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Legionella in drinking water: the detection method matters

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ABSTRACT

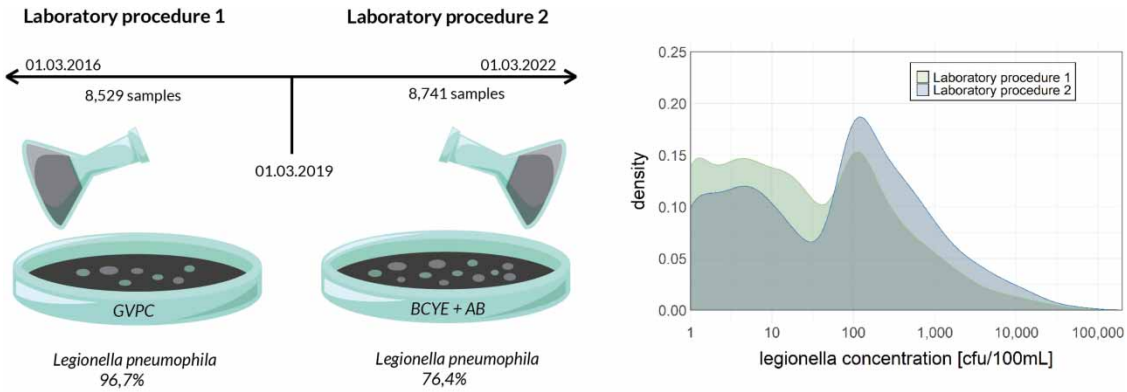
Legionella concentrations in drinking water have been regulated for decades and are evaluated with regard to their concentrations in drinking water plumbing systems (DWPS). The respective action levels differ at the international level. In Germany, the Federal Environment Agency (UBA) specifies the application of ISO 11731 for the detection of legionella in drinking water and gives a binding recommendation for the methods to be used for culturing and evaluation. Effective from 01 March 2019, the UBA recommendation was revised. The utilized culture media in the culture approach were altered, consequently affecting the spectrum of legionella colonies detected in drinking water. Using data from a routine legionella monitoring of a large laboratory, over a period of 6 years and 17,270 individual drinking water samples, allowed us to assess the impact of the alteration on the assessment of DWPS. By comparing the amount of action level exceedances before and after the method change, it could be demonstrated that exceedances are reported significantly more often under the new method. Consequently, the corresponding action level for evaluation of legionella contamination and the resulting risk to human health needs to be revised to avoid the misleading impression of increased health risk.

Key words: action level, guideline, human health, *Legionella* spp., risk

HIGHLIGHTS

- Culture media directly impact reported *Legionella* spp. concentrations.
- Culture media directly impact isolated *Legionella* spp. spectra.
- Over-reporting is mainly due to detection of *Legionella* non-*pneumophila* strains.

GRAPHICAL ABSTRACT



INTRODUCTION

Legionella bacteria are the causative agents of legionellosis, a severe form of pneumonia. Legionellosis outbreaks occur worldwide in community and hospital-like settings and are associated with warm, technical potable water systems where fogging occurs (Breiman *et al.* 1990; Kistemann *et al.* 2010). Due to the high health risk posed by legionella in the technical environment, preventive protective measures against the proliferation of these bacteria in hot potable water systems are established in many countries.

The bacterial genus *Legionella* includes at least 50 species that differ concerning their cell surface antigens, summing up to 70 different serogroups (Fields *et al.* 2002). With a reported incidence of 1.7 cases per 100,000 inhabitants in 2018, Germany is slightly below the European average of 1.8 cases per 100,000 inhabitants (ECDC 2019; RKI 2019). Since not all cases of pneumonia are tested for infection with legionella, significant under-reporting has to be assumed. The vast majority of reported legionellosis cases are traced back to *L. pneumophila* serogroup 1 (Lück & Steinert 2006; Lück 2011; Buchholz *et al.* 2020; Lehfeld *et al.* 2022). *Legionella* spp. are assumed to persist and proliferate in drinking water by occurring in three different forms: (i) most frequently they take advantage of their ability to reside in free-living protozoa (Abu Kwaik *et al.* 1998), (ii) they frequently colonize biofilms lining the inner surface of man-made water systems (Moritz *et al.* 2010; Wingender 2011) and (iii) even if less frequent, legionella can survive as a planktonic form within the water phase. This might explain the discrepancy between their fastidious lifestyle in axenic cultures and their natural oligotrophic environment such as drinking water (Devos *et al.* 2005).

