Susceptibilities of *Escherichia coli* from Gut of Pigs against Animal Feed Additive Colistin under Anaerobic condition

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The gut of animals which is the habitat of intestinal bacterial flora is under highly anaerobic and redox conditions, and the oxidation-reduction potential in these has been showed to be -350 mV to -50 mV^{1-2} . So, the antibacterial activity of the antibiotic in the gut of animals has to be reacted under anaerobic condition. However, procedures of the antibiotic susceptibility testing has been usually examined under the aerobic condition³. It is known a little about the relation of susceptibilities of bacteria against antibiotics under aerobic and anaerobic conditions⁴. However, it is not known about these against colistin as feed additive for livestock. Colistin is antibiotic agent belonging to the polymyxin group of which chemical structure is cyclic polypeptide, and is known to exert its potent antibiotic efficacy against the gram-negative bacteria, such as *Escherichia coli*, *Salmonella* and *Bordetella*⁵⁻⁷. Colistin is widely used in feed additive for domestic animals in Japan. Present studies were carried out to make clear the susceptibilities of several strains of *E. coli* isolated from gut of pigs against colistin under aerobic and anaerobic conditions.

Materials and Methods

Strains: One hundred and ninety-four strains of *E. coli* isolated from 126 pigs reared in Tohoku and Kanto areas of Japan were employed for this study. Standard strain for the susceptibility tests was *E. coli* strain NIHJ JC-2 maintained in our laboratory.

Isolation of *E. coli*: One gram of the rectum contents of the pigs was stirred and homogenized. Then, they were diluted gradationally from 10^{-1} to 10^{-6} with tenfold dilution. Each 0.1 ml of them was spread onto DHL agar (Nissui) plates. The plates were incubated overnight at 37°C . These screened strains were identified as *E. coli* according to the biochemical tests by Ewing and Martin⁸.

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Determination of minimal inhibitory concentration (MIC): The procedures of the Nippon Kagaku Ryoho Gakkai (Japan Society of Chemotherapy) were followed⁹⁾. Trypticase soy broth (BBL) was used as the medium for growing culture. Each culture was incubated at 37°C for 24 hours, and prepared the cell suspension with the density of 10^8 cell/ml. Determination of the susceptibility tests was done by using the medium of Heart infusion agar (Nissui). Colistin (19400 U/mg. Asahi Chemical Industry, Co., Ltd. Tokyo) was diluted gradationally from 25 $\mu\text{g/ml}$ to 0.10 $\mu\text{g/ml}$ in 10 grades. The MIC was recorded as the lowest concentration of antibiotic that completely inhibited the growth of each strains. The anaerobic culture system used were the steel wool jar¹⁰⁾.

Results and Discussion

Table 1 shows the distribution of the number of tested strains on the susceptibility to colistin under aerobic and anaerobic conditions. Under the anaerobic condition, the susceptibilities of 105 strains (54%) were elevated, those in 20 strains (10%) were reduced, and those in 69 strains (36%) were the same under the both conditions. Accordingly, the susceptibility of various strains of *E. coli* from pigs against colistin was elevated under the anaerobic condition. The susceptibility was, however, strongly related with the strain specificity.

The result of this experiment showed that the susceptibilities of *E. coli* from pigs against colistin were not always same under the aerobic and the anaerobic conditions. ARAT⁴⁾ reported that the susceptibilities of bacteria against various antibiotics were different in accordance with the difference of the test conditions. Aminoglycoside antibiotics, such as streptomycin and kanamycin, are less effective under anaerobic condition than aerobic condition. β -lactam antibiotics, such as ampicillin are, however, more effective under anaerobic condition than aerobic condition. Chloramphenicol and tetracycline have different effectiveness in accordance with the difference of the strains. Erythromycin, furazolidone and moenomycin have same effectivity both under aerobic

Table 1. Number of *E. coli* on the minimal inhibitory concentration (MIC) to colistin under aerobic and anaerobic conditions.

MIC ($\mu\text{g/ml}$)	MIC of colistin to <i>E. coli</i> strains under aerobic condition ($\mu\text{g/ml}$)							
	12.5	6.25	3.13	1.56	0.78	0.39	0.20	0.10
MIC of colistin to <i>E. coli</i> strains under anaerobic condition								
12.5	1							
6.25								
3.13								
1.56								
0.78				1	10	18		
0.39				2	48	45	2	
0.20					5	49	13	
0.10								

and anaerobic conditions. The preparation of culture media and the cultivation techniques employed in the present study were rather moderate anaerobic condition¹¹⁻¹²⁾. So the different results might be obtained with the media prepared by the pre-reduced methods¹³⁾ which have been applied for the anaerobic cultivation of *Bacteroides*, *Selenomonas* and other intestinal bacteria.

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