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Short communication

Population structure of *Legionella* spp. from environmental samples in Gabon, 2013

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ABSTRACT

Aquatic environments are the most important source for *Legionella* spp. infections such as Legionnaires' disease and Pontiac fever. The reservoirs of *Legionella* spp. are mostly unclear in sub-Saharan Africa. The aim of this study, conducted in 2013, was to identify geographical areas of an increased risk for exposure to *Legionella* spp., and to describe the population structure of *Legionella* spp. from different water sources in a cross-sectional study in Gabon. Fresh water samples ($n = 200$) were cultured on *Legionella* selective agar; species were confirmed by MALDI-TOF, a *Legionella pneumophila* specific real-time PCR and 16S RNA gene sequencing. Serogroups were identified by agglutination test. The population structure was assessed by multilocus sequence typing (MLST).

Legionella spp. isolates ($n = 29$) were frequently found in the hospital setting particularly in hot water systems. Open water bodies (i.e. rivers, lakes) were not contaminated with *Legionella* spp. Isolated *L. pneumophila* mainly belonged to serogroups 2–14 ($n = 19$) and MLST sequence type ST1, ST75 (and related STs) and ST1911.

In conclusion, hospitalized patients might have an increased risk to become infected with *Legionella* spp. in the studied areas in Gabon, particularly if they have risk factors such as comorbidities. Both broadly extended (ST1, ST75) and local lineages (ST1911) were present in our setting.

1. Introduction

Natural reservoirs of *Legionella* spp. are aquatic environments. Humans usually get infected by the inhalation of contaminated aerosols and might develop the severe pneumonic Legionnaires' disease or the self-limited Pontiac fever (Whiley et al., 2014). Risk factors are comorbidities, immunosuppression and age (Whiley et al., 2014). *Legionella pneumophila* is the most common species causing legionellosis with a predominance of serogroup 1 which accounts for almost 80% of isolates from Legionnaires' disease (Helbig et al., 2002). Other *Legionella* spp. (i.e. *Legionella dumoffii*, *Legionella anisa*) can also cause pneumonic infections (Muder, 2000).

Epidemiological data on *Legionella* spp. from environmental and clinical samples are limited and partly contradicting in sub-Saharan Africa. Few studies point towards a high prevalence in surface water in South Africa (73–75%) and Nigeria (67.3%) (Alli et al., 2011; Bartie et al., 2003; Dobrowsky et al., 2014). In contrast, legionellosis is infrequently reported. No case of *Legionella* infection was detected in patients with community acquired pneumonia in Gabon and only one case was detected in patients with influenza-like illness or severe acute respiratory infection in Kenya respectively (Kim et al., 2012; Lassmann et al., 2008). This is in contrast to South Africa where *Legionella* spp. was the fifth most frequent cause of pneumonia in patients that required hospital admission (Potgieter and Hammond, 1992).

Knowledge about reservoirs of *Legionella* spp. is important to assess the potential risk of infection particularly in older people with comorbidities and to develop strategies for infection control and prevention. As this information is missing for Central Africa, we performed a cross sectional study on the burden of *Legionella* spp. in water bodies in Gabon. The aim was to identify

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geographical areas of increased risk for exposure and to describe the population structure of *Legionella* spp. in the aquatic environment in Gabon.

2. Materials and methods

2.1. Water samples

Water samples were drawn from randomly selected water sources at seven sites in four provinces in Gabon (Estuaire, Moyen Ogooué, Ogooué-Ivindo, Ngounié) from April to October 2013 (Table 1). Samples were taken from households or institutions after receiving permission from the person in charge.

From each sampling site, 500 ml of water were collected in sterile, screw capped glass bottles containing a final concentration of 0.16 mM sodium thiosulphate. Samples from water taps and standpipes were aseptically collected after water was allowed to run for at least 1 min. In case of hot water systems, two samples were drawn (first cold then hot water). Samples were stored in a cool box and analyzed within 4 h after sampling. For each sample, the type of water source (e.g. tap water, shower, standpipe, borehole, open water body) and temperature of the water sample were recorded. The study protocol was approved by our local scientific review committee (SRC 2013.01).

