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RELATIONSHIP BETWEEN PRODUCTION OF ENTEROTOXIN AND VARIOUS DRUG RESISTANCE PATTERNS IN STRAINS OF ESCHERICHIA COLI ISOLATED FROM PIGLETS SUFFERING FROM COLIENTEROTOXAEMIA*

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LIVEN, EIVIND: *Relationship between production of enterotoxin and various drug resistance patterns in strains of Escherichia coli isolated from piglets suffering from colienterotoxaemia.* Acta vet. scand. 1979, *20*, 396—403. — The relationship between production of enterotoxin and drug resistance patterns in 92 E. coli strains isolated from the jejunal content from piglets suffering from colienterotoxaemia was investigated. The drugs included were oxytetracycline, streptomycin, sulphaisodimidin, neomycin, ampicillin and chloramphenicol. The frequency with which transmission of drug resistance occurred as well as the O-groups of these strains were also recorded.

Fifty-one E. coli strains produced enterotoxin. Of these, 42 (approx. 82 %) produced the LT. The same number of strains also produced the ST. Strains simultaneously producing both enterotoxins thus constituted approx. 64 % of the enterotoxin-producing strains. The E. coli strains were divided in 7 groups, each representing different drug resistance patterns. Statistical analysis showed that the various categories of enterotoxin production mentioned above were more frequently found in strains simultaneously resistant to oxytetracycline, streptomycin and sulphaisodimidin than in strains belonging to the other drug resistance groups in general.

Transmission of drug resistance was demonstrated in all resistance pattern groups. Approx. 50—70 % of the enterotoxin-producing strains showed drug transmission. The O-groups 138 and 149 constituted 8 and 54 out of the 62 typable strains, respectively.

Escherichia coli; enterotoxin; drug resistance; pig.

The significance of enterotoxin production in intestinal disorders caused by Escherichia coli in man and animals is well documented (*Smith & Halls* 1967, *Kohler* 1968). Chemoterapeu-

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tics and antibiotics are often used in the treatment of such infections, although reports on the occurrence of drug resistance in enteropathogenic E. coli strains indicate that the therapeutic effect of drugs might be limited (*Smith & Halls* 1966, *Larsen & Larsen* 1972, *Liven* 1979). This is also supported by practical experience.

The production of enterotoxins by E. coli strains as well as their ability to develop drug resistance, seem to be controlled by transmissible plasmids (Ent and R plasmid) (*Smith & Halls* 1966, 1968). The possibility that E. coli strains harbour both Ent and R plasmids has not been investigated widely, although the possible danger of a simultaneous occurrence of these plasmids in the cell has been emphasized (*Tschäpe & Rische* 1974). *Walton* (1977) suggested that there was no relationship between the prevalence of Ent and R plasmids in field strains of E. coli. He also concluded, in the light of in vitro experiments, that there seemed to be no co-transfer of the Ent plasmid with R plasmids controlling resistance to streptomycin, neomycin and chloramphenicol.

The purpose of the present work was to test for enterotoxin production in E. coli strains isolated from piglets suffering from colienterotoxaemia, and to correlate the ability to produce enterotoxin with the resistance patterns of the strains.

MATERIALS AND METHODS

Strains

Ninety-two strains of E. coli isolated from jejunal content taken from piglets suffering from colienterotoxaemia were used in the experiment. The strains were classified into O-groups*.

Enterotoxins

Production of enterotoxins from the E. coli strains was performed as described by *Sack & Sack* (1974) using the syncase medium of *Kohler* (1968). Tests for the presence of heat-labile enterotoxin (LT) were carried out using Y1 adrenal cells (*Donta et al.* 1974) by adding 0.2 ml culture supernatant to a tissue culture grown in Petri dishes (60 mm in diameter). Test cells

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* The serological classification of the strains in O-groups was carried out at the National Veterinary Institute in Oslo by Dr. L. Sørum to whom I express my sincere gratitude.

were maintained and cultivated as recommended by *Sack & Sack*. The Y1 adrenal cell test was read after 24 hrs. incubation at 37° C.

The suckling mouse test described by *Dean et al.* (1972) was used to detect the presence of heat-stable enterotoxin (ST). Two to 4 days old mice were inoculated with 0.1 ml of the culture supernatants of the strains to be tested. Evan Blue was added to the supernatant which was then injected through the body wall directly into the stomach using a No. 30 hypodermic needle. Four mice were inoculated with each strain. The mice were sacrificed after 3 hrs. The intestines and bodies of the 4 mice were weighed separately and the ratio of intestine weight and body weight calculated. Ratios of 0.090 or more were considered as being positive and ratios less than 0.090 were considered as being negative as regards the presence of ST in the supernatant injected.

