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The *in vitro* Drug Resistance of *Escherichia coli* Isolated from Piglets, Calves, Lamb and Goats with Diarrhoea

Bong Hwan Kim, D.V.M., M.S., Ph.D., Jai Chin Rhee, D.V.M. and Dong Sung Kim, D.V.M., Ph.D.

Institute of Veterinary Research, Office of Rural Development

Introduction

The association of episome mediated transfer of drug resistance[13,16,17,21,22] and the immergence of resistant mutants[11] are generally attributed to a constantly changing pattern in susceptibility of organisms to the antimicrobial drugs in use. The excessive use of antibiotics in livestock, particularly continuous administration of sublethal doses in the form of feed supplements, are also responsible for the free dissemination of multiple drug resistance among bacteria [12,16]. In recent years, an increased incidence of antibiotic resistant strains of *Escherichia coli* has been reported in domestic animals in many part to the world[1,5-10,14,15,19]. Moreover, the resistance observed was commonly multiple in nature, thus complicating therapy and prevention of many important bacterial diseases, in particular neonatal enteric infections, of domestic animals[18,23].

The most fundamental principle in the treatment of any infection is that therapy should be determined by isolation and determination of the causative organisms and *in vitro* testing of thir sensitivity to antimicrobial drugs. However, under most clinical situations, rule of thumb treatment is instituted without proper microbiological laboratory investigation.

This study was designed to obtain an information on the prevalence of the *in vitro* drug resistance and drug resistance patterns of *E. coli* of clinical importance isolated from young animals with diarrhoea.

Materials and Methods

A total of 126 *E. coli* strains isolated from scouring infant animals varying in age from a few days to 4 weeks during the year 1978 were tested for sensitivity to 15 antimicrobial drugs; 62 strains were from piglets, 32 from calves and the remaining 32 from goats and lamb. All the *E. coli* strains from scouring piglets were isolated from the upper part of small intestine. The strains from calves, lamb and goats were recovered from rectal swabs taken from clinical cases of diarrhoea.

Using aseptic technique, intestinal contents or rectal swabs were streaked on to 5% sheep blood agar and Mac Conkey agar plates. After overnight incubation at 37°C, where there was a predominance of lactose fermenting colonies, 2 to 3 colonies were selected and their identification as *E coli* was confirmed by the standard biochemical tests[3].

For sensitivity testing, Muller-Hinton agar(Difco) was used throughout the work without supplementation. An overnight nutrient broth culture of the test organism was diluted to one in 1,000 with sterile physiological saline. Two mililitters of diluted inoculum was flooded onto dried Muller-Hinton agar plates of 9 cm diameter and distributed evenly over the agar surface by gently rocking the plate. The plates were tipped and the excess fluid was allowed to drain and decanted. The agar surface was then allowed to dry for 15 minutes at 37°C. Upon drying, the sensitivity discs were applied to the agar surface and were lightly pressed against the agar with forceps. After application of 8 discs on one plate, the plates were allowed to stand st room temperature (18-25°C) for 30 minutes. The plates were then incubated aerobically at 37°C for 18 hours. The zones of bacterial inhibition surrounding the discs were measured to the nearest milimeter and inter-

preted by the zone interpreting chart of Bryant[2]. The antimicrobial agents used with the respective disc potencies are listed in Table 1.

Results

The prevalence of antimicrobial drug resistance to 15 agents of 126 strains of *E. coli* recovered from scouring livestock in the republic during 1978 is given in Table 2. It can be seen from the table that all the strains isolated from piglets were resistant to erythromycin and lincomycin while none of them were resistant to gentamicin. The majority of the pig isolates were resistant in order of prevalence to novobiocin (98.4%), penicillin(96.%), ampicillin (95.2%). tetracycline (93.5%), streptomycin (77.4%), and sulfaisodimidin(64.5%), while only 3.2% of them were resistant to colistin. The percentage of pig isolates resistant to kanamycin, neomycin, chloramphenicol, trimethoprim with sulfamethoxazole and cephalosporin were 6.5%, 8.1%, 24.2%, 35.5%, and 50%, respectively. All the calf isolates were resistant to erythromycin and lincomycin as pig isolates and also to novobiocin and penicillin, while all of them were shown to be sensitive to gentamicin. The majoity of calf strains were resitant to cefalosporin, tetracycline and streptomycin, their resistance rates being 96.9%, 90.0% and 87.5%, respectively, As much as 62.5% of calf isolates were resistant to neomycin and 40.6% of them resistant to chloramphenicol and kanamycin. Goat isolates were tested against 14 antimicrobial drugs and all of them were resistant to cephalosporin, erythromycin, lincomycin, novobiocin and penicillin, while all of them were sensitive not only to gentamicin but to colistin. Only 3.1% of goat isolates were resistant to chloramphenicol and 9.4% of them were resistant to both kanamycin and neomycin. Their resistance to tetracycline and ampicillin was domparable to those of pig and calf isolates but the resistance to streptomycin and sulfaisodimidin was significantly low when compared with those of pig and calf strains.

