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Research Article

IN VITRO ANTIBIOGRAM OF BACTERIAL ISOLATES FROM PREPUTIAL WASHINGS AND COW BULL SEMEN

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ABSTRACT

The present study was conducted to assess the antibiogram of bacterial isolates from preputial washings and different semen samples of cow bull used for frozen semen production in Himachal Pradesh. A total of 120 samples (30 each of preputial washings, neat, extended and frozen cow bull semen) were collected out of which 96 samples showed bacterial growth. The *in vitro* antibiogram of samples was done by disc diffusion method using antibiotics discs of streptomycin (S), ampicillin (AMP), amoxicillin (AMX), penicillin (P), enrofloxacin (Ex), ciprofloxacin (Cip), ofloxacin (Of), gentamicin (Gen), oxytetracycline (O), lincomycin (LIN), ceftriaxone (CTR) and marbofloxacin (MAR). Highest sensitivity was recorded for marbofloxacin, streptomycin and ciprofloxacin whereas penicillin showed highest resistance.

Keywords: Extended semen, Frozen semen, Neat semen, Preputial washings

Presently, around 85 million frozen semen doses (FSDs) are being produced per year in 44 A and B graded semen stations of the country to fulfill the requirement for artificial insemination (AI) in the country (Rath *et al.*, 2016). Superior class and infection free semen production along with its spread is one of the key objectives of semen stations in India and throughout the world (Meena *et al.*, 2015). It is, therefore, of paramount importance that to meet the growing frozen semen demand, some necessary steps should be taken to ensure quality of the semen (NDDB, 2016).

The procedure of semen collection invites bacterial contamination through various sources (Meena, 2008). Bacteria gain access into semen from diseased bulls, preputial cavity, dilutors and unhygienic practices followed during collection, processing and packaging of semen. Microbiological analysis of semen is required at each and every stage of semen production for quality semen production (Navya, 2012). Semen straws should be checked periodically for microbial load for bacteriological quality control (NDDB, 2016). Therefore, the present study was conducted with an objective of isolating the bacterial pathogens and their antibiogram at different stages of semen processing.

MATERIALS AND METHODS

The present study was carried out from July 2018 to January 2019 in fifteen adult healthy bulls (13 Jersey and 2 Red Sindhi) aged between two to six years maintained at Sperm Station, Himachal Pradesh Livestock Development Board, Palampur and Department of Veterinary Gynaecology and Obstetrics, College of Veterinary & Animal Sciences, CSK Himachal Pradesh Agricultural University, Palampur.

About 20 ml of NSS was introduced into the preputial sheath by a sterilized AI sheath and a sterile 20 ml syringe. Further by closing the orifice, the preputial sheath was massaged and preputial washings were aspirated. After discarding the first few drops, rest was transferred into a sterile sample collection tube.

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Semen samples collected at different stages were cultured directly in nutrient broth and incubated at 37ºC overnight. Bacterial isolation was done on blood agar, nutrient agar, MacConkey lactose agar and Eosin methylene blue agar. A sterile swab dipped in nutrient broth culture was spread over sterile Mueller–Hinton agar Petri plates and *in vitro* antibiogram was carried out as per standard single disc diffusion method (Bauer *et al.*, 1996) using 13 antibiotic discs (Hi- media, Mumbai, India) viz. streptomycin (10mcg/disc), ampicillin (10mcg/disc), amoxicillin (10mcg/disc), penicillin (10units/disc), enrofloxacin (10 mcg/disc), ciprofloxacin (5 mcg/disc), ofloxacin (5 mcg/disc), gentamicin (10 mcg/disc), oxytetracycline (30 mcg/disc), lincomycin (2 mcg/disc), ceftriaxone (30 mcg/disc) and marbofloxacin (1 mcg/disc). The antibiotic discs were then laid on the dried inoculums with the help of a dispenser. The zone of inhibition was estimated using antibiotic zone measuring scale in mm.

