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Resistance of gram-negative bacteria to antibiotics in large calf agglomerations

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In memoriam Oscar Felsenfeld

Summary

The antibiotic resistance of *E. coli*, *Citrobacter*, *Enterobacter-Klebsiella* and *Pseudomonas aeruginosa* strains isolated from calves was tested. A high proportion of multiresistance was found even in *E. coli* strains isolated from newborn calves. Gram-negative bacteria isolated from animals in three large calfhouses were almost 100% resistant to ampicillin, tetracyclines and sulphonamides. Multiresistance was general and varied from 5 to 12 antibiotics among different strains. Initial high sensitivity to antibiotics which had never been used before was observed. Antibiotic resistance rapidly increased after use started. The usefulness of antibiotics in *E. coli* induced diarrhea is questioned and oral rehydration is appraised.

Key words: calf diarrhea; *E. coli*; resistance; antibiotics.

Diarrhea in the youngest age group is a serious problem not only for human neonates and infants. In modern husbandry with great agglomerations of young animals diarrhea of bacterial and viral origin is responsible for considerable economic losses. Among enterobacteria enterotoxin producing strains of *E. coli* (Rašková et al., 1977) are the most common source of bacterial enteric infection.

Numerous human studies deal with the challenging increase of antibiotic resistance of enterobacteria. The health hazard due to this phenomena is of great concern in human medicine (WHO, 1978).

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Table 1. Cowhouses and calfhouses

Place	Number of housed cows	Housing of newborn calves	Hygienic level	Antibiotic administration
B	174	individual cages	average	massive
T	174	individual cages	poor	massive
S	96	in small groups	poor	frequent
C	80	in small groups	poor	frequent

Place	Number of housed calves	Housing	Hygienic level	Antibiotic administration
S	2000	in large groups agglomerated from 30 different cowhouses. Age 14–21 days	average	massive
T	300	two weeks individual, then agglomerated in groups from 15 cowhouses. Age 14–21 days	very poor	massive
V	2000	two weeks individual, then in groups agglomerated from over 30 cowhouses. Age 14–21 days	average	massive

In husbandry antibiotics are indiscriminately used for prevention and treatment of enteric infections. In spite of warnings they are still widely administered as growth promoting feed additives (Jukes, 1972). Although *E. coli* is the predominant bacterial cause of diarrhea in calves and pigs only few studies are concerned with animal strains (WHO, 1978).

Diarrhea in large agglomerations has been studied by our group. This gave the opportunity to collect material on antibiotic-resistant enterobacteria in field conditions.

Material and methods

The study consisted of several parts:

1. Successive testing of antibiotic-resistant *E. coli* strains from newborn calves in cowhouse B. During four months rectal swabs were taken from calves one day after delivery (between March and June 180 calves were born). The epizootological situation in the cowhouse was very bad. Chloramphenicol, tetracyclines and neomycin were used indiscriminately. Furadantin was introduced for the first time while the investigation was in progress. A mastitis program was also started. Streptomycin was administered into the udder of dry cows.
2. The antibiotic resistance of *E. coli* strains was tested in three calfhouses. The calves were agglomerated at the age of 10–21 days. Rectal swabs were taken within the first week after arrival. In calfhouse S. *Klebsiella-Enterobacter*, *Citrobacter* and *Pseudomonas aeruginosa* became prevalent. The antibiotic resistance to these organisms was also tested.

