

尿路コリネバクテリア分離用選択培地を用いての牛外陰部, 膣前庭からの本菌の検出

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Selective Medium for Isolation of Urinary Corynebacteria and Detection of the Organisms from Bovine Vulva and Vaginal Vestibule using the Medium

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ABSTRACT. Selective medium for the isolation of urinary corynebacteria from samples contaminated with feces or urine of cows was prepared by incorporating 200 µg/ml fosfomycin and 2 µg/ml amphotericin B into standard blood agar. Using this medium, distribution of the organisms was examined from the vulva and vaginal vestibule of 210 apparently healthy cows. *C. pilosum* was isolated from the vulva and vaginal vestibule at the ratio of 16.2%, and *C. renale* was isolated from the same sites at 5.7% and 6.6%, respectively. Number of colonies detected from the vulva was greater than that from the vaginal vestibule. The findings suggest that bovine vulva may be a suitable habitat for *C. pilosum* and *C. renale*, and the results correlated with the fact that these organisms adhered best to the epithelial cells of the vulva in *in-vitro* experiments.—**KEY WORDS:** *C. pilosum*, *C. renale*, distribution, selective medium, vulva.

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Results of a previous *in-vitro* experiment indicated that *Corynebacterium renale* and *C. pilosum* adhered best to epithelial cells of the vulva among various parts of the bovine urinary tract examined¹⁾. Though the distribution of *C. pilosum* and *C. renale* in meatus urinarius and the vaginal vestibule in apparently healthy cows was reported by the authors³⁾, no investigation has been made on the presence of the organisms in the bovine vulva. Because of contamination with feces or urine, isolation of the organisms from the bovine vulva has been unsuccessful, showing the need for a selective medium for efficient recovery of the organisms.

The aim of the present study was to prepare a selective medium for isolation of urinary corynebacteria and to detect the organisms from the bovine vulva and vaginal vestibule using the medium.

MATERIALS AND METHODS

Bacterial strains: Seven strains were used for examination of drug susceptibility: *C. renale* (ATCC 19412), *C. pilosum* (ATCC 29592), *C. cystitidis* (ATCC 29593), *Staphylococcus aureus* (Kitami-3-9D), *Streptococcus agalactiae* (ATCC 27956), *Bacillus subtilis* (ATCC 633) and *Escherichia coli* (NIHJ).

Medium: Heart infusion (HI) agar (BBL, Cockeysville, U. S. A.) containing 5% horse blood was used for the susceptibility test.

Assay of antibiotic susceptibility: As a screening test, the following 15 disks (Sho-wa Yakuhin Co., Tokyo), each impregnated with a different antibiotics, were used; fosfomycin (FOM), aminobenzylpenicillin, benzylpenicillin, sulbenicillin, tetracycline, oxytetracycline, kanamycin, gentamycin,

Table 1. Minimum inhibitory concentration (MIC) of bacterial species to FOM

Bacterial species	MIC ($\mu\text{g/ml}$)
<i>C. renale</i>	50,000
<i>C. pilosum</i>	75,000
<i>C. cystitidis</i>	25,000
<i>E. coli</i>	25.0
<i>S. aureus</i>	12.5
<i>S. agalactiae</i>	12.5
<i>B. subtilis</i>	25.0

streptomycin, josamycin, cefalothin, chloramphenicol, novobiocin, colistin, and amikacin.

After the screening test, minimum inhibitory concentration (MIC) was examined by an agar dilution method for FOM. One loopful of bacteria grown at 37°C for 24 hrs, except for corynebacteria, which was grown for 48 hrs, in Trypticase soy broth (BBL) was streaked in a straight line onto an HI agar plate containing different concentrations of FOM. The MIC of FOM was recorded after incubation at 37°C for 24 to 48 hrs aerobically.

Isolation of urinary corynebacteria from bovine vulva and vaginal vestibule: The survey was conducted on 210 apparently healthy cows from 5 herds in and around Ebetsu City in Hokkaido, from March to May in 1986. Of the 5 herds, 3 had a history of occurrence of cystitis or pyelonephritis, and 2 were free of the diseases. The surface of the vulva and vaginal vestibule were wiped with sterile swabs (Culturette, Marison Scientific, Missouri). The swabs were placed in ice and taken to the laboratory for culturing within 3 hrs after sampling. They were then put into 5 ml of sterile saline and washed thoroughly. Serial 10-fold dilution was made and 0.1 ml of 10^{-1} to 10^{-3} dilution was inoculated onto the selective medium, respectively. After incubation at 37°C for 48 hrs colonies were counted and 4 to 8 colonies per specimen were examined

Table 2. Isolation of urinary corynebacteria from vulva and vaginal vestibule in 210 apparently healthy cows

Bacterial species	Isolated from	
	vulva	vaginal vestibule
<i>C. renale</i>	12(5.7%)	14(6.6%)
<i>C. pilosum</i>	34(16.2%)	34(16.2%)
Total	46(21.9%)	48(22.8%)

for the presence of urinary corynebacteria.

RESULTS

Of the 15 antibiotics tested, only FOM did not interfere with urinary corynebacteria growth, but instead inhibited growth of the bacterial species other than corynebacteria.

The strains of urinary corynebacteria showed a marked resistance to FOM; MIC of the strains to FOM ranged from 25,000 to 75,000 $\mu\text{g/ml}$. On the contrary 4 strains other than urinary corynebacteria were susceptible to FOM; the MIC ranged from 12.5 to 25.0 $\mu\text{g/ml}$ (Table 1).

