

BACTERIOLOGICAL STUDIES ON THE ENTEROPATHOGENIC ESCHERICHIA COLI

THE ENTEROPATHOGENIC ESCHERICHIA COLI ISOLATED FROM MAN, ANIMALS, AND
OTHER NATURAL SOURCES, AND ITS SEROLOGICAL PROPERTIES

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INTRODUCTION

The important objects of food sanitation are production and supply of safe and innocuous foods. Among them, the relationship between foods and microorganisms, especially, as bacteria are related to bacterial food poisoning, oral infectious disease, and spoilage (putrefaction and deterioration), is attaching great importance to food sanitation.

It is well known that Escherichia coli (E. coli) is to be distributed extensively in the intestinal contents of man and animals as normal microflora. The E. coli does not yet recognized the pathogenic activity, but several types of E. coli are inferring to be pathogenic to man or animals, these types of E. coli are called "Enteropathogenic E. coli".

On the pathogenicity of E. coli, Jensen (1897) was firstly reported that the causative agent of the "white scours" of a calf was E. coli, and Adam's report (1927) of infants gastroenteritis associated with E. coli was the first one for the present enteropathogenic E. coli. Goldschmidt (1933)

was studied strains of E. coli isolated from infants gastroenteritis thoroughly by serological and ecological methods, but the most investigators were consigned these works to oblivion at that time. By the bacteriological report of Bray (1945), it was responded to gastroenteritis-E. coli relationship, consequently, these reports of Adam and Goldschmidt were realized. Bray (1945) isolated E. coli "bacterium coli neapolitanum in classificational name of E. coli at that time" from many inpatients at the prevalence of infants gastroenteritis in a hospital in England. Soon after Bray's report, there were similar reports of Varela et al (1946), Giles et al (1948), Smith (1949) and Taylor et al (1949). But the serological classification of E. coli by Kauffmann and his co-workers in Northern Europe (especially in Copenhagen).were really great to the cause of investigation of the enteropathogenic E. coli. Thus, from 1950, the enteropathogenic E. coli was isolated from infants gastroenteritis by many investigators, and was found 18 serotypes of the enteropathogenic E. coli untill today. But, unknown serotypes of the enteropathogenic E. coli existent, in 1973, 10 new serotypes of the enteropathogenic E. coli were supplemented.

The enteropathogenic E. coli isolated from infants gastroenteritis was recognized the pathogenicity to adults. In the last 10 years in Japan, the studies of the enteropathogenic E. coli have been made progress for contribution to the direction of food poisoning.

Several types of the enteropathogenic E. coli show not only pathogenic to man but also cause mastitis in cattle and diarrhoea or septicaemia in calf, fowl and young pig. These bacterial types are recognized in common in the both man and animals. But the report on the distribution of the enteropathogenic E. coli in healthy animals and in the nature except humans are little in Japan and other foreigners up to now. Because, there is no selective medium of the enteropathogenic E. coli, and the identification for the enteropathogenic E. coli and non-pathogenic E. coli has to test by serological method.

In this time, I report on the distribution of the enteropathogenic E. coli in man, animals and other sources and its pollution movement.

MATERIALS AND METHODS

1. Isolation of the enteropathogenic E. coli in feces, waters and oysters.

From August 1967 to February 1970, a total of 15,044 E. coli strains isolated from feces of healthy humans, cattle, horses, hogs, pet dogs, stray dogs, fowls, cats, sheep and rabbits, and river water, sea water, well water, septic tank water, sewages in the Nagasaki City abattoir, waters from digestion tank of the Nagasaki City abattoir, oysters in market and natural oysters, were examined for isolation of the enteropathogenic E. coli.

The serological identification of the enteropathogenic E. coli were as follows:

- 1) At first, agglutination were carried out with OK mixed antiserum on a slide glass, and next, the agglutinated strains firmly within 30 seconds were examined similarly with single OK antiserum.
- 2) About the positive strains of No. 1), agglutinations were carried out with O antiserum on a slide glass, and were ascertained the existence of K antigens.
- 3) About the negative strains of No. 2), the organisms were incubated at 37°C for 15 hours in Nutrient broth and heated at 100°C for 1 hour, and a drop of concerned O antiserum added to 0.5 ml of this broth, were examined agglutinations in a test tube at 50°C overnight. The positive strains with the naked eye were examined its quantitative agglutinations of antigen in vitro.