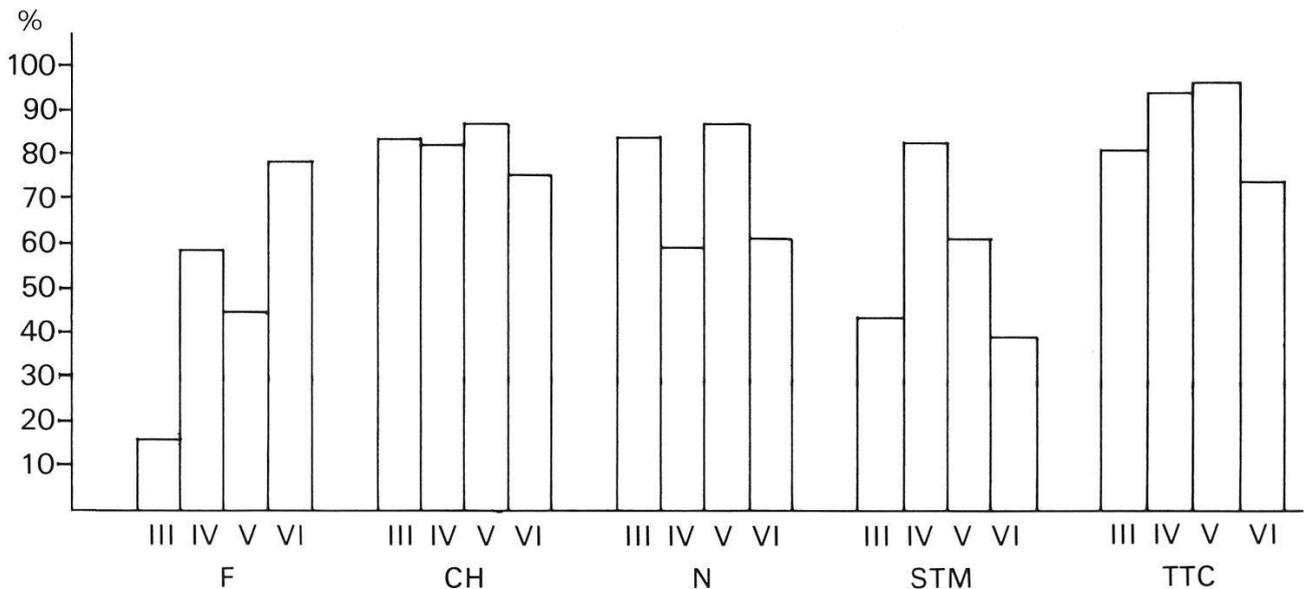


Fig. 1. Resistance of *E. coli* strains antibiotics. Cultivation from rectal swabs 24 h after birth from calves successively born in the same cowhouse. III = March, IV = April, V = May, VI = June F = furadantin, CH = chloramphenicol, N = neomycin, STM = streptomycin, TTC = tetracycline. Ordinate: Antibiotic resistance in percent.

3. To discriminate between the antibiotic resistance in domestic stables and the consequences of agglomeration a special experiment was performed.

Prior to transport to calfhouse T rectal swabs were taken from calves in three cowhouses of different size.

Basic data about the different stables are presented in Table 1. Cultivation and differentiation was performed according to standard procedures (Ewing, 1973). Resistance to antibiotics was established by the method of Ericsson et al. (1971) using internal reference standards. The disks (Messrs Lachema, Brno) contained the following drugs: novobicin (NOV), furadantin (FUR), kanamycin (KAN), nalidixic acid (NAL), sulphametoxydin (SU), cephalosporin (CEPH), chloramphenicol (CHP), colistin (COL), neomycin (N), streptomycin (STM) and tetracycline (TTC).

In the vast majority of cases resistance was complete, i.e. no inhibition zone was found. The diameter of the zone of inhibition of the partly resistant strains measured only a few mm.

Results

1. Study in the cowhouse B

Very high antibiotic resistance of *E. coli* strains from one-day-old calves was found. None of these animals had received any antibiotic. In this establishment chloramphenicol, tetracyclines and neomycin were constantly and widely used. Furadantin was introduced only after the start of the study. And so was streptomycin for local mastitis treatment.

Fig. 1 demonstrates the very high antibiotic resistance immediately after birth to tetracyclines, chloramphenicol and neomycin. After their introduction the resistance to furadantin and streptomycin increased rapidly.