RESULTS AND DISCUSSION

Bacteria isolated from preputial washings (n=30) and from semen at different stages of semen collection and processing (n=30 each) have been shown in Table 1. Each of 30 samples of preputial washings and neat semen showed bacterial growth while 8 (26.67%) and 17 (56.67%) samples of extended semen and frozen semen were devoid of bacterial growth, respectively. In a total of 183 isolates, number of bacterial isolates was 71, 62, 27, and 22 in preputial washings, neat semen, extended semen and frozen semen straw, respectively. Balqis *et al.* (2018) recovered *Escherichia* spp. from the preputial washings of bull. *Staphylococcus epidermis*, *Escherichia coli*, *Proteus*

Table 1

Bacteria isolated from preputial washings and semen collected at different stages of semen processing

Sr. No.	Samples collected from	Total Samples	Total bacterial isolates	Bacterial isolates									
				Staphylococcus spp.		*Bacillus* spp.		*Micrococcus* spp.		*Streptococcus* spp.		*E.coli*	
				n	%	n	%	n	%	n	%	n	%
1.	Preputial washing	30	71	20	28.16	20	28.16	16	22.53	10	14.08	5	7.04
2.	Neat semen	30	62	30	48.38	10	16.12	10	16.12	10	16.12	2	3.2
3.	Extended semen	30	27	20	74.07	-	-	2	7.40	5	18.51	-	-
4.	Frozen Semen straws	30	22	18	81.81	-	-	-	-	4	18.18	-	-

Table 2

Sensitivity of bacteria against various antibiotics isolated from different samples (preputial washings and semen) of cow bulls

Sr. No.	Bacteria isolated	No. of isolates	S			AMP			AMX			P		
			HS	S	R	HS	S	R	HS	S	R	HS	S	R
1.	*Staphylococcus* spp.	88	63	25	0	3	5	80	55	0	33	0	5	25
	%		71.5	28.4	0	34.09	5.68	90.90	5.68	56.81	37.5	0	56.81	28.40
2.	*Bacillus* spp.	30	2	28	0	9	11	10	10	9	11	0	5	25
	%		6.66	93.33	0	30	36.66	33.33	33.33	30	36.66	0	16.66	83.33
3.	*Streptococcus* spp.	29	14	15	0	19	10	0	10	9	10	0	5	83
	%		48.27	51.7	0	65.51	34.48	0	34.48	31.03	34.4	0	17.24	34.93
4.	*Micrococcus* spp.	28	11	17	0	4	4	20	4	17	7	0	2	26
	%		39.28	60.71	0	14.28	14.28	71.42	14.28	60.71	25	0	7.14	92.85
5.	*E. coli*	07	7	0	0	1	6	0	7	0	0	0	0	7
	%		100	0	0	14.28	85.71	0	100	0	0	0	0	100
	Total isolates	182	97	85	0	36	36	110	36	85	61	0	12	170
	%		53.33	46.66	0	20	20	60	20	46.66	33.33	0	6.66	93.33

Sr. No.	Bacteria isolated	No. of isolates	EX			CIP			OF			GEN		
			HS	S	R	HS	S	R	HS	S	R	HS	S	R
1.	*Staphylococcus* spp.	88	40	41	7	8	80	0	24	50	14	78	0	10
	%		45.4	46.5	7.9	9	90.9	0	27.2	56.81	15.9	88.6	0	11.4
2.	*Bacillus* spp.	30	25	5	0	10	20	0	3	17	10	30	0	0
	%		83.33	16.66	0	33.33	66.66	0	10	56.66	33.33	100	0	0
3.	*Streptococcus* spp.	29	15	11	3	25	4	0	5	20	4	19	10	0
	%		51.7	37.9	10.3	86.2	13.7	0	17.24	17.2	13.7	65.5	34.4	0
4.	*Micrococcus* spp.	28	10	15	3	23	5	0	3	17	8	16	10	2
	%		35.71	53.57	10.71	82.14	17.8	0	10.71	60.71	28.57	57.14	35.17	7.14
5.	*E. coli*	07	7	0	0	7	0	0	2	5	0	5	2	0
	%		100	0	0	100	0	0	28.57	71.4	0	71.4	28.57	0
	Total isolates	182	97	72	13	73	109	0	37	109	36	146	24	12
	%		53.33	40	6.66	40	60	0	20	60	20	80	13.33	6.6

