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Garvey, Mark; Wilkinson, Martyn A. C.; Holden, Kerry; Martin, Tracey; Parkes, Jane; Holden, Elisabeth

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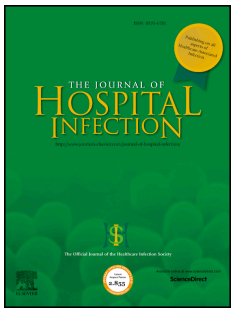

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Tap out: reducing waterborne *Pseudomonas aeruginosa* transmission in an intensive care unit.

Mark I. Garvey[1,2*], Martyn A. C. Wilkinson[1], Kerry Holden[1], Tracey Martin[1], Jane Parkes[1] and Elisabeth Holden[1]

[1]University Hospitals Birmingham NHS Foundation Trust, Queen Elizabeth Hospital Birmingham, Edgbaston, Birmingham, B15 2WB

[2]Institute of Microbiology and Infection, The University of Birmingham, Edgbaston, Birmingham

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[*]Corresponding author

Email: mark.garvey@uhb.nhs.uk or m.i.garvey@bham.ac.uk

Tel: 0121 371 3787

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SUMMARY

Background: *Pseudomonas aeruginosa* is a ubiquitous and important opportunistic pathogen in immunocompromised or critically ill patients. Nosocomial *P. aeruginosa* outbreaks have been associated with hospital water sources. Here we describe engineering interventions to minimise contamination of water outlets and the subsequent clinical impact.

Methods: New tap outlets were fitted at selected outlets across the intensive care unit (ICU). Laboratory testing demonstrated that, following artificial contamination with *P. aeruginosa,* these taps could be effectively decontaminated using a thermal washer disinfector. Water samples were collected weekly from new outlets on the ICU over an 8-month period and tested for the enumeration of *P. aeruginosa* via membrane filtration. surveillance of *P. aeruginosa* from clinical specimens was routinely undertaken.

Results: Prior to the interventions, water sampling on ICU indicated that 30% of the outlets were positive for *P. aeruginosa* at any one time and Whole Genome Sequencing data suggested at least 30% transmission from water to patient. Since their installation, weekly sampling of the new tap outlets has been negative for *P. aeruginosa*, and the number of *P. aeruginosa* clinical isolates has fallen by 50%.

Conclusions: Installation and maintenance of tap outlets free of *P. aeruginosa* can substantially reduce the number of *P. aeruginosa* clinical isolates in an ICU.

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INTRODUCTION

Pseudomonas aeruginosa is a ubiquitous and important opportunistic pathogen in immunocompromised or critically ill patients.[1-3] In the UK, *P. aeruginosa* is responsible for 3% of all reported monospecies bacteraemias.[4] Due to its natural ability to survive in the moist environment, nosocomial *P. aeruginosa* outbreaks have been associated with hospital water sources.[5-6] In 2013 the Department of Health (DH) in England published guidance that included recommendations for sampling of water and implementing control measures where *P. aeruginosa* is found in the water.[7]

Water outlets, particularly taps and associated pipework, are a recognised reservoir for micro-organisms, providing a large surface area for biofilms to harbour potential pathogens, such as *P. aeruginosa*.[6,8] Between November 2011 and January 2012, 25 babies admitted to neonatal intensive care units in Northern Ireland acquired *P. aeruginosa*;[6,9] these incidents were attributed to contaminated tap outlets. Further nosocomial *P. aeruginosa* outbreaks associated with hospital water sources have been reported.[10-12] Other potential routes of transmission include cross-infection, for example, carriage on the hands of healthcare workers, and through contaminated medical equipment.[13-14] The DH has recently updated the Health Technical Memorandum (HTM) 04-01 which emphasises the role of water in nosocomial infections and suggests a risk-management approach to the safety of water is pivotal in the control of infection in a healthcare setting.[15-16]

We have previously shown that holistic measures, including appropriate tap cleaning and waste water disposal, combined with installation of point of use filters have resulted in reduction of waterborne transmission of *P. aeruginosa* in a tertiary referral hospital intensive care unit (ICU).[17] Here we describe the use of an engineering

intervention to control *P. aeruginosa* on our ICU. We specifically investigated whether installation of new tap outlets would have an impact on the number of outlets colonised with *P. aeruginosa* and whether this would have a clinical impact. Secondary aims of the study were to investigate whether *P. aeruginosa* could be removed from contaminated taps, and to determine how often water sampling needed to be undertaken in a setting where contamination of tap outlets with *P. aeruginosa* is high.