Table 2. Resistance of *E. coli* strains isolated from rectal swabs of calves in three calfhouses (S, T and V)

Locality	Ampicillin		Furadantin		Kanamycin		Nalidixic acid		Novobiocin		Sulpha-metoxydin							
	R	PR	S	R	PR	S	R	PR	S	R	PR	S						
S	91	9	0	20	52	28	25	5	70	12	10	78	100	0	0	100	-	0
T	100	0	0	10	52	38	25	0	75	3	78	19	100	0	0	96	4	0
V	100	0	0	0	63	37	56	7	37	0	62	38	100	0	0	87	13	0

Locality	Cephalo-sporin		Chlor-amphenicol		Colistin		Neomycin		Streptomycin		Tetracycline							
	R	PR	S	R	PR	S	R	PR	S	R	PR	S						
S	37	63	0	63	1	36	14	86	0	16	16	68	86	10	4	94	3	3
T	67	33	0	40	0	60	4	96	0	20	0	80	88	4	8	90	5	5
V	75	25	0	94	0	6	0	91	9	67	0	33	69	24	7	100	0	0

R = complete resistance, PR = resistance in part, S = sensitivity.

Table 3. Antibiotic resistance of *E. coli* strains from calves in cowhouses T, S and C

Origin *	AMP	FUR	KAN	NAL	NOV	SU	CEPH	CHP	COL	N	STM	TTC	
T	33	100	66.6	30.3	66.6	100	100	94	42.4	66.6	24	94	97
S	9	100	33	44	0	100	100	100	55	11	44	78	78
C	5	100	20	0	40	100	100	100	0	20	0	40	100

Rectal swabs were taken before transport from the maternal cowhouses. AMP = ampicillin, FUR = furadantin, KAN = kanamycin, NAL = nalidixic acid, NOV = novobiocin, SU = sulphamethoxydin, CEPH = cephalosporin, CHP = chloramphenicol, COL = colistin, N = neomycin, STM = streptomycin, TTC = tetracycline. * = number of animals.

Table 4. Analysis of multiresistance of *E. coli* strains to antibiotics in percent

	0	1	2	3	4	5	6	7	8	9	10	11	12
S1 N = 80	0	0	0	1	0	5	13	16	22	16	12	10	5
S2 N = 43	0	0	0	0	0	0	7	11	4	19	35	17	7
V N = 16	0	0	0	6	0	0	0	12.5	12.5	12.5	18.5	38	0
T N = 52	0	0	0	0	0	6	4	17.5	19.2	36.5	5.7	9.5	0
Average	0	0	0	1.7	0	2.7	6	14.2	14.4	21	17.8	18.6	3

0–12 = Resistance to 0–12 antibiotics; S1 = First sampling at calfhouse S; S2 = Second sampling one month later; N = number of calves from which sample was taken.

2. Calfhouses S, T and V

The results of the studies in calfhouses S, T and V are presented together. The antibiotic resistance of *E. coli* from the three calfhouses is summarized in Table 2. From the data it is evident that almost all investigated strains from the three calfhouses were resistant to the most frequently administered antibiotics, i.e. tetracyclines, streptomycin and sulphonamides.

There were variations in the use of other antibiotics in the calfhouses. Neomycin was scarcely administered in the calfhouses S and T. On the other hand neomycin was given to all animals for prophylaxis of diarrhea in V. The comparatively low resistance of *E. coli* strains from S and T and the high resistance of strains from V is in good agreement with the difference of administration. These differences were even more significant when sensitivity tests were performed in several cowhouses. The size of the cowhouse and the local practices of antibiotic administration are reflected in differences of antibiotic resistance as demonstrated in Table 3. Calves born in the smallest cowhouse were completely resistant only to tetracyclines and sulphamethoxydin, while the resistance to other antibiotics was much lower than in large agglomerations.

The multiresistance of one batch of *E. coli* strains from the three calfhouses was analyzed. After 4 weeks the investigation was repeated in S. The results are shown in Table 4. The numbers 0–12 indicate to how many antibiotics the *E.*

Table 5. Comparison of resistance of *E. coli*, *Citrobacter* and *Enterobacter-Klebsiella* (calthouse S)

	<i>E. coli</i>	<i>Citrobacter</i>	<i>Enterobacter-Klebsiella</i>
Ampicillin	94	98	100
Furadantin	69	27	36
Kanamycin	33	2.5	35
Nalidixic	24	38	43
Novobiocin	100	100	100
Sulphametoxydin	100	77	98
Cephalosporin	90	93	78
Chloramphenicol	52	32	80
Colistin	72	79	90
Neomycin	38	2.5	37
Streptomycin	96	57	98
Tetracycline	96	86	76

Resistance to antibiotics expressed in percent.

coli strains were resistant. For clearer understanding the data were transformed to percentage which caused small inaccuracies. The multiresistance was extremely high. Most strains were resistant to 7–12 of the 12 assayed antibiotics. In the calthouse S after 4 weeks of agglomeration the *E. coli* strains isolated from the same animals showed further increase in multiresistance. The S calthouse agglomerated animals instantly upon arrival. A very high morbidity and mortality resulted. Antibiotics were continuously applied to prevent and treat diarrheas and also as food additives.

