

# S-R Variation and Antimicrobial Susceptibility of *Escherichia coli* Isolated from Mastitic Cows, Diarrheal Pigs and Diarrheal Chickens

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## 젖소유방염, 돼지 및 닭의 설사증에서 분리한 대장균의 S-R 변이와 항균 요법제의 감수성

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### 국문요약

1992년부터 1995년까지 약 4개년 동안 전남 지역에서 사육하고 있는 가축의 유병률을 조사한 결과, 유방염으로 의심이 되는 젖소 970두 중에서 180두(18.6%)가 유방염에 이환되었으며, 돼지 2,400두 중에서 84두(3.5%)가 설사증에 이환되었고, 닭 1,010수중에서 9수(0.9%)가 설사증에 이환되었다. 대장균의 분리율을 조사한 결과, 유방염에 이환되어 있는 젖소 180두중 37두(20.6%)에서 대장균이 분리되었으며, 설사증에 이환되어 있는 돼지 84두중 51두(60.7%)에서 대장균이 분리되었고, 설사증에 이환되어 있는 닭 9수중 5수(55.6%)에서 대장균이 분리되었다. Acriflavine을 이용하여 대장균의 S-R변이를 조사한 결과, S-R변이는 mercuric chloride, cysteine, caffeine, glucose, nicotine 등의 순으로 나타났는데, 이 중 mercuric chloride가 가장 감수성이 높았다. 대장균의 항균요법제에 대한 감수성은 naxcel, linsmycin, cephalixin, gentamycin등이 높은 감수성을 나타내었는데, S-R변이 후에는 감수성이 일반적으로 저하되는 경향이였다.

**Keywords :** *Escherichia coli*, Prevalence, S-R Variation, Antimicrobial Susceptibility

## I. Introduction

*Escherichia coli* was first isolated from the feces of neonates and described by Escherich as *Bacterium coli commune*<sup>10)</sup>, and have become the most fully documented organisms currently known. Gram-negative mastitis caused by organisms such as *E. coli* is a serious disease among dairy cattle. Affected cows often develop signs of systemic illness, including depression, anorexia, and rumen stasis 8,12,24. Most investigators agree that the signs of systemic illness are due to endotoxin in the udder that cause the release of leukocytic factors<sup>27)</sup> or possibly are absorbed into the blood stream directly.<sup>41)</sup> In mid-August 1981, a student at a Missouri School for mentally retarded adults and children was hospitalized for a dysentery-like illness, and a slowly lactose-fer-

menting *E. coli* O124 was isolated from her stool specimen.<sup>15)</sup>

Although the strategy of ecological analysis was introduced in the social sciences over 40 years ago<sup>26)</sup>, specialized epidemiologic discussions have become common only in the last decade.<sup>1,14,19,23,25,28)</sup> These discussions may have been inspired by the recognition that, under special circumstances, ecologic studies can supply estimates of individual-level relative risks.<sup>3)</sup> Much of the work which has investigated the influence of the environment on the rate of new intramammary infections refers more generally to coliform organisms, however Bramley<sup>5)</sup> reported that, in a survey of a large UK dairy farm, 77% of this grouping was represented by *E. coli*.

This study describes the prevalence, isolation rate, susceptibility test and S-R variation of *E.*

*coli* isolated from mastitic cows, diarrheal pigs and diarrheal chickens by cysteine hydrochloride, glucose, caffeine sodium benzoate, mercuric chloride, and nicotine. Furthermore, this study hopes to help increase the productivity of animal farms through control of mastitic cows, diarrheal pigs and diarrheal chickens due to *E. coli*, in addition to promote the nation's health by preventing food poisoning due to *E. coli*.

## II. Materials and Methods

### 1. Bacterial strains

A total of 93 samples including raw milk from 37 mastitic cows, and feces specimens from 51 diarrheal pigs and 5 diarrheal chickens, were taken and examined for isolatable causative agents from 1992 to 1995 in Korea. The control group of *E. coli* O148:H28(ETC 81, ST<sup>+</sup>, LT<sup>+</sup>), O78:H1(ETC 82, ST<sup>+</sup>, LT<sup>+</sup>), O15:H11(ETC 83, ST<sup>+</sup>, LT<sup>+</sup>), and O1:H7(ETC 84, ST<sup>+</sup>, LT<sup>+</sup>) were supplied by Cho, M.J. from the Department of Microbiology, Kyung Sang University, Medical College, Korea.