Sr. No.	Bacteria isolated	No. of isolates	O			LIN			CTR			MAR		
			HS	S	R	HS	S	R	HS	S	R	HS	S	R
1.	*Staphylococcus* spp.	88	29	29	30	8	20	60	25	43	20	88	0.	0
	%		32.95	32.95	34.09	9.09	22.7	68.18	28.40	48.86	22.7	100	0	0
2.	*Bacillus* spp.	30	26	0	4	4	6	20	25	3	2	30	0	0
	%		86.66	0	13.33	13.33	20	66.6	83.33	10	6.66	100	0	0
3.	*Streptococcus* spp.	29	16	8	5	1	8	19	14	5	10	29	0	0
	%		55.17	27.5	17.2	3.4	27.58	65.5	48.27	17.24	34.48	100	0	0
4.	*Micrococcus* spp.	28	0	18	10	6	12	10	4	20	4	28	0	0
	%		0	64.28	35.71	21.4	42.85	35.7	14.28	71.42	14.28	100	0	0
5.	*E. coli*	07	2	5	0	4	3	0	5	2	0	7	0	0
	%		28.57	71.4	0	57.14	42.8	0	71.4	28.57	0	100	0	0
	Total isolates	182	73	60	49	24	49	109	73	73	36	182	0	0
	%		40	33.33	26.66	13.18	26.92	60	40	40	20	100	0	0

mirabilis, *Enterobacter cloacae*, *Staphylococcus epidermis* and *Staphylococcus aureus* have been isolated from neat semen samples of cow bulls (Yaniz *et al.*, 2010). Another study conducted by Jasial *et al.* (2000) concluded that *Staphylococcus* spp., *Micrococcus* spp., *Corynebacterium* spp. and members of Enterobacteriaceae were the main bacterial isolates in neat semen of cow and buffalo bulls which is in agreement with our findings. *Staphylococcus* spp., *Micrococcus* spp., *Escherichia coli*, *Pseudomonas* spp., *Corynebacterium* spp., *Proteus* spp., *Klebsiella* spp. (Mitra *et al.*, 2016), *Moraxella bovis* (Gandhi *et al.*, 2008), *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa* (Najee *et al.*, 2012) have been recovered from frozen bovine semen.

Perusal of Table 2 indicates that bacterial isolates recovered from preputial washings and semen collected during various stages of semen processing were found to be 100% sensitive for marbofloxacin, streptomycin and ciprofloxacin each, gentamicin (93.33%), enrofloxacin (93.33%), ofloxacin (80.0%), ampicillin (40.0%) and lincomycin (39.99%). Most of the bacterial isolates (93.33%) were resistant to penicillin. In concurrence of our study, Akhter *et al.* (2013) concluded that streptomycin and ciprofloxacin were the most effective antibiotics for controlling bacterial load of cryopreserved bull semen. In contrary to our findings, Navya (2012) concluded that lincomycin is the most effective drug (86.10%), followed by spectinomycin (81.94%), tylosin (79.16%), gentamicin (76.38%) for reducing bacterial load of cow bull semen. In other studies, penicillin, streptomycin and amikacin combination of antibiotics was found to be the most effective for controlling bacterial population (Meena, 2008), whereas, most of the isolates were resistant to ampicillin (Patel and Patel, 2012). However, gentamicin, tylosin, lincomycin and spectinomycin (GTLS) combination has been reported as a worthy option of reducing bacterial load in semen samples by other researchers (Andrabi *et al.*, 2016; Meena *et al.*, 2017).

In conclusion, despite prolonged use of streptomycin as antibiotic during semen processing, streptomycin is still competent in reducing the bacterial load of semen. Since bacterial load and type of bacterial microflora is a subject to variation, regular antibiogram of bacterial isolates of semen should be obtained. However, bacteria can acquire resistance to antibiotics; therefore, focus should shift towards maintaining better hygienic condition rather than exclusively relying on addition of antibiotics for controlling bacterial contamination.

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