial pathogens was 7 (Figure 2)

Discussion
Pathogenic bacteria are present in mild to severe samples of eye infections. These infections include Conjunctivitis, Corneal ulcer and the most severe cases of Endophthalmitis [16,17]. Different bacterial species belonging to the genus *Staphylococcus* sp, *Haemophilus* sp., *Pseudomonas* sp. and *Treponema* sp. have been isolated from the above mentioned diseases [18,19]. Mostly gram negative strains of bacteria were isolated as compared to gram positive strains. On average basis non-spore forming and non-motile bacteria were identified.
Different biochemical tests were performed in the laboratory to analyze the biochemical characteristics of these isolated bacterial species [20]. The effect of antibiotics on the isolated bacterial species was also observed during research work (Table 3). Mostly Catalase positive strains of bacterial isolates were observed. Identification of all these bacterial strains was done with the help of Bergey's Manual. From the mild samples, species of genus Staphylococcus and *Haemophilus* have been identified which are responsible for causing Conjunctivitis in patients. Staphylococcus aureus and Haemophilus inflenzae are the two bacterial species which are isolated from the mild samples of eye infections [21,22].
Staphylococcus aureus are gram positive coccus. These bacteria are facultative anaerobes. These are non-motile and do not form spores. These bacteria are parasites, occurring on the skin and mucous membranes of humans. S. aureus is the major pathogenic specie. These are catalase positive and oxidase negative bacteria. S.aureus forms a fairly large yellow colony on rich medium. They are strongly resistant to antibiotics such as Penicillin and Ampicillin. *H. influenzae* are facultative anaerobic bacteria. They are small, gram negative and rod shaped bacterium. They do not form spores and are non-motile. There are some strains of *H. influenzae* which contain a polysaccharide capsule. These are pathogenic bacteria and cause different diseases in humans [23]. Invasive disease caused by *H. influenzae* can affect many organ systems. The most common types of invasive disease are pneumonia, otitis media, purulent pericarditis, occult febrile bacteremia, osteomyelitis, epiglottitis, septic arthritis, cellulitis, and other less common infections such as endocarditis, and meningitis. They give positive results for certain biochemical tests performed in the laboratory such as: Oxidase and Catalase tests. They are Indole-Positive bacteria. These bacteria show resistance against Penicillin [24,25].
From the severe eye infections, species of genus *Treponema* have been identified which are responsible for causing Endophthalmitis. Treponema primitia is the respective bacterial species that have been isolated. They are motile, helically coiled organisms having a corkscrew-like shape. They stain very poorly because their thickness approaches the resolution of the light microscope. *Treponema* are delicate organisms requiring pH in the range 7.2 to 7.4, temperatures in the range 30°C to 37°C and a microaerophilic environment. The structure of these organisms is somewhat different:

the cells have a coating of glycosamino-glycans, which may be host-derived, and the outer membrane covers the three flagella that provide motility. In addition, the cells have a high lipid content (cardiolipin, cholesterol), which is unusual for most bacteria [26].

Declarations

There is no conflict of interest among authors.

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TABLE 1: MORPHOLOGICAL CHARACTERIZATION OF BACTERIA ISOLATED FROM EYE INFECTION SAMPLES

Samples	Gram Staining	Endospore Staining	Motility	Bacterial Genus
MS2A	+ve	-ve	-ve	S. aureus
MS2B	-ve	-ve	-ve	H. influenza
MO1A	+ve	-ve	-ve	S. aureus

MO1B	-ve	-ve	+ve	P. aeruginosa
MO2	+ve	-ve	-ve	S. aureus
SS1	-ve	-ve	+ve	T. primitia
SS2	-ve	-ve	+ve	T. primitia

+ve =POSITIVE
-ve =NEGATIVE

TABLE 2: BIOCHEMICAL CHARACTERIZAION OF BACTERIAL ISOLATES

+ve = POSITIVE
-ve = NEGATIVE

Tests	MS2A	MS2B	MO1A	MO1B	MO2	SS1	SS2
Oxidase	-ve	+ ve	- ve	+ve	- ve	+ ve	+ ve
Gelatin	+ ve	- ve	+ ve	-ve	+ve	+ ve	+ve
Indole	+ ve	+ ve	+ ve	+ve	+ ve	+ ve	+ ve
Carbohydrate Fermentation	+ve	-ve	+ve	+ve	-ve	+ve	+ ve
Litmus	+ve	-ve	+ve	+ve	+ve	+ve	+ve