To assess and prevent the public health risk of legionella in drinking water plumbing systems (DWPS), numerous national and international guidelines have been developed. Van Kenhove *et al.* (2019) give a comprehensive overview of the different regulations for legionella control worldwide. Several European countries such as Germany, the UK, the Netherlands and France have implemented rules, requesting actions to reduce the concentrations of legionella in case a certain limit (action level) is exceeded. These guidelines focus on constructional and operational aspects, which are expected to have the potential to limit the colonization and proliferation of pathogens within DWPS. Germany applies the ALARA principle (As Low As Reasonably Achievable), as originally implemented and applied in radiation protection (Hendee & Marc Edwards 1986), in the regulations to monitor drinking water in general. In order to circumvent the usage of disinfectants in the preparation and supply of drinking water, Germany implemented strict action levels to assess any potential risks.

According to the German Drinking Water Ordinance, *Legionella* spp. concentrations above 100 cfu/100 mL (action level) demand resampling of the contaminated DWPS paired with its technical inspection and measures to reduce legionella occurrence (BMG 2019). Controlling, inhibiting or eliminating legionella might comprise strict water temperature management, measures to reduce biofilm growth and sedimentation and measures to reduce water flow stagnancy (DVGW 2004).

In the laboratory, culturing of legionella is rather tedious, due to their slow growth rate and fastidious nutritional requirements, which stand in great contrast to the oligotrophic environment they are naturally encountered in. For instance, they are unable to synthesize a range of essential amino acids (e.g. L-cysteine), which must therefore be provided by culture media (George *et al.* 1980). Regular mandatory legionella laboratory testing of DWPS in public buildings such as hospitals, retirement homes, as well as properties, which supply drinking water as part of a commercial or public activity, is strictly regulated in Germany.

German laboratories are required to adhere to a set of national law-like rules and international standards: (i) the German Drinking Water Ordinance (BMG 2019), (ii) the German Code of Practice W 551 of the German Association of Gas and Water (DVGW), (iii) the ISO 11731 and additionally (iv) the recommendation of the Federal Environment Agency (UBA).

The UBA recommendation of 2012 stated that 100 mL of a drinking water sample must be examined by membrane filtration and 1 mL of the sample must be plated twice by spreading a volume of 0.5 mL both onto GVPC agar (ISO 11731-2:2008; UBA 2012). The amendment of the UBA recommendation in 2018, enforced since March 2019, resulted in changes to the culturing method, especially concerning the choice of culture media for the direct plating of the samples (Table 1). It demands that 50 or 80 mL are to be examined using membrane filtration on GVPC agar or BCYE + AB agar and the direct plating (2×0.5 mL) must be done on BCYE + AB agar instead of GVPC medium.

The changes enforced in 2019, particularly concerning the culture media to be used, might influence the results obtained by culturing. It is virtually certain that two media with different chemical compositions lead to different isolation and quantification patterns. It is hypothesized that the new BYCE + AB agar, with slightly different nutrient composition and different antibiotics/antimycotics, added to prevent the growth of the accompanying flora, could allow for the growth of another

Table 1 | Culturing procedure according to the recommendations of the Federal Environment Agency for the testing of DWPS drinking water samples for legionella according to the German Drinking Water Ordinance

	Up to 28 February 2019			Since 1 March 2019		
	Recommendation 2012 in regard to ISO 11731-2:2008 (LP 1)			Recommendation 2018 in regard to ISO 11731:2017 (LP 2)		
	GVPC	BCYE	BCYE + L-cysteine	GVPC	BCYE	BCYE + AB
Direct plating (without acid treatment)	Required	Optional	Optional	Optional	Optional	Required
Membrane filtration (with acid treatment)	Required	Optional	Optional	Optional	Optional	Optional

spectrum of *Legionella* spp. per volume. A study by Scaturro *et al.* (2020) already demonstrated that using GVPC and BCYE agar for the same sample resulted in a higher abundance of *L. pneumophila* SG 1 isolates on the GVPC agar than the BCYE agar. No changes concerning the evaluation of individual findings and the action level have been made in the UBA recommendation. Consequently, the currently applied action levels are based on methods that are no longer in use.

