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ON THE ANTIGENIC RELATIONSHIP OF « VIBRIO CHOLERÆ » TO « ENTEROBACTERIACEÆ »

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RÉSUMÉ

RELATIONS ANTIGÉNIQUES ENTRE « VIBRIO CHOLERÆ » ET LES ENTÉROBACTÉRIES.

Nous avons examiné 120 souches de type Salmonella et 71 de type Arizona, représentatives du groupe Salmonella/Arizona, 33 Escherichia coli, 30 Citrobacter et 75 Proteus, souches qui toutes sont sérologiquement définies, afin de mettre en évidence une relation antigénique entre celles-ci et les Vibrio cholerae Inaba et V. cholerae Ogawa.

Nous avons noté que 25 Salmonella du groupe ON (= 30) ont été agglutinées (à > 1/320) par de l'immunsérum anti-O aussi bien Ogawa qu'Inaba. Toutes les bactéries du type Salmonella ayant seulement l'antigène 30, ont montré une coagglutination avec les vibrions cholériques, alors que les souches ayant un puissant facteur 30, ont réagi plus faiblement. S. bergen, S. bere et S. alexanderplatz (groupe 47₁, 47₂) ont montré aussi une forte relation antigénique avec V. cholerae Inaba et Ogawa. De même, 9 sérotypes du groupe 0:23 de Arizona, qui correspond aux Salmonella 47₁ et 47₂, ont été agglutinés par les sérums anticholérique.

Les souches 9a et 9b:48 de Citrobacter, connues pour être sérologiquement apparentées au groupe 0:30 des Salmonella (30₁ et 30₂) ont également montré une réaction d'agglutination nettement positive à l'égard des deux immunséums anticholériques (à un titre de 640).

Les types 0:43, 0:85, 0:104, 0:105 et 0:150 de E. coli ont été agglutinés très nettement par les sérums anticholériques en particulier les types 0:43 et 0:85 dont les titres ont atteint respectivement 320 et 640.

Trois types de Shigella ont présenté une faible agglutination (à 1/10-1/20). Aucun des Proteus n'a réagi positivement.

MOTS-CLÉS : Vibrio cholerae, Salmonella, Shigella, Escherichia coli, Citrobacter, Proteus, Antigénicité ; Agglutination.

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INTRODUCTION

In August 1944, in the «Kurmarkische Zellwolle und Zellulose A. G.» factory, Wittenberge (Germany) a lot of people was taken ill with diarrhoea and morbid vomiting [9]. Later, it was established that this mass-infection was due to food poisoning by *Shigella flexneri* type 6 (at the time called L-dysentery). Before a bacteriological diagnosis was made, the works medical officer suspected cholera in view of the case history. He wrote «the following can be said about the symptoms of the sickness: morbidly frequent and fluid evacuation of the bowels, similar to cholera cases, accompanied by acute vomiting. Particularly evident is the sometimes enormous loss of water which, for example, in one of my patients amounted to approx. 5 lbs»(*)

Before these people were taken ill, a large amount of dry yeast had been added to the ready cooked canteen meal («on account of its vitamin content») which means that any chance bacteria were not destroyed by the cooking process. For that reason the works medical officer sent to the department of communicable diseases at the Robert-Koch-Institute (Berlin) not only samples of blood, stool, and urine taken from numerous patients but also a sample of the yeast with the request «to examine all samples also for cholera». He considered this request especially justified as he had heard of an outbreak of cholera epidemic at the eastern front near the Donez-basin during 1942-1943 [2, 3].

At that time, when making cholera tests it was customary to suspend the faecal samples in a pepton solution (pH 7.8) and then to spread them on alkaline Pilon plates. Simultaneously, the same material was inoculated on bromthymolblue-metachromeyellow plates, according to Winkle [8]. This method was commonly employed for the diagnosis of typhoid, paratyphoid, enteritis, and dysentery.

By analogy, the yeast was also examined. After an incubation period of 24 hours, the Pilon plates were found to be sterile, except one. The green bromthymolblue-metachromeyellow plates, however, showed a growth of yellow non-suspicious colonies, but also a growth of dubious green colonies.

