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Review Article

Review on the Epidemiology, Milk Composition Changes, and Antimicrobial Susceptibility of Causative Agents of Bubaline Mastitis in Asia

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ABSTRACT

Mastitis is one of the diseases that cause economic losses worldwide due to the reduction in milk yield and the high treatment costs in dairy buffaloes. Although antibiotics are the mainstay treatment for this disease, the overuse of antibiotics has resulted in the emergence of antimicrobial resistance in animals and humans. Hence, this study aims to review and assess the available literature on bubaline mastitis in Asia. The prevalence of subclinical mastitis was higher in dairy buffaloes than in clinical mastitis, especially in Pakistan. Bubaline mastitis was commonly detected using the California mastitis test, surf field mastitis test, somatic cell count, and bacterial culture. In Asia, farm management and host factors were the primary causes of bubaline mastitis risk factors. Mastitis in buffaloes caused alterations in milk composition, such as increasing lactose levels, somatic cell count, and the presence of bacteria in the milk. However, protein, fat, and solid non-

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antimicrobial susceptibility of causative agents in Asia varies depending on the usage of common antibiotics to treat bubaline mastitis in each country. This review will help to understand bubaline mastitis better, although studies are limited in many Asian countries.

Keywords: Antimicrobial resistance, Asia, bubaline mastitis, buffalo, epidemiology, milk composition

INTRODUCTION

Mastitis is the inflammation and infection of the udders area, a global issue in the dairy buffaloes farming industry. Mastitis is classified as either subclinical mastitis (SCM) or clinical mastitis (CM). The risk factors of bubaline mastitis include milking practices, farm and milk management, age, number of parity, stage of lactation, udder and leg hygiene, and teat end morphology of dairy buffaloes (Salvador et al., 2012). Common mastitis treatments in dairy animals are streptomycin, ampicillin, cloxacillin, penicillin, and tetracycline (Bhosale et al., 2014). The consequences of mastitis in dairy animals included a reduction of milk yield, an increase in the spread of mastitis within the herd, high treatment costs, and high economic losses (Fareed et al., 2017; Fetrow, 2000; Jingar et al., 2017).

Although mastitis can be treated with antibiotics, the misuse and overuse of antibiotics can lead to another serious issue: antimicrobial resistance (AMR). The common causative agents of AMR in bubaline mastitis are *Staphylococcus* spp. (36.04%), *Enterococcus* spp. (19.52%), *Escherichia coli* (9.26%), and *Streptococcus* spp. (4.39%) (Patel et al., 2019). The consequences of AMR in mastitis are harmful to animals and humans due to the causative pathogens resistant to antibiotic treatment, increased mortality, high cost of treatment, and high economic losses.

The buffaloes are considered to be less susceptible to mastitis than cows (Fagiolo & Lai, 2007). However, once the buffaloes are infected, the causative pathogens can multiply rapidly due to the high nutrient content in buffalo milk. Buffaloes have tighter teat sphincters, which is one of the reasons the occurrence of mastitis in buffaloes is lower than in bovine mastitis, as well as contributes to a better barrier to pathogens invasion (Krishnaswamy et al., 1965), and the mucin-1 (MUC1) gene that mainly to protect the cell surface from the environment pathogens (da Rosa et al., 2020). Since the occurrence of mastitis in buffaloes is lower than in bovine mastitis, the information about the risk factors, milk composition changes, occurrence, and antimicrobial susceptibility of mastitis in dairy buffaloes, especially in Asian countries, is very limited.

For this review, the main objective was to review and assess available literature on the epidemiology, milk composition changes, and antimicrobial susceptibility of causative agents of bubaline mastitis in Asia.

Dairy Industry

The dairy industry consists of dairy farming and the processing industry. Dairy farming is an agricultural class of animal husbandry that involves long-term milk production, mainly from cows and followed by buffaloes, goats, sheep, and camels. Cows, buffaloes, goats, sheep, and camels are the important dairy animals that contributed 81.05, 15.14, 2.25, 1.20, and 0.35%, respectively, to the world milk production in 2019 (Food and Agriculture Organization of the United Nations [FAO], n.d.).

Due to the high consumption of dairy products, especially cheese, there is still insufficient supply to satisfy the world's demand for dairy products, despite increases in milk production, dairy product

Table 1World dairy buffaloes population and milk productionin 2019

Countries	Population of dairy buffaloes	Production of milk
countries	(heads)	(tonnes)
Total (World)	69,924,520	133,752,296
Total (Asia)	68,328,457	131,363,080
Bangladesh	90,934	35,790
Bhutan	261	261
Brunei	178	183
China	5,818,607	2,928,369
(Mainland)		
Georgia	10,177	6,180
India	45,000,000	92,000,000
Indonesia	117,441	85,474
Iran	45,000	128,000
Iraq	59,738	35,981
Malaysia	5,555	7,691
Myanmar	458,523	204,750
Nepal	1,560,584	1,372,905
Pakistan	14,959,000	34,371,000
Sri Lanka	91,790	73,566
Syria	3,940	6,378
Turkey	79,333	79,341
Vietnam	27,396	27,211

manufacture, and the number of dairy animals. India and Pakistan are expected to contribute to milk production in the world over the next ten years, which includes milk production from cows and buffaloes (Organisation for Economic Co-operation and Development/Food and Agriculture Organization of the United Nations [OECD/ FAO], 2020).

India and Pakistan have produced more milk from buffaloes than cows (FAO, n.d.). Buffaloes are the second-highest milk producer in the world, producing approximately 133,752,296 tonnes of milk, and have the fourth-highest dairy animal population in the world, with about 69,924,520 heads. A total of 98.21% of buffalo milk is produced in Asia countries, especially in India (70.03%) and Pakistan (26.16%). The world and Asia countries' dairy buffalo population and milk production in 2019 are shown in Table 1 (FAO, n.d.).