- 4) At the quantity test of K antigen, the organisms incubated at 37°C for 15 hours in Nutrient broth used for K antigen directly, and quantitative agglutinations were examined with K antiserum in vitro (after 2 hours reacted at 37°C, let it alone overnight in dark and cold place).
- 5) At the quantity test of O antigen, the organisms incubated at 37°C for 15 hours in Nutrient broth heated at 100°C for 1 hour and used for O antigen, and quantitative agglutinations were examined with O antiserum in vitro at 50°C overnight.
- 6) The antiserum did dilute by two-fold method. Dilution ranges were from 5X to 640X in case of K antiserum, O antiserum were from 100X to 6,400X. In this time, the agglutinin titers for the commercial antiserum were 160X in the K antiserum and 1,600X in the O antiserum, I concluded to the enteropathogenic E. coli strains that the isolates indicated the titer of 80X and over in case of K antiserum and 800X and over in case of O antiserum.

2. 1) Selective effect of dihydrostreptomycin sulfate (DHS) on isolation of the enteropathogenic E. coli

The 18 standard strains of enteropathogenic E. coli consisting of 18 serotypes (E1-E18), the 114 enteropathogenic E. coli isolated strains belonging to 14 serotypes, and 104 common E. coli strains isolated from human feces, 62 common E. coli strains isolated from river waters, and 110 common E. coli strains isolated from hog feces, were examined its sensitivity of dihydrostreptomycin sulfate (DHS).

In the present study, the basal medium used was MacConkey agar, and various concentrations of DHS added to MacConkey agar. And these enteropathogenic and common E. coli strains incubated at 37°C overnight in Nutrient broth. The cultures were inoculated on the DHS MacConkey agar and incubated at 37°C for 20 hours. The states of bacterial growth were observed.

2) The effect on growth in dihydrostreptomycin sulfate (DHS) broth of the enteropathogenic E. coli

18 standard strains and 114 isolated strains of enteropathogenic E. coli, and 20 common E. coli strains isolated from human feces, were examined its sensitivity of DHS broth. The used media were Nutrient, LB, BGLB and EC broths in commercial.

3. Antibiotics sensitivity of the enteropathogenic E. coli isolates

A total of the 187 enteropathogenic E. coli strains isolated from various sources were examined its sensitivity to 9 different antibiotics. Each 1 ml of Nutrient broth cultures of the 187 strains incubated at 37°C overnight was inoculated again to Nutrient broth and incubated at 37°C 4-5 hours. A drop of the cultures dropped on a Heart Infusion agar plate by 1 ml pipette and extended by sterile-L glass stick. After dried, disc containing each antibiotics placed on the plate gently and let it alone room temperature 1 hour, and incubated at 37°C overnight. The diameter of a produced inhibitory zone was measured. The used 9 different antibiotics were as follows: Tetracycline (T), Demethylchlortetracycline (Td), Oxytetracycline (O), Chloramphenicol (C), Colistin (K), Streptomycin (S), Polymyxin B (Xp), Kanamycin (Ka), and Paromomycin (H).

4. Analysis of the antigen of the enteropathogenic E. coli isolates

Absorption tests of 57 strains consisting of 5 serotypes isolated from various sources were examined.

Serotype O-111:K58(B4) strain isolated from cattle, O-112a.c:K66 (B11) isolated from hog, O-125:K70(B15) isolated from human, O-128:K67(B12)

isolated from hog and O-136:K78(B22) isolated from cat, were prepared antisera by hyperimmunization in 5 rabbits. These 5 antisera were absorbed K and O agglutinins by the enteropathogenic E. coli standard strains. These absorbed antisera used for quantitative agglutinations in vitro of 57 strains consisting of 5 serotypes. The dilution of antisera was carried out by two-fold serial dilutions from 3X in case of K antisera and from 10X in case of O antisera. The agglutinin titers of 5 different rabbit antisera were about 1,600X in case of O antisera and about 160X in case of K antisera.

RESULTS

1. Distribution of the enteropathogenic E. coli in feces, waters and oysters

15,044 E. coli strains isolated from various sources were examined for identification of the enteropathogenic E. coli. The results are summarized as follows:

- 1) Number of the enteropathogenic E. coli positive samples were 109 (5.34%), and 187 strains (1.24%) consisting of 15 serotypes were isolated.
- 2) The distribution of the 15 enteropathogenic E. coli serotypes were as follows; O-112a.c:K66(B11) (15.5%), O-128:K67(B12) (15.0%), O-136:K78(B22) (11.2%), O-125:K70(B15) (10.7%), O-26:K60(B6) (9.6%), O-111:K58(B4) (8.6%), O-127a:K63(B8) (6.4%), O-143:KX₁(B) (5.9%), O-28a.c:K73(B18) (5.3%), O-86a:K61(B7) (3.7%), O-55:K59(B5) (2.7%), O-124:K72(B17) (2.1%), O-119:K69(B14) (1.6%), O-126:K71(B16) (1.1%) and O-86:K62(L) (0.5%). Both types O-112a.c:K66(B11) and O-128:K67(B12) were isolated in higher rate. In this time, O-44:K74(L), O-144:KX₂(B) and O-146:K89(B) were not isolated.
- 3) Serotype O-128:K67(B12) was isolated from 9 kinds of samples, O-125:K70(B15)

