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Research Article

Studies on bacterial flora of Murrah buffalo semen and their antibiogram

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ABSTRACT

A total of 37 bacterial isolates were obtained from 28 neat semen samples of Murrah buffalo bulls. Out of 37 isolated bacteria, 15, 20 18 were gram positive and 19 were gram negative. The isolated bacteria included *Bacillus* s 29, 20p., *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus* sp., *Alcaligenes faecalis, Serratia* sp., *Salmonella* sp., *Pseudomonas aeruginosa, Shigella* sp. and *Klebsiella* sp. Antibiotic sensitivity test was performed against 12 different antibiotic discs (Amikacin, enrofloxacin, gentamicin, chloramphenicol, ciprofloxacin, norfloxacin cephalexin, streptomycin, amoxycillin, ampicillin, erythromycin and penicillin). The results of sensitivity test revealed that gram positive bacteria were more susceptible as compared to gram negative bacteria with antimicrobials used in the study. *Bacillus sp.* was found to be susceptible to all the antimicrobials used, whereas *Pseuodomonas aeruginosa* was sensitive to Amikacin and Enrofloxacin only. Amikacin was found to be most effective (100.00%) against the isolates followed by enrofloxacin (97.30%), genatamicin (97.30%), chloramphenicol (97.30%), ciprofloxacin (97.30%), norfloxacin (94.60%), cephalexin (91.90%) ampicillin (75.70%), penicillin (67.20%), erythromycin (62.20%), amoxycillin (59.50%) and streptomycin (56.80%).

Key words: Buffalo, neat semen, bacteria, antibiotic sensitivity

Artificial insemination in cattle and buffaloes is widely practiced in India and other parts of the world. The exchange of genetic material between countries has gained global importance. At present millions of semen doses are exchanged annually on national and international basis. This unfortunately has increased the risk of transmitting disease caused due to micro-organisms through semen. Bacteria present in the semen are Bacterial flora of Murrah buffalo semen responsible for causing lethal effects on the spermatozoa due to their toxins and metabolic end products (Boryozko *et al.*, 1981 and Jovicin *et al.*, 1991) and farther may cause change in EH, compete for same nutrients present in the extender leading to deterioration of semen quality and reproductive disorders (Eaglesome *et al.*, 1992) in the inseminated females. The present study was undertaken to determine the types of bacteria in neat semen of buffalo bulls and to provide guidelines for proper antibiotic treatment of semen dilutors so as to control microbial load of semen and to control infectious infertility due to contaminated or infected semen.

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MATERIALS & METHODS

Seven breeding buffalo bulls stationed at semen production centre of the College of Vety. and Animal Sciences, G. B. P. U. A. & T., Pantnagar were used in present study. A Total of 28 neat semen samples were taken for study. The bulls from which semen were collected were in good general and reproductive health and maintained under optimum managemental condition.

Loop fall of pooled semen was inoculated on blood agar and incubated for 48 hr at 37° C for primary isolation. The organisms isolated from semen samples were identified on the basis of morphological, cultural and biochemical characteristics as per Cruick Shank *et al.* (1975).

The bacterial isolates were tested *in vitro* against 12 different antibiotic disc *viz.* Amikacin (30 mcg),

Enrofloxacin (10 mcg), Gentamicin (30 mcg), Chloramphenicol (30 mcg), Ciprofloxacin (10 mcg), Norfloxacin (10 mcg), Cephalexin (30 mcg), Streptomycin (25 mcg), Amoxycillin (30 mcg), Ampicillin (25 mcg), Erythromycin (15 mcg) and *Penicillin* (10 mcg), supplied by Hi-Media Laboratories, Mumbai, India as per method recommended by Bauer *et al.* (1966).

RESULTS & DISCUSSION

The results obtained in the present study are given in Table 1 and 2. A total of 28 semen samples were examined and 37 bacterial isolates were obtained. The isolates included 18 gram positive and 19 gram negative bacteria. The bacteria isolated were viz. Bacillus sp., *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus* sp., *Alcaligenes faecalis, Serratia* sp., *Salmonella* sp., *Pseudomonas aeruginosa, Shigella* sp. and *Klebsiella* sp., Naidu *et al.*(1982) reported Bacillus sp., *Staphylococcus aureus Alcaligenes faecalis, Pseudomonas aeruginosa, Micrococcus* sp. *E. coli, Corynebacteriumpseudo-diphtheriticum, Corynebacterium equi, Corynebacterium renale, Corynebacterium xerosis* and *Proteus* sp. 42 samples of Murrah bulls.

Table 1: Types of bacteria present in neat semen samples of Murrah buffalo bulls.