Buffaloes

Buffaloes can be categorized into river and swamp buffaloes. Both are used as human food sources such as meat and milk, byproducts such as hide, fertilizer, and draught power (Deb et al., 2016), and river buffaloes produce more milk than swamp buffaloes, which each produce 1,800 to 2,000 kg per lactation and 500 to 800 kg per lactation, respectively (Borghese, 2011; Nanda & Nakao, 2003). The breeds of Asian swamp buffalo (Moioli & Borghese, 2005; Mokhber et al., 2018; Mukesh et al., 2009; Sethi, 2003) are listed in Table 2. Based on Table 2, the common breeds of river buffalo are

Table 2		
Buffalo b	reed i	in Asia

Breed	Countries	Milk yield (kg/lactation)	Milk fat (%)
Sambalpuri	India	2,200-2,400	-
Kundi	Pakistan	2,000	7.0
Jafarabadi	India	1,800-2,700	8.5
Meshana	India	1,800-2,700	6.6-8.1
Murrah	India (common in Asian countries)	1,800-2,000	7.2
Nili Ravi	Pakistan, India (common in Asian countries)	1,800-2,000	6.5
Pandharpuri	India	1,502	-
Surti	India	1,300-2,090	6.6-8.1
Kuzestani	Iran, Iraq	1,300-1,865	6.6
Azeri	Iran, Azerbaijan	1,200-1,300	6.6
Paralakhemudi	India	1,200	-
Anatolian	Turkey	700–1,000	4.2-4.6
Bhadawari	India	780-1,150	7.2
Lime	Nepal	875	7.0
Parkote	Nepal	875	7.0

Murrah and Nili Ravi due to their high milk yields and high-fat content in milk (Siddiky & Faruque, 2017; Zhou et al., 2018).

Buffaloes can be readily acclimatized to varied climates and reared in Southeast Asian countries with hot and humid climates (Ayalew & Taye, 2004). They are known to be less prone to disease than cows and have better productivity with a high milk yield (Siddiky & Faruque, 2017). Buffalo's milk contains low water content and cholesterol levels as well as high total solids, lactose, proteins, and fat content, which are suitable for producing fat-based and solid non-fat (SNF) based dairy products such as cheese, butter, and ghee (Fundora et al., 2001).

Mastitis and the Effect of Bubaline Mastitis on the Dairy Industry

Dairy animal diseases can affect milk production in the dairy industry. Mastitis

is one of the major economic diseases in dairy animals worldwide. The repercussions of mastitis in the dairy industry can be decreased milk quality and yield, high treatment costs and veterinary services, discarded milk, and milk withholding times, leading to economic losses in the dairy industry. One previous study on economic losses due to mastitis in Pakistan (2015) stated that the total daily cost of milk losses and mastitis treatment for dairy cows is \$8.02 and \$26.31, respectively (Fareed et al., 2017). India (2011) and the United States (US) (2009) reported that the annual economic losses on dairy cows were \$47.14 and \$2 billion, respectively (Jingar et al., 2017; Viguier et al., 2009). Due to a lower incidence of mastitis among buffaloes in India, the total loss in buffaloes (\$18.77) was lower than in cows (\$21.23) (Jingar et al., 2017).

Mastitis is a mammary gland or udder inflammation caused by several diverse pathogens, including bacteria, fungi, viruses, and others (Dalanezi et al., 2020). Pathogen invasion on the udder is followed by pathogen multiplication and the production of harmful substances, resulting in inflammation, decreased milk production, and altered milk quality (Oliver & Calvinho, 1995). Based on the clinical examination of dairy cattle for mastitis symptoms such as redness and swelling of the udders, altered milk consistency, and appearance; reduced milk production; lack of appetite, and fever, clinical mastitis can be characterized (Harmon, 1994). It is readily visible and easy to detect. In comparison, subclinical mastitis is difficult to distinguish from CM because there are no visible symptoms on the udder or milk. However, the number of somatic cells increases, and milk production diminishes, usually diagnosed based on somatic cell counts and inflammatory markers in milk (Schukken et al., 2003; Varshney & Mukherjee, 2002).

Prevalence and Detection of Bubaline Mastitis

Several methods that can be used to detect bubaline mastitis include the California mastitis test (CMT), surf field mastitis test (SFMT), white side test (WST), somatic cell count (SCC), electrical conductivity (EC), bromothymol blue (BTB), N-acetyl glucosaminidase (NAGase), and bacteria culture (BC). Combining CMT and SCC to detect mastitis in dairy animals is the best method than SCC, CMT, WST, and SFMT alone because the result is more precise and accurate (Hoque et al., 2014). SFMT was suggested as a cheaper and more user-friendly method for poor countries to use as SCM diagnostic test (Akhtar et al., 2012). BC is a gold standard in the detection method for determining the prevalence of causative pathogens in bubaline mastitis (Anirban et al., 2012). However, BTB and NAGase are rarely used to detect mastitis in dairy animals. These methods can be used for early diagnosis and prevention of SCM in dairy animals, which can prevent mastitis infection, boost milk quality and quantity, and prevent economic losses in the future (Panchal et al., 2016).

The prevalence of bubaline mastitis in Asia (2005 to 2021) is listed in Table 3. In Asia, the prevalence range of animal-based SCM and CM varies between 36.38 and 77.98% and between 10.20 and 24.60%, respectively. Meanwhile, the prevalence ranges for quarter-based SCM and CM are 9.77 to 64% and 2.81 to 9.33%, respectively. The prevalence of SCM was higher in dairy buffaloes than in CM, especially in Pakistan (Ashfaq & Muhammad, 2008). However, the prevalence of bubaline mastitis in Asia varies due to risk factors such as animal breed, lactation stage, immunological response, season, temperature, detection methods, and management practices, including poor environmental hygiene, improper hygiene, improper milking handling, and milking method (Aliul et al., 2020; Eberhart, 1986; Jingar et al., 2014; Kavitha et al., 2009; M. Z. Khan & Khan, 2006; R. Hussain et al., 2013; Salvador et al., 2012; Tapdasan et al., 2018).