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MATERIALS AND METHODS

Setting. Queen Elizabeth Hospital Birmingham (QEHB) is a tertiary referral National Health Service teaching hospital in Birmingham, UK. QEHB has one of the largest co-located ICUs in the world with 100 beds and 231 water outlets, of which 130 are clinical outlets. The hospital is funded via a Private Finance Initiative (PFI), and was opened in 2010.

Patient surveillance. Surveillance of *P. aeruginosa* in clinical specimens was undertaken as previously described.[17]

Water sampling and microbiological methods. Water samples from all outlets on the ICU were collected every 6 months. These were cultured and *P. aeruginosa* identified as previously described.[17] Additional water sampling was performed from the eight test outlets (see below), and from eight randomly selected control outlets, also on ICU A. All of the test and control outlets were clinical hand wash basins.

Engineering interventions. On ICU A 8 new taps (Marwik 21, Armitage Shanks, UK; Figure 1) were fitted at selected bed spaces (Figure 2). These replaced the previous Rada therm 3 (Rada, UK) tap outlets that were installed across QEHB at the time of construction. The new taps were selected on the basis of recommendations from colleagues in other centres that they may be associated with fewer contamination issues with *P. aeruginosa*. ICU The 8-bedded bay on ICU A was chosen because it had a dedicated sluice to facilitate disposal of patient waste water.

Test taps. The Markwik21 tap assembly (Figure 1) is designed to be detached from the water supply, dismantled and subjected to a decontamination procedure. The spout and mixing valve can be removed and access to the pipework achieved by removal of a cover from each end. The components can withstand high temperatures and can be processed in a benchtop thermal washer disinfector.

Removal of artificial contamination. The initial assessment of the ease of decontamination of the test taps was undertaken with artificial contamination. Two strains of *P. aeruginosa* were used: NCTC 6749 and PS-1054, a clinical isolate from QEHB known to readily form biofilm.[18] A suspension of the test bacteria containing approximately 10^8/ml was prepared in sterile distilled water. The spout, thermostatic mixing valve (TMV) and pipework (Bar) were flushed / immersed in the culture for 30 seconds. Excess culture was discarded, the components were placed into a sealed plastic bag to retain humidity and were stored at 21℃ for 7 days to allow biofilm formation. Microbial counts on the components were determined before and after disinfection using a Medisafe Pico thermal washer-disinfector with 3E-Zyme enzymatic cleaner (Medisafe, Stortford, UK). The spout and mixing valve were sampled by placing them in a sterile bag containing ¼ strength Ringer's solution (Oxoid, Basingstoke, UK) and processing in an ultrasonic water bath for 1 minute. The wash fluid was then cultured to determine the number of viable bacteria. The pre-disinfection samples were serially diluted in Ringer's solution, whilst the post disinfection samples were filtered, and the filter placed on the surface of a culture plate. The pipework was too large to sample in this way, so a manual washing/flushing method was used. This entailed aseptically transferring the bar to a sterile plastic bag, followed by manual elution with 100 ml Ringer's solution. The pre and post samples were plated onto tryptone soya agar plates and incubated for 42 - 48 hours at 37^{o}C, and the numbers of colony forming units were enumerated. Tests were performed in triplicate.

The sampling methods were validated by performing a pre-count sampling procedure on a contaminated tap, followed by a further sampling procedure on the

same (still contaminated) tap. This allowed the reduction in bioburden due to the sampling procedure to be measured.

Removal of natural contamination. At the conclusion of the 33-week sampling period, two test taps (from ICU A) were removed and disassembled into their component parts. The spout, TMV and bar were sampled for pre-counts, processed in a washer-disinfector, and then sampled for post counts in the same manner as for the artificial contamination detailed above.

Holistic interventions. In February 2016 (Figure 2), a revised tap cleaning method was implemented, as previously described.[19] Patient waste water was disposed of directly into the sluice, or into a macerator after addition of absorbent gel sheets (Figure 2).[17]

Statistics. TVCs and *P. aeruginosa* CFUs between the test and control taps were compared using negative binomial regression models in the MASS package in R version 3.5.0.[20] To determine the frequency of water sampling, Bayesian analysis was used to determine the probability, θ, of a tap being contaminated with *P. aeruginosa*. The Bayesian analysis was performed by hand and subsequently ratified in R.[20] Patient acquisition rates of *P. aeruginosa* per 100,000 bed days in ICU overall, and in ICU A separately, were analysed using segmented Poisson regression models in R, using a similar technique to Gebski *et al.,*[20-21] Data from the period August 2013 to December 2017 were included in the analysis.

Audits. Monthly audits were undertaken by the Infection Prevention and Control Team during the period of this report. These included monitoring the correct disposal of patient waste water and tap cleaning.

