RESEARCH ARTICLE

Antibiogram Pattern of Bacterial Isolates from Bovine and Bubaline Semen in Karnataka

Nithyananda V.C.[1], Shivaraj Murag[2], Chandrashekar K.M.[3]*, Shivashankar B.P.[2], Isloor S.[4], Rathnamma D.[4]

Abstract

To avert bacterial contamination in semen, antibiotics are added. Inspite of addition of conventional antibiotics, extended and frozen semen samples from bulls have been reported to contain a number of bacterial contaminants which may be due to the antimicrobial resistance because of indiscriminate usage of antibiotics. The present study was conducted to know the antibiotic susceptibility pattern of the bacterial isolates from neat semen and frozen semen straws of cattle and buffalo bulls, collected from three semen stations (Farm A, B & C) of Karnataka. A total of twenty eight Holstein Friesian (HF) bulls, fourteen Murrah and two Surti buffalo bulls were selected for the study. Three time sample collections from each farm at a gap of 15 days were carried out. A total of 205 bacterial isolates were obtained from 264 samples of neat and frozen semen from three different farms (Farm A 49, Farm B 63 and Farm C 93). All isolates were, highly sensitive to newer generation antibiotics Piperacillin-Tazobactam, Imipenem and completely resistant to routinely used antibiotics Penicillin G, and Streptomycin.

Key Words: Antibiotics, Frozen semen, Neat semen, Penicillin, Streptomycin.

Ind J Vet Sci and Biotech (2024): 10.48165/ijvsbt.20.4.06

Introduction

The prime aim of semen stations is to produce good quality semen for artificial insemination. The bacterial contaminants in the semen are a major concern for most of the semen production laboratories because they adversely affect the semen quality. Antibiotics are routinely added to the extenders before semen extension to check the bacterial contamination, if any. However, in spite of addition of conventional antibiotics like Penicillin and Streptomycin, there have been reports of a number of bacterial contaminants in the diluted and frozen semen samples from bulls (Ramaswamy *et al*., 1994; Hasan *et al*., 2001). This can be attributed to the antimicrobial resistance due to its indiscriminate usage. Several studies have established that, the most of frozen semen bacterial isolates are resistant to commonly used antibiotics such as Penicillin and Streptomycin (Rathnamma *et al.,* 1997; Ramaswamy *et al*., 2002). In view of such ever growing multi-drug resistance, introduction of new generation antibiotics for reducing the bacterial load in semen, drug sensitivity of the bacterial isolates of semen is imperative (Sandeep *et al*., 2000). Current international standards with regard to the antibiotic components of semen extenders have made it necessary to look for alternatives for the Strepto-Penicillin containing extender (Hasan *et al*., 2001). The production of frozen semen of a larger dimension needs a quality control service to maintain supply of good quality frozen semen, so microbial analysis should be carried out at each and every step of processing. In view of the above, the present study was carried out to identify the antibiotic susceptibility profile of bacterial isolates from semen to know the efficacy of presently used antibiotics in semen stations.

[1]Department of Animal Husbandry & Veterinary Services, Govt. of Karnataka, India

[2]Southern Regional Disease Diagnostic Laboratory (SRDDL), IAH&VB, Hebbal-560024, Bengaluru, KVAFSU, Bidar, India

[3]Department of Veterinary Microbiology, Veterinary College, Gadag-582101, KVAFSU, Bidar, India

[4]Department of Veterinary Microbiology, Veterinary College, Hebbal-560024, Bengaluru, KVAFSU, Bidar, India

Corresponding Author: Chandrashekar K.M., Department of Veterinary Microbiology, Veterinary College, Gadag-582101, KVAFSU, Bidar, India. e-mail: dr.chandruvk112@gmail.com

How to cite this article: Nithyananda, V. C., Murag, S., Chandrashekar, K. M., Shivashankar, B. P., Isloor, S., & Rathnamma, D. (2024). Antibiogram Pattern of Bacterial Isolates from Bovine and Bubaline Semen in Karnataka. Ind J Vet Sci and Biotech. 20(4), 25-28.