This study aimed to assess the impact of the culture medium and therefore the new recommendation (UBA 2018) on the isolation and quantification of legionella from drinking water to alert authorities to consider future changes in guidelines. Against this background, our study compares the routine findings of legionella in drinking water in a German laboratory 3 years before the change of culture media by the new recommendation (UBA 2012: laboratory procedure/LP 1) with data generated in the following 3 years after alteration (UBA 2018: laboratory procedure/LP 2) to evaluate practical implications.

MATERIALS AND METHODS

Laboratory testing for legionella

Laboratory testing of water samples was carried out by the Technical Hygiene Department of the Institute of Hygiene and Public Health in Bonn, Germany. The testing was done according to the recommendation 'Systemic testing of drinking water installations for legionella according to the Drinking Water Ordinance' of the German Federal Environment Agency of 23 August 2012 (UBA 2012), as well as the amendment of 18 December 2018. The version of ISO 11731, which was valid at the respective time, was used (see Table 1). This study focuses on laboratory results generated between 1 March 2016 and 28 February 2022. 1 March 2019 was used as a cut-off date to distinguish the laboratory results of this period, based on the utilized laboratory protocols: (i) procedure 'LP 1' (recommendation of the German Federal Environment Agency up to that date) and (ii) procedure 'LP 2' (recommendation of the German Federal Environment Agency starting at that date and ongoing).

Laboratory procedure 1, according to the UBA recommendation of 2012, followed a testing protocol as follows: 100 mL of a drinking water sample were examined by membrane filtration on GVPC agar (OXOID, Wesel, Germany) and 1 mL of the sample was spread (2×0.5 mL) onto GVPC agar (ISO 11731-2:2008; UBA 2012).

The amendment of the UBA recommendation in 2018, enforced since March 2019, changed the procedure to LP 2, in which the concentrated water by filtration was examined using the GVPC agar (OXOID, Wesel, Germany) and the direct plating (2×0.5 mL) was done on BCYE + AB agar instead of GVPC medium (Table 1).

Suspicious colonies grown on the membrane filters or by direct plating in both procedures (LP 1 and LP 2) were streaked onto BCYE medium (must show characteristic legionella growth) and Colombia sheep blood agar (must show no growth). The thus verified colonies were then subjected to an agglutination antibody test (Legionella Latex Test, OXOID, Wesel, Germany) to distinguish between *Legionella* non-*pneumophila*, *L. pneumophila* serogroup 1 and *L. pneumophila* serogroups 2–14.

Both cultural results, i.e. from membrane filtration and direct plating, are offset against each other and the higher colony count per 100 mL is to be stated as the final result (UBA 2012, 2018). Furthermore, according to both UBA recommendations (2012, 2018), values <3 cfu per plate must be extrapolated, contradicting the specifications of ISO 8199.

Data collection and cleaning

Only complete data sets (containing information on the concentration of legionella, water temperature at sampling, water temperature at constancy, sampling site, sampling date and sampling method corresponding to the recommendation) were

included in the statistical analysis. Follow-up samples, where sampling sites were re-sampled after exceeding the action level (i.e. >100 cfu/100 mL), were removed from the data set, as they might distort the comparison of the two LPs. Therefore, results for individual sampling sites, which reoccurred in a timeframe of 120 days, were excluded. Since *Legionella* spp. is only obliged to be monitored in potable water hot systems (PWHs) of DWPSs (according to the guidelines), samples from potable water cold systems (PWC) were excluded.