The broadest spectrum of inhibitory activity against all *E. coli* strains used is demonstrated by gentamicin, followed by colistin, kanamycin, neomycin and chloramphenicol. The range of resistance of the remaining 10 drugs varies from 35.1% for trimethoprim with sulfamethoxazole to 100% for erythromycin and lincomycin.

The types of resistance patterns against 10 drugs in common use such as ampicillin, cefalosporin, chloramphenicol, colistin, gentamicin, kanamycin, neomycin, streptomycin, tetracycline and sulfonamides are shown in Table 3. All the strains were resistant to more than two drugs and 33 different patterns of multiple resistance were noted among 126 strains. The most common multiple resistance patterns were AM, CE, SM, TC, & SU pattern(18.3%) and AM, CE & TC pattern (11.9%). The most prevalent multiple resistance pattern of goat isolates was three-fold pattern of AM, CE & TC while those of pig or calf strains were five-fold pattern of AM, CE, SM TC & SU and eight-fold pattern of AM, CE, CP, KM, NM, TC & SU, respectively. In goat and lamb strains, some 40.6% of them were resistant to 3 drugs and 25% to 2 drugs indicating that much low resistance was occurred in goat and lamb isolates in contrast to those of pig and calves.

Table 1. Antimicrobial Discs Used for Diffusion Sensitivity Test

Antimicrobial Drugs	Concentration
Ampicillin (AM)*	10μg
Bacitracin (BA)*	10iu
Cephalosporin (CE)*	30μg
Chloramphenicol (CP)**	30μg
Colistin (CL)*	30μg
Erythromycin (EM)**	15μg
Gentamicin (GM)*	30μg
Kanamycin (KM)**	30μg
Lincomycin (LM)*	15μg
Neomycin (NM)**	30μg
Novobiocin (NB)**	30μg
Penicillin (PC)**	10iu
Streptomycin (SM)**	10μg
Tetracycline (TC)**	30μg
Sulfaisodimidin (SU)*	2.5mg
Sulfamethoxazole-trimethoprim (SXT) @	25μg

*: AB Biodisk, **: Difco multodisk, @: Oxoid
Letters in the parentheses indicate the abbreviation used for the drugs.

Table 2. Drug Resistance of *Escherichia coli* Isolated from Piglets, Calves, Goats and Lamb with Diarrhoea

Drugs	Drug Resistant Strains(%)			
	Piglets (62)	Calves (32)	Goats & Lamb (32)	Total (126)
Ampicillin	95.2	68.8	78.1	84.1
Cephalosporin	50	96.9	100	74.6
Chloramphenicol	24.2	40.6	3.1	23.0
Colistin	3.2	12.5	0	4.8
Erythromycin	100	100	100	100
Gentamicin	0	0	0	0
Kanamycin	6.5	40.6	9.4	15.9
Lincomycin	100	100	100	100
Neomycin	8.1	62.5	9.4	22.2
Novobiocin	98.4	100	100	99.2
Penicillin	96.8	100	100	98.4
Streptomycin	77.4	87.5	28.1	67.5
Tetracycline	93.5	90.6	93.8	92.9
Sulfaisodimidin	64.5	56.3	28.1	53.2
Sulfamethoxazoletrimethoprim	35.5	31.3	—	35.1

Figures in the parentheses indicate the number of strains tested.