Source of support: Nil

Conflict of interest: The authors declare that there is no conflict of interest

Submitted 08/02/2024 **Accepted** 22/03/2024 **Published** 10/07/2024

Materials and Methods

Sample Collection and Animal Selection

Semen samples (neat semen, semen extender and frozen semen straws) were collected every fortnight for three collections in the month of January and February 2022 from semen collection centre (Farm A, B & C) located in Bengaluru, Karnataka, India. Extender samples were collected on the day of semen sample collection. Frozen semen straws of the bulls from which neat semen was collected, were collected once the freezing process was completed. The samples were collected aseptically and transported in cold chain and liquid

nitrogen container and processed in Diagnostic Bacteriology and Mycology Section, Southern Regional Disease Diagnostic Laboratory (SRDDL), Institute of Animal Health and Veterinary Biologicals, Hebbal, Bangaluru. A total of 28 Holstein Friesian (HF) bulls (Farm A - 08, Farm B - 12 & Farm C - 08) and 14 Murrah (Farm A & C), two Surti (Farm C) buffaloes were selected for the study. All the selected animals were healthy and regularly used for semen collection.

Identification by Cultural and Biochemical Tests

The isolates were identified by cultural characters by inoculating them in enrichment broth and on selective media. Further, isolates were identified by grams staining technique for their morphological characters and biochemical tests.

Antibiogram

Antimicrobial sensitivity test of the identified bacterial isolates was performed by Kirby Bauer disc diffusion method (Bauer *et al*., 1966) on Muller Hinton agar (Himedia, India) based on standards of the Clinical and Laboratory Standards Institute (CLSI, 2020). The isolates were tested for their susceptibility to the antibiotics, *viz.,* Amoxicillin-clavulanic acid (AMC) 10 µg, Ciprofloxacin (CIP) 10 µg, Chloramphenicol (C) 10 µg, Cefotaxime (CTR) 10 µg, Enrofloxacin (EX) 10 µg, Gentamicin (GEN) 10 µg, Imipenem (I) 10 µg, Oxytetracycline (O) 30 µg, Ofloxacin (OF) 5 µg, Penicillin G (P) 10 units, Piperacillin & Tazobactam (PIT) 30 µg and Streptomycin (S) 10 µg. *Escherichia coli* ATCC 25922 was used as a quality control strain. The zone of inhibition was interpreted based on the CLSI guidelines by using antibiotic zone measuring scale in mm and inferred as SSS - Highly sensitive, SS - Moderately sensitive, S - Susceptible, R - Resistant.

Results and Discussion

A total of 205 bacterial isolates were obtained from 264 samples of neat and frozen semen from three different farms and they were typed up to genus level by staining and culture methods (Table 1). Of the 205 isolates, the numbers of isolates from different farms were Farm A - 49, Farm B - 63 and Farm C - 93.

The bacteria isolated from the neat semen samples were *Staphylococcus* spp. (34), *Bacillus* spp. (31), *Klebsiella* spp. (23), *Corynebacterium* spp. (19), *Serratia* spp. (3) and *Proteus* spp. (31), which were similar to the findings of Ronald and Prabhakar (2001), Dhruti and Rajesh (2012), and Pankaj and Madhumeet (2020). The number and species of bacterial genera isolated in the present study were much lesser when compared to the number of isolates obtained by Rathnamma *et al.* (1997), which can be attributed to the health status of the animals and strict hygienic management practices during collection of semen. The extender samples from all the three semen stations showed no bacterial growth this may be ascribed to the hygienic precautions followed during preparation of extenders.

The bacteria isolated from frozen semen samples in the present study were *Staphylococcus* spp. (12), *Bacillus* spp. (16), *Klebsiella* spp. (12), *Corynebacterium* spp. (7), *Serratia* spp. (3) and *Proteus* spp. (14), which were similar to the studies of Rathnamma *et al*. (1997), Ronald and Prabhakar (2001), Abro *et al*. (2015), Joyjit *et al*. (2016), Sadeq *et al*. (2017) and Reda *et al*. (2020). The number of bacterial genera obtained from the frozen semen were much lesser when compared to the studies of Rathnamma *et al*. (1997), Abro *et al*. (2015) and Joyjit *et al*. (2016), which can be attributed to hygienic practices followed during semen processing and the sensitivity of the antibiotics used for semen extension. Presence of microorganisms in the frozen semen also indicates that they can survive the freezing temperatures of liquid nitrogen and pose a question to the sensitivity of these isolates towards the antibacterial agents used in semen extenders.

