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Susceptibility of ruminal bacteria isolated from large and small ruminant to multiple conventional antibiotics

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ABSTRACT

The pivotal aim of the present context was to isolate diversified group of bacteria from the ruminants and to evaluate their antibiogram pattern against 22 antibiotics of 14 different classes. The bacterial isolates from small and large ruminant (sheep, cattle and calves) were isolated from the rumen based on various colonies morphology, and subjected for preliminary antibiotics susceptibility assay using disc diffusion method. The most sensitive isolates (based on zone of inhibition) were selected for determining the minimum inhibitory concentration (MIC) of each antibiotic ranging from 1 to 256 μg/mL. Results revealed the concentration dependent growth inhibitory property of antibiotics a species-specific process. The maximum tolerable concentration (MTC) of each antibiotic was further determined using disc diffusion method, and results exhibited that the tolerance nature of ruminal isolates to antibiotics is a species-specific mechanism. Based on the MIC and MTC values of antibiotics, amikacin, ciprofloxacin, and amoxicilline were observed to be the most potent antibiotics in terms of inhibiting the growth of ruminal isolates. In brief, the findings of the current study showed that despite the overexploitation of antibiotics as additives in the animal's feed, most of the ruminal isolates are sensitive to multiple conventional antibiotics tested. The growth inhibitory trait of antibiotics proves these antimicrobials a propitious agent against the pathogenesis of ruminal isolates in livestock.

1. Introduction

The supplementation of antibiotics in ruminant's feed has undoubtedly manifested their combative role against clinical pathogens. In spite of the major role of antibiotics in promoting animal growth, unfortunately, the antibiotic therapy resulted into the emergence of multi-drug resistant ruminal microflora due to its indiscriminate uses [1]. In fact, bacteria have genetic attribute to acquire antibiotic resistant trait, thereby posing enormous threat worldwide in livestock industries, particularly in developing countries. Considering this, the indiscriminate use of antibiotics was banned in Europe as a precautionary step to prevent the emergence of antibiotics resistance bacteria. Study revealed that the routine supplementation of antibiotics in animal feed creates pressure for resistance that eventually spreads to humans [2].

In the last few years, plethora of antibiotics has been supplemented at sub-therapeutic levels in ruminant production systems in order to optimize rumen fermentation patterns [3,4]. Variations in ruminal fermentation are often attributed to shifts in microflora counts [5]. Previous studies reported that organic acids such as acetic acid, formic acid, lactic acid, and butyric acid producing bacteria tend to be sensitive to antibiotics, while succinic acid producing and lactic acid fermenting bacteria tend to be resistant [5,6].

In general, the bacterial growth inhibitory characteristics of antibiotics could be mainly because of their influences on peptidoglycan synthesis, ribosome activity, DNA replication, mRNA transcription, nucleotide synthesis, and/or membrane stability [7]. It should be noteworthy that the susceptibility and resistant nature of ruminal microflora depend not only on the source of isolation but also on the type of strains exploited. Since, the influence of diversified antibiotics on specific ruminal bacterial species of varied ruminants are undetermined, hence, the present context was investigated to demonstrate the susceptibility/resistivity trait of ruminal bacterial isolates to various classes of commercial antibiotics.

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2. Materials and methods

2.1. Bacterial isolation

Approximately 100 mL of rumen content (liquor and feed particles) were collected from five varied sites of respective animals. Thioglycollate agar medium (g/L: casein enzymic hydrolysate 15.0, L-Cystine 0.5, dextrose 5.5, yeast extract 5.0, sodium chloride 2.5, sodium thioglycolate 0.5, resazurin 0.001, and agar 20.0) was autoclaved and poured into the sterilized petriplates aseptically for the isolation of ruminal bacteria. The freshly collected rumen contents were homogenized and 1 mL of the fluid was serially diluted. One milliliter of the diluted rumen content was spread on the thioglycollate agar medium and incubated at 37 °C for 72 h under anaerobic condition. Diversified colonies were further streaked on freshly prepared thioglycollate agar medium in order to obtain the purified cultures. The purified bacterial cultures were preserved at −80 °C in glycerol stock for further studies.