Descriptive analysis

The data of each LP were analysed with regard to sample sizes, the number of buildings and the number of samples per building to investigate for an even distribution. As a first step, distributions of positive legionella findings per LP were compared with each other. The following categories were compared in the data sets of both LPs: results with (i) low legionella concentrations (1–99 cfu/100 mL), (ii) legionella concentrations, which exactly met the action level of 100 cfu/100 mL and (iii) legionella exceedances of the action level (>100 cfu/100 mL). As mentioned above, if 1, 2 or 3 cfu were found by direct plating, results were extrapolated to a volume of 100 mL, resulting in 100, 200 and 300 cfu/100 mL as reported legionella concentration. Since this approach contradicts the ISO 8199, such results were emphasized in the statistical analysis. Furthermore, data were differentiated and compared with regard to the detected species and, if applicable, the serogroup of *L. pneumophila*. Samples with mixed colonies and thus showing more than one of the specifications '*L. pneumophila* serogroup 1', '*L. pneumophila* serogroups 2–14' and '*L.* non-*pneumophila*' were always evaluated in favour of *L. pneumophila* and if applicable *L. pneumophila* serogroup 1.

Statistical analysis

A multi-field table (by category) was created to show the distribution of legionella concentrations from both LPs. This multi-field table was analysed with the chi-square homogeneity test ($\alpha = 5\%$). The same procedure was applied to the species and, if applicable, to the serogroups. A regression analysis was carried out to model relationships between the variable 'concentration of legionella' with the variables 'water temperature at sampling', 'water temperature at constancy', 'LP (1 or 2)' as well as the 'sample location (central or periphery)'. Central sampling points are the outlet of the water heater as well as the inlet of the water heater at the recirculation pipe, whereas peripheral means sampling location at the water tap (point of use).

Whether a variable has a significant influence on the legionella concentration was tested with the *F*-test ($\alpha = 5\%$). Adjusted *R*-squares were calculated to describe the fit of the model to the data.

RESULTS

Data collection and cleaning

The data set comprised a total of 24,564 drinking water samples, which were analysed for the presence of *Legionella* spp. from 01 March 2016 up to 28 February 2022. Overall, 17,270 samples from 1,209 buildings met the inclusion criteria and were included in the statistical analysis. In LP 1, 8,529 (49.4%) samples from 947 different buildings and in LP 2, 8,741 (50.6%) samples from 879 different buildings met all inclusion criteria, reflecting an even sample distribution in both data sets.

Furthermore, we examined the number of samples drawn from each building to approximate the building sizes of both LPs. Figure 1 shows the distribution of sample counts per building per LP data set. Most of the buildings were sampled less than 10 times, and this is true for both LP data sets. Buildings with more than 25 samples were excluded in Figure 1 but remained in the data set ($n = 22$ in LP 1, $n = 19$ in LP 2).

Distribution of legionella concentrations

In total, 83.1% of the results were found to be 0 cfu/100 mL with 82.4% in LP 1 and 83.8% in LP 2, respectively. For the remaining legionella-positive samples, *Legionella* spp. concentration was between 1 and 288,000 cfu/100 mL in LP 1 and between 1 and 70,800 cfu/100 mL in LP 2, respectively. The density of measured legionella concentrations exceeding the action level (100 cfu/100 mL) was higher in LP 2 than in LP 1 (Figure 2).

The total share of positive samples might be higher in LP 1 with 17.5% in comparison to 16.1% in LP 2 (Figure 2), but the proportion of legionella concentrations >100 cfu/100 mL (exceeding the action level) was greater in LP 2 (6.5%) than in LP 1 (4.9%) (Table 2).

