THE SUSCEPTIBILITY OF ENTEROPATHOGENIC AND NON-ENTEROPATHOGENIC PORCINE E. COLI STRAINS TO POLYMYXINS AND OTHER ANTIBIOTICS.

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An important approach in eliminating enteropathogenic E. coli from the intestinal tract is the use of effective antibiotics (Nielsen et al., 1976). The prevalence of antibiotic resistance, especially to broad-spectrum antibiotics, is increasing (Sørensen, 1977). This development has been accellerated by the emergence of R-factors. The aims of this study have been to compare the incidence of resistance among 100 enteropathogenic and 100 non-enteropathogenic porcine E. coli strains to the following antibiotics: Ampicillin, chloramphenicol, colistin (polymyxin E), neomycin, polymyxin B, streptomycin, sulphonamides, and tetracycline and in detail to study the susceptibility of the strains to the polypeptide antibiotics colistin and polymycin B. It was also attempted to develop resistance in-vitro by passage transfers in sub-inhibitory concentrations on neomycin, tetracycline, colistin, and polymyxin B.

The pathogenic strains were isolated from cases of neonatal or postweaning diarrhoea and found to be enterotoxin producing in the gut loop test in pigs. The non-pathogenic strains were isolated from healthy 9-12-weeks old pigs in markets.
Initially, the resistance patterns of the strains were determined by agar diffusion. Inocula were prepared as described by WHO (Ericsson and Sherris, 1968). The strains were classified as: Sensitive, moderately sensitive, relatively resistant, or resistant. The minimal inhibitory concentrations (M.I.C.) were tested against colistin and polymyxin B by a broth dilution method. Doubling dilutions of the two antibiotics (0.1 - 48 ug/ml) were used.

Ten of the pathogenic strains and 5 non-pathogenic strains were studied as to development of resistance in-vitro by repeated transfers in broth containing sub-inhibitory concentrations of colistin (0.1 mcg/ml), polymyxin B (0.1 mcg/ml) neomycin (2.5 mcg/ml) and tetracycline (0.25 mcg/ml). The strains were sensitive to 5-10 times these concentrations.
During 6 months, subcultures to fresh antibiotic containing broths were made twice every week (52 transfers). The strains were then tested for M.I.C. against the 4 antibiotics.
The prevalence of resistance to individual antibiotics within the two groups of strains (pathogenic and non-pathogenic) did not differ statistically. 50-60 per cent of all strains were resistant to streptomycin, sulphonamides, and tetracycline. Resistance to chloramphenicol occurred only rarely (2.5 per cent) whereas 11.5 per cent were resistant to ampicillin. All strains were sensitive to colistin, polymyxin B, and neomycin.
80.5 per cent of the strains were resistant to one or more antibiotics. Mono-, di-, and multiresistance occurred in 17.5, 32.5, and 30.5 per cent, respectively.
In the M.I.C. studies with colistin and polymyxin B it appeared that the in-vitro activity of the two chemically closely related antibiotics was identical. 50 per cent of E. coli strains were sensitive to 0.5 mcg/ml or less. 95 per cent were inhibited by 2.4 mcg/ml or less.

After 52 repeated transfers of the 15 E. coli strains in sub-inhibitory concentrations of colistin, polymyxin B, neomycin, and tetracycline the M.I.C.'s were determined. A significant rise in M.I.C. was defined as an increase of more than 4 two-fold steps. According to this definition, development of resistance to tetracycline was demonstrated in two strains at a high level (80 and 160 ug/ml). One strain became resistant to colistin (20 ug/ml). Development of resistance to neomycin and polymyxin B was not demonstrated.
The present study clearly indicates that the incidence of resistance to a number of antibiotics is so high (> 50 per cent) that a successful outcome of therapy is doubtful.
A number of alternative antibiotics are available for prevention and therapy of E. coli diarrhoea. Colistin and polymyxin B represent such alternatives for several reasons: The drugs are bactericidal at quite low concentrations. They are not absorbed from the GI-tract and the level of resistance in animal E. coli is negligible (Smith, 1980, Mercer 1971). In addition, polymyxin antibiotics have no effect on the gram-positive intestinal flora.

Emergence of colistin/polymyxin-resistant organisms is due to selection of phenotypically resistant variants in a population of normally sensitive cells (Greenwood, 1975). This adaptation, however, is unstable, and the strains revert to sensitivity when no longer exposed to antibiotic (Gilleland & Murray, 1976). This phenomenon in addition to the fact that R-factors transferring polymyxin/colistin resistance has not been demonstrated yet in enterobacteria, probably explains why the level of resistance to these antibiotics remains low.
In animal husbandry, antibiotics are sometimes used prophylactically at low concentrations. Under such conditions, sub-inhibitory concentrations may be present occasionally. Our study has revealed the findings of others, that the potential of low concentrations of polymyxins to select resistant variants is low (Hirsch et al., 1960).

Conclusions:

The drug resistance potential of colistin and polymyxin B appears to be low compared to a number of other drugs. This suggests that the use of these drugs in E. coli intestinal infections is a rational choice.

Selected references: Nielsen, N.C., Bille, N., Svendsen, J., and Riising, H.-J.; The Royal Veterinary and Agricultural University, Copenhagen, 1976; Sørensen, M.; Nord. Vet-Med. 1979, 31 : 25; Ericsson, H. and Sherris, J.C.; Acta Path. Microbiol. Scand. 1971, Supplementum no. 217; Smith, H.W.; J. Hyg., Cambr. 1980, 84 : 467; Mercer, H.D., Pocurull, D., Gaines, S., Wilson, S., and Bennett, J.V.; Appl. Microbiol. 1971, 22 : 700; Gilleland, H.E. and Murray, R.G.E.; J. Bact. 1976, 125 : 267; Hirsch, H.A., McCarthy, C.G., and Finland, M.; Proc. Soc. Exp. Biol. Med. 1960, 103 : 338.