2.2. Antibiogram assay

The susceptibility test of ruminal isolates against commercially available antibiotics was determined using disc diffusion method [8]. Mueller Hinton agar (g/L: meat, infusion solids 2.0, casein acid hydrolysate 17.5, starch. 1.5, and agar 20.0) plates were prepared and allowed to cool aseptically. Overnight grown isolates were swabbed on the agar media. Meanwhile, 22 antibiotics of 14 classes were dissolved in DMSO (dimethylsulfoxide) and sterile double distilled water based on their solubility for antibiotic susceptibility test. Antibiotics used in this study were - Aminoglycoside - Amikacin (amik), Gentamicin (gen), Neomycin (neo), Tobramycin (tbra); Cephalosporin 1st - Cepharadin (cfrad), Cefadroxil (cdro); Cephalosporin 3rd - Cefoperazone (cper), Cefotaxime (cftax); Chloramphenicol - Chloramphenicol (clor); Fluoroquinolone 3rd - Ciprofloxacin (cipr); Glycopeptide - Vancomycin (van); Macrolide - Erythromycin (ery), Roxithromycin (rox), Streptomycin (str); Penicillin Amino - Amoxicilline (amox), Ampicillin (ampi); Penicillin ESP Anti PS - Piperacillin (pipe); Penicillin Natural - Penicillin G (pen); Peptide - Bacitracin (bacit); Polymyxin - Colistin (col); Sulfonamide - Sulphaguanidin (sg); Tetracycline - Oxytetracycline (tet). Antibiotics (5 μg; 10 μL) were impregnated on filter paper discs and placed on culture swabbed agar medium using ethanol wiped and flamed forceps. Plates were incubated at 37 °C and zone of inhibition was observed after 24 h of incubation in order to determine the antibiotics susceptibility profile of isolates. Experiments were carried out in duplicate.

2.3. Minimum inhibitory concentration (MIC) determination

The MIC of antibiotics against the sensitive isolates of respective animals was determined using disc diffusion assay [8]. Antibiotics were prepared at varied concentrations (1, 2, 4, 8, 16, 24, 32, 48, 64, 96, 128, 192, and 256 μg/mL). The antibiogram assay was determined according to the methodology as described earlier. The minimum concentration of antibiotic exhibiting the zone of inhibition against the sensitive isolates was considered as MIC of particular antibiotic. All the experiments were carried out in triplicate.

2.4. Maximum tolerable concentration (MTC) determination

The MTC of antibiotics for isolates were determined according to the disc diffusion assay [9]. The MTC of antibiotics was estimated as the highest concentration of particular antibiotic that allows the growth of isolate after required period of incubation.

2.5. Statistical analyses

The sensitivity variation based on the zone of inhibition of antibiotics against ruminal isolates were analyzed according to the factorial design [10]. Differences among ruminal isolates were tested for each antibiotic using MIXED of SAS [11].

3. Results

3.1. Antibiogram assay

The bacterial isolates from small and large ruminants were isolated from animal's rumen based on various colonies morphology i.e. the maximum percentage of isolates belong to cattle (26.5%) followed by sheep (22.9%) and calves (22.1%). All the isolates were subjected to susceptibility tests against multiple antibiotics. From the bacteria isolated from sheep's rumen, the R11-1, R11-2, R11-3, R11-4, R11-5, R01-1, R01-2, R01-3, and R01-4 exhibited high susceptibility against all the antibiotics tested based on the measurement of zone of inhibition. Further, antibiotics showed maximal growth inhibition property against isolates of cattle's rumen viz. R05-1a, R05-1b, R05-2, R05-3a, R05-3b, R07-1a, R07-2, R07-3, R07-4, R14-1, R14-2, R15-1, R15-2, R7-1b, R03-1, R03-2, R04-1, R04-2, and R04-3. Likewise, isolates from calves such as R02-1, R12-1, R12-2, R12-3, R18-1, R18-2, R19-1, R19-2, and R2-3 were observed to exhibit higher susceptibility to tested antibiotics (Tables not shown).

3.2. Minimum inhibitory concentration determination

In sheep, among all the antibiotics tested, amoxicilline exhibited potent activity against all bacterial isolates with lower MICs values ranging from 4 to 24 μg/mL. On the other hand, cepharadin, cefadroxil, bacitracin, colistin, and sulphaguanidin revealed low susceptibility towards the isolates with MICs values of 256, 96–256, 128–256, 192–256, and 256 μg/mL, respectively (Table 1- Figs. 1–2).

The MICs values of antibiotics against the bacteria isolated from the rumen of cattle are shown in Table 2 and Figs. 1–2. The isolates were

Table 1
MICs values of sensitive bacteria isolated from the rumen of sheep to antibiotics.

Isolates	amik	gen	neo	tbra	cfrad	cdro	cper	cftax	clor	cipr	van	ery	rox	str	amox	pipe	ampi	pen	bacit	col	sg	tet
R11-1	64	96	256	96	256	256	96	256	256	24	96	64	48	64	16	48	96	64	256	256	256	16
R11-2	32	64	96	48	256	256	64	64	256	32	64	16	32	64	16	192	96	64	256	256	256	96
R11-3	48	32	64	48	256	256	96	256	96	32	48	8	32	192	8	192	64	64	192	192	256	32
R11-4	48	64	96	96	256	256	48	8	64	32	48	128	64	48	16	192	64	16	256	192	256	256
R11-5	32	96	96	64	256	256	48	8	96	48	96	128	96	64	24	64	24	16	256	192	256	32
R01-1	24	8	96	48	256	192	48	256	192	24	48	4	8	256	4	64	48	32	192	256	256	128
R01-2	8	4	256	64	256	96	32	32	192	8	64	32	16	256	4	32	32	16	256	256	256	256
R01-3	24	4	128	48	256	256	32	48	256	4	48	2	8	256	4	32	24	16	256	256	256	192
R01-4	24	4	192	64	256	128	32	24	64	4	32	2	24	192	16	96	32	24	128	192	256	192

