Serological Cross-Reaction between *Legionella* spp. and *Capnocytophaga ochracea* by Using Latex Agglutination Test

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Cross-reactivity between *Legionella* spp. and *Capnocytophaga ochracea* was noted by latex agglutination tests (Serobact Legionella; Disposable Products, Adelaide, Australia). Four of 11 (36%) *C. ochracea* isolates agglutinated with latex reagents designed to identify *Legionella pneumophila* serogroups. *C. ochracea* isolated on buffered charcoal yeast extract media may give false-positive results in this *Legionella* latex agglutination assay.

Serological assays for the detection and identification of *legionellae* include immunofluorescent and agglutination techniques (1, 10, 13). These methods use pooled monoclonal or polyclonal antisera which react with *Legionella* antigens. The specificities of these tests are high for isolates from individuals with pneumonia (12), but for those from patients with other clinical symptoms, the specificities are unknown. Organisms that have been reported to cross-react with polyclonal anti-*Legionella* immunofluorescent reagents include various *Pseudomonas* spp., *Bordetella pertussis*, and *Bacteroides* spp. (4, 7, 11). In addition, apparently significant rises in antibody titers to *Legionella pneumophila* in serum have been reported for a variety of conditions including rickettsial infections and sepsis from *Bacteroides* spp. and *Citrobacter freundii* (6, 11).

An organism cultured from the sputum of a patient with chronic obstructive lung disease who was receiving prednisone at 15 mg daily was provisionally identified as a *Legionella* organism in our laboratory by a latex agglutination test (Serobact Legionella; Disposable Products, Adelaide, Australia). Colonies were visible macroscopically after 72 h of incubation on supplemented buffered charcoal yeast extract (BCYEα) medium (11) and displayed pink iridescence with the characteristic ground-glass appearance under a dissecting microscope. Further testing indicated that this isolate did not require cysteine for growth and was therefore not a *Legionella* spp.; it was identified as *Capnocytophaga ochracea*. We subsequently investigated potential cross-reactivity with *Legionella* antisera by examining an additional 12 isolates of *Capnocytophaga* spp. by this technique.

Stock isolates of *Capnocytophaga* spp., including the index organism, were obtained from a collection in our laboratory. These had previously been identified as *C. ochracea* (11 isolates) or *Capnocytophaga canimorsus* (2 isolates) by standard methods (9). Six *C. ochracea* strains were obtained from cultures of gingival specimens and three were obtained from sputum. Two *C. ochracea* and two *C. canimorsus* isolates were from cultures of blood. None of the patients from whom the isolates were obtained had clinical or radiological evidence of pneumonia. The isolates were subcultured onto BCYEα medium and were incubated at 36°C in a humidified aerobic atmosphere for 48 to 72 h. The organisms were additionally subculutred onto horse blood agar (HBA); isolates were then prepared for examination by latex agglutination.

Serobact Legionella comprises two latex test suspensions: one contains a polyclonal *L. pneumophila* serogroup (SG) 1 antibody and the other contains a mixture of polyclonal antibodies against *L. pneumophila* SG 2 to 14. The polyclonal *Legionella longbeachae* latex antibody was obtained from the Institute of Medical and Veterinary Science, Adelaide, Australia. One colony was selected from the BCYEα plates and was emulsified in isotonic saline onto a Serobact slide. The manufacturer's instructions for slide agglutination were followed. Each isolate was tested with all three reagents. A scale was used to grade the agglutination intensity from 1+ (just visible agglutination) to 4+ (maximal agglutination). Reactions were scored by comparison with the results obtained with suspensions of known *L. pneumophila* SG 1, *L. pneumophila* SG 4, and *L. longbeachae*. Isolates that gave weak reactions were retested immediately. Organisms grown on HBA were also tested on the same day. The organisms were then examined with a polyvalent (MarDx; Baxter Diagnostics, Archerfield, Australia) and a monoclonal (Genetics Systems; Sanofi Diagnostics Pasteur, Sydney, Australia) direct immunofluorescent-antibody test according to the manufacturer's directions. The entire procedure was performed on two separate occasions to ensure reproducibility.

