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Evaluation of an MPN test for the rapid enumeration of *Pseudomonas aeruginosa* in hospital waters

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ABSTRACT

In this study, the performance of a new most probable number (MPN) test (Pseudalert®/Quanti-Tray®) for the enumeration of *Pseudomonas aeruginosa* from hospital waters was compared with both international and national membrane filtration-based culture methods for *P. aeruginosa*: ISO 16266:2006 and UK *The Microbiology of Drinking Water – Part 8* (MoDW Part 8), which both use *Pseudomonas* CN agar. The comparison based on the calculation of mean relative differences between the two methods was conducted according to ISO 17994:2014. Using both routine hospital water samples (80 from six laboratories) and artificially contaminated samples (192 from five laboratories), paired counts from each sample and the enumeration method were analysed. For routine samples, there were insufficient data for a conclusive assessment, but the data do indicate at least equivalent performance of Pseudalert®/Quanti-Tray®. For the artificially contaminated samples, the data revealed higher counts of *P. aeruginosa* being recorded by Pseudalert®/Quanti-Tray®. The Pseudalert®/Quanti-Tray® method does not require confirmation testing for atypical strains of *P. aeruginosa*, saving up to 6 days of additional analysis, and has the added advantage of providing confirmed counts within 24–28 hours incubation compared to 40–48 hours or longer for the ISO 16266 and MoDW Part 8 methods.

Key words | hospital waters, MPN enumeration, Pseudalert, *Pseudomonas aeruginosa*

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INTRODUCTION

Pseudomonas aeruginosa is an important cause of hospital-acquired infections, particularly for patients in intensive care and neonatal units and those suffering cystic fibrosis, burns and similar immunocompromised conditions (Bertrand *et al.* 2000; Berthelot *et al.* 2001). It can also infect immunocompetent people, commonly resulting in skin, ear and eye infections (Botzenhart & Doring 1993; Mena & Gerba 2009). Tap water in hospitals has been implicated in several studies as a significant source of colonisation by *P. aeruginosa* (Ferroni *et al.* 1998; Trautmann *et al.* 2001; Reuter *et al.* 2002; Aumeran *et al.* 2007; Rogues *et al.* 2007; Cholley *et al.* 2008) resulting in infection either by direct contact with the tap water or via contamination of materials (e.g. face cloths) or staff hands. During late 2011 and early 2012, four premature babies died in Northern Ireland after becoming infected with *P. aeruginosa* via contaminated tap water (RQIA 2012; Walker *et al.* 2014). A recent literature review has highlighted the importance of healthcare water systems as sources for *P. aeruginosa*, although the actual transmission routes still need to be elucidated (Loveday *et al.* 2014). *Pseudomonas aeruginosa* has also been isolated from recreational and hydrotherapy pools that were associated with infections, most notably folliculitis (Moore *et al.* 2002). *Pseudomonas aeruginosa* is found widely in the natural and built environment, especially in water and other moist environments (Mena & Gerba 2009) and is commonly associated with biofilms in water systems (Trautmann *et al.* 2005). These biofilms may be difficult to eradicate in hospital systems using standard water disinfection doses (Suman *et al.* 2008).

As infection control specialists become more aware of the potential for water used within the healthcare environment to be the source of hospital-acquired infections (not just from legionellae) and begin to develop more holistic water safety plans as recommended by the World Health Organization (WHO 2011), the role of *P. aeruginosa* in causing infections, and especially its ability to act as a reservoir of antibiotic resistance, is increasingly being recognised. As a consequence, monitoring for *P. aeruginosa* in hospital water systems is increasingly being undertaken to identify potential sources of infection and also to validate and verify that control measures are effective. In France, mandatory control of *P. aeruginosa* in waters used in hospitals was introduced in 2005 (Ministère de la Santé 2005). In the UK, guidance on monitoring for *P. aeruginosa* in augmented care areas of hospitals was introduced in 2013 (Department of Health 2013).