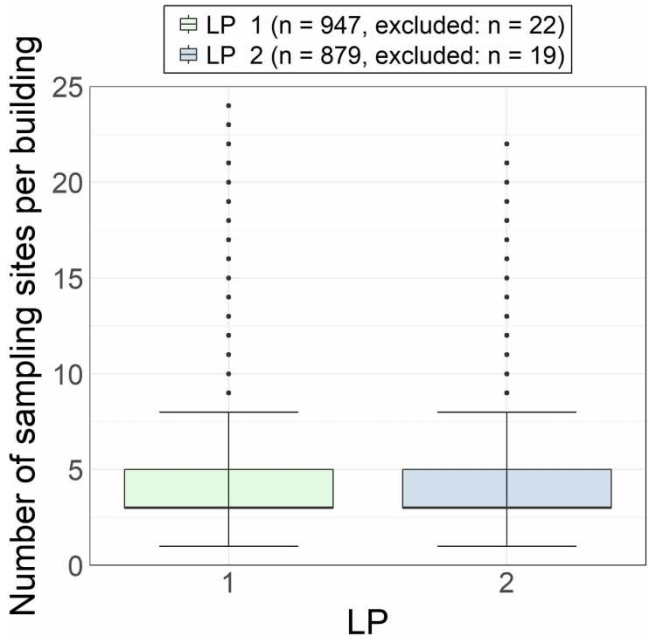


Figure 1 | Comparison of the numbers of samples taken in the respective buildings during the compared periods (LP 1 and LP 2).

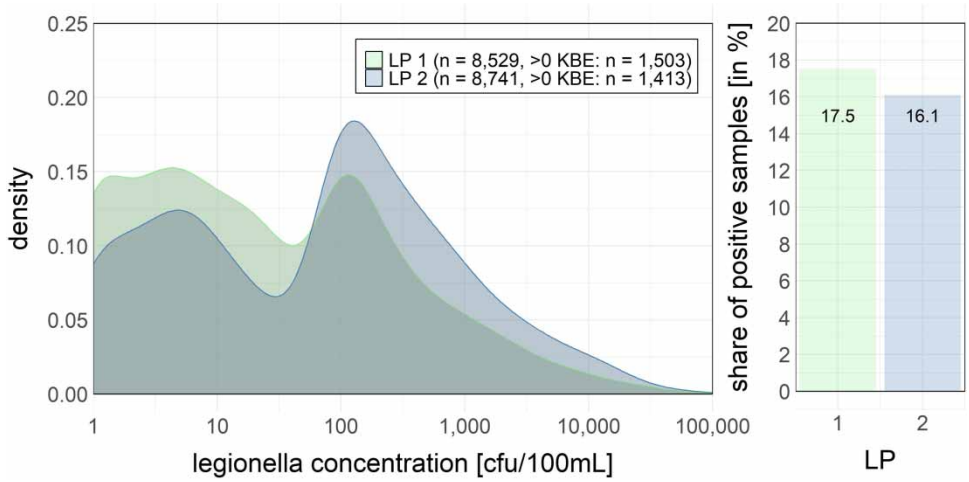


Figure 2 | Density of *Legionella* spp. concentrations before and after the amendment of the UBA recommendation for testing of DWPS for legionella, according to the German Drinking Water Ordinance. Values above 100,000 cfu/100 mL were removed to improve clarity.

Table 2 | Categories regarding the concentration level of results obtained during LP 1 and LP 2, before and after the amendment of the UBA recommendation for testing of DWPS for legionella

	LP 1 regime		**LP 2 regime**	
Category	***N***	**%**	***N***	**%**
0 cfu/100 mL	7,026	82.4	7,328	83.8
1–99 cfu/100 mL	946	11.1	626	7.2
100 cfu/100 mL	135	1.6	220	2.5
>100 cfu/100 mL	422	4.9	576	6.5
Total	**8,529**	**100.0**	**8,741**	**100.0**

Consequently, to obey the German drinking water ordinance, 4.9% of sampling sites had to be re-evaluated by resampling and/or undergoing risk assessment during the LP 1 period. After the amendment of the UBA recommendation (i.e. during the LP 2 period), resampling had to be conducted 1.3 times more often.

The isolation of a single colony obtained by direct plating extrapolates to a concentration of exactly 100 cfu/100 mL. This was the case in 1.6% of the cases in LP 1 (UBA 2012) and in 2.5% of the cases in LP 2 (UBA 2018). The final legionella concentration of 200 cfu/100 mL, resulting from 2 cfu, occurred in 0.8% (LP 1) and 1.2% (LP 2) of the samples. Analogously, three colonies and a final legionella concentration of 300 cfu/100 mL were detected in 0.5% (LP 1) and 0.7% (LP 2) of the samples, respectively. In total, the proportion of results based on 1–3 detected cfus (by direct plating of 1 mL) increased by 59.0% for one colony, by 42.3% for two colonies and by 55.1% for three colonies after the change from LP 1 to LP 2.

