

Research Article

***Listeria monocytogenes*; A Cause of Unconstrained Miscarriages in Humans.**

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ABSTRACT:

Listeriosis is a serious type of infection that is caused mainly by the bacterium, *Listeria monocytogenes*. This infection seems harmless at first with so mild symptoms that the infected person does not recognize, but it can cause serious problems among individuals such as miscarriages, stillbirth, gastrointestinal problems, as well as serious damages among infants, the elderly as well as those who are immunocompromised. *Listeria* was regularly found in handled and prepared foodstuffs and Listeriosis was related with high morbidity and mortality. This is basically a food-borne infection that mainly comes with poorly cooked meats and dairy products. Motile through flagella, *Listeria monocytogenes* can, however move to the interior of animal cells through unstable chemical action of actin fibers (known as comet tails or actin rockets). In the current study, In order to find the links between *Listeria monocytogenes* and miscarriages, 50 blood samples were collected from patients that have recently undergone through miscarriages. Samples were collected from Bahawalpur Victoria Hospital, gynae ward and were analyzed using various tests in order to get identified and isolated from other bacterial agents, including biochemical tests, microscopy and CAMP test.

After isolation and identification, 10 out of the 50 samples were found to be *Listeria* positive, whereas 8 out of those 10 samples were *Listeria monocytogenes*. This research is a hope in preventing further unconstrained miscarriages in the future as well as infant and elderly deaths, especially among the illiterate and poor community. A more prolonged work has to be done in this field, moreover the preventive measures like proper hygienic conditions, properly cooked food, screening of food handlers and food processing plants may protect the population from this serious disease. The idea given in this research invites future researchers and health authorities to continue the research work for improvement of public health.

Keywords: *Listeria monocytogenes*, Listeriosis, Haemolysis, Miscarriages, Neonatal infections.

[I] INTRODUCTION

Listeria is a bacterium present almost everywhere like soil, food items, humans and even animals for example cattle and poultry. It can be accessible in rough deplete and sustenance created utilizing rough deplete. It can moreover live in food processing plants and defile an assortment of prepared meats. It is not in the slightest degree like various distinctive germs since it can turn out to be even exposed temperature of the cooler. *Listeria* is killed by high heat and purification techniques [19]. *Listeria monocytogenes* is a kind

of disease causing microorganisms that causes the disease, called Listeriosis. It happens to be a facultative pathogenic bacterium, which can grow and live without, or with oxygen. It can grow and duplicate inside the host cells. The bacterium *Listeria monocytogenes*, which is gram-positive in nature, from the division Firmicutes, named after the great biologist in history of science, Joseph Lister is considered as omnipresent pathogen which lives inside the host cells and which has been ensnared inside the previous decade as the

reason of life form in a few episodes of foodborne sickness[14]. There are six distinct types of the bacterium *Listeria*. Just a single of these animal categories *Listeriamonocytogenes* (*L. monocytogenes*) causes human ailment – the others are generally un-effective for people[11].

Miscarriage is a term used for a pregnancy that ends suddenly, between the underlying 20 weeks of fetus growth. Miscarriage or unexpected labor is the common-most and broadly perceived kind of abortion, woman may not understand that she conceived when she experiences a synthetic pregnancy [6, 20]. Generally, pregnant ladies infected with *listeria* don't feel sick themselves, rather, they can unconsciously pass *Listeria sp.* on to their unborn infants. In any case, if the infection occurs as the pregnancy proceeds, at that point the mother is more in danger. If an unborn infant is infected with *Listeria* later on in the pregnancy, the child could create concerning medical problems, including paralysis, seizures, visual deficiency, intellectual disability, or impairments of the kidney, heart, and mind. The reason the mother is more vulnerable is not really in light of the fact that her immune system is compromised, but rather on the grounds that the microbes that got into her placenta infected her and miscarriages that occur from these infections could be a body's natural defensive mechanism to disperse this source of disease [1].