Typically, the method employed to detect *P. aeruginosa* in the laboratory is either the International Standards Organisation (ISO) 16266 (ISO 2006) procedure or the UK *The Microbiology of Drinking Water – Part 8* (MoDW Part 8) (SCA 2010) method, both of which use the membrane filtration procedure and *Pseudomonas* CN agar (PACN agar) for the isolation of *P. aeruginosa*. For typical pyocyanin-producing colonies, confirmed counts are obtained by both of these methods after 44 ± 4 hours incubation at 36 ± 2 °C. However, atypical presumptive colonies (i.e. fluorescent non-pyocyanin-producing colonies or reddish brown colonies) require supplementary testing, which may take up to 6 days before confirmation of identity is achieved. Additionally, the ISO 16266 confirmation procedure uses acetamide broth (acetamide is carcinogenic) and Nessler's reagent (which contains toxic mercuric chloride). An alternative method employing the Quanti-Tray® system (IDEXX Laboratories, Westbrook, ME, USA) provides a confirmed most probable number (MPN) count of *P. aeruginosa* within 24–28 hours incubation at 38 ± 0.5 °C. This is achieved by the use of the Pseudalert® reagent (IDEXX Laboratories, Westbrook, ME, USA), which is a bacterial enzyme detection reagent that signals the presence of *P. aeruginosa* through the hydrolysis of a diagnostic fluorogenic substrate present in the reagent.

The aim of this study was to evaluate the performance of Pseudalert®/Quanti-Tray® for the enumeration of *P. aeruginosa* from hospital waters in comparison to the two reference PACN agar membrane filtration methods. Both routinely analysed hospital waters and hospital waters spiked with reference and environmental strains (obtained from hospital water samples) of *P. aeruginosa* were examined. The data were analysed according to the principles outlined in *The Microbiology of Drinking Water – Part 3* (SCA 2002) for the preliminary analysis of the data from each participating laboratory, and ISO 17994:2014 (ISO 2014) to assess the comparability of performance between the Trial Method and the Reference Method.

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METHODS

Initially, it was planned to use only samples of hospital waters that were being routinely analysed and suspected of being contaminated with *P. aeruginosa* for the study. However, this became unrealistic as the number of positive samples from most of the participating laboratories tended to be very low, resulting in great difficulty in achieving a sufficiently large database for comparative analysis. Consequently, it was decided to achieve sufficient positive results through the spiking of hospital water samples with a selection of 10 reference and environmental strains of *P. aeruginosa*.

Phase 1 – samples from hospital systems

Six European laboratories (from France, Germany, Italy and the UK) participated in the initial study utilising routine samples taken from hospital water systems. The laboratories were asked to retain these routine samples at 5 ± 3 °C after analysis by their normal method and to examine the plates after 20–24 hours incubation. Any samples that showed positive growth for *P. aeruginosa* were retrieved and processed again by both the Pseudalert/Quanti-Tray method and by membrane filtration using PACN agar. This generated a data set of paired counts by the methods.

Phase 2 – artificially contaminated samples

Five European laboratories participated in this part of the study. A selection of isolates of *P. aeruginosa* was supplied to each laboratory on nutrient agar slopes. A spiking protocol was designed so as to achieve four levels of spike for each isolate, within a target range of 10–100 *P. aeruginosa* per 100 mL from a 200 mL sample. Each strain was grown up overnight on a non-selective agar at 37 °C and inoculated into 5 mL of quarter-strength Ringer's solution to create an initial suspension equivalent to a McFarland No. 1 standard. This was used to generate a final volume of spiking suspension through serial dilution. Each final suspension was used to spike water samples to four levels of contamination (0.1, 0.2, 0.3 and 0.4 mL of spiking suspension) per isolate. One laboratory also spiked samples with 0.5 mL of spiking suspension. All laboratories had on-site training in the protocol. Laboratories retained routine samples at 5 ± 3 °C, and where the results were negative for *P. aeruginosa*, these were used for the spiking procedure. Each spiked sample was analysed by Pseudalert®/Quanti-Tray® and by membrane filtration using PACN agar. As cultures of known strains of *P. aeruginosa* were being used, confirmation testing was not undertaken. The sources of the selected strains are detailed in Table 1. All these strains produced typical colonies on PACN agar and fluorescence in Pseudalert®. A strain of *Pseudomonas fluorescens* was included to act as an atypical control.