The linear regression model with the variables: (1) concentration of legionella, (2) water temperature at sampling, (3) water temperature at constancy, (4) laboratory procedure (LP 1 versus LP 2) and (5) sample location revealed that the variable 'laboratory procedure' had a significant influence on the legionella concentration ($p < 0.001$). In addition, only the water temperature at constancy (3) also had a significant influence ($p = 0.003$).

Distribution of *Legionella* species

The variable '*Legionella* species' was subsequently split into three sub-variables to distinguish between *L. pneumophila* serogroup 1, serogroups 2–14 and *L.* non-*pneumophila* ($n = 2{,}902$). In some samples, mixed *Legionella* spp. population occurred, resulting in more than one type of *Legionella* spp. Consequently, we obtained 3,178 data points on this parameter.

For the data set LP 1, 99.3% of the overall positive samples contained information on *Legionella* species, whereas in LP 2, information was given in 99.7% of the positive samples. Table 3 shows the distribution of *Legionella* spp. with regard to the *L. pneumophila* serogroups (1, 2–14) and *L.* non-*pneumophila* strains among LP 1 and LP 2. The proportion of samples containing only *L.* non-*pneumophila* strains is 7.2 times higher during the LP 2 (UBA 2018) period (26.6%) than during the LP 1 (UBA 2012) period (3.3%) (Table 3). The percentage of *L. pneumophila* serogroup 1 isolates within the group of *L. pneumophila* isolates was 41% during the LP 1 period and 38.1% during the LP 2 period.

If only considering samples that exceeded the action level (>100 cfu/100 mL), the data show that after the amendment in LP 2, 39.3% of the exceedances were caused exclusively by *L.* non-*pneumophila* strains. In LP 1, this proportion was only 9.8% (Table 4). In other words, exceedances of the action level were caused rather by *L. pneumophila* strains during the LP 1 period (UBA 2012) and were caused by approximately 40% by *L.* non-*pneumophila* in the LP 2 period (UBA 2018).

Table 3 | Comparison of the distribution of *Legionella* spp. in the positive samples during LP 1 and LP 2 regimes, i.e. before and after the amendment of the UBA recommendation for testing of DWPS for legionella

	LP 1 regime		LP 2 regime	
***Legionella* species**	***N***	**%**	***N***	**%**
L. pneumophila	1,443	96.7	1,077	76.4
L. pneumophila serogroup 1	592	41.0	410	38.1
L. pneumophila serogroups 2–14	851	59.0	667	61.9
L. non-*pneumophila*	50	3.3	332	23.6
Total	**1,493**	**100.00**	**1,409**	**100.00**

Table 4 | Comparison of the distribution of *Legionella* spp. in the samples exceeding the threshold action during LP 1 and LP 2 regimes, i.e. before and after the amendment of the UBA recommendation for testing of DWPS for legionella

	LP 1 regime		LP 2 regime	
***Legionella* species**	***N***	**%**	***N***	**%**
L. pneumophila	367	90.2	340	60.7
L. non-*pneumophila*	40	9.8	220	39.3
Total	**407**	**100.00**	**560**	**100.00**

DISCUSSION

Practical implications of the amendment of the UBA recommendation concerning the change of culture medium have not yet been evaluated with regard to the culture-based results.

Legionellosis cases are subclassified into three categories, taking into account the infection source: (1) travel-associated, (2) nosocomial and (3) community-acquired. Community-acquired cases take the largest proportion of 70% of reported cases in Germany (RKI 2019).

Even though an infectious dose-response relationship for *L. pneumophila* infections has been studied (Armstrong & Haas 2007; Hamilton *et al.* 2019), a relationship between the concentration of legionella in drinking water and the incidence of disease can often not be established. This is partly because a case of legionellosis is usually only assigned to an infectious source when a genotypic match, based on identical sequence types, has been detected, typically happening in outbreak-scenarios. According to Orkis *et al.* (2018), residential potable water, large building water systems and car travel (car air-conditioner water leakage) substantially contribute to sporadic Legionnaires' disease cases. However, they acknowledged that in most cases, the sources of sporadic disease could not be clearly identified.

