VETERINARSKI ARHIV 82 (5), 423-432, 2012

Antibiotic resistance and virulence genes in *Streptococcus agalactiae* isolated from cases of bovine subclinical mastitis

Beenu Jain[1]*, Anuj Tewari[2], Bharat B. Bhandari[1], and Mayur K. Jhala[1]

[1]*Department of Veterinary Microbiology, Veterinary College Anand, AAU Anand, Gujrat, India*
[2]*Department of Veterinary Microbiology, Veterinary College Mathura, India*

JAIN, B., A. TEWARI, B. B. BHANDARI, M. K. JHALA: Antibiotic resistance and virulence genes in *Streptococcus agalactiae* isolated from cases of bovine subclinical mastitis. Vet. arhiv 82, 423-432, 2012.

ABSTRACT

Twenty-seven *S. agalactiae* isolates obtained after screening 89 cows for subclinical mastitis were studied for antibiotic resistance and virulence genes. The highest resistance was found by Disc diffusion method for Streptomycin (85.1%) whereas the lowest resistance was found for Gentamicin (3.7%). Resistance against Tetracycline, Erythromycin, Co-trimoxazole, Ampicillin and Enrofloxacin was 55.5%, 33.3%, 11.1%, 11.1%, and 7.4% respectively. PCR based detection revealed three isolates carrying the *tet*O gene associated with Tetracycline resistance and seven isolates carrying the *Erm*B gene associated with Erythromycin resistance. Molecular detection of virulence associated genes revealed that out of 27 isolates of *S. agalactiae,* none was carrying the *bca* gene, while 6, 7, 8 and 6 isolates were positive for *Scp*B, *rib*, *lmb* and *cyl*E genes respectively.

Key words: chronic, contagious, disc diffusion, mastitis, molecular detection, resistance, virulence

Introduction

S. agalactiae, the lone member of the Lancefield group B, is an important cause of chronic, contagious bovine mastitis. It also causes mastitis and invasive disease in camels and is an occasional cause of disease in dogs, cats, fish, and hamsters. Its presence is frequently associated with high somatic cell counts in milk and decreased milk yield. Unidentified carrier cows are responsible for spreading the pathogen to herdmates (KEEFE, 1997). *S. agalactiae* infections have major consequences for public health, because they may cause neurological problems in newborn humans and endometritis and sterility in

*Corresponding author:
Dr. Beenu Jain (M.V.Sc. Veterinary Microbiology), 5/233 Vipul khand Gomtinagar, Lucknow. 226010, Uttar Pradesh, India, Phone: +945 5555 890; E-mail: venusjain31@gmail.com

ISSN 0372-5480
Printed in Croatia

the mothers. The major component of the Streptococcal cell surface is a polysaccharide coat, which is serotype specific. A complement of proteins is embedded throughout this coat. To date, only a few such proteins have been identified. Predominant amongst these are the tandem repeat proteins, including Cα protein (WILKINSON and EAGON, 1971), R protein (LANCEFIELD and PERLMANN, 1952), Rib protein (WÄSTFELT et al., 1996), the Cβ protein (RUSSELL et al., 1992) and X protein (RAINARD et al., 1991). Other identified outer surface proteins include glutamine synthetase (SUVOROV et al., 1997) and α enolase (PANCHOLI and FISCHETTI, 1998).

