APPLIED MICROBIOLOGY, Feb. 1968, p. 417-418

Vol. 16, No. 2
Printed in U.S.A.

NOTES

Identification of Unclassified *Escherichia coli* Strains[1]

PAUL J. GLANTZ

Department of Veterinary Science, The Pennsylvania State University, University Park, Pennsylvania 16802

Received for publication 16 October 1967

The importance of *Escherichia coli* strains in diseases of man and animals is due primarily to the serological classification of the strains. Most *E. coli* strains can be classified with the standard O group sera; however, the number of *E. coli* isolates that do not react with the standard O groups is sometimes larger than would be expected. It was perplexing to isolate *E. coli* strains believed to be associated with a disease and to find that the strains could not be classified serologically.

When a large number of unclassified *E. coli* strains isolated from various sources had accumulated, it was decided to determine their possible serological identity, since the occurrence of the same serogroups in similar pathological conditions might lead to the identification of pathogenic *E. coli* strains.

Strains of *E. coli* that would not react with any of the standard *E. coli* O antisera, even after heating the antigens at 121 C for 2.5 hr, were designated O-negative or unclassified (OX). The O and K antisera were prepared in rabbits according to standard methods (P. R. Edwards and W. H. Ewing, *Identification of Enterobacteriaceae*, Burgess Publishing Co., Minneapolis, 1962), and were tested for homologous and heterologous reactions by tube titer agglutination.

Cultures that deteriorated when heated at 121 C for 2.5 hr were difficult to use in agglutination tests. Therefore, the cultures were streaked on Veal Infusion Agar (Difco) plates, and thin transparent colonies were selected for O antigen; thick mucoid colonies were used for K antigens.

A total of 45 OX sera were prepared in rabbits and utilized for serological examination of nonreacting *E. coli* isolates. Of the 45 original OX sera, 15 were eventually eliminated. Table 1 presents data relating to the strain, animal, source, and diagnosis of the animals from which 30 of the OX strains were originally isolated during 1961 to 1965. Although most strains were associated with disease, some were isolated from the feces of animals with no illness.

In Table 2, the homologous and heterologous agglutination titers of cross-reacting OX serogroups are listed. Although several OX strains were found to have similar serological reactions, their O sera were used individually to confirm the identity of *E. coli* antigens reacting with them. When O and K antisera of *E. coli* OX strains 28, 29, 30, 32, 39, and 40 were tested for homologous and heterologous reactions, they were found to have identical O and K antigens. The six strains will be referred to as the OX28 group. Other OX strains found to have the same *E. coli* O antigen were: OX18 and OX24, OX25 and OX42, and OX21 and OX45. The *E. coli* OX18 and OX24 sera cross-reacted, and, when tested with reciprocally cross-absorbed O antisera, were found to be identical. The O antigens of OX25 and OX42 shared antigenic factors with OX21 and OX26. The OX25 and OX42 cross-reacted to identical O titers, and, when tested with reciprocally cross-absorbed sera, had the same O antigen. The O antigen of OX45 had relatively low homologous and heterologous O antiserum titers (160 to 640). In addition, the O antigen of OX21, but not OX45, shared a common minor antigenic factor with OX42, and there was a minor nonreciprocal cross-reaction with OX22. When the OX21 and OX45 antigens were tested with reciprocally cross-absorbed O sera, they were not identical. The reactions obtained indicated an intra-O group relationship of the *a-a,b* variety. OX21 would have the *a,b* factor and OX45 the *a* factor.

The antigens prepared from "thin" colonies and heated at 100 C for 2 hr gave good reactions (1:5,120) with their respective O antisera. In contrast, unheated antigen from autoclaved "thick" colonies did not give good O agglutination reactions (1:320). Unheated thick-colony antigen also had a definite inhibitory effect on O agglutination, and readily produced K agglutination with K (thick colony) serum. As a result, two strains, OX31 and OX35, previously un-

[1] Authorized for publication 9 October 1967 as paper No. 3311 in the journal series of the Pennsylvania Agricultural Experiment Station.