Table 3

Prevalence and detection of bubaline mastitis in Asia (2005 to 2021)

Countries	Prevalence	Methodology	Sample size	Reference
Bangladesh	Animal-wise SCM: 56.66% (17/30 buffaloes) Quarter-wise SCM: CMT: 32.50% (39/120 milk samples)	CMT, WST, SFMT	30 buffaloes, 120 milk samples	Kisku and Samad (2013)
	WST: 29.16% (35/120 milk samples) SFMT: 26.66% (32/120 milk			
	samples)			
	SCM: 70% (21/30)	CMT	30 buffaloes	Talukder et al. (2013)
	SCM: 20% (14/70 buffaloes)	CMT	70 buffaloes	Biswas et al. (2020)
India	SCM: 32.90% (1878/5707 milk samples) CM: 18.74% (1070/5707 milk samples)	BC	2,057 buffaloes, 5,707 milk samples	Sharma and Sindhu (2007)
	Animal-wise SCM: CMT:	CMT, EC,	57 buffaloes, 228	Neelesh et al. (2009)
	14.03% (8/57 buffaloes)	SCC, BC	milk samples	
	EC: 10.53% (6/57 buffaloes) SCC: 12.28% (7/57 buffaloes) BC: 15.78% (9/57 buffaloes)			
	SCM: 13.60% (17/125 buffaloes)	SCC, CMT	125 buffaloes, 1,000 milk	Kavitha et al. (2009)
	CM: 3.10% (31/1000 milk samples)		samples	
	78.10% (1883/2411 milk samples)	СМТ	2,411 milk samples	Bhanot et al. (2012)
	Animal-wise SCM: BC: 28.78% (19/66) SCC: 13.63% (9/66) Quarter-wise SCM: BC: 18.30% (48/262) SCC: 7.25% (19/262)	BC, SCC	66 buffaloes, 262 milk samples	Charaya et al. (2013)
	CM: 47.83% (22/46 milk samples)	CMT	46 milk samples	Sagi (2014)
	SCM: SCC: 48.40% EC: 40% CMT: 45.80% BTB: 61.10%	SCC, EC, CMT, BTB, NAGase	57 buffaloes, 190 milk samples	Preethirani et al. (2015)
	NAGase: 61.60%			
	20% (48/240)	CMT, BC	240 milk samples	Maurya and Joshi (2021)
Iran	SCM: CMT: 34.82% (70/201 milk samples) SCC: 45.77% (92/201 milk	CMT, SCC	51 buffaloes, 201 milk samples	Beheshti et al. (2011)
	samples)			

Pertanika J. Trop. Agric. Sci. 46 (2): 541 - 570 (2023)

Bubaline Mastitis in Asia

Table 3 (continue)

Countries	Prevalence	Methodology	Sample size	Reference
	Animal-wise SCM: 23.66% (71/300 buffaloes) Quarter-wise SCM: 13.87% (166/1200)	CMT	300 buffaloes, 1,200 milk samples	Vajdi et al. (2011)
Iraq	CM: 36.25% (29/80 buffaloes)	CMT, SCC	80 lactating buffaloes	Al-saadi and Alshakh (2015)
	CM: 12% (12/100 milk samples) SCM: 24% (24/100 milk samples)	СМТ	25 buffaloes, 100 milk samples	Wahid et al. (2017)
Turkey	SCC: SCM: 9.77% (160/1637 milk samples) CM: 2.81% (46/1637 milk samples) CMT (CM and SCM): 12.58% (206/1637 milk samples)	SCC, CMT	1,637 milk samples	Özenç et al. (2008)
Nepal	SCM: 6.48% (23/355 buffaloes) CM: 93.52% (332/355 buffaloes)	EC	355 buffaloes	Dhakal et al. (2007)
	55.15% (75/136 buffaloes)	CMT	136 buffaloes	Ng et al. (2010)
Philippines	24.22% (93/384 buffaloes)	CMT	384 buffaloes	Badua et al. (2020)
Pakistan	SCM: 27% (54/200 milk samples) CM: 4% (8/200 milk samples)	SFMT	50 buffaloes, 200 milk samples	Z. Khan and Muhammad (2005)
	Animal-wise SCM: 77.98% (234/300 buffaloes) Quarter-wise SCM: 58.75% (705/1200 milk samples)	SFMT	300 buffaloes, 1200 milk samples	Bachaya et al. (2005)
	Quarter-wise CM: 9.33% (7/75 milk samples) SCM: 64% (48/75 milk samples)	SFMT	75 milk samples	Ashfaq and Muhammad (2008)
	SCM: 44% (264/600 buffaloes)	WST	600 buffaloes	M. A. Ali et al. (2011
	Animal-wise: SCM: 36.38% (139/382 buffaloes) CM: 24.60% (94/382 buffaloes) Quarter-wise: SCM: 16.04% (222/1528 milk samples) CM: 8.04% (123/1528 milk	SFMT	382 buffaloes, 1,528 milk samples	Hameed et al. (2012)

Nor'Amira Mohd Amin, Md Zuki Abu Bakar, Sharina Omar and Rozaihan Mansor

Countries	Prevalence	Methodology	Sample size	Reference
	SCM: 18.46% (240/1300 milk samples)	SFMT	1,300 milk samples	A. Hussain et al. (2013)
	CM: 4.62% (60/1300 milk samples)			
	SCM: 41.80% (163/390 buffaloes) CM: 13.60% (53/390 buffaloes)	СМТ	390 buffaloes	T. Ali et al. (2014)
	SCM: 38.80% (402/1036 buffaloes) CM: 10.20% (106/1036 buffaloes)	CMT	1,036 buffaloes	A. Hussain et al. (2018)

Note. SCM = Subclinical mastitis; CM = Clinical mastitis; CMT = California mastitis test; SFMT = Surf field mastitis test; WST = White side test; SCC = Somatic cell count; EC = Electrical conductivity; BTB = Bromothymol blue; NAGase = N-acetyl glucosaminidase; BC = Bacteria culture

One previous study about the prevalence of bovine and bubaline mastitis stated that the prevalence of bubaline mastitis is lower than bovine mastitis because the buffaloes have a tighter teat sphincter that provides a better barrier to the invasion of causative pathogens (Krishnaswamy et al., 1965), a longer teat and teat canal than cows so that pathogens must travel further through the teat to establish the infection within the mammary gland (Thomas, 2004), and the *MUC1* gene that mainly to protect the cell surface from the environmental pathogens (da Rosa et al., 2020).

Causative Agents of Bubaline Mastitis

There are several methods, such as BC, polymerase chain reaction (PCR), and metagenomics pyrosequencing, are utilized to detect the causative pathogens of bubaline mastitis (SCM and CM) (Patel et al., 2019; Preethirani et al., 2015). Using BC, bacteria are detected based on the morphology and biochemical tests, consequently allowing antibiotic susceptibility testing. At the same time, PCR utilizes primers to detect the conserved DNA sequences of bacterial genes that encode ribosomal RNA (Järvinen et al., 2009). PCR can also detect all genotypic resistance and underlying genetic mechanisms. The advantage of PCR is simple, less time-consuming (it can take less than 24 hr to complete compared to BC), and a less expensive method for processing milk samples (Abd El-Razik et al., 2010).

