O-I Phage Lysis in *Salmonella* Species in India*

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A total of 794 *Salmonella* strains, of 26 serotypes prevalent in India, were examined for sensitivity to O-I phage. Of 597 *Salm. typhi* strains, 13.6% proved resistant to phage lysis. Other serotypes which contained resistant strains are *Salm. anatum*, *Salm. paratyphi A*, *Salm. typhimurium*, *Salm. chester*, *Salm. colombo* and *Salm. matopeni*. The specificity of O-I phage lysis against other Enterobacteriaceae was also tested. The results of the study suggest that resistance to O-I phage lysis in *Salmonella* is more frequent than earlier studies have indicated, and that O-I phage lysis is highly specific for *Salmonella* and should be useful for the detection of new *Salmonella* serotypes.

Susceptibility to phage lysis has been frequently used for the determination of *Salmonella* species. Felix & Callow (1943) found that *Salmonella paratyphi B* O-I phage lysed salmonellae belonging to several other somatic groups. Cherry et al. (1954) stated that O-I phage can be successfully employed in the identification of the genus *Salmonella*. They advocated a rapid and simple technique for its detection with this phage. *Salmonella* infections are very common in India. The causative organism is usually *Salm. typhi* or *Salm. paratyphi A*; the former is responsible for about 88% of enteric infections while the latter is responsible for 6% (Agarwal, Singh & Shrivastav, 1961). The present investigation was undertaken to investigate the utility of phage O-I lysis in detection of *Salmonella* serotypes commonly found in India.

METHODS AND MATERIAL

Organisms examined

Bacterial strains belonging to the family Enterobacteriaceae had been obtained during the previous four years at the National *Salmonella* and *Escherichia* Centre, Kasauli, for identification and serotyping from various hospitals, public health laboratories and veterinary colleges in different parts of the country. The strains were obtained both from human and animal sources. They were isolated from cultures made from blood, urine and faeces. The organisms were identified completely, both biochemically and serologically, and were placed in the appropriate groups: *Salmonella*, *Arizona*, *Shigella*, *Escherichia*, *Klebsiella*, *Aerobacter* and *Proteus*. The main group of organisms tested is *Salmonella*. Some of the *Salmonella* serotypes were tested and identified at the *Salmonella* Reference Laboratory, Colindale, London. Other members of the family Enterobacteriaceae were tested only to determine the specificity of the phage. All the strains had been stored in freeze-dried form.

Media

Phage broth of pH 7.0 was prepared by adding 20 g Lab-Lemco and 7 g sodium chloride to one litre of distilled water and autoclaving at 15 pounds pressure per square inch (1.05 atm.) for 20 minutes. Agar plates were made by adding 2.5% agar to phage broth. These plates were used for testing phage susceptibility. MacConkey plates were employed for isolating single colonies of lactose and non-lactose fermenters.

Transfer and propagation of phages

O-I phage and its propagating strain *Salm. paratyphi B* (phage type I) were the same as used by Cherry et al. (1954). The phage was propagated to a higher titre in broth on its parent host *Salm. paratyphi B*. For this, the broth was inoculated with the parent strain and incubated at 37°C for 6 hours; at the end of this period O-I phage was added to it. The phage-inoculated growth was incubated at 37°C for 18 hours and later kept at 0-4°C for 24 hours. It was filtered through a sintered glass funnel having a poro-
Phage-typing technique

The phage activity was tested using the technique advocated by Cherry et al. (1954). Freeze-dried cultures were opened and 0.5 ml of phage broth was put into the culture tube. A MacConkey's plate was inoculated with the strain so as to give single isolated colonies. This procedure was necessary to select single non-lactose fermenter and lactose fermenter colonies. A single isolated colony was inoculated into 3 ml of phage broth. The inoculated broth was incubated at 37°C for 4-6 hours. Slight turbidity appeared in it at the end of this period. Lawns measuring approximately 2×2 cm were made from these cultures on 2.5% agar plates. Each agar plate was sufficient for making 10-12 lawns. After the lawns had been made the plates were left to dry for five minutes. Each lawn was inoculated in the centre with 0.03 ml of phage suspension by means of sterile fine-bore Pasteur pipettes. The plates were incubated at 37°C for 18 hours. At the end of this incubation period the results were read as lysis (+), thinning (±) and no lysis (—). Strains showing specific biochemical and serological reactions for the Salmonella group but no lysis with phage O-I were seeded again in agar plates and four colonies were retested for phage lysis. A strain was labelled as resistant to phage O-I only when it did not lyse any of the four colonies on retesting.

