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In vitro Antibiotic Susceptibility of Bacterial Species, Identified from Uteri of Slaughtered Goats

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Abstract: Antibiogram susceptibility of various bacterial species was determined. Among the eight different antibiotics: gentamycin, chloramphenicol, tetracycline, kanamycin and ampicillin were found highly effective against most of the species of bacteria. The species, highly sensitive to gentamycin were *S.intermedius*, *S.epidermidis*, *C.pyogenes*, *S.aureus*, *P.aeroginosa*, *K.aerogenes*, *L.monocytogenes*, *P.mirabilis* and *E.coli and their sensitivity recorded was* 96.6, 93.3, 93.3, 86.6, 86.6, 80.5, 80, 80, and 80% respectively. On the other hand, *P.multocida*, *S.epidermidis*, *S.intermedius*, *S.aureus*, *C.pyogenes*, *P.aeroginosa*, *L.monocytogenes*, *K.aerogenes*, *M.citreus* and *E. coli* were observed highly sensitive to chloramphenicol and their sensitivity recorded was 93.3, 86.6, 86.6., 86.6, 86.6, 86.6, 93.3, 80, 80, and 73.3% respectively. The species *P.multocida* also showed sensitivity to tetracycline (96.6%). The species *K.aerogenes*, *L.monocytogenes*, *C.pyogenes*, *S.aureus* and *M.citreus* were also sensitive to tetracycline with 80% sensitivity. However, the species *S.intermedius*, *S.epidermidis*, *C.pyogenes*, *P.aeroginosa* and *S.aureus* were highly sensitive to kanamycin and its efficacy against above organisms was 93.3, 90, 90, 80 and 73.3% respectively. While *S.epidermidis*, *S.intermedius* and *K.aerogenes* were highly susceptible to ampicillin and their sensitivity was 80%.

Key words: Antibiotic susceptibility, bacterial spp., uteri of goats

Introduction

Susceptibility of bacterial organisms against antibiotics is one of the most controversial issues in treating uterine infection in animals. The multiplicity of drugs available and the numerous production-management systems in which they are utilized, confuse the treatment discussions. The confusion is compounded by the complexity of bacterial infections and constantly changing information available. This confusion produces scientific, ethical, moral and legal dilemmas for veterinarians and economic questions for producers. A lot of investigations have been carried-out throughout the world and many valuable suggestions have already been made by various workers on sensitivity of bacterial species to different antibiotics (Singh *et al.*, 1992; Nizamani, 1999 and Rind and Khan, 2000). Nizamani (1999) isolated similar bacterial species from the uterine of sheep. On testing the sensitivity of the organisms to different antibiotics by disc diffusion technique, he found most of the species, highly sensitive to tetracycline, sulfamethoxazole trimethoprim and neomycin (100%) but a few species showed less susceptibility against the drugs. Keeping in view the sensitivity and resistance of bacterial species to antibiotics, the present study was designed to investigate the susceptibility of the organisms to antibiotics that cause uterine infections in goats.

Materials and Methods

One hundred uteri of slaughtered goats were collected from different slaughter houses of Hyderabad and Tando Jam in sterilized polythene bags with the help of sterile scissors, forceps and were kept in ice box immediately, and brought to the Central Veterinary Diagnostic Laboratory, Tando Jam for isolation and identification of bacterial species.

Glassware such as Petri dishes, pipettes and flasks etc. were kept in 1% HCL solution overnight and washed well with distilled water for several times then dried in oven at 65°C. Then sterilization was carried-out in hot air oven at 180°C for one and half hour.

The media were prepared and used for detailed investigation of bacterial organisms as a formula adapted by Difco (1969). Both solid and broth media were used. In solid: nutrient, blood and MacConkey's agars, while in broth: nutrient broth were cultured and colony characteristics were recorded.

A pure colony from cultured dishes was picked-up and smear was made on a cleaned glass slide and stained by Gram's method of staining and staining reaction was observed.

A few biochemical tests like oxidase, coagulase, indole, Voges Proskauer, urease, methyl red, gelatin liquefaction, Simmon citrate, H_2S production, catalase and TSI tests were also carried- out to confirm their chemical properties (Cruickshank, 1970).

Eight different sugars were prepared and used for fermentation properties of isolated organisms as described by Cruickshank (1970). The sugars used were: glucose, sucrose, lactose, maltose, mannitol, inositol, arabinose and raffinose.

