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Research Article

Bacterial flora in frozen semen of Murrah buffalo bulls and their antibiogram

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ABSTRACT

A total of 28 frozen semen samples were screened for bacteria and 29 bacterial isolates were obtained. Out of 29 isolated bacteria, 6 were identified as *Staphylococcus aureus* (20.68%), 4 as *Bacillus sp.* (13.79%), 4 as *Escherichia coli* (13.79%), 4 as *Alcaligenes faecalis* (13.79%), 4 as *Proteus sp.* (13.79%), 4 as *Citrobacter sp.* (13.79%) and 3 as *Pseudomonas aeruginosa* (10,34%). Antibiotic sensitivity test was performed against 12 different antibiotic discs (Amikacin, enrofloxacin, gentamicin, chloramphenicol, ciprofloxacin, cephalexin, streptomycin, amoxycillin, ampicillin, erythromycin, norfloxacin 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. and Alcaligenes faecalis* were found to be susceptible to all the antimicrobials used, whereas *Pseudomonas aeruginosa* was sensitive to ciprofloxacin, gentamicin, amikacin and enrofloxacin only. Amikacin and ciprofloxacin were found to be most effective (89.65%) against the isolates, followed by enrofloxacin (86.20%), genatamicin (82.75%), norfloxacin (79.31%), chloramphenicol (72.41%), cephalexin (68.97%) ampicillin (48.27%), streptomycin (44.82%) penicillin (37.93%), amoxycillin (31.03%) and erythromycin (20.68%).

Key words: Buffalo, Frozen semen, Bacteria, Antibiotic sensitivity.

INTRODUCTION

Artificial insemination technology employing frozen semen has gained great acceptance in India. The hygienic status of semen is important for vitality of spermatozoa and for the fertility of inseminated cows. Inspite of utmost sanitary precautions, bacterial flora can become semen-borne during collection, processing and storage of semen (Korudzhiiski, 1979). At present millions of semen doses are exchanged annually on national and international basis and this has increased the risk of transmitting disease due to micro-organisms in semen. Bacteria present in the semen are 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 further may cause change in pH, compete for nutrients present in the extender leading to deterioration of semen quality and reproductive disorders (Eaglesome *et al.*, 1992) n the inseminated females. The present tudy was undertaken to determine the types of bacteria in frozen semen of buffalo bulls and to find out proper treatment of semen dilutors so as to control microbial load of semen and to control infectious infertility due to contaminated or infected semen.

MATERIALS & METHODS

Seven breeding buffalo bulls stationed at Semen Production Centre of the College of Vety. and Animal Sciences, G. B. P, U. A. and T, Pantnagar were used in present study. A Total of 28 frozen semen

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samples were taken for study. The bulls from which semen samples were collected had good physical and reproductive health and were maintained under optimum managemental conditions. The semen samples were collected using artificial vagina.

Loopful of pooled semen was inoculated on blood agar and incubated for 48 hours 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

A total of 28 semen samples were examined and 29 bacterial isolates were obtained (Table 1). The isolates included 10 gram positive and 19 gram negative bacteria. The various bacteria isolated from frozen semen were identified as *Staphylococcus aureus, Bacillus sp., Proteus sp., Escherichia coli, Alcaligenes faecalis, Citrobacter sp.* and *Pseudomonas aeruginosa.* One or other types of bacteria isolated in the present study have also been reported by other workers (Raghavan *et al.*, 1982; Flatcher and Holzmann, 1984; Gangadhar *et al.*,1986; Bindra, 1991; Ahmed, 1996; Jasial *et al.*, 2000).

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

Name of organisms	Number of bacterial isolates
Bacillus sp. Staphylococcus aureus	4
Alcaligenes faecalis	6
Pseudomonas aeruginosa,	4
E.aeruginosa, E. coli	3
Citrobacter sp.	4
Proteus sp.	4
Total	25

The higher incidence of *Staphylococcus sp.* observed in the present study was in agreement with findings of Ahmed (1996) and Jasial *et al.* (2000). 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 (Weisser, 1981; Shah & Dholakia, 1983). These findings make it imperative to follow proper hygienic measures during collection and preservation of semen using effective antibiotics.