All *Capnocytophaga* isolates grew on BCYEα medium after 48 to 72 h of incubation. Four (one from blood culture, one from a gingival specimen, two from sputum specimens) of 11 (36%) isolates of *C. ochracea* produced strong agglutination (4+) with one or both reagents directed against *L. pneumophila* (Table 1); four with *L. pneumophila* SG 1 antibody and two with *L. pneumophila* SG 2 to 14 antibodies. The reaction with the *L. longbeachae* reagent observed with strain 93-74076 was weak (1+). Positive reactions occurred within 30 s in all cases; autoagglutination did not occur. Identical reactions were seen with isolates grown on HBA. Neither *C. canimorsus* isolate produced agglutination. Fluorescence was not observed on subsequent examination of the isolates by either of the direct immunofluorescent-antibody tests.

The cross-reactivity of bacteria with *Legionella* antisera has been described previously, predominantly with the use of polyclonal anti-*Legionella* immunofluorescent reagents used for the detection of *Legionella* spp. (7, 11). This is believed to occur as a result of shared lipopolysaccharide antigenic determinants on the surface of *L. pneumophila* and other gram-negative bacteria (3). These reactions are serogroup specific and are not detected with the negative control antibody. Rapid
identification of \textit{L. pneumophila} by the alternate technique of latex agglutination was first described in 1984 (2). Cross-reactivity between \textit{L. pneumophila} serogroups 1 and 12 was observed in an evaluation of one commercial latex product (MicroScreen Legionella Latex; Mercia Diagnostics, Guildford, United Kingdom) (10); non-\textit{Legionella} isolates including several \textit{Pseudomonas} spp. and \textit{C. freundii} did not react with \textit{Legionella} antisera. Our observations, however, indicate that \textit{C. ochracea} can cross-react with \textit{L. pneumophila} antisera. Serobact Legionella has recently been launched in Australia as MicroScreen Legionella (7a); laboratories intending to use this kit should be aware of possible cross-reactivity with \textit{Capnocytophaga} spp. The reaction with \textit{L. longbeachae} was equivocal. 

In our hands, testing of a single colony produced visible agglutination of latex particles. The manufacturer’s protocol suggests that several colonies be used for each test; however, more than one \textit{Legionella} species or serogroup may be present on culture plates, leading to unreliable results. It has been proposed previously that one colony be examined when suspected legionellae are tested from direct culture (10). Our findings support this recommendation.

\textit{C. ochracea} is a gram-negative, capnophilic organism which is a member of the indigenous oral flora. It has been implicated as a primary cause of gingivitis, lung abscess, and empyema (8) and can be isolated from the sputum of immunocompromised hosts (5). It is capable of growing on media used primarily for the enhanced recovery of \textit{Legionella} spp. and resembles legionellae morphologically and in its growth characteristics. Isolation of \textit{Capnocytophaga} spp. from respiratory specimens may cause confusion in the microbiology laboratory, although it is isolated at a low frequency. To our knowledge, this is the first description of \textit{C. ochracea} cross-reacting with \textit{L. pneumophila} antisera. Potential cross-reactive epitopes common to \textit{Capnocytophaga} and \textit{Legionella} spp. have not been identified. The latex agglutination assay is quick, sensitive, and easy to use. However, as in the case of fluorescent-antibody staining, it should not be used as the only method of identification of legionellae. Laboratories that use these tests to screen isolates should regard positive results as preliminary pending culture confirmation by conventional methods.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
Isolate & Source & \textit{Latex} \textit{pneumophila} & \textit{Latex} \textit{pneumophila} & \textit{Latex} \textit{longbeachae} \\
& & SG 1 & SG 2 to 14 & \\
\hline
91-32619 & Gingival & - & - & - \\
91-34123 & Gingival & - & - & - \\
91-61758 & Gingival & - & - & - \\
91-92379 & Gingival & 4+ & 4+ & - \\
93-48315 & Sputum & 4+ & - & - \\
93-73020 & Blood & 4+ & - & 1+ \\
93-74076 & Blood & - & - & - \\
93-79301 & Sputum & 4+ & 4+ & - \\
91-1220 & Gingival & - & - & - \\
91-4980 & Gingival & - & - & - \\
\hline
\multicolumn{2}{|l|}{* – and +, absence and presence of agglutination, respectively.}
\end{tabular}
\caption{Cross-reactivities of \textit{C. ochracea} isolates with latex agglutination reagents}
\end{table}

\textbf{REFERENCES}


7a. Oxoid Australia (Melbourne, Australia). Personal communication.


