We evaluated the contribution of amoebic coculture to the recovery of *Legionella* spp. from 379 respiratory samples. The sensitivity of axenic culture was 42.1%. The combination of axenic culture with amoebic coculture increased the *Legionella* isolation rate to 47.1%. Amoebic coculture was particularly efficient in isolating *Legionella* spp. from respiratory samples contaminated with oropharyngeal flora.

*Legionella* spp. are facultative intracellular Gram-negative bacteria that are ubiquitous in natural and man-made aqueous environments, in which they survive as free-living bacteria or, more commonly, as intracellular forms in amoebae (1). When humans inhale contaminated aerosols, legionellae can infect and replicate within lung macrophages and cause a severe pneumonia referred to as Legionnaires’ disease (LD).

Urinary antigen detection is the first-line diagnostic test, although this test is limited to *Legionella pneumophila* serogroup 1 (Lp1) (7). Molecular techniques improve LD diagnosis by detecting other serogroups and species. Nevertheless, isolation of *Legionella* strains is required to perform further epidemiological investigations. The sensitivity of axenic culture ranges from 15% to 90%, depending on the *Legionella* inoculum, the level of contamination of the samples with oropharyngeal flora, the prior use of antibiotics, and the experience of the laboratory members (2, 4). Several authors have described amoebic coculture as a method to recover *Legionella* spp. from culture-negative specimens (3, 8, 10–12). However, large-scale studies evaluating the benefit of amoebic coculture in routine laboratory practice are lacking.

In this work, we evaluated the contribution of amoebic coculture to the recovery of *Legionella* spp. from 379 respiratory samples collected over a period of 32 months. This prospective study included 348 patients with suspected LD who were admitted to 98 French hospital facilities from April 2008 to November 2010. An LD case was defined as a patient with clinical and/or radiological findings compatible with pneumonia from Respiratory Samples: Prospective Analysis over a Period of 32 Months

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Identification of *Legionella* spp. was performed by latex agglutination (Oxoid, bioMérieux); the Lp1 isolates were typed using Dresden monoclonal antibody (MAb) subgrouping (6) and sequence-based typing (5, 9).

Among 348 patients, 222 cases of LD corresponding to 240 samples were confirmed. The *Legionella* urinary antigen assay was positive in 217 cases (sensitivity, 98%). A total of 113 *Legionella* isolates were recovered by axenic culture and/or amoebic coculture (Table 1). Axenic culture isolated 101 isolates were recovered by axenic culture only. These results suggest that amoebic coculture recovered 12 additional strains (11 Lp1, 1 Lp8). Among these 12 strains, amoebic coculture demonstrated lower sensitivity than axenic culture (33.8% versus 42.1%, *P* = 0.004; chi-square test). Performing the amoebic coculture weekly and refrigerating and pretreating the samples may have impaired *Legionella* viability and reduced the actual coculture isolation rate. However, amoebic coculture recovered 12 additional strains (11 Lp1, 1 Lp8) and increased the global isolation rate to 47.1%. The 12 corresponding samples were all highly contaminated with oropharyngeal flora, in comparison to 12% (6/52) of the samples recovered by axenic culture only. These results suggest that amoebae supporting *Legionella* growth eliminated the interfering oropharyngeal flora (10). Among these 12 strains, amoebic coculture recovered 2 from samples from patients negative for antigenuria (1 Lp1 Knoxville, 1 Lp8). No significant difference between the performance of *A. castellanii* and that of *A. polyphaga* was observed (the sensitivities of cocultures with these amoeba species were 30% and 36%, respectively [*P* = 0.29; chi-square test]). The type of sample significantly impacted the performance of axenic culture and amoebic coculture (Table 2). The highest sensitivities were obtained with BAL fluid by both methods. No significant difference in the sequence type or MAb subgrouping of the strains isolated by one or the other method was observed.

In this study, we showed that the combination of amoebic coculture with axenic culture enhanced the rate of *Legionella* isolation from respiratory samples and allowed further epidemiological investigations. Amoebic coculture was particularly efficient in isolating *Legionella* spp. from respiratory samples contaminated with oropharyngeal flora and may be systematically applied to such samples.

**REFERENCES**