The severity of neonatal disease in Group B streptococcus (GBS) infections may be determined mostly by the number of virulence factors encoded, among others by the *cps* gene cluster coding for the capsule and the *scpB* gene coding for surface enzyme ScpB (a C5a peptidase), which causes impairing of neutrophil recruitment and binds fibronectin to promote bacterial invasion of epithelial cells (BECKMANN et al., 2002). Previously, C5a peptidase genes were found only in group B streptococci (*scpB* gene), group A streptococci (*scpA* gene) and group G streptococci (*scpG* gene) (FRANKEN et al., 2001). The *ScpB* gene, being extremely homologous to the *scpA* and *scpG* genes, has a 51 bp deletion at the 3'-end of the gene (CHMOURYGUINA et al., 1996). The *bca* gene codes for alpha-C protein, a surface protein that helps the bacteria to enter the host cells (BOLDUC et al., 2002), the *lmb* gene codes for lmb (laminine-binding protein), a surface protein that plays a role in the invasion of the damaged epithelium (SPELLERBERG et al., 1999), the *cylE* gene codes for *β*-hemolysin, a toxin that plays a role in tissue injury and the systemic spread of the bacteria and contributes to meningitis (DORAN et al., 2003), and the *rib* gene encodes the surface Rib protein mostly present in invasive strains (STALHAMMAR et al., 1993). The CAMP factor is a 23.5 kDa ceramide binding protein of *S. agalactiae* that potentiates the action of staphylococcal sphingomyelinase (beta toxin). The lethal properties of the CAMP factor for cell cultures and for rabbits and mice suggest that it may have a cytotoxic action for mammary tissue. *S. agalactiae* is an obligate parasite of the epithelium and tissues of ruminant mammary glands, and eradication of the organism from herds is, therefore, possible by identification of animals with mammary infection followed by treatment or culling.

Treatment with intramammary infusion of antibiotics is the main approach to deal with the infection, and a number of studies on *in vivo* and *in vitro* trials to assess the antibiotic sensitivity/resistant pattern have been documented. However, there are few reports focusing upon the genes involved in resistance especially for *S. agalactiae* isolates of bovine origin. GBS resistant to penicillin has not been reported so far. However, rare GBS clinical strains with reduced sensitivity for penicillin have been recorded. The present study aims to identify antibiotic resistant and virulence genes in *Streptococcus agalactiae* isolated from subclinical mastitis cases.

Materials and methods

The present study focused on the isolation and identification of the *S. agalactiae,* antibiotic sensitivity pattern of *S. agalactiae* isolates, PCR based detection of *S. agalactiae* and genes associated with virulence and antibiotic resistance. All the 27 isolates were from cattle. The antibiotic discs were obtained from HiMedia Laboratories Ltd. Mumbai. Isolates were tested against commonly used antibiotics viz: Streptomycin, Tetracycline, Co-Trimoxazole, Enrofloxacin, Erythromycin, Gentamicin and Ampicillin. All the confirmed *S. agalactiae* isolates were subjected to an antibiotic sensitivity test by the Disc diffusion method using MH media.

Table 1. Zone size interpretative chart for the *in vitro* antibiotic sensitivity test

S. No. 1.	Antimicrobial agent	Symbol	Antibiotic concentration (in mcg)	Diameter of zone of inhibition (mm)*		
				R	I	S
1	Streptomycin	ST	300 mcg	11	12-14	15
2	Tetracycline	T	10 mcg	14	15-18	19
3	Ampicillin	A	10 mcg	18	19-21	22
4	Enrofloxacin	En	10 mcg	15	16-20	21
5	Cotrimoxazole	CO	25 mcg	15	16-20	21
6	Erythromycin	E	15 mcg	12	13-16	17
7	Gentamicin	G	10 mcg	12	13-14	15

R - Resistant; I - Intermediate; S - Sensitive

PCR was carried out to identify the isolates by using primer sagF 432 and sagR1086 as per RIFFON et al. (2001). For detection of antibiotic resistance genes from the confirmed *S. agalactiae* isolates, PCR was carried out using primer pairs as per DOGAN et al. (2005) (Table 2).

Table 2. Details of primers used for antibiotic resistance genes

Primers	Sequences (5'- 3')	Target Gene	Size of amplified product (bp)
ErmB(F) ErmB(R)	GAAAAGGTACTCAA CCAAATA AGTAACGGTACTTAAATTGTTTAC	*ErmB*	442
tetO(F) tetO(R)	AGCGTCAAAGGGGAATCACTATCC CGGCGGGGTTGGCAAATA	*tetO*	1723

The initial denaturation temperature was 94 °C for 5 min. Denaturation was done for 30 sec. for the *ErmB* gene and 1 min. for the *tetO* gene. Annealing was done at 50 °C for the *ErmB* gene and at 55 °C for the *tetO* gene for 30 sec. and 1 min. respectively. Extension was done for 1 min. and 1 min. 30 sec. for the *ErmB* and *tetO* genes respectively.

