SHORT ARTICLES

ANTIBIOTIC-CONTAINING ENRICHMENT MEDIA FOR CERTAIN *ESCHERICHIA COLI* STRAINS OF PORCINE ORIGIN

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RECTAL swabs from pigs affected with enteric diseases attributable to *Escherichia coli* are likely to yield large numbers of organisms of certain strains of *E. coli* that are haemolytic on 5 per cent. sheep blood agar. However, after pigs recover from the disease, the dominance of the intestinal flora by the haemolytic organisms wanes and it may be impossible to detect them by direct cultural examination because they are overgrown by other strains of *E. coli*. Bacteriological studies of these infections would, therefore, be greatly assisted by the availability of an effective selective or enrichment medium for the pathogenic strains.

Enrichment media were developed for pathogenic strains of *E. coli* in three different herds of pigs. In these herds, the pathogenic strains of *E. coli* were resistant to some of the antibiotics that had been used for the therapy of porcine diarrhoea, whereas the majority of commensal strains of *E. coli* from the same herds were sensitive to the same antibiotic. The enrichment media proved useful for obtaining an estimate of the distribution of potentially pathogenic strains of *E. coli* in healthy pigs and their environment.

MATERIALS AND METHODS

The antibiotic-sensitivity of isolates of *E. coli* was assessed *in vitro* by the application of a paper disc impregnated with antibiotics (Multodisk, Oxoid) to a suspension of the organism inoculated on the surface of a sensitivity test agar plate. The plate was incubated aerobically at 37°C overnight.

Three strains of *E. coli*, namely strain G205 {O8: K87(B), K88a,c(L)}, strain Abbotstown {O149: K91, K88a,c(L)} and strain G4/66 {O45: K88a,c(L), K?} were isolated from three different herds of pigs in which they were associated with some, but not all, of the outbreaks of diarrhoea. These three strains were isolated from rectal swabs and all the isolates of each strain were haemolytic and almost all were resistant to an antibiotic that had been used for the treatment of diarrhoea in herds (table I). Strains of non-haemolytic *E. coli*, which were not typable with the *E. coli* antisera available, were isolated from rectal swabs of other pigs in the same herd and the majority of these strains were sensitive to the same antibiotic.

Development of the enrichment media

The inclusion in nutrient broth of the antibiotic to which the pathogenic strain of *E. coli* was resistant offered a possible means of increasing the ratio of the numbers of the pathogenic strain relative to the numbers of organisms of the untyped strains. The growth of the pathogenic strains G205, Abbotstown and G4/66 in broth containing various concentrations of the antibiotic to which each was resistant was compared with the growth of antibiotic-sensitive strains of untyped *E. coli* from the same herd in order to determine the concentrations of antibiotic that would impair the growth of the commensal strains of *E. coli* but not that of the pathogenic ones. Because Dixon (1959) had found that incubation of broth cultures

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of faeces at 44°C, instead of at 37°C, favoured the growth of *E. coli* as against that of other organisms, the enrichment media were incubated for 24 hr at 44°C in a thermostatically controlled waterbath.

Rectal swabs were examined both by direct plating and by plating after preliminary

TABLE I
The antibiotic sensitivity of pathogenic and commensal strains of Escherichia coli of porcine origin

Antibiotic (10 µg on a disc)	Strain of <i>E. coli</i>	No. of isolates of <i>E. coli</i> from different rectal swabs		
		total tested	sensitive to antibiotic	resistant to antibiotic
Streptomycin	G205 Untyped *	70	1	69
		121	68	53
Neomycin	Abbotstown Untyped *	119	1	118
		11	10	1
Chloramphenicol	G4/66 Untyped *	31	0	31
		12	12	0

* Untyped strains were strains not typable with the *E. coli* antisera available and they were not associated with diarrhoea. They were isolated from other pigs in the same herds as those in which the pathogenic strains occurred.

TABLE II
Comparison of efficiency of direct plating, and plating after enrichment for the isolation of E. coli organisms of pathogenic, antibiotic-resistant types

Pathogenic strain of <i>E. coli</i> in swab	No. of rectal swabs	No. of swabs yielding patho- genic strain		Antibiotic and concentration (µg per ml) in broth
		in direct culture	after enrichment	
G205	13	2	11	Streptomycin, 500
Abbotstown	4	0	4	
G4/66	4	1	4	Chloramphenicol, 50

culture in the enrichment medium. The swabs were first plated on 5 per cent. sheep blood agar. They were then shaken in and left in the enrichment medium. Subcultures were made from the enrichment medium after it had been incubated for 24 hr at 44°C. The numbers and proportions of haemolytic *E. coli* organisms on swabs were estimated from counts of colonies in direct platings, whereas the use of the enrichment media provided a

more sensitive means of detecting small numbers of these organisms in swabs from which none had been isolated by direct culture.

The concentrations of antibiotics finally adopted in the enrichment broths were, per ml nutrient broth, 500 μ g streptomycin, 40 μ g neomycin and 50 μ g chloramphenicol.

RESULTS

The results of preliminary trials indicated the potential usefulness of the antibiotic-containing enrichment media (table II). The pathogenic strains of *E. coli* were isolated from a larger proportion of rectal swabs when preliminary enrichment was done in the antibiotic broth than when the swabs were plated directly on the plating medium, which was sheep blood agar.

All three enrichment media were more effective than direct plating for detecting the smaller numbers of the organisms for which they were devised, but they were not completely effective in this respect. The minimum concentration of organisms of the pathogenic strains of *E. coli* consistently detected in faeces by direct plating was antilog_{10} 4.9 organisms per g of faeces, whereas the minimum concentration of the same organisms consistently detected by the enrichment media was antilog_{10} 2.9 organisms per g of faeces.

The enrichment media were found to be useful for obtaining a picture of the distribution of potentially pathogenic strains of *E. coli* in healthy pigs and their environment. For example, the G205 strain of *E. coli* was found in the faeces of a sow and in that of 7 of her 9 piglets during their 1st wk of life by culturing rectal swabs in streptomycin broth as an enrichment medium, whereas no isolations were made of this strain by direct plating of the same rectal swabs. These media also made it possible to determine the distribution of pathogenic strains of *E. coli* in the environment of newborn piglets. The Abbotstown strain of *E. coli* was found in 11 out of the 15 pens in the farrowing house of one herd by culturing swabs taken from the pens in neomycin broth, whereas this strain was found in only 4 pens by direct plating of the same swabs.

DISCUSSION

Although these enrichment media were useful for conducting epidemiological investigations into the occurrence of pathogenic strains of *Escherichia coli* in a few herds of pigs, they would not be suitable for general diagnostic application because their enriching ability depended on the differential antibiotic sensitivity of pathogenic and commensal strains of *E. coli* within the same herd. Such differential antibiotic sensitivity is probably determined by the antibiotic therapy employed within a herd, and the antibiotic regime used will vary considerably from herd to herd.

SUMMARY

Enrichment media were developed for the detection of small numbers of organisms of certain pathogenic strains of *Escherichia coli* in porcine faeces. The media contained antibiotics to which the bacteria were resistant.

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REFERENCE

- DIXON, J. M. S. 1959. *J. Hyg., Camb.*, **57**, 174.