Metagenomics pyrosequencing of 16rDNA was performed to characterize the bacterial communities associated with milk samples from healthy, CM, and SCM in buffaloes. Quantitative Insights into Microbial Ecology (QIIME) analyzed the obtained sequencing data (Caporaso et al., 2010), whereas Paleontological Statistics (PAST) was used for statistical analysis (Hammer et al., 2001). Pyrosequencing produced 47.3 million base pairs reads (Patel et al., 2019). Phylogenetic profiles using the ribosomal database resulted in the

different genus-level prevalence of bacterial communities in healthy, CM, and SCM samples. Healthy samples are prevalent with Lactobacillus, Paenibacillus, and Pseudomonas; CM samples are prevalent with Staphylococcus, Enterococcus, Escherichia, and Streptococcus; and SCM samples are prevalent with Staphylococcus, Ralstonia, Enterococcus, and Bacillus (Patel et al., 2019). Genomic pyrosequencing can detect highly diverse and complex bacterial communities in three milk samples simultaneously. However, the bacterial communities included causative bacteria and non-causative bacteria. In the future. it can contribute to an increase in general insights into the pathogenesis of bubaline mastitis, which can lead to improved diagnostics (Patel et al., 2019).

The prevalence of causative pathogens of bubaline mastitis in Asia is listed in Table 4. The causative pathogens of bubaline mastitis can be divided into two types: contagious pathogens such as Staphylococcus aureus and Streptococcus agalactiae, and environmental pathogens such as Streptococcus uberis, Streptococcus dysgalactiae, Escherichia coli, Klebsiella pneumonia, and Pseudomonas aeruginosa. Environmental pathogens are present in feces, bedding surfaces, milking equipment, and milkers' hands (Fagiolo & Lai, 2007). However, Streptococcus agalactiae can be either contagious or environmental pathogens. The common causative pathogens in bubaline mastitis are Staphylococcus spp. including S. aureus and coagulase-negative staphylococci (CNS), including (Staphylococcus albus, Staphylococcus epidermidis, Staphylococcus simulans, Staphylococcus hyicus, Staphylococcus lentus, Staphylococcus xylosus, Staphylococcus *hominis*, and *Staphylococcus intermedius*); Streptococcus spp. including S. agalactiea, S. dysgalactiae, S. uberis, and Streptococcus mitis; Escherichia spp. such as E. coli; and Entrococcus spp.

Table 4

Countries	Prevalence	Methodology	Total of isolated bacteria	References
Bangladesh	Single infection: Staphylococcus spp. (30.77%) Streptococcus spp. (20.51%) Bacillus spp. (15.39%) Escherichia coli (12.82%) Mixed infection (12.82%) Unclassified bacterial growth (7.69%)	BC	39 isolated bacteria	Kisku and Samad (2013)
	Staphylococcus spp. (50%) Escherichia coli (28.57%) Enterobacter (14.29%) Bacillus spp. (4.76%) Proteus spp. (2.38%)	BC	42 isolated bacteria	Talukder et al. (2013)

Prevalence of causative pathogens of bubaline mastitis in Asia

Table 4 (continue)

Countries	Prevalence	Methodology	Total of isolated bacteria	References
	Bacteria culture: Staphylococcus aureus (44.83%) Escherichia coli (31.03%) Bacillus spp. (13.79%) Streptococcus spp. (10.34%) PCR: Staphylococcus aureus Escherichia coli	BC, PCR	29 isolated bacteria	Biswas et al. (2020)
Turkey	Candida spp. (41.91%) CNS (20.59%) Staphylococcus aureus (16.91%) Penicillium spp. (2.21%) Bacillus spp. (1.47%) Mixed infections (16.91%)	BC	136 isolated bacteria	Özenç et al. (2008)
India	Staphylococcus spp. (coagulase positive) (30%)Escherichia coli (18.75%)Coagulase-negative staphylococci (17.50%)Streptococcus agalactiae (7.50%)Corynebacterium pyogenes (6.25%)Klebsiella pneumonie (6.25%)Corynebacterium bovis (5%)Bacillus cereus (5%)Pseudomonas aeruginosa (3.75%)	BC	80 isolated bacteria	Das and Joseph (2005)
	Staphylococcus spp. (38.81%)Streptococcus spp. (32.40%)Escherichia coli (11.80%)Corynebacterium spp. (5.20%)Bacillus spp. (1.36%)Klebsiella spp. (2.03%)Pseudomonas aeruginosa (0.78%)Proteus (0.14%)Mixed infections (7.33%)	BC	3,447 isolated bacteria	Sharma and Sindhu (2007)
	Staphylococcus spp. (20.11%)Staphylococcus aureus (11.73%)Escherichia coli (16.76%)Streptococcus spp. (11.17%)Streptococcus dysgalactiae (5.03%)Streptococcus uberis (3.91%)Streptococcus agalactiae (2.23%)Mixed infections (29.05%)	PCR, BC	179 isolated bacteria	Kumar (2009)
	Staphylococcus spp. (43.60%) Streptococcus spp. (21.80%) Escherichia coli (16.3%) Klebsiella spp. (5.40%)	BC	-	Bhanot et al. (2012)

Bubaline Mastitis in Asia

Table 4 (continue)

Countries	Prevalence	Methodology	Total of isolated bacteria	References
	Corynebacterium pyogenes (5.40%) Pseudomonas aeruginosa (3.60%) Bacillus spp. (3.60%)			
	Staphylococcus spp. (64%) Streptococcus spp. (36%)	BC	50 isolated bacteria	Charaya et al. (2013)
	Coagulase-negative staphylococci (64.80%) Streptococcus spp. (18.10%) Escherichia coli (9.80%) Staphylococcus aureus (7.30%)	BC, Monoplex PCR, multiplex PCR	195 isolated bacteria	Preethirani et al. (2015)
	Staphylococcus spp. (51.16%) Streptococcus spp. (37.94%) Escherichia coli (8.41%) Corynebacterium pyogenes (1.62%) Pseudomonas aeruginosa (0.46%) Klebsiella (0.19%) Mixed infections (10.38%)	BC	2,580 isolated bacteria	Sharma et al. (2018)
	CM causative pathogens: Staphylococcus (25.95%) Enterococcus (10.80%) Escherichia (8.88%) Streptococcus (3.97%) SCM causative pathogens: Lactococcus (23.96%) Staphylococcus (10.09%) Ralstonia (12.72%) Enterococcus (8.72%) Bacillus (4.29%)	Metagenomic pyrosequencing	-	Patel et al. (2019)
	<i>Escherichia coli</i> (29.17%) <i>Staphylococcus aureus</i> (54.17%)	BC	48 isolated bacteria	Maurya and Joshi (2021)
Iran	Staphylococcus spp. (48.55%) CNS (36.18%) Staphylococcus aureus (14%) Lactobacillus (14%) Corynebacterium bovis (8%) Bacillus subtilis (7%)	BC	173 isolated bacteria	Beheshti et al. (2011)
	CNS (38.24%) Corynebacterium bovis (11.75%) Bacillus subtilis (11.75%) Streptococcus agalactiae (5.87%) Staphylococcus aureus (2.95%) Mixed infections (29.41%)	BC	34 isolated bacteria	Vajdi et al. (2011)
	CNS (66.08%) Staphylococcus aureus (33.91%)	BC, PCR	171 isolated bacteria	Ahmadi et al. (2020)