PCR was carried out for the virulence associated genes using primers as given in Table 3.

Table 3. Primers used for the virulence associated genes were as follows

Primers	Sequences (5'- 3')	Target Gene	Size of amplified product (bp)	References
bca (F) bca (R)	TAACAGTTATGATACTTCACAGAC ACGACTTTCTTCCGTCCACTTAGG	*bca*	535	MANNING et al. (2006)
ScpB (F) ScpB (R)	ACAACGGAAGGCGCTACTGTTC ACCTGGTGTTTGACCTGAACTA	*scpB*	255	DMITRIEV et al. (2004)
rib (F) rib (R)	CAGGAAGTGCTGTTACGTTAAAC CGTCCCATTTAGGGTTCTTCC	*rib*	369	MANNING et al. (2006)
lmb(F) lmb(R)	ACCGTCTG AAATGATGTGG GATTGACGTTGTCTTCTGC	*lmb*	572	SPELLERBERG et al. (1999)
CylE(F) Cyl(R)	TGACATTTACAAGTGACGAAG TTGCCAGGAGGAGAATAGGA	*cylE*	248	BERGSENG et al. (2007)

Initial denaturation was done at 94 °C for the *scpB* gene (1 min.) and at 95 °C for the *rib, bca, CylE* and *lmb* genes for 5 min., 5 min., 10 min. and 5 min. respectively. Denaturation was done at 94 °C for 1 min. Annealing of the *ScpB* gene was done at 47 °C for 1 min., the *rib* gene at 55 °C for 30 sec., the *bca* gene at 55 °C for 30 sec., the *CylE* gene at 55 °C for 1 min. and for the *lmb* gene at 54 °C for 1 min. Extension was done at 72 °C for 2 min. Total cycles were kept 35.

Results

All the 27 isolates of *S. agalactiae* were tested for *in vitro* sensitivity to seven antibacterial drugs and the sensitivity/resistance of individual isolates to various drugs was interpreted according to the manufacturer's instructions (Hi Media Ltd., Mumbai, India). In the present study, *S. agalactiae* isolates were found variably resistant to the antibiotics tested. Overall, the highest percentage of the isolates were resistant to Streptomycin (85.1%), followed by Tetracycline (55.5%), Erythromycin (33.3%), Co-trimoxazole (11.1%), Ampicillin (11.1%), Enrofloxacin (7.4%) and Gentamicin (3.7%).

Out of 27 isolates tested for identification of Tetracycline resistance genes, three isolates could be identified as Tetracycline resistant as they yielded an amplification product of 1723 bp and seven isolates could be identified as Erythromycin resistant as they yielded an amplification product of 442 bp (Fig. 1).

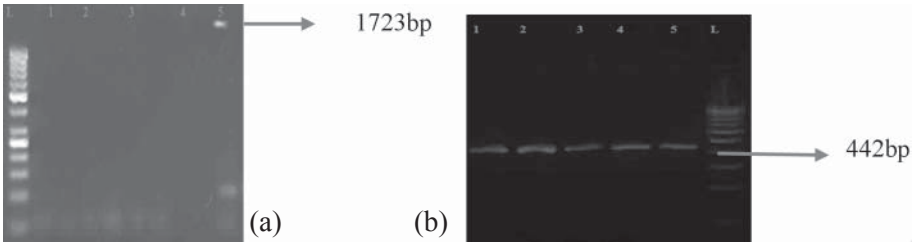

Fig. 1 (a) and (b): Agarose gel electrophoresis of PCR amplified Tetracycline and Erythromycin resistant genes, product showing 1723 bp using primer set tet(O) and 442 bp using primer set ErmB respectively

Molecular detection of virulence associated genes in 27 *S. agalactiae* isolates revealed that none of the isolate was carrying the *bca* gene. Six isolates were positive for the *Scp*B gene, seven isolates for the *rib* gene, eight isolates for the *lmb* gene and six isolates were positive for the *cyl*E gene (Fig. 2).

