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Determination of virulence genes and antibiotic resistance profiles of *Streptococcus agalactiae* isolated from buffalo milk

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Abstract: Mastitis is a contagious disease that causes significant milk loss and serious economic damage in dairy animals. *Streptococcus agalactiae* causes clinical and subclinical mastitis in animals and infections in humans, especially adults, and newborns. This study aimed to confirm the identification of *S. agalactiae* isolates by PCR in buffalo milk; to determine the antibiotic resistance and virulence genes by multiplex PCR. The material of this study consisted of 30 (12%) *S. agalactiae* strains identified by phenotypic, biochemical, and PCR methods (rRNA-16S) from 250 isolates with bacterial growth from milk samples of buffaloes with mastitis. In addition, the Kirby-Bauer disc diffusion method was used to test the antibiotic susceptibility of isolates. The antibiotic resistance genes (*tetA*, *tetB*, *tetK*, *tetM*, *tetO*, *ermA*, *ermB*, *ermC*, *mefA/E*, *strA*, and *strB*) and the virulence genes (*fbsA*, *hylB*, *Cluster PI-1*, *cps* C, D, E and *cps J*, *K*, *e neu B*) were investigated by the multiplex PCR method. As a consequence of the study, 13 strains were found to have *ermA* (43.3%), one strain *ermB* (3.3%), 15 strains *mefA/E* (50%), one strain *tetO* (3.3%), and one strain both *tetM* and *tetK* (3.3%). The study conducted in terms of virulence genes showed that 12 strains carried both *PI-1* (40%) and *cps C*, *D*, *E* genes (40%), 25 strains had *hylB* (83.3%), and 13 strains *cps J*, *K*, *e neu B*. Finally, it was concluded that molecular methods are correct and quicker to determine the pathogenic agent, susceptibility and resistance profiles to antibiotics, and the presence of virulence genes at routine intervals in the prevention and control studies for mastitis in farm animals.

Key words: Antibiotic resistance, buffalo, mastitis, *Streptococcus agalactiae*, virulence genes

1. Introduction

Mastitis is a contagious disease that causes significant milk losses and serious economic losses in dairy animals. Bacteriae are the most important causative agents of mastitis. Bovine mastitis cases are frequently caused by *Escherichia coli*, *Staphylococcus aureus*, *Mycoplasma bovis*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, and *Streptococcus agalactiae* [1].

The agents of mastitis are classified as environmental and contagious according to the primary host and routes of transmission. *S. agalactiae* (Group B *Streptococcus*, GBS) is a bacteria among the contagious agents and causes chronic mastitis [2]. *S. agalactiae* is a Lancefield group B member and can cause chronic and contagious bovine mastitis, invasive diseases along with mastitis in camels, and various diseases in cats, dogs, fish, and hamsters. *S. agalactiae* infections are also important for public health, as they are behind neurological problems in newborns and endometritis and infertility in mothers [3]. It has also been reported that the source of infection in cows may be the infection in humans [4].

S. agalactiae is a Gram-pozitive, catalase-negative, CAMP (Christie, Atkins, and Munch-Peterson) positive, esculin hydrolysis negative, sodium hippurate hydrolysis positive cocci-form bacterium. *S. agalactiae* is a common mastitis agent in cows and well-adapted to the mammary gland. It has the ability to adhere to the udder, and the microenvironment of the udder is important for the growth of bacteria [1].

There is an important relationship between the severity of Group B *Streptococcus* (GBS) infections and the virulence factors of the agents. Among these, the *fbsA* gene codes the fibrinogen-binding protein; the *hylB* gene, the hyaluronate lysate enzyme; the *cps* gene, the protein behind the formation of the polysaccharide capsule; the *neuB* gene, the protein controlling the production of sialic acid; and the *IP-1* gene, the codification of the pili protein [5].

The use of antibacterial drugs for therapeutic and prophylactic purposes in cases of mastitis in farm animals depends on the etiological agent, the appropriate use of drugs, sanitation practices, and the stage of the disease.

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Monitoring the antibiotic resistance profiles of bacteria is very important for clinical and public health [6]. Beta-lactam group antibiotics are among the group of drugs that should be considered in the first place for the treatment. Slightly elevated Minimal Inhibitory Concentrations (MIC) detected in these drugs may indicate the presence of resistant Streptococci [1].

Nowadays, the interest in buffalo milk and dairy products is increasing. Studies on mastitis, antibiotic resistance, and virulence genes in buffaloes are very limited in our country. This study aimed to determine the phenotypic and biochemical characteristics of virulence genes and antibiotic resistances of buffalo-originated *S. agalactiae* isolates.

2. Materials and methods

2.1. Bacterial isolation and identification

The identification of 30 (12%) *S. agalactiae* strains from 250 isolates from milk samples of buffalo with mastitis brought to the Microbiology Laboratory of the Selçuk University Faculty of Veterinary Medicine between June 2021 and June 2022, presenting bacterial growth was performed by phenotypic, biochemical, and PCR methods (rRNA-16) [3]. In addition, the presence of biofilm in the isolates was examined, and the CAMP test was performed [7].