Only a small number of people who consume *Listeria* get sick or get side effects. For the ones who get the side effects because of their contamination, the disease is either mild or very extreme, though is in a few cases insinuated as a "bimodal dispersion of seriousness". On the mellow side, Listeriosis ordinarily consists of the sudden rise in body temperature, chills, serious migraine, spewing, and other flu-like side effects [19]. Although, the historical backdrop of Listeriosisbackpedals approximately 60 years. In any case known as an explanation behind plagues and sporadic cases in approximately 50 types of creatures, the ailment now shows up with expanded recurrence in human populaces in danger. The infective agent

Listeriamonocytogenes is essentially a psychrophilic bacterium with a wide pathogenic potential along these lines inciting basically pyaemia, cerebromeningitis and intrauterine infections. Late perceptions demonstrate definite sorts of sustenance as the guideline mode for transmission of Listeriosis[9].

Motile through flagella, *Listeria monocytogenes* can, however move to the interior of animal cells through unstable chemical action of actin fibers (known as comet tails or actin rockets). Research suggests that even up to 10% of human alimentary canal would have been populated by *Listeria monocytogenes*[4]. This bacterium is oxygen consuming and not obligate anaerobic. Sugars are fermented which produce acid, not gas [12]. The event of diseases in man is sporadic inspite of the fact that a couple of pandemics have been accounted for remarkably in Germany[18]. Uterus-related disease of the embryo brings about death, or an intensely sick baby with a purulent dispersed type of infection, Listeriosis. Popular injuries of the skin might be seen in Listeriosis in the infant [2]. A comparable cutaneous form is accounted for in people working with infected creatures [3]. The intracellular existence-cycle of the bacterium shields it from hosts' both innate and adaptive immune reactions.

Anti-microbial remedy calls for agents, capable of infiltrating, distributing and staying stable inner host cells [8]. In spite of the giant statistics on *L. Monocytogenes* pathogenesis, key focuses with recognizing to Listeriosis amid pregnancy and the perinatal duration stay difficult to understand. This audit compresses Listeriosis in human beings and home creatures amid being pregnant, and creature fashions used to have a look at the route of pathogenesis and safe response to *L. Monocytogenes* contamination amid those periods[17, 7]. This sickness has a lesser prevalence of infection, no matter the truth that this became undeniably expanding, with an excessive casualty charge among the ones inflamed. In expectant women, this infection may also motive miscarriage, fetal death, or infant morbidity as septicemia and meningitis

[13]. Listeriosis could be analyzed by means of the placental examination on its own, and this examination as regularly as feasible fortified the clinical willpower [21]. Treatment of Listeriosis changes with time, depending upon the extent of the signs and symptoms. The vast majority with mellow manifestations require no treatment generally. Progressively common infections can be treated with antibiotics. During pregnancy, incite anti-microbial treatment may, sometimes, help in protecting the infant from the disease [16].

[II] MATERIAL & METHODS

2.1. Place of work

All the experimental work was carried out at IMBB department at the University of Lahore. It was basically a cross sectional study consuming a 6 month period. Samples were selected on the basis of inclusion and exclusion criteria. The age range of females was from 15-45 and this was inclusion criteria. Exclusion criteria: Patients not undergoing sudden miscarriages. Repeated sample from same patient during the same episode of illness.

2.2. Sample Collection

A total of 50 blood donors (n=50) which were all women undergone through a miscarriage in the respective week from the sexually active group of 18-45 years were accepted during the period from gynae ward at Bahawalpur Victoria Hospital Bahawalpur, Punjab, Pakistan. Whole blood was collected by venipuncture aseptically in such way as to avoid haemolysis.

2.3. Sample Storage

Blood serum was stored at 2 to 8 degrees C. The bacterial samples were stored in Glycerol stock for preservation for up to 4 months if the test is to be run within seven days of a collection. If test is delayed more than seven days, then the specimen should be frozen at -20 degrees C or colder. Specimen showing particulate turbidity or matter will be centrifuges before testing in order to avoid providing erroneous results. The total 50 samples were used in the study either females, voluntarily,

between the age group 18 to 45 from Bahawalpur Victoria Hospital according to standard methodology.