MICs – Minimum inhibitory concentrations; amik – Amikacin; gen – Gentamicin; neo – Neomycin; tbra – Tobramycin; cfrad – Cepharadin; cdro - Cefadroxil; cper – Cefoperazone; cftax - Cefotaxime; clor - Chloramphenicol; cipr - Ciprofloxacin; van - Vancomycin; ery - Erythromycin; rox – Roxithromycin; str - Streptomycin; amox – Amoxicilline; pipe - Piperacillin; ampi – Ampicillin; pen - Penicillin G; bacit - Bacitracin; col - Colistin; sg - Sulphaguanidin; tet - Oxytetracycline.

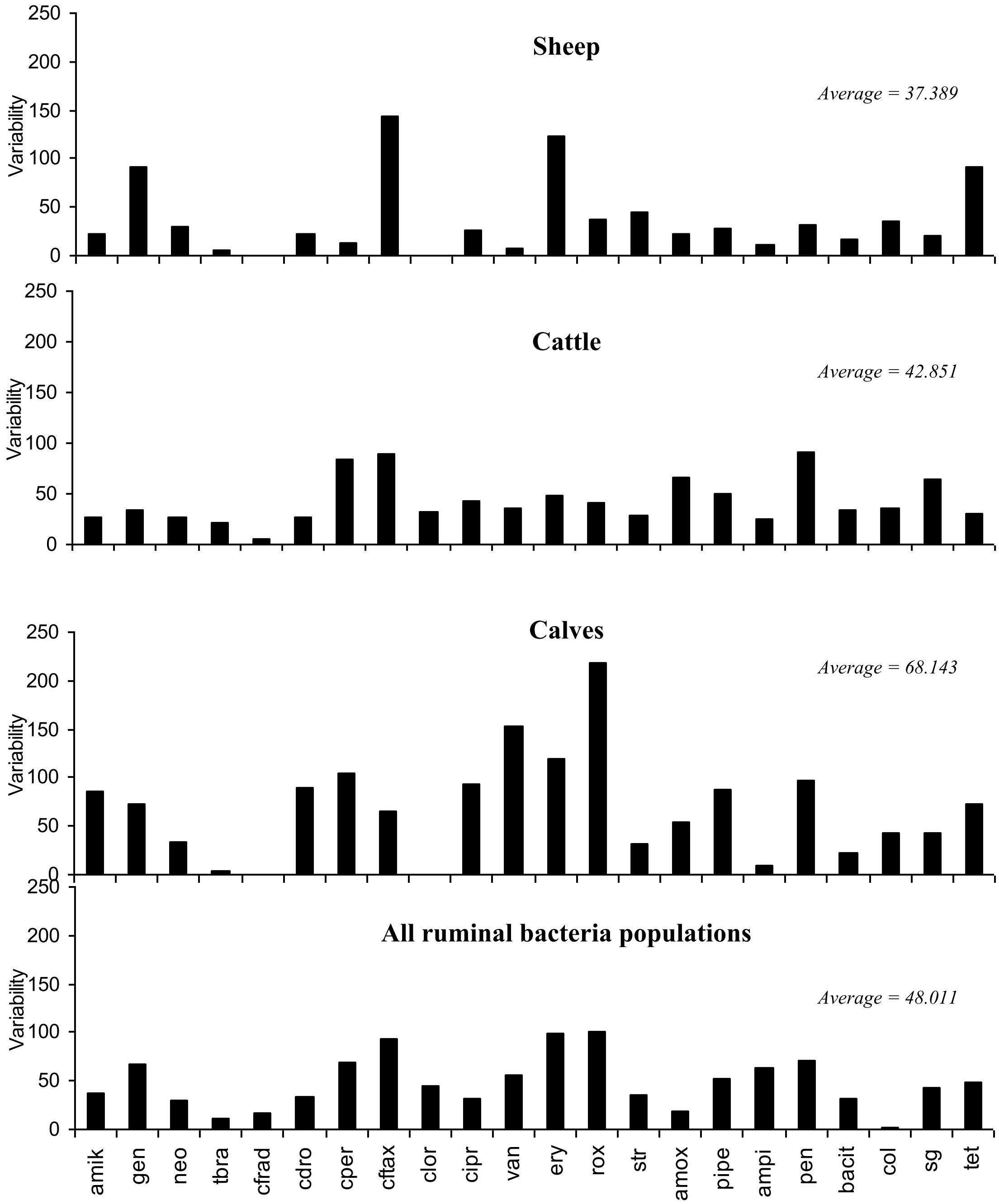

Fig. 1. Variability among the ruminal bacteria population isolated from ruminant in response to various antibiotics tested.
amik – Amikacin; gen – Gentamicin; neo – Neomycin; tbra – Tobramycin; cfrad – Cepharadin; cdro - Cefadroxil; cper – Cefoperazone; cftax - Cefotaxime; clor - Chloramphenicol; cipr - Ciprofloxacin; van - Vancomycin; ery - Erythromycin; rox – Roxithromycin; str - Streptomycin; amox – Amoxicilline; pipe - Piperacillin; ampi – Ampicillin; pen - Penicillin G; bacit - Bacitracin; col - Colistin; sg - Sulphaguanidin; tet – Oxytetracycline.

observed to very sensitive to antibiotics viz. amikacin, gentamicin, ciprofloxacin, erythromycin, roxithromycin, amoxicilline, and oxytetracycline with MICs values of 8–96, 16–96, 2–96, 8–192, 8–256, 1–64, and 24–256 μg/mL, respectively.