2.2. Antibiotic susceptibility test

The Kirby-Bauer disc diffusion test was used to determine the antibiotic susceptibility of *S. agalactiae* isolates. For this purpose, discs containing penicillin, amoxicillin-clavulanic acid, erythromycin, azithromycin, cephalothin, novobiocin, spiramycin, cefepime, enrofloxacin, cefixime, neomycin, pristinamycin, clindamycin, lincomycin, tetracycline, levofloxacin, oxacillin, ampicillin, cephalexin, vancomycin, chloramphenicol, teicoplanin, linezolid, daptomycin, quinupristin-dalfopristin, rifampicin, sulfazotrim, streptomycin, gentamicin, and kanamycin were used. The results of antibiotic susceptibility were evaluated in accordance with EUCAST criteria.

2.3. Molecular studies

2.3.1. DNA Isolation

For the DNA extraction, a commercial DNA isolation kit (Thermo Scientific GeneJET Genomic DNA Purification Kit, Lot No:01074848) was used according to the manufacturers' instructions.

2.3.2. Multiplex PCR

The rRNA-16S (ribosomal ribonucleic acid) gene was used to confirm by PCR the isolates identified as *S. agalactiae*. The antibiotic resistance genes were investigated with multiplex PCR. The gene regions used for antibiotic resistance genes were as follows: tetracycline resistance: *tetA*, *tetB*, *tetK*, *tetM*, *tetO*; macrolide and lincosamide resistance: *ermA*, *ermB*, *ermC*, *mefA/E*; and aminoglycoside resistance: *strA* and *strB*. The virulence genes of *S. agalactiae* (*fbsA*, *PI-1*, *hylB*, *cps C*, *D*, *E*, *cps J*, *K*, *e neu B)* were also investigated by PCR. The primer sequences and amplicon sizes of the genes used in the PCR are given in Table 1.

Table 1. Primer sequences and amplicon sizes of the genes used in the PCR.

Gene	Sequence (5'-3')	Amplicon size (bp)
rRNA-16S (aga16)	F: TTACCAGGTCTTGACATCCTTCT	116
	R: GACTTAACCCAACATCTCACGAC	
strA	F: CCGTCAATCCCGACTTCTTA	263
	R: CCAGTTCTCTTCGGCGTTAG	
strB	F: CGGTCGTGAGAACAATCTGA	313
	R: ATGATGCAGATCGCCATGTA	
sulI	F: GACGAGATTGTGCGGTTCTT	350
	R: AGGGTTTCCGAGAAGGTGAT	
sulII	F: CCGTCTCGCTCGACAGTTAT	399
	R: ATTTGCGCGAAACAGACAG	
tetA	F: TGTCCGACAAGTTGCATGAT	178
	R: CCTTGAACGGCCTCAATTT	
tetB	F: CTCCTTGGCTTGGAAAAATG	229
	R: AACCAACCGAACCACTTCAC	
tetO	F: TAGCGGAACATTGCATTTGA	290
	R: TTTCTGTAAGTGCCCCAAGC	

tetM	F: AGGGCATCAAGCAACATTTC	366
	R: TCGAGGTCCGTCTGAACTTT	
tetK	F: CCCACCAGAAAACAAACCAA	439
	R: CCCTTCACTGATTATGGTGGT	
mefA/E	F: CGTATTGGGTGCTGTGATTG	248
	R: TATGCACAGGCGTTCCATTA	
msrA	F: AAGGCTTGTCCGCAATACAC	320
	R: CCATTACCCCCAATAAGTGC	
ermB	F: TTTTTGAAAGCCATGCGTCT	201
	R: CTGTGGTATGGCGGGTAAGT	
ermA	F: TCAATGGTTGATGTCGTTCA	165
	R: AGAAGGGATTTGCGAAAAGA	
ermC	F: CAAAACGCTCATTGGCATTA	257
	R: ATCGTCAATTCCTGCATGTT	
Cluster PI-1	F: CTCATCAGTTGACGATTGTTC	751
	R: CCATTGCCTGTTGCTCAC	
hylB	F: GCAACAGCCACTCATAGCA	1180
	R: GAGCGAGGGACACCGAT	
fbsA	F: GCTTTGGCTTTATATGGGAG	1662
	R: GCTACATTAGTAACCTGAGA	
cps C, D, E	F: GCTAATGCTTGCGATGGTT	1852
	R: CTGGTCTTTCTTTTCTAAGGA	
cps J, K, e neu B	F: GGATTAGCCTTTATCACACTT	668
	R: GCAACTTCTTTAGTATTGTATA	

In the amplification of antibiotic resistance genes, a total volume of 10 µL was obtained with 5 µL PPP master mix, 1.4 µL primer mix (0.1 µL (10 pmol) of each primer), 3.1 µL PCR water, and 0.5 µL genomic DNA (20 ng/µL). The amplification process; initial denaturation at 95 °C for 1 min, 35 cycles of denaturation at 95 °C for 30 s, 30 s of annealing at 60 °C and 30 s of extension at 72 °C, concluding with a 7 min final extension step at 72 °C [7]. The PCR products were submitted to electrophoresis (200 V, 150 A, 1 h) in ethidium bromide (10 mg/mL)-containing 2% agarose gel. Finally, the PCR products were visualized and evaluated in an ultraviolet lamp [7].