Starch	+ve	-ve	+ve	-ve	-ve	+ve	+ve
MacConkey Agar	+ ve	+ ve	-ve	+ ve	+ ve	+ve	+ ve
Catalase	+ ve	+ ve	+ ve	+ve	+ ve	-ve	- ve
Urease	+ve	-ve	+ve	-ve	+ve	+ve	+ve
Casein Hydrolysis	+ve	+ve	+ve	-ve	+ve	+ve	+ve
Optimization IN COLD TEMP (4˚C) AT ROOM TEMP (40˚C)	No growth	No growth	No growth	No growth	NO growth	No growth	No growth
In Incubator (37˚C)	+ ve -ve	-ve +ve	-ve +ve	+ ve +ve	+ ve +ve	-ve +ve	+ve +ve

TABLE 3: ANTIBIOTIC RESISTANCE OF ISOLATED BACTERIAL STRAINS

Strains	Doxycycline (Do 30µg) (mm)	Oxytetracycline (T 30µg) (mm)	Penicillin (P 10µg) (mm)	Ampicillin (Am 10µg) (mm)
MS2A	S (5.57)	S (2.25)	R (0)	R (0)

mm = millimeter

S = Sensitive (zone of inhibition)

MS2B	S (4.5)	S (7.25)	R (0)	S (4)
MO1A	S (5.57)	S (2.25)	R (0)	R (0)
MO1B	S (6.25)	S (6.6)	R (0)	R (0)
M02	S (6.0)	S (2.25)	R (0)	R (0)
SS1	R (0)	S (7)	S (11)	R (0)
SS2	S (0)	S (6)	S (11.5)	R (0)

R = Resistance

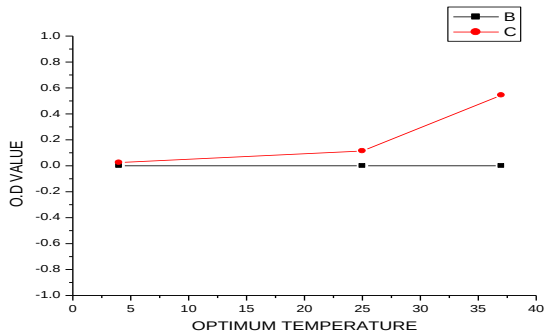

Figure 1: Graph showing optimum temperature of bacterial pathogen

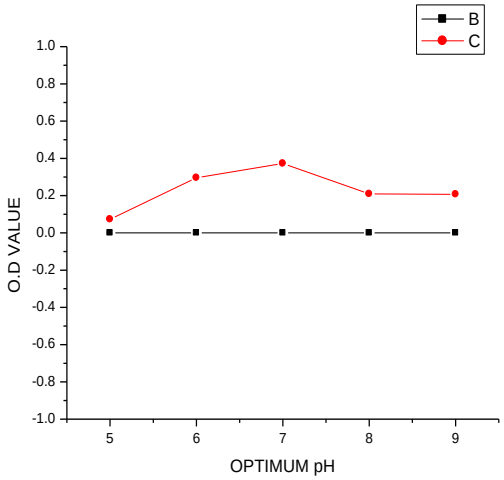

Figure 2 Graph showing optimum pH of bacterial pathogen

Figure 3: Sample tubes containing culture sticks

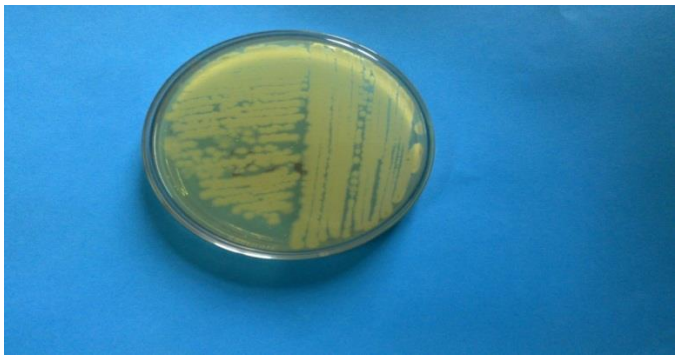

Figure 4: Growth of bacterial isolate ss1 (Pure culture)