Pertanika J. Trop. Agric. Sci. 46 (2): 541 - 570 (2023)

Table 4 (continue)

Countries	Prevalence	Methodology	Total of isolated bacteria	References
Iraq	Escherichia coli (27.78%)	BC	36 isolated bacteria	Wahid et al. (2017)
Nepal	CNS (36.96%) <i>Micrococcus</i> (21.74%) <i>Staphylococcus aureus</i> (20.65%) <i>Clostridium</i> spp. (8.69%) <i>Bacillus subtillis</i> (7.61%) <i>Escherichia coli</i> (5.43%) <i>Klebsiella pneumonia</i> (2.17%) <i>Providencia</i> spp. (2.17%) <i>Pseudomonas</i> spp. (2.17%) <i>Streptococcus mitis</i> (1.09%) <i>Corynebacterium diphtheria</i> (1.09%) Mixed infections (4.35%)	BC	92 isolated bacteria	Dhakal et al. (2007)
	CNS (35.70%) <i>Micrococcus</i> (23.20%) <i>Clostridium</i> spp. (10.70%) <i>Bacillus subtillis</i> (8.90%) <i>Escherichia coli</i> (7.10%) <i>Klebsiella pneumonia</i> (3.60%) <i>Providencia</i> spp. (3.60%) <i>Pseudomonas</i> spp. (3.60%) Mixed infections (3.60%)	BC	56 isolated bacteria	Dhakal and Nagahata (2018)
Pakistan	Staphylococcus aureus (45.16%) Streptococcus agalactiae (22.58%) Escherichia coli (17.74%) Bacillus spp. (14.52%)	BC	62 isolated bacteria	A. Z. Khan an Muhammad (2005)
	Staphylococcus aureus (44%) Streptococcus agalactiae (22%) Escherichia coli (16%) Bacillus spp. (4%) Mixed growth (14%)	BC 50 isolated bacteria		Kisku and Samad (2013)
	Staphylococcus aureus (48.08%) Streptococcus agalactiae (21.15%) CNS (13.45%) Corynebacterial spp. (3.9%) Undifferentiable (nontypable) Coagulase-negative staphylococci (3.8%) Bacillus spp. (3.8%) Staphylococcus hominis (1.92%) Escherichia coli (1.9%)	BC	52 isolated bacteria	Ashfaq and Muhammad (2008)
	Staphylococci (28.32%) Escherichia coli (16.18%) Pseudomonas (13.29%)	BC	364 isolated bacteria	M. A. Ali et a (2011)

Bubaline Mastitis in Asia

Countries	Prevalence	Methodology	Total of isolated bacteria	References
	Bacillus (12.42%)			
	Streptococci (7.51%)			
	Salmonellae (7.22%)			
	Corynebacterium (6.64%)			
	Klebsiella (5.20%)			
	Enterococci (3.17%)			
	CNS (61.67%)	BC	180 isolated	A. Hussain et
	Staphylococcus aureus (38.33%)		bacteria	al. (2013)
Philippines	Staphylococcus aureus (41.94%)	BC	93 isolated bacteria	Badua et al. (2020)

Table 4 (continue)

Risk Factors of Bubaline Mastitis

In Asia, the risk factors of bubaline mastitis can be divided into 3 main categories, which are climatic and seasonal, host, and management. In countries with hot and humid climates, such as Southeast Asia, the prevalence of bubaline mastitis is higher than in countries with cold and humid and hot and dry climates, especially in the rainy season. In temperate countries, the prevalence of bubaline mastitis is higher in winter than in other seasons due to the low temperature-humidity index (THI)associated with cold stress during the night as the buffaloes are in loose housing systems (Aliul et al., 2020; Jingar et al., 2014).

The host factor consists of animal age, breed, number of parities, stage of lactation, the shape of udders, teat end morphology, teat and udder lesion, leakage from the teats, and position of quarters. The prevalence of bubaline mastitis is higher in buffalo aged 7 to 18 than in those aged 3 to 6 due to the structural changes in the udder and teats and the gradual suppression of the buffalo immune system (Tapdasan et al., 2018). The prevalence of bubaline mastitis is higher in crossbred buffalo than in indigenous breed buffaloes, as the crossbred buffaloes can produce more milk (Tapdasan et al., 2018). Buffaloes with more than 4 parities had a higher chance of getting infected with mastitis than buffaloes with less than 3 parities (Kavitha et al., 2009; T. Ali et al., 2014). The prevalence of bubaline mastitis in the early lactation stage (14 to 100 days) is higher than in the late lactation stage (more than 200 days) and the mid-lactation stage (100 to 200 days) due to a gradual increase in milk production (Kavitha et al., 2009; T. Ali et al., 2014; Tapdasan et al., 2018).

It will lead to physiological stress in newly calved buffalo, with various contaminations of causative pathogens of mastitis during parturition. The prevalence of bubaline mastitis is higher in buffaloes with bowl- or round-shaped udders than cup shape udders and higher in cylindrical and round teat ends than pointed teat ends (R. Hussain et al., 2013). The presence of teat and udder lesions leads to a high prevalence of bubaline mastitis because the causative pathogens enter easily through the teat canal. The prevalence of bubaline mastitis is also high in the presence of leakage from the teats due to incomplete milk removal in the udders after milking. It is important to completely remove the milk from the udder to decrease the bubaline mastitis prevalence (R. Hussain et al., 2013). The hind-quarters are more easily infected with bubaline mastitis than the fore-quarters because the hind-quarters have greater chances of getting soiled with tail and urine (Kavitha et al., 2009; R. Hussain et al., 2013; Salvador et al., 2012; T. Ali et al., 2014).