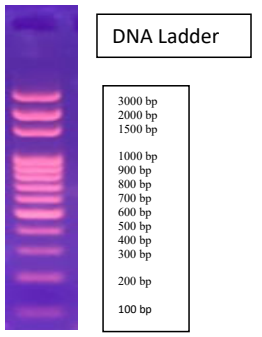
In the amplification of virulence gene regions, the total mix consisted of 30 µL: 0.9 µL each primer (10 pmol), 6 µL master mix, 7.5 µL genomic DNA (50 ng/µL), and 7.5 µL PCR water. The amplification consisted of a 5 min initial denaturation at 94 °C, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing for 1 min at 57 °C, 2 min at 72 °C of extension, and completed with a final extension step at 72 °C for 10 min. The PCR products were submitted to electrophoresis (200 V, 150 A, 1 h) in ethidium bromide (10 mg/mL)-containing 1% agarose gel. The PCR products were visualized and evaluated in an ultraviolet lamp [5].

3. Results

Thirty isolates identified as *S. agalactiae,* according to their phenotypic and biochemical properties, were confirmed as *S. agalactiae* by PCR (rRNA-16S) (Figure 1). In addition, it was observed that 13 of the isolates produced biofilm, and 3 of them had a negative CAMP test.

The antibiotic-resistance genes of *S. agalactiae* isolates were detected by PCR. It was determined that 13 of the isolates presented the gen *ermA* (43.3%) (Figure 2); 15 *mefA/E* (50%) (Figure 2), 1 had both *tetM* and *tetK* genes (3.3%) (Figure 2), 1 *ermB* (3.3%) (Figure 3) and one *tetO* (3.3%) (Figure 4).

The multiplex PCR targeting virulence genes (*fbsA*, *hylB*, *Cluster PI-1*, *cps C*, *D*, *E* and *cps J*, K, *e neu B*) determined that 12 isolates carried both *PI-1* (40%) and *cps C*, *D*, *E* gene (40%), 25 isolates carried *hylB* (83.3%) and 13 isolates carried *cps J*, *K*, *e neu B* gene (43.3%). The *fbsA* gene could not be detected in any of the isolates (Figure 5).



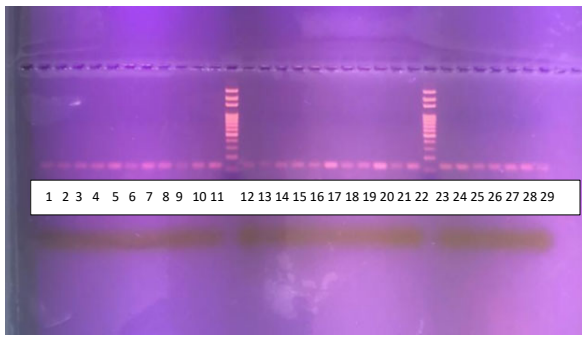


Figure 1. Gel image of the rRNA-16S (aga16) gene region of *S. agalactiae* isolates.

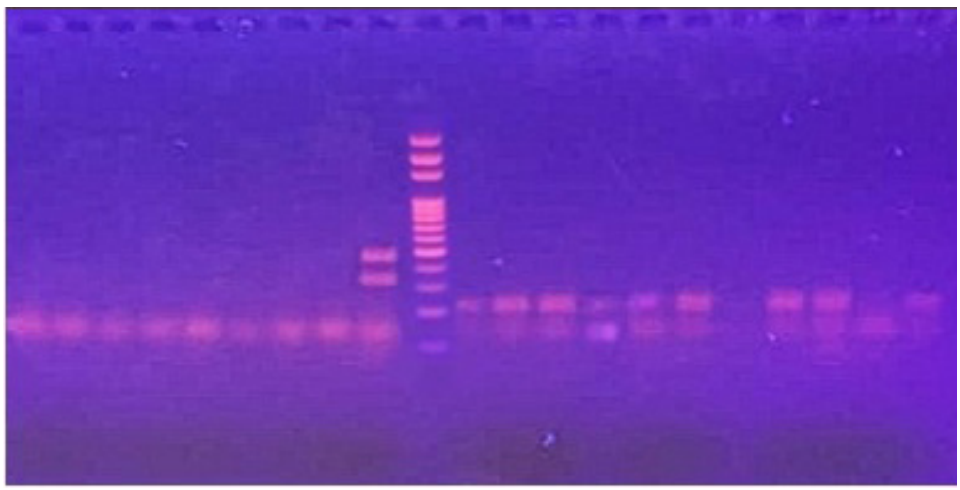


Figure 2. Gel image of *tetK*, *tetM*, *mefA/E*, and *ermA* gene regions of *S. agalactiae* isolates.

