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Aerobic Microbes of Cervico-Vaginal Mucus from Repeat Breeder Bovines and their Antibiogram

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ABSTRACT

Ninety-two cervical mucus samples from repeat breeding bovines were screened for the presence of aerobic micro-organisms. From 63 samples, 73 organisms were isolated in pure culture, of which the organisms of importance were *Bacillus* sp, *Escherichia coli, Pseudomonas* sp, *Streptococcus* sp, and *Staphylococcus* sp. The isolates were resistant to penicillin, streptomycin, polymyxin-β among other antimicrobials. The isolates were sensitive mostly to gentamicin, kanamycin, and amikacin. Forty-four out of 68 isolates exhibited multiple drug resistance to 50% or more of the drugs tested.

INTRODUCTION

Repeat breeder syndrome is a condition in which female bovines fail to become pregnant even after 3 or more breedings. The problem is worldwide with an incidence rate of 10–25% (Bartlett, Kirk and Mather, 1986). Of the estimated $800 m loss to cattle industry in USA due to reduced breeding efficiency, 10 to 15% is due to repeat breeders (Mackay, 1981). In India, the incidence was 19.01% among Gir cows and their crosses (Shukla and Pandit, 1989).

Infertility due to infections is a common cause of reproductive failures in bovines. The presence of non-specific organisms in the female genital tract with resultant mild infections lead to impaired fertility. Pathogenic or potentially pathogenic micro-organisms have been isolated from cervical mucus of repeat breeding cows (Murthy, Nanjiah and Murthy, 1974) and from endometritis cases of bovines (Sinha, Arnjea and Singh, 1977). The present study was undertaken to isolate microbes from repeat breeding bovines in this part of the country and to find their *in vitro* susceptibility to various drugs.

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The wool from the crossbred sheep has been found to be acceptable on the world market albeit at relatively low prices. A local wool processing plant is prepared to pay between M$1.80 to $2.30 per kilogram for greasy wool depending on the grade. This will contribute additional income to the farmers.

Sheep dung that has been processed by composting and drying finds a ready market in the horticulture and market gardening sector. It currently fetches 20 cents per kilogram sold in 50 kilogram bags.

RESEARCH AND DEVELOPMENT

Various agencies are involved in ongoing studies in areas of fertility, nutrition and improvement of pastures in plantations with the use of shade tolerant species. Work is also being carried out at breeding hair (woolless) sheep. There are also attempts at encouraging utilisation of wool in the cottage industries by small scale entrepreneurs in the rural areas.

CONCLUSION

There is a large potential for sheep production in Malaysia. This optimistic outlook is based on the use of readily available labour and forage resources in the plantation areas that are not in direct competition with other agricultural activities. The development of a new "Malaysian" breed that is adapted to the local environment will enhance productivity in the future. There is a lot of confidence that Malaysia will have a sheep population of 1 million by the year 2000.

MATERIALS AND METHODS

Cervical mucus samples were collected from a total of 92 bovines which includes 30 heifers from Nagercoil area of Kanya Kumari district of Tamil Nadu, India. They were screened by using simple and specific media. The isolated microbes were grouped to generic or species level as per Bergey's manual of Determinative Bacteriology (Buchanam and Gibbons, 1974).

The organisms except fungi were then subjected to antibiogram studies by disc diffusion method (Bauer, Kirby, Sherris and Turk, 1966) using 16 different antimicrobial agents (Span diagnostics/Pasteur Biologicals, India). They were Amikacin (30 μg), Amoxicillin (10 μg), Cephalexin (30 μg), Chloramphenicol (30 μg), Clindamycin (2 μg), Colistin (10 μg), Erythromycin (15 μg), Gentamycin (10 μg), Kanamycin (30 μg), Neomycin (30 μg), Nitrofurantoin (300 μg), Penicillin (10 μg), Polymyxin-β (300 μg), Streptomycin (10 μg), Tetracycline (30 μg) and Tobramycin (10 μg).

RESULTS

A total of 73 organisms were obtained in pure culture from 63 samples (68.47% out of the 92 examined. Details of various organisms isolated and their antibiotic sensitivity to different antimicrobial agents are presented in Table 1. The organisms isolated in high frequency include *Bacillus* sp. (13.69%), *Escherichia coli* (12.32%), *Pseudomonas* sp. (13.69%), Streptococci (13.69%) and Staphylococci (10.95%).

In the present antibiogram study the organisms were resistant to polymyxin-β, colistin, penicillin, clindamycin, cephalexin, streptomycin and nitrofurantoin in a descending order from 98.52% to 57.35% (Table 1).

The isolates were resistant to a minimum of 2 or more of the 16 drugs used. Fifty per cent or more of the organisms of each genera except *Bacillus* and *Proteus*, exhibited resistance to 8 or more of the drugs tested (Table 2). *Pseudomonas* sp., *Escherichia* sp., *Staphylococcus* sp. and *Corynebacterium* sp. showed multiple drug resistance to 100%, 77.77%, 75% and 71.42% of the drugs tested respectively.

