INTRODUCTION

Ascitic fluid infection (AFI) is a serious and life threatening complication of liver cirrhosis, it has a high morbidity and mortality ***(Kamani et al., 2008)***. Spontaneous Bacterial Peritonitis (SBP) is defined as an infection of initially sterile ascitic fluid (AF) without a detectable, surgically treatable source of infection ***(Balan et al., 2011)***. It causes a sudden increase of ascitis or worsening of patient's general condition ***(Lata et al., 2009)***.

The percentage of SBP in hospitalized cirrhotic patients with ascitis ranges between 10% and 30% and the mortality rate was reported to be around 20% ***(Riggio and Angeloni, 2009)***. The organisms that cause SBP are predominantly enteric, Escherichia coli is the most frequent recovered pathogen, and Gram-positive bacteria, mainly Staphylococcus species ***(Horner et al., 2010)***.

It is common for ascitic fluid cultures to be sterile, even when the leukocytic count are more than 500 cells/mm^3. This entity is called culture negative neutrocytic ascitis (CNNA). It is a variant form of SBP and has a high incidence reaching up to 34% with polymorphnuclear leukocytic count (PMNL)>250 cells/mm^3 ***(Agarwal et al., 2008)***. Therefore, the antibiotic therapy is not guided by antimicrobial susceptibility test results ***(Iyer and Kappor, 2009)***.

Diagnosis of Spontaneous Bacterial Peritonitis (SBP) using conventional cultures as well as automated systems is not always successful. So, supplementation of the media with non - anionic surfactant agents such as Tween 80 or treatment of specimen by

Triton X-100 gives better growth of organisms as compared with direct plating methods and thus guides antimicrobial therapy in such patients ***(Runyon, 2002)***. These agents act by liberating intracellular bacteria before they are killed and/or separation of bacteria by a reduction in surface tension, thus allowing growth of more colony forming units. But the concentration at which these agents are used is important as higher concentrations may prove toxic to bacteria ***(Iyer and Kappor, 2009)***.

The utility of these agents in facilitating the growth of pathogens in other body fluids has remained unexplored. ***Iyer and Kappor (2009)*** reported the use of these agents in processing the ascitic fluid and hope that these results would pave the way for their use in other body fluids in clinical laboratories.

AIM OF THE WORK

The aim of this work is to compare between the utility of conventional cultures, culture of ascitic fluid from blood culture bottles, culture of specimen on media supplemented with non-anionic surfactant agents such as Tween 80 and culture of specimen treated with Triton X-100 in diagnosis of ascitic fluid infection.

SURFACTANTS

Surfactants (surface active agents) are compounds that lower the surface tension (or interfacial tension) between two liquids or between a liquid and a solid. They may act as detergents, wetting agents, emulsifiers, foaming agents, and dispersants ***(Rosen and Kunjappu, 2012)***. They are usually organic compounds that are amphiphilic, meaning they contain both hydrophobic groups (their tails) and hydrophilic groups (their heads). Therefore, a surfactant contains either a water insoluble (or oil soluble) component and a water soluble component ***(Linke, 2013)***.

Surfactants will diffuse in water and adsorb at interfaces between air and water or at the interface between oil and water, in the case where water is mixed with oil. The water-insoluble hydrophobic group may extend out of the bulk water phase, into the air or into the oil phase, while the water-soluble head group remains in the water phase ***(Hartmann et al., 2006)***.

Mechanism of action:

Detergents used in biology and biochemistry laboratories are mild surfactants that are used for disruption of the membrane of cells (cell lysis) and the release of the intracellular material in soluble form. Their main applications are for the breakage of protein-protein, protein-lipid and lipid-lipid interactions, denaturation of protein structure, prevention of unspecific binding in immunochemical approaches and protein crystallization ***(Rabilloud, 2013)***. Most non-ionic detergents will interfere with ultra-violet (UV) spectrophotometry, especially Triton X-100, as they contain a phenyl ring and they absorb UV light ***(Chae et al., 2010)***.

Classification:

Surfactants are classified according to polar head group into:-

1. Anionic (head carries a negative charge).
    a. Sulfate, sulfonate, and phosphate esters
    b. Carboxylates
2. Nonionic surfactant (has no charge groups in its head).
3. Cationic (head carries a positive charge).
4. Zwitterionic surfactants (contain a head with two oppositely charged groups) ***(Rosen and Kunjappu, 2012)***.

Safety and environmental risks:

Most anionic and nonionic surfactants are nontoxic ***(Misik et al., 2008)***.

Toxic effects of surfactants are usually presented after a direct contact with eyes and skin, mainly characterized by irritation, redness, itching (eyes), erythema, contact dermatitis (skin), or harmful effects if swallowed e.g. damage of intestinal wall and internal organs (liver, kidneys etc...) ***(Ho-Yeon et al., 2012)***.

