Legionnaires' disease pseudoepidemic due to falsely-positive urine antigen tests.

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Legionella pneumophila (Lp) is an opportunistic human pathogen that can cause a severe respiratory syndrome known as Legionnaires’ Disease (LD). Lp accounts for 96% of all LD cases notified in Europe (1). Although culture of respiratory secretions and serology are methods also used for diagnosing LD, urinary antigen test (UAT) is easy and fast to perform, consequently ca. 77% of cases are diagnosed using this method (1). Several UATs have been developed with high specificity but variable sensitivity, most of them are only able to reliably detect Lp serogroup 1 antigen (2-4). In this brief report we describe a pseudo LD outbreak due to false positive UAT results. Between December 2012-January 2013, 18 community-acquired LD cases were notified by a University hospital in Rome.

All cases were living in Rome and were diagnosed using one particular UAT assay (ThermoFisher Oxoid Xpect®, Basingstoke, UK) in two independent laboratories of the same university hospital (the emergency department laboratory – hereby Lab 1- and the central hospital laboratory- hereby Lab 2).

Alerted by an increased number of notified cases, the Local Health Authority started an epidemiological investigation in December 2012. A questionnaire was used to interview each patient, collecting clinical data, individual risk factors, and possible exposures. Interviews showed that the patients' houses were located all over the city, and no common exposures were identified. By the end of January 2013 the outbreak was considered improbable since no common source or epidemiological link was found among the 18 LD cases and no further cases were reported in any other hospital in Rome.

An incorrect laboratory diagnosis was hypothesized. The Hospital Infection Control Team (HICT) was requested to verify which assay was used for the laboratory diagnosis of each case. Furthermore, a careful revision of clinical records of all LD cases was recommended. The in-depth analysis highlighted that only 9 out of the 18 reported cases fulfilled the current case definition (clinical and laboratory criteria). Despite the presence of clinical criteria, nine patients did not fulfill the case definition because the same UAT assay (ThermoFisher Oxoid Xpect®, Basingstoke, UK) performed by...
both Lab 1 and Lab 2 gave discordant results (Lab 1 positive and Lab 2 negative). For some patients, both Labs used the same UAT lots.

Respiratory secretions, sera and urine samples were available from 4 of the 9 patients with discordant test results. In order to confirm the diagnosis, they were sent to the National Reference Laboratory for Legionella (NRL). Urine samples were boiled, centrifuged and then tested by both Xpect® and Binax NOW® (Alere, USA) UATs. All samples gave negative results. Therefore, only 9 cases were classified as confirmed.

As a precautionary measure the HICT decided to withdraw the Xpect® UAT from the laboratories and to replace it with two other commercially available kits. Following this, the incidence of LD later decreased to the expected hospital values. In mid February 2013, an urgent security warning was issued by the ThermoFisher Oxoid Company about the use of 3 Xpect® lots associated with possible false positive results, inviting the users to re-evaluate the results obtained and consider running the tests again. One of the 3 lots was found to be among those used by the two laboratories of the university hospital. Specificity of UATs in detecting Lp 1 is known to be very high (97-100%), with a low probability of false positive results (2-4). Enhanced surveillance allowed the prompt identification of this false cluster and the implementation of corrective measures earlier than the official communication from the UAT producer.

This experience highlighted two important issues in the diagnostics of LD that should be considered when suspecting an outbreak. Firstly, the reliability of a test is never absolute. Results obtained can be impaired depending on the quality of the test but also on the procedures applied by different operators, while the application of standard operating procedures might help to achieve uniformity of performance of the test. Therefore, during the outbreak investigation, the NRL as per the recommendations of the ECDC Legionella Reference Laboratory at Public Health England-London, provided laboratories with
a modified protocol to have a better performance of immune-chromatographic tests (unpublished data).

All positive urine samples should be re-tested after boiling for 5min and centrifugation (5’ at 12000×g) and only the supernatant should be tested for Legionella antigen (5). Secondly, that the diagnosis of LD should always be based on complementary assays such as culture, serology, and in future PCR, once this method will have been validated in inter-laboratory studies (6,7).

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