Enumeration of *P. aeruginosa* by ISO 16266 and MoDW Part 8 (the Reference Method)

One hundred millilitres of sample was filtered through a 47 mm diameter 0.45 μm cellulose ester membrane filter, which was then placed onto a PACN agar (Oxoid, Basingstoke, UK) plate. PACN plates were incubated for 44 ± 4 hours at 36 ± 2 °C before counting of confirmed *P. aeruginosa* (blue/green pyocyanin-producing) colonies and subculturing of presumptive *P. aeruginosa* (fluorescent non-pyocyanin-producing or reddish brown) colonies. Any required confirmation tests were conducted according to ISO 16266 and MoDW Part 8.

Table 1 | Sources of *Pseudomonas aeruginosa* isolates used for spiking hospital water samples

Isolate	Identification	Source
1	*P. aeruginosa*	Wash basin, Germany
2	*P. aeruginosa*	High dependency unit sluice basin, UK
3	*P. aeruginosa*	Ambulatory surgery tap, France
4	*P. aeruginosa*	Hot water tap, Italy
5	*P. aeruginosa*	Wash basin, Germany
6	*P. aeruginosa*	En-suite room tap, UK
7	*P. aeruginosa*	Multi-surgery shower, France
8	*P. aeruginosa*	Hot water tap, Italy
9	*P. aeruginosa*	WDCM[a] 00024 (=ATCC[b] 10145)
10	*P. aeruginosa*	WDCM 00025 (=ATCC 27853)
11	*P. fluorescens*	Drinking water, UK (negative control)

[a]World Data Centre for Microorganisms.
[b]American Type Culture Collection.

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Enumeration of *P. aeruginosa* by Pseudalert®/Quanti-Tray® (the Trial Method)

One hundred millilitres of sample was added to a sterile 120 mL vessel containing an antifoam reagent (IDEXX Laboratories, Westbrook, ME, USA). To this, one snap pack of Pseudalert® reagent was added, the vessel capped and the sample shaken to dissolve the reagent before being left to stand for any foam to settle. The sample was then poured into a 51-well Quanti-Tray® pouch (IDEXX Laboratories, Westbrook, ME, USA), sealed and incubated at $38 \pm 0.5\,^{\circ}$C for 24–28 hours. After incubation, the Quanti-Trays® were examined under UV irradiance (365 nm), and all wells demonstrating blue fluorescence compared to a negative blank sample were counted as positive for *P. aeruginosa*. MPN counts were derived using the manufacturer's table of MPN values for the number of positive wells.

Confirmation of identity of *P. aeruginosa* from hospital water samples

A selection of positive wells from Pseudalert®/Quanti-Tray® and positive colonies from PACN agar was subcultured to nutrient agar and subjected to confirmation testing according to ISO 16266 (oxidase test, production of ammonia from acetamide and production of fluorescence on King's B agar) and MoDW Part 8 (oxidase test and hydrolysis of casein on milk cetrimide agar) to confirm identities as *P. aeruginosa*.

Statistical methodology

Prior to statistical analysis, results were excluded from the data analyses when the results for one or both methods exceeded the upper count limit of either method, and when both methods resulted in zero counts. MPN counts from Pseudalert®/Quanti-Tray® were converted to nearest whole integers as required by ISO 17994 (ISO 2014). Preliminary statistical analyses (tests for normality by the Kolmogorov–Smirnov Goodness of Fit test, outliers according to ISO 17994 and non-parametric statistics by the Wilcoxon signed rank test) were undertaken using MINITAB statistical software (release 14.20, Minitab Inc., State College, PA, USA), and analyses of comparative count mean relative differences were calculated according to ISO 17994. The normality test was only applied for the preliminary analysis of the data as the ISO 17994 mean relative difference analysis assumes normality. Mean relative difference analyses were performed using a program written in Excel 2007 (Microsoft, Redmond, Washington, USA).