Inhalation of aerosols leads to respiratory tract irritation. Carcinogenic, teratogenic and mutagenic effects are considered after chronic exposure but there is usually a lack of experimental data ***(Misik et al., 2008)***.

Cationic and anionic surfactants act as harmful agents mainly in microorganisms and water organisms, thus are chiefly used as disinfectants or inhibitors of the fungal or algae growth ***(Madaan and Tyagi, 2008)***.

Tween family:

Tween family variants are TW 20, 60 and 80, they are nonionic surfactants and emulsifiers derived from polyethoxylated sorbitan and oleic acid. The hydrophilic groups in these compounds are polyethers also known as polyoxyethylene groups which are polymers of ethylene oxide ***(Chou et al., 2013)***. They do not affect protein activity and they are effective in solubilization ***(Rabilloud, 2013)***. Brand name (commercial name) is Aranesp, AlkestTW80, Tween 80 and Canarcel. Generic name is darbepoetin alfa, and there are other names for polysorbate 80 which are:

- Polyoxyethylene (20) sorbitan monooleate.
- Sorbitan mono-9-octadecenoate poly (oxy-1,2 ethanediyl).
- POE (20) sorbitan monooleate.
- E433 ***(Chou et al., 2013)***.

Physical and chemical properties of Tween 80:

- **Molecular formula:** $C_{64}H_{124}O_{26}$.
- **Molar mass:** 1310 g/mol.
- **Appearance:** Amber yellow colored viscous liquid.
- **Density:** 1.06-1.09 g/ml, oily liquid.
- **Boiling point:** > 100°C.
- **Solubility in water:** Very soluble 5-10 g/100 mL at 23 ºC.
- **Solubility in other solvents**: soluble in ethanol, cottonseed oil, corn oil, ethyl acetate, methanol, toluene.
- **Stability:** Stable except with excessive heating.
- **Viscosity:** 300-500 centistokes at 25°C.
- **Hazards**: Irritant.
- **Flash point:** >113°C ***(Linke, 2013)***

Applications and mechanism of action:

1) Tween 80 not so common ingredient of cell lysis buffers ***(Rabilloud, 2013)***, but the usual applications are as washing agent in ELISA and immunoblotting because it helps in minimizing unspecific binding of antibodies and in removing unbound moieties ***(Scholler et al., 2008)***.

2) Tween 80 significantly increased the rate of positive cultures and the growth indices as compared with conventional plate culture techniques. This agent acts by liberating intracellular bacteria before they are killed and/or separation of bacteria by a reduction in surface tension, thus allowing growth of more colony forming units (CFUs) ***(Iyer and Kappor, 2009)***.

3) The Lecithin and Tween 80 added as supplements to Tryptic Soy Agar (a nutritious base) to inactivate some preservatives that may inhibit bacterial growth. Tryptic Soy Agar / Lecithin and Tween 80 are recommended for determining the sanitation efficiency of containers, equipment, and work area (environmental monitoring) ***(Leavitt et al., 2011)***.

4) It acts as an excipient (pharmacologically inactive substance) that is used to:

a. Stabilize aqueous formulations of medications for parenteral administration.

b. An emulsifier in the manufacture of the popular anti-arrhythmic amiodarone ***(Koocheki et al., 2011)***.

c. In some vaccines as human papilloma virus (HPV), Rotavirus and H1 N1 swine flu vaccine ***(Badiu et al., 2012)***.

d. In the culture of *Mycobacterium tuberculosis* in Middlebrook 7H9 broth ***(Murli et al., 2007)***.

e. Stabilize purified protein derivative (PPD) solution used in skin testing for tuberculosis exposure.

f. In some eye drops (redness reliever/lubricant) ***(Oz et al., 2014)***.

5) Used as a solubilizer, meaning that it aids in dissolving ingredients that would not otherwise dissolve or that would not fully dissolve, in vitamins and many medication ***(Ratz-Bravo et al., 2014)***.

6) Used as a spermicide in vaginal gels ***(James, 2011).***

7) Tween 80 has been widely used to increase secretion of enzymes or other exoproteins in both bacteria and fungi, it is gentle in its action, and most enzymes retain their activity after exposure to Tween 80 ***(Stutzenberger, 2011)***.

8) Tween 80 implicated in the suppression of the immunological response. ***Barnett (2011)*** reported that the primary IgE and IgG, suppressed in mice pretreated with Tween 80 followed by an immunizing dose of albumin adsorbed to aluminium hydroxide (an antigen adjuvant combination known to produce high levels of IgE and IgG), so the immuno-suppression caused by Tween 80 is restricted to the primary humoral response ***(Barnett, 2011)***.

