The Indian Journal of Animal Reproduction; 23(2): 117-119; December 2002

Aerobic bacteria in buffalo semen and their antibiogram

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Received : May 12, 2000
Accepted : June 15, 2002

ABSTRACT

Atotal of 29 buffalo frozen semen samples were screened and 23 microorganisms including 2 yeasts were isolated. Sixteen organisms of the 21 bacteria isolated belonged to Gram-positive group. The antibiogram studies on 15 bacteria with 17 antimicrobial agents revealed that all the isolates were sensitive to chloramphenicol, ciprofloxacin, gentamycin and neomycin. All of them were found to be resistant to ampicillin, amoxycillin, carbenicillin, oxytetracycline and penicillin-G. A combination of streptomycin and another drug or combination of other drugs has been suggested to control bacteria in buffalo frozen semen.

Key words : Buffalo, frozen semen, bacteria, sensitivity, resistance

The microbes because of their ubiquitous presence have ample access to contaminate the semen during collection, processing and preservation stages. The semen though free from specific pathogens gets contaminated subsequently sometimes even under strict hygienic conditions. Presence of opportunistic pathogenic organisms may compete along with spermatozoa for nutrients (Salisbury *et al.*, 1978) and may cause reproductive disorders (Eaglesome *et al.*, 1992) when used. The addition of Penicilin and streptomycin in semen may control the contaminating bacteria, but there are increasing reports of resistance to these drugs by bacteria present in semen (Rahman *et al.*, 1983; Kumar *et al.*, 1994). This study was undertaken to determine the types of organisms in Murrah buffalo frozen semen and also to know the feasibility of using different antimicrobial agents in reducing the contaminants in semen.

MATERIALS AND METHODS

A total of 29 frozen semen samples of Murrah buffalo bulls, in duplicate, procured from Buffalo Frozen Semen Station, Erode, Tamil Nadu were used for this study. They were brought to the laboratory in liquid nitrogen containers. The bulls from which semen were frozen were in good general and reproductive health and were free from any known specific diseases.

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Loopful of pooled semen was inoculated on blood agar and MacConkey agar plates and were later incubated for 48 hours at 37 degree celsius. Further identification of the isolates was carried out as per Bergey's manual of determinative bacteriology (Buchanum and Gibbons, 1974). *In vitro* drug sensitivity test was carried out with 17 antimicrobial agents by paper disc diffusion technique (Bauer *et al.*, 1966) using biodiscs (Hi-Media Laboratories, Mumbai, India).

RESULTS AND DISCUSSION

In the present study 12 out of 29 samples (41.39%) were devoid of organisms and from the rest, 23 microbes were isolated (Table 1). The bacteria isolated viz. *Pseudomonas* sp., *Corynebacterium* sp., *Staphylococcus* sp. and *Escherichia coli* and *Streptococcus* sp., have earlier been isolated from buffaloes (Ghanghadar *et al.*, 1986) as well as from bovine semen (Naidu *et al.*, 1982; Singh *et al.*, 1990).

Kumar *et al.* (1994) have reported the presence of *Corynebacterium* sp., *Streptococcus* sp., *Staphylococcus* sp. and *Escherichia coli* in fresh bovine semen. *E. coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus* sp. were isolated from bull semen in Bangladesh (Hossain *et al.*, 1990) and in Pakistan (Aleem *et al.*, 1990) from buffalo semen among other microbes, and thus the kinds of organism isolated in the present study were similar to those

Table 1. Types of microorganisms present in semen samples of murrah buffalo bulls

Name of the organism	Number	No. for which antibiogram done
Bacillus sp.	5	3
Corynebacterium sp.	5	3
Staphylococcus sp.	5	4
Pseudomonas aeruginosa	2	1
Escherichia coli	2	2
Aeromonas sp.	1	1
Streptococcus sp.	1	1
Yeast	2	–
Total	**23**	**15**

reported earlier. These organisms are opportunistic pathogens (Eagelsome *et al.*, 1992) and have been found to lower the motility and viability of the spermatozoa, when present in sufficient numbers. In the present study yeasts have been isolated besides the bacteria. Yeasts are not part of the natural flora of bull semen but must have entered during collection and processing of semen. The addition of antimicrobial agents in extenders might have resulted in the unchecked growth of these mycotic agents (Richard *et al.*, 1975; Kodagali, 1979).

Sensitivity observed to chloramphenicol, ciprofloxacin, gentamicin, neomycin, streptomycin, tobramycin, co-trimoxazole and erythromycin decreased from 100% to 53.33% and for the remaining nine drugs resistance increased from 86.62% to 100% (Table 2).

