INTRODUCTION

The dairy cattle has a pivotal role to serve as reservoir of multiple bacteria and is an important subject to study as it affects major geographical regions and are also responsible for important zoonosis. Pakistan being a developing country has multiple concerns in the food and health sectors. The increasing rate of modernization in the medication field has made life more safe and healthy, but it has also lead to other challenges in life, one of which is the antibiotic resistance.
Raw milk before reaching the consumer undergoes through series of processing steps, which involves; extraction, storage and transportation. Milk gets contaminated at each step with several kinds of bacteria, which can cause serious public health issues. The bacteria are usually acquired as a result of negligence of the worker or due to the environment in which the animal is being kept. The use of improper washed utensils, presence of dung/mud in the area where the animal is kept can contaminate the teats and the udder. While, the unhygienic practices adopted during milking by the worker are examples quoting and directing to the non-serious behavior in the livestock management. Common bacteria that are found in the dairy farm belong to Pseudomonas, Escherichia, Micrococcus, Streptococcus, Staphylococcus, Bacillus, Clostridium, Enterobacteriaceae, Listeria, Salmonella, Corynebacteria, Klebsiellae, Coliforms, Pasturella and Brucella etc.

One of the most important infection found and reported in cattle is “Mastitis”. It is characterized as the inflammation of the mammary glands of the animal, caused by bacteria (both gram-positive and gram-negative), mycoplasmas and algae. Bovine mastitis refers to the mastitis caused to the cattle (Zadok et al., 2011). Mastitis occurs both in clinical and sub-clinical forms. The mastitis causes a reduction in the milk production i.e., product yield and thus it is of utmost importance that the mastitis infection must be effectively controlled to prevent causing any harm to the herd (Hogan and Smith, 2012).

Mastitis is defined as “The inflammation of the mammary gland, primarily as a result of the invasion caused by the pathogenic microbes through the teat canal, affecting the quality and production of the milk” (Erskein et al., 1989). The incidence can range from 0 to 200 cows per 100 cows per year (Cha et al., 2011). It is found in all dairy herds, it is caused by Staphylococcus aureus, Escherichia coli, Klebsiella spp and Streptococci (S.uberis, S. dysgalactia) etc.

**Table 1: Epidemiology**

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Country</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Iran</td>
<td>Hosseiniazadeh and Saei, 2014; Huth et al., 2015</td>
</tr>
<tr>
<td>2.</td>
<td>Mexico</td>
<td>Infante-Martinez et al., 1999</td>
</tr>
<tr>
<td>3.</td>
<td>Brazil</td>
<td>Condas et al., 2013</td>
</tr>
<tr>
<td>4.</td>
<td>Ethiopia</td>
<td>Getahun et al., 2008</td>
</tr>
<tr>
<td>5.</td>
<td>Pakistan</td>
<td>Bachaya et al., 2011; Ali et al., 2011; Bachaya et al., 2005; Sharif and Ahmed, 2007</td>
</tr>
<tr>
<td>6.</td>
<td>India</td>
<td>Ranjan et al., 2011</td>
</tr>
<tr>
<td>7.</td>
<td>Bangladesh</td>
<td>Rahman et al., 2010;</td>
</tr>
<tr>
<td>8.</td>
<td>Nepal</td>
<td>Khanal and Pandit, 2013; Dhungana et al., 2011</td>
</tr>
<tr>
<td>9.</td>
<td>Korea</td>
<td>Moon et al., 2007; Nam et al., 2010</td>
</tr>
<tr>
<td>10.</td>
<td>Japan</td>
<td>Nagase et al., 2002; Hata et al., 2006</td>
</tr>
<tr>
<td>11.</td>
<td>China</td>
<td>Wang et al., 2015; Gao et al., 2012</td>
</tr>
<tr>
<td>12.</td>
<td>Russia</td>
<td>Vard and Popov, 1993; Fursova et al., 2018</td>
</tr>
<tr>
<td>13.</td>
<td>Canada</td>
<td>Reyher et al., 2011; Riekerink et al., 2008; Sabour et al., 2004;</td>
</tr>
</tbody>
</table>
Clinical cases of mastitis are usually thought to be the “tip” of an iceberg. This clearly shows that a larger portion of the herd goes unrecognized as sub-clinical cases. Sub-clinical mastitis is “The presence of a microorganism along with an elevation observed in the somatic cell count (SCC) in the milk”. Sub-clinical mastitis is considered as the economic burden causing infection. Hence, Mastitis offers complication in treatment and medication. Epidemiology of the disease, microbe interaction with the host and the environmental factors greatly affect incidence and prevalence of the disease.