All the calves isolates revealed higher sensitivity to amikacin, tobramycin, cefoperazone, cefotaxime, ciprofloxacin, amoxicilline, piperacillin, ampicillin, penicillin G, and oxytetracycline with MICs values of 8–192, 48–96, 8–256, 8–96, 2–48, 1–32, 16–128, 16–256, 8–256, and 24–256 μg/mL, respectively (Table 3; Figs. 1–2).

3.3. Maximum tolerable concentration determination

The maximum tolerable concentrations (MTCs) values of antibiotics against bacteria isolated from the rumen of sheep are shown in Table 4. The isolates were able to tolerate high concentrations of antibiotics viz. cepharadin (192 μg/mL), cefadroxil (64–192 μg/mL), chloramphenicol (48–192 μg/mL), streptomycin (32–192 μg/mL), piperacillin (24–128 μg/mL), bacitracin (96–192 μg/mL), colistin (128–192 μg/mL), sulphaguanidin (192 μg/mL), and oxytetrcycline (8–192 μg/mL).

Table 5 shows the tolerance trait of bacteria isolated from rumen of cattle to antibiotics. Isolates were able to tolerate neomycin, tobramycin, cepharadin, cefadroxil, chloramphenicol, bacitracin, colistin, and sulphaguanidin with high concentrations of 24–192, 16–192, 16–192, 64–192, 16–192, 24–192, 96–192, and 32–192 μg/mL, respectively.

The tolerance ranges of antibiotics towards varied rumen bacterial

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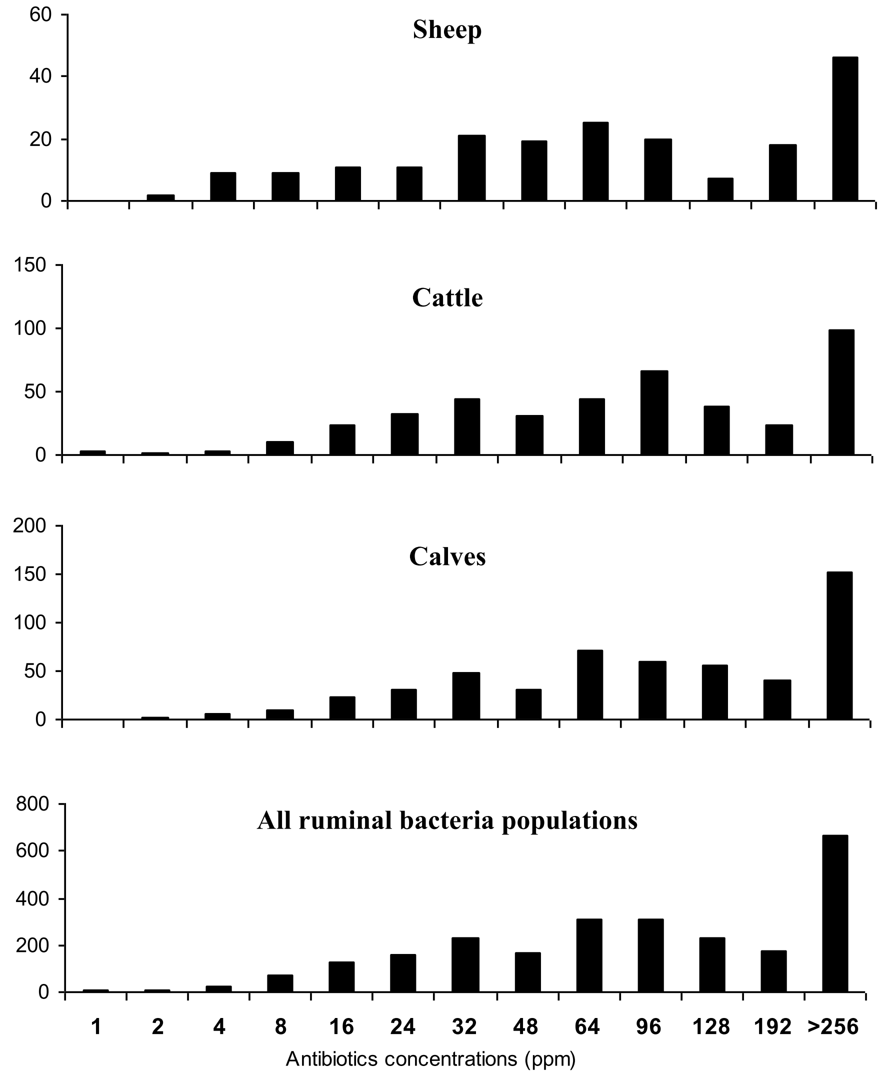