Agglutination with Vi serum and polyvalent Salmonella serum

All the Salmonella strains were tested for agglutination with Vi serum and polyvalent Salmonella serum during the serological typing.

RESULTS

Susceptibility of various Salmonella serotypes to lysis with O-Phase I

Altogether 794 Salmonella strains were examined; these belonged to 26 Salmonella serotypes. The maximum number of strains was of Salm. typhi (597). Salm. paratyphi A (69) constituted the next largest group. The rest of the serotypes were rather small in number. Serotypes containing strains resistant to lysis were Salm. typhi, Salm. paratyphi A, Salm. anatum, Salm. typhimurium, Salm. chester, Salm. colombo and Salm. matopeni. The results are shown in the table, which also gives the percentage of phage-resistant strains in each serotype. It is significant that among the 597 Salm. typhi strains,

PREVALENCE OF O-I PHAGE RESISTANT STRAINS AMONG SALMONELLA SEROTYPES COMMONLY ENCOUNTERED IN INDIA

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Antigenic structure</th>
<th>Total No. of strains tested</th>
<th>First isolation</th>
<th>Retesting for lysis on 4 colonies</th>
<th>Percentage of resistant strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salm. typhi</td>
<td>9, 12, Vi, d</td>
<td>597</td>
<td>497</td>
<td>100 (16.8%)</td>
<td>13.6%</td>
</tr>
<tr>
<td>Salm. paratyphi A</td>
<td>1, 2, 12 : e</td>
<td>69</td>
<td>65</td>
<td>4</td>
<td>[4.3%]</td>
</tr>
<tr>
<td>Salm. anatum</td>
<td>3, 10 : e, h - 1, 6</td>
<td>8</td>
<td>3 b</td>
<td>2 b</td>
<td>[37.5%]</td>
</tr>
<tr>
<td>Salm. typhimurium</td>
<td>4, 5, 12 : i - 1, 2</td>
<td>24</td>
<td>22</td>
<td>2</td>
<td>4.2%</td>
</tr>
<tr>
<td>Salm. chester</td>
<td>4, 12 : eh - e, n, x</td>
<td>7</td>
<td>6</td>
<td>1</td>
<td>[14.3%]</td>
</tr>
<tr>
<td>Salm. colombo</td>
<td>38 : y - 1, 6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>[100%]</td>
</tr>
<tr>
<td>Salm. matopeni</td>
<td>30 : y - 1, 6</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>[100%]</td>
</tr>
<tr>
<td>Serotypes susceptible to lysis*</td>
<td>86</td>
<td>86</td>
<td>0</td>
<td>...</td>
<td>0</td>
</tr>
</tbody>
</table>

Total | 794 | 681 | 113 (14.2%) | 21 | 92 (11.6%) |

* Percentages in square brackets are based on fewer than 10 strains tested.  
  b Thinning.  
  c Listed in the text.
81 (13.6%) were resistant to phage lysis. The importance of this observation is enhanced when it is realized that in India Salm. typhi are isolated from 88% of patients with Salmonella infection. Among Salm. paratyphi A strains, the next most common serotype in India, 4.3% were resistant to phage lysis. Hence, Salm. paratyphi A strains are less likely to be missed when O-I phage lysis is used for detection of Salmonella species. The proportion of O-I phage resistant strains in other less common Salmonella species—namely, Salm. anatum, Salm. chester, Salm. colombo and Salm. matopeni—seems to be greater, but the numbers of strains tested for species were small. Serotypes susceptible to lysis are Salm. aberdeen (2), Salm. bareilly (4), Salm. bovis-morbificans (5), Salm. brunei (4), Salm. champaign (2), Salm. cholera suis (1), Salm. dublin (1), Salm. enteritidis (7), Salm. newport (2), Salm. paratyphi C (3), Salm. pomona (1), Salm. poona (12), Salm. richmond (13), Salm. salford (1), Salm. sandiego (3), Salm. stanley (2), Salm. virchow (1), Salm. weltevreden (20) and Salm. worthington (2).