Singh *et al.* (1990) recorded similar sensitivity to gentamicin and streptomycin earlier for microbes of frozen bull semenand in buffalo frozen semen by Sharma *et al.* (1994). Kumar *et al.* (1994) reported a lesser sensitivity to

Table 2. Percentage of sensitivity and resistance of bacterial isolates

S.No.	Antimicrobial with concentration (in µg)	Sensitivity in %	Resistance in %
1.	Chloramphenicol (30)	100.00	0.00
2.	Ciprofloxacin (5)	100.00	0.00
3.	Gentamicin (10)	100.00	0.00
4.	Neomycin (30)	100.00	0.00
5.	Streptomycin (10)	86.62	13.33
6.	Tobramycin (10)	80.00	20.00
7.	Co-trimoxazole (25)	73.33	26.67
8.	Erythromycin (15)	53.33	46.67
9.	Polymyxin-B (300 µ)	13.33	86.62
10.	Cephalexin (30)	6.67	93.33
11.	Nitrofurantoin (300)	6.67	93.33
12.	Tetracycline (30)	6.67	93.33
13.	Ampicillin (10)	0.00	100.00
14.	Amoxicillin (10)	0.00	100.00
15.	Carbenicillin (50)	0.00	100.00
16.	Oxytetracycline (30)	0.00	100.00
17.	Penicillin-G (10 U)	0.00	100.00

chloramphenicol, gentamicin and streptomycin (67% each) whereas in the present study the sensitivity was more than their report, but the present sensitivity agreed with them with respect to ciprofloxacin (100%). In a previous study on frozen semen samples from the same place (Ramaswamy *et al.*, 1990), the sensitivity and resistance of the previous isolates differed with the present findings. Lower sensitivity with respect to gentamicin, streptomycin and tobramycin and also lower resistance to polymyxin-B, tetracycline and penicillin were observed in that study. This shows that both sensitivity and resistance of the isolates for some drugs were increased over a period of five years.

Most of the bacteria were resistant to polymyxin-B, cephalexin, nitrofurantoin, tetracycline, ampicillin, amoxicillin, carbenicillin, oxytetracycline and penicillin in the present study. Singh *et al.* (1990) have recorded higher resistance to ampicillin, polymixin-B, cephaloridine and tetracycline which is in line with the present findings. However, tetracycline resistance has been reported earlier as 50% (Kumar *et al.*, 1994) but in the present study the tetracycline resistance was 93.33%. Resistance to penicillin noticed was 100% in this study. This is in conformity with the observations of earlier workers (Wayda, 1991; Kumar *et al.*, 1994). The higher resistance noticed to these drugs may be due to their wide and indiscriminate use in the treatment of various infections of buffaloes.

Owing of the increased resistance exhibited by the isolates to penicillin and with higher susceptibility to streptomycin, it is suggested to use either a combination of streptomycin with any other drug or combination of few other drugs to reduce the bacterial load in buffalo frozen semen.

REFERENCES

Aleem, M., Chaudhry, R.A., Naeem-Ullahkhan, Rizvi, Atta-ur-Rahman and Ahmad, R. (1990). Occurrence of pathogenic bacteria in buffalo semen. Buffalo, 6: 93-98.

Bauer, A.W., Kirby, W.M.M., Sherris, S.C. and Turk, M. (1966). Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol., 49: 493.

Buchanum, R.E. and Gibbons, N.S. (1974). Bergey's Manual of Determinative Bacteriology. 8th edn. The Williams and Wilkins Co., Baltimore, USA.

Eaglesome, M.D., Gargia, M.M. and Stewart, R.B. (1992). Microbial agents associated with bovine genital tract infections and semen. Part II. *Haemophilus somnus*, *Mycoplasma* sp. and *Ureaplasma* sp. *Chlamydia*; pathogens and semen contaminants; treatment of bull semen with antimicrobial agents. Vet. Bull., 62: 887-910.

Ganghadhar, K.S., Rao, A., Krishnaswamy, B. and Rao, S. (1986). Bacterial and fungal types and their load in the frozen semen of buffalo bulls. Indian Vet. J., 63: 48-53.

Hossain, M.F., Alam, M.G.S., Ahmed, J.U., Rahman, M.M. and Rahman, A. (1990). Bacteriological examination of semen as an aid to sexual health control in bulls. Bangladesh Microbiol., 7: 85-88.

Kodagali, S.B. (1979). Prevalence of fungi in buffalo semen. Indian Vet. J., 56: 807-809.

Kumar, G., Raju, P.V. and Nair, P. (1994). Type of bacteria and its load in fresh semen of cross-bred bulls. Indian J. Anim. Reprod., 15: 48-49.

Naidu, K., Rao, A. and Rao, V. (1982). Microbial flora of semen of Jersey and Murrah bulls. Indian Vet. J., 59: 91-95.

Rahman, H., Dutta, J.C., Boro, B.R. and Rajkumar, C.K. (1983). Studies on bacterial flora of bulls semen and their antibiotic spectra. Indian J. Comp. Pathol. Microbiol. Infect. Dis., 4: 110-112.

Ramaswamy, V., Saravanabava, K., Andrew, M.J., Venugopal, A.T. and Venkatesan, R.A. (1990). Microbial flora of buffalo frozen semen and their antibiogram. Indian J. Anim. Sci., 61: 843-845.

Richard, J.U.L., Fitchtner, R.E. and Pier, A.C. (1975). Yeasts in bovine semen. Cornell Vet., 66: 362-368.

Salisbury, G.W., Van Demark, N.C. and Lodge, J.R. (1978). Semen collection. In: Physiology of reproduction and artificial insemination of cattle. 2nd edn. W.H. Freeman and Co., San Francisco, USA, pp 385-483.

Singh, A.K., Singh, L.C., Tiwary, B.W. and Singh, M.P. (1999). Efficacy of specific antibiotics on microbial load in chilled and frozen semen of crossbred bulls. Indian Vet. J., 67: 531-536.

Sharma, R.K., Pangawkar, G.R. and Mathanoo, I.S. (1994). Efficacy of certain antibiotics on the bacterial load of buffalo semen during cryopreservation. Indian J. Anim. Sci., 61: 468-470.