Fig. 2. Frequency of the sensitivity and resistance of each ruminal bacteria to each antibiotic concentration levels.
amik – Amikacin; gen – Gentamicin; neo – Neomycin; tbra – Tobramycin; cfrad – Cepharadin; cdro - Cefadroxil; cper – Cefoperazone; cftax - Cefotaxime; clor - Chloramphenicol; cipr - Ciprofloxacin; van - Vancomycin; ery - Erythromycin; rox – Roxithromycin; str - Streptomycin; amox – Amoxicilline; pipe - Piperacillin; ampi – Ampicillin; pen - Penicillin G; bacit - Bacitracin; col - Colistin; sg - Sulphaguanidin; tet – Oxytetracycline.

isolates of calves are shown in Table 6. The isolates exhibited high tolerance against antibiotics viz. gentamicin, neomycin, cepharadin, cefadroxil, chloramphenicol, vancomycin, roxithromycin, streptomycin, bacitracin, colistin, and sulphaguanidin with maximal concentration of 8–192, 16–192, 8–192, 96–192, 32–192, 8–192, 8–192, 48–192, 64–192, 128–192, and 96–192 µg/mL, respectively.

4. Discussion

The emergence of antibiotic resistance property in animals and its subsequent transfer to humans are considered to be due to the supplementation of antibiotics in animal's feed at sub-therapeutic concentrations. However, there is lack of clear report revealing the transfer of antibiotic resistance genes between two bacteria. Based on the previous study, it has been partially reported that the rumen has the potentiality to act as a site for gene transformation between bacteria [12].

According to Sengupta et al. [13], gram-negative bacteria in anaerobic microflora consist of screened integrons and transposons. However, they are not responsible for the development of resistance among gram-positive bacteria. Currently, the reports on the antibiotics susceptibility properties of diversiform strains of bacteria isolated from the ruminants are scarce. In the present context, we isolated several bacteria from the rumen of sheep, cattle, and calves. All the isolates were subjected to antibiogram assay using 22 antibiotics and results showed that isolates from the rumen of cattle were highly susceptible to antibiotics, followed by isolates from calves, and sheep. The variations in the susceptibility properties to antibiotics might be because of the differences in types of bacterial strains [14].

In the present study, antibiotics might have inhibited the growth of ruminal isolates by blocking the several metabolic mechanisms such as the synthesis of peptidoglycan, DNA replication, protein synthesis, ribosomal transcription, and synthesis of nucleotides [15]. In addition to this, the disruption of cellular membrane is another crucial mode of action of antibiotics in order to inhibit the growth of bacteria.

Bacteria isolated from the rumen of animals exhibited varied MICs and MTCs values. Few antibiotics tested were highly effective even at low doses. Some of the isolates had capability to resist higher concentrations of antibiotics tested. The variations in the MICs and MTCs concentrations of these antibacterial agents might be because of the varied influences of the antibiotics on the ions and proton movement across the cellular membrane [16]. In fact, organisms have the ability to maintain the higher potassium concentration inside cells, and release

Table 2
MICs values of sensitive bacteria isolated from the rumen of cattle to antibiotics.