Table 3. Distribution of Drug Resistance Patterns of 126 Strains of *Escherichia coil* Isolated from Piglets, Calves, Goats and Lamb with Diarrhoea

Multiple Drug Resistant Patterns*	Strains with This Pattern	Swine(62) %	Bovine(32) %	Ovine(32) %	Total(126) %
AM, CE, CP, KM, NM, SM, TC, SU	9	0	28.1	0	7.1
AM, CE, KM, NM, SM, TC, SU	5	3.2	0	9.4	4.0
AM, CE, CP, SM, TC, SU	8	11.3	3.1	0	6.3
AM, CE, SM, TC, SU	23	24.2	12.5	12.5	18.3
AM, CP, SM, TC, SU	6	9.7	0	0	4.8
AM, SM, TC, SU	9	9.7	9.4	0	7.1
AM, CE, SM, TC	4	4.8	0	3.1	3.2
AM, CE, TC	15	3.2	0	40.6	11.9
AM, SM, TC	4	6.5	0	0	3.2
AM, TC	4	6.5	0	0	3.2
CE, TC	8	0	6.3	18.9	6.3

* The most frequent of 33 patterns found.
Figures in the parentheses indicate total number of strains tested from each group.

Discussion

Diarrhoea syndrome attributed to *E. coli* infection in neonatals has become one of the most troublesome diseases of livestock in Korea[9]. The economic losses incurred by the condition has gradually been increasing as no adequate control measures are available. To minimize the losses, antimicrobial agents are often used without any laboratory assessment in the prevention and treatment of the infection, resulting in the emergence of antibiotic resistant strains[12,17]. The fact further complicates the treatment of the disease and will in the long run lead to a change in the bacterial ecology which may prove unfavorable

Table 4. Multiple Drug Resistance of *Eschericha coli* Isolated from Piglets with Diarrhoea

Resistant to	Number Resistant	% Resistant	% Accumulated
7 Drugs	2	3.2	3.2
6 Drugs	9	14.5	17.7
5 Drugs	22	35.5	53.2
4 Drugs	12	19.4	72.6
3 Drugs	8	12.9	85.5
2 Drugs	4	6.5	92.0
1 Drug	4	6.5	98.5
0	1	1.6	100.1

Table 5. Multiple Drug Resistance of *Escherichia coli* Isolated from Calves with Diarrhoea

Resistant to	Number Resistant	% Resistant	% accumulated
9 Drugs	1	3.1	3.1
8 Drugs	9	28.1	31.2
7 Drugs	2	6.3	37.5
6 Drugs	3	9.4	46.9
5 Drugs	6	18.8	65.7
4 Drugs	4	12.5	78.2
3 Drugs	3	9.4	87.6
2 Drugs	4	12.5	100.1

Table 6. Multiple Drug Resistance of *Escherichia coli* Isolated from Lamb and Goats with Diarrhoea

Resistant to	Number Resistant	% Resistant	% Accumulated
7 Drugs	3	9.4	9.4
5 Drugs	4	12.5	21.9
4 Drugs	4	12.5	34.4
3 Drugs	13	40.6	75.0
2 Drugs	8	25.0	100.0

to human being as well. In this respect, the continuous monitoring for drug resistance of *E. coli* isolated from animals would constitute an important bases for assessing future trends in the antimicrobial resistance pattern.

The present finding of a higher incidence of antibiotic resistance in *E. coli* isolated from neonates with diarrhoea is generally in agreement with the previous work on *E. coli* of piglet origin[9]. Similar findings have also been observed by other workers from many part of the world[5-8,10,14,15,19]. It is worthy to note that *E. coli* from ovine animal is much more susceptible to such antibiotics as chlora mphenicol and streptomycin than pig and calf isolates. In general, calf isolates are more resistant to chloramphenicol, kanamycin and neomycin. This may be a refraction of the difference in the frequency of the use of antimicrobial drugs in different species of animals. Excessive use of antibiotics in the treatment of animal diseases, in particular cattle diseases, and large scale administration in the form of feed additives in pig industry could be responsible for free desemination of multiple drug resistance among *E. coli* isolates from these animals. All the strains sensitive to gentamicin and the majority of them were susceptible to colistin. Ensley and others[4] reported that gentamicin is drug of choice for the prevention and treatment of colibacillosis in piglets and Kim[9] also found that all the *E. coli* strains isolated from cases of colibacillosis in piglets in Korea were susceptible to gentamicin. Colistin was reported to be effective for the control of *E. coli* infection[9].

