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Antigenic Variation in *Salmonella typhimurium* (37817)

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(Introduced by A. G. Osles)

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During an epidemic at two hospitals in Curitiba–Parana, Brazil, several strains of *Salmonella typhimurium* were isolated; some of these cross-reacted with anti-a sera.

The serological study of these strains is described and evidence is presented to show that these strains possess three immunologic phases, one of which cross-reacts with *S. paratyphi* A.

Materials and Methods. The bacteriologic and serologic methods described by Edwards and Ewing (1) were followed. Somatic and flagellar antigens were prepared as in Edwards and Ewing (1). Unless otherwise stated, culture media and antisera were obtained from Difco Laboratories, Detroit, MI.

TABLE I. Distribution of Isolated Strains According to Species.

Salmonella sp	423
S. typhimurium	407
S. paratyphi A	13
S. typhi	2
S. cholera-suis	1
Shigella	
Sh. flexnerii	58
Sh. dysenteriae	1
Enteropathogenic *E. coli*	240
E. coli—0111:B_4	201
E. coli—0119:B_{14}	18
E. coli—026:B_6	4
E. coli—055:B_5	17
Mixed infections	27
Salmonella + *E. coli*—0111:B_4	22
Salmonella + *Shigella flexnerii*	1
Salmonella + *E. coli*—0119:B_{14}	2
Shigella flexnerii + *E. coli*-0011:B_4	2
Total	748

Results. Isolation of strains. During the years 1967–1970, several species of pathogenic enterobacteria were isolated from patients in two hospitals at Curitiba–Parana. A total of 749 pathogenic strains were isolated from 2,103 patients. The frequency with which the different species were isolated is shown in Table I. As can be seen, *S. typhimurium* and enteropathogenic *E. coli* 0:111 B_4 were the most frequent isolates. The incidence of infection with these two species in hospitals A and B is shown in Figs. 1 and 2, respectively. Simultaneous epidemics of *S. typhimurium* and *E. coli* occurred first in hospital A during the second half of 1967. During this period, no infection by these microorganisms was detected in hospital B, suggesting that the outbreak at hospital B may have been transmitted by the medical staff working at both hospitals.

Some of the *S. typhimurium* strains isolated at both hospitals reacted with anti-a sera. These strains have been studied by the following methods.

Serological testing. Slide agglutination tests were carried out with certified Difco antisera. Agglutination tests were also carried out with reference cultures.

The data in Table II summarize the findings with two isolated strains. Strain 1,576 behaved exactly as did the reference *S. typhimurium* (CDC), whereas strain 481 reacted with somatic group B sera as well as with both anti-a and anti-1,2 sera.

Smooth colonies of strains 481, 1,576, and *S. paratiphy* A were isolated from nutrient agar plates and transplanted to semisolid

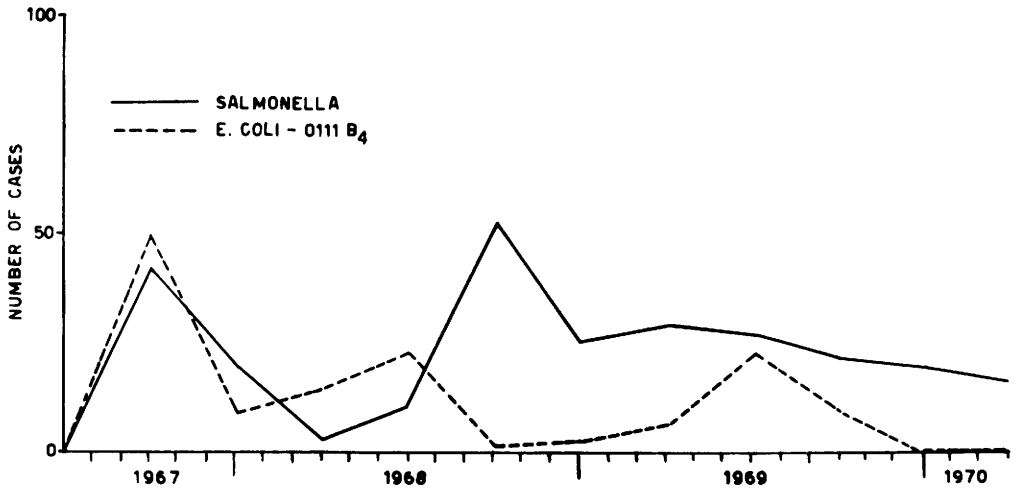

FIG. 1. Incidence of *E. coli*-0111B$_4$ and *Salmonella* in hospital A.

nutrient agar. Somatic and flagellar antigens were prepared from these strains and used for immunization of rabbits.

