

## OCCURRENCE OF BACTERIA IN FROZEN BUFFALO SEMEN AND THEIR ANTIBIOGRAM

V. Ramaswamy, N. Latha, T. Gnanasubramanian and A.T. Venugopalan

Centre for Animal Health Studies, Madhavaram Milk Colony,  
Chennai - 600 051, Tamil Nadu, India.

### SUMMARY

Frozen semen samples from 29 Murrah buffalo bulls were examined for microbial contamination. Seventeen (58%) samples yielded 23 microorganisms which included two yeasts. The predominant bacteria isolated were *Bacillus* sp, *Corynebacterium* sp, and *Staphylococcus* sp. An antibiogram study was conducted on 15 bacteria isolates using 17 antimicrobial agents. The results revealed that all the bacteria were sensitive to neomycin, gentamicin, ciprofloxacin and chloramphenicol. However, they were resistant to ampicillin, amoxycillin, carbenicillin, oxytetracycline and penicillin. Addition of alternative antibiotics in place of conventional antibiotics to control bacterial flora of frozen buffalo semen is discussed.

Keywords: Buffalo, frozen semen, bacteria, sensitivity, resistance

### INTRODUCTION

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 pathogenic organisms, gets contaminated subsequently, sometimes even under strict hygienic conditions. Presence of opportunistic pathogenic microbes 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 penicillin and streptomycin in semen may control the contaminating bacteria, but there are increasing reports of resistance to these drugs by the 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 antimicrobials in reducing the bacterial contaminants in semen.

### MATERIALS AND METHODS

A total of 29 frozen semen samples from 29 Murrah buffalo bulls, in duplicate, procured from Buffalo Frozen Semen Station, Erode, Tamil Nadu, India were used for this study. They were brought to the laboratory in liquid nitrogen containers. The bulls from which the semen were frozen, were in good

general health and were free from any known specific diseases.

Loopful of pooled semen was inoculated onto blood agar MacConkey and Sabouraud's dextrose agar plates and were later incubated for 48 h or more at 37°C. Further identification of isolates were carried out as per Bergey's manual of determinative bacteriology (Buchanan and Gibbons, 1974).

*In vitro* drug sensitivity test was carried out with 17 antimicrobial agents by Bauer-Kirby method using biodiscs (HI Media, India). The resistance/sensitivity was recorded by measuring the zone of inhibition and interpreting it with zone-size interpretative chart of M/s. Span Diagnostics, India.

### RESULTS

Of the 29 frozen semen samples screened, 12 samples (41.39%) were free from contamination. The remaining 17 samples yielded 23 microorganisms including two yeasts (Table 1). Among the isolates, gram positive group constituted 76.19% of the organisms.

The *in-vitro* sensitivity and resistance of the bacterial isolates to 17 antimicrobial agents are also presented in Table I. Broadly, the sensitivity of isolates ranged from 53 to 100% with respect to eight agents. All isolates tested were sensitive to four agents namely neomycin, gentamicin, ciprofloxacin and chloramphenicol.

Table 1. Drug sensitivity and resistance of buffalo frozen semen bacteria

	Organism							Total	Resistance (%)	Sensitivity (%)
	Staph. sp.	Strept. sp.	Baci. sp.	Coryne. sp.	Pseud. sp.	Aero. sp.	Yeast			
Total no. of isolates	5	1	5	7	2	1	2	23		
Antibiogram done	4	1	3	5	1	1		15		
Number of organisms resistant to antimicrobials										
Ampicillin (10 µg)	4	1	3	5	1	1	x	15	100	0.0
Amoxycillin (10 µg)	4	1	3	5	1	1	x	15	100	0.0
Carbenicillin (50 µg)	4	1	3	5	1	1	x	15	100	0.0
Oxytetracycline (30 µg)	4	1	3	5	1	1	x	15	100	0.0
Penicillin (10 µg)	4	1	3	5	1	1	x	15	100	0.0
Cephalexin (30 µg)	4	1	2	5	1	1	x	14	93.3	6.7
Nitrofurantoin (300 µg)	4	1	3	4	1	1	x	14	93.3	6.7
Tetracycline (30 µg)	4	1	3	4	1	1	x	14	93.3	6.7
Polymyxin-B (300 µg)	3	1	3	4	1	1	x	13	86.7	13.3
Erythromycin (15 µg)	2	-	2	2	1	-	x	7	46.7	53.3
Co-trimoxazole (25 µg)	1	-	1	2	-	-	x	4	26.7	73.3
Tobramycin (10 µg)	-	-	1	2	-	-	x	3	20.0	80.0
Streptomycin (10 µg)	1	-	1	-	-	-	x	2	13.7	86.6
Chloramphenicol (30 µg)	-	-	-	-	-	-	x	-	0.0	100
Ciprofloxacin (5 µg)	-	-	-	-	-	-	x	-	0.0	100
Gentamicin (10 µg)	-	-	-	-	-	-	x	-	0.0	100
Neomycin (30 µg)	-	-	-	-	-	-	x	-	0.0	100
Staph. -	Staphylococcus		Strept. -	Streptococcus		Baci. -	Bacillus			
Coryne. -	Corynebacterium		Pseud. -	Pseudomonas		Aero. -	Aeromonas			
x - Antibiogram not done										