9) Treating chronic renal failure and some kinds of cancer. It stimulates bone marrow to make red blood cells. It is similar to the naturally occurring human protein erythropoietin ***(Steel et al., 2011)***.

10) Used in oral pain relief, diuretics and in treatment of yeast infection ***(You et al., 2014)***.

11) Gold nanoparticles conjugated with DNA represent an attractive and alternative platform for broad applications in biosensors, medical diagnostic and biological analysis. Using Tween 80 as protective and stabilizing agent for rapid conjugation with a fluorescence-based technique ***(Xu et al., 2011)***. The resulted particles exhibit an excellent stability, non-toxic and water-soluble with a high level of purity over a wide pH range ***(Hormozi-Nezhad et al., 2013)***.

12) Antibacterial activity of silver nanoparticles coated with different functionalizing agents i.e., polyethylene glycol, Tween 80 and sodium dodecyl sulphate were evaluated on both normal and multi-drug resistant strains of bacteria. Under the same reaction conditions, these agents added separately to coat silver nanoparticles. Among these, polyethylene glycol coated nanoparticles were most effective in killing all the bacterial strains which include *(Escherichia coli, Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus* and multi-drug resistant clinical isolates of *Shigella spp. (flexneri, boydii, sohnea)* and *Vibrio cholera* ***(Bhattacharya et al., 2012)***.

Side effects:

Side effects range from mild to severe and include:

1. **Allergic reaction** - sneezing, coughing, swelling, wheezing, shortness of breath, rashes, itching and pain.
2. **Dermatological reaction**- skin dryness, irritation, contact dermatitis and eczema ***(Lewis, 2010)***.
3. **Gastrointestinal problems** - nausea, vomiting, diarrhea

4. **Heart problems** - arrhythmia, heart attack and stroke.
5. **Fertility problems** - infertility in women, especially those who have taken vaccines containing polysorbate 80, such as swine flu H1N1 and HPV vaccines ***(Oser and Oser, 2013)***.
6. **Headache and dizziness**.
7. **Tumor growth and cancer** - Tween 80 increase the probability of forming tumors in susceptible patients ***(Steel et al., 2011)***.

Triton family:

Triton X-100 is a nonionic surfactant which has a hydrophilic polyethylene oxide chain (on average it has 9.5 ethylene oxide units) and an aromatic hydrocarbon lipophilic or hydrophobic group. Triton X-100 is often warmed prior to use due to its high viscosity at room temperature ***(Sjögren et al., 2013)***. It is 100% active ingredient, which is often used in biochemical applications to solubilize proteins, it has no antimicrobial properties and considered a comparatively mild non-denaturing detergent ***(Grassia, 2013)***.

Members of this family (Triton X-100, TritonX-114, Nonidet P40, Igepal® CA-630) are quite similar and differ only in their average number (n) of monomers per micelle (9.6, 8.0, 9.0, and 9.5, respectively) and in the size distribution of the polyethylene glycol (PEG) based headgroup ***(Arnold and Linke, 2013)***.

There are other names of Triton X-100 which are:

- Polyethylene glycol p-(1,1,3,3-tetramethylbutyl)-phenyl ether.
- Octyl phenol ethoxylate.
- Polyoxyethylene octyl phenyl ether.
- 4-Octylphenol polyethoxylate.

- Mono 30.
- TX-100.
- T-octylphenoxypolyethoxyethanol.
- Octoxynol-9 **(*Shanshan et al., 2013)***

Physical and chemical properties of Triton X-100:

- **Molecular formula:** $C_{14}H_{22}O(C_2H_4O)_n$ (n=9-10).
- **Physical state:** Viscous fluid.
- **Appearance:** Clear yellow to slightly hazy.
- **Odor:** aromatic odor.
- **PH:** 6-8.
- **Vapor pressure:** < 1 mmHg at 20 ^{o}C.
- **Vapor density:** 7.11 (Air=1) ***(Medina-Ortiz et al., 2013)***.
- **Evaporation rate:** Negligible.
- **Viscosity:** 240 centistokes at 25^{o}C.
- **Cloud point:** 64^{o}C.
- **Flash point:** 483.8 °F/ 251°C.
- **Freezing/ melting Point:** 7^{o}C.
- **Solubility:** Soluble in water.
- **Specific gravity/ density:** 1.082.
- **Molecular weight:** 625.
- **Typical working concentration:** About 0.1% solution in water for lysing cells ***(Sjögren et al., 2013)***

- **Stability and Reactivity:**
  - **Chemical stability:** Stable under normal temperatures and pressures.
  - Susceptible to auto oxidation with exposure to bright light, air, moist air or water ***(Chae et al., 2010)***.

Mechanism of action:

Triton X-100 is a detergent, mild surfactant, used for the disruption of cell membranes (cell lysis) and the release of intracellular materials in a soluble form. Detergents break the protein-protein, protein-lipid and lipid-lipid associations, denature proteins and other macromolecules, and prevent unspecific binding in immunochemical assays and protein crystallization ***(Chae et al., 2010)***.