A large-scale study in Germany (LeTriWa) evaluated all reported cases of community-acquired legionellosis and tried to identify the source of infection, using a tailored evidence matrix (Buchholz *et al.* 2022; Lehfeld *et al.* 2022). For this, cases reported to the Robert Koch-Institute were assigned to either an external source of infection, a domestic non-drinking water source or a domestic drinking water source (Buchholz *et al.* 2022; Lehfeld *et al.* 2022). In 27% of cases, domestic drinking water was identified as the source of infection. The authors describe a significant association with the presence of Mab (monoclonal antibody) 3-1-positive *L. pneumophila* serogroup 1 strains in the residential drinking water samples and the legionellosis cases. They did not find a significant association with the presence of *Legionella* spp. or the exceedance of the action level in standard household water samples. Lehfeld *et al.* (2022) calculated that the odds ratio augmented with increasing specificity of the parameter ('any legionella' < 'over 100 cfu/100 mL' < *L. pneumophila* < *L. pneumophila* serogroup 1). Studies by Harrison *et al.* (2009) conducted in England and Ditommaso *et al.* (2014) conducted in Italy support the same thesis that the risk of infection increases if a Mab 3-1-positive isolate is found. Studies by Borchardt *et al.* (2008), Harrison *et al.* (2009) and Reimer *et al.* (2010) show that fewer variant diversities are observed among clinical isolates compared to environmental isolates. Thus, Lück (2011) concluded that only a part of the *Legionella* spp. occurring in nature are virulent and logically derives that *Legionella* spp. virulence differs among variants and that Mab 3-1-positive *L. pneumophila* strains should be given a higher focus instead of focussing on all *Legionella* spp. in DWPS. However, it should be noted that urinary antigen tests predominantly used to diagnose legionellosis most reliably detect *L. pneumophila* SG 1, even though some assays have shown to be capable of detecting non-serogroup 1 *L. pneumophila* and other *Legionella* spp. (Benson *et al.* 2000). Some infections with *L.* non-*pneumophila* could stay unrecognized since sputum samples for *Legionella* spp. culture are not frequently analysed to examine community-acquired pneumonia (Muder & Victor 2002; Yáñez *et al.* 2005; Miyashita *et al.* 2020).

It is generally accepted that the composition of culture media significantly determines the spectrum of isolated microorganisms. This is their intended purpose and a fundamental microbiological principle of bacterial culture. Thus, the change in the UBA recommendation regarding the culture medium to be used for direct plating (LP 2) has a direct influence on the number and diversity of *Legionella* spp. colonies found in routine analysis, as proven by our analyses. A study conducted by Scaturro *et al.* (2020) demonstrated that the GVPC agar was more efficient in detecting legionella than the BCYE agar (without added antibiotics), but could not demonstrate that the isolation of *L.* non-*pneumophila* isolates was improved using the BCYE agar.

The action level, as established in the German guidelines, is based on experience and expert opinions while including a 'safety buffer'. It is not based on evidence derived from epidemiological or infectiologic data. Even though the action level has always been applied to *Legionella* spp., our statistical analysis shows that the use of GVPC agar (in accordance with the German regulation for the detection of legionella in drinking water up to 2019) might culturally focus and select for the detection of *L. pneumophila* (96.7% of detected isolates). The empirically derived values that led to the definition of the action level in the past may therefore be seen as the result of data that showed predominantly *L. pneumophila* colonies.

The presented results demonstrate that the detected (and reported) concentration of *L.* non-*pneumophila* in routine sampling is significantly higher since the change of the utilized culture media in the application of the UBA recommendation, in force since 1 March 2019 (LP 2). Even though the general amount of reported legionella-positive samples has slightly decreased during the last years (Figure 2), exceedances of the action level were reported more frequently. Additionally, the

results demonstrate that the percentage of *Legionella* non-*pneumophila* colonies in positive samples is significantly higher when using the BCYE + AB agar (LP 2) than the GVPC agar (LP 1).

Applying the established action level, which is empirically derived from *L. pneumophila* data (96.7%) decades ago, to a data set containing much higher amounts of *L.* non-*pneumophila* strains (23.6%), necessarily results in higher exceedance frequencies. According to the reviewed literature, this does not indicate an increased risk to public health.