[III] EXPERIMENTAL WORK

Different confirmatory tests including gram staining, microscopy, biochemical tests, and CAMP test were used to confirm the presence of the bacterium *Listeria monocytogenes* in the blood of patients undergoing a miscarriage.

3.1. Gram staining

Principle of Gram Staining: This differential type of recoloring technique disintegrates most microbes into two categories based on their cell wall content: Gram positive bacteria have a thick layer of peptidoglycan due to which 90% of cell wall retains the color purple. Gram negative microbes having thin layer of peptidoglycan, i.e 10% of cell wall and high lipid content – is colored pink.

Procedure of Gram staining:

1. A clean, oil free glass slide was taken.
2. Then the spread of mixture on the clean slide with a loop full of the sample was set.
3. This slide was then air dried and then was heat fixed.
4. Crystal Violet was then poured on this slide and was kept for around 30 seconds to 1 minute and washed with distilled water.
5. Surged the gram's iodine for 1 moment and washed with distilled water.
6. At that point, washed with 95% liquor or $(\text{CH}_3)_2\text{CO}$ for around 10-20 seconds and flushed with distilled water.
7. Then safranin was added for around 1 moment and washed with water.
8. Air dried, Blot dried and Observed under the Microscope.

3.2. Catalase test:

Principle of Catalase test: The enzyme catalase, intervenes the disintegration of H_2O_2 into oxygen and water. The presence of this enzyme in a bacterial sample is obvious when a little inoculum is brought into H_2O_2 , and a quick rise of oxygen bubbles happen. The absence of this enzyme, catalase is clear by an absence of or a little bubble

creation. The culture under observation ought not to be over 24 hours old.

Microbes consequently shield themselves from the deadly impact of H₂O₂ which is collected as a final result of aerobic carbohydrate metabolism.

3.3. CAMP test:

Principle of CAMP test: The CAMP test identifies a creation of diffusible, heat-stable, an extracellular protein known as a CAMP factor, delivered by the Group B Streptococcus. This factor acts antagonistically with the beta lysin created by *Staphylococcus aureus* to deliver an area of improved lysis of sheep or cow Red blood cells. The authentic CAMP test relies upon the progress of two toxins amid development to create a typical arrowhead or some flame shaped clearing at the intersection of the two different bacterial cultures when they are placed opposite to one another.

The quick test uses a concentrate of Staphylococcal beta-lysin that acts upon the CAMP factor directly, which is already diffused in the medium around the *S. agalactiae* colony. The positive CAMP response is demonstrated by a visible hemolysis in 30-60 minutes of including a drop of CAMP factor reagent.

3.4. The use of selective media

Principle of selective media: *Listeria monocytogenes* can develop effectively in Oxford Listeria Agar base (7428) and also Brain Heart Infusion Agar (BHI). Brain Heart Infusion Agar is exceedingly nourishing and can bolster amazing development of a huge assortment of microbes. It can additionally be improved by the option of blood or provide particular properties by including diverse antimicrobials. It is a broadly useful medium utilized for essential disconnection of vigorous microorganisms from medical examples. Expansion of 50 mg/l chloramphenicol or 40mg/l streptomycin or a blend of 50mg/l gentamicin and 50mg/l chloramphenicol alongside 5-10% sterile de-fibrinated blood is frequently suggested for the hindrance of microorganisms and disconnection of pathogenic foundational growths.

Proteose peptone and imbuements utilized as a part of the media fills in as wellsprings of carbon, nitrogen, vitamins, amino acids, alongside basic development points. Dextrose is the vitality origin. NaCl keeps up the osmotic balance of the medium while disodium phosphate cushions the medium. Sheep blood with removed fibrin added to the basal medium gives fundamental development variables to the more exacting contagious living beings.