DISCUSSION

Species of *Pseudomonas, Escherichia, Klebsiella, Proteus, Staphylococcus, Corynebacterium, Bacillus* and *Micrococcus* were recorded from repeat breeding bovines in India (Murthy *et al*, 1974; Verma and Tyagi, 1974 and Dholakia, Shah, Purohit and Kher, 1987), and in other countries (Osman, El-Naggar and El-Timawi, 1983; Panangala, Fish and Barnum, 1978). The isolation of these organisms including fungi from endometritis cases of bovines have been reported by Murthy and Rao (1978). Most of the organisms of the present study were found to be associated with abortion and reproductive disorders

Table 1

Antibiogram of Bacterial Isolates from Repeat Breeding Bovines

Name of the Organism	Antimicrobials and Number of Resistant Organisms															
		AK	AM	CP	CH	CM	CS	EM	GM	KM	NM	NF	PC	PM	SM	TC	TM
Acinetobacter calcoaceticus	1	–	1	1	1	1	–	1	–	–	–	1	1	1	1	–	–
Bacillus sp.	10	6	2	6	–	4	10	1	2	5	4	4	5	10	9	–	3
Corynebacterium sp.	7	2	1	4	3	4	7	3	2	1	1	4	6	7	3	5	3
Enterobacter sp.	2	–	1	1	2	1	2	2	–	–	1	2	2	2	–	1	1
Escherichia coli	9	3	6	7	6	8	9	9	2	2	3	5	9	8	4	7	1
Micrococcus sp.	6	1	1	3	2	6	6	5	1	2	2	3	6	6	3	3	2
Proteus sp.	5	–	3	3	3	4	5	4	1	2	1	4	4	5	2	2	–
Pseudomonas sp.	10	1	10	10	5	9	9	6	2	5	3	10	10	10	9	7	4
Staphylococcus sp.	8	4	4	5	4	5	8	4	5	1	4	2	4	8	6	5	6
Streptococcus sp.	10	3	2	8	5	8	10	4	2	2	3	6	7	10	5	2	1
Yeast	2							Antibiogram not done									
Aspergillus sp.	3							Antibiogram not done									
Total	73	20	31	48	31	50	66	39	17	20	22	39	54	67	42	32	21
Percentage Resistance		29.41	45.58	70.58	45.58	73.52	97.05	57.35	24.99	29.41	32.35	57.35	79.41	98.52	61.76	47	30.88
Susceptible		70.59	54.42	29.42	54.42	26.48	2.95	42.65	75.01	70.59	67.65	42.65	20.59	1.48	38.24	33	69.12

AK: Amikacin
AM: Amoxicillin
CP: Cephalexin
CH: Chloramphenicol
CM: Clindamycin
CS: Colistin
EM: Erthromycin
GM: Gentamycin
KM: Kanamycin
NM: Neomycin
NF: Nitrofurantoin
PC: Penicillin
PM: Polymycin
SM: Streptomycin
TC: Tetracycline
TM: Tobramycin

Table 2

Multiple Drug Resistance of Repeat Breeder Isolates

S. No.	Organism	No. of drugs to which resistant out of 16 drugs	Total no. of organisms tested	Percentage of organisms showing resistance to 8 (50%) or more drugs
1.	*Acinetobacter calcoaceticus*	9	1	100.00 (1)*
2.	*Bacillus* sp.	5, 5, 5, 6, 6, 7, 8, 9, 9, 11	10	40.00 (4)
3.	*Corynebacterium* sp.	2, 6, 8, 9, 9, 9, 13	7	71.42 (5)
4.	*Enterobacter* sp.	4, 10	2	50.00 (1)
5.	*Escherichia coli*	6, 7, 8, 8, 10, 12, 12, 13, 13	9	77.77 (7)
6.	*Micrococcus* sp.	5, 7, 7, 10, 12, 13	6	50.00 (3)
7.	*Proteus* sp.	5, 6, 7, 11, 14	5	40.00 (2)
8.	*Pseudomonas* sp.	9, 9, 10, 10, 10, 12, 12, 12, 14, 15	10	100.00 (10)
9.	*Staphylococcus* sp.	5, 6, 9, 9, 11, 11, 12, 13	8	75.00 (6)
10.	*Streptococcus* sp.	4, 5, 5, 6, 7, 9, 10, 10, 11, 12	10	50.00 (5)

* Number of organisms.

among cattle in the USA (Siddique, Grant, Blackwell and McKenzie, 1976). *Acinetobacter calcoaceticus* has been isolated from cases of abortion in water buffaloes (Das and Paranjape, 1986).

In the present study resistance noticed to penicillin and streptomycin were 79.41% and 61.76% respectively. The organisms were also resistant to a number of other antimicrobials (Table 1). Murthy and Rao (1979) have recorded resistance, to penicillin and streptomycin from microbes of uterine infections in buffaloes.

Of the antimicrobials tested, gentamycin was found to be most effective (75.01%) followed by kanamycin, amikacin (each 70.59%), tobramycin (69.12%) and neomycin (67.65%). Similar sensitivities of isolates have been reported earlier to gentamycin and kanamycin (Dholakia, Shah, Purohit and Kher, 1987) to gentamycin and neomycin (Shah and Dholakia, 1983) in addition to other antimicrobials.

Forty-four out of 68 isolates (64.70%) exhibited simultaneous resistance to 8 or more drugs. One culture of *Pseudomonas* sp. showed resistance to 15 out of 16 drugs used. Sharda, Moghe and Tanwami (1991) reported the existence of multiple drug resistance among isolated procured from repeat breeding animals.

This study records the ineffectiveness of penicillin and streptomycin for treating genital tract infections of repeat breeding bovines. The isolation of potentially pathogenic organisms and their varied susceptibility pattern to antimicrobials in this study suggests that a routine bacteriological examination with sensitivity profile is useful before selecting a treatment regime of 2 or 3 drugs is used for the repeat breeder bovines.

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