was isolated from 7, O-127a:K63(B8) was isolated from 6, O-112a.c:K66(B11) was isolated from 5, O-26:K60(B6) and O-28a.c:K73(B18) were isolated from 4, O-55:K59(B5), O-86a:K61(B7), O-124:K72(B17), O-136:K78(B22) and O-143:KX_i(B) were isolated from 3, and O-111:K58(B4) and O-126:K71(B16) were isolated from 2.

- 4) Serotype O-86:K62(L) was isolated only from dog, and O-119:K69(B14) was isolated only from humans.
- 5) Serotypes O-112a.c:K66(B11) and O-125:K70(B15) were isolated from 2 hogs, O-55:K59(B5) and O-128:K67(B12) were isolated from 1 hog, O-86:K62(L) and O-128:K67(B12) were isolated from 1 dog, O-55:K59(B5) and O-128:K67(B12) were isolated from 1 dog, O-26:K60(B6) and O-136:K78(B22) were isolated from 1 cat, and O-111:K58(B4) and O-128:K67(B12) were isolated from 1 cat.

From March 1969 to February 1970, the enteropathogenic E. coli strains isolated from normal and watery contents in the hog caeca, and sewages and waters from digestion tank in the Nagasaki City abattoir, were examined for its seasonal variation and quantitative relationship of coli-aerogenes group. The results are summarized as follows:

- 1) Of normal and watery contents, the organism-positive contents were more than negative contents, and of the organism-negative contents, watery contents were more than normal contents in number of coli-aerogenes group. But, of the organism-positive contents, the difference of number of coli-aerogenes group was not recognized between normal and watery contents.
- 2) Of normal contents, the isolation rate of the organisms was higher in spring (from March to May) through the year.
- 3) Sewages and waters from digestion tank in the abattoir, the isolation rate of the organisms were higher in summer (from July to August) through the year.

2. Selective effect of dihydrostreptomycin sulfate (DHS) on isolation of the enteropathogenic E. coli

The present study was carried out to confirm selective effect of dihydrostreptomycin sulfate on the isolation of the enteropathogenic E. coli from humans, animals and river water, in the light of Ramirez's report that media containing DHS were highly effective as selective substance for isolation of the enteropathogenic E. coli from normal feces and sewage. The results are summarized as follows:

- 1) Of the enteropathogenic E. coli standard strains, the growth of serotypes O-26:K60(B6), O-28a.c:K73(B18), O-44:K74(L), O-86:K62(L), O-111:K58(B4), O-119:K69(B14), O-124:K72(B17), O-125:K70(B15), O-126:K71(B16), O-127a:K63(B8), O-136:K78(B22), O-144:KX₂(B) and O-146:K89(B) were normal, but O-55:K59(B5), O-86a:K61(B7), O-112a.c:K66(B11), O-128:K67(B12) and O-143:KX₁(B) were slightly inhibited on the DHS MacConkey agar (2-10 µg. level per ml).
- 2) Of the enteropathogenic E. coli isolated strains, almost of strains grew on the DHS MacConkey agar (10 µg. level per ml). Of these, the growth of O-55:K59(B5), O-86a:K61(B7), O-128:K67(B12) and O-143:KX₁(B) as well as O-26:K60(B6), O-28a.c:K73(B18), O-86:K62(L), O-111:K58(B4), O-119:K69(B14), O-124:K72(B17), O-127a:K63(B8) and O-136:K78(B22) were normal, but O-112a.c:K66(B11) and O-125:K70(B15) were slightly inhibited on the DHS MacConkey agar.
- 3) Of non-pathogenic E. coli isolates, 42.3% of strains from human were inhibited by 4 µg. level per ml of DHS, 76.0% by 6 µg. per ml and 77.9% by 8 µg. per ml, and none of strains from river waters were inhibited by 4 µg. level per ml, 45.2% by 6 µg. per ml and 58.1% by 8 µg. per ml; on the other hand, strains of hogs were slightly inhibited by 4-8 µg. level per ml of DHS MacConkey agar.