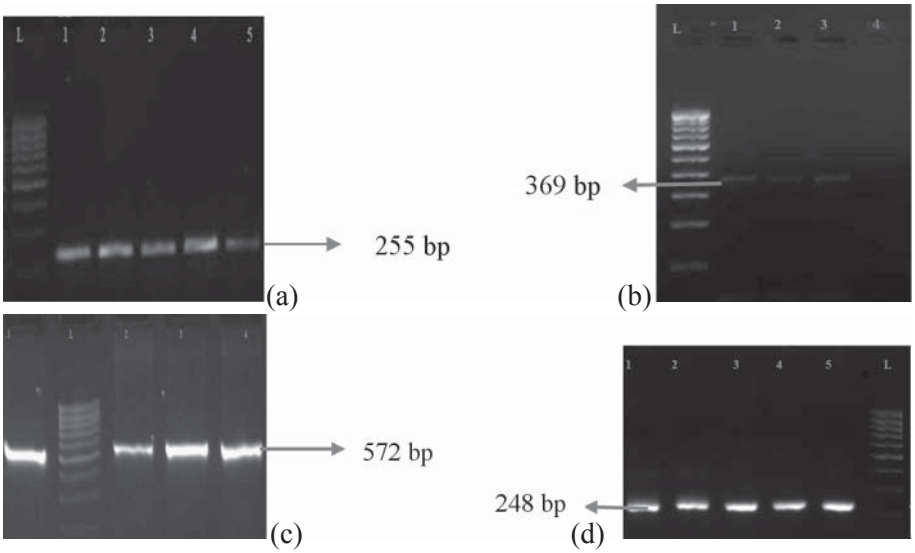

Fig. 2 (a), (b), (c) and (d): Agarose gel electrophoresis of PCR amplified *ScpB* gene, *rib* gene, *lmb* gene and *cylE* genes showing product size 255bp, 369bp, 572bp, 248bp respectively

Discussion

S. agalactiae infections in both humans and bovines are treated by administration of antibiotics. Penicillin is the drug of choice for treatment of both human and bovine *S. agalactiae* infections (SCHRAG et al., 2000). For penicillin-allergic individuals, erythromycin and clindamycin are recommended. The prevalence of resistance to erythromycin and clindamycin has been increasing in *S. agalactiae* (UH et al., 2001); recently, resistance to erythromycin has been associated with human *S. agalactiae* serotype III and V isolates (LIN et al., 2000). Extensive use of antibiotics in medicine and animal husbandry results in increased antibiotic resistance among bacterial populations. Several studies have suggested that antimicrobial use in animals causes the development of antibiotic resistance among pathogens in humans.

In the present study 15 out of 27 isolates were resistant to Tetracycline based on the disc diffusion method, whereas four isolates were found sensitive. Out of 15 resistant isolates, only two isolates were found positive for Tetracycline resistance gene (*tet*O) and thirteen isolates were negative. All the four Tetracycline sensitive isolates were also negative for the presence of the Tetracycline resistance gene (Table 4).

Table 4. Comparative analysis of the tetracycline resistance profile

PCR based detection	Disc diffusion method				
	R	S	I	Total
+ve	2	0	1	3
-ve	13	4	7	24
Total	15	4	8	27

R- Resistant, S- Sensitive, I-Intermediate

Out of nine Erythromycin resistant isolates shown by the disc diffusion method, two isolates had the gene associated with the Erythromycin resistance. A total of five isolates had the Erythromycin resistance gene of 18 Erythromycin sensitive isolates, whereas the remaining 13 isolates were negative for the presence of the Erythromycin resistance gene (Table 5).