For the ISO 17994 analyses, the relative difference (x) of each pair of counts was calculated using the equation $x = 100(\ln(a) - \ln(b)$, where $\ln(a)$ is the natural logarithm of the count by the Trial Method (Pseudalert®/Quanti-Tray®) and $\ln(b)$ is the natural logarithm of the count by the Reference Method (ISO 16266 or MoDW Part 8), for each sample for each analysis. Data with a zero count by one method had plus one (i.e. count +1) added to each pair of counts prior to log-transformation. Since the objective of the study was to show there was no difference between the Trial Method (Pseudalert®) with an established Reference Method, it was considered that the 'two-sided' comparison according to ISO 17994 was appropriate. The percentage value of the upper and lower limits was set at +10 and −10, respectively, as suggested by ISO 17994 for potable water samples. In this context, a significant difference would be when the mean relative difference and its 'confidence interval' are wholly above or below the value of zero. A conclusive outcome is when the mean relative difference and its 'confidence interval' are statistically significant, while an 'inconclusive' outcome is typically associated with the lower value of the 'confidence interval' being less than zero but not below the set limit of −10 and the upper value is greater than the set limit of +10, usually by an insufficient number of samples being analysed.

RESULTS

For both phases of the study, preliminary statistical analysis of the data indicated that they were not normally distributed (Kolmogorov–Smirnov Goodness of Fit test, $p = <0.010$). Log_{10}-transformation, however, did not improve normality (Kolmogorov–Smirnov Goodness of Fit test, $p = <0.010$), so it was decided to use a non-parametric analysis on untransformed data. This revealed no significant differences in relative performance of the methods between the

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participating laboratories for the routine hospital water samples (Wilcoxon signed rank test) (Table 2). For the artificially contaminated samples, Pseudalert®/Quanti-Tray® produced significantly higher counts at three of the five laboratories (Wilcoxon signed rank test) (Table 3). The data from both sets of samples were tested for mean relative difference analyses according to ISO 17994.

The data were also analysed by the ISO 17994 mean relative difference method to determine if there was any difference in recoveries between the 10 isolates used in generating artificially contaminated samples.

Phase 1 – samples from hospital systems

There were 80 samples with counts within the acceptable count range for statistical analysis (Table 2). These were analysed according to ISO 17994 for individual laboratories and for combined data (Table 4). For three of the laboratories, there were very few samples resulting in unreliable ISO 17994 analysis and 'inconclusive' outcomes. For the remaining laboratories and the combined data, the outcomes were also 'inconclusive'. This is not unexpected as the data sets are rather small, and the number of samples insufficient for a conclusive outcome to be determined. However, there does appear to be a tendency for higher counts being achieved by Pseudalert®/Quanti-Tray® as the lower value of the 'confidence interval', X_L, of -1.8 is markedly closer to zero than the upper value of the 'confidence interval', X_U, of $+23.8$.

A total of 585 positive wells from Pseudalert®/ Quanti-Tray® tests were subcultured and subjected to confirmation

Table 2 | Non-parametric statistics of untransformed paired sample results where Pseudalert/Quanti-Tray gave lower, equal or higher *P. aeruginosa* counts than PACN agar from routine hospital water samples

	Pseudalert					
Laboratory	**Lower**	**Equal**	**Higher**	**Total**	p^a	**Median paired difference (95% c.i.)**
1	2	0	5	7	0.453	+16.0 (–6.5 to +47.7)
2	2	0	4	6	0.688	+1.0 (–13.7 to +3.9)
3	9	3	18	30	0.122	+1.5 (0.0 to +3.0)
4	5	3	6	14	1.000	0.0 (–2.0 to +3.0)
5	11	3	7	21	0.481	–2.0 (–3.3 to +4.3)
6	1	0	1	2	1.000	–1.0 (–8.0 to +6.0)
All data	30	9	41	80	0.235	+1.0 (0.0 to +2.0)

[a]Binomial probability, parameter $p = 0.5$.