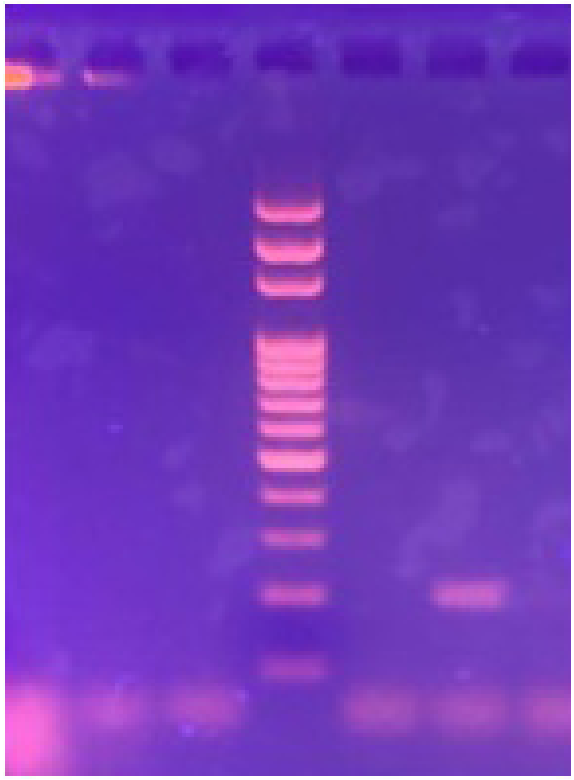


Figure 3. Gel image of the *ermB* gene region of the *S. agalactiae* isolates.

Figure 4. Gel image of the *tet O* gene region of *S. agalactiae* isolates.

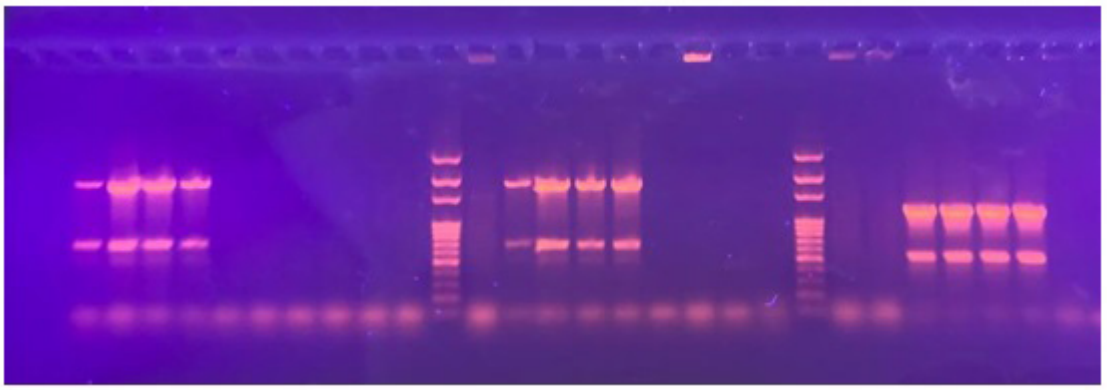


Figure 5. Gel image of virulence gene regions of *S. agalactiae* isolates.

Concerning the antibiotic susceptibilities of *S. agalactiae* isolates, all the isolates were found susceptible to cephalosporins (cephalexin, cefixime, cephalothin, cefepime), penicillins (ampicillin, amoxicillin-clavulanic acid, oxacillin) and vancomycin. Two isolates were found as resistant to spiramycin, 9 isolates to erythromycin, 6 isolates to clindamycin, 2 isolates to azithromycin, 17 isolates to lincomycin, 2 isolates to tetracycline, 8 isolates to chloramphenicol, 2 isolates to glycopeptides (teicoplanin), 6 isolates to quinolones (levofloxacin), 1 isolate to linezolid, and 3 isolates to rifampicin. The susceptibility results of neomycin (8–21 mm), gentamicin (12–19 mm), streptomycin (9–20 mm), kanamycin (8–22 mm), enrofloxacin (18–30 mm), daptomycin (18–38 mm), novobiocin (11–28 mm) and quinupristin-dalfopristin (8–26 mm) were given as information. The results of the disc diffusion test of the isolates of *S. agalactiae* whose antibiotic resistance genes were investigated are given in Table 2.

Table 2. Disc diffusion test and antibiotic resistance gene results of *S. agalactiae* isolates.

