Comparison of the Rates of Motility of *Salmonella* with Those of Other Enteric Bacteria

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Comparison of the rates of motility through a semisolid medium of 16 common *Salmonella* sp., 14 *Escherichia coli* serotypes, 4 *Arizona* strains, 2 *Escherichia freundii* (*Citrobacter*) isolates, 2 *Proteus* sp., and 2 *Pseudomonas* sp. revealed the following.

(i) Very closely related bacteria could demonstrate markedly different rates of progression. (ii) All of the salmonellae tested advanced faster than the *Proteus* and *Pseudomonas* test cultures but some *Salmonella* sp., notably *S. choleraesuis* and *S. typhi*, progressed relatively slowly compared to many other test cultures. (iii) The mean rate of motility for the fastest 14 *Salmonella* sp. (1.49 cm/hr) was not statistically greater than the mean value for the 14 *E. coli* serotypes (1.31 cm/hr) at the 1% level of significance. Selective motility procedures may not be a reliable means of isolating all *Salmonella* sp. from materials contaminated with other bacteria.

Very early in the study of bacteria, it was recorded that some organisms are capable of spontaneous motion whereas others are not, and it was noted that not all motile bacteria exhibit a uniform rate of progression. Bibb (3) reported that the rate of motility is a characteristic of each bacterial species. Later, he recorded (4) that certain paratyphoid salmonellae move more rapidly through semifluid media than do some other members of *Enterobacteriaceae*. Jones and Handley (6) introduced a mixed culture of *Salmonella* and *Pseudomonas* into one arm of a U tube containing a semisolid medium, and they reported that the salmonellae migrated rapidly to the second arm from which they could be isolated in pure culture. Stuart and Pivnick (7) devised a modified U-tube apparatus which contained a semisolid (0.6% agar) selenite-Brilliant Green enrichment medium which encouraged the selective migration of certain salmonellae.

More recently, Banwart (1) described a glassware apparatus with three side U tubes projecting into a central chamber. Semisolid (0.6% agar) media were introduced into the arms only, lactose broth was added into the central chamber, and Brain Heart Infusion broth was introduced above the semisolid media in the side arms. Low numbers of salmonellae and higher numbers of competitive bacteria were introduced into the lactose broth, and within 24 hr of incubation at 37 C the presence of salmonellae could be detected serologically in the fluid above the semisolid agar in at least one of three side arms. In a further study (2), a semisolid medium incorporating 0.8% agar and Brilliant Green was used in the side arms and selenite cystine broth was added into the central chamber. In this form, the apparatus was used to detect *Salmonella* contamination in turkey rolls and fresh chicken parts.

Studies have shown that the comparatively rapid motility of certain salmonellae facilitates their recovery from specimen materials contaminated with competitive organisms. The study presented was initiated to compare the rates of motility of 16 common *Salmonella* sp. with the rates of progression for *Arizona* types, *Escherichia coli* serotypes, *E. freundii* (*Citrobacter*) strains, *Proteus* sp., and *Pseudomonas* sp. to determine whether common salmonellae are more rapidly motile than the contaminant bacteria with which they are often associated.

**MATERIALS AND METHODS**

Simple glass U tubes, with a bore of 2 cm, were obtained, and a line was etched on each arm 10.0 cm from the midpoint of the bend. A cap was placed over each open end.

Motility Test Medium (BioQuest) was used, and 16.5 g was dissolved in 1 liter of distilled water. The prepared medium contained 0.3% agar which had been found in previous trials to be the most suitable value for comparative tests. The sterilized medium was dispensed while still very hot into sterile U tubes to a level approximately 2 cm from the top of each arm. The hot tubes were placed immediately into a refrig-
erator at about 6 C to ensure rapid setting and to discourage any settling of the agar component. Medium was also dispensed in 8-ml lots, in capped test tubes (16 by 150 mm), each containing an open-ended, narrow-bore glass cylinder 8 cm in length, and all cultures were maintained in such motility tubes and subcultured daily for 5 days before testing to ensure a uniform procedure and optimum motility. A test culture was collected from a 24-hr motility tube in a Pasteur pipette, and one drop was deposited onto the medium in one arm of each of five U tubes. By using a sterile wire, the drop was mixed into the top 1 cm of medium. The rack of five U tubes was placed in an incubator at 37 C. Each test isolate was permitted to migrate some 5 to 7 cm through the medium before the position of the leading edge was marked and the time was recorded. The leading edge of each motile culture was clearly visible as a line perpendicular to the wall of the tube dividing the clear amber medium and the turbid zone of bacterial growth. Each culture was allowed to progress for approximately 30 cm, after which its leading edge location was marked on each of the five test U tubes and the time was recorded. Each culture was reisolated from the test medium, its purity was checked, and its identity was reconfirmed biochemically or serologically, or by both methods.