Management factors for bubaline mastitis include herd size, type of bedding, cleanliness of the farm, milking method, milking hygiene practices, history of the postparturient disease, as well as dry cow management and antibiotic therapy (Biswas et al., 2020; Kaur et al., 2015; T. Ali et al., 2014). Due to the differences in the farm management systems, bubaline mastitis is higher in big herds than in medium or small herds (Aliul et al., 2020). The prevalence of bubaline mastitis in freerange feeding areas is higher than in stallfeeding areas (Aliul et al., 2020), and there is a higher prevalence for buffaloes raised on the floor without bedding compared to concrete and sand flooring (Hameed et al., 2012; Kavitha et al., 2009). It is due to the close contact of the buffaloes with pathogens from the environment. The poor condition and cleanliness of the farm also lead to contaminated environments with increased exposure to causative pathogens of bubaline mastitis (Biswas et al., 2020; T. Ali et al., 2014). The hand milking method, including full hand, knuckling, and stripping, contributed to a high prevalence of bubaline mastitis rather than machine milking (Kaur et al., 2015; Kavitha et al., 2009; T. Ali et al., 2014). The milking method is closely related to milking hygiene practices themselves. Improper milking handling and cleaning of milking equipment, as well as poor milker hygiene habits, also cause a higher prevalence of bubaline mastitis (Biswas et al., 2020; Sharif & Ahmad, 2007) due to the transmission of causative pathogens from equipment and humans to buffaloes and from buffaloes to humans through the milk. Post antiseptic teat dipping, cleaning the milking equipment and milker hand with hot water and detergent, and drying it before milking can help to prevent and reduce bubaline mastitis (Aliul et al., 2020; M. Z. Khan & Khan, 2006; Rathva et al., 2019; Sah et al., 2020). However, the knowledge of good milking practices, such as postantiseptic teat dipping in Asia countries, is very poor and limited. The presence of dry cow management and antibiotic therapy can reduce bubaline mastitis prevalence by eliminating 70% of environmental causative pathogens such as Streptococcus spp. (Eberhart, 1986; M. Z. Khan & Khan, 2006).

Milk Composition Changes in Bubaline Mastitis

Bubaline mastitis causes a reduction in milk quantity and alteration in milk composition, such as increased lactose levels, somatic cell count, and bacteria in the milk. Variations in protein level, fat level, and solid non-fat level were also affected by other factors such as stage of lactation, breed, and age. Some studies found that mastitis in buffaloes changes milk composition, especially in protein, lactose, fat, and SNF (M. Singh et al.,2017; R. Hussain et al., 2012; Swami et al., 2017; Uallah et al., 2005). Low lactose concentration is due to the lactose efflux from milk to blood, and increased tissue permeability between the udder milk duct and blood results in blood components leakage into the udder and milk composition changes (Sharif et al., 2007). One previous study done on the difference in mineral and traces element profiles in milk between healthy buffaloes and buffaloes infected with SCM stated that there is an increase in sodium (Na) and chloride (Cl) content and a decrease in protein, fat, and zinc (Zn), iron (Fe), potassium (K), calcium (Ca), and selenium (Se) content. The value of protein, fat, Zn, Fe, Na, K, Ca, Cl, and Se in healthy buffaloes and buffaloes infected with SCM are listed in Table 5 (M. Singh et al., 2017). Fat percentage decrease in

bubaline mastitis due to an increase in lipolysis rate. Na, Cl, and K decrease due to intramammary infection (IMI) response, causing the breakdown of ductal cells and secretory epithelium. Ca decreases due to the unbalanced permeability of calcium transport from milk to blood and the disruption of a junctional complex of mammary epithelium by udder pathogens (Aslam & Tucker, 1998). The IMI causes an increase in somatic cells, which leads to changes in milk's mineral and trace element profile, as well as oxidative stress due to free radical production and reactive oxygen that leads to mammary gland damage (K. V. Singh et al., 2017).

Treatment and Prevention for Bubaline Mastitis

The common treatment for bubaline mastitis is β -lactam antibiotics such as penicillin, tetracycline, streptomycin, ampicillin, and cloxacillin (Bhosale et al., 2014; Preethirani et al., 2015), and dry antibiotic therapy are enrofloxacin and oxytetracycline (Kashif et al., 2013). Both have a high cure rate

Table 3	Tal	ble	5
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Λ	lutritional	valı	ue l	between	heali	thy	buffal	oes	and	bц	ffa	loes	inf	ected	wit	h S	C	ľ	1
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Nutritional value	Healthy buffaloes	Buffaloes infected with SCM
Protein	4.44±0.10%	3.65±0.08%
Fat	$7.08 \pm 0.14\%$	6.38±0.24%
Zn	5.96±1.44 mg/L	4.22±0.69 mg/L
Fe	6.99±1.37 mg/L	4.97±0.76 mg/L
Na	450±52 mg/L	606±152 mg/L
Κ	1063±90 mg/L	856±156mg/L
Ca	566±90 mg/L	475±77 mg/L
Cl	616±47 mg/L	828±113 mg/L
Se	0.22±0.04 mg/L	0.14 ± 0.06 mg/L

Pertanika J. Trop. Agric. Sci. 46 (2): 541 - 570 (2023)

of 91.67 and 70%, respectively (Kashif et al., 2013), administered intramammary to eliminate the existing IMI and prevent the new IMI. In return, dry therapy helps to control mastitis (Kashif et al., 2013). Another study was done on non-antibiotic and non-proprietary formulations containing trisodium citrate, vitamin C, zinc sulfate, and copper sulfate, which were equivalently effective in treating CM compared to antibiotic treatment with cure rates were 70.21 and 72.09%, respectively (Manzoor et al., 2020).

The prevention of bubaline mastitis is by introducing the latest technologies for mastitis diagnosis and implementing good milking hygiene and practice guidelines provided by National Mastitis Council (NMC) to the farmers. The mastitis-field test kit, such as CMT, SFMT, and WST, can be introduced to the farmers, which can help in the early detection of bubaline mastitis. One example of milking hygiene and practice guidelines are washing their hands with water and soap and drying them properly, washing the teats and udder of dairy animals with sanitizing solution and drying it with paper towels or individual towels, using the chlorhexidine gluconate to clean the udders, dipping the teats using germicidal teat dip solution to provide a protective germicidal barrier film on teats skin before and after milking, drying and wiping off the excess germicidal teat dip solution on the teat with paper towels or individual towels, wipe the end teat with a soaked cotton swab of 70% alcohol to kill the pathogens, and always start to cleaning

the farthest teat to the nearest teat from the milkers to prevent contamination at the teat ends (A. Singh & Ramachandran, 2020; NMC, n.d.). The importance of knowledge of good milking hygiene and practices guidelines can benefit the farmers, such as reducing mastitis occurrence on farms and increasing milk yield and quality (Sah et al., 2020).