TABLE 1. *Strain number, animal, diagnosis, and source of Escherichia coli OX cultures*

OX antigens (OX:H)	Strain no.	Animal	Source	Diagnosis
14:19	17006A	Calf	Feces	No illness
15:21	17031	Calf	Feces	Diarrhea
16:8	PF6-10G	Pig	Feces	No illness
17:46	PF6-11D	Pig	Feces	No illness
18:12	PF1912	Sow	Feces	No illness
19:21	PDL-39A	Poultry	Heart	CRD[a]
20:6	EA11961	Poultry	Cecum	Coligranuloma
21:14	Pur 599	Pig	Colon	Diarrhea
22:31	Pur 652	Pig	Jejunum	Diarrhea
23:38	PF10-3J	Pig	Feces	No illness
24:2	PF10-7H	Pig	Feces	No illness
25:6	F6432	Calf	Feces	No illness
26:46	F6433J	Calf	Feces	No illness
28:NM	7026N	Calf	Feces	Diarrhea
29:NM	7029J	Calf	Feces	Diarrhea
30:NM	5-518	Calf	Lung	Septicemia-toxemia
31:NM	5-569	Calf	Liver	Septicemia-toxemia
32:NM	5-633	Calf	Lymph node	Septicemia-toxemia
33:NM	5-324	Calf	Lung	Septicemia-toxemia
35:NM	5-583	Calf	Lymph node	Septicemia-toxemia
36:9	PF7-10C	Pig	Feces	No illness
37:21	Pur 589	Pig	Jejunum	Diarrhea
38:47	PF11-6E	Pig	Feces	Diarrhea
39:NM	7020C	Calf	Feces	Diarrhea
40:NM	7020A	Calf	Brain	Septicemia-toxemia
41:10	PDL31B	Poultry	Air sac	CRD
42:6	8582	Pig	Intestine	Dermatitis
43:19	8547	Pig	Intestine	Pericarditis
44:27	8531	Cow	Milk	Mastitis
45:32	9998-1	Pig	Intestine	Peritonitis

[a] CRD = chronic respiratory disease.

TABLE 2. *Unclassified Escherichia coli (OX), serological reactions*

O antigen	Reactions with OX antisera[a]
OX-18	18;8[b] *24*;5
OX-21	*45*;8 21;8 22;4 *42*;2
OX-22	43;2 45;3 22;7
OX-24	*18*;7 24;8
OX-25	21;6 25;8 26;3 *42*;8
OX-28	28;5 *29*;7 *30*;7 *32*;5 *39*;7 *40*;8
OX-29	*28*;6 29;7 *30*;7 *32*;6 *39*;7 *40*;8
OX-30	*28*;5 *29*;8 30;7 *39*;7 *40*;8 *32*;5
OX-31	31;8 *35*;7 *37*;6
OX-32	*28*;5 *29*;8 *30*;7 32;6 *39*;7 *40*;8 2;1
OX-33	33;5 35;6 *37*;8
OX-35	*31*;8 35;5
OX-36	31;1 35;2 36;8
OX-37	*31*;7 *33*;5 35;6 37;7
OX-39	*28*;6 *29*;8 *30*;7 *32*;6 39;8 *40*;8
OX-40	*28*;4 *29*;8 *30*;7 *32*;6 *39*;8 40;8
OX-42	*21*;6 *25*;8 26;3 42;8
OX-45	45;5 *21*;3

[a] Reciprocal reactions are shown in italics.

[b] Titers: 1 = 40, 2 = 80, 3 = 160, 4 = 320, 5 = 640, 6 = 1,280, 7 = 2,560, 8 = 5,120. 18;8 = O group OX18 reacts with OX-18 to a titer of 8 (1:5,120).

classifiable, were found to belong to standard *E. coli* O group 101 and to have the same K antigen. Two strains, OX33 and OX37, also belong to O group 101 but do not have the same K antigen as OX31 and OX35.

The OX antisera have been used for more than a year to identify *E. coli* strains that would not react with standard O and K antisera. During this time, the most frequently isolated OX groups have been OX28 and OX31 (101). These isolates, from natural and experimental infection of animals, sometimes occurred in polluted water. Without the use of the OX sera, these *E. coli* strains would remain unclassified and their significance would remain unknown. A report of the distribution of the unclassified *E. coli* in animals and polluted water is in preparation.

The assistance of W. H. Ewing, National Escherichia Center, Atlanta, Ga., and F. Ørskov, International Escherichia Center, for examining representative OX strains, and Sara R. Rearick for the serological procedures is gratefully acknowledged.

This investigation was supported by Public Health Service research grant HD-02568-1 from the National Institute of Child Health and Human Development.