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Occurrence of mastitis and associated pathogens with antibiogram in animal population of Peshawar, Pakistan

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Occurrence of mastitis and associated pathogens with antibiogram in animal population of Peshawar, Pakistan

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Abstract

Mastitis is a devastating disease of dairy animals in Pakistan that occurs in clinical and subclinical forms. Various bacterial pathogens are associated with mastitis and the susceptibility of these pathogens remains inconsistent to various antibacterial drugs. This study was aimed to assess the prevalence of mastitis in and around the city of Peshawar, Pakistan.

A total of 2,791 milk samples (Bovine, Ovine and Caprine) were subjected to White side test and Surf field mastitis test. Positive milk samples for mastitis were cultured on different selective culture media for identification of prevalent bacterial pathogens. Antibiogram of these organisms was evaluated against different commonly used antibiotics by disc diffusion method. Out of the 2,791 milk samples, 2,253 (81%) were positive for mastitis using conventional screening tests. From all positive samples different bacterial pathogens were isolated; *Escherichia coli* was 54.5%, *Proteus* spp. 12%, *Staphylococci* spp. 7.5%, *Klebsella* spp. 6%, *Pasteurella* spp. 3%, *Pseudomonas* spp. 2.25%, *Salmonella* spp. 1.49% and *Streptococci* spp. 1.5%. Fungus was 0.75%, whereas mixed infections were 11.2%. In antibiogram studies the sensitivity of antibiotics, enrofloxacin, gentamycin, norfloxacin, ciprofloxacin and streptomycin were found to be 69.4, 60.5, 57.5, 49.3 and 48.5 percent, respectively. It was found that *Escherichia coli* and *Proteus* spp. were the most prevalent pathogens responsible for mastitis in the animals of Peshawar and surrounding areas. Enrofloxacin and gentamicin were found relatively more effective and sensitive against mastitis, providing better outcome in the treatment of mastitis.

Keywords: mastitis, antibiogram, *E. coli*, *Proteus*, Peshawar

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Introduction

In developing countries such as Pakistan, mastitis is one of the most economically important diseases of dairy herds. Generally, it affects all domesticated animals, affecting the physical, chemical and microbiological properties of milk and at the same time influencing the pathological changes in udder parenchyma cells (Chishty et al., 2007; Beheshti et al., 2010).

Mammary gland infection can alter the nutritional composition of milk secretion. Mastitis affects flavor and shelf life of milk that contains pathogens along with toxins and its consumption may lead to increased risk of milk-borne diseases (Sharif and Muhammad, 2008). The prevalence of subclinical mastitis is 15-40 times higher than clinical forms and is responsible for great economic losses (Jarassaeng et al., 2012). In the subcontinent, the prevalence of subclinical mastitis was reported 17-93% in cows and 4-48% in buffaloes, respectively (Allore, 1993). The risk of global warming and climate change is imitable and several alarming manifestations of destruction have occurred (Ali et al., 2014). Animals may be affected by the climate change in four ways: extreme weather events, heat stress and diseases, animal adaptation to production systems in new environments, and emergence or recurrence of infectious diseases. Vector-borne diseases significantly depend on climatic and environmental conditions (Forman et al., 2008).

In Khyber Pakhtunkhwa province, the prevalence of clinical and subclinical mastitis has been recorded at 8.95% and 34.95% in cattle and buffaloes, respectively, causing estimated loss of 15 billion rupees per year (PARC, 2009). Different organisms such as *Escherichia coli*, *Staphylococci*, *Streptococci*, *Pseudomonas*, *Proteus* spp., *Pasteurella*, *Salmonella* and *Bacillus* spp. are involved in causing mastitis (Iqbal et al., 2004; Jarassaeng et al., 2012). The risk of transmission of zoonotic diseases like tuberculosis, brucellosis, leptospirosis and streptococcal sore throat to human being is also associated with mastitis (Radostits et al., 2000). This study was designed to investigate the common microorganisms responsible for mastitis and their susceptibility pattern to various antibiotics.

Materials and Methods

Study area and population: The study was conducted in the diagnostic laboratory in Peshawar, Khyber Pakhtunkhwa (KP), Pakistan. The city is the provincial capital, located at 4.00°N 71.32°E. Animal samples were submitted to the laboratory, referred by veterinarians from periphery or even from remote areas (<http://www.pbs.gov.pk>). Lactating cattle, buffaloes, sheep and goats were investigated during the study.