Eight different antibiotics were placed on cultured plate and zone of sensitivity was recorded from center of disc to the zone made by inhibition of the growth of organism in mm according to the method of Bauer *et al.* (1966). The antibiotics used were: Gentamycin(GM), Kanamycin (K), Neomycin (N), Chloramphenicol (CP), Tetracycline (TC), Sulphamethoxazole (S), Ampicillin (AM) and Polymyxin B (PB).

Testing the antibiotic susceptibility of the bacterial species, following materials were brought in use:

1. Muller-Hinton agar plates
2. 150X15mm, 4-6mm deep medium
3. Sterile saline
4. Barium chloride standard
5. Sterile cotton wool or sterile swabs
6. Sterile forceps
7. Ruler
8. Sensitivity chart

Procedure: The surface of Muller-Hinton agar was dried by incubating at 37°C for 30 minutes. The isolated colonies were selected and suspended in normal saline and then colour was

matched with barium chloride to record the bacterial cell population. The sterile cotton swab was dipped in the bacterial suspension and then rolled over the surface of the agar medium and covered evenly with the bacterial suspension and placed in incubator for 30 minutes to get dried. Eight different antibiotics were placed over the surface of agar plate. The culture was incubated for 24 hours and results were recorded with the annotations and percentage of susceptibility was calculated as described by Bauer *et al.* (1966).

Results and Discussion

The results are shown in Plate 1 and Table 1.

Micrococcus citreus: It was highly sensitive to gentamycin, chloramphenicol and tetracycline with sensitivity of 80%. The organism was also found quite sensitive to ampicillin and kanamycin and its sensitivity recorded as 66.6 and 46.6% respectively. Moderate effects of other antibiotics like neomycin and polymyxin B were also observed and their level of sensitivity were 33.3 and 20.0% respectively. No effect of sulphamethoxazole was seen during investigation (Plate 1-a and Table 1). Being a species of the same genus, Rind and Khan (2000) recorded *Micrococcus luteus* highly sensitive to chloramphenicol, tetracycline and ampicillin, also noted in present survey as well.

Staphylococcus epidermidis,intermidius* and *aureus: The tendency of sensitivity of the antibiotics against *S. epidermidis* is presented in Plate 1-b and Table 1. Gentamycin, kanamycin, chloramphenicol and ampicillin were recorded most effective drugs and showed average effect as 93.3, 90.0, 86.6 and 80.0% respectively. While quite sensitive action of tetracycline and sulphamethoxazole was also observed and their sensitivity was recorded as 66.6%. The efficacy of other antibiotics to *S. epidermidis* is also presented in Plate 1-b and Table 1.

The results about antibiotic sensitivity of *S. epidermidis* to various drugs are not in accordance to that of Poornima and Upadhye (1995), who collected samples from 25 apparently healthy and 100 infected birds from 20 different broiler farms in and around Banglore. On testing antibiotic sensitivity of *S. epidermidis* to various drugs, they found multiple resistance against ampicillin, amoxycillin, lincomycin, gentamycin and chloramphenicol, while we found highly sensitive to most of the drugs, this variation might be due to host species. Other reason may be that we isolated from uteri of goats and they obtained from birds. Generally, it is noted by some workers that some resistance was observed in sensitivity to various antibiotics, although the species was isolated from different host species.

A similar trend of sensitivity for various antibiotics against *S. intermedius* was also observed (Plate 1-c and Table 1*). S. intermedius* showed low resistance to gentamycin, kanamycin, chloramphenicol and ampicillin and its sensitivity noted was 96.6, 93.3, 86.6 and 80% respectively. However, next highly effective antibiotics recorded were tetracycline and sulphamethoxazole which inhibited the growth of 66.6% organisms.

Rind and Khan (2000), who recorded susceptibility to gentamycin, neomycin, chloramphenicol, tetracycline and ampicillin as 35.3, 53.3, 46.6, 66.6, and 80% respectively were not in close accordance to our results because we had recorded some higher percentage of sensitivity to antibiotics as compared to above author. Very low percentage of sensitivity was also observed by Awad *et al.* (1988) who tested gentamycin, neomycin, tetracycline, chloramphenicol, and ampicillin and recorded susceptibility to the above species as 4, 8, 11, 28, 39.5, and 16.9% respectively.