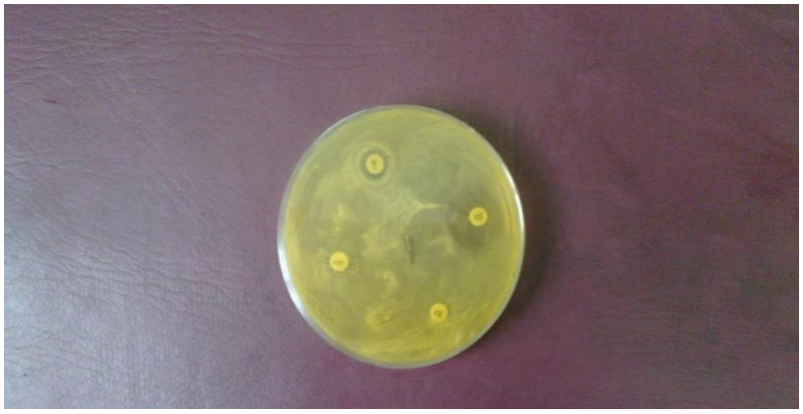

Figure 5: Antibiotic resistance of bacterial isolate MO1B (Disc diffusion method)

Figure 6: Antibiotic resistance of bacterial isolate of SS2 (Disc diffusion method)

Figure 7: Gelatin hydrolysis test, positive result

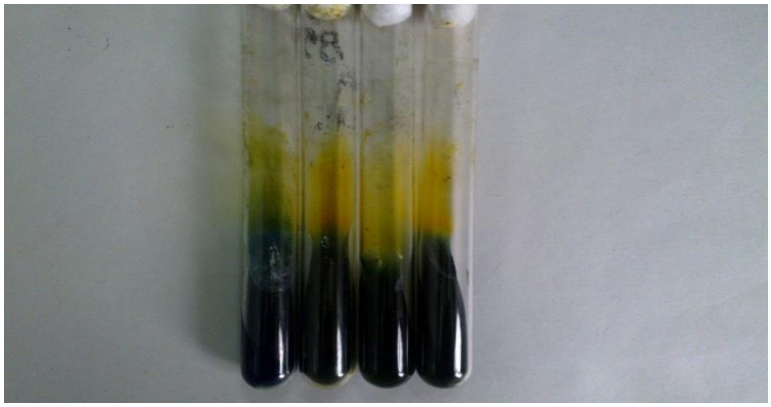

Figure 8: Citrate test, positive result

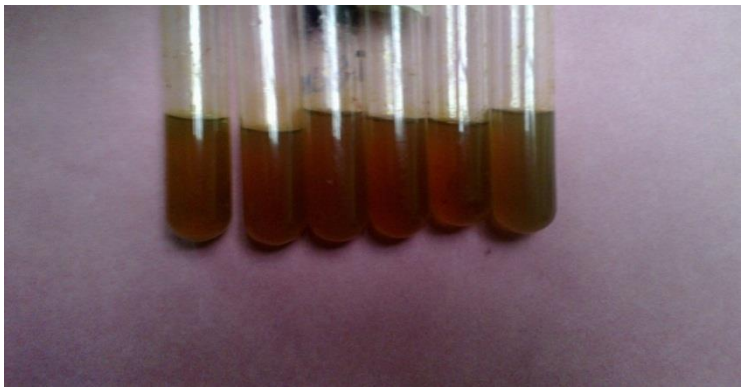

Figure 9 : Indole test, positive result

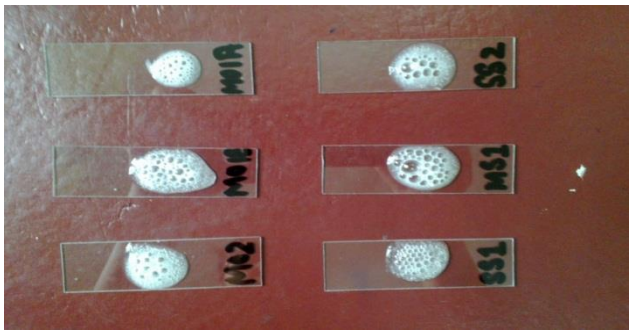

Figure 10: Catalase test, positive result