The results of the antibiotic sensitivity (Table 2) of different isolates revealed that amikacin and ciprofloxacin were found to be most effective (89.65%) followed by enrofloxacin (86.20%), gentamicin (82.75%), norfloxacin (79.31%), chloramphenicol (72.41%), cephalexin (68.97%), ampicillin (48.27%), streptomycin (44.82%), penicillin (37.93%), amoxycillin (31.03%) and erythromycin (20.68%). These findings point out that ampicillin, streptomycin, amoxycillin, erythromycin and penicillin were less effective than the other antibiotics used. Ramaswamy *et al.*, (2002) observed higher sensitivity to chloramphenicol (100.00%), Ciprofloxacin (100.00%) and Gentamicin (100.00%).

Table-2. Sensitivity and resistance of bacterial isolates.

S.NO.	Antimicrobial agents(mcg)	Sensitivity %	Resistance %
1	Amikacin(30mcg)	89.65	10.35
2	Ciprofloxacin(lOmcg)	89.65	10.35
3	Enrofloxacin(lOmcg)	86.20	12.80
4	Gentamicin(30mcg)	82.75	17.25
5	Norfloxacin(1 Omcg)	79.31	20.69
6	Chloramphenicol(30mcg)	72.41	17.59
7	Cephalexin(30mcg)	68.97	31.03
8	Ampicillin(25mcg)	48.27	51.73
9	Streptomycin(25mcg)	44.82	55.18
10	Penicillin(lOmcg)	37.93	62.07
11	Amoxycillin(30mcg)	31.03	68.97
12	Erythromycin(1 5mcg)	20.68	79.32

Sensitivity recorded in the present study to Cephalexin, Ampicillin, Amoxycillin and Penicillin-G were higher as compared to the report of Ramaswamy *et al.,* (2002). Kumar *et al.,* (1994) reported a lesser sensitivity to chloramphenicol and gentamicin (67.00% each), whereas in the present study the sensitivity recorded to chloramphenicol and gentamicin were 72.41% and 82,75%, respectively. But the present sensitivity agreed with *Kumar et al.* (1994) with respect to Ciprofloxacin. Resistance to Penicillin recorded in this study was 62.07 %, which is less with the observation of other workers (Kumar *et al.,* 1994; Gangadhar *et al.,* 1986; Ahmed 1996). These workers observed 100.00% resistance to Penicillin. Ampicillin was found moderately effective antibiotic (48.27%) against isolates in the present study, however, this was found to be most effective (86.40%) by Gupta and Maurya (1993). Ronald and Prabhakar (2001) reported very less (16.66%) sensitivity to ampicillin. Sensitivity observed to erythromycin was least in the present study. It was recorded as 20.68per cent. However, Ahmed (1996) and Ramaswamy *et al.* (2002) reported higher sensitivity (76.47% and 53.33%) to erythromycin which is not in agreement with the findings of the present study. Gupta and Maurya (1993) reported that 40.60% of the isolates were sensitive to erythromycin.

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.

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ISSAR NEWS

Dr. S.K. Agarwal, Principal Scientist, Division of Animal Reproduction , IVRI, Izatnagar U.P. has been awarded a Project entitled 'Antiluteolytic Strategies- a novel approach to enhance fertility in buffalos' for a period of 5 years, with a budget allocation of 270.859 Lac rupees. The project is financed by the National fund for basic and strategic research under the Union Ministry of Agriculture, New Delhi.

Dr. S.K.Agarwal will be the Principal Investigator and the lead institution for the project will be Indian Veterinary Research Institute, Izatnagar, and U.P. The collaborating institutions and Co-PI are as follows-

1. Indian Institute of Science, Bangalore - Dr. Medhamurti
2. Guru Angad Dev Veterinary and animal Science University, Ludhiana-Dr. G .S Dhariwal
3. National Institute of Animal Nutrition and Physiology, Bangalore- Dr. S. Senvaraju.
4. G.B. Pant University of Agri. & Technology, Pantnagar, U.A. –Dr. Shiv Prasad.

ISSAR takes pleasure in heartily felicitating Dr. S.K. Agarwal for the winning of the prestigious project and awaits its successful completion with fruitful results in respect of Augmentation of fertility in buffalo.