invasive GAS infections are necessary to confirm this observation.

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References


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Preservation of Vibrio cholerae by Suspension in Normal Saline

Dear editor,

Sporadic cases and small outbreaks of cholera continue to occur in Asian countries. Epidemiological and microbiological studies demand preservation of the isolated strains in the region. Vibrio cholerae can be preserved by lyophilisation and at ultra low temperature [1,2] however, this facility may not exist in most laboratories. Members of Enterobacteriaceae family can be well preserved on egg media or by stab method [3] but not V. cholerae (personal observation). Survival of V. cholerae for more than a month is not possible even on nutrient agar slants stored at 4°C. Our accidental observation that V. cholerae suspended in normal saline pH 8 and kept at 4°C retained viability for more than six months prompted us to study preservation of suspension of V. cholerae in normal saline with pH 8 solution stored at 4°C. The pH of the normal saline is adjusted to 8 because vibrios prefer alkaline pH and are susceptible to acid pH.

Classical V. cholerae 569B Inaba procured from Haffkine institute, Mumbai, India was received as lyophilized culture. Nine isolates of V. cholerae El Tor Ogawa isolated during 2003-2006 from cases of cholera in Indore city and preserved in 15% glycerol containing nutrient broth (Hi Media, India) at −70°C were included in the study. The nutrient agar (Hi Media, India) slants were inoculated with cultures and incubated overnight at 37°C. The growth on nutrient agar slant was harvested in 5 ml of the normal saline pH 8 and the opacity adjusted to 0.5 at 635 nm using colorimeter (ERBA Japan). The suspensions were aliquoted in 9 mL amount in 10 mL injection vials in triplicate. The vials were capped with rubber bungs, sealed with aluminium caps and stored in domestic refrigerator. The temperature was recorded every morning and evening round the year and was in the range of 4-8°C.

The initial mean count for standard strain of V. cholerae 569B Inaba and other clinical isolates of V. cholerae were 0.8-1.5 × 10⁶ CFU/mL in normal saline. The suspension of standard strain of V. cholerae 569B Inaba and isolates number 4 and 8 (randomly selected) were subjected to viable counts [1] on the day one and subsequently once a month for 12 consecutive months. One hundred micro litre of the ten fold dilutions were plated on nutrient agar plates in duplicate and the plates showing colonies 50-200 were used for the viable count estimation. The remaining culture suspensions were subjected to viable count on day one and then after 12 months. The viability study results for the standard strain 569B and isolate number 4 and 7 were similar (Figure).

The colony morphology remained typical after revival from normal saline and the subculture at the end of one year did not show any change in staining and biochemical characters. The antigenic properties with regards to agglutinability with the antisera remained the same. The mean counts of surviving bacteria among the nine isolates of V. cholerae were 0.8-1.8 × 10⁴ CFU/mL in normal saline.
Thus, consistent survival of \textit{V. cholerae} was seen in normal saline with pH 8.

The suspensions in normal saline showed almost four log fall in 1 month of storage. However, subsequently there was no marked fall in the viability during an observation period of 12 months. Initial fall in viable count from $10^9$ to $10^4$ CFU/mL at the end of 1 month is possibly due to accumulation of lethal metabolites and/or depletion of nutrients. However, the subsequent loss in viability in normal saline was minimal till 12 months. The survival of vibrios could be due to very low metabolic activity without autolysis in the suspending fluid. The storage in domestic refrigerator is practical for any average microbiological laboratory in the economically developing countries that do not have freezers or lyophilizing equipment for preservation and the simple method will prove valuable for preservation of \textit{V. cholerae}.

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Prevalence of Syphilis and Biological False Positive Reactions in VDRL Test among Injecting Drug Users: A Preliminary Study

Dear editor,

Injecting drug users (IDUs) are a population at increased risk of developing syphilis. In developing countries screening for syphilis is done by non-treponemal tests (VDRL/RPR). Asymptomatic persons and patients with atypical presentations require confirmation by treponemal tests like \textit{Treponema pallidum} haemagglutination (TPHA). VDRL is a standardised, economical, sensitive and easy to perform test but one major problem with this test is that it is associated with false positive reactions. Foreign studies on IDUs have reported more than 10% incidence of false positive results. There is paucity of similar studies in India. Therefore the present study was conducted to address this issue.

Serum samples collected from 150 IDUs (April 2003-March 2005) were subjected to VDRL test by standard method. Samples reactive in VDRL test were then subjected to TPHA test (TPHA-200 Kit, Kentford, UK). The samples reactive in both the VDRL and TPHA tests were interpreted as positive for syphilis.

Out of 150 serum samples tested, 6 (4%) were found to be reactive by VDRL test. The antibody titre was >8 in two (R32; R128) and <8 in the remaining four. TPHA test was reactive in 2(1.3%). These two TPHA reactive samples were also reactive in VDRL test in titre >8. Therefore, the