Communication

Virulence factors in *Escherichia coli* strains isolated from pigs in the Ribeirao Preto region, State of Sao Paulo, Brazil

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**Materials and Methods**

Three-hundred rectal swabs were taken from pigs with diarrhoea aged 1 to 10 and 25 to 35 days (post-weaning period) in farms in various areas of the Ribeirao Preto region in the State of Sao Paulo (SP).

**Isolation and chemical identification**

The faecal samples were inoculated into McConkey agar and incubated at 37 °C for 24 h. Typical *E. coli* colonies were identified by the following tests: lactose fermentation, indole production, methyl red and Voges & Proskauer reactions, citrate utilization, urease formation and hydrosulphide gas (*H₂S*) production. Readings were made after 24 hours of incubation at 37 °C.

**Serological identification**

*E. coli* strains were identified serologically using polyvalent OK rabbit antiserum by the slide agglutination test using three different serum pools:

- pool I consisted of sera against strains E 681 (0141 : K85ab : K88ab), E 145 (0141 : K85ab), P 155 (0149 : K91 : K60ac), E 60 II (0141 : K65ab : K66ac), and P 307 (08 : K87 : 88ab);
- pool II consisted of P 104 (0139 : K82), E 65 (045 : K'65), V 17 (0157 : "V17" : K88ac), E 57 (0138 : K81), G 491 (0138 : K81 : 88ac) and G 1253 (0147 : K89 : 88ac);
- pool III consisted of P 16 (09 : K103), Moon 637 (054 : K?), Troyer (09 : K35) and Moon 431 (0101 : K30 : K99).

**Enterotoxin detection**

STa : *E. coli* strains were cultured in a brain heart infusion (BHI) broth on a water bath with shaking at 150-200 rpm, at 37 °C for 18 h and then centrifuged. Evans blue (2%) was added to the supernatant and 0.1 ml of the mixture was inoculated intragastrically into groups of 4 mice aged 3 to 4 days leaving one mouse as a control, according to the technique of DEAN (4).
**STb**: strains were cultured in BHI broth on a water bath with shaking at 150-200 rpm, at 37 °C for 18 h and then centrifuged for the determination of STb enterotoxin. The method used was that of the ligated loop from 6-8 week old piglets (9). The animals were submitted to laparotomy, the small intestine exposed, the intestinal lumen washed with physiological saline, the strains inoculated and the intestinal loops ligated. Ten ml of culture supernatant per loop were inoculated into a total of 15 to 20 10 cm long loops. After 18 h, the animals were sacrificed and loops were examined for the presence of dilatation and the ratio of fluid volume to loop length was calculated for each loop. Values of 1.0 or more were considered as positive.

**LT**: thermolabile enterotoxin was detected by radial immunohaemolysis as described by YANO (17).

**Detection of colonization factors**

For the detection of F4 adhesin, the *E. coli* strains were cultured in phosphated glucose-agar (12). Cultures to be examined for F5 adhesin were inoculated in Minca medium (12) and those to be examined for F6 adhesin were cultured on blood-agar (14). All media were incubated at 37 °C for 24 h. Five colonies per plate were tested individually against purified anti-F4, anti-F5 and anti-F6 sera by slide agglutination.

**Mannose-resistant haemagglutination (MRHA)**

*E. coli* strains were cultured in Minca agar and incubated at 36 and 16 °C for 24 and 72 h, respectively, and the bacterial cells were resuspended in PBS. Sheep, pig, guinea pig, horse, chicken and human red cells were resuspended at a concentration of 3 %. One drop of bacterial suspension was mixed with one drop of red cells, with or without a 1.5 % mannose solution. The mixture was incubated at 0 °C for a few minutes and a reading of the results was undertaken (1).

**Bacterial sensitivity to antibiotics and chemotherapeutic agents**

All *E. coli* strains were submitted to the bacterial sensitivity test in the presence of the following antibiotics and chemotherapeutic agents: nalidixic acid, ampicillin, cephalotin, chloramphenicol, erythromycin, streptomycin, gentamicin, kanamycin, lincomycin, penicillin, novobiocin, neomycin, nitrofurantoin and tetracycline. The method used was that of BAUER (2).

**Results**

Of the 300 faecal swabs obtained from pigs with diarrhoea in the Ribeirao Preto region (SP), 100 *E. coli* strains were isolated. Table I shows the distribution of virulence factors among strains. Only three (3.0 %) of the 100 strains examined produced enterotoxin STA, 24 (24 %) produced STb, and five produced enterotoxin LT. Table I also shows that colonization factor F4 was present in 3 of the 5 strains that produced enterotoxin LT, in 6 of the STB+ strains, and in 2 of the STA+ strains. Eight F5+ strains produced enterotoxin STb and 1 produced enterotoxin STA. Five F4+, 12 F5+ and 1 F6+ strains were not enterotoxigenic. Twelve strains without colonization factors produced enterotoxins, and 50 (50.0 %) of all *E. coli* strains examined were not enterotoxigenic.