The antibiogram pattern for all the bacterial isolates observed in the present study in descending order is summarized in Tables 2 to 5. All isolates were highly sensitive to Piperacillin-Tazobactam and Imipenem, whereas *Staphylococcus* spp. in addition with latter antibiotics also showed high sensitivity to Ofloxacin; moderately sensitive to Ciprofloxacin, Ceftriaxone (except *Proteus* spp.), Enrofloxacin and Ofloxacin (except *Staphylococcus* spp), and least sensitive to Chloramphenicol, Oxytetracycline, Amoxyclav and Gentamicin. In addition, with above antibiotics *Corynebacterium* spp., *Klebsiella* spp., *Staphylococcus* spp. and *Proteus* spp., showed least sensitivity to Streptomycin and Ceftriaxone. Isolates showed complete resistance to Penicillin G and Streptomycin (except *Staphylococcus* spp., *Corynebacterium* spp. and *Klebsiella* spp.).

Table 1: Farm wise isolates from neat semen and frozen semen straws (FSS)

Species	Farm A		Farm B		Farm C		
	Neat	FSS	Neat	FSS	Neat	FSS	Total
Staphylococcus spp.	11	3	8	4	15	5	**46**
Bacillus spp.	8	3	11	5	12	8	**47**
Klebsiella spp.	9	2	6	4	8	6	**35**
Corynebacterium spp.	4	-	6	3	9	4	**26**
Serratia spp.	-	-	-	-	3	3	**06**
Proteus spp.	5	4	12	4	14	6	**45**
Total	**37**	**12**	**43**	**20**	**61**	**32**	**205**

Table 2: Antibiogram of *Staphylococcus* spp.

Antibiotic	SSS (%)	SS (%)	S (%)	R (%)
Imipenem	43 (93.48)	3 (6.52)	-	-
Piperacillin+ Tazobactam	42 (91.3)	4 (8.7)	-	-
Ceftriaxone	03 (6.52)	41 (89.13)	2(4.35)	-
Ciprofloxacin	3 (6.52)	39 (84.78)	4 (8.7)	-
Enrofloxacin	8 (17.39)	37 (80.34)	1 (2.17)	-
Ofloxacin	29 (63.04)	13 (21.67)	4 (8.7)	-
Chloramphenicol	-	4 (8.7)	38 (82.6)	4 (8.7)
Gentamicin	-	4 (8.7)	38 (82.6)	4 (8.7)
Streptomycin	-	-	40 (86.96)	6(13.04)
Penicillin-G	-	-	3 (6.52)	43 (93.48)
Amoxyclav	-	5 (10.87)	35 (82.61)	6 (13.04)
Oxytetracycline	-	2 (4.35)	42 (91.3)	2 (4.35)

Table 3: Antibiogram of *Bacillus* spp.

Antibiotic	SSS (%)	SS (%)	S (%)	R (%)
Imipenem	45(95.74)	2(4.26)	-	-
Piperacillin+ Tazobactam	42(89.36)	5(10.64)	-	-
Ceftriaxone	2(4.26)	40(85.10)	5(10.64)	-
Ciprofloxacin	1(2.13%)	40(85.10)	6(12.76)	-
Enrofloxacin	-	39(82.98)	8(17.02)	-
Ofloxacin	3(6.38)	35(74.47)	9(19.15)	-
Chloramphenicol	-	4(8.51)	41(87.23)	2(4.26)
Gentamicin	-	2(4.26)	42(89.36)	3(6.38)
Streptomycin	-	-	4(8.51)	43(91.49)
Penicillin-G	-	-	1(2.13)	46(97.87)
Amoxyclav	-	4(8.51)	43(91.49)	-
Oxytetracycline	-	22(46.81)	23(48.93)	2(4.26)

Table 4: Antibiogram of *Klebsiella* spp.

Antibiotic	SSS (%)	SS (%)	S (%)	R (%)
Imipenem	32(91.43)	3(8.57)	-	-
Piperacillin+ Tazobactam	31(88.57)	4(11.43)	-	-
Ceftriaxone	15(42.86)	17(48.57)	3(8.57)	-
Ciprofloxacin		23(65.71)	12(34.29)	-
Enrofloxacin		27(77.14)	8(22.86)	-
Ofloxacin		32(91.43)	3(8.57)	-
Chloramphenicol	-	7(20.00)	26(74.29)	2(5.71)
Gentamicin	-	13(37.14)	22(62.86)	-
Streptomycin	-	-	24(68.57)	11(31.43)
Penicillin-G	-	-	2(5.71)	33(94.29)
Amoxyclav	-	-	32(91.43)	3(8.57)
Oxytetracycline	-	-	28(80.00)	7(20.00)

Table 5: Antibiogram of *Corynebacterium* spp.