RESULTS AND DISCUSSION

A total of 40 motile pure cultures were tested, and the mean rate of motility for each culture was calculated from the results of five individual tests. Figure 1 shows the rates of progression of the organisms studied. An E. coli strain proved to be the most rapidly motile culture tested, but 7 of the fastest 10 test cultures were common Salmonella species. A strain of S. blockley which progressed at 1.72 cm/hr was the most rapidly motile Salmonella culture, whereas S. typhi was slower than any E. coli tested and progressed at only 0.78 cm/hr. Stuart and Pivnick (7) recorded that S. typhi took more than twice the period of time to traverse the test medium in their apparatus than did nontyphoid salmonellae. S. choleraesuis migrated at the comparatively slow rate of 1.01 cm/hr. Conspicuous variation in the migration rates of different E. coli serotypes and Salmonella sp. was noticed, and neither genus clearly appeared to be more rapidly motile than the other. The mean rate of progression of the 16 salmonellae (1.37 cm/hr) was not found to be statistically significantly greater than the mean rate of the 14 E. coli (1.31 cm/hr). When the results for S. choleraesuis and S. typhi were omitted from the data, statistical analysis revealed that the mean rate for the remaining 14 Salmonella sp. (1.49 cm/hr) was not significantly greater than the mean rate for the E. coli strains tested at the 1% level of significance.

The rate of motility appeared to be a characteristic of individual strains rather than a characteristic of a species or genus. Two isolates of S. typhimurium which were different phage types showed dissimilar rates of progression, as did serotypes of E. coli with like somatic antigens, two E. freundii (Citrobacter) strains, and two strains of Arizona 7:1,7,8. A motility method for differentiating salmonellae from Citrobacter and Arizona strains did not seem feasible.

Motility systems might prove useful for isolating salmonellae from specimen materials contaminated with Proteus or Pseudomonas, or both, because results indicated that Proteus mirabilis, P. vulgaris, Pseudomonas fluorescens and P. aeruginosa moved very slowly through the semisolid medium. Such slow progression might be due to the conditions of relatively low oxygen tension which prevail deep within the medium and which discourage the metabolism of Proteus and Pseudomonas, whereas the activities of salmonellae are not inhibited.

Multiple passage before inoculation of test U tubes encouraged enhanced motility of all cultures. Within the test apparatus, motile bacteria progressing into fresh medium might not maintain a uniform motility due to an individual sensitivity to oxygen tension or due to an inherent ability to evolve highly motile strains during passage through the semisolid agar. The rate of progression of several cultures was recorded for the initial 4 hr of a test and compared with the rate for the final 4 hr. Results indicated that whereas some cultures displayed an enhanced final rate (S. paratyphi B, 5%; E. coli 055:K59:H6, 3%), the final rate of others was depressed (E. coli 086:K62:H2, -3%; E. coli 08:K45:H9, -14%).

S. gallinarum, S. pullorum (standard and variant strains), and Shigella sonnei are nonmotile bacteria. These cultures were inoculated into the motility test apparatus and were found to have progressed no more than a few millimeters after several days of incubation.

In a pilot project, prior to the study presented, tests were made with the selected medium to determine the agar content most suitable for comparative motility studies. A medium with an agar content of 0.4% or higher provided poor adhesion between the medium and the glass wall. Use of a semisolid medium containing 0.3% agar was found to be satisfactory for comparative motility studies because no seeping of a test culture was observed.

Specimen materials submitted to a diagnostic laboratory for the isolation of Salmonella or Arizona are often grossly contaminated with E. coli strains and other competitive enteric bacteria. Results of this study showed that many extraneous organisms progressed more rapidly than
certain common Salmonella sp. and Arizona strains. The test procedure provided useful comparative results but could not be recommended for effecting extensive isolation of Salmonella sp. from mixed cultures. Use of a suitable semisolid medium which selectively encourages the growth of motile salmonellae could be a useful method of isolating certain salmonellae efficiently, but the agar content of the medium and the length of the column proved to be factors which require careful consideration. Effective use of comparative motility for efficient isolation of fastidious members of the Salmonella group remains to be demonstrated. Recovery of important nonmotile species, such as S. gallinarum, S. pullorum, and some nonmotile strains which occur occasionally, could not be accomplished in motility systems.

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LITERATURE CITED

1. Banwart, G. J. 1968. Glassware apparatus for determining motile bacteria. Poultry Sci. 47:1209-1212.

2. Banwart, G. J. 1969. Rapid detection of salmonella in turkey rolls and on fresh chicken parts. Poultry Sci. 48:1528-1532.

3. Bibb, L. B. 1925. Long tube method of cultivating microorganisms with observations on mobile colonies in liquid medium. J. Bacteriol. 10:561-567.

4. Bibb, L. B. 1927. Differentiation of intestinal organisms by means of semifluid sugar media. J. Bacteriol. 13:123-128.

5. Ino, J., and C. D. Graber. 1955. Recovery of salmonella from contaminated cultures. U.S. Armed Forces Med. J. 6:586-587.

6. Jones, R. F., and W. R. C. Handley. 1945. A selective medium for the isolation of salmonella bacilli from heavily contaminated material. Mon. Bull. Min. Health Lab. Serv. 4:107-111.

7. Stuart, P. F., and H. Pivnick. 1965. Isolation of salmonellae by selective motility systems. Appl. Microbiol. 13:365-372.