Isolates	amik	gen	neo	tbra	cfrad	cdro	cper	cftax	clor	cipr	van	ery	rox	str	amox	pipe	ampi	pen	bacit	col	sg	tet
R05-1a	64	24	96	96	256	128	128	128	256	24	192	32	32	128	32	128	192	48	256	256	256	48
R05-1b	32	16	64	256	256	256	64	128	256	24	96	8	24	96	24	48	96	256	256	192	256	64
R05-2	64	32	96	96	256	256	192	96	192	32	64	32	24	128	16	96	128	96	256	256	256	24
R05-3a	48	24	192	96	256	128	96	64	128	32	96	24	32	64	48	128	96	32	256	192	256	32
R05-3b	16	16	192	32	256	128	64	16	24	8	64	32	8	32	1	32	192	256	256	128	256	64
R07-1a	32	96	96	96	256	256	256	256	64	32	32	16	24	64	48	192	96	64	128	192	256	96
R07-2	96	96	256	64	256	256	96	256	256	16	64	16	48	64	8	128	96	32	96	256	256	64
R07-3	64	64	128	64	256	256	32	32	96	4	48	8	16	64	24	64	32	16	256	256	256	96
R07-4	8	96	96	96	256	256	96	24	256	2	256	96	192	64	8	48	64	256	256	192	256	32
R14-1	48	32	96	64	256	256	96	256	256	96	64	16	32	96	24	192	192	96	256	192	256	32
R14-2	48	48	32	24	24	256	16	16	256	24	32	128	256	256	24	1	2	4	32	192	256	32
R15-1	96	96	128	96	256	256	96	128	128	48	64	192	64	96	16	64	256	256	256	256	256	96
R15-2	64	64	128	128	256	128	128	96	96	64	32	8	16	48	32	48	96	24	256	192	96	256
R7-1b	48	96	96	64	256	256	128	128	192	16	128	32	64	64	16	96	128	96	96	256	64	96
R03-1	16	16	256	128	256	96	64	64	96	8	64	48	24	256	32	48	96	64	256	192	256	48
R03-2	32	24	96	48	256	192	24	32	192	16	48	32	32	128	24	48	24	24	256	256	256	48
R04-1	64	24	96	96	256	128	96	96	96	16	128	24	32	96	24	128	256	96	256	256	48	32
R04-2	48	24	96	96	256	256	16	4	256	32	16	32	64	128	24	48	128	128	256	256	256	48
R04-3	48	24	256	96	256	256	1	24	256	8	32	48	48	128	64	32	256	128	256	256	192	48

MICs – Minimum inhibitory concentrations; amik – Amikacin; gen – Gentamicin; neo – Neomycin; tbra – Tobramycin; cfrad – Cepharadin; cdro - Cefadroxil; cper – Cefoperazone; cftax - Cefotaxime; clor - Chloramphenicol; cipr - Ciprofloxacin; van - Vancomycin; ery - Erythromycin; rox – Roxithromycin; str - Streptomycin; amox – Amoxicilline; pipe - Piperacillin; ampi – Ampicillin; pen - Penicillin G; bacit - Bacitracin; col - Colistin; sg - Sulphaguanidin; tet - Oxytetracycline.

Table 3
MICs values of sensitive bacteria isolated from the rumen of calves to antibiotics.

Isolates	amik	gen	neo	tbra	cfrad	cdro	cper	cftax	clor	cipr	van	ery	rox	str	amox	pipe	ampi	pen	bacit	col	sg	tet
R02-1	8	16	96	48	16	256	8	8	128	2	32	8	16	192	1	16	96	48	256	192	256	64
R12-1	64	128	96	64	256	256	256	64	256	32	256	256	256	256	24	64	48	48	192	192	256	24
R12-2	48	32	128	96	256	256	64	32	192	48	128	256	256	64	24	64	48	128	256	192	256	64
R12-3	32	64	256	96	256	128	96	96	64	32	256	96	256	256	24	64	64	256	256	192	256	256
R18-1	192	256	256	96	256	256	256	48	256	32	256	256	256	256	32	64	256	256	256	192	256	256
R18-2	24	128	128	64	256	128	8	32	48	16	16	16	24	64	8	32	16	16	256	256	256	64
R19-1	32	192	128	96	256	256	64	64	128	48	16	24	32	96	24	64	96	96	96	256	256	64
R19-2	8	256	24	64	256	256	64	16	256	8	256	128	256	192	24	128	96	96	96	256	128	64
R2-3	64	128	128	64	256	256	96	48	192	24	192	128	192	192	8	64	16	8	256	256	256	24

MICs – Minimum inhibitory concentrations; amik – Amikacin; gen – Gentamicin; neo – Neomycin; tbra – Tobramycin; cfrad – Cepharadin; cdro - Cefadroxil; cper – Cefoperazone; cftax - Cefotaxime; clor - Chloramphenicol; cipr - Ciprofloxacin; van - Vancomycin; ery - Erythromycin; rox – Roxithromycin; str - Streptomycin; amox – Amoxicilline; pipe - Piperacillin; ampi – Ampicillin; pen - Penicillin G; bacit - Bacitracin; col - Colistin; sg - Sulphaguanidin; tet - Oxytetracycline.

Table 4
MTCs values of sensitive bacteria isolated from the rumen of sheep to antibiotics.