The high frequency of multiply resistant *E. coli* recovered from calves and piglets is worth attention, although similar findings have been reported by Walton[20], especially in view of the problems that have arisen in the treatment of colibacillosis in these animals.

Conclusion

Resistance to fifteen antimicrobial drugs was determined by the use of disc diffusion technique in 126 strains of *Escherichia coli* isolated from piglets, calves, goats and lamb with diarrhoea during the year 1978. All the strains used was resistant to erythromycin and lincomycin while none of them were resistant to gentamicin. An assessment of the extent of resistance to the remaining 12 drugs revealed a gradient of the following order: novobiocin 99.2%, penicillin 98.4%, tetracycline 92.9%, ampicillin 84.1%, cephalosporin 74.6%, streptomycin 67.5%, sulfaisodimidin 53.2%, trimethoprim with sulfamethoxazole 35.1%, chloramphenicol 23.0%, neomycin 22.2%, kanamycin 15.9% and colistin

4.8%. A high prevalence of multiple drug resistance was observed and the two most common resistant patterns among 33 patterns noted were AM, CE, SM, TC & SU pattern(18.3%) and AM, CE & TC pattern (11.9%).

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대장균 설사증에 이환된 소, 돼지, 양에서 분리한 대장균의 약제감수성

김 봉 환·이 재 진·김 동 성
가축위생연구소

초 록

대장균 설사증에 걸린 어린 돼지, 송아지, 어린 양에서 분리한 대장균 126주의 항생제와 화학요법제(15종)에 대한 감수성을 disc diffusion technique로 조사한 성적은 다음과 같다.

1. 어린 돼지에서 분리한 62주의 대장균은 gentamicin(GM)에 100%, colistin(CL)에 96.8%, kanamycin(KM)에 93.5%, neomycin(NM)에는 91.9%가 감수성을 가지고 있었으나 ampicillin(AM), erythromycin(EM), lincomycin(LM), novobiocin(NB), penicillin(PC), streptomycin(SM), tetracycline(TC), sulfaisodimidin(SU)에는 내성을 가지고 있었다. chloramphenicol(CP), sulfamethoxazole-trimethoprim(SXT), cephalosporin(CE)에는 각각 75.8%, 64.5%, 50%가 감수성이 있었다.

2. 송아지에서 분리한 32주의 대장균은 GM에 100%, CL에는 87.5%, SXT에는 66.7%가 감수성이 있었으나 CP와 KM에는 각각 40.6%, SU에는 56.2% NM에는 62.5%, SM에는 87.5%가 내성을 가지고 있었다. EM, LM, NB, PC에는 전혀 감수성이 없었으며 AM, SM, TC에도 고도의 내성을 가지고 있었다.

3. 어린 양에서 분리한 32주의 대장균은 GM과CL에 100%, CP에 96.9%, KM과 NM에 90.6%가 감수성이 있었다. SM과 SU에도 71.9%나 감수성이 있었으나 CE, EM, LM, PC, TC에는 대부분 내성을 가지고 있었다. AM에는 21.9%가 감수성이 있었다.

4. 2종류 이상의 약제에 내성을 가진 대장균의 AM, CE, CP, CL, GM, KM, NM, SM, TC, SU등 10종의 약제에 대한 multiple drug resistance pattern(MDRP)을 조사한 바 돼지 유래 약제내성 대장균의 MDRP는 18가지였으며 이중 가장 빈도가 높은 것은 AM, CE, SM, TC, SU 내성형으로 전체의 24.2%나 되었다. 송아지 유래 약제내성 대장균의 MDRP는 17가지였으며, AM, CE, CP, KM, NM, SM, TC, SU 내성형이 28.1%로 가장 빈도가 높았다. 반면에 어린 양에서 분리한 대장균의 MDRP는 9가지였으며 AM, CE, SU의 3약제 내성형이 40.6%로 가장 많았다.