### 2. Test for variation by chemical compounds

Tryptic soy broth(DIFCO) was prepared, consisting of 0.1 percent, 0.25 percent, 0.5 percent, and 1.0 percent of cysteine hydrochloride (Sigma); dextrose (Junsei); caffeine sodium benzoate (Sigma); mercuric chloride (Junsei); and nicotine (Fluka), respectively.

Two hours incubation in the water-bath might have made it possible to discern strong positive reactions, but that much time was not enough to enable moderate and weak reactions. Therefore, overnight setting at room temperature after the 2 hours incubation at 37°C was required to produce definite results, from the strong positives to the

weak ones.

Leaving the mixture for longer than one night did not bring about any particular change in the results.

The slide agglutination test was performed using a 0.2% acriflavine solution (Sigma) and normal saline (control) for confirmation of the test organisms, normal S-forms, intermediate variant SR-forms, and variant R-forms.<sup>1,2)</sup>

### 3. Susceptibility test

Antimicrobial susceptibility was measured using a standardized disc susceptibility test.<sup>11,12)</sup>

Antimicrobials were chosen based on their effectiveness against *E. coli* isolated from mastitic cows, diarrheal pigs and diarrheal chickens. They included cephalixin (CEP, Dae Sung, Korea), enrofloxacin (ENR, DIFCO), gentamycin (GM, BBL), linsmycin (LIN, A/S ROSCO, Denmark), naxcel (XNL, A/S ROSCO, Denmark), neomycin (NM, A/S ROSCO, Denmark) and norfloxacin (NOR, DIFCO).

## III. Results

The prevalence was observed in 180 cases of mastitis among 970 dairy cattle (18.6%), 84 cases of diarrhea among 2,400 pigs (3.5%) and 9 cases of diarrhea among 1,010 chickens (0.9%). *E. coli* was isolated from 37 of the 180 mastitic cows (20.6%), 51 of the 84 diarrheal pigs (60.7%) and 5 of the 9 diarrheal chickens (55.6%) as shown in Table 1.

Table 2 shows the results of the agglutination of *E. coli* in the presence of various concentration of cysteine, glucose, caffeine, mercuric chloride and nicotine. In general, R and SR variants by 0.2% acriflavine solution were agglutinated of

**Table 1.** The outbreaks patterns of mastitic cows, diarrheal pigs and diarrheal chickens.

Cases	Total number of animals in survey	Number of cases(%)	Number of isolated <i>E. coli</i> strains(%)
Mastitic cows	970	180(18.6)	37(20.6)
Diarrheal pigs	2,400	84( 3.5)	51(60.7)
Diarrheal chickens	1,010	9( 0.9)	5(55.6)
Total	4,380	273( 6.2)	93(34.1)

⊕ Prevalence

**Table 2.** Influence on the S-R variation of *Escherichia coli* by chemical compounds.

Cases (strains)	Cysteine			Glucose						Caffeine											
	Chemical compounds			0.1%		0.5%		1.0%		0.1%		0.5%		1.0%		0.1%		0.25%			
	Sa	Ac	Sa	Sa	Ac	Sa	Ac	Sa	Ac	Sa	Ac	Sa	Ac	Sa	Ac	Sa	Ac	Sa	Ac		
Control (EC 82, ST, LT) S SR R	-	-	-	-	-	-	±	-	-	-	-	-	-	-	-	-	-	-	-		
Mastitic Cows (n=10 strains) S SR R	10	6	10	6	10	6	10	6	10	8	10	9	10	9	10	10	10	9	10		
Diarrheal pigs (n=3 strains) S SR R	3	2	3	2	3	1	3	2	3	2	3	3	3	3	3	3	3	3	3		
Diarrheal chickens (n=3 strains) S SR R	3	3	3	1	2	1	3	2	3	2	3	3	3	3	3	3	3	3	3		
Cases (strains)	Caffeine			Mercuric chloride						Nicotine											
	Chemical compounds			0.5%		1.0%		0.1%		0.25%		0.5%		1.0%		0.1%		0.25%		0.5%	
	Sa	Ac	Sa	Sa	Ac	Sa	Ac	Sa	Ac	Sa	Ac	Sa	Ac	Sa	Ac	Sa	Ac	Sa	Ac		
Control (EC 82, ST, LT) S SR R	-	-	-	±	++	++	++	++	++	++	++	++	++	++	++	±	-	-	-		
Mastitic Cows (n=10 strains) S SR R	10	3	10	10	10	9	10	5	7	10	10	10	9	10	9	10	9	10	9		
Diarrheal pigs (n=3 strains) S SR R	3	3	3	3	3	1	3	1	3	1	3	3	3	3	3	3	3	3	3		
Diarrheal chickens (n=3 strains) S SR R	3	3	3	3	3	1	3	1	3	1	3	3	3	3	3	3	3	3	3		