Sampling and screening for mastitis: A total of 2,791 freshly obtained milk samples were collected during 2010-2013 from suspected mastitis infected animals and were initially checked by Surf field mastitis test (SFMT) for subclinical mastitis. SFMT-positive samples were submitted to the mastitis section of Veterinary Research Institute Laboratory, Peshawar, KP. The collected milk samples were mostly from milch

animals, while a few milk samples were from goats and sheep. SFMT was used as screening test following Muhammad et al. (1995).

Culturing milk samples: The milk samples positive for SFMT were further subjected to pathogen isolation and identification. A portion (1 mL) of the selected positive sample was inoculated on Tryptone soy agar (Merck, Germany), MacConkey's agar (Himedia, India) and Blood agar plates (Himedia, India) and incubated at 37 °C for 24-48 h. Isolated organisms were identified on the basis of morphology, culture characteristics and biochemical profiling as proposed by Soomro et al. (2002).

Antibiogram profiling: Microbial isolates were tested for their antibiotic sensitivity and resistance pattern, using disk diffusion assay following Akbar and Anal (2015), to 17 different antibiotic discs (Oxoid), i.e. enrofloxacin, gentamicin, norfloxacin, ciprofloxacin, streptomycin, kanamycin, chloramphenicol, oxytetracycline, flumequine, amoxicillin, cephradine, tobramycin, ampicillin, cloxacillin, neomycin, lincomycin and penicillin.

Results and Discussion

The total of 2,791 milk samples from four different animal species were tested in the laboratory for mastitis. Amongst them, 2,253 were found positive. The overall occurrence of mastitis was 81% as shown in Figure 1. This shows a very high degree of infection that ranged from 75% to 85% in all three years. The findings are different from an earlier report of 23-50% mastitis cases in cows, buffaloes and sheep/goat (Iqbal et al., 2004). Our findings are also in contrast to those of PARC (2009), in which 9% and 35% overall prevalence of clinical mastitis and subclinical mastitis was recorded in Peshawar, respectively. The high prevalence of mastitis cases in our study could be due to the referral of suspected clinical and subclinical cases by veterinarians.

Mastitis is an economically important disease and is a great challenge for the dairy industry. It affects the industry in terms of decreased milk production, treatment and labor costs, risk of culling, mortality and reduced milk production and quality. The presence of microbial load turns milk more risky for human consumption (Indurr et al., 2008).

The prevalence of mastitis in the years 2010-11, 2011-12 and 2012-13 in cows was 75%, 85% and 84%; in buffaloes 75.7%, 85.9% and 75.3%; in goats 36.4%, 81.2% and 100%; and in sheep 50%, 100% and 100%, respectively. The overall prevalence of mastitis was recorded at 81%, 79%, 66 % and 75% in cows, buffaloes, goats and sheep, respectively (Table 1). Out of the 2,253 positive milk samples, only 213 (9.5%) were cultured (considering the history of medication and farmers' consent), in which 134/213 (62.9%) showed bacterial growth. Khan et al. (2015) reported 20.95% clinical mastitis cases in Peshawar. This contradiction might be due to random evaluation of flocks (diseased and healthy), while in our study only apparently diseased and physically altered milk samples were included.

In the present study, the SFMT-positive milk samples from cattle, buffaloes, goats and sheep; 94/149 (63%), 37/60 (62%), 2/3 (97%) and 1/1 (100%), respectively; produced microbial growth on the culture media. The relative occurrence of microbial isolates in the milk samples from cattle, buffaloes, goats and sheep is indicated in Table 2. A total of 94

(63%) microorganisms were isolated from the cattle comprising *Escherichia coli* 51/94 (54%), *Proteus* spp. 9/94 (9%), *Staphylococci* spp. 8/94 (9%), *Klebsiella* spp. 6/94 (6%), *Pasteurella* spp. 3/94 (3%), *Pseudomonas* spp. 3/94 (3%), *Salmonella* spp. 2/94 (2%), *Streptococci* spp. 2/94 (2%) and mixed growth infection 10/94 (11%).