Drug resistance

The drug resistance pattern of the strains was investigated as described by *Liven* (1979). The method employed was in principle identical to the methods described by *Larsen & Larsen* (1972) and *Wierup* (1975). The resistance pattern of the strains was tested on PDM*-Antibiotic Sensitivity Medium** using discs (AB-Biodisk) containing the following drugs: oxytetracycline — 30 μg (Te), streptomycin — 30 μg (S), sulphaisodimidin — 250 μg (Su), neomycin — 30 μg (N), ampicillin — 10 μg (A) and chloramphenicol — 30 μg (C). Demonstration of transmissible drug resistance (R-factors) was performed as described by *Larsen & Larsen*.

RESULTS

Production of enterotoxins was demonstrated in 51 out of the 92 strains tested (55.4 %). Production of LT was demonstrated in 42 strains (approx. 82 % of the toxin-producing strains). Production of ST was also found in 42 strains. The simultaneous production of LT and ST was thus found in approx. 64 % of the enterotoxin-producing strains.

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* Paper disc method.

** AB-Biodisk, Pyramidvägen 7, S-17136 Solna, Sweden.

The relation between the production of enterotoxins and drug resistance patterns is shown in Table 1. Strains were grouped into 7 groups according to resistance patterns. Entero-

Table 1. Relationship between enterotoxin production and drug resistance in 92 strains of E. coli isolated from piglets suffering from colienterotoxaemia. (Statistical analysis* was carried out between Group 1 and the other groups separately).

Resistance pattern groups**	Number of strains tested	Number of enterotoxin-producing strains	Number of LT-producing strains	Number of ST-producing strains	Number of strains producing both LT and ST	Number of toxin-producing strains showing drug transmission
Group 1 Te S Su	28	21	20	18	17	14
Group 2 Te S Su N Te S Su A Te S Su C	13	4 ($P < 0.01$)	3 ($P < 0.01$)	4 ($P < 0.05$)	3 ($P < 0.05$)	2
Group 3 Te S A Te Su N S Su A S Su N Su A C	11	6	4 ($P < 0.05$)	5	3	5
Group 4 Te S Te Su S Su	22	10	9 ($P < 0.05$)	7 ($P < 0.05$)	6 ($P < 0.001$)	6
Group 5 Te N S N Su C	6	3	2	2	1	2
Group 6 Te S Su	12	7	5	5	3	1
Group 7 Total of Groups 2 3 4 5 and 6	64	30 ($P < 0.02$)	23 ($P < 0.02$)	23 ($P < 0.02$)	16 ($P < 0.001$)	16

* Statistical analysis is based on the Fischer Irwin-test.

** Te: Oxytetracycline; S: Streptomycin; Su: Sulphaisodimidin; N: Neomycin; A: Ampicillin; C: Chloramphenicol.

toxin production was demonstrated in 21 (75 %) of the 28 strains comprising Group 1 (simultaneously resistant to Te, S and Su). LT and ST were found in, respectively, approx. 95 % and 86 % of the toxin-producing strains in this group. The simultaneous production of both enterotoxins was thus demonstrated in approx. 81 % of the enterotoxin-producing Group 1 strains. Enterotoxin production was less common in strains with other resistance patterns, both in general, and also with regard to the specific production of either LT or ST or both. When comparing the frequency of these categories of enterotoxin production, statistical analysis showed that there were significant differences between Group 1 and Groups 2, 3, 4 and 7. In Groups 2 and 7 the frequency of all 4 categories of enterotoxin production was significantly less than in Group 1. In Groups 3 and 4 production of LT was significantly less than in Group 1. In Group 4 also the frequency of ST production and the simultaneous production of LT and ST was significantly less than in Group 1. Table 1 shows that transmission of drug resistance was demonstrated within strains in all the resistance pattern groups. In Group 6 only 1 out of 7 enterotoxin-producing strains showed transmission of drug resistance. In all other groups, transmission of drug resistance was found in approx. 50—70 % of the enterotoxin-producing strains.

As to the O-groups, 54 out of 62 typable strains belonged to O-group 149, while 8 strains belonged to O-group 138. Thirty out of the 92 strains were not typable.

