Introduction – Monitoring and culturing

Since the amendment of the German drinking water ordinance (German DWO) in 2012, the occurrence of Legionella spp. has to be monitored in drinking water plumbing systems (DWPS) of buildings where hot water is stored centrally and offered to the public, or is used commercially. The monitoring has to be done by standard culturing methods and is rated with reference to a technical threshold level (TTL) of 100 CFU/100mL. The culturing method is known to potentially underestimate contamination in a DWPS, because it does not take into account the possibility of Legionella occurring in viable-but-nonculturable (VBNC) states. It is known that bacteria are able to leave this VBNC state and regain cultivability and infectiousness. Gene detection by classic real-time PCR enables to find all DNA of a species, even hidden contamination of bacteria in the VBNC state.

Methods

- DWPS of nine buildings with known Legionella contamination were monitored according to German law and by following DIN EN ISO 19458 (2006) for the analysis of water quality at an outlet in the DWPS (objective b).
- Monitoring over a period of six month in order to determine long term effects following a fixed sampling schedule.
- Short term variations of Legionella contamination were investigated in one building by sampling every two hours (morning till late afternoon) in summer and winter.
- Cultivation of Legionella spp. in 100mL according to German DWO (2001 and 2012) and DIN EN ISO 11731-2 (2008).
- Determination of isolates using the latex agglutination test kit (Oxoid) for species and serogroup differentiation (L. pneumophila SG 1, L. pneumophila SG 2-14, other Legionella species).
- For comparison with the cultivation results, the TTL of 100 CFU/100mL, it was necessary to detect relatively low L. pneumophila concentrations using qPCR.
- One litre water sample filtrated through a cellulose nitrate filter (figure 1).
- Cell lysis using Proteinase K and chelator Chexin Resin 100, followed by heat incubation (1h 55°C, 10 min 95°C) and centrifugation (7,700g) according to Walsh et al. (1991). In pre-tests, this method showed the highest DNA recovery (50%).
- Reduction of sample volume in a SpeedVac to an end volume of 50µL.
- Primer and probe sequences for amplification of the L. pneumophila by macrophage infectivity potentiator (mip) gene (Shannon et al., 2007) (table 1).
- Ratio of qPCR reaction components (total volume 25µL) and temperature protocol were determined by the polymerase mixture used (Maxima Probe qPCR Master Mix, Fermentas).
- The final detection limit was 200 Gene Units L. pneumophila DNA in 1L sample, considering the detection limit of the qPCR standard curve as 20 GU/µL DNA-extract (figure 2; using the Light Cycler, Roche), the reduced sample volume (50µL), and the mean recovery rate of the DNA extraction method (50%).

Results

- Cultivation shows high spatial and temporal variability (up to 4 log_{10} of Legionella concentrations in all buildings over a half year period (n=777, maximum 64,400 CFU/100mL, figure 4) as well as over the course of a day (n=32, figure 5).
- Only 10 of 105 sampling points showed continuously high Legionella concentrations exceeding the TTL during long term monitoring.
- Positive qPCR results were determined in 303 of 732 (41%) samples. They showed a variation of 3 to 6 log_{10} within a building, and up to 3 log_{10} within one tap (figure 4).
- Only 0.6 to 16.7% of taps within a building show positive Legionella results in all samples. Three buildings showed no permanent detectable contamination.
- Statistically high significant (p<0.001), but weak (r=0.319) Pearson correlation for all data pairs (n=647) of L. pneumophila (cultivation) and L. pneumophila (qPCR).

Conclusions

- Detected long-term and short-term variations of Legionella concentrations in DWPS should result in a re-evaluation of guidelines which propose sampling strategies for monitoring bacterial contamination in DWPS.
- Exclusion of DNA from dead bacteria cells from detection with qPCR with e.g. propidium monozadie (PMA) to select cells with intact membrane integrity.

References