Remark : 1) Sa : 0.9% saline solution, and Ac : 0.2% acriflavine solution. 2) S ( ) : normal, SR (±, +) : intermediate variant, and R(+++, +++) : variant.

**Table 3.** Antimicrobial susceptibility of S-R variation of *Escherichia coli* by chemical compounds.

Antimicrobials	Disc potency	Diameter of susceptible zone (mm more)	No. of susceptible strains before variant (n=93), (%)	No. of susceptible strains after variant(%)				
				Cysteien (n=27)	Glucose (n=6)	Caffeine (n=9)	Mercuric chloride (n=49)	Nicotine (n=4)
Cephalexin(CEP)	30 mcg	18	86(92.5)	24(88.9)	6(100.0)	8(88.9)	45(91.8)	4(100.0)
Enrofloxacin(ENR)	10 mcg	21	71(76.3)	20(74.1)	4(66.7)	6(66.7)	37(75.5)	3(75.0)
Gentamycin(GM)	10 mcg	18	86(92.5)	24(88.9)	5(83.3)	8(88.9)	44(90.0)	4(100.0)
Linsmycin(LIN)	19 mcg	23	87(93.5)	25(92.6)	6(100.0)	9(100.0)	46(93.9)	4(100.0)
Naxcel(XNL)	30 mcg	24	88(94.6)	26(96.3)	6(100.0)	9(100.0)	46(93.9)	4(100.0)
Neomycin(NM)	30 mcg	17	79(84.9)	22(81.5)	5(83.3)	7(77.8)	41(83.7)	3(75.0)
Norfloxacin(NOR)	10 mcg	17	72(77.4)	21(77.8)	4(66.7)	6(66.7)	37(75.5)	3(75.0)

mercuric chloride, cysteine, caffeine, glucose and nicotine, in that order. Mercuric chloride ions were most sensitive in differentiating the variation degree of *E. coli*.

The selection of pure S-form from doubtful S-form is possible only through the tube agglutination technique with mercuric chloride ions, even through the slide agglutination test with acriflavine may provide the rough difference between S and SR. Besides, the acriflavine reaction on the slide agglutination test was influenced by the high concentration of chemical compounds which were stronger than low concentration generally. The titers of this reaction were almost parallel with degree of acriflavine reaction on the slide.

*E. coli* was observed to be most susceptible to XNL (94.6%), LIN (93.5%), and both CEP (92.5%) and GM (92.5%) as shown in Table 3. In after S-R variation by cysteine, glucose, caffeine, mercuric chloride and nicotine, the antimicrobial susceptibility of *E. coli* appeared to decrease somewhat (Table 3).

#### IV. Discussion

*E. coli* is considered to be a part of the normal microflora of the intestinal tract of human and most other warm-blooded animals. It is generally present in human and animal feces, and also in milk from mastitic animals. Most strains of *E. coli* are harmless, but a few are pathogenic.<sup>6,16,17)</sup>

The objective of this study was to define influence of antimicrobial susceptibility in after S-R variation of *E. coli* isolated from mastitic cows,

diarrheal pigs and diarrheal chickens by cysteine, glucose, caffeine, mercuric chloride and nicotine.

Isolation studies have found *E. coli* in 16.6% of the milk from mastitic cows in rural areas, and in 23.9% of the milk from mastitic cows in urban areas, according to Jung.<sup>16,17)</sup>

Yoon *et al.*<sup>20)</sup> reported the incidence of diarrhea in 20.9% of the piglets in his study and typical isolation rates of *E. coli* from diarrheal piglets can run as high as 144.5%, 137.2%, and 131% according to Kim<sup>19,20)</sup>, Kim and Jung<sup>18)</sup>, respectively.