Antibiotics	Disc diffusion test		Antibiotic resistance genes										
	S	R	*tetA*	*tetB*	*tetK*	*tetM*	*tetO*	*ermA*	*ermB*	*ermC*	*mefA/E*	*strA*	*strB*
Tetracycline	28	2	-	-	1	1	1						
Spiramycin	28	2											
Erytromycine	21	9											
Clindamycin	24	6						13	1	-	15		
Azitromycin	28	2											
Lincomycine	13	17											
	Growth inhibition zone diameter												
Neomycin	8–21 mm											-	-
Gentamycin	12–19 mm											-	-
Streptomycin	9–20 mm											-	-
Kanamycin	8–22 mm											-	-
-: Not detected, S: susceptible, R: resistant													

4. Discussion

Buffaloes are livestock raised for the production of both milk and meat. Buffaloes, known as black gold in Asia, are an important dairy animal, especially in countries such as Pakistan, which ranks second in world buffalo milk production. Italy is one of the countries in Europe where buffalo production has gained popularity in recent years. The reason behind this is the attractiveness of Mozzarella cheese and buffalo milk [8]. The buffalo population in Türkiye is approximately 192,000 buffaloes, Anatolian buffalo, which is among the Mediterranean buffalo breeds, is mostly bred in the Central Black Sea Region [9].

Mastitis is among the most widespread infectious diseases that cause milk loss in farm animals worldwide [10]. Ali et al. [11] reported that the prevalences of clinical and subclinical mastitis were 13.6% and 41.8%, respectively, and that the highest incidence (43.3%) was observed at the beginning of lactation. In 1637 buffalo milk in Afyonkarahisar, the rates of isolation of *Candida* species/coagulase-negative *Staphylococcus* (CNS) and *S. aureus* and mixed infections were 41.91%, 20.59%, and 16.91%, respectively [12]. In our country, Hadimli et al. [13] reported that out of 104 catalase-negative cocci isolates obtained from cattle with subclinical mastitis, 32 were *Enterococcus faecalis* (30.76%); 21, *Aerococcus viridans* (20.19%); 18, *E. faecium* (17.30%); 13, *S. agalactiae*. (12.50%); 11, *S. uberis* (10.57%); 3, *Lactococcus garviae/lactis* spp. *lactis* (2.88%); 2, *L. lactis* spp. *latis* (1.92%); and 1 was (0.96%) *L. garviae*. In a study that determined the prevalence of subclinical mastitis in Egypt, subclinical mastitis was determined in 71.6% and 43.5% of the milk of 340 cows and buffaloes, respectively. The bacteriological analysis showed a 38.3% *S. aureus* and 20% *S. agalactiae*, while these rates were 41% and 22% by PCR. In the same study, it was reported that in the nose swap samples obtained from 50 volunteer animal owners, a 50% of *S. aureus* positivity was determined by PCR, while the presence of *S. agalactiae* was not found [14]. In a study conducted in China, the pathogens isolated from cows with mastitis were *E. coli* (14.4%), *Klebsiella* spp. (13.0%), CNS (11.3%), *S. dysgalactiae* (10.5%), *S. aureus* (10.2%), *S. agalactiae* (2.8%), and *S. uberis* (2.1%); *S. agalactiae* and other *Streptococcus* strains were reported to be frequently isolated in the winter season (October–March) [15].

Bzdil [16] reported that an aetiological agent in cow mastitis cases between 2000 and 2010 was the *S. agalactiae* strain, with a rate of 5.6% [16].

In a study conducted in 1566 cows and buffaloes (1096+470) with mastitis in Pakistan, it was reported that clinical mastitis was found at a higher rate in cows (20%) than in buffaloes (11%); buffaloes showed a higher rate (66%) of subclinical mastitis than cows (53%); the etiological agents were *Staphylococcus* spp. (34%), *E. coli* (19%), *Streptococcus* spp. (9%), *Klebsiella* spp. (8%), *Salmonella* spp. (2%), *Proteus* spp. (1%), and *Candida* spp. (0.6%); 92% of the isolates presented susceptibility to gentamicin, 88% to enrofloxacin, and 79% to ciprofloxacin, but they were found resistant to sulfamethoxazole (99%), lincomycin (98%), oxytetracycline (89%), ampicillin (86%), and doxycycline (85%) [11]. In another study conducted in buffaloes in Pakistan, the prevalence of *Streptococcus* spp. was determined as 23% [17].

Evren et al. [18], in their antibiotic susceptibility tests performed by disc diffusion method on 166 GBS human isolates, showed sensitivity in all the isolates to penicillin, tigecycline, vancomycin, and linezolid; to nitrofurantoin, levofloxacin and norfloxacin in 94.5%, 78.3%, and 77.7%, respectively; they also reported that the resistance to erythromycin and clindamycin was 35.5% and 30.7%, respectively, and the rate of iMLSB (inducible clindamycin resistance) was 73%.