The infection occurs when the pathogen crosses the barrier of the host; physical barrier; i.e., teat, while prompting the host defenses and substituting the colonizing in the quarter of the cow. The infection is usually categorized as environmentally acquired or contagious, but these two terms are now-a-days debatable (Aitken et al., 2011; Zadoks, 2014). The contagious pathogen can readily spread from one infected quarter to another quarter of the same or the neighboring cow (Bradley, 2002; Atalla et al., 2010; Contreras and Rodriguez, 2011). The infection furnished by a number factors of the host, including (Kehrli and Shuster, 1994; Steeneveld et al., 2008): Age, Lactation stage and Somatic cell score history. Pathogen-dependent responses are highly crucial for the host to combat or stop an invasion by the pathogen.

**Symptoms:**

The symptoms observed, (from time of onset to the level that the disease becomes severe in nature) have been summarized in the Table 2.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Stage</th>
<th>Symptoms</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Sub-clinical Stage</td>
<td>No visible signs in the udder or inflammation is observed superficially</td>
<td>Cady et al., 1983; Khan et al., 1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Milk production decreases</td>
<td>Cady et al., 1983; Khan et al., 1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Somatic cell count increases, affecting older lactating animals</td>
<td>Cady et al., 1983; Khan et al., 1991</td>
</tr>
<tr>
<td>2.</td>
<td>Clinical Stage</td>
<td>Sudden onset of inflammation</td>
<td>Cady et al., 1983</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Swelling</td>
<td>Khan et al., 1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Redness of the udder</td>
<td>Philpot, 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pain</td>
<td>Cady, et al., 1983</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduced and altered milk secretion from the quarters that are infected</td>
<td>Cady et al., 1983; Philpot, 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clots, flakes and water like inconsistency in</td>
<td>Khan et al., 1991</td>
</tr>
</tbody>
</table>
**Challenge of Bovine Mastitis**

<table>
<thead>
<tr>
<th>the milk</th>
<th>Hillerton, 1999; Shearer and Harris, 2003</th>
</tr>
</thead>
<tbody>
<tr>
<td>The value of somatic cell count (SCC) is above 300,000 somatic cells/ml indicating the inflammation in the udder.</td>
<td></td>
</tr>
</tbody>
</table>

**Etiology:**

Mastitis may be caused by pathogens that are contagious or are part of the environment.

Mastitis spreads through microorganisms that are contagious and found as potential pathogens in the environment. Contagious mastitis usually occurs from the infected quarter to the other quarters of the same animal, or it can get transferred to other animals of the herd during milking process. Environmental organisms on the other hand are generally ubiquitous and often regarded as opportunistic organisms of the environment (Erskine, 2001). Infection can be caused at any stage during the process of milking. It may occur at any time, for example, during milking, between consecutive milkings, during the dry period, or prior to the first calving. Employment of proper hygiene is essentially important to reduce mastitis cases caused by contagious pathogens. On the other hand, clinical cases due to environmental pathogens are of great concern for well-managed herds as the pathogens remain persistently, with low SCC (Hogan and Smith, 2003). Environmental pathogens are not only responsible for Intra mammary infections but also cause clinical mastitis in heifers at calving. With the improvement in the management strategies to control the frequency of the clinical cases, a visible decline has been observed in cases due to contagious pathogens while the relative frequency of cases due to environmental pathogens has increased in the recent years. This indicates the persistent nature of these organisms, serving as a potential threat and an economic burden in the livestock sector of any country (Hogan and Smith, 2003; Fox and Gay 1993).

**Contagious Mastitis:**

A contagious mastitis is defined as the Intra mammary Infection (IMI) transmitted by a pathogen, directly from one animal to the other (Erskine 2001). The incidence of contagious mastitis depends on the dose and the type of microbe to which a cow is exposed. The physical barriers and the innate as well as the acquired defense mechanisms contribute towards the incidence of the mastitis. The pathogens that are highly prevalent in causing intra mammary infections during mastitis are usually of major interest for the dairy farms. The most notorious organisms for contagious mastitis include *Streptococcus agalactiae*, *Staphylococcus aureus*, *Corynebacterium bovis* and Mycoplasma species (spp.). *S. aureus* is the major bacteria responsible for high prevalence of the intra-mammary infection in the herd. The high prevalence of *S. aureus* can be well demonstrated by the fact that mastitis cases due to this pathogen are about 7-40% of all cows at any given time (the breed and geographical location of the herd may differ in each incidence) (Fox and Gay 1993).