Selective medium was prepared based on the results. The composition was shown as follows:

Trypticase soy agar (BBL),
FOM 200 $\mu\text{g/ml}$
Amphotericin B 2 $\mu\text{g/ml}$
Defibrinated blood of horse 5%

FOM, amphotericin B, which was used to inhibit growth of fungi, and horse blood were added to Trypticase soy agar, which was kept at 50°C after autoclaving. The mixture was poured into Petri dishes, and the plates were used for isolation of urinary corynebacteria from cows.

As shown in Table 2, *C. renale* was isolated from 12 (5.7%) samples from the vulva and 14 (6.6%) from the vaginal vestibule out of the 210 cows examined. *C.*

Table 3. Number of colonies of urinary corynebacteria isolated from the vulva and vaginal vestibule in 210 apparently healthy cows

Number of colonies	Isolated from	
	vulva	vaginal vestibule
+++ ^{a)}	23(10.9%)	10(4.7%)
++	18(8.5%)	16(7.6%)
+	5(2.3%)	22(10.5%)

a) Organisms isolated from 10^{-3} (+++), 10^{-2} (++), or 10^{-1} (+) dilution of samples.

pilosum was isolated from 34 (16.2%) samples from the vulva and vaginal vestibule, respectively. *C. cystitidis* was not isolated from the materials of apparently healthy cows.

The numbers of colonies of urinary corynebacteria detected from the materials were indicated in Table 3 as grades +, ++, and +++. In the vulva, the rate of isolation of the organisms corresponding to grade +++ in number of colonies was higher than that at grades ++ or +. In contrast, the rate of colonies in the vaginal vestibule corresponding to grade ++ was lower than that corresponding to ++ or +.

DISCUSSION

FOM is known as an antibiotic that inhibits bacterial peptidoglycan synthesis. Kahan *et al.*⁴⁾ established that FOM attacks pyruvyl transferase, which is responsible for the first step in the synthesis of bacterial cell walls.

The present studies showed that urinary corynebacteria were markedly resistant to FOM, whereas *S. aureus*, *S. agalactiae*, *B. subtilis* and *E. coli* were susceptible to it. It was speculated that there are differences in the step of synthesis of bacterial cell walls between urinary corynebacteria and bacterial species other than those examined here.

The presence of *C. pilosum* and *C. renale* in the bovine vulva was revealed by using a selective medium containing FOM, though it was necessary to distinguish whether or not the colonies grown in the selective medium were urinary corynebacteria, since the medium did not inhibit the growth of urinary corynebacteria and other species of corynebacteria. The number of organisms isolated from the vulva was greater than that in the vaginal vestibule, suggesting that the vulva is a suitable habitat for *C. pilosum* or *C. renale*, and that it may be the first entry site of the agent among the organs of the urinary tract, as discussed by Hayashi *et al.*¹⁾ The cows affected with cystitis or pyelonephritis excreted urine containing vast amounts of urinary corynebacteria on the barn floor and in the soil of the paddock. The spray of such urine probably contaminated directly or indirectly the vulva of neighboring cows.

Survival of urinary corynebacteria in soil was reported by Hayashi *et al.*²⁾ In the autoclaved soil from a paddock, *C. renale* and *C. cystitidis* survived for 56 and 63 days, respectively, and *C. pilosum* for a longer period of at least 210 days. However, in non-autoclaved soil from a paddock, *C. renale* and *C. pilosum* survived for at least 18 days, showing 10^5 bacteria, and some of the surviving bacteria could not be estimated thereafter because of the increase in the number of contaminants. We are now conducting experiments to enable survival of the agent in non-autoclaved soil, saw-dust and feces of cows using the selective medium.

The selective medium for isolation of urinary corynebacteria described here may be useful not only to clarify the distribution of the organisms in cows, but to detect the organisms in the soil and the barn floor, which are considered as an important source of contamination by the organisms.

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要 約

尿路コリネバクテリア分離用選択培地を用いての牛外陰部、膻前庭からの本菌の検出：平棟孝志・工藤竜大・菊地直哉・梁川 良¹⁾（酪農学園大学獣医学科家畜伝染病学教室，¹⁾北海道大学獣医学部家畜衛生学講座）——*C. renale*, *C. pilosum* および *C. cystitidis* はホスホマイシン（FOM）に抵抗性で、その最小発育阻止濃度（MIC）は25,000-75,000 $\mu\text{g/ml}$ であったが、*S. aureus*, *S. agalactiae*, *E. coli*, および *B. subtilis* のそれは12.5-25.0 $\mu\text{g/ml}$ であった。トリプチケースソイ寒天培地にFOMを200 $\mu\text{g/ml}$ 、抗真菌剤として、アムホテリシンBを2 $\mu\text{g/ml}$ 、馬脱線血を5%に加えた選択培地を用いて、江別市近郊で飼育されている210頭の健康牛から尿路コリネバクテリアの検出を行ったところ、12頭（5.7%）の外陰部および14頭（6.6%）の膻前庭から *C. renale* が、また、34頭（16.2%）の外陰部および膻前庭から *C. pilosum* が分離され、外陰部からの尿路コリネバクテリア菌数が、膻前庭からのそれよりも多かった。上記選択培地を用いることにより、糞尿で汚染されている牛の外陰部に *C. renale*, *C. pilosum* が多数存在することが明らかにされ、この選択培地は牛舎の床、放牧場の土などにおける尿路コリネバクテリアの分布を調べるために有用と思われた。