Table 3 | Non-parametric statistics of untransformed paired sample results where Pseudalert/Quanti-Tray gave lower, equal or higher *P. aeruginosa* counts than PACN agar from artificially contaminated hospital water samples

	Pseudalert					
Laboratory	**Lower**	**Equal**	**Higher**	**Total**	p^a	**Median paired difference (95% c.i.)**
1	7	0	28	35	<0.001	+16.0 (+7.3 to +28.0)
2	12	0	28	40	0.017	+11.5 (+3.8 to +16.8)
4	6	2	30	38	<0.001	+14.0 (+6.0 to +19.0)
5	17	1	21	39	0.627	+2.0 (−7.1 to +8.2)
6	18	6	16	40	0.864	+0.0 (−1.6 to +1.6)
All data	60	9	123	192	<0.001	+6.0 (+3.9 to +11.1)

[a]Binomial probability, parameter $p = 0.5$.

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Table 4 | Mean relative difference analysis (Trial Method – Reference Method) of paired sample results from the Trial Method (Pseudalert/Quanti-Tray) and the Reference Method (ISO 16266 and MoDW Part 8 PACN agar) for routine hospital water samples analysed for *P. aeruginosa*

Laboratory	Number of results	Mean relative difference	Standard deviation	W^a	X_L^b	X_U^c	Outcome
1	7	54.7	103.3	78.1	–23.4	132.7	Inconclusive
2	6	12.0	59.4	48.5	–36.7	60.5	Inconclusive
3	30	9.2	42.9	15.7	–6.5	24.9	Inconclusive
4	14	5.5	51.2	27.4	–21.8	32.9	Inconclusive
5	21	–5.2	42.3	18.5	–23.7	13.3	Inconclusive
6	2	91.2	146.2	206.7	–115.5	298.0	Inconclusive
Combined data	80	11.0	57.2	12.8	–1.8	23.8	Inconclusive

[a]Half width of the 'confidence interval' around the mean relative difference.
[b]Value of the relative difference at the lower 'confidence limit'.
[c]Value of the relative difference at the upper 'confidence limit'.

Table 5 | Mean relative difference analysis (Trial Method – Reference Method) of paired sample results from the Trial Method (Pseudalert/Quanti-Tray) and the Reference Method (ISO 16266 and MoDW Part 8 PACN agar) for artificially contaminated hospital water samples analysed for *P. aeruginosa*

Laboratory	Number of results	Mean relative difference	Standard deviation	W^a	X_L^b	X_U^c	Outcome
1	35	43.7	51.2	17.3	26.4	61.0	Trial method: higher recovery
2	40	20.8	32.4	10.2	10.6	31.1	Trial method: higher recovery
4	38	41.9	52.7	17.1	24.8	59.0	Trial method: higher recovery
5	39	–1.8	41.5	13.3	–15.1	11.5	Inconclusive
6	40	–2.0	59.1	18.7	–20.7	16.7	Inconclusive
Combined data	192	19.8	51.7	7.5	12.3	27.3	Trial method: higher recovery

[a]Half width of the 'confidence interval' around the mean relative difference.
[b]Value of the relative difference at the lower 'confidence limit'.
[c]Value of the relative difference at the upper 'confidence limit'.

tests according to ISO 16266 and MoDW Part 8. Of these, 581 (99.3%) were confirmed as *P. aeruginosa* by at least one procedure. Correspondingly, of 510 colonies from PACN agar, 489 (95.9%) were confirmed as *P. aeruginosa* by at least one confirmation procedure.

Phase 2 – artificially contaminated samples

The spiking protocol was successful in generating data from 210 artificially contaminated samples, of which 18 had paired count results in which the count by at least one method exceeded the maximum count range and, therefore, were excluded from statistical analysis leaving a data set of 192 samples with paired counts (Table 3). The results of the mean relative difference analyses are summarised in Table 5. In three laboratories (1, 2 and 4), these resulted in the Trial Method (Pseudalert®/Quanti-Tray®) yielding significantly higher counts of *P. aeruginosa* compared with those for the ISO 16266 PACN or MoDW Part 8 method. For the remaining two laboratories (5 and 6), the outcomes were 'inconclusive'. However, the spread of the confidence intervals (X_L and X_U) indicates that the two methods would be comparable at these two laboratories if sufficient samples had been analysed. Combining the data from all five laboratories Pseudalert®/Quanti-Tray® produced overall significantly higher counts of *P. aeruginosa*.