In India, Sharma et al. [19], in a study conducted on 1299 buffaloes in lactation, *Staphylococci* (51.16%), *Streptococci* (37.94%), *E. coli* (8.41%), and *Corynebacterium pyogenes* (1.62%) were identified from milk; except for *E. coli*, the others were highly susceptible to ceftriaxone and cefoperazone. It was reported that *Streptococci* isolates were susceptible at 94.28% and 95.23%, respectively. In the same region, Thakur et al. [20] reported that, from 51 buffalo milk samples with mastitis and bacterial growth, they isolated 35.29% *S. aureus* and 25.49% *S. agalactiae*, and they did not isolate coliform microorganisms.

In Egypt, of 87/310 cows and 39/240 buffalo presenting clinical mastitis, *Streptococcus* spp. was isolated from 39.7% of cows and 60% of buffalo, of which, respectively, 10 (13.7%) and 15 (20.5%) were *S. agalactiae*; 5 (6.8%) and 10 (23.7%), *S. dysgalactiae;* 8 (10.6%) and 7 (13.7%), *S. uberis*; 3 (4.1%) and 10 (13.7%), *E. fecalis*; 3 (4.1%) and 2 (2.7%), *S. lactarius*. The antimicrobial susceptibility test showed a 100% resistance to gentamicin, penicillin, doxycycline, and streptomycin, while on the contrary, 100% sensitivity to ciprofloxacin, ceftriaxone, and cotrimoxazole. In the biofilm test performed on YESCA CR agar, all *S. agalactiae* and *S. dysgalactiae* strains, 78%, 50%, and 75% *S. uberis*, *S. lactarius,* and *E. fecalis,* respectively, were reported positive [21].

In a study conducted in China, it was reported that, according to the biochemical, phenotypic and 16S rRNA sequence characteristics of the isolates identified in 313 farm animals with mastitis, 105 (33.6%) were *S. agalactiae*; 100% susceptible to aminoglycosides (gentamicin, kanamycin, tobramycin, and neomycin); 98.1% resistant to beta-lactams (penicillin, ceftazidime, amoxicillin, and piperacillin), therefore they carry the *TEM* gene; and that all the isolates carry *cfb*, *cylE*, *fbsA*, *fbsB*, *hylB,* and *α-enolase* genes [22].

In a study conducted in New York by Doğan et al. [23] in which 52 human and 83 bovine *S. agalactiae* strains were investigated, it was reported that tetracycline and erythromycin resistance was 84.6%–26.9% and 14.5%–3%, respectively, and this resistance had most likely developed independently in humans and cattle; that although human isolates may rarely be transmitted to animals, resistance genes may occasionally be transferred between species.

In a study conducted in Brazil in 2014, the presence of *S. agalactiae* was determined in 1042 buffalo milk samples, 28.8% during the dry period and 9.24% during the lactation period [24]. In the following years, in the bacteriological study performed in buffaloes with subclinical mastitis; of 20 milk samples, the presence of *Streptococcus* spp. was determined by biochemical tests in 11 (5.5%) and *S. agalactiae* in 8 by molecular methods. High sensitivity to gentamicin and oxacillin has been reported in molecular studies [25].

In this study, 30 (12%) *S. agalactiae* were identified. The presence of biofilm was determined in 43.3% of these isolates, and CAMP test positivity was determined in 90%. It was determined by the method of disc diffusion that 100% of the isolates were sensitive to penicillin, cephalosporin, and vancomycin; on the contrary, 63.3% were resistant to macrolides, 6.3% to tetracycline, 56.6% to lincosamide, 26.6% to chloramphenicol, 6.6% to teicoplanin, 20% to levofloxacin, 3.3% to linezolid, and 10% to rifampicin.

Nowadays, to determine mastitis agents and their antibiotic resistance profiles and virulence genes, molecular methods are of preference in many studies [5, 26].

Ruegg et al. [27] reported that the strains of *Streptococcus* spp. isolated from cattle with mastitis presented an antibiotic resistance of 52.6%, being 47.7% *tetM*, 20.9% *tetO,* and 18.6% *tetK*. Rato et al. [28] reported a higher presence of *tetK* in cattle with mastitis.

In light of the literature, studies involving different regions and their results are very important as antibiotic resistance profiles may differ from region to region.

Zhang et al. [29] isolated from 1122 cow milk samples collected from 27 farms in 2012–2018 26.2% *S. agalactiae*, 23.2% *E. coli,* and 10.6% *S. aureus*; resistance using the disk diffusion method to kanamycin (93.8%), gentamicin (49.4%), vancomycin (49.4%), tetracycline (35.8%), clindamycin (34.6%), and erythromycin (32.1%) was determined; by PCR method the presence of *ermA* (53.1%) and *ermB* (85.2%) genes was reported, along with the virulence genes *scpB* (81.4%), *cyl* (100%), *glnA* (76.6%), *cfb* (98.8%), *hylB* (98.8%), and *scaA* (69.1%).