**Environmental Pathogens:**

Environmental organisms for mastitis are usually gram-negative bacteria, which includes the coliforms and Streptococci species. The gram-negative bacteria include *Escherichia coli*, Klebsiella spp., Enterobacter spp., Citrobacter spp., Seratia, Pseudomonas spp., Proteus and *Actinomyces pyogenes*. The environmental Streptococci include species like *S. uberis*, *S. dysgaladliae,*
and *S. equinus*. Coliforms contribute in acute cases of mastitis, while Streptococci contribute in the short lived infections (those having duration of less than seven days). While *A. pyogenes*, generates heavy losses (Hogan and Smith, 2003). The Streptococcus infection lasts for a period less than 30 days (Hogan and Smith, 2003). *E. coli* and Klebsiella are the most common bacteria isolated as the environmental pathogen for mastitis. Approximately 80% of Gram-negative and 50% of environmental Streptococci are reported in clinical mastitis cases (Hogan and Smith, 2003).

The dairy industry faces economic loss worth millions every year due to high cost spent on antibiotic treatment and culling (Zaragoza et al., 2011). *E.coli* and *S.aureus* are considered as the most notorious and well-studied pathogens. *Staphylococcus aureus* is considered as common pathogen as it contributes to 25-50% of mastitis cases (Demasures *et al.*, 1997; Demasures and Gueguen, 1997; Oladipo and Omo-Adua, 2011). While, the rest of the cases are caused by bacteria such as *Streptococcus agalactiae*, *Streptococcus uberis*, *Corynebacterium pyogenes*, Pseudomonas, Pasteurella and *Brucella abortus*.

Table 3: List of the potential “mastitis-causing” micro-organisms referred at various occasions in the literature.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Micro-organism</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Bacteria</td>
<td><em>Escherichia coli</em></td>
<td>Hill et al., 1979; Lam et al., 1996; Wenz et al., 2006; Leimbach et al., 2017; Roussel et al., 2017; Liu et al., 2018</td>
</tr>
<tr>
<td>2.</td>
<td><em>Klebsiella pneumonia</em></td>
<td>Jasper et al., 1975; Rose et al., 1989; Kikuchi et al., 1995; Munoz et al., 2006; Munoz et al., 2007; Klibi et al., 2019</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td><em>Streptococcus agalactiae</em></td>
<td>McDonald and McDonald, 1976; Erskine and Eberhart, 1990; Keefe, 1997; Gao et al., 2012; Cobo-Angel et al., 2019</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td><em>Staphylococcus aureus</em></td>
<td>Lam et al., 1996; Smith et al., 1998; Vasudevan et al., 2003; Monistero et al., 2018</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td><em>Streptococcus uberis</em></td>
<td>Zadoks et al., 2001; Gillespie and Oliver, 2005; Almeida et al., 2015; Davies et al., 2016; Collado et al., 2018; Kappeli et al., 2019</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td><em>Streptococcus dysgalactiae</em></td>
<td>Merin et al., 2008; Lundberg et al., 2016; Reyes et al., 2017</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td><em>Klebsiella oxytoca</em></td>
<td>Zadoks et al., 2011; Hoque et al., 2019</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Osborne et al., 1981; Scaccabarozzi et al., 1997; <em>Brucella abortus</em></td>
<td></td>
</tr>
</tbody>
</table>
### Challenge of Bovine Mastitis