[IV] RESULTS

4.1. Isolation of *Listeria monocytogenes*

The microscopic and biochemical investigation of 50 samples from 50 patients with unconstrained miscarriages uncovered 10 samples resembling *Listeria* species. These 10 isolates were recuperated from 10 different cases. Out of these 10, 8 isolates were distinguished as *L.monocytogenes* and were taken from the whole blood of miscarriage patients. The remaining listeriae could be described as *L. seeligeri* and *L. welshimer*

Microscopy:

Different bacterial structures were obtained. The structures under observation were Gram positive Rods, the specific shape of the bacterium *Listeria monocytogenes*. [Fig.1]

CAMP Test:

10 out of 50 samples were CAMP test positive. Obvious hemolysis was demonstrated by an arrow point molded zone of beta-hemolysis at the intersection of the two cultures.

In CAMP Spot test positive outcome is shown by the appearance of clear zone (circular segment or circle) of improved hemolysis. [Fig. 2, 3]

Catalase test: 19 out of 50 samples were catalase positive. Catalase Positive response was Evident by prompt bubble formation. [Fig.4]

Selective media:

Different species of *Listeria* was seen on the surface of selective media, Hence its presence was confirmed in some cases.[Fig.5]

4.2. Final Results

In our study, 50 blood samples from the patients of sudden miscarriages were taken and their

bacterial contents were isolated. After various biochemical tests and procedures, it was concluded that a total of 10 samples among those [Table-1.1]

50, contained the different species of bacterium *Listeria* while 8 of these 10 were confirmed to be *Listeria monocytogenes*.

Sample No.	Microscopy	Selective media	CAMP test	Catalase test	<i>Listeria</i> Spp.
1	+ve Rods	+ve	+ve	+ve	+ve
2	+ve Rods	+ve	-ve	+ve	-ve
3	+ve Rods	+ve	-ve	+ve	-ve
4	-ve Rods	+ve	-ve	+ve	-ve
5	+ve Rods	+ve	+ve	+ve	+ve
6	+ve Rods	-ve	-ve	+ve	-ve
7	-ve Rods	+ve	-ve	-ve	-ve
8	+ve Cocci	-ve	-ve	-ve	-ve
9	+ve Rods	+ve	+ve	+ve	+ve
10	+ve Rods	+ve	+ve	+ve	+ve
11	Spirilla	+ve	-ve	-ve	-ve
12	Coccobacilli	Mixed	-ve	-ve	-ve
13	+ve Rods	+ve	+ve	+ve	-ve
14	-ve Rods	Mixed	-ve	-ve	+ve
15	-ve Rods	-ve	+ve	+ve	+ve
16	Cocci	+ve	-ve	+ve	-ve
17	-ve Rods	+ve	-ve	-ve	-ve
18	+ve Rods	+ve	+ve	+ve	+ve
19	Vibrio	-ve	-ve	-ve	-ve
20	Unidentified	Mixed	-ve	-ve	-ve
21	-ve Rods	+ve	+ve	+ve	-ve
22	Unidentified	-ve	-ve	-ve	-ve
23	+ve Rods	+ve	+ve	+ve	+ve
24	-ve Cocci	+ve	-ve	-ve	-ve
25	+ve Rods	+ve	+ve	+ve	+ve
26	Cocci	-ve	-ve	-ve	-ve
27	-ve Rods	+ve	-ve	-ve	+ve
28	+ve Rods	+ve	-ve	+ve	-ve
29	Vibrio	+ve	-ve	-ve	-ve
30	Unidentified	-ve	-ve	-ve	-ve
31	-ve Rods	Mixed	-ve	-ve	-ve
32	Unidentified	+ve	-ve	-ve	-ve
33	+ve Rods	-ve	-ve	-ve	-ve
34	-ve Cocci	Mixed	-ve	-ve	-ve
35	+ve Cocci	-ve	-ve	-ve	-ve
36	Vibrio	+ve	-ve	-ve	-ve
37	Unidentified	Mixed	-ve	-ve	-ve
38	-ve Rods	-ve	-ve	-ve	-ve
39	Unidentified	+ve	-ve	-ve	-ve
40	+ve Rods	+ve	-ve	+ve	-ve
41	-ve Cocci	+ve	-ve	-ve	-ve
42	+ve Cocci	-ve	-ve	-ve	-ve
43	Cocci	Mixed	-ve	-ve	-ve
44	-ve Rods	+ve	-ve	+ve	-ve