Analysis of combined data sets

Analysis of the data from both the naturally contaminated and the artificially contaminated data sets reveals no

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significant difference between the two populations. A scatter plot of the paired counts for these combined data is presented in Figure 1, showing a greater number of data points on the Pseudalert®/Quanti-Tray® side of the line of equivalence. The two data sets were combined giving 272 paired counts for further analysis according to ISO 17994. This resulted in mean relative difference of +17.3 (standard deviation = 53.4) with a lower value of the 'confidence limit', X_L, of +10.9 and an upper value of the 'confidence limit', X_U, of +23.8, indicating that Pseudalert®/Quanti-Tray® gave significantly higher counts of *P. aeruginosa*.

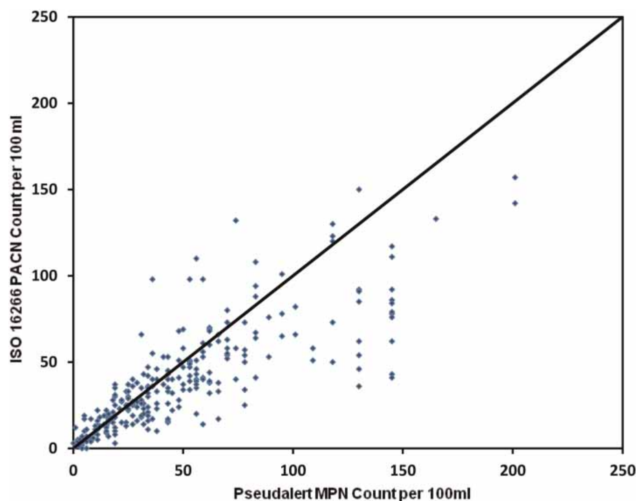

Figure 1 | Scatter plot of the counts from paired sample results from the Trial Method (Pseudalert/Quanti-Tray) and the Reference Method (ISO 16266 and MoDW Part 8 PACN agar) for the combined data sets of routine and artificially contaminated hospital water samples.

Variation in recovery of strains of *P. aeruginosa*

The analysis of relative recoveries of the strains of *P. aeruginosa* used to artificially contaminate samples is set out in Table 6. Significantly higher recoveries of six of the 10 strains were obtained by Pseudalert®/Quanti-Tray®. Relative recoveries of two of the remaining strains (6 and 8) were inconclusive, but with a tendency for higher recoveries by Pseudalert®/Quanti-Tray®. The data for strain 1 indicate potentially equivalent performance, whilst those for strain 4 indicate potentially higher recoveries by ISO 16266 and MoDW Part 8. There was no obvious microbiological reason to explain the different response of strain 4.

Recovery of *P. fluorescens*

All laboratories reported the strain of *P. fluorescens* as not producing fluorescence in Pseudalert®/Quanti-Tray®. However, three laboratories reported limited growth on PACN

Table 6 | Mean relative difference analysis (Trial Method – Reference Method) of paired sample results from the Trial Method (Pseudalert®/Quanti-Tray®) and the Reference Method (ISO 16266 and MoDW Part 8 PACN agar) for artificially contaminated hospital water samples analysed for *P. aeruginosa* based on strains

Strain of *P. aeruginosa*	Number of results	Mean relative difference	Standard deviation	W[a]	X_L^b	X_U^c	Outcome
1	20	–1.9	30.6	13.7	–15.6	11.7	Inconclusive
2	20	36.8	42.2	18.9	17.9	55.7	Trial method: higher recovery
3	21	20.2	36.8	16.1	4.1	36.3	Trial method: higher recovery
4	19	–21.8	88.3	40.5	–62.3	18.6	Inconclusive
5	20	18.4	22.9	10.3	8.1	28.7	Trial method: higher recovery
6	19	17.8	44.4	20.4	–2.5	38.2	Inconclusive
7	19	27.5	48.6	22.3	5.2	49.8	Trial method: higher recovery
8	19	23.8	66.1	30.3	–6.6	54.1	Inconclusive
9	17	37.4	43.9	21.3	16.2	58.7	Trial method: higher recovery
10	18	43.3	41.4	19.5	23.8	62.8	Trial method: higher recovery

[a]Half width of the 'confidence interval' around the mean relative difference.
[b]Value of the relative difference at the lower 'confidence limit'.
[c]Value of the relative difference at the upper 'confidence limit'.