Table 5. Comparative analysis of the erythromycin resistance profile

PCR based detection	Disc diffusion method			
	R	S	I	Total
+ve	2	5	0	7
-ve	7	13	0	20
Total	9	18	0	27

Out of 27 isolates of *S. agalactiae,* none of the isolates carried the *bca* gene as evident from the absence of the 183 bp PCR product. HANNOUN et al. (2009) observed 56.5% isolates positive for the *bca* gene. In the present study the *Scp*B gene was found in 22.2% (6/27) isolates. According to DMITRIEV et al. (2004), all the strains isolated from humans and only 9% of bovine isolates had the *ScpB* gene. DMITRIEV et al. (2004) observed that the presence of the *ScpB* gene is more frequent in bovine isolates. The majority of *S. agalactiae* strains express one or more surface-anchored proteins, such as the Rib protein, that vary by strains. These proteins are characteristic for *S. agalactiae*, and are able to induce the formation of protective antibodies. In the present study, 25.9% (7/27) isolates were found positive for the presence of the *rib* gene. MAELAND et al. (2005) observed 93% (56/60) and VIDOVA et al. (2009) 73% isolates positive for the presence of the *rib* gene. Adherence to extracellular matrix proteins is considered an important factor in the pathogenesis of Streptococcal infection. The *lmb* gene (laminin binding protein) plays an important role in the adherence of *S. agalactiae*. 29.6% (8/27) isolates were found to contain the *lmb* gene in the present study. According to SPELLERBERG et al. (1999), the *lmb* gene was present in the common serotypes of *S. agalactiae.* The *cyl*E genes of *S. agalactiae* are required for the production of hemolysin and in the present study, 22.2% (6/27) isolates had the *cylE* gene. SPELLERBERG et al. (2000) observed 23% isolates positive for the *cyl*E gene, while BERGSENG et al. (2007) found 34.3% positivity.

The study resulted in obtaining the *S. agalactiae* isolates, which were identified and characterized by conventional as well as molecular approaches. The real output was regarding the antibiotic resistance profile, especially the PCR based detection of genes associated with resistance and also the molecular detection of a few important genes associated with virulence of *S. agalactiae.* More elaborate studies are recommended, including a much larger sample of subclinical mastitis cases and *S. agalactiae* isolates, as well as targeting more genes associated with antibiotic resistance and virulence, to understand the mechanism that plays a role at the genetic level so as to understand further the pathobiology of *S. agalactiae* and the menace of subclinical mastitis.

In conclusion it may be logically inferred that there was no definite pattern observed in antibiotic resistance by the phenotypic and genotypic methods. As with Tetracycline, two of the three *S. agalactiae* isolates found to be Tetracycline resistant genotypically, were also observed as Tetracycline resistant phenotypically, but the other *S. agalactiae* isolates, which were Tetracycline resistant phenotypically, were not found to contain a gene associated with resistance to Tetracycline. In the case of Erythromycin, five of the *S. agalactiae* isolates which were positive for the presence of a gene associated with resistance, were observed to be sensitive to Erythromycin by the Disc diffusion method and a few phenotypically resistant isolates were found to be negative for the presence of a gene associated with resistance to Erythromycin. This is possible as there are other

factors playing a role in conferring resistance, including the role of other genes such as: *tet*(M), *tet*(S), *tet*(W), *tet*(Q) and *tet*(B) for Tetracycline and *erm*(A) and *mef*(A) for Erythromycin.

References

BECKMANN, C., J. D. WAGGONER, T. O. HARRIS, G. S. TAMURA, C. E. RUBENS (2002): Identification of novel adhesins from Group B streptococci by use of phage display reveals that c5a peptidase mediates fibronectin binding. Infect. Immun. 70, 2869-2876.

BERGSENG, H., L. BEVANGER, M. RYGG, K. BERGH (2007): Real-time PCR targeting the *sip* gene for detection of group B streptococcus colonization in pregnant women at delivery. J. Med. Microbiol. 56, 223-228.

BOLDUC, G. R., M. J. BARON, C. GRAVEKAMP, C. S. LACHENAUER, L. C. MADOFF (2002): The alpha C protein mediates internalization of group B streptococcus within human cervical epithelial cells. Cell. Microbiol. 4, 751-758.