Antibiotic	SSS (%)	SS (%)	S (%)	R (%)
Imipenem	16(61.54)	9(34.62)	1(3.84)	-
Piperacillin+ Tazobactam	19(73.08)	4(15.38)	3(11.54)	-
Ceftriaxone	-	24(92.31)	2(7.69)	-
Ciprofloxacin	06(23.08)	16(61.54)	4(15.38)	-
Enrofloxacin	01(3.84)	12(46.16)	13(50.00)	-
Ofloxacin	-	14(53.84)	12(46.16)	-
Chloramphenicol	-	-	24(92.31)	2(7.69)
Gentamicin	-	-	21(80.77)	5(19.23)
Streptomycin	-	-	22(84.62)	4(15.38)
Penicillin-G	-	-	4(15.38)	22(84.62)
Amoxyclav	-	-	7(26.92)	19(73.08)
Oxytetracycline	-	-	22(84.62)	4(15.38)

Table 6: Antibiogram of *Proteus* spp.

Antibiotic	SSS (%)	SS (%)	S (%)	R (%)
Imipenem	40(88.89)	5(11.11)	-	-
Piperacillin+ Tazobactam	33(73.33)	12(26.67)	-	-
Ceftriaxone	-	12(26.67)	28(62.22)	5(11.11)
Ciprofloxacin	2(4.44)	39(86.67)	4(8.89)	-
Enrofloxacin	-	22(48.49)	23(51.51)	-
Ofloxacin	-	25(55.56)	14(31.11)	6(13.33)
Chloramphenicol	-	6(13.33)	31(68.89)	8(17.18)
Gentamicin	-	2(4.44)	40(88.89)	3(6.67)
Streptomycin	-	-	14(31.11)	31(68.89)
Penicillin-G	-	-	8(17.18)	37(82.22)
Amoxyclav	-	-	41(91.11)	4(8.89)
Oxytetracycline	-	2(4.44)	40(80.89)	3(6.67)

The *Serratia* spp. isolated from neat and frozen semen (3 each) of bulls of only Farm C were highly sensitive to Piperacillin-Tazobactam and Imipenem; moderately sensitive to Ceftriaxone, Ciprofloxacin, Ofloxacin, and least sensitive to Chloramphenicol, Amoxyclav, Oxytetracycline, Gentamicin, Enrofloxacin and complete resistant to Penicillin G and Streptomycin.

The fourth generation antibacterial agents like Imipenem and Piperacillin-Tazobactam showed highest sensitivity against all the isolates in comparison to other antibiotics due to their less usage. On contrary, the routinely used antibiotics like Penicillin-G and Streptomycin were fully resistant to many isolates due to their extensive usage for semen extension, which might have led to the development of a high degree of resistance. The lower sensitivity pattern of Chloramphenicol, Gentamicin, Amoxyclav and oxytetracycline recorded in the present study was similar to Sharma *et al*. (1994), Sandeep *et al*. (2000), and was contrary to the studies of Ahmed and Greesh (2002), and Ronald and Prabhakar (2002), who

reported high sensitivity to the above antibiotics. The least sensitivity to Chloramphenicol and Gentamicin to many isolates in the present study can be due to the contamination of above drug resistance bacteria from the animal handlers or environmental contamination as the above two drugs are not routinely used in bull stations for treatments or in semen extenders.

Conclusion

The present study concluded that, in the event of development of antimicrobial resistance to the routinely used antibiotics like penicillin-G and streptomycin, the best results can be obtained by studying the antibiogram of bacterial isolates from semen and changing the antibiotics and their dose during processing of frozen semen. Based on the results of this study it was suggested to use higher generation antibiotics, *viz.,* Imipenem and Piperacillin-Tazobactam in extender instead of regularly used antibiotics to reduce the bacterial contamination of the semen and to increase the success rate in dairy breeding by artificial insemination.