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agar producing colonies, which were not typical of those produced by *P. aeruginosa*.

DISCUSSION

Pseudomonas aeruginosa is commonly found in moist environments and can be detected in domestic, thermal and hospital water networks. Also, although this bacterium does not cause significant infection in healthy people, *P. aeruginosa* has emerged as a major pathogen in nosocomial infections due to both the number and severity of the infections it causes in immunocompromised people. This depends not only on the pathogenicity of *P. aeruginosa* and the susceptibility of patients, but also on the multi-resistance in *P. aeruginosa* to antimicrobial agents. In 2006, a point prevalence survey in France indicated that *P. aeruginosa* was responsible for 10% of all nosocomial infections, only slightly fewer than for those attributable to *Escherichia coli* and *Staphylococcus aureus* (InVS 2009). The role of the water environment as a reservoir and vector of *P. aeruginosa* is indisputable, and contamination is often difficult to control. Several authors assume that priority should be given to improvement of local diagnosis of the water network and to rapid monitoring of *P. aeruginosa* in hospital water systems (Rogues *et al.* 2007; Baghal Asghari *et al.* 2013) to control tap water contamination. Polymerase chain reaction (PCR) detection can provide simple, rapid and reliable identification of *P. aeruginosa* in hospital water systems (Baghal Asghari *et al.* 2013), but when genotyping is needed as part of conducting epidemiological surveillance and to identify the source and reservoir of contamination, cultural methods remain essential and cannot be replaced by PCR (Trautmann *et al.* 2001; Cholley *et al.* 2008).

This study compared the Pseudalert®/Quanti-Tray® method for the MPN enumeration of *P. aeruginosa* from contaminated hospital water and artificially contaminated hospital water samples to the ISO 16266:2006 and MoDW Part 8 PACN agar membrane filtration methods. The Pseudalert®/Quanti-Tray® method has the advantage of providing confirmed counts of *P. aeruginosa* within 24 hours compared to the 48 hours needed for the two reference methods. This is a significant advantage for assessing water safety in hospital environments where susceptible patients may be at risk of infection. There are also significant health and safety benefits of using the trial method compared to ISO 16266 as the ISO confirmation procedure requires acetamide broth (containing acetamide, which is carcinogenic) and Nessler's reagent (containing mercuric chloride, which is toxic). It would be advantageous to avoid the use of such chemicals in laboratories.

ISO 17994 (ISO 2014) recommends analysing a method comparison by using mean relative differences. For the hospital water samples, because of the difficulties in getting sufficient numbers of naturally positive samples, this analysis resulted in an inconclusive assessment as to whether the Pseudalert®/Quanti-Tray® method was equivalent to the ISO 16266 and MoDW Part 8 PACN methods for the enumeration of *P. aeruginosa*. However, the data do indicate a tendency for higher counts being achieved by Pseudalert®/Quanti-Tray®. More samples would need to be analysed to confirm this. For artificially contaminated samples, the outcome of ISO 17994 analysis was that the recovery of *P. aeruginosa* was significantly higher using the Pseudalert®/Quanti-Tray® method. The same outcome was achieved when the data from both naturally contaminated and artificially contaminated samples were combined for analysis. Part of the higher recovery of *P. aeruginosa* by Pseudalert®/Quanti-Tray® may be related to better recovery of stressed bacteria in liquid media compared to isolation on solid agar media. The Pseudalert® reagent also demonstrated good specificity with 99.3% of tested positive wells confirming *P. aeruginosa*.

CONCLUSIONS

From this study, it is concluded that the Pseudalert®/Quanti-Tray® method is an acceptable alternative to the ISO 16266:2006 and MoDW Part 8 PACN agar methods for the enumeration of *P. aeruginosa* from hospital waters and has the additional benefits of giving a more rapid confirmed result. This is particularly important during the investigation of possible hospital-acquired infections enabling appropriate action to be taken to protect patients within as short a time scale as possible while also enabling the more rapid identification of uncontaminated outlets, so they can be safely returned to use within 24 hours.

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