CHMOURYGUINA, I., A. SUVOROV, P. FERRIERI, P. P. CLEARY (1996): Conservation of C5a peptidase genes in group A and B streptococci. Infect. Immun. 64, 2387-2390.

DMITRIEV, A., A. SUVOROV, A. SHEN, Y. H. YANG (2004): Clinical diagnosis of group B streptococci by *scpB* gene based PCR. Indian J. Med. Res. 119, 233-236.

DORAN, K. S., G. Y. LIU, V. NIZET (2003): Group B streptococcal beta-hemolysin/cytolysin activates neutrophil signaling pathways in brain endothelium and contributes to development of meningitis. J. Clin. Invest. 112, 736-744.

DOGAN, B., Y. H. SCHUKKEN, C. SANTISTEBAN, K. J. BOOR (2005): Distribution of serotypes and antimicrobial resistance genes among *Streptococcus agalactiae* isolates from bovine and human host. J. Clin. Microbiol. 43, 5899-5906.

FRANKEN, C., G. HAASE, C. BRANDT, J. WEBER-HEYNEMANN, S. MARTIN, C. LAMMLER, A. PODBIELSKI, R. LUTTICKEN, B. SPELLERBERG (2001): Horizontal gene transfer and host specificity of beta-haemolytic streptococci: the role of a putative composite transposon containing *scpB* and *lmb*. Mol. Microbiol. 41, 925-935.

HANNOUN, A., M. SHEHAB, M.T. KHAIRALLAH, A. SABRA, R. ABI-RACHED, T. BAZI, K. A. YUNIS, G. F. ARAJ, G. M. MATAR (2009): Correlation between group B streptococcal genotypes, their antimicrobial resistance profiles, and virulence genes among pregnant women in Lebanon. Int. J. Microbiol. 01/2009; 2009:796512.

KEEFE, G. P. (1997): *Streptococcus agalactiae* mastitis: A review. Can. Vet. J. 38, 429-437.

LANCEFIELD, R. C., G. E. PERLMANN (1952): Preparation and properties of a protein (R antigen) occurring in streptococci of group A, type 28 and in certain streptococci of other serological groups. J. Exp. Med. 96, 83-97.

LIN, F. Y., P. H. AZIMI, L. E. WEISMAN, J. B. PHILIPS, J. REGAN, P. CLARK, G. G. RHOADS, J. CLEMENS, J. TROENDLE, E. PRATT, R. A. BRENNER, V. GILL (2000): Antibiotic susceptibility profiles for group B streptococci isolated from neonates, 1995-1998. Clin. Infect. Dis. 31, 76-79.

MAELAND, J. A., L. BEVANGER, R.V. LYNG (2005): Immunological markers of the R4 protein of *Streptococcus agalactiae.* Clin. Diagn. Lab. Immunol. 12, 1305-1310.

MANNING, S. D., M. KI, C. F. MARRS, K. J. KUGELER, S. M. BORCHARDT, C. J. BAKER, B. FOXMAN (2006): The frequency of genes encoding three putative group B streptococcal virulence factors among invasive and colonizing isolates. BMC Infect. Dis. doi:10.1186/1471-2334-6-116

PANCHOLI, V., V. A. FISCHETTI (1998): Alpha-enolase, a novel strong plasmin(ogen) binding protein on the surface of pathogenic streptococci. J. Biol. Chem. 273, 14503-14515.

RAINARD, P., Y. LAUTROU, P. SARRADIN, B. POUTREL (1991): Protein X of *Streptococcus agalactiae* induces opsonic antibodies in cows. J. Clin. Microbiol. 29, 1842-1846.

RIFFON, R., K. SAYASITH, H. KHALIL, P. DUBREUIL, M. DROLET, J. LAGACE (2001): Development of a rapid and sensitive test for identification of major pathogens in bovine mastitis by PCR. J. Clin. Microbiol. 39, 2584-2589.

RUSSELL, R. B., J. BREED, G. J. BARTON (1992): Conservation analysis and secondary structure prediction of the sh2 family of phosphotyrosine binding domains. FEBS Lett. 304, 15-20.