45	+ve Cocci	-ve	-ve	+ve	-ve
46	-ve Rods	+ve	-ve	-ve	-ve
47	Unidentified	Mixed	-ve	-ve	-ve
48	Unidentified	Mixed	-ve	-ve	-ve
49	Unidentified	Mixed	-ve	-ve	-ve
50	Unidentified	Mixed	-ve	-ve	-ve

Table. 1.1 Identification of *Listeria monocytogenes*.

[V] DISCUSSION

In our study, 50 blood samples from the patients of sudden miscarriage were taken and their bacterial contents were isolated. After various biochemical tests and procedures, it was concluded that a total of 10 samples among those 50, contained the different species of bacterium *Listeria* while 8 of these 10 were confirmed to be *Listeria monocytogenes*. The patients were from the rural areas of southern Punjab, Pakistan, where basic facilities lack and the people are mostly illiterate. This is somehow, why the patients had no idea of the severity of the disease, while considering the mild symptoms as “Normal”.

Listeria monocytogenes during the period of pregnancy is directly linked to miscarriage, premature birth, and neonatal, including septicemia and cerebromeningitis. However the, chance of the above symptoms is believed to be most prominent during the last three months of pregnancy [23]. In our present study, we observed the women in hospital undergoing through miscarriage in the third trimester of pregnancy, where 8 out of 50 women had *Listeria monocytogenes* reported in their blood. *Listeriosis* is the most prominent infection among the most deadly bacterial infections for babies and infants. In any case, pregnant women who get infected with *Listeria* generally go through simply mild symptoms, making it hard to diagnose, despite when the baby is mortally infected and at high risk of death [5]. In our study we witnessed that women infected with *Listeriosis* themselves did not realize the severity of the disease. They told us they did not feel sick and suffered through a miscarriage spontaneously.

Listeria was regularly found in handled and prepared foodstuffs and *Listeriosis* was related with high morbidity and mortality [8]. Our patients told us that they used to have meat poultry and artificial foodstuffs on daily basis and that they didn’t know they could cause such harm to their babies.

Clinical statistics were gathered on 722 isolates of *Listeria monocytogenes* infection in humans. 248 cases (34%) were related to being pregnant (maternal, fetal, and neonatal), and contained 9 isolates (4%) of maternal infection with no disorder of the infant; 42 samples (19%) of the intra-uterine passing; 118 samples (54%) of infant ailment analyzed inside 2 days child blues; and 50 isolates (23%) of infant sickness analyzed as sick following 2 days infant blues. A preferred mortality of half was recorded [10]. In our study, 10 out of 50 cases were preferred to be positive having the organism *Listeria* and 8 of these were undoubtedly *Listeria monocytogenes*.

Listeria's unusual microbiologic highlights make it a troublesome infection to identify and deal with: it is miles an intracellular creature that covers up interior cells of the host. As a result of the viable drastic results, it is vital that gynaecologists understand about the diagnosis, treatment, and prevention of this contamination [22]. It was observed that almost 50% of sudden miscarriages due to *Listeria monocytogenes* occurred due to negligence of the gynecologists, who did not take the mild symptoms being told, seriously. Moreover, in rural areas like southern Punjab, people do not prefer going to the doctors for their regular checkups until it goes beyond the limits, and never really realize the issues going on.

4597 placentas with medical signs and symptoms for observation were reflectively pondered for morphologic affirmation of Listeriosis. Seven placentas have been distinguished as having Listeriosis. Listeriosis could be analyzed by means of the placental examination on its own and this examination as regularly as feasible fortified the clinical willpower. At the point when the

contamination was no longer suspected clinically, the exam helped in accomplishing the proper finding [21]. We identified 8 cases out of 50 as *Listeria* positive, but if we go to direct placental examination, we could find more of the cases being positive, as the bacterium commonly resides inside the placental host cells. Therefore, results could be improved [21, 15].