In the first place the suspicious colonies of the Pilon plates were examined by a slide-agglutination test. They showed a strong agglutination in cholera serum diluted at the ratio of 1/50. A repeat of the agglutination test with other colonies of the Pilon plates gave the same results. There was no agglutination in the polyvalent *Salmonella* and *Shigella* serum nor in the normal serum used for control. Therefore, there was an indisputable agglutination in a diagnostic cholera serum which, however, contained not only O- but also H-agglutinins.

Since *V. cholerae* do not ferment lactose promptly, the green colonies,

(*) The medical officer's suspicions were strengthened by the fact that the blood samples of many patients «were on account of the enormous loss of water only coagulum».
which grew without changing colour on the indicator plate containing lactose (bromthymolblue-metachromeyellow plate), were examined. Contrary to all expectation they proved negative in the slide agglutination with cholera antiserum (*). As against that, the subsequent examination of the non-suspicious lactose-positive yellow colonies gave a strong agglutination in cholera antiserum. This, at least, proved that the suspicious looking germ on the Pilon plate was not V. cholerae, despite of its strong agglutination. After a thorough biochemical analysis the cholera paragglutinative strain proved to be an Escherichia coli with an unusual alkali resistance.

Unfortunately, the strain got lost in the general upheaval at the end of the war so that the reciprocal serological relations could not be defined exactly. One thing, however, was certain, that the possibility of an antigenic affinity exists between cholera vibrios and other enteric bacteria. This is a factor which ought to be taken into consideration every time a serological cholera diagnosis is made.

During the last pandemic we received numerous cholera cultures from various countries affected, and during their examination we were able to observe the presence of such co- or paragglutinations in several cases. Since Enterobacteriaceae exist everywhere and as food poisoning agents, occasionally, produce symptoms similar to cholera, a serologically incorrect diagnosis is quite easy made on account of such agglutinations at the time of an acute cholera scare. A such erroneous diagnosis could have serious consequences.

Having regard to this fact we decided to examine thoroughly and systematically all serologically determined types of the Enterobacteriaceae family with a view to their probable relationship to cholera vibrios.

**MATERIAL AND METHODS**

The following strains were used in this work.

**Salmonella**: 69 serotypes representative of all O-groups and subgroups (1-61) of the Kauffmann-White scheme.

**Arizona**: 42 serotypes representative of all O-groups and sub-groups (1-2-34) of the Arizona-schema after Edwards, Fife and Ewing.

**Citrobacter**: 25 serotypes representative of 23 O-groups of the Citrobacter-schema after Sedlack.

**Shigella**: 32 serotypes representative of all Shigella serotypes with exception of Sh. flexneri X, Sh. dysenterie 8, 9, 10, from the collection of our Institute.

**Escherichia**: 145 strains from the International Escherichia Center, representative of O-groups 01-0150.

**Proteus**: 75 serotypes representative of all groups and sub-groups (01-049) of the Proteus-schema after Kauffmann.

(*) The lactose-negative colonies were Shigella flexneri type 6 about which Winkle has reported in a comprehensive monograph [9].
Primarily, the examination was made by slide-agglutination. We tested 24 hours old agar-cultures in Ogawa and Inaba O-antisera diluted 1/5 (*) in order to check even the slightest antigenic relation. Ogawa O-antisera contains antibodies against factors A and B, and Inaba O-antisera has antibodies against factors A and C.

Incidentally, the O-antigen structure of the El Tor strains, which played such a prominent part in the last pandemic, does not differ from the classical V. cholerae. The serotypes of the El Tor cultures belonging to Ogawa or Inaba cannot be differentiated serologically by agglutination. This means that up till now their differentiation from the classical cholera-types is only possible by biochemical tests (details see Bockemühl [1]).

In cases of positive slide agglutinations, a titration was made in the tube-agglutination, separately employing Ogawa and Inaba O-antisera (1/10-1/1280). The tubes were then incubated in a water bath («bain-marie») for 24 hours at 50° C and then read from the agglutinoscope.