**TABLE I** Virulence factors in 100 Escherichia coli strains isolated from pigs with diarrhoea in the Ribeirao Preto region, State of São Paulo.

| Enterotoxin | No. of positive strains/total no. of strains (%) | Colonization Factors |
|-------------|-----------------------------------------------|---------------------|
| STA         |                                               | F4⁺ F5⁺ F6⁺ F4⁺ F5⁺ F6⁺ |
| STB         |                                               | 5/100               | 3 0 0 2 |
| STA         |                                               | 24/100              | 6 8 0 10 |
| STA         |                                               | 3/100               | 2 1 0 0 |
| EcET⁺       |                                               | 68/100              | 5 12 1 50 |
| Total       |                                               | 100                 | 16 21 1 62 |

* Enterotoxigenic Escherichia coli.

On the basis of the MRHA test, 100 *E. coli* strains were divided into four groups (table II). Group A included a single strain which reacted positively with sheep, chicken, horse and human red cells. Group B included a single strain that reacted positively with sheep and chicken red cells and was positive in the slide agglutination test for

**TABLE II** MHRH patterns in Escherichia coli strains isolated from pigs with diarrhoea in the Ribeirão Preto region, State of São Paulo.

| Group | No. of isolated strains | Guinea pig | Sheep | Bovine | Chicken | Horse | Human |
|-------|-------------------------|------------|-------|--------|---------|-------|-------|
| A     | 1                       | -          | +     | -      | +       | -     | -     |
| B     | 1                       | -          | +     | -      | +       | -     | -     |
| C     | 1                       | +          | +     | -      | +       | -     | +     |
| D     | 97                      | -          | -     | -      | -       | -     | -     |
were resistant to at least one antibiotic, the highest percentages being observed for resistance to penicillin (82%), tetracycline (93%) and cephalotin (72%).

Discussion
On the basis of slide serum agglutination with the serum pool against the Escherichia coli serotypes that are enteropathogenic for pigs, only 100 E. coli strains were considered to be the primary cause of diarrhoea among 300 piglets examined.

The frequency of LT enterotoxin-producing strains (5%), although relatively low did not differ from that reported by OLIVEIRA (15) in a study of 700 E. coli strains isolated from pig with diarrhoea in the city of Sao Paulo. Contrary to the high percentage (18%) of STb strains isolated by CASTRO (3) from pigs in the Concordia region in the State of Santa Catarina, the present results showed that only 3.0% of the isolated E. coli strains produced STa enterotoxin. The high frequency of STb+ strains (24.0%) in relation to the other enterotoxins shows the importance of this enterotoxin in the pathogeny of E. coli-induced enteritis in the Ribeirao Preto region. It should be pointed out that the frequency of enterotoxigenic strains with colonization factors F4, F5 or F6 represented almost two times the frequency of enterotoxigenic strains without these colonization factors. Of the 68 non-enterotoxigenic strains, 5 produced the F4 antigen, 12 produced the F5 antigen and one produced the F6 antigen. The fact that no STa+ strain produced colonization factor F6 contradicts GAASTRA and DE GRAAF (6) who reported that this colonization factor F6 contradicts GAASTRA and DE GRAAF (6) who reported that this colonization factor F6 represented almost two times the frequency of enterotoxigenic strains with colonization factors F4, F5 and F6. The strains were also tested for sensitivity to 14 antibiotics and chemotherapeutic agents. Twenty-four Escherichia coli strains produced enterotoxin STb, 5 produced LT and 3 produced STa. In the mannose-resistant haemagglutination reaction, one strain reacted positively with sheep, chicken, horse and human red blood cells and another reacted positively with guinea pig, sheep, chicken, horse and human red cells. However, both strains were negative for colonization factors F4, F5 and F6 when submitted to the slide agglutination test. All Escherichia coli strains were resistant to at least one antibiotic, the highest percentages being observed for resistance to penicillin (82%), tetracycline (93%) and cephalotin (72%).

Analysis of the results showed that most of the E. coli strains studied were simultaneously resistant to two or more drugs. No strain was sensitive to all drugs. Penicillin, tetracyclin and cephalotin were the antibiotics to which the strains were most resistant. This may possibly be due to the abusive and indiscriminate utilization of these drugs for veterinary purposes.

Two samples belonging to groups A and C, respectively of the MRHA patterns were negative in the slide agglutination test for colonization factors F4, F5 and F6. This fact suggests the possible existence of new colonization factors other than F4, F5 and F6, participating in the pathogeny of E. coli-induced pigs enteritis in the Ribeirao Preto region.

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