Name of organism	Number
Bacillus sp.	1
Staphylococcus aureus	7
Staphylococcus epidermidis	7
Streptococcus sp.	3
Alcaligenes faecalls.	11
Serratia sp.	3
Salmonella sp.	2
Pseudomonas aeruginosa,	1
Shigella sp.	1
Klebsiella sp.	1

Table 2: Percentage of sensitivity and resistance of bacterial isolates.

S.No.	Antimicrobial agents (mcg)	Sensitivity %	Resistance %
1	Amikacin (30 mcg)	100	0
2	Enrofloxacin (10 mcg)	97.3	2.7
3	Gentamicm (30 mcg)	97.3	2.7
4	Chloramphenicol (30 mcg)	97.3	2.7
5	Ciprofloxacin (10 mcg)	97.3	2.7
6	Norfloxacin (10 mcg)	94.6	5.4
7	Cephalexin (30 mcg)	91.9	8.1
8	Streptomycin (25 mcg)	56.8	43.2
9	Amoxycillin (30 mcg)	59.5	40.5
10	Ampicillin (25 mcg)	75.7	24.3
11	Erythromycin (15 mcg)	62.2	37.8
12	Penicillin (10 mcg)	67.2	32.8

Jasial *et al.* (2000) isolated staphylococci, Micrococci, Corynebacterium, members of Enterobacteriaceae, and Anthracoids from fresh ejaculates of bovine & buffalo bulls. Most of the bacteria isolated in present study were also isolated by other workers. These organisms have been reported to be associated with wide variety of reproductive disorders in bovine (Shah & Dholakia, 1983). Therefore, it becomes imperative to follow proper hygienic measures during collection and preservation of semen.

The results of the antibiotic sensitivity of different isolates revealed that amikacin *was found* to be most effective (100%) followed by ciprofloxacin (97.30%), enrofloxacin (97.30%), gentamicin (97.30%), chloramphenicol (97.30%), norfloxacin (94.60%), cephalexin (91.90%), ampicaiin (75.70%), penicillin (67.20%), erythromycin (62.20%), amoxycillin (59.50%) and streptomycin (56.80%). These findings point out that streptomycin, amoxycillin, erythromycin and penicillin were less effective than the other antibiotics. Ramanswamy *et al.* (2002) observed almost similar sensitivity to chloramphenicol (100.00%), ciprofloxacin (100.00%) and gentamicin (100.00%). Resistance to Penicillin recorded in this study was 32.40 %, which is very less with the observation of other workers (Kumar *et al.*, 1994; Gangadhar *et al.*, 1986; Ahmed 1996). They observed 100.00% resistance to Penicillin.

On the basis of antibiogram of the isolates obtained in the present study, amikacin, Gentamicin, Enrofloxacin, Ciprofloxacin, Norfloxacin, can be effectively used in dilutor to control the bacterial load in processed semen provided that the drug concentration that in used should not have harmful effect on spermatozoa. Penicillin in combination with Streptomycin can be used in semen dilutor because Penicillin is effective against most of the gram positive isolates and Streptomycin is effective

against some gram positive and most of the gram negative isolates obtained in the present study.

REFERENCES

Ahmed, S.K. (1996). Studies on bacterial load in fresh and preserved semen .of buffalo bulls and effect of antibiotics for improvement of semen quality . Ph.D. Thesis submitted to I.V.R.I., Izatnagar.

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. Patho., **49:**493.

Boryozko, Z., Ossowicka Stepinska, J., Udala, J. and Lasota, B. (1981). Effect of Penicillin and Streptomycin on metabolism and survival of bull spermatozoa. Medycyna Weterynaryjna. **37:** 493-495. (c.f. Vet. Bull. **52**: 1946).

Cruick Shank, R., Duguid, J.P., Marmion, B. P. and Swain, R. H. A. (1975). Medical Microbiology. 12th edition. Churchill Livingstone, Edinburg, London and New York.

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.

Jasial, S., Katoch, R.C., Chachara, D. and Mahajan, A. (2000). Evaluation of bacterial load in fresh ejaculates of bovine and buffalo bull semen in Himachal Pradesh. Indian J. Anim. Sci., **70**: 465-467.

Jovicin,M., Pupavac, V. and Vaselinovic, S. (1991). Study of the effect of Pseudomonasaeroginosa on bull spermatozoa in vitro. Veterinarski Glasnik. **45**: 453-455.

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.

Ramaswamy, V., Latha, N., Gnanasubramanian, T. and Manickam, R. (2002). Aerobic bacteria in buffalo semen and their antibiogram. The Indian J. Anim. Reprod., **23**: 117-119.

Shah, N.M. and Dholakia, P.M. (1983). Microflora of the cervicovaginal mucus of Surti buffaloes and their drug resistance pattern. Indian J. Anim. Sci., **53**: 147.