In this study, the presence of 43.3% *ermA* gene, 3.3% *ermB* gene, 50% *mefA/E* gene, and 6.6% *tetK*, *tetM,* and *tetO* genes were determined by PCR.

In cases of subclinical and clinical mastitis caused by *S. agalactiae* in cow herds in Brazil, genes *fbsA* (85.7%), *hylB* (38.8%), *cps C*, *D*, *E* (4.48%), *cpsJ*, *K*, *neuB (*79.1%), and *Pl-1* (1.49%) were determined in 16 isolates [5].

In this study, the presence of 40% *PI-1* and *cps C*, *D*, *E*; 83.3% *hylB*, and 43.3% *cps J*, *K*, *e neuB* genes was determined.

5. Conclusion

In this study, the resistance to antibiotics and virulence gene profiles of *S. agalactiae* isolates from buffalo herds with mastitis were revealed by the multiplex PCR method. It is important to detect resistance genes in isolates that phenotypically did not show resistance. In addition, determining the result more accurately and in a shorter time will ensure that the measures and treatment to be taken in terms of herd health are carried out with the right approach and faster, preventing the unnecessary usage of antibiotics.

As a result, the identification of the pathogenic agent, along with the determination of the susceptibility and resistance profiles to antibiotics at routine intervals and in different regions, will be of great benefit for determining the prevention and control actions against mastitis in farm animals in addition to the treatment protocols, and, therefore, in choosing a quicker and more accurate treatment approach.

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References

1. Keefe GP. Streptococcus agalactiae mastitis. Canadian Veterinary Journal 1997; 38 (7): 429-437.
2. Jain B, Tewari AA, Bhandari B, Jhala MK. Antibiotic resistance and virulence genes in Streptococcus agalactiae isolated from cases of bovine subclinical mastitis. Veterinarski Arhiv 2012; 82 (5): 423-432.
3. Markey B, Leonard F, Archambault M, Cullinane A, Maguire D. Clinical Veterinary Microbiology E-Book (Amsterdam: Elsevier Health Sciences) 2013; 857.
4. Zadoks RN, Middleton JR, McDougall S, Katholm J, Schukken YH. Molecular epidemiology of mastitis pathogens of dairy cattle and comparative relevance to humans. Journal of Mammary Gland Biology and Neoplasia 2011; 16 (4): 357-72. https://doi.org/10.1007/s10911-011-9236-y
5. Arpini CM, Cardoso PG, Paiva IS, Costa Custódio DA, Costa GM. Virulence genes of the Streptococcus agalactiae associated with bovine mastitis in Minas Gerais livestock herds, Brazil. Applied Microbiology 2016; 2 (3): 1-7. https://doi.org/10.4172/2471-9315.1000119