SCHRAG, S. J., S. ZYWICKI, M. M. FARLEY, A. L. REINGOLD, L. H. HARRISON, L. B. LEFKOWITZ, J. L. HADLER, R. DANILA, P. R. CIESLAK, A. SCHUCHAT (2000): Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis. N. Engl. J. Med. 342, 15-20.

SPELLERBERG, B., E. ROZDZINSKI, S. MARTIN, J. WEBER-HEYNEMANN, N SCHNITZLER, R. LUTTICKEN, A. PODBIELSKI (1999): Lmb, a Protein with Similarities to the LraI Adhesin Family, Mediates Attachment of *Streptococcus agalactiae* to Human Laminin. Infect. Immun. 67, 871-878.

SPELLERBERG, B., S. MARTIN, C. BRANDT, R. LUTTICKEN (2000): The cyl genes of Streptococcus agalactiae are involved in the production of pigment. FEMS Microbiol Lett. 188, 125-128.

STALHAMMAR-CARLEMALM, M., L. STENBERG, G. LINDAHL (1993): Protein Rib: a novel group B streptococcal cell surface protein that confers protective immunity and is expressed by most strains causing invasive infections. J. Exp. Med. 177, 1593-1603.

SUVOROV, A., A. DMITRIEV, I. USTINOVITCH, C. SCHALEN, A.A. TOTOLIAN (1997): Molecular analysis of clinical group B streptococcal strains by use of alpha and beta gene probes. FEMS Immunol. Med. Microbiol. 17, 149 -154.

UH, Y., I. H. JANG, G. Y. HWANG, K. J. YOON, W. SONG (2001). Emerging erythromycin resistance among group B streptococci in Korea. Eur. J. Clin. Microbiol. Infect. Dis. 20, 52-54.

VIDOVA, B., M. CHOTAR, J. TIMKO, A. GODANY (2009): Multiplex PCR method for detection of variability in genes encoding the alpha *Streptococcus agalactiae.* Nova Biotechnologica 9, 63-70.

WÄSTFELT, M., M. STALHAMMAR-CARLEMALM, A. M. DELISSE, T. CABEZON, G. LINDAHL (1996): Identification of a family of streptococcal surface proteins with extremely repetitive structure. J. Biol. Chem. 271, 18892-18897.

WILKINSON, H. W., R. G. EAGON (1971): Type-specific antigens of group B type Ic streptococci. Infect. Immun. 4, 596-604.

Received: 28 September 2011
Accepted: 17 April 2012

JAIN, B., A. TEWARI, B. B. BHANDARI, M. K. JHALA: Otpornost na antibiotike i geni za virulenciju sojeva vrste *Streptococcus agalactiae* izdvojenih iz krava kod supkliničke upale mliječne žlijezde. Vet. arhiv 82, 423-432, 2012.

SAŽETAK

Ukupno je 27 izolata vrste *S. agalactiae* bilo izdvojeno iz 89 krava sa supkliničkom upalom mliječne žlijezde te pretraženo na otpornost na antibiotike i gene za virulenciju. Difuzijskim postupkom ustanovljeno je da su izolati najčešće bili otporni na streptomicin (85,1%), a najrjeđe na gentamicin (3,7%). Otpornost na tetraciklin dokazana je u 55,5% izolata, na eritromicin u 33,3%, na kotrimoksazol u 11,1%, na ampicilin u 11,1% te na enrofloksacin u 7,4% izolata. Lančanom reakcijom polimerazom u tri je izolata dokazan gen *tetO* povezan s otpornošću na tetraciklin, a u sedam gen *ErmB* povezan s otpornošću na eritromicin. Molekularnim dokazivanjem gena odgovornih za virulenciju ni u jednog od 27 pretraženih izolata vrste *S. agalactiae* nije bio dokazan gen *bca*, dok je u šest izolata bio dokazan gen *ScpB*, u sedam gen *rib*, u osam gen *lmb* te u šest izolata gen *cylE*.

Ključne riječi: mastitis, *Streptococcus agalactiae*, antibiotici, otpornost, virulencija, geni