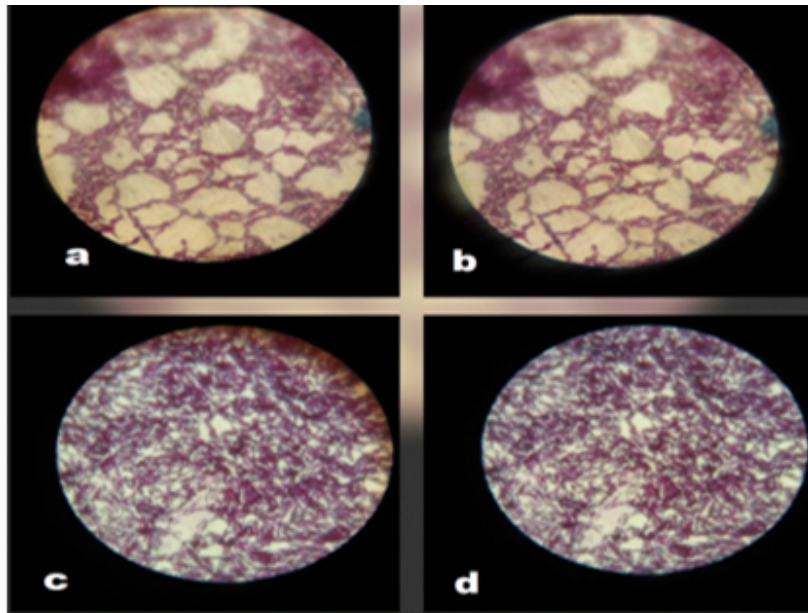


Fig. 1. Microscopic view of *Listeria monocytogenes*.