As a deleterious consequence of this malpractice *Klebsiella*, *Citrobacter* and *Pseudomonas aeruginosa* strains became prevalent, Table 5 reproduces the resistance data to *E. coli* (EC), *Citrobacter* (C) and *Enterobacter-Klebsiella* (EKL) strains. The *Pseudomonas aeruginosa* strains were completely resistant to all tested antibiotics. While the percentage of resistance for *E. coli* and *Enterobacter-Klebsiella* is comparable, *Citrobacter* strains were more sensitive especially to kanamycin and neomycin.

Discussion

In human medicine and husbandry the importance of *E. coli* in enteric infections and gram-negative bacteremia is now a textbook knowledge. Antibiotics are widely used for both types of infection. Gram-negative bacteremia is a matter of great concern in humans (Wolff and Bennett, 1974) and in calves (Fey, 1971).

The indiscriminate use of antibiotics for weight gain, presumed prophylactic and therapeutic effects is common in husbandry. Reports about antibiotic resistance of enterobacteria differ. To quote only some recent publications: Pohl

(1977) reported that only 20% of newborn calves were resistant to antibiotics. Brophy et al. (1977) described high resistance to antibiotics in youngest calves, which persisted in the calfhouse. When the animals were transferred to free grazing, resistance decreased. These experiences were gained from smaller herds. On the other hand Sato et al. (1977) tested antibiotic resistance in a large feedlot similar to the conditions described in this paper. They found 213 of 214 isolates of *Salmonella* Dublin strains resistant to antibiotics and multiresistance in all *E. coli* strains. In agreement with our material the multiresistance of *E. coli* and other gram-negative bacteria is extremely high in large agglomerations. High antibiotic resistance of *E. coli* makes the use of these drugs questionable. In our own experience as in that of Oxender et al. (1973) and Shull et al. (1978) the use of antibiotics rather aggravates the situation. Calf diarrhea like infant diarrhea leads to water and electrolyte losses, followed in severe cases by acidosis and death due to dehydration shock.

Since 1973 excellent results have been achieved by our group in diarrheal disease of calves by oral rehydration. A balanced electrolyte solution with glucose is used. This can be offered to the animals ad libitum (Rašková et al., 1976). For non cholera diarrhea predominantly oral rehydration without antibiotics (WHO, 1976) is now advocated by the World Health Organization. Thus the treatment of choice according to our experience is early and adequate rehydration. In spite of the analogous recommendation of WHO for human diarrheas, the superfluous and sometimes dangerous administration of antibiotics continues both in human medicine and husbandry.

High *E. coli* antibiotic resistance in calves has another challenging site. A possible transfer of resistant animal strains to humans. A number of older reports gave mainly circumstantial evidence for this possibility.

The recent paper of Hirsch and Wiger (1977) brings direct experimental proof about the transfer of plasmid coded *E. coli* strains from calves to man. In human medicine gram-negative resistant bacteria present a steeply increasing danger especially in hospitals (Whitehead, 1973; Wood and Bennett, 1974; Nichols, 1977; WHO, 1978). Polyresistance of gram-negative gut microbes from newborn infants is comparable to our data from newborn calves. The increase in resistance and multiresistance of *E. coli* in time has recently been described for TTC in humans (Moller et al., 1977). TTC resistance increased from 50% to 88% after 4 weeks of treatment. Multiresistance originally absent, raised to 50% after 4 weeks of treatment.

High multiresistance to antibiotics of gram-negative bacteria isolated from calves is a serious problem in husbandry. The same is true for humans. The feedback between animals and man may be so far moderate. But the rational use of antibiotics is imperative both for animal and man.

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