The flagellar antisera prepared in the laboratory were absorbed with heavy suspensions of *S. derby* C.D.C. (1,4,5,12,-f,g) to avoid interference with somatic antibodies. No agglutination was observed when these absorbed sera were tested with the somatic antigens of strains 481, 1,576, *S. paratyphi* A (C.D.C.), *S. typhimurium* (CDC), and *S. derby* (CDC).

The results of typical slide agglutination tests with these flagellar antisera are presented in Table III. It can be seen that antibodies specific for the flagella of *S. paratyphi* A react with the flagellar antigen of strain 481. The antibodies specific for the flagella of strain 481 also react with *S. paratyphi* A. Nothwithstanding these findings, and despite some similarities between the three antisera, each serum has distinctive properties.

Titrations of these sera with different antigens were also performed, and these results are shown in Table IV. The data indicate that strains 481 and 1,586 can be considered as typical *S. typhimurium* since similar titers were obtained when these antigens were tested with antisera to strains 481 and 1,576. The low titer observed in cross-reactions between *S. paratyphi* A and strain

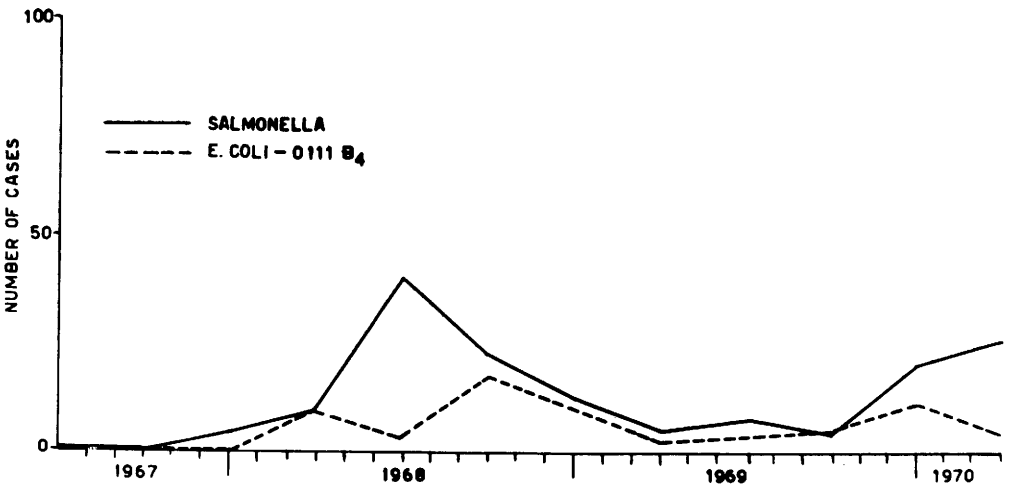

FIG. 2. Incidence of *E. coli*-0111B$_4$ and *Salmonella* in hospital B.

TABLE II. Serologic Reactions of Isolated Strains in Slide Agglutination Tests.

Antigen	*Salmonella* 0 antiserum group A (496311)	*Salmonella* 0 antiserum group B (496311)	*Salmonella* H antiserum group a (496045)	*Salmonella* H antiserum group i (496045)	*Salmonella* H antiserum 1,2 (473871)
Strain 481	0	+++	+++	+++	+++
Strain 1576	0	+++	0	+++	0
S. paratyphi A (C.D.C.)	+++	0	+++	0	0
S. typhimurium (C.D.C.)	0	+++	0	0	0
S. paratyphi B (C.D.C.)	0	+++	0	+++	+++
S. derby (C.D.C.)	0	+++	0	0	0

481 cannot be ascribed to nonspecific reactions since consistent results were obtained with several strains similar to 481. Moreover, the cross-absorption experiments, presented in Table V, indicated that strain 481 possesses an antigen not represented either in *S. typhimurium* or strain 1,576.