RESULTS AND DISCUSSION

Salmonella/Arizona

The Salmonella serotypes representing groups N (= 30), V (= 44), X (= 47), and group 58 gave slide agglutination with the cholera antisera diluted 1/5. The various serotypes belonging to groups V and 58 and also the corresponding Arizona types with the O-formula 1,3, and 1,33 agglutinated in the cholera-O-antisera in tubes to a 1/20 dilution. Therefore, this is regarded as an unspecific antigenic relationship.

Since S. landau and S. urbana, both belonging to group N, agglutinated in the cholera antisera to a titre 320 and 80 respectively, we subsequently tested 25 other Salmonella serotypes of group N (0 = 30) in our Ogawa and Inaba O-antisera. As expected, all the Salmonella types tested gave a positive slide agglutination but the reaction was not equally strong in every strain. In fact, the nature of O-antigen 30 is complex, and according to Kauffmann and Petersen ([7]) consists of the partial antigens 30₁, 30₂, 30₅. The majority of Salmonella types belonging to the group N have only the factor 30₁.

All species whose O-antigen contains both factors, 30₁ and 30₂, are lysogenic (Le Minor [5]) and contain a converting phage. With the aide of this phage all Salmonella strains with the O-antigen 30₁ can be converted into a 30₅⁺ form, thereby obtaining the complex O-antigen 30₁, 30₂. We observed that all Salmonella types with only the O-antigen 30₁, were strong

(*) The Ogawa and Inaba anti-O — sera employed for the slide — and tube-agglutinations were prepared with strains No. NIH 41 (Ogawa) and No. 35 A 3 (Inaba). We gratefully acknowledge Dr Gallut from the Institut Pasteur, Paris, as supplier of these strains.

For the control of our results, we also tested all co-agglutinative strains with different cholera antisera which we received from other institutes.

An Hikojima O-antiserum was not used, because the type Hikojima holds an intermediate position between Ogawa and Inaba, i. e. it not only has the mutual antigen A but also O-factors B and C specific to Ogawa and Inaba.
cholera co-agglutinative. Strains with a well developed factor 30 reacted proportionately weak.

We observed another strong antigenic relationship between Salmonella O-antigen 47 and the cholera types Inaba and Ogawa. According to Kauffmann and Petersen [4] the Salmonella group 0:47, to which the Arizona groups 0:23 and 0:28 correspond, can be divided into two sub-groups, 47; 47 (S. bergen = 47,47; i.e., n, z15) and 47, 47 (S. kaolack = 47, 47; z1:6). The first corresponds to the Arizona group 0:23 and the latter to Arizona 0:28. It is noteworthy that the serological cross-reactions to cholera referred only to the group 47, 47 and Arizona 0:23 respectively. Groups 47, 47 and Arizona 0:28 reacted practically negative despite their mutual factor (47).

The three Salmonella types belonging to sub-group 47, 47, i.e. S. bergen (47,47; i.e., n, z15), S. alexanderplatz (47,47; z35; — ), and S. bere (47,47; z4, z3: z6) and 9 representative serotypes of the Arizona group 0:23 showed a very strong and characteristic O-agglutination. They formed typical membranes which covered the top of the test-tube and subsequently rolled away from the rim into sausage-like structures. These crumbled into shreds and small fragments when shaken. However, all representatives of the sub-group 47, 47 and of Arizona 0:28 reacted negative in the tube-agglutination (1:10). This agglutination was so typical as it seemed that both cholera-sera were pure factor sera against 0:47, and Arizona 0:23 which for want of a suitably absorbed Salmonella or Arizona antiserum could be used for the group diagnosis 47, and Arizona 0:23 respectively.

On the other hand, suspensions of V. cholerae Ogawa and Inaba, which had been boiled for two hours, showed an agglutination in the antisera of S. bergen and also of Arizona 23:33:25 up to a titre 1:80+ + and 1:160++. Since both Vibrio-types (Ogawa and Inaba) agglutinated positive, it can be assumed that these cross-reactions are due to an antigenic relation to the mutual V. cholerae factor A.

Le Minor et al. [6] found a serological relationship between Yersinia enterocolitica 0:12 and the Salmonella O-antigen factor 47. We received such a Yersinia culture from Le Minor after the termination of our experiments, and as expected proved a positive agglutination in the Ogawa and Inaba antisera.