Isolates	amik	gen	neo	tbra	cfrad	cdro	cper	cftax	clor	cipr	van	ery	rox	str	amox	pipe	ampi	pen	bacit	col	sg	tet
R11-1	48	64	192	64	192	192	64	192	192	16	64	48	32	48	8	32	64	48	192	192	192	8
R11-2	24	48	64	32	192	192	48	48	192	24	48	8	24	48	8	128	64	48	192	192	192	64
R11-3	32	24	48	32	192	192	64	192	64	24	32	4	24	128	4	128	48	48	128	128	192	24
R11-4	32	48	64	64	192	192	32	4	48	24	32	96	48	32	8	128	48	8	192	128	192	192
R11-5	24	64	64	48	192	192	32	4	64	32	64	96	64	48	16	48	16	8	192	128	192	24
R01-1	16	4	64	32	192	128	32	192	128	16	32	2	4	192	2	48	32	24	128	192	192	96
R01-2	4	2	192	48	192	64	24	24	128	4	48	24	8	192	2	24	24	8	192	192	192	192
R01-3	16	2	96	32	192	192	24	32	192	2	32	1	4	192	2	24	16	8	192	192	192	128
R01-4	16	2	128	48	192	96	24	16	48	2	24	1	16	128	8	64	24	16	96	128	192	128

MTCs – Maximum tolerable concentrations; amik – Amikacin; gen – Gentamicin; neo – Neomycin; tbra – Tobramycin; cfrad – Cepharadin; cdro - Cefadroxil; cper – Cefoperazone; cftax - Cefotaxime; clor - Chloramphenicol; cipr - Ciprofloxacin; van - Vancomycin; ery - Erythromycin; rox – Roxithromycin; str - Streptomycin; amox – Amoxicilline; pipe - Piperacillin; ampi – Ampicillin; pen - Penicillin G; bacit - Bacitracin; col - Colistin; sg - Sulphaguanidin; tet - Oxytetracycline.

further protons and sodium accordingly [17]. Antibiotics exploited in the present investigation are known to affect not only the flow of ions but also played role as antiporters by binding effectively with protons as well as sodium and potassium ions. It resulted into the free movement of ions and protons through the cellular membrane [16].

The dose dependent antibiotics tolerance characteristics of bacteria are particularly a strain specific property. In the present study, the variations in the antibiotics tolerance nature of ruminal isolates were due to the different types of bacterial species and strains isolated from the rumen of sheep, cattle, and calves. In view of the previous research activities, there are undoubtedly limited reports on the ruminal isolates and their antibiotics susceptibility assay for understanding the antibiotics resistance nature of bacteria isolated from ruminants. According to the study of Hassanain [18], a number of *Campylobacter* strains were isolated from ruminant's fecal samples and tested for their sensitivity to antibiotics. Findings showed that > 55% of isolates were resistant to ampicillin, streptomycin, chloramphenicol, erythromycin, and tetracycline. In the line of previous report, the present investigation also

Table 5
MTCs values of sensitive bacteria isolated from the rumen of cattle to antibiotics.

Isolates	amik	gen	neo	tbra	cfrad	cdro	cper	cftax	clor	cipr	van	ery	rox	str	amox	pipe	ampi	pen	bacit	col	sg	tet
R05-1a	48	16	64	64	192	96	96	96	192	16	128	24	24	96	24	96	128	32	192	192	192	32
R05-1b	24	8	48	192	192	192	48	96	192	16	64	4	16	64	16	32	64	192	192	128	192	48
R05-2	48	24	64	64	192	192	128	64	128	24	48	24	16	96	8	64	96	64	192	192	192	16
R05-3a	32	16	128	64	192	96	64	48	96	24	64	16	24	48	32	96	32	24	192	128	192	24
R05-3b	8	8	128	24	192	96	48	8	16	4	48	24	4	24	ND	24	128	192	192	96	192	48
R07-1a	24	64	64	64	192	192	192	192	48	24	24	8	16	48	32	128	64	48	96	128	192	64
R07-2	64	64	192	48	192	192	64	192	192	8	48	8	32	48	4	96	64	24	64	192	192	48
R07-3	48	48	96	48	192	192	24	24	64	2	32	4	8	48	16	48	24	8	192	192	192	64
R07-4	4	64	64	64	192	192	64	16	192	1	192	64	128	48	4	32	48	192	192	128	192	24
R14-1	32	24	64	48	192	192	64	192	192	64	48	8	24	64	16	128	128	64	192	128	192	24
R14-2	32	32	24	16	16	192	8	8	192	16	24	96	192	192	16	ND	1	2	24	128	192	24
R15-1	64	64	96	64	192	192	64	96	96	32	48	128	48	64	8	48	192	192	192	192	192	64
R15-2	48	48	96	96	192	96	96	64	64	48	24	4	8	32	24	32	64	16	192	128	64	192
R7-1b	32	64	64	48	192	192	96	96	128	8	96	24	48	48	8	64	96	64	64	192	48	64
R03-1	8	8	192	96	192	64	48	48	48	4	48	32	16	192	24	32	64	48	192	128	192	32
R03-2	24	16	64	32	192	128	16	24	128	8	32	24	24	96	16	32	16	16	192	192	192	32
R04-1	48	16	64	64	192	96	64	64	64	8	96	16	24	64	16	96	192	64	192	192	32	24
R04-2	32	16	64	64	192	192	8	2	192	24	8	24	48	96	16	32	96	96	192	192	192	32
R04-3	32	16	192	64	192	192	ND	16	192	4	24	32	32	96	48	24	192	96	192	192	128	32