The findings in context with *S. aureus* recorded are presented in Plate 1-d and Table 1. This species showed low resistance to gentamycin, chloramphenicol, tetracycline, kanamycin and sulphamethoxazole and their efficacy was recorded as 86.6, 86.6, 80, 73.3 and 73.3 % respectively. The sensitivity percentages of *S. aureus* to other drugs are summarized in Plate 1-d and Table 1.

Rind and Khan (2000) recorded the percentage sensitivity of the species to gentamycin, chloramphenicol, tetracycline, kanamycin, and sulphamethoxazole trimethoprim as 53.3, 53.3, 80, 66.6 and 73.3 respectively. Chaudhry *et al.* (1994) also found gentamycin to be the most effective drug against *S. aureus*. In their study, gentamycin showed 93.68%; kanamycin 91.43%, chloramphenicol 87.20% and oxytetracycline 40.90%, efficacy against the above species. Whereas Rakesh *et al.* (1991) collected cervico-vaginal mucus samples from 50 repeat breeding cattles and buffaloes and examined, *S. aureus* was identified from 13 samples. *In-vitro* antibiotic sensitivity test revealed that gentamycin was the most effective drug against the species followed by penicillin, chloramphenicol, oxytetracycline, triple sulfa, streptomycin and nitrofurantoin in the decreasing order while ampicillin was observed least effective against the above species.

Proteus mirabilis: These organisms were detected highly sensitive to gentamycin and susceptibility was recorded as 80% while other drugs were failed to act against it (Plate 1-e and Table 1). Dinev *et al.* (1987) also found gentamycin highly effective against *P.vulgaris*. The other antibiotics observed unlikely successful were chloramphenicol, tetracycline and streptomycin. The results of the present survey on the susceptibility to antibiotics are in complete agreement with those of Dinev *et al.* (1987). However, we made comparison of *mirabilis* with *vulgaris* through the action of gentamycin, because both species belong to the same genus *Proteus*. Generally, the species of the same genus are very close to each other in their physio-chemical nature, therefore the action of the drug should be similar to both the species. Dinev *et al.* (1987) and Haque (1978) studied antibiotic sensitivity of the bacterial isolates from cases of metritis in cattle. It was found that chloramphenicol was 100% effective against *P. vulgaris*. The results of the present study are not in agreement to the findings of Haque (1978) who recorded chloramphenicol as most effective for *vulgaris* and we recorded gentamycin for *mirabilis*, though both the species belong to the same genus i.e., *Proteus*.

Escherichia coli: It was observed highly sensitive to gentamycin and chloramphenicol and susceptibility was 80.0 and 73.3% respectively. The sensitivity of *E. coli* to different antibiotics are presented in Plate 1-f; Table 1. The drugs observed ineffective were tetracycline, sulphamethoxazole and ampicillin and showed 0% action against the above species. Singh *et al.* (1992) also assessed the susceptibility of *E. coli* by 14 different antibiotics through the disc diffusion technique and found tetracycline most ineffective against *E. coli*. The present findings also agreed with the results of Ishimaru *et al.* (1996), Burrows *et al.* (1989), Roy *et al.* (1986), Melhotra *et al.* (1984) Adetosoye and Ojo (1983) who found *E. coli* resistant to tetracycline, sulphamethoxazole and

Table 1: *In-vitro* antibiotic susceptibility of the organisms identified from the uteri of goats.