Isolation of phases. In order to see if the novel antigen of 481 corresponded to a new phase different from those observed in *S. typhimurium,* the following experiments were performed.

Strain 481 was replated in nutrient agar and well-isolated smooth colonies were transplanted to diphasic semisolid agar. Suspensions were then prepared and tested by slide agglutination with the specific antisera anti-a, anti-1, and anti-2.

From a random sample of 400 isolated colonies, every colony tested except two reacted with these three antisera. The two exceptions reacted only with anti-a and somatic group B antisera. Subsequent replating of these isolated colonies yielded mixed phases reacting with anti-i, anti-a, and anti-1,2 antisera, the isolation by chance of a single phase being extremely improbable.

TABLE III. Slide Agglutination Tests with Anti-Flagellar Sera Prepared in the Laboratory.

Flagellar antigens	Antisera: *S. paratyphi* A (C.D.C.)	Antisera: Strain 1576	Antisera: Strain 481
Strain 481	+++	+++	+++
Strain 1576	0	+++	+++
S. paratyphi A (C.D.C.) (1,2,12-a)	+++	0	+
S. typhimurium (C.D.C.) (1,4,5,12-i)	0	+++	+++
S. paratyphi B (C.D.C.) (1,4,5,12-b-1,2)	0	0	+++
S. derby (C.D.C.) (1,4,5,12-f,g)	0	0	0

Strain 481 was then cultured in a medium containing 3.9 ml of nutrient broth and 0.1 ml of a mixture of equal amounts of anti-i and anti-1,2. Each serum had an agglutination titer of 1/2,000. Overnight cultures in this medium were transplanted to a similar medium consisting of 1.8 ml of nutrient broth and 0.2 ml of the antisera. After five successive transplants in identical media, cultures were transplanted to nutrient agar. Slide testing of suspensions of these agar cultures showed agglutination only with anti-a and anti-group B somatic sera. This phase will be designated hereafter as phase a′.

This phase is not very stable when cultured in the absence of anti-i and anti-1,2 antisera. Subcultures of the phase a′, in nutrient agar without antisera, become reactive with anti-i, anti-1,2, and anti-a sera after 3–5 transplants.

Antibodies specific for phase a′ were prepared by immunizing rabbits with the flagellar antigens derived from isolated phase a′ and absorbing the immune sera with group B somatic antigen (*S. derby* suspension). These antibodies reacted only with strain 481 and *S. paratyphi* A and, after absorption with *S. paratyphi* A suspensions, still ag-

TABLE IV. Agglutination Titers of Flagellar Anti-Sera Tested with Different Antigens.

Flagellar antigens	Flagellar antisera		
	S. paratyphi A (C.D.C.)	Strain 481	Strain 1576
Strain 481	1/1	1/40,000	1/30,000
Strain 1576	0	1/40,000	1/40,000
S. paratyphi A (C.D.C.)	1/2,000	1/1	0
S. typhimurium (C.D.C.)	0	1/40,000	1/40,000

glutinated strain 481.

Typical phases i and 1,2 organisms were also isolated from strain 481 by using the appropriate mixtures of anti-a antibodies and anti-i or anti-1,2 antibodies. Isolated phases on successive subcultures readily yielded organisms pertaining to other phases.

Discussion. The results presented in this report indicate that several strains isolated during an outbreak of *S. typhimurium* infection in two hospitals have three different phases, one of which is not found in typical diphasic *S. typhimurium.*

In a routine laboratory, these strains would be classified as typical *S. typhimurium,* since the results of perfunctory biochemical and serological examinations were identical with those for the reference strains. Moreover, identical titers were obtained when *S. typhimurium* and the isolated strains were tested with anti-i, anti-1,2 sera. These findings provide further evidence for a close relationship between these two organisms. The reactions observed between anti-a sera and some isolated strains could be considered nonspecific since they were extremely low in titer. In fact, those strains have been labeled as *S. typhimurium* by us as well as in other laboratories specializing in studies of Enterobacteria to whom samples of these strains were sent for characterization.

The consistent slide agglutination results obtained with anti-a sera in tests with the isolated strains indicated the need for further study.