This study found 180 cases of mastitis among the 970 dairy cattle surveyed (18.6%), 84 cases of diarrhea among 2,400 pigs (3.5%) and 9 cases of diarrhea among 1,010 chickens (0.9%). *E. coli* was isolated from the milk of 37 of the 180 mastitic cows(20.6%), 51 of the 84 diarrheal pigs (60.7%) and 5 of the 9 diarrheal chickens (55.6%). Differences were due to year, area, and surveyed animal. The isolation rate was calculated on the basis of strains per pigs and chickens in these results.

Ahn<sup>12)</sup> reported that the titers of acriflavine reaction were almost parallel with the degree of acriflavine reaction on slides, and that Pb ions are most sensitive in differentiating the variation degree of *Shigella flexneri*. According to Youmans<sup>30)</sup>, many of the properties of small-colony variants can be accounted for by an over-all lowering of the metabolic rate.

Clowes and Rowley<sup>7)</sup> suggested that this explanation might account for the isolation of many of these variants from toxic environments, and proposed that the inhibitor (antibiotics, metal

ions etc.) permeates the variants at a lower rate, thus permitting their survival for a longer time than the corresponding, normally metabolizing, organisms. They have further suggested that the general lowering of the metabolic rate might be due to a decrease in cell wall permeability.

In general, R and SR variants by 0.2% acriflavine solution were agglutinated by mercuric chloride, cysteine, caffeine, glucose and nicotine, in order of effectiveness. Mercuric chloride ions were especially sensitive in differentiating the variation degree of *E. coli*. The titers of this reaction were almost parallel with the degree of acriflavine reaction on slides in these results, which is in agreement with the findings of Ahn<sup>1,2)</sup>

*E. coli* was observed to be most susceptible to ENR (81.3%), GM (68.8%), NM (62.5%), and KM (59.4%) by Jung.<sup>17)</sup> AN, amoxicillin/clavulanic acid, ceftiofur, colistin and nitrofurantoin showed of 100% effectiveness against all isolates, and many of the isolates were also highly sensitive to CF, GM, NM and NOR. The bacteria showed a complete or substantial level of resistance to LM, clindamycin, tiamulin, tylosin, EM, chlortetracycline/tiamulin, TC, triple sulfur, PP and SM by Kim.<sup>20)</sup> The effectiveness of antimicrobial drugs against *E. coli* were tested, and GM was found to be the most effective (96.1%), whereas the susceptibilities to the other drugs were below 50% by Kim.<sup>20)</sup> *E. coli* has also been observed to be highly susceptible to XNL (94.6%), LIN (93.5%), and CEP (92.5%), as well as GM (also 92.5%). After S-R variation by cysteine, glucose, caffeine, mercuric chloride and nicotine, these figures decrease somewhat.

The same antimicrobials, are studied with similar results by Jung<sup>16,17)</sup>, Kim<sup>19)</sup> and Kim.<sup>20)</sup>

The antimicrobial susceptibility was decreased current due to antimicrobials misused and abused, generally. Although antimicrobials and growth hormone can be misused when overfed to young animals to promote growth, the also to treat and prevent animal disease. Their misuse or abuse is thus an important issue for human health.

This study is to suggest that treat and prevent animal disease, to help reduce the accuracy of food-borne diseases and increase the productivity of animal farming.

## V. Conclusion

The prevalence was observed in 180 cases of mastitis (18.6%) among 970 dairy cattle, 84 cases of diarrhea (3.5%) among 2,400 pigs and 9 cases of diarrhea (0.9%) among 1,010 chickens. *E. coli* was isolated from 37 cases (20.6%) among the 180 mastitic cows, 51 cases (60.7%) among the 84 diarrheal pigs and 5 cases (55.6%) among the 9 diarrheal chickens.

Varied R and intermediate varied SR of *E. coli* by acriflavine were agglutinated by mercuric chloride, cysteine, caffeine, glucose and nicotine, in that order. Mercuric chloride ions were most sensitive in differentiating the variation degree of *E. coli*. *E. coli* was observed to be highly susceptible to naxcel, linsmycin, cephalixin and gentamycin, but in after, normal S-varied R of *E. coli* by cysteine, glucose, caffeine, mercuric chloride and nicotine, appeared to decrease somewhat.

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