<table>
<thead>
<tr>
<th>No.</th>
<th>Pathogen</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.</td>
<td><em>Serratia marcescens</em></td>
<td>Isaksson and Holmberg, 1984; Todhunter et al., 1991; Reugg et al., 1992; Zadoks et al., 2011; Azevedo et al., 2016</td>
</tr>
<tr>
<td>10.</td>
<td><em>Streptococcus equi</em> subsp.</td>
<td>Las Heras et al., 2002; Kuusi et al., 2006; Pelkonen et al., 2013</td>
</tr>
<tr>
<td></td>
<td><em>Zooeoidemicus</em></td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td><em>Streptococcus canis</em></td>
<td>Hassan et al., 2003; Tikofsy and Zadoks, 2005; Hassan et al., 2005</td>
</tr>
<tr>
<td>12.</td>
<td><em>Escherichia faecalis</em></td>
<td>Koskinen et al., 2009; Elhadidy and Elsayyad, 2013; Rozanska et al., 2019</td>
</tr>
<tr>
<td>13.</td>
<td><em>Escherichia facium</em></td>
<td>Petersson-Wolfe et al., 2009; Zadoks et al., 2011</td>
</tr>
<tr>
<td>14.</td>
<td><em>Staphylococcus chromogenes</em></td>
<td>Hosseinzadeh and Saei, 2014; Dos Santos et al., 2016; Piccart et al., 2016</td>
</tr>
<tr>
<td>15.</td>
<td><em>Staphylococcus epidermidis</em></td>
<td>Oliveira et al., 2006</td>
</tr>
<tr>
<td>16.</td>
<td><em>Staphylococcus haemolyticus</em></td>
<td>Hosseinzadeh and Saei, 2014; Dolder et al., 2017</td>
</tr>
<tr>
<td>17.</td>
<td><em>Staphylococcus simulans</em></td>
<td>Hosseinzadeh and Saei, 2014; Dolder et al., 2017</td>
</tr>
<tr>
<td>18.</td>
<td><em>Staphylococcus equorum</em></td>
<td></td>
</tr>
<tr>
<td>19.</td>
<td><em>Staphylococcus xylosus</em></td>
<td>Hosseinzadeh and Saei, 2014; Dolder et al., 2017</td>
</tr>
<tr>
<td>20.</td>
<td><em>Staphylococcus hyicus</em></td>
<td>Roberson et al., 1996</td>
</tr>
<tr>
<td>22.</td>
<td><strong>Protheca</strong> (Yeast like micro-algae)</td>
<td><strong>Protheca zopfii</strong> Janosi et al., 2001; Moller et al., 2007; Osumi et al., 2008; Huth et al., 2015</td>
</tr>
<tr>
<td>23.</td>
<td><strong>Protheca blaschkae</strong></td>
<td>Huth et al., 2015; Marques et al., 2008; Thompson et al., 2009</td>
</tr>
<tr>
<td>24.</td>
<td><strong>Protheca wickerhamii</strong></td>
<td>Moller et al., 2007</td>
</tr>
<tr>
<td>25.</td>
<td><strong>Mycoplasma</strong></td>
<td><strong>Mycoplasma bovis</strong> Jasper, 1977; Bennett and Jasper, 1977; Punyapornwithaya et al., 2010</td>
</tr>
<tr>
<td>26.</td>
<td><strong>Mycoplasma californicum</strong></td>
<td>Mackie et al., 1986; Infante-Martinez et al., 1999</td>
</tr>
<tr>
<td>27.</td>
<td><strong>Mycoplasma hyopnemoniae</strong></td>
<td>Aebi et al., 2012; Haapala et al., 2018</td>
</tr>
</tbody>
</table>

**Pathogenesis:**

The dairy animals get infected by mastitis and the animal after the exposure goes through sub-clinical and clinical stages, where the microbe propagates and flourishes to cause the disease. The pathogenesis of mastitis occurs as follows:

1. The udder becomes infected with the aetologic agent.
2. The agent multiplies with in the body, producing toxins.
3. The milk secreting tissues get damaged by the effect of toxins.
4. The leucocytes increase in amount also known as the somatic cell count in the milk, affect its quality.

5. The teat comprises of a sphincter of smooth muscles, a teat canal. The muscle functions to keep the canal close, to stop milk from secreting and to avoid any possible invasion of bacteria. The teat therefore is the first line of defense against the infection (Murphy et al., 1988).

6. The teat canal is lined with keratin derived from stratified squamous epithelium. The damage to the keratin living the teat canal can cause high susceptibility of bacterial invasion and colonization (Bramley and Dodd, 1984).

7. Keratin contains esterified and non-esterified fatty acids e.g., myristic acid, palmitoleic acid and linolinic acid which are bacteriostatic (Treece et al., 1966).

**Diagnosis:**
Mastitis can be diagnosed using several test. A few include;

1. Using direct isolation and identification of the organism from the suspected milk.
2. Culturing the organism on various media as listed in Table 4.

**Table 4: Types of Media**

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Nature of Media</th>
<th>Name of Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Solid Media</td>
<td>Blood Agar Media</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Modified Edward’s Media</td>
</tr>
<tr>
<td>2</td>
<td>Liquid Media</td>
<td>Veal infusion broth</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Digest infusion broth</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pancreatic digest broth</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Meat infusion broth</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blood broth</td>
</tr>
</tbody>
</table>

3. Serological tests can be conducted for the confirmation and rapid testing of the organism. Tests like slide agglutination test, precipitation test and plasma-coagulase test can be done to detect the pathogen (Hogan and Smith, 2003).