Bacterial species	Antibiotic discs used	Zone around discs	Indication of sensitivity	Sensitivity %	Degree of sensitivity
M.citreus	Gentamycin	12mm	+ + + +	80.0	Highly sensitive
	Kanamycin	7mm	+ + +	46.6	Quite sensitive
	Neomycin	5mm	+ +	33.3	Moderately sensitive
	Chloramphenicol	12mm	+ + + +	80.0	Highly sensitive
	Tetracycline	12mm	+ + + +	80.0	Highly sensitive
	Sulphamethoxazole	0mm	-	00.0	Resistant
	Ampicillin	10mm	+ + +	66.6	Quite resistant
	Polymyxin B	4mm	+ +	20.0	Moderately sensitive
S.epidermidis	Gentamycin	14mm	+ + + +	93.3	Highly sensitive
	Kanamycin	13.5mm	+ + + +	90.0	Highly sensitive
	Neomycin	5mm	+ +	33.3	Moderately sensitive
	Chloramphenicol	13mm	+ + + +	86.6	Highly sensitive
	Tetracycline	10mm	+ + +	66.6	Quite sensitive
	Sulphamethoxazole	10mm	+ + +	66.6	Quite sensitive
	Ampicillin	12mm	+ + + +	80.0	Highly sensitive
	Polymyxin B	5mm	+ +	33.3	Moderately sensitive
S.intermedius	Gentamycin	14.5mm	+ + + +	96.6	Highly sensitive
	Kanamycin	14mm	+ + + +	93.3	Highly sensitive
	Neomycin	8mm	+ + +	53.3	Quite sensitive
	Chloramphenicol	13mm	+ + + +	86.6	Highly sensitive
	Tetracycline	10mm	+ + +	66.6	Quite sensitive
	Sulphamethoxazole	10mm	+ + +	66.6	Quite sensitive
	Ampicillin	12mm	+ + + +	80.0	Highly sensitive
	Polymyxin B	5mm	+ +	33.3	Moderately sensitive
S.aureus	Gentamycin	13mm	+ + + +	86.6	Highly sensitive
	Kanamycin	11mm	+ + + +	73.3	Highly sensitive
	Neomycin	4mm	+ +	26.6	Moderately sensitive
	Chloramphenicol	13mm	+ + + +	86.6	Highly sensitive
	Tetracycline	12mm	+ + + +	80.0	Highly sensitive
	Sulphamethoxazole	11mm	+ + + +	73.3	Highly sensitive
	Ampicillin	8mm	+ + +	53.3	Quite sensitive
	Polymyxin B	0mm	-	0.0	Resistant
P.mirabilis	Gentamycin	12mm	+ + + +	80.0	Highly sensitive
	Kanamycin	0mm	-	0.0	Resistant
	Neomycin	0mm	-	0.0	Resistant
	Chloramphenicol	0mm	-	0.0	Resistant
	Tetracycline	0mm	-	0.0	Resistant
	Sulphamethoxazole	0mm	-	0.0	Resistant
	Ampicillin	0mm	-	0.0	Resistant
	Polymyxin B	0mm	-	0.0	Resistant
E.coli	Gentamycin	12mm	+ + + +	80.0	Highly sensitive
	Kanamycin	10mm	+ + +	60.0	Quite sensitive
	Neomycin	6mm	+ +	40.0	Moderately sensitive
	Chloramphenicol	11mm	+ + + +	73.3	Highly sensitive
	Tetracycline	0mm	-	0.0	Resistant
	Sulphamethoxazole	0mm	-	0.0	Resistant
	Ampicillin	0mm	-	0.0	Resistant
	Polymyxin B	5mm	+ +	33.3	Moderately sensitive
C.pyogenes	Gentamycin	14mm	+ + + +	93.3	Highly sensitive
	Kanamycin	13.5mm	+ + + +	90.0	Highly sensitive
	Neomycin	4mm	+ +	26.6	Moderately sensitive
	Chloramphenicol	13mm	+ + + +	86.6	Highly sensitive
	Tetracycline	12mm	+ + + +	80.0	Highly sensitive
	Sulphamethoxazole	11mm	+ + + +	73.3	Highly sensitive
	Ampicillin	5mm	+ +	33.3	Moderately sensitive
	Polymyxin B	0mm	-	0.0	Resistant
P.aeroginosa	Gentamycin	13mm	+ + + +	86.6	Highly sensitive
	Kanamycin	12mm	+ + + +	80.0	Highly sensitive
	Neomycin	4mm	+ +	26.6	Moderately sensitive
	Chloramphenicol	13mm	+ + + +	86.6	Highly sensitive
	Tetracycline	10mm	+ + +	66.6	Quite sensitive
	Sulphamethoxazole	12mm	+ + + +	80.0	Highly sensitive
	Ampicillin	4mm	+ +	26.6	Moderately sensitive
	Polymyxin B	5mm	+ +	33.3	Moderately sensitive
L.monocytog.	Gentamycin	12.5mm	+ + + +	80.0	Highly sensitive
	Kanamycin	10mm	+ + +	66.6	Quite sensitive
	Neomycin	12mm	+ + + +	80.0	Highly sensitive
	Chloramphenicol	12mm	+ + + +	80.0	Highly sensitive
	Tetracycline	12mm	+ + + +	80.0	Highly sensitive
	Sulphamethoxazole	8mm	+ + +	53.3	Quite sensitive
	Ampicillin	5mm	+ +	33.3	Moderately sensitive
	Polymyxin B	0mm	-	0.0	Resistant