- 4) All of the enteropathogenic E. coli standard strains grew by 4 and 6 µg. level per ml, and 72.2% of strains grew 8 µg. per ml of DHS MacConkey agar. Of the enteropathogenic E. coli isolated strains, 99.1% of strains grew by 4 µg. per ml, 96.5% of strains grew by 6 µg. per ml, and 93.0% of strains grew by 8 µg. level per ml of DHS MacConkey agar.
- 5) Of non-pathogenic E. coli isolates, 60% of strains were inhibited by 4 µg. level per ml of DHS Nutrient broth.
- 6) Of the enteropathogenic E. coli standard strains, the growth of 88.9% of strains were normal by 4 µg. level per ml of DHS Nutrient broth.
- 7) In the examination of fecal specimens of hogs, the combined use of DHS Nutrient broth and DHS MacConkey agar resulted in normal average rate of detection of the enteropathogenic E. coli.
- 8) From these results, it is presumed that the use of a combination of DHS Nutrient broth (4 µg. level per ml) and DHS MacConkey agar (4, 6 and 8 µg. level per ml) will yield a higher percentage for isolation of the enteropathogenic E. coli from human feces and waters except hog feces than do any other medium alone.

3. Antibiotics sensitivity of the enteropathogenic E. coli isolates

A total of the 187 enteropathogenic E. coli strains isolated from various sources were examined its sensitivity to 9 different antibiotics.

The results are summarized as follows:

- 1) The highly effective compounds in the sensitivity tests were chloramphenicol, colistin, polymyxin B, kanamycin and paromomycin. Moderative inhibition was shown in tetracycline, demethylchlortetracycline, oxytetracycline and streptomycin.
- 2) There was little difference in the sensitivity to all antibiotics between the strains from humans and from animals.

- 3) It is noted that tetracyclines- and streptomycins-resistant strains were obtained from only feces of hogs.
- 4) Of all the strains tested, 81 strains (43.3%) were resistant to antibiotics, 1 strain (0.5%) was resistant to 5 kinds of antibiotics, 12 strains (6.4%) were resistant to 4, 18 strains (9.6%) were resistant to 3, 11 strains (5.9%) were resistant to 2, and 39 strains (20.9%) were resistant to 1.
- 5) The serotypes of antibiotics-resistant strains in the isolates were as follows; serotypes O-26:K60(B6) and O-112a.c:K66(B11) isolated from hogs were resistant to tetracyclines and streptomycin. O-111:K58(B4) isolated from cattle, O-136:K78(B22) isolated from cats and O-143:KX₁(B) isolated from human and dogs, were resistant to streptomycin.

4. Analysis of the antigen of the enteropathogenic E. coli isolates

Absorption tests of 57 isolated strains consisting of 5 serotypes were examined. The results are summarized as follows:

- 1) As a results of the agglutinins were observed completely by the enteropathogenic E. coli standard strains, both O and K negative agglutinations were found in 10 strains consisting of 3 serotypes.
- 2) Other 47 strains were slightly recognized the difference of a certain sections of O only or K antigen structures from its enteropathogenic E. coli standard strains.
- 3) In both O and K agglutinations, 10 negative strains consisting of 3 serotypes were as follows; 2 strains of serotype O-111:K58(B4) isolated from cats, 5 strains of O-125:K70(B15) isolated from hogs and 3 strains of O-128:K67(B12) isolated from hogs.

DISCUSSION AND CONCLUSION

There exists considerable literatures on ecological investigation of the enteropathogenic E. coli in man, but little work has been done to study its distribution in animals and in the natural world. In this time, numerous strains of E. coli isolated from animals and the natural world were examined serotypes of the enteropathogenic E. coli which causes diarrhoea or gastroenteritis and food poisoning in man. They have distributed widely in human living environments and in animals, especially the cat and dog. It was recognized the relationship between the serotypes isolated from many sources and the serotypes isolated from diarrhoea, gastroenteritis and food poisoning, on that account, it is presumed that these are important problems for food sanitation.

In case of compared with the antigen structure of the enteropathogenic E. coli standard strains and the enteropathogenic E. coli strains isolated from animals or in the natural world were recognized to be slightly different, in a certain portion of the antigen structure (for example, parts of O or K antigen) from the antigen structure of the standard strains. These points are attached great importance to the ecology of the enteropathogenic E. coli. Moreover, the pathogenicity of these strains are attached great importance for further study on the enteropathogenic E. coli.

In conclusion, on account of the ecological study on the enteropathogenic E. coli which are behind than the present ecological knowledge of other Enterobacteriaceae. It must be investigated thoroughly by many investigators for public health. For accomplishment of the object, I hope that the final selective isolation medium may be developed for the enteropathogenic E. coli appear in nearly future, because many hours and prodigious labor need for identification of the enteropathogenic E. coli compared with other Enterobacteriaceae today.