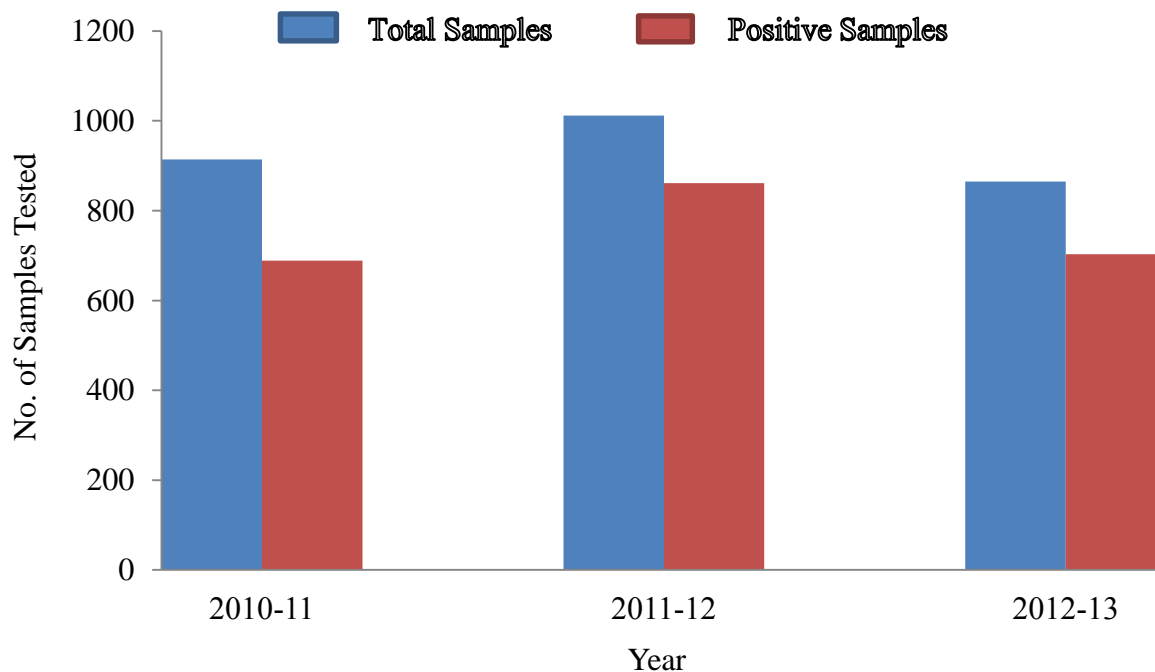


Figure 1 Year wise occurrence of mastitis in tested animals of Peshawar, Pakistan

Table 1 Bacterial species detected in healthy and periodontitis dogs

| Year | Cattle | | Buffalo | | Goat | | Sheep | |
|---------|----------------|--------------|----------------|--------------|----------------|--------------|----------------|--------------|
| | Samples tested | Positive (%) | Samples tested | Positive (%) | Samples tested | Positive (%) | Samples tested | Positive (%) |
| 2010-11 | 701 | 524 (75) | 189 | 143 (76) | 22 | 08 (36) | 02 | 01 (50) |
| 2011-12 | 678 | 578 (85) | 311 | 267 (86) | 22 | 18 (82) | 01 | 01 (100) |
| 2012-13 | 568 | 477 (84) | 287 | 216 (75) | 09 | 09 (100) | 01 | 01 (100) |
| Total | 1947 | 1579 (81) | 787 | 626 (79) | 53 | 35 (66) | 4 | 03 (75) |

Table 2 Relative prevalence of microbial agents isolated from total mastitic milk samples

| Microbes | Cattle | | Buffalo | | Goat | | Sheep | | Overall | |
|---------------------------|--------|-----|---------|------|------|-----|-------|-----|---------|------|
| | No. | % | No. | % | No. | % | No. | % | Total | % |
| <i>Escherichia coli</i> | 51 | 54 | 22 | 59 | 0 | 0 | 0 | 0 | 73 | 54 |
| <i>Proteus</i> spp. | 9 | 10 | 6 | 16 | 1 | 50 | 0 | 0 | 16 | 12 |
| Mixed growth | 10 | 11 | 4 | 10 | 1 | 50 | 0 | 0 | 15 | 11 |
| <i>Staphylococci</i> spp. | 8 | 9 | 2 | 5 | 0 | 0 | 0 | 0 | 10 | 7 |
| <i>Klebsiella</i> spp. | 6 | 6 | 2 | 5 | 0 | 0 | 0 | 0 | 8 | 5 |
| <i>Pasteurella</i> spp. | 3 | 3 | 0 | 0 | 0 | 0 | 1 | 100 | 4 | 3 |
| <i>Pseudomonas</i> spp. | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 2 |
| <i>Salmonella</i> spp. | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 |
| <i>Streptococci</i> spp. | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 |
| Fungus | 0 | 0 | 1 | 2.70 | 0 | 0 | 0 | 0 | 1 | 0.75 |
| Total | 94 | 100 | 37 | 100 | 2 | 100 | 1 | 100 | 134 | 100 |