K.aerogenosa	Gentamycin	12.5mm	+ + + +	80.5	Highly sensitive
	Kanamycin	10mm	+ + +	66.6	Quite sensitive
	Neomycin	12mm	+ + + +	80.0	Highly sensitive
	Chloramphenicol	12mm	+ + + +	80.0	Highly sensitive
	Tetracycline	12mm	+ + + +	80.0	Highly sensitive
	Sulphamethoxazole	0mm	-	0.0	Resistant
	Ampicillin	12mm	+ + + +	80.0	Highly sensitive
	Polymyxin B	0mm	-	0.0	Resistant
P.multocida	Gentamycin	10mm	+ + +	66.6	Quite sensitive
	Kanamycin	10mm	+ + +	66.6	Quite sensitive
	Neomycin	15mm	+ + + +	100.0	Highly sensitive
	Chloramphenicol	14mm	+ + + +	93.3	Highly sensitive
	Tetracycline	14.5mm	+ + + +	96.6	Highly sensitive
	Sulphamethoxazole	8mm	+ + +	53.3	Quite sensitive
	Ampicillin	8mm	+ + +	53.3	Quite sensitive
	Polymyxin B	8mm	+ + +	53.3	Quite sensitive

Absence of zone around discs – , Clear zone with 1-2mm diameter around discs +, Clear zone with 2-5mm diameter around discs + +, Clear zone with 6-10mm diameter around discs + + +, Clear zone with 11-15mm diameter around discs + + + +