Specificity of O-I phage lysis

O-I phage was tested on organisms belonging to several other genera of the family Enterobacteriaceae for its ability to produce lysis. These organisms were Shigella (26), Escherichia coli (106), Klebsiella (28), Aerobacter (40) and Proteus (17).1 O-I phage lysis was observed only in one strain each of Escherichia coli and Aerobacter. The Shigella organisms tested included Sh. boydi (types 1, 2, 3, 4, 5), Sh. flexneri (types 1a, 2a, 3, 4a, 5a, 6, X, Y) Sh. shigae, Sh. schmitzii and Sh. sonnei. Klebsiella strains included the non-motile Aerogenes strains also. Proteus strains included Proteus 0X2, Proteus X19/H, Proteus X19 (warsaw), Proteus 0XK, Proteus 0X19, and Proteus mirabilis. Hence it appears that O-I phage lysis is highly specific for Salmonella organisms.

DISCUSSION AND CONCLUSIONS

Salmonella strains resistant to phage O-I have been reported only infrequently. Cherry et al. (1954) found that some serotypes having a common antigen 35 belonging to somatic group E were resistant to O-I phage. Thal & Kallings (1955) noticed only nine O-I phage resistant strains among 1811 Salmonella strains belonging to 169 different serotypes. Fulton (1955) reported a few phage-resistant cultures of Salm. gatow, while Silliker & Taylor (1957) found that Salm. derby, Salm. typhimurium, Salm. mikawasima, Salm. tennessee and Salm. oregon were resistant to phage lysis. Sharma (1961) encountered a few phage-resistant strains of Salm. pomona and Salm. matopeni. Fulton, Szafran & Lesco (1961) observed that some Salm. telaviv and Salm. plympmouth cultures were negative to lysis by phage O-I while all Salm. champaign and Salm. ramatgan cultures were sensitive to lysis. In our series 14.2% of strains are resistant to O-I phage lysis on single colony isolation. This number is, however, reduced to 11.6% when four colonies of each strain are retested for phage lysis. Thus the over-all incidence of phage-resistant strains in the genus Salmonella is considerably higher than that reported elsewhere. This may be due to a difference in the serotypes tested by us and by other workers. The maximum number of Salmonella strains in our series is of Salm. typhi and 16.8% of them are resistant to phage lysis on first single-colony isolation and 13.6% on retesting on four colonies. Among Salm. paratyphi A and Salm. typhimurium, 4.3% and 4.2% respectively of the strains are resistant to phage lysis. Hence these strains are less likely to be missed when it is sought to identify them with phage O-I. The numbers of certain other resistant Salmonella strains (Salm. colombo and Salm. matopeni) are rather small, and it is difficult to say whether all strains belonging to these serotypes will be resistant to phage lysis. Hence, it seems that if phage O-I is used for the detection of Salmonella species in India about 13.6% of Salm. typhi strains will not be lysed and consequently missed. Therefore, if an organism suspected to be Salmonella does not show lysis with phage O-I, it is necessary that it should be tested serologically with Vi and Salmonella polyclonal O sera. If, however, a positive lytic reaction is present there is a high degree of certainty that the organism is a Salmonella, as the phage O-I lysis is highly specific for Salmonella. Thus we have observed that among 106 strains of E. coli tested for O-I phage lysis there was only one which was susceptible to the phage. Similarly, out of 40 strains of Aerogenes tested lysis occurred in one only. Phage O-I is also non-lytic for the Shigellae, Klebsiella and Proteus groups.

In view of what has been said, the following conclusions seem warranted.

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1 Figures in parentheses indicate the number of strains tested.
1. Sole reliance should not be placed on O-I phage lysis for detection of Salmonella serotypes.

2. Doubtful strains of salmonellae giving no lysis with phage O-I should be tested with Salmonella Vi serum and polyvalent O serum.

3. A strain giving a positive lytic zone with phage O-I is highly likely to be Salmonella, and that should be confirmed by other biochemical and serological tests.

4. O-I phage should be very useful for the detection of new Salmonella serotypes.

ACKNOWLEDGEMENTS

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RÉSUMÉ


La spécificité de lyse du phage O-I contre d’autres organismes appartenant à la famille des Enterobacteriaceae a été également soumise à l’examen.

Les résultats de cette étude incitent à penser que: 1) la résistance des salmonellae à la lyse par le phage O-I est plus fréquente qu’on ne le pensait jusqu’ici; 2) la lyse par le phage O-I est hautement spécifique des salmonellae et devrait être utilisée pour la détection de nouveaux sérotypes.

REFERENCES