Note: % = percentage

The buffalo milk samples showed the growth of *E. coli* 22/37 (59%), *Proteus* spp. 6/37 (16%), *Staphylococci* spp. 2/37 (5%), *Klebsiella* spp. 2/37 (5%), fungus 1/37 (3%) and mixed growth 4/37 (11%). Similarly, in the goat milk samples, only *Proteus* spp. and mix growth were found, while there was only one *Pasteurella* spp. growth in the sheep milk samples in the ratio of 1/37 (3%). The overall relative percentage of microorganisms was recorded as *E. coli* 73/134 (54%), *Proteus* spp. 16/134 (12%), *Staphylococci* spp. 10/134 (7%), *Klebsiella* spp. 8/134 (6%), *Pasteurella* spp. 4/134 (3%), *Pseudomonas* spp. 3/134 (2%), *Salmonella* spp. 2/134 (1.5%), *Streptococci* spp. 2/134 (1.5%), fungus 01/134 (0.75%) and mixed growth 15/134 (11.19%). Our results correspond with that of Iqbal et al. (2004), which showed the highest incidence of *E. coli* (40.7 %). The high prevalence of *E. coli* in mastitis milk samples has also been reported in some previous studies (Deborah et al., 1991; Balakrishnan et al., 2004; Soomro et al., 2002). However, the results of Behishti et al. (2011) differ; they recorded 48.6% prevalence of *Staphylococci* spp. This might be due to good management practices at their farms, as *E. coli* is an

environmental pathogen resulting from poor management practices and general unhygienic conditions in farms (PARC, 2009).

Although a small number of milk samples, 213 (9.5%), were subjected to culture for bacterial growth, 134 (63%) yielded growth. A total of 93 (63%) microorganisms were isolated from the cattle, mostly contaminated by *E. coli* followed by *Proteus* spp., *Staphylococci* spp., *Klebsiella* spp., *Pasteurella* spp., *Pseudomonas* spp., *Salmonella* spp., *Streptococci* spp. and mixed growth infections.

In this study, seventeen common antibiotics in use were evaluated against the isolates, in which enrofloxacin, gentamicin, norfloxacin, ciprofloxacin and streptomycin were the most effective antibiotics showing efficacy of 69.4, 60.5, 57.5, 49.3 and 48.5 percent respectively. The antibiogram profile of the isolated microorganism is depicted in Table 3. Our findings substantiate a previous study conducted in Lahore, Pakistan reporting ciprofloxacin and gentamicin being the most sensitive drugs in mastitis (Mustafa, 2011).

Table 3 Antibacterial susceptibility of individual isolates in percentage

| Isolates | <i>E. coli</i> | <i>Proteus</i> spp. | <i>Staphylococcus</i> spp. | <i>Klebsiella</i> spp. | <i>Pasteurella</i> spp. | <i>Pseudomonas</i> spp. | <i>Salmonella</i> spp. | <i>Streptococcus</i> spp. | Fungus | Mixed growth | Total |
|----------------------------|----------------|---------------------|----------------------------|------------------------|-------------------------|-------------------------|------------------------|---------------------------|--------|--------------|-------|
| No. of isolates | 73 | 16 | 10 | 8 | 4 | 3 | 2 | 2 | 1 | 15 | 134 |
| Antibiotics applied | | | | | | | | | | | |
| Enrofloxacin | 68 | 62 | 70 | 87.5 | 25 | 100 | 50 | 50 | 0 | 80 | 69.40 |
| Gentamicin | 63 | 69 | 30 | 50 | 50 | 100 | 50 | 50 | 0 | 60 | 60.45 |
| Norfloxacin | 56 | 50 | 40 | 75 | 50 | 66 | 100 | 0 | 0 | 73 | 57 |
| Ciprofloxacin | 47 | 62 | 40 | 50 | 0 | 66 | 0 | 50 | 0 | 66 | 49 |
| Streptomycin | 56 | 31 | 60 | 37 | 75 | 33 | 50 | 0 | 0 | 33 | 48 |
| Kanamycin | 45 | 50 | 50 | 50 | 25 | 33 | 100 | 0 | 0 | 33 | 45 |
| Chloramphenicol | 36 | 37 | 40 | 37 | 25 | 0 | 50 | 100 | 0 | 20 | 34 |
| Oxytetracycline | 23 | 37 | 50 | 25 | 0 | 33 | 50 | 100 | 0 | 33 | 30 |
| Flumequine | 32 | 31 | 10 | 13 | 25 | 0 | 50 | 0 | 0 | 20 | 27 |
| Amoxicillin | 14 | 6 | 40 | 13 | 0 | 0 | 50 | 0 | 0 | 27 | 16 |
| Cephadrine | 11 | 6 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 26 | 13 |
| Tobramycin | 10 | 31 | 0 | 13 | 0 | 33 | 0 | 0 | 0 | 0 | 10 |
| Ampicillin | 4 | 13 | 20 | 0 | 25 | 0 | 50 | 0 | 0 | 7 | 8 |
| Cloxacillin | 3 | 0 | 30 | 13 | 0 | 0 | 0 | 50 | 0 | 7 | 6 |
| Neomycin | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 4 |
| Lincomycin | 0 | 12 | 20 | 0 | 25 | 0 | 0 | 50 | 0 | 0 | 4 |
| Penicillin | 0 | 6 | 10 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |