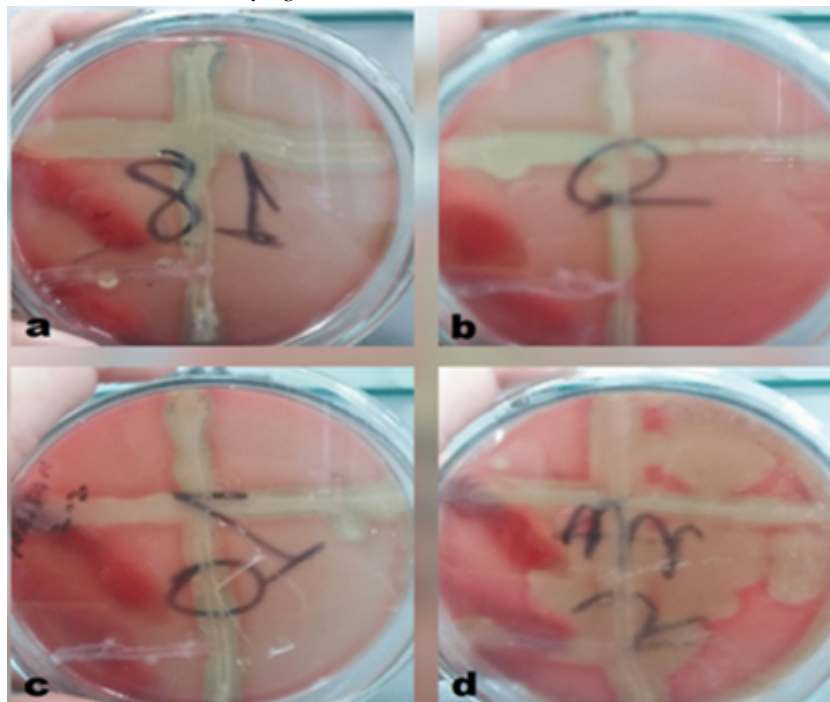


Fig. 2. Positive CAMP test

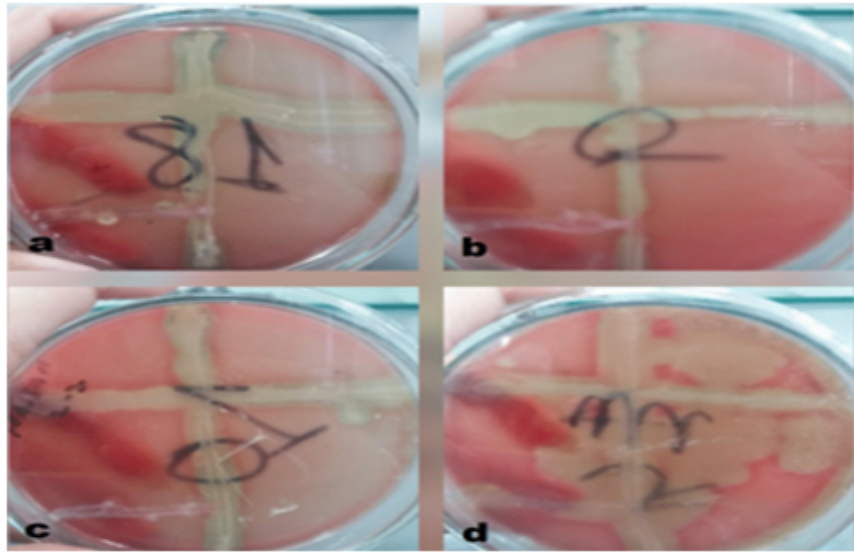


Fig. 3. Positive CAMP test

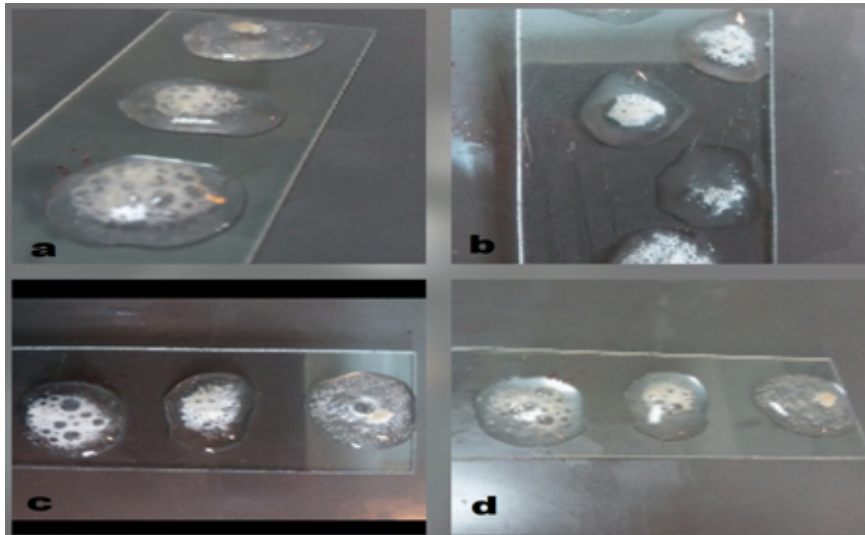


Fig. 4. Positive catalase test



Fig. 5. Growth on BHI agar.

[VI] CONCLUSIONS

Based on the findings of the current study, it was concluded that the relatively high prevalence of *Listeria monocytogenes* was found at Bahawalpur Victoria Hospital Bahawalpur, where 50 blood samples were taken, and 8 *Listeria monocytogenes* positive cases among 50 patients were seen. It is now concluded that the bacteria, *Listeria monocytogenes* mostly attack immunocompromised patients, as seen in the case of pregnant women, whose immunity is not as strong as a normal human. This bacterium is a great threat to fetuses as well as the infants, as it can cause serious health issues if the baby still survives. As per study and research, the pregnant women should avoid poorly cooked meat and poultry, as well as unpasteurized dairy products and processed cheeses because this bacterium is hard to kill. Regular checkups and blood tests during pregnancy are a plus.

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