Antimicrobial Resistance (AMR) and the Impact of AMR on the Buffalo Dairy Industry

To control mastitis in dairy animals, antibiotics treatment is commonly used. It has raised another global concern on AMR due to the overuse and misuse of antibiotics, inadequate infection prevention and control measures, and lack of surveillance and monitoring. The development of AMR reduces the effectiveness of existing treatments, with the need to develop new antibiotics. The development of AMR in dairy animals leads to high potential resistant infections to humans, high pressure in the antimicrobial selection, high mortality in dairy animals, and high economic losses (Loo et al., 2019).

The World Health Assembly of the World Health Organization (WHO) endorsed a global action plan to tackle AMR in May 2015. The global action plan on AMR has five strategic objectives, including improving awareness and understanding of antimicrobial resistance through effective communication, education, and training; strengthening the knowledge and evidence base through surveillance and research; reducing the incidence of infection through effective sanitation, hygiene, and infection prevention measures; to optimize the use of antimicrobial medicines in human and animal health; and to develop the economic case for sustainable investment that takes account of the needs of all countries and increase investment in new medicines, diagnostic tools, vaccines, and other interventions. All countries are expected to develop national action plans on AMR (WHO, 2015).

Antimicrobial Susceptibility of Causative Agents of Bubaline Mastitis

There are several methods for determining the antimicrobial susceptibility of causative pathogens of bubaline mastitis, such as the disk diffusion method, microdilution assay, and PCR (Clinical Laboratory Standards Institute [CLSI], 2019; Hoque et al., 2022). Disk diffusion method is commonly used to determine the antimicrobial susceptibility causative pathogens of bubaline mastitis in Asia countries because it is simple, reliable, and can detect the isolated resistant colonies (Mayrhofer et al., 2008; Milici et al., 2007). PCR detected the virulence genes in isolated bacteria (Hoque et al., 2022).

Kirby-Bauer disk diffusion assay was performed to determine the antimicrobial susceptibility of the isolated pathogen colonies of mastitis in buffaloes in which the commercially prepared antimicrobial disks containing common antibiotics used to treat bubaline mastitis were put on each inoculated plate (Maurya & Joshi, 2021). The inhibition zones are then measured using a precision caliper and interpreted as susceptible or sensitive, intermediate, or resistant following the suggested breakpoints diameter according to the Clinical Laboratory Standards Institute standard (CLSI, 2019).

The antimicrobial susceptibility of causative pathogens of bubaline mastitis in Asia is listed in Table 6. Most showed high resistance towards penicillin, amoxicillin, ampicillin, and streptomycin, except for cefoxitin (Gram-positive bacteria), ceftriaxone, and cefotaxime (Gram-negative bacteria). In Asia, the antimicrobial susceptibility of causative agents of bubaline mastitis countries varies based on the common antibiotics used to treat mastitis. The isolated bacteria are resistant to the common antibiotics used to treat mastitis and susceptible to antibiotics not commonly used to treat bubaline mastitis. The antibiotics susceptible to isolated bacteria can potentially become the new antibiotics to treat bubaline mastitis (Biswas et al., 2020).

CONCLUSION

The prevalence of SCM was higher in dairy buffaloes than in CM, especially in Pakistan. SFMT and CMT are usually used to detect subclinical mastitis in dairy buffaloes since both methods are cheaper, reliable, and can be used in the field. In Asia, the main risk factors of bubaline mastitis are caused by farm management, including improper milking handling and host factors such as lactation stage, age, and quarters position. Farmers can be educated on proper milking

Countries	Isolated bacteria	Antimicrobial susceptibility	Number of antibiotics used	Methodology	References
India	Streptococcus agalactiae	Resistant: Penicillin	8 antibiotics	Disk diffusion method	Das and Joseph (2005)
	Klebsiella pneumonie, Pseudomonas aeruginosa	Resistant: Penicillin and erythromycin			
	Staphylococcus spp. (CPS and CNS), Corynebacterium bovis, Corynebacterium pyogenes, E. coli, Bacillus cereus	Susceptible: Ciprofloaxacin, penicillin, chloramphenicol, gentamycin, erythromycin, kanamycin, ampicillin, and streptomycin			
	Staphylococcus spp.	Susceptible: Ampicillin, cloxacillin, amoxycillin, ceftriaxone, cefoperazone, penicillin, chloramphenicol, tylosin, and enrofloxacin	14 antibiotics	Disk diffusion method	Charaya et al. (2013)
	Streptococcus spp.	Susceptibility: Chloramphenicol, ampicillin, enrofloxacin, amoxycillin, ceftriaxone, and cefoperazone			
	Staphylococcus aureus	Disk diffusion method: Resistant: Penicillin, methicillin, amoxycillin, ceftriaxone, amoxycillin + clavulanic acid, and ceftriaxone + tazobactum	6 antibiotics (disk diffusion method), 3 antibiotics (microdilution assay)	Disk diffusion method, microdilution assay	Sagi (2014)
		Microdilution assay: Resistant: Amoxycillin and amoxycillin + clavulanic acid microdilution assay			

Pertanika J. Trop. Agric. Sci. 46 (2): 541 - 570 (2023)

Countries	Isolated bacteria	Antimicrobial susceptibility	Number of antibiotics used	Methodology	References
	Staphylococcus aureus	Resistant: Penicillin, vancomycin, and nalidixic acid Intermediate: Cefixime, methicillin, novobiocin, amoxiclav, colistin, pipemidic arcid, ofloxacin, streptomycin, arnotycaci, streptomycin, acid, ofloxacin, streptomycin, sulphamethizole, ampicillin/sulbacta, cefalexin, cefazolin, cefoperazone, enrofloxacin, floxidin, and meropenem Susceptible: Cefuroxim, ciprofloxacin, and tetracycline tetracycline	38 antibiotics	Disk diffusion method	Nigam (2015)
	CNS	Resistant: Methicillin, amoxycillin/sulbactam, and penicillin G Internediate: Ceftriaxone/sulbactam, cefoxitin, and cefotaxime Susceptible: Co-trimoxazole, chloramphenicol, and gentamicin	15 antibiotics	Disk diffusion method	Preethirani et al. (2015)
	Staphylococcus aureus	Resistant: Cefoxitin, penicillin G, and ceftriaxone/sulbactam Intermediate: Enroftoxacin Susceptible: Co-trimoxazole and oxacillin			