References

Abro, S.H., Abro, R., Tunio, M., Rind, R., & Bughio, S. (2015). Evidence of bacterial contamination in the frozen bovine semen. *Pakistan Journal of Agriculture, Agricultural Engineering and Veterinary Science*, *31*(1), 102-108.

Ahmed, K., & Greesh, M. (2002). Effect of antibiotics on the bacterial load and quality of semen of Murrah buffalo bulls at different stages of freezing. *Indian Journal of Animal Sciences*, *72*(2), 138-139.

Bauer, A.W., Kirby, W.M.M., Sherris, S.C., & Turk, M. (1966). Antibiotic susceptibility testing by a standardized single disc diffusion method. *American Journal of Clinical Pathology*, *45*(4), 493-496.

CLSI (Clinical and laboratory Standards Institute) (2020). Performance Standards for Antimicrobial Susceptibility Testing, M100S, 30th ED., CLSI Vol.-40, N0. 1, CLSI, Wayne, PA 19087 USA.

Dhruti, Y.P., & Rajesh, K.P. (2012). Estimation of biochemical activities of microbial load isolated from the frozen semen of HF and HF crossbred cattle bulls. *Current Trends in Biotechnology and Pharmacology*, *6*(3), 328-339.

Hasan, S., Andrabi, S.M.H., Muneer, R., Anzar, M., & Ahmad, N. (2001). Effects of a new antibiotic combination on post-thaw motion characteristics and membrane integrity of buffalo and Sahiwal bull spermatozoa and on the bacteriological quality of their semen. *Pakistan Veterinary Journal*, *21,* 6-12.

Joyjit, M., Sumit, C., Santanu, P., Mrityunjay, C., & Anup, S. (2016). Microbiological evaluation of bovine frozen semen samples in West Bengal, India. *Exploratory Animal Medical Research, 6*(2), 185-191.

Pankaj, R., & Madhumeet, S, (2020). Prevalence of bacteria in different stages of semen processing before and after semen collection of cow bulls of Himachal Pradesh. *Himachal Journal of Agricultural Research*, *46*(1), 91-94.

Ramaswamy, V., John, K.J., Joseph, A.J.M., Parimal, R., & Venugopalan, A.T. (1994). Prevalence of microbes in frozen cattle semen and their antibiotic spectra. *Indian Journal of Animal Reproduction, 15*(1), 50-52.

Ramaswamy, V., Latha, N., Gnanasubramanian, T., & Manickam, R. (2002). Aerobic bacteria in buffalo semen and their antibiogram. *Indian Journal of Animal Reproduction, 23,* 117-119.

Rathnamma, D., Rao, M.S., Ramanatha, K.R., & Raghavan, R. (1997). Assessment of bacterial load in semen of Holstein Friesian bulls. *Current Research in University of Agricultural. Sciences*, *Bangalore, 26*(11), 205- 207.

Reda, A.A., Almaw, G., Abreha, S., Tadeg, W., & Tadesse, B. (2020). Bacteriospermia and sperm quality of cryopreserved bull semen used in artificial insemination of cows in South Wollo zone, Ethiopia. *Veterinary Medicine International, 202,* 1-11.

Ronald, B.S.M., & Prabhakar, T.G. (2001). Bacterial analysis of semen and their antibiogram. *Indian Journal of Animal Sciences*, *71*(9), 829-831.

Ronald, B.S.M., & Prabhakar, T.G. (2002). Effect of *Pseudomonas aeruginosa* and *Proteus mirabilis* on spermatozoa. *Indian Journal of Animal Sciences*, *72*(2), 140.62.

Sadeq, J.Z., Kreem, I.A., Ban, N.N., Ahmed, A., Ahmed, S., & Israa, A. (2017). Comparative study of physical properties and bacterial contamination between local and imported straws frozen semen. *Journal of Entamology and Zoological Studies*, *5*(4), 1969-1973.

Sandeep, J., Katoch, R.C., Deepti, C., & Arvind, M. (2000). The drug resistant bacteria associated with frozen semen technology of cow and buffalo bulls. *Indian Journal of Animal Sciences*, *70,* 488-489.

Sharma, R., Pangawkar, G.R., & Matharoo, J.S. (1994.) Efficacy of certain antibiotics on the bacterial load of buffalo semen during cryopreservation. *Indian Journal of Animal Sciences*, *64,* 468-470.