DISCUSSION

The present investigation showed that 30 out of 51 (58.8 %) E. coli strains isolated from piglets suffering from colienterotoxaemia possessed both the ability to produce enterotoxins and to transfer drug resistance. Furthermore, enterotoxin production was demonstrated significantly more frequently in oxytetracycline/streptomycin/sulphaisodimidin resistant strains than in strains showing other resistance patterns.

Although all the E. coli strains were isolated from piglets which showed microbiological and pathological findings consistent with colienterotoxaemia, only 56 % of the strains could be shown to produce enterotoxin. *Søderlind et al.* (1976) using the loop test in pigs and rabbits as well as the Y1 adrenal cell

test, demonstrated enterotoxin production in 35 % of the strains tested. *Sack et al.* (1971) found LT production in 16 out of 27 (59 %) strains isolated from humans with a severe cholera-like disease, while *Pesti & Lukács* (1978) had evidence that more than 80 % of E. coli strains isolated from piglets with diarrhoea produced ST.

Aeration of the culture, which should be favourable for ST production (*Sack* 1975) was not a part of the cultivation technique used for the production of enterotoxins in this investigation. This might explain the failure to demonstrate ST in some of the strains. Furthermore, an intestine/body weight ratio of 0.085 has been used instead of 0.090 by some workers as the lower positive limit for ST production in E. coli strains (*Phillips & Kelly* 1978). In the present work this would have resulted in an increase of approx. 8 % in the number of ST producing E. coli strains. The fact that progenies of enterotoxigenic E. coli cells might lose the plasmid which controls enterotoxin production may further explain the absence of demonstrable enterotoxins in some strains.

Although the number of strains in the various resistance pattern groups was not very high, significant differences in the frequency of enterotoxin production could be shown. Of special interest are Groups 2 and 7, in which the frequency with which all categories of enterotoxin production occurred proved to be significantly different from the frequency with which these same categories occurred in Group 1.

The relationship between enterotoxin production and drug resistance in enteropathogenic strains of E. coli needs to be further elucidated. The present work, however, seems to indicate that enterotoxins are most frequently produced in E. coli strains simultaneously resistant to oxytetracycline, streptomycin and sulphaisodimidin than in E. coli strains with other resistance patterns. According to *Skovgaard* (1978), enterotoxigenic E. coli strains do not show multiple drug resistance. It was suggested that an incompatibility between the Ent plasmid and the R plasmids existed in the bacterial cell. However, the present investigation demonstrated that 44 out of 51 (86.2 %) enterotoxin-producing strains isolated from pigs showed double or multiple resistance. In strains simultaneously resistant to oxytetracycline, streptomycin and sulphaisodimidin, 21 out of 28 strains (75 %) produced enterotoxin. Transmission of drug resistance was

demonstrated in ⅔ of the strains showing this particular resistance pattern. The danger of a possible recombination of Ent and R plasmids as stressed by *Tschäpe & Rische* (1974) should therefore not be excluded.

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SAMMENDRAG

Sammenheng mellom produksjon av enterotoksin og forskjellige resistensmønstre hos stammer av Escherichia coli isolert fra smågris med kolienterotoksemi.

Forholdet mellom resistens mot antibiotika (oxytetracyklin, streptomycin, sulfaisodimidin, neomycin, ampicillin, chloramphenicol) og produksjon av enterotoksiner ble undersøkt i 92 E. coli-stammer som ble isolert fra tynntarminnhold fra smågriser døde av kolienterotoksemi. Frekvens av overførbar antibiotikaresistens samt stammenes O-grupper ble også undersøkt.

Femtien E. coli-stammer produserte enterotoksin. Av disse produserte 42 (ca. 82 %) LT. Det samme antall stammer produserte ST. Ca. 64 % av de enterotoksinproduserende stammer produserte dermed begge enterotoksiner. E. coli-stammene ble inndelt i 7 ulike grupper hvor hver gruppe representerte et resistensmønster. Ved statistisk beregning viste det seg at de ulike typer av enterotoksinproduksjon som er nevnt ovenfor var hyppigere i stammer som var samtidig resistent mot oxytetracyklin, streptomycin og sulfaisodimidin enn i stammer som hadde et annet resistensmønster.

Overføring av antibiotikaresistens ble påvist innen alle grupper av resistensmønster. I ca. 50—70 % av de enterotoksinproduserende E. coli-stammer ble overførbar antibiotikaresistens påvist. Av de 62 typbare E. coli-stammene tilhørte 8 O-gruppe 138 og 54 O-gruppe 149.

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