Pertanika J. Trop. Agric. Sci. 46 (2): 541 - 570 (2023)

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Countries	Isolated bacteria	Antimicrobial susceptibility	Number of antibiotics used	Methodology	References
	Streptococcus spp.	Resistant: Methicillin, streptomycin, cefoxitin, and penicillin G Intermediate: Gentamicin and ceftriaxone Susceptible: Chloramphenicol and Oxacillin			
	Escherichia coli	Resistant: Amikacin, amoxycillin/sulbactam, ampicillin, cefotaxime, cefoxitin, ceftriaxone/sulbacta, methicillin, penicillin G, and streptomycin Intermediate: Enrofloxacin Susceptible: Cotrimazole and chloramphenicol			
	Staphylococcus aureus	Susceptible: Gentamicin and streptopenicillin	12 antibiotics	Disk diffusion method	Maurya and Joshi (2021)
	Escherichia coli	Susceptible: Tetracycline, gentamicin, enrofloxacin, streptopenicillin, ceftriaxone + sulbactum, streptomycin, ceftriaxone, and methicillin	I		
Bangladesh	Staphylococcus spp., Streptococcus spp., Bacillus spp., E. coli	Resistant: Ampicillin, Amoxycillin and streptomycin High and intermediate susceptible: Gentamicin, ciprofloxacin, Endrofloxacin and chloramphenicol	7 antibiotics	Disk diffusion method	Kisku and Samad (2013)

Table 6 (continue)

560

Pertanika J. Trop. Agric. Sci. 46 (2): 541 - 570 (2023)

Countries	Isolated bacteria	Antimicrobial susceptibility	Number of antibiotics used	Methodology	References
	All isolated bacteria	Resistant: Amoxicillin Susceptible: Chloramphenicol and ciprofloxac	10 antibiotics	Disk diffusion method	Talukder et al. (2013)
	Staphylococcus spp.	Resistant: Amoxicillin, erythromycin, and azithromycin Susceptible: Ceftriaxone, trimethoprim, chloramphenicol, gentamycin, levofloxacin, and amoxicillin			
	Escherichia coli	Resistant: Ceftriaxone, trimethoprim, Azithromycin and nitrofurantoine Susceptible: Ciprofloxacin, azithromycin, trimethoprim, chloramphenicol, and levofloxacin			
	Staphylococcus aureus, E. coli, Streptococcus spp., Bacillus spp.	Resistant: Penicillin, amoxicillin, cefoxitin, and tetracycline Intermediate: Ceftriaxone, enrofloxacin, vancomycin, and erythromycin Susceptible: Gentamicin	12 antibiotics	Disk diffusion method	Biswas et al. (2020)
Iraq	Escherichia coli	Resistant: Resistant: Cefotaxime and ampicillin Susceptible: Tetracycline, amikacin, trimethoprim/ sulfamethaxol, gentamicin, and ciprofloxacin	7 antibiotics	Disk diffusion method	Wahid et al. (2017)

Bubaline Mastitis in Asia

Pertanika J. Trop. Agric. Sci. 46 (2): 541 - 570 (2023)

561

Countries	Isolated bacteria	Antimicrobial susceptibility	Number of antibiotics used	Methodology	References
Nepal	Escherichia coli	Resistant: Ceftriaxone and ciprofloxacin		Disk diffusion method	Bhandari et al. (2021)
Pakistan	All isolated bacteria	Resistant: Ampicillin, streptomycine, chloramphenicol, penicillin, and amoxicillin Susceptible: Ciprofloxacin Sensitive: Gentamicin and norfloxacine	8 antibiotics	Disk diffusion method	Mustafa et al. (2013)
	Staphylococcus aureus	Susceptible: Co-trimaxazole, oxytetracycline, ciprofloxacin, chloramphenicol, amoxicillin, gentamycin, ampicillin, novobiocin, and enrofloxacin	9 antibiotics	Disk diffusion method	A. Hussain et al. (2013)
	Staphylococcus aureus	Susceptible: Trimethoprim, erythromycin, ciprofloxacin, doxycycline, streptomycin, gentamycin, tylosin, kanamycin, amoxicillin, and chloramphenicol	10 antibiotics	Disk diffusion method	A. Hussain et al. (2020)
Philippines	Staphylococcus aureus	Resistant: Penicillin Intermediate: Erythromycin Susceptible: Chloramphenicol, clindamycin, trimethoprim-sulfamethoxazole, tetracycline, rifampicin, ciprofloxacin, and gentamycin	9 antibiotics	Disk diffusion method	Badua et al. (2020)

Table 6 (continue)

Pertanika J. Trop. Agric. Sci. 46 (2): 541 - 570 (2023)

562

practices and farm management to help reduce the prevalence of bubaline mastitis. Mastitis in buffaloes causes changes in milk composition, such as increased lactose levels, SCC, and bacteria in the milk. However, protein, fat, and solid nonfat level variations were also affected by other factors such as the stage of lactation, breed, and age. The most prevalent bacteria in bubaline mastitis milk samples were CNS, S. aureus, and Streptococcus spp., including S. agalactiae and S. uberis, and E. coli were detected using bacterial isolation and identification and PCR. Penicillin, amoxicillin, ampicillin, and streptomycin are highly resistant to the isolated bacteria, according to previous studies, but not cefoxitin for Gram-positive bacteria or ceftriaxone for Gram-negative bacteria. However, the antimicrobial susceptibility of causative agents in Asia varies depending on the usage of common antibiotics to treat bubaline mastitis in each country. This review will help better understand bubaline mastitis, although studies are limited in Asia except for Pakistan and India, which both have the most buffalo and produce the highest amount of buffalo milk. However, Southeast Asian countries, except Thailand, have conducted no studies on bubaline mastitis. More studies on the epidemiology, diagnostic tests, milk composition changes, and antimicrobial susceptibility of causative agents of bubaline mastitis in Asian countries are needed to fill the knowledge gap in the future.

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