ND = Not Determined; MTCs – Maximum tolerable concentrations; amik – Amikacin; gen – Gentamicin; neo – Neomycin; tbra – Tobramycin; cfrad – Cepharadin; cdro - Cefadroxil; cper – Cefoperazone; cftax - Cefotaxime; clor - Chloramphenicol; cipr - Ciprofloxacin; van - Vancomycin; ery - Erythromycin; rox – Roxithromycin; str - Streptomycin; amox – Amoxicilline; pipe - Piperacillin; ampi – Ampicillin; pen - Penicillin G; bacit - Bacitracin; col - Colistin; sg - Sulphaguanidin; tet - Oxytetracycline.

Table 6
MTCs values of sensitive bacteria isolated from the rumen of calves to antibiotics.

Isolates	amik	gen	neo	tbra	cfrad	cdro	cper	cftax	clor	cipr	van	ery	rox	str	amox	pipe	ampi	pen	bacit	col	sg	tet
R02-1	4	8	64	32	8	192	4	4	96	ND	24	4	8	128	ND	8	64	32	192	128	192	48
R12-1	48	96	64	48	192	192	192	48	192	16	192	192	192	192	16	48	32	32	128	128	192	16
R12-2	32	24	96	64	192	192	48	24	128	24	96	192	192	48	16	48	32	96	192	128	192	48
R12-3	24	48	192	64	192	96	64	64	48	16	192	64	192	192	16	48	48	192	192	128	192	192
R18-1	128	192	192	64	192	192	192	32	192	16	192	192	192	192	24	48	192	192	192	128	192	192
R18-2	16	96	96	48	192	96	4	24	32	4	8	8	16	48	4	24	8	8	192	192	192	48
R19-1	24	128	96	64	192	192	48	48	96	24	8	16	24	64	16	48	64	64	64	192	192	48
R19-2	4	192	16	48	192	192	48	8	192	2	192	96	192	128	16	96	64	64	64	192	96	48
R2-3	48	96	96	48	192	192	64	32	128	8	128	96	128	128	4	48	8	4	192	192	192	16

ND = Not Determined; MTCs – Maximum tolerable concentrations; amik – Amikacin; gen – Gentamicin; neo – Neomycin; tbra – Tobramycin; cfrad – Cepharadin; cdro - Cefadroxil; cper – Cefoperazone; cftax - Cefotaxime; clor - Chloramphenicol; cipr - Ciprofloxacin; van - Vancomycin; ery - Erythromycin; rox – Roxithromycin; str - Streptomycin; amox – Amoxicilline; pipe - Piperacillin; ampi – Ampicillin; pen - Penicillin G; bacit - Bacitracin; col - Colistin; sg - Sulphaguanidin; tet - Oxytetracycline.

demonstrated the susceptibility attribute of diversified ruminal isolates to various antibiotics.

The antibiotics tolerance trait of few ruminal bacterial strains is considered due to extracellular polysaccharide too. However, there is limited information revealing the molecular mechanisms for the production of extracellular polysaccharide in ruminal bacteria. But the production of extracellular polysaccharide in non-ruminal bacteria is obtained due to the presence of a variety of inducible genes [19]. Additionally, the end products obtained after the fermentation process play a paramount role as markers in order to evaluate the resistance or sensitive nature of bacteria residing rumen. Generally, lactic acid, butyric acid, and formic acid are indication for susceptibility attribute of bacteria, while succinic acid is an indication for the resistant nature of ruminal bacteria [20].

5. Conclusion

The present study clearly exhibited the antibiotics susceptibility patterns of diverse bacteria isolated from the rumen of sheep, cattle, and calves. Isolates showed higher range of sensitivity to multiple conventional antibiotics based on the zone of inhibition. Further, the minimum inhibitory concentrations and maximum tolerable concentrations determination of antibiotics to the most susceptible isolates revealed the growth inhibitory concentrations of antibiotics a species-specific process. Among all the antibiotics tested, amikacin, ciprofloxacin, and amoxicilline were observed to be the most active antibiotics in terms of inhibiting the growth of ruminal isolates.

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