## DISCUSSION

The organisms isolated in the present study viz. *Pseudomonas* sp., *Corynebacterium* sp., *Staphylococcus* sp., *Escherichia coli*, and *Streptococcus* sp. have been earlier reported in semen of buffaloes (Gangadhar *et al.*, 1986) and from bovine semen (Naidu *et al.*, 1982; Singh *et al.*, 1990). Kumar *et al.* (1994) reported the presence of *Corynebacterium* sp., *Streptococcus* sp., *Staphylococcus* sp., and *Escherichia coli* in fresh bovine semen. In Bangladesh, Hossain *et al.*, 1990 and in Pakistan, Aleem *et al.*, 1990 isolated the same organisms (observed in the present study) from bull and buffalo semen, among other microbes, *E. coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus* sp.

These organisms are opportunistic pathogens. (Eaglesome *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 were also isolated. 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.*, 1976; Kodagali, 1979).

In the present investigation 100% sensitivity was recorded to four drugs including gentamicin and chloramphenicol and 86% to streptomycin.

In an earlier study Gangadhar *et al.* (1986) observed that the chloramphenicol-kanamycin combination fared better than other combinations including penicillin - streptomycin in controlling bacterial load of frozen semen of buffalo bulls. Ahmed *et al.* (1989) found that frozen bull semen samples containing gentamicin had no bacterial growth in contrast to 45% bacteria positive samples with streptopenicillin.

Similarly, Singh *et al.* (1990) noted the addition of gentamicin and chloramphenicol led to maximum decline in bacterial load of frozen bull semen as compared to conventional antibiotics in the extender. These two antimicrobials were found to be more beneficial than others at every stage of cryopreservation of buffalo frozen semen (Sharma *et al.*, 1994).

Though Kumar *et al.* (1994) observed a lower sensitivity to gentamicin, chloramphenicol and streptomycin (67% each) by bacteria of fresh semen of crossbred bulls, their observation of 100% sensitivity to ciprofloxacin is similar to our findings.

The resistance of the bacteria to ampicillin, polymyxin-B and streptomycin was similar to that observed by Singh *et al.* (1990), but the resistance to



cephaloridine and tetracycline were lower than in the present study.

Resistance to penicillin was 100% in this study as was observed by earlier workers (Wayda, 1991, Kumar *et al.*, 1994).

In a previous study of 60 semen samples from 60 Murrah bulls (same Murrah bull station) (Ramaswamy *et al.*, 1990), the organisms were found resistant to six drugs including penicillin (88.9%) tetracycline (74.1%) and polymyxin-B (55.6%). The previous isolates were sensitive to eight drugs including tobramycin (77.8%), gentamicin (70.1%), streptomycin (66.7%) and neomycin (66.7%).

Comparing the present study, in which only 11 animals and eight antimicrobials were common, with the previous study, there was an increase in resistance to amoxycillin, penicillin, polymyxin-B, and tetracycline. There was also an increased susceptibility to gentamicin, neomycin, and streptomycin. There was no change in the susceptibility to tobramycin suggesting that both sensitivity and resistance of the isolates for some drugs were increased over a period of five years.

The higher resistance to few drugs might be due to their wide and frequent use in the treatment of various infections of buffaloes, conversely, the least used drugs were found to be more sensitive.

This study revealed that it is better to replace the conventional antibiotics of frozen semen namely penicillin - streptomycin with a combination of any two of the following drugs viz., gentamicin, chloramphenical, ciprofloxacin, tobramycin and streptomycin to control bacterial flora especially in frozen buffalo semen.

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## RINGKASAN

### KEMUNCULAN BAKTERIA DALAM SEMEN SEJUK BEKU KERBAU DAN ANTIBIOGRAMNYA

Sampel semen sejuk beku daripada 29 ekor kerbau Murrah telah diperiksa untuk pencemaran mikrob. Tujuh belas (58.6%) sampel menghasilkan 23 mikroorganisma termasuk 2 yis. Bakteria paling banyak dipencilkan ialah *Bacillus* sp, *Corynebacterium* sp dan *Staphylococcus* sp. Satu kajian antibiogram dilakukan terhadap 15 isolat bakteria mengguna 17 agen antimikrob dan hasilnya telah menunjukkan kesemua peka terhadap neomisin, gentamisin, siprofloksasin dan kloramfenikol sambil kesemuanya tahan terhadap ampicilin, amoksisilin, karbenisilin, oksitetrasiklin dan penisilin. Penambahan antibiotik gantikan kepada antibiotik biasa untuk mengenal flora bakteria.