**Citrobacter.**

The Citrobacter strains 9a, 9b:48, known to be related serologically to the Salmonella group 0:30 [7], showed a distinct positive agglutination in Ogawa and Inaba 0-antiserum. This was verified by the tube-test up to a titre of 640 in both cholera antisera. We noted that these Citrobacter strains showed a strong agglutination in the Salmonella antiserum 30, but not in 30.

**Shigella.**

The Shigella cultures S. flexneri 3, S. flexneri Y and S. boydii 10— had a weak co-agglutination in Inaba antiserum diluted 1:5. The titration
in the test tube with increasing serum dilutions demonstrated an insignificant agglutination of *S. flexneri* Y in the Inaba O-serum to a dilution 1/10 and 1/20 +. Similar results were also obtained with 2½ hours boiled suspensions of the *Shigella* strains for destroying the surface of the antigens.

*Escherichia coli.*

The *E. coli* cultures — 043, 085, 0104, 0105 and 0150 — showed a positive reaction in the agglutination test with Inaba-Ogawa-O-antiserum. Especially types 043 and 085 achieved a high agglutination titre (320 and 160 respectively). For the tube-agglutination tests we used *E. coli* suspensions boiled for 2½ hours to destroy the surface antigens.

*Proteus.*

None of the 75 different serologically determined *Proteus* types gave a positive slide agglutination using a 1/5 dilution of cholera Ogawa and Inaba antisera.

We have already stated that *Enterobacteriaceae*, especially *Salmonella*, frequently produce symptoms similar to cholera in cases of food poisoning. The presence of cholera-paragglutinative gram-negative *Enterobacteriaceae* (in cases of combined diarrhoea and vomiting) could, therefore, lead to diagnostic difficulties and a false diagnosis, particularly in such areas where cholera outbreak is suspected or raging. An additional factor is that cholera-paragglutinative *Salmonella* and *Citrobacter* strains are motile and that the latter are in part lactose negative.

This co-agglutinability of numerous *Enterobacteriaceae* cultures must, therefore, be considered seriously, particularly as it frequently happens that, in suspected cholera infections, relatively inexperienced laboratory assistants have to identify *V. cholera*. The nervousness during such scares and the pressure from the authorities for a quick identification can easily lead to the premature diagnosis of cholera being made only on the basis of a serological examination of the strains.

This knowledge that some bacteria of different *Enterobacteriaceae* genera give significant agglutination in *V. cholerae* antisera means that presumptive identification, made solely on serology, must be confirmed by biochemical identification of the bacteria.

A false cholera alarm can cause unpredictable confusion in the country affected and has most unpleasant restrictions for air traffic and world trade, as well as stringent consequences for human beings like unnecessary isolation and quarantine.

**SUMMARY**

We have examined 120 *Salmonella*— and 71 *Arizona*-types, representatives of the entire Salmonella/Azirona group, 33 *Shigella*, 142 *E. coli*, 30 *Citrobacter*, and 75 *Proteus* types — all defined serologically — for evi-
Evidence of an antigenic relationship with Vibrio cholerae Inaba and Ogawa.

In either Ogawa and Inaba O-antisera, 25 Salmonella types of the O-group N (= 30) showed agglutination (titre up to 320). All Salmonella types having only the O-antigen 30, were cogently cholera-co-agglutinative, whereas strains with a well developed factor 30 reacted proportionally weakly. S. bergen, S. bere and S. alexanderplatz (O-group: 47, 47\textsubscript{b}) also showed a strong antigenic relationship to the V. cholerae Inaba and Ogawa. Similarly, 9 serotypes of the Arizona O-group 23, which correspond to Salmonella 47\textsubscript{a}, 47\textsubscript{b}, agglutinated with cholera-antisera.

The Citrobacter strain 9a,9b:48, known to be related serologically to the Salmonella O-group 30 (30\textsubscript{1}, 30\textsubscript{2}—), also showed a distinctly positive agglutination (= 640).

The E. coli types 0:43, 0:85, 0:104, 0:105 and 0:150 showed a distinct reaction in Cholera antisera. Especially the types 0:43 and 0:85 reached titres of 320 and 640, respectively.

Three Shigella types showed only weak agglutination (at 1/10-1/20). None of the Proteus types reacted positive.


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