6. Saini V, McClure JT, Léger D, Dufour S, Sheldon AG et al. Antimicrobial use on Canadian dairy farms. Journal of Dairy Science 2012; 95 (3): 1209-1221.
7. Pyatov V, Vrtková I, Knoll A. Detection of selected antibiotic resistance genes using multiplex PCR assay in mastitis pathogens in the Czech Republic. Acta Veterinaria Brno 2017; 86 (2): 167-174. https://doi.org/10.2754/avb201786020167
8. Moroni P, Sgoifo Rossi C, Pisoni G, Bronzo V, Castiglioni B et al. Relationships between somatic cell count and intramammary infection in buffaloes. Journal of Dairy Science 2006; 89 (3): 998-1003. https://doi.org/10.3168/jds.S0022-0302(06)72165-8
9. Ugurlu M. Buffalo breeding and buffalo breeds in Türkiye and world and their production characteristics. Turkiye Klinikleri Animal Nutrition and Nutritional Diseases - Special Topics 2017; 3 (2): 77-83.
10. De Vliegher S, Fox LK, Piepers S, McDougall S, Barkema HW. Invited review: mastitis in dairy heifers: nature of the disease, potential impact, prevention, and control. Journal of Dairy Science 2012; 95 (3): 1025-1040. https://doi.org/10.3168/jds.2010-4074
11. Ali T, Rahman A, Qureshi MS, Hussain MT, Khan MS et al. Effect of management practices and animal age on incidence of mastitis in Nili Ravi buffaloes. Tropical Animal Health and Production 2014; 46 (7): 1279-1285. https://doi.org/10.1007/s11250-014-0641-2
12. Özenç E, Vural M, Şeker E, Uçar M. An evaluation of subclinical mastitis during lactation in anatolian buffaloes. Turkish Journal of Veterinary & Animal Sciences 2008; 32 (5): 359-368.
13. Hadimli HH, Sayin Z, Erganis O, Kav K. Identification and antibiotic susceptibility of catalase negative Gram positive cocci isolated from dairy cows with subclinical mastitis. Eurasian Journal of Veterinary Sciences 2013; 29 (3): 153-158.
14. Elhaig MM, Selim A. Molecular and bacteriological investigation of subclinical mastitis caused by Staphylococcus aureus and Streptococcus agalactiae in domestic bovids from Ismailia, Egypt. Tropical Animal Health and Production 2015; 47: 271-276. https://doi.org/10.1007/s11250-014-0715-1
15. Gao J, Barkema HW, Zhang L, Liu G, Deng Z et al. Incidence of clinical mastitis and distribution of pathogens on large Chinese dairy farms. Journal of Dairy Science 2017; 100 (6): 4797-4806. https://doi.org/10.3168/jds.2016-12334
16. Bzdil J. Prevalence of selected pathogens of mammary gland in cattle during the years 2000 to 2010. Veterinarstvo 2011; 61: 7-11.
17. Khan AZ, Muhammad G, Quarter-wise comparative prevalence of mastitis in buffaloes and crossbred cows. Pakistan Veteterinary Journal 2005; 25 (1): 9-12.
18. Evren K, Yardibi Demir HA, Mutlu Sarıgüzel F, Dinç B. Klinik örneklerden izole edilen streptococcus agalactiae izolatlarinin antibiyotik duyarliliklari. Türk Mikrobiyoloji Cemiyeti Dergisi 2021; 51 (3): 239-244. https://doi.org/10.5222/TMCD.2021.71601
19. Sharma A, Chhabra R, Singh M, Charaya G. Prevalence, etiology and antibiogram of bacterial isolates recovered from mastitis of buffaloes. Buffalo Bulletin 2018; 37 (3), 313-320.
20. Thakur S, Singh M, Aseri GM, Verma A, Khan SS. Isolation and characterization of mastitis pathogens and milk composition changes in Murrah buffaloes (Bubalus bubalis) during winter season. Indian Journal of Animal Research 2018; 52 (2): 276-280. https://doi.org/10.18805/ijar.11320
21. Raheel I, Mohammed A, Mohamed A. Phenotypic and genotypic characterization of streptococci associated with clinical bovine mastitis. Journal of Veterinary Medical Research 2022; 29 (1): 13-20. https://doi.org/10.21608/JVMR.2022.121460.1050
22. Han G, Zhang B, Luo Z, Lu B, Luo Z et al. Molecular typing and prevalence of antibiotic resistance and virulence genes in Streptococcus agalactiae isolated from Chinese dairy cows with clinical mastitis. PLoS One 2022; 17 (5): e0268262. https://doi.org/10.1371/journal.pone.0268262
23. Dogan B, Schukken YH, Santisteban C, Boor KJ. Distribution of serotypes and antimicrobial resistance genes among Streptococcus agalactiae isolates from bovine and human hosts. Journal of Clinical Microbiology 2005; 43 (12): 5899-5906. https://doi.org/10.1128/JCM.43.12.5899-5906.2005
24. Pizauro LJL, Silva DG, Santana AM, Clemente V, Lara GHB et al. Prevalence and etiology of buffalo mastitis and milk somatic cell count in dry and rainy seasons in a buffalo herd from Analandia, São Paulo State, Brazil. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 2014; 66 (6): 1703-1710. https://doi.org/10.1590/1678-7259
25. Vásquez-García A, dos Santos Silva T, de Almeida Queiroz SR, Godoy SHS, Fernandes AM et al. Species identification and antimicrobial susceptibility profile of bacteria causing subclinical mastitis in buffalo. Pesquisa Veterinária Brasileira 2017; 37 (5): 447-452. https://doi.org/10.1590/S0100-736X2017000500004
26. Malhotra-Kumar S, Lammens C, Piessens J, Goossens H. Multiplex PCR for simultaneous detection of macrolide and tetracycline resistance determinants in streptococci. Antimicrobial Agents and Chemotherapy 2005; 49 (11): 4798-4800. https://doi.org/10.1128/AAC.49.11.4798-4800.2005
27. Ruegg, PL, Jin LW, Okwumabua O. Phenotypic antimicrobial susceptibility and occurrence of selected resistance genes in gram-positive mastitis pathogens isolated from Wisconsin dairy cows. Journal of Dairy Science 2015: 98 (7): 4521-4534. https://doi.org/10.3168/jds.2014-9137
28. Rato MG, Bexiga R, Florindo C, Cavaco LM, Vilela CL et al. Antimicrobial resistance and molecular epidemiology of streptococci from bovine mastitis. Veterinary Microbiology 2013; 161 (3-4): 286-294. https://doi.org/10.1016/j.vetmic.2012.07.043
29. Zhang Z, Yang F, Li X-pu, Luo J-yin, Liu L-hai et al. Distribution of serotypes, antimicrobial resistance and virulence genes among Streptococcus agalactiae isolated from bovine in China. Acta Scientiae Veterinariae 2019; 47: 1699. https://doi.org/10.22456/1679-9216.97254