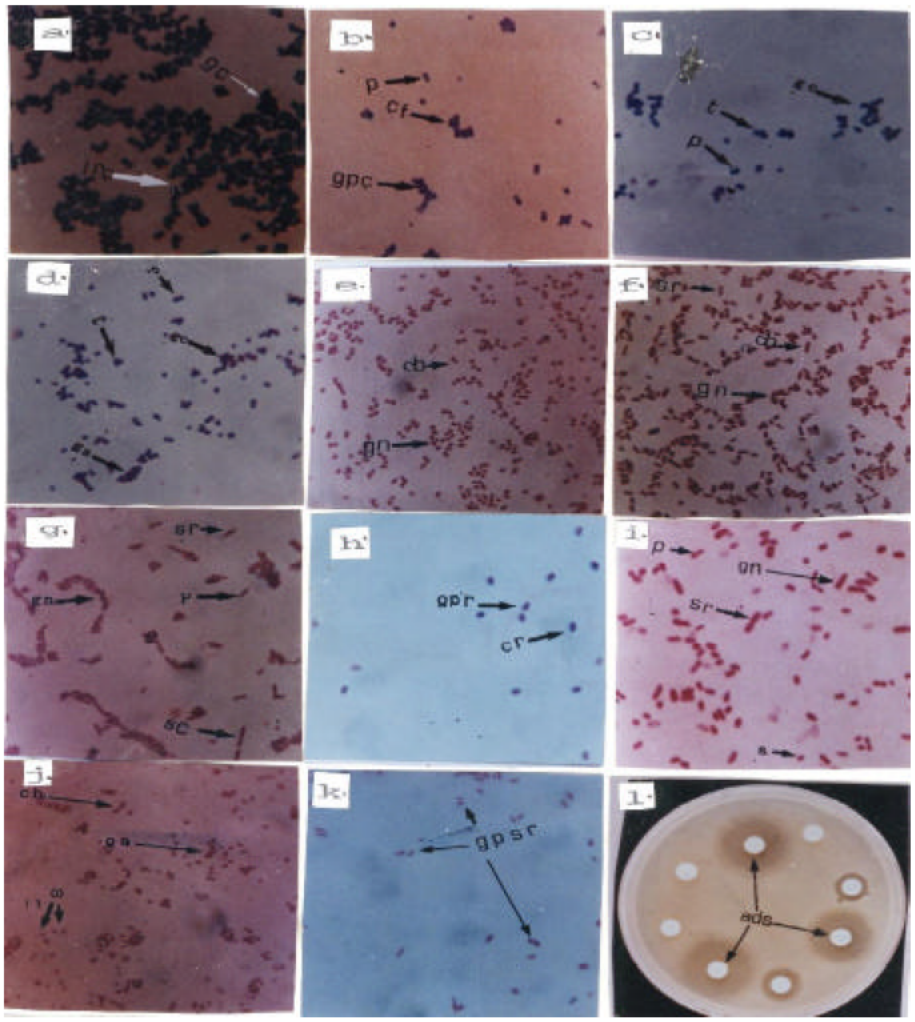

Plate 1: Shows bacterial organism identified from the uteri of goats and it's pattern of sensitivity to different antibiotics. a) *M.citreus:* gc, gram positive; irc, irregular culuster. b) *S.epidermidis;* gpc, gramþpositive cocci; p, pair; cf, cluster form c) *S.intermidius;* gc, gram-positive cocci; t, tetrads; p, pair. d) *S.aurenus;* gc, gram-positive cocci; t, tetrads; gs, grape lime structure. e) *P.mirabili;* gn, gram-negative; cb, cocco-bacilli. f) *E.coli;* gn, gram-negative; cb. cocco-bacilli. g) *P.aeroginosa:* gn, gram-negative; p, pair; sc, short chains. h) *C.pyogenes:* gpr, gram-positive rods; cr, cocci rods. I) *K.aerogenes:* gn, gram-negative; p, pair; sr, short rods. j) *P.multocida:* gn, gram-negative; cb, cocco-bacilli. k) *L.monocytogenes;* gpsr, gram-positive short rods. l) *E.coli:* ads, antibiotic discs sensitivity

ampicillin while sensitive to chloramphenicol.

Pseudomonas aeroginosa: The results about drug sensitivity of *P. aeroginosa* are presented in the same Plate 1-g, Table 1. Neomycin, ampicillin, polymyxin B were moderately effectiveMay 25, 2001 against *P. aeroginosa* and its susceptibility was recorded as 26.6, 26.6 and 33.3% respectively. However, the most active antibiotics against organisms of *P. aeroginosa* were gentamycin, kanamycin, chloramphenicol, sulphamethoxazole and its susceptibility was recorded as 86.6, 80.0, 86.6, 80.0% respectively and were also demonstrated by Roy *et al.* (1986), but at the same time *P. aeroginosa* was also observed highly sensitive to gentamycin.

Corynebacterium pyogenes: This species was observed highly sensitive to gentamycin, kanamycin, chloramphenicol, tetracycline and sulphamethoxazole (Plate 1-h, Table 1). Similar results were also reported by Malik *et al.* (1987) and Zahid *et al.* (1986). Haque (1978) also found gentamycin as 93.67%, kanamycin 88.61% and chloramphenicol 87.33% active against *C. pyogenes*.

Klebsiella aerogenes: The most effective antibiotics against *K. aerogenes* were gentamycin, neomycin, chloramphenicol, tetracycline and ampicillin and their sensitivities were 80.5, 80.0, 80.0, 80.0 and 80.0% respectively. While sulphamethoxazole and polymyxin B had 0% action against the species (Plate 1-l, Table 1). Burrows *et al.* (1989) also tested *Klebsiella* species sensitivity against chloramphenicol, furazolidone, neomycin and oxytetracycline, while Roy *et al.* (1986) found it highly sensitive to chloramphenicol only but not to gentamycin.

Pasteurella multocida: The species was 100% sensitive to neomycin. Other antibiotics chloramphenicol and tetracycline were also observed highly effective against the species and their effect was recorded as 93.3 and 96.6% respectively. Gentamycin was found quite active and its sensitivity was 66.6% (Plate 1-j, Table 1). Burrows *et al.* (1989) tested the sensitivity of 678 strains of *Pasteurella* species recognized from cattle, horses, goats, sheep, dogs, poultry and other animals to various antibiotics. *Pasteurella* species were found highly sensitive to chloramphenicol, furazolidone, neomycin and oxytetracycline. On the other hand, according to Roy *et al.* (1986), *P. multocida* was found highly sensitive to gentamycin only. So the results of the present investigation agree with the findings of the above authors.

Listeria monocytogenes: This was quite sensitive to ampicillin and susceptibility recorded was 33.3% while ineffective drug noted was polymyxin B, that showed 0% action against above species while most effective drugs were gentamycin and chloramphenicol and their susceptibility was 12.5 and 12% as shown in Plate 1-k, Table 1. Rakesh *et al.* (1991) examined cervico-vaginal mucus samples from 50 repeated breeding cattle and buffaloes. Besides these, three isolates of *L. monocytogenes*, *In-vitro* antibiotic sensitivity tests revealed that gentamycin was to be the most effective antibiotic followed by penicillin, chloramphenicol, oxytetracycline, triple sulfa, streptomycin and nitrofurantoin to the isolates in the decreasing order, whereas ampicillin was least effective against the isolates of *L. monocytogenes*.

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