The initial step in most bacterial infections is the attachment of the responsible organism to surfaces of the target cells in question. This attachment is mediated by surface structures of which two morphologically distinct types are defined. The first type consists of filamentous appendages generally found in enterobacteria (Dagley & Old, 1980), while hairy projections called fimbriae occur in streptococci (Buchel, 1981). The fimbriae and fimbriae-like structures of E. coli are also referred to as pilus (Dagley & Old, 1980). As such structures confer adhesive properties to the microorganism, they are called adhesins, or because of their involvement in the colonization of mucous membranes, colonization factors (CF).

Interest in the adhesion or colonization process has increased during the last few years, since prophylactic measures based on antibacterial or antibiotic therapy in many infections important for economy in animal production have been unsuccessful.

Buchel (1981) has suggested three new approaches in prevention of serious bacterial infections: 1) use of purified adhesin or receptor material to interfere with the adhesion, 2) use of antibiotics with influence on development of the adhesin, 3) use of vaccines against the adhesins.

This paper deals with the effect of colistin on the adhesive properties of the K88 antigen of a porcine enteropathogenic E. coli and the K99 antigen of a calf enteropathogenic E. coli.

Both strains had been isolated from animals which had succeeded to diarrhoea. The calf strain, K73/81, with K99 antigen belonged to serogroup 0139. The porcine K88-positive strain belonged to serogroup 0149, was isolated from a 5-week-old piglet.

The susceptibility of the strains to ampicillin, chloramphenicol, colistin, gentamicin, neomycin, polymyxin B sulphate, and tetracycline was determined by an agar dilution technique. The tetracycline resistant strain was resistant to streptomycin (MIC 128 μg/ml) and the porcine strain was resistant to tetracycline (MIC 192 μg/ml).

The MIC of colistin was 0.5 μg/ml (15 units/ml) for the K99-positive strain and 1.0 μg/ml (30 units/ml) for the K88-positive strain.

The strains were kindly supplied by Drs. H.v. Angph and H.H. Christiansen, The State Veterinary Serum Laborato, (Copenhagen).

Preparation of bacterial cells, erythrocytes and saline solution for the hemagglutination test was performed according to Jones & Rubier (1974).

In preliminary experiments mannose-resistant hemagglutination (MRHA) with erythrocytes from the following animal species was tested: Guinea pig, pig, calf, horse, chicken, sheep.

Both strains reacted with pig erythrocytes, whereas the calf strain reacted weakly with horse erythrocytes. In the following experiments only pig erythrocytes were tested.

From the standardized bacterial suspensions doubling dilutions were prepared in HI-W with 1.5% D-mannose. 0.25 ml of the suspension and the dilution were transferred to plastic microtiter trays with U-shaped holes. To each dilution equal volumes of red cell suspension were added and the trays were incubated at 3-4°C for 2 hr. The degree of hemagglutination was recorded as 0, ±, +, or ++. As negative control 0.025 ml of PBS without bacteria was mixed with the erythrocytes.

Hemagglutination-inhibition test with colistin.

Three bacterial suspensions of each strain with 3 x 10⁸ cells per ml were prepared in PBS containing 1% D-mannose and 0.1, 0.5, and 0.25 μg colistin per ml, respectively. The colistin concentrations indicate base activity of the antibiotic corresponding to 30, 15, and 7.5 units per ml (1 μg colistin base = 30 units).

In preliminary experiments doubling dilutions of the bacteria were prepared in two ways: HI-W with 1% D-mannose and in PBS with 1% D-mannose supplemented with 3 different concentrations of colistin. Since no difference could be detected, PBS without colistin was used as dilution medium. The undiluted bacterial suspensions were left approximately 10 minutes before dilution and transfer to the microtiter trays.

The technical procedure and reading of results in the hemagglutination-inhibition test was identical with the hemagglutination test procedure.

Studies by Roland and Heelan (1979) have shown that the antibiotics: Tetracycline, minocycline, chloramphenicol, and neomycin are able to inhibit the hemagglutinating effect of E. coli. In the probiotics of travellers diarrhoea it has been proved that doxycycline was an effective drug and the activity attributed to colistin resistance inhibition (Sack et al., 1978, Roland & Heelan, 1979). It should actually be emphasized that the inhibitory dose of tetracyclines was 400-1,600 μg/ml.

The results of the present study show that colistin even at sub-inhibitory concentrations is able to inhibit hemagglutinating pig erythrocytes by E. coli strains from pig and calf possessing the K88 and K99 antigen respectively. The suggestion could be made that colistin is much more potent than tetracyclines in preventing coliform bacteria from attaching cell surfaces. If this suggestion could be proven in vivo conditions colistin has two different effects: An antibacterial and an anticolonization one.

Although further studies are necessary to elucidate the real nature of the hemagglutination inhibition effect some perspectives are raised. If the low dose is able to inhibit or prevent attachment, this treatment will apply to no specific selection of antibiotic resistant bacteria is essential. In the occurrence of a dominant strain, a flora as a result. Generally repeated subculturing of E. coli strains in broth containing sub-inhibitory concentrations of colistin gave only minimal response concerning development of resistance (Larsen & Sayegh, 1981).

Additionally, a low dosage of colistin will have minimal ecological implications which means that the inhibitory effect of the normal flora on parasitic microorganisms remain unaltered.