**Treatment and Clinical Management:**
The treatment and management of the clinical cases is highly important in making a significant decline in the rate of the reaction (Hogan and Smith, 2003). Following actions can be adopted during handling of clinical cases:

1. Affected animal should be separated, from the rest of the herd.
2. Healthy quarter should be milked first.
3. The affected quarter should be free from secretion/milk, and if necessary use of sterile syphon should be done.
4. Culturing and drug-sensitivity testing should be conducted as it is very important to suggest a rational therapy.
5. Supportive therapy should be provided i.e., use of isotonic fluid containing antihistamines, glucose and corticosteroids etc. it helps in checking fibrosis in the mammary glands.
6. Use of enzymes such as streptodornase, hylase helps in digesting the copious pus that is being produced in the mammary gland.
7. Fomentation to relief inflammation is done using magnesium sulfate.
8. Intra-mammary infusion drugs such as Pendistrin-SH, Aureomycin, Nefuran, Tilox, Fluclox, Alciclox, Kefelong and Wochadine may be used Deb et al., 2013.
**Challenge of Bovine Mastitis**

**Control and Prevention:**
Control of mastitis can be achieved by the following methods Deb et al., 2013:

1. Reducing the duration of infection by:
   a. Culling the chronic cases that are non-responsive to treatment.
   b. Treatment of the clinical cases.
   c. Treatment of all quarters at drying off.
2. Reducing the rate of new infections which is usually achieved by:
   a. Washing the udder before and after milking with antiseptic lotion.
   b. Proper washing of hands by the milker before and after the milking.
   c. Dipping of all teats after milking.
   d. Cleaning and disinfecting the milking machines and the cup after each milking.
   e. Use of infusion in each cows during drying off as there are more chances of resurgence of infection.
   f. Sealing the teat with a solution of acrylic latex helps in reduction of udder infection rate.
   g. Using bedding of sand is ideal as sand has lower bacterial content.
   h. Proper disposal of infected milk. Use of 5% of phenol in the infected milk ensures “safe discardment” of the milk.
   i. Control of fly population to avoid spread and transfer of the infection. For this, insecticide repellants can be used in the area and its surroundings.

**Future Challenges:**
Antimicrobial drugs especially the antibiotics have been exclusively used; in the animal feed as additives to prevent the invasion of bacterial infections. It has been used as a preventive measure as well as to control disease in the dairy cattle (Holmes and Zadoks, 2011). Although this preventive strategy was mainly employed to avoid any socio-economic loss but it has recently raised concerns and questions regards food safety and public health as resistant bacteria are frequently making their appearance. Therefore, it is essential to study the incidence and the prevalence of resistance among bacteria isolated from raw milk (Gao et al., 2012).

**CONCLUSION**
Mastitis is considered as one of the most devastating disease; with high economic loss, globally. The etiological agents vary depending on the geographical region, the climate, animal species and animal husbandry. Mastitis can be bacterial or mycotic in nature (Deb et al., 2013). The characteristic attributes of the disease include the reduction in the production, discarded milk, early culling, labor costs and veterinary services. If antibiotics are not astutely applied, it can cause potential spread of antibiotic resistance. In spite of the implementation of preventive measures, management practices, genetics and the genetic selection continues to be inadequate. Mastitis causing pathogens are highly adapted to the host, causing clinical symptoms and sometimes sub-clinical infection, followed by the persistence in the mammary glands. The invasion and persistence in the mammary gland can lead to the recurrent infections and can cause an increase in the somatic cell count. It would be just to conclude, that raw milk serves as a reservoir, for a variety of bacteria, making them a potential threat as they act as potential pathogens. Long term and the indiscriminate use of antibiotics has led to enrichment of multi-drug resistant bacteria.
REFERENCES


Challenge of Bovine Mastitis


51. Käppeli N, Morach M, Zurfluh K, Corti, S, Nüesch-Inderbinen M and


Murphy SC, Cranker K, Senyk GF, Barbano DM, Saeman AI and Galton


87. Reyes J, Chaffer M, Rodriguez-Lecompte JC, Sánchez J, Zadoks RN,
Challenge of Bovine Mastitis


113. Zaragoza CS, Olivares RAC, Watty AED, de la Peña Moctezuma A and
Challenge of Bovine Mastitis


Challenge of Bovine Mastitis


138. Larsen HD, Aarestrup FM and Jensen NE (2002). Geographical