2.2. Sample processing and culture

Trained laboratory technicians performed the microbiological analysis of samples. The laboratory successfully participates in regular external quality assessments (Alabi et al., 2013). Water samples were analyzed using a modified protocol of the International Organization for Standardization (ISO 11731 and ISO 11731-2) (International Organization for Standardization (ISO), 1998). Briefly, 500 ml of each water sample was filtered using a 0.45 µm membrane filter (Millipore Merck, Billerica, Massachusetts, USA). To suppress the growth of non-*Legionella* spp. organisms, the filter was treated with 20 ml of 0.2 M hydrochloride/sodium chloride buffer (pH 2.2) for 5 min and was rinsed with sterile water. The membrane was shaken vigorously in 3 ml of sterile water to wash the organisms from the membrane. Of this, a volume of 0.75 ml was cultured on GVPC agar (Oxoid, Wesel, Germany) at 37 °C for up to 10 days under microaerophilic conditions. Fluorescence of colonies was examined under UV-light (254 nm/366 nm) after 3, 6, and 10 days. Contamination with *Legionella* spp. was reported as colony forming units (CFU) per 100 ml of unfiltered water.

As concentrations of non-*Legionella* spp. and suspended matters were high in samples from open water bodies (i.e. borehole, river, lake), we filtered 100 ml instead of 500 ml of these samples.

2.3. Identification of Legionella spp.

Presumptive *Legionella* spp. colonies were sub-cultured on GVPC agar and Columbia blood agar. Colonies that grew on Columbia blood agar were considered cysteine non-dependent and reported as non-*Legionella* spp. (Centers For Disease Control and Prevention, 2005). Serogrouping was performed on pure cultures using a latex agglutination test which differentiates between serogroup 1, serogroup 2–14 and seven non-*L. pneumophila* species (*Legionella longbeachae*, *Legionella bozemanii*, *Legionella dumoffii*, *Legionella gormanii*, *Legionella jordanis*, *Legionella micdadei* and *Legionella anisa*, Oxoid, Wesel, Germany). Species identification was done in Germany using MALDI-TOF mass spectrometry (Biotyper, Bruker, Bremen, Germany) and the MALDI Biotyper library (Version 3.3.2.0). Species of *L. pneumophila* was confirmed by a *L. pneumophila* specific real-time PCR (GeneProof, medac Diagnostika, Wedel, Germany). All non-*L. pneumophila* species were confirmed with 16S RNA gene sequencing (Becker et al., 2004).

2.4. Genotyping

From all confirmed *Legionella* spp. isolates, DNA was extracted with QIAmp DNA Mini Kit (Qiagen, Herne, Germany). Multilocus sequence typing (MLST) was performed for all *L. pneumophila* isolates using the protocol developed by the European Working Group for *Legionella* Infections (EWGLI) published by the ESCMID study group for *Legionella* infections (Gaia et al., 2005; Mentasti et al., 2014). MLST sequence types were deduced using the sequence based typing (SBT) Database of *L. pneumophila* (www.hpa-bioinformatics.org.uk/legionella/legionella_sbt/php/sbt_homepage.php) hosted at the Health Protection Agency, England. The SBT scheme has a good correlation with genetic lineages based on whole genome sequencing (Underwood et al., 2013).

To assess the phylogenetic relation of our *L. pneumophila* isolates with African isolates published in the *Legionella* SBT Database (assessed 9 January 2015, one isolate per ST per country), we constructed a neighbor joining tree using the concatenated sequences of the seven MLST genes of the *Legionella* typing scheme and MEGA5 (www.megasoftware.net).

2.5. Statistics

Comparisons between groups were performed by means of the Chi^2-test and the Fisher exact test for binominal variables (i.e. contamination rates, hot/cold-water systems) where appropriate. Odds ratios were reported as measures of association. Student's *t*-test and Mann–Whitney-*U*-test were calculated to detected differences between numerical variables according to the underlying distribution. The significance level was set at 0.05. Statistical analysis was performed with PASW Statistics Version 18 (SPSS Inc., Chicago, Illinois, USA).

2.6. Ethics statement

Permits are not required to collect water samples from public water sources in Gabon.

Table 1
Samples screened for contamination with *Legionella* spp. in Gabon in 2013.

Place	Province	Number of samples (improved/unimproved)	Mean temperature of water (°C) ± SD	Detection of *Legionella* sp. (*n*)
Bengue	Moyen-Ogooué	4 (2/2)	28.5 ± 1.2	1
Fougamou	Ngounié	10 (7/3)	26.7 ± 1.5	0
Lambaréné	Moyen-Ogooué	175 (169/6)	32.8 ± 11.8	22
Libreville	Estuaire	2 (1/1)	27.8 ± 0.4	0
Lopé	Ogooué-Ivindo	2 (1/1)	28.3 ± 0	0
Makouké	Moyen-Ogooué	4 (3/1)	28.4 ± 1.4	0
Sindara	Ngounié	3 (1/2)	27.9 ± 1.3	0