Immunization of rabbits with the flagellar antigen of the epidemic strains which cross-reacted with anti-a sera led to the production of antisera reactive with *S. typhimurium,* phase i and phase 1,2, *S. paratyphi* A, and *S. paratyphi* B. These antisera did not react with the somatic antigens of either group A or group B salmonellae. Reactivity of the strains which cross-reacted with anti-a sera persisted even after absorption of the serum with *S. typhimurium* phases i and 1,2, thus indicating the existence of strain-specific antibodies.

The antigenic composition of the flagella of the strains which cross-reacted with anti-a sera can be viewed as composed of three different components. Two of these are common to *S. typhimurium* phase i and phase 1,2. The third component cross-reacted with antigen a from *S. paratyphi.* Not only did the epidemic strains react with anti-a sera but immunization of rabbits induced the production of antibodies which

TABLE V. Slide Agglutination—Reactions of Anti-Flagellar Sera after Specific (Cross) Absorption.

Flagellar antigens	Anti-strain 481 sera absorbed with *S. typhimurium* (C.D.C.)	Anti-strain 481 sera absorbed with strain 1576	Anti-strain 1576 sera absorbed with strain 481	Anti-*S. paratyphi* A sera absorbed with strain 481
Strain 481	+++	++	0	0
Strain 1576	0	0	0	0
S. typhimurium (C.D.C.)	0	0	0	0
S. paratyphi A (C.D.C.)	+	+	0	+
S. paratyphi B (C.D.C.)	0	0	0	0
S. derby (C.D.C.)	0	0	0	0

cross-reacted with the flagellar antigen of *S. paratyphi* A.

In agreement with these results, the isolation of phases from strains reactive with anti-a sera permitted the demonstration of three different phases; namely i, 1,2, and a′, the last one (provisionally designated as a′ for sake of simplicity) by its capacity to cross-react with antigen *a* of *S. paratyphi* A. However, further studies are needed in order to establish its place among the other *Salmonella* antigens.

The data indicate that these epidemic strains possess a novel flagellar antigen. Immunization of rabbits with formolized cultures of phase a′ organisms elicited antibodies that agglutinated only *S. paratyphi* A, and epidemics *phase a′* organisms. No reaction could be observed with group A or B somatic antigens either with phases 1,2 and i organisms obtained from epidemic strains or from typical diphasic *S. typhimurium*. Moreover, preliminary experiments in which flagellar antigens were isolated by the method of Koffler (2) confirmed the flagellar nature of the antigen.

Phase *a′* flagellar antigen is not identical to *S. paratyphi* a antigen, since the organism cannot absorb the *anti-a′* antibodies completely. However, a complete characterization of this novel antigen requires further studies.

Several of the present findings deserve some speculation. With what justification, for instance, is it valid to apply a new name to a given strain of the recently isolated Enterobacteria. Although classification has been highly useful in the past, the practice of ascribing a name to each novel combination of antigens (White and Kauffman) is viewed critically by more and more investigators (3, 4). According to present use, a new name should be ascribed to those strains having the three phases a′, i, and 1,2. However, we feel that the relationship between *S. typhimurium* and the strains under study is so close that assigning a new name to these strains may be premature.

The conventional view that *Salmonella* are diphasic is being revised since Edwards *et al.* (5) showed the natural occurrence of several reversible phases in *S. mikawashima*. Consequently, the presence of a third reversible phase in a strain of *Salmonella* would not be unusual unless, as is here the case, it refers to a widely prevalent and intensively studied species like *S. typhimurium*. The failure to observe this third phase could be explained as follows:

(a) The view that *Salmonella* are diphasic excludes systematic research for other phases, and serological characterization ends when the known phases are encountered. This does not seem to be the case, since in the present investigation only some strains among the 408 which were isolated presented the third phase.

(b) It seems that only some strains isolated from a particular ecological environment may present a third phase. It would therefore be of interest to know whether this finding might not reflect genetic interaction between two particular strains of enteric bacteria, as has been demonstrated to occur *in vitro* (6).

Summary. Strains of *S. typhimurium* possessing three different phases have been isolated from human cases. The flagellar antigen of one of these isolated phases cross-reacts with *S. paratyphi* A flagellar antigen.

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