We demonstrated with our analysis, that relatively and also absolutely, fewer samples contained *L. pneumophila* colonies (Table 3) than prior to the amendment of the UBA recommendation. The relative proportion decreased from 16.9% of all samples examined to 12.3%. Because we have introduced a bias in favour of *L. pneumophila* when evaluating mixed samples that contained more than one *Legionella* species, the proportion of exceedances of the action level due to *L.* non-*pneumophila* findings, in mixed samples, is even greater (39.3%) than the proportion determined due to solely *L.* non-*pneumophila* positive samples (23.5%).

According to both UBA recommendations (2012, 2018), values <3 cfu per plate must be extrapolated, contradicting the specifications of ISO 8199. The ISO 8199 'Water quality – General requirements and guidance for microbiological testing by culture methods' is generally applicable to microbiological testing of water samples. It focuses on the statistical validation of the indicated concentration when stating the result, and not primarily on the protection of human health in the sense of a worst-case scenario as done in the UBA recommendations (2012, 2018). A previous evaluation by Zacharias *et al.* (2020) has shown that the statistical uncertainty of extrapolating <4 cfu per plate as requested by the UBA recommendation up to 2019 logically resulted in more exceedances of the action level. This directly affects action obligations concerning the sanitization of DWPS (Zacharias *et al.* 2020). According to the results presented here, the majority of *Legionella* spp. considered by Zacharias *et al.* (2020) have most likely been *L. pneumophila*.

We have shown that the amendment of the UBA recommendation has impacted the spectrum of *Legionella* spp. and concentration detected in routine analysis, which is most likely attributed to the used culture medium.

Data interpretation, action levels and requests concerning control measures have so far not been adapted to the new cultural method. As a result, health authorities act based on rules that have not been adapted to the new method. We therefore call for an evidence-based policy discussion, on past and future changes, with regard to the detection method for legionella in drinking water and action levels.

The currently applicable legal requirements are altered again in 2023 with a revision of the German Drinking Water Ordinance due to the new EU Drinking Water Directive. The EU Drinking Water Directive states that values equal to 100 cfu/100 mL must also be reported as an exceedance. If we would use our utilized data set to recalculate the proportion of exceedances affected from the new definition, the proportion of reported exceedances would additionally increase from 6.5 to 9.0% of the samples. This would increase the reported exceedances by 84% in comparison to the number of exceedances before the amendment of the UBA recommendation in 2018. We recommend that in the future mainly *L. pneumophila* should be taken into account, when evaluating DWPS, as it was majorly done by applying the GVPC medium under the LP 1 regime. From a laboratory point of view, the serological differentiation required for this distinction can easily be carried out in routine analysis. Only under this condition could it justifiably maintain the currently valid action level.

The ISO 11731 recommends a range of culture media, which reduces comparability of legionella contamination in drinking water in an international context, in case different culture media are used. The presented results demonstrate that the comparison of data on *Legionella* spp. in drinking water can hardly be done on an international scale. The specification of the culture media and laboratory procedure by the Federal Environment Agency at least ensures national comparability within Germany. Nevertheless, the changeover in 2018 had far-reaching consequences, as described above.

CONCLUSION

It is a basic microbiological principle that the choice of culture media and the nutrient and/or antibiotic composition influence the spectrum of isolated bacteria. According to our data, the change in the UBA recommendation, which is decisively influenced by the medium to be used, had a significant effect on the isolated spectrum of legionella detected. The action level for *Legionella* spp. (100 cfu/100 ml), which has been empirically derived and established decades ago, applied to data which, due to the utilized medium, mostly selected *L. pneumophila* isolates. According to our data, reporting of more exceedances to the action level in drinking water, when using the new medium, only means that more samples are detected that exclusively contain *L.* non-*pneumophila* strains. Evidence-based clinical and epidemiological studies underpin that the vast majority of

clinical legionellosis cases are linked to *L. pneumophila*. Against this background, it does not seem justified to attribute virtually the same virulence to all strains of environmental *Legionella* spp., to initiate control measures without considering strain-specific health risks.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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