Mastitis is a global problem in dairy herds, especially in low resource settings. It has also been reported in Iran with 42.5% prevalence (Hashemi et al., 2011). The result of the present study clearly indicates that the microbial quality of raw milk supplied to cities is unhygienic and unsatisfactory. Mastitis in dairy farms ultimately causes financial burden on dairy

owners, leading to severe economic losses (Samiullah et al., 2000).

This study identifies the need for early diagnosis and prevention of subclinical mastitis performed by dairy holders in order to acquire good quality milk and prevent economical losses. The control and prevention measure for mastitis is

necessary and can be achieved through continuous monitoring of animals' health.

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บทคัดย่อ

อุบัติการณ์ของเต้านมอักเสบและความไวต่อยาต้านจุลชีพของเชื้อก่อโรคในสัตว์ ในเขต เพชวาร์ ประเทศปากีสถาน

ราฟีลาห์¹ มิซ่า อาลี ซ่าน² มุฮัมหมัด ซารีฟ³ อาลี อักกา⁴ อันวา อาลี⁵
มุฮัมหมัด โซอ็อบ⁶ ไฟซา อัครอฟ¹ นีมาซูลา ซ่าน⁶

โรคเต้านมอักเสบแบบแสดงอาการ และไม่แสดงอาการ เป็นโรคที่มีความสำคัญในโคนมในประเทศปากีสถาน โดยมีเชื้อแบคทีเรียหลายชนิดเป็นสาเหตุของการเกิดโรคเต้านมอักเสบ และมีความไวของเชื้อต่อยาต้านจุลชีพที่หลากหลาย การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาอุบัติการณ์ของโรคเต้านมอักเสบ ในเขตเพชวาร์ ประเทศปากีสถาน โดยเก็บตัวอย่างน้ำนมจำนวน 2,791 ตัวอย่าง จาก โค แกะ และแพะ และนำมาตรวจด้วยวิธี White side test และ Surf field mastitis test จากนั้นนำตัวอย่างน้ำนมที่ให้ผลบวกมาเพาะแยกเชื้อในอาหารเลี้ยงเชื้อจำเพาะ เพื่อตรวจพิสูจน์ชนิดของเชื้อแบคทีเรีย และตรวจหาความไวต่อยาต้านจุลชีพของเชื้อแบคทีเรีย ด้วยวิธี disc diffusion method ผลการศึกษาพบว่า น้ำนมจำนวน 2,253 ตัวอย่างคิดเป็น 81% ให้ผลบวกต่อการตรวจคัดกรองโรคเต้านมอักเสบ และสามารถเพาะแยกเชื้อแบคทีเรียได้หลายชนิด ได้แก่ *Escherichia coli* 54.5%, *Proteus* spp. 12%, *Staphylococci* spp. 7.5%, *Klebsella* spp. 6%, *Pasteurella* spp. 3%, *Pseudomonas* spp. 2.25%, *Salmonella* spp. 1.49%, *Streptococci* spp. 1.5% และพบเชื้อรา Fungus 0.75% และมีการติดเชื้อร่วมระหว่างแบคทีเรียและเชื้อรา 11.2% ผลการศึกษาความไวต่อยาต้านจุลชีพพบว่า เชื้อแบคทีเรียไวต่อยา enrofloxacin, gentamycin, norfloxacin, ciprofloxacin และ streptomycin คิดเป็น 69.4, 60.5, 57.5, 49.3 และ 48.5 เปอร์เซ็นต์ ตามลำดับ โดยพบว่าเชื้อ *Escherichia coli* และ *Proteus* spp เป็นเชื้อแบคทีเรียที่เป็นสาเหตุของการเกิดโรคเต้านมอักเสบสูง ในเขตเพชวาร์ ประเทศปากีสถาน และพบว่ายา enrofloxacin และ gentamicin เป็นยาที่ให้ผลในการต้านเชื้อแบคทีเรียและเหมาะสำหรับการรักษาโรคเต้านมอักเสบ

คำสำคัญ: เต้านมอักเสบ ความไวต่อยาต้านจุลชีพ *E. coli* *Proteus* เพชวาร์

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