Applications:

1) Ingredient in Influenza vaccine (Fluzone) ***(Jonges et al., 2012)***.

2) Dissolve primary antibodies for immunocytochemistry ***(Saito and Dahlin, 2013)***.

3) Permeabilizing unfixed (or lightly fixed) cells in immuno-cytochemistry e.g. eukaryotic cell membranes ***(Peralta-Ramirez et al., 2013)***.

4) Triton X-100 significantly increases the solubility of recombinant human growth hormone (HGH) produced in *Escherichia coli*. (High-level expression of recombinant human growth hormone (HGH) in *E.coli* leads to the formation of insoluble aggregates as inclusion bodies devoid of biological activity) ***(Kim et al., 2013)***.

5) Solubilizing membrane proteins in their native or denatured state in conjunction with zwitterionic surfactant for successful electrophoresis ***(Kami et al., 2013)***.

6) Solublize tissue samples for western blots ***(Dilly and Rajala, 2013)***.

7) Part of the lysis buffer (usually in a 5% solution in alkaline lysis buffer) in DNA extraction.

8) Blocking buffer for immunohistochemistry ***(Fang et al., 2013)***.

9) Various chemical surfactants could affect permeability of yeast cells. Scanning electron micrographs of yeast cells showed that the cells treated with Triton X-100 had altered yeast cell structure and were smaller and narrower compared with the non-treated ones ***(Mirbagheri et al., 2011)***.

PERITONITIS

Peritonitis is defined as an inflammation of the peritoneum the membrane lining the abdominal cavity and contains all organs of the abdominal cavity ***(Pavlidis, 2013)***.

Classification:

Peritonitis can be classified into primary, secondary and tertiary peritonitis (Table 1) ***(Wachsmuth, 2010)***.

Table (1): Classification of peritonitis ***(Wachsmuth, 2010)***.

I) Primary peritonitis: Diffuse bacterial peritonitis in the absence of disruption of intra -abdominal hollow viscera	**A)** Spontaneous peritonitis in children **B)** Spontaneous peritonitis in adults **C)** Peritonitis in patients with continuous ambulatory peritoneal dialysis (CAPD). **D)** Tuberculous and other granulomatous peritonitis.
II) Secondary peritonitis: Localized(abscess) or diffuse peritonitis originating from a defect in abdominal viscous	**A) Acute perforation peritonitis** 1. Gastro-intestinal perforation 2. Intestinal ischemia 3. Pelvi-peritonitis 4. Infected pancreatic necrosis **B) Postoperative peritonitis** 1. Anastomotic leak 2. Accidental perforation and devascularization **C) Post-traumatic peritonitis** 1. After blunt abdominal trauma 2. After penetrating abdominal trauma
III) Tertiary peritonitis Peritonitis-like syndrome occurring late due to disturbance in the host's immune response	**A)** Peritonitis without evidence for pathogens **B)** Peritonitis with fungi **C)** Peritonitis with low-grade pathogenic bacteria

Epidemiology:

The incidence of peritoneal infection is difficult to establish and varies with the underlying abdominal disease processes ***(Lata et al., 2009)***. As regard primary peritonitis it is the rarest type of peritonitis, less than one percent of all types of peritonitis but the most common infection in patients with ascites and cirrhosis as represent 10% -30% of ascitic fluid infections ***(Han and Hyzy, 2007)***. Secondary peritonitis is the most common type of peritonitis ***(Pavlidis, 2013)***. Tertiary peritonitis incidence in patients requiring ICU admission for severe abdominal infections may be as high as 50- 74%, while the incidence of abscess formation after abdominal surgery is less than 1- 2 % ***(Nouri - Majalan et al., 2010)***.

Etiology:

1. **Primary peritonitis**: It is the result of the spread of an infection from the blood stream and lymph nodes into the peritoneal cavity. It's most often spontaneous bacterial peritonitis (SBP) caused by chronic liver disease ***(Runyon, 2013)***.

2. **Secondary peritonitis:** It occurs when an infection enters the peritoneum via the gastrointestinal or biliary tract. Any disease of the viscera (chest, abdominal cavity, and pelvis) may end in peritonitis ***(Cavallaro et al., 2008)***. It presents with a surgically treatable intra-abdominal source of infection and is nearly always polymicrobial ***(Kelley and Kerlakian, 2011)***.

Two of the following three findings of ascitic fluid are present: glucose < 50 mg/dl (2.78 mmol/l) (due to bacterial glucose utilization), protein >1 g/dl (in contrast to SBP), and lactate dehydrogenase values exceed normal serum levels >225 mU/ml ***(Wiest et al., 2012)***. Once