RESULTS

Water sampling. From the water sampling undertaken on the ICU, 27% of the outlets were positive for *P. aeruginosa* in 2017 (Table 1). Since 2013, there has been a 37% increase in the number of water outlets colonized with *P. aeruginosa* across the ICU (Table 1).

Test Tap decontamination. Validation of the sampling method showed that the mean reduction in contamination over the serial sampling procedures, calculated using the three components of two taps was found to be 1.11 $\log_{10}$ units (data not shown). Sampling an artificially contaminated tap showed mean $\log_{10}$ reduction factors after thermal disinfection for the different components of 6.36 for the bar, 6.98 for the TMV and 6.20 for the spout.

Total viable counts of test tap samples. Approximately one week after the installation of the test taps, weekly sampling commenced. The mean TVC isolated from the 264 control samples was 15402 CFUs, whilst the mean from the same number of test samples was 10360 CFUs. Using a negative binomial regression model the TVCs of test taps were significantly lower than for control taps ($p = 0.000232$).

P. aeruginosa isolated from tap samples. Substantial levels of *P. aeruginosa* were recovered from the control taps. However, there were only two instances of contamination of test taps with *P. aeruginosa*; 2 CFUs were recovered from one test tap, and 1 CFU from another, as isolated occurrences (subsequent samples from both taps were negative). The mean *P. aeruginosa* CFU's isolated from the two sample types were highly divergent (3313.6 and 0.011 for control and test samples, respectively), and a negative binomial regression model confirmed that *P. aeruginosa* counts from the test taps were significantly lower ($p < 2 \times 10^{-16}$).

Tap decontamination redux. After 8 months two test taps were removed and sampled. While there was no contamination recovered from the spout of one of the taps, the other components contained extensive levels of bacteria. The $\log_{10}$ pre-counts per 100ml were 5.04 for the other spout, 4.56 and 5.34 for the bars, and 6.68 and 6.78 for the TMV's. The components were then processed through the washer-disinfector and then resampled. All of the post-counts were zero.

Water testing frequency. Bayesian analysis (beta-binomial conjugate model) was used to predict the probability of detecting contamination with *P. aeruginosa* from each control or test tap from water sampling (Table 2). The model suggests monthly sampling detects all the *P. aeruginosa* positive outlets more assuredly than six-month sampling regimes over any one six month period.

Clinical isolates. The Poisson regression model used to analyse the clinical isolates from ICU as a whole suggests that the two most important interventions were the fitting of filters to selected taps across ICU, and the alteration of the disposal of waste water and cleaning protocols ('holistic factors') (Figure 3A). The model provides very strong evidence that the provision of filters was coincident with a marked reduction in the acquisition of *P. aeruginosa* ($p = 3.73 \times 10^{-8}$). The trend term in the model suggests that over the 12-month period following the introduction of filters, there was a 67% decrease in the acquisition of *P. aeruginosa* across ICU. Somewhat counterintuitively, the model provides good evidence that the introduction of 'holistic factors' across the whole of ICU was associated with an increase in the acquisition of *P. aeruginosa* ($p = 0.00135$). In contrast, the regression model used to analyse ICU A alone, suggests that the only important intervention was the fitting of the new taps (Figure 3B). The model provides marked evidence that the new taps were associated with a noticeable decrease in the incidence of *P. aeruginosa*

acquisition on ICU A ($p = 1.98 \times 10^{-5}$). The model suggests that the introduction of the new taps was associated with an immediate and sustained 72% decrease in the acquisition of *P. aeruginosa* on ICU A.

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DISCUSSION

P. aeruginosa can persist in hospital water systems for long periods and has resulted in a number of hospital outbreaks.[22-23] We previously reported an outbreak of *P. aeruginosa* in an ICU setting with the source being a colonised tap outlet.[2] Moreover, the number of tap outlets testing positive for *P. aeruginosa* increased from 2013 to 2017 by 37%.[17] We subsequently demonstrated that point of end filters, together with holistic measures, reduced the number of clinical isolates of *P. aeruginosa*.[17] Point of use filters are not the ideal solution, because the filters themselves can become contaminated with *P. aeruginosa* if used inappropriately.[24] Breakpoint models indicated the engineering and holistic interventions resulted in a 50% reduction in the number of *P. aeruginosa* clinical patient isolates over a year.[17] We have continued our pioneering work in this area by focusing on new engineering control measures and the effect these have controlling the transmission of *P. aeruginosa* in an ICU setting.

The HTM 04-01 guidance details that when an outlet is positive for *P. aeruginosa,* risk reduction and preventive measures should be considered.[15] These include engineering considerations such as removal of flow straighteners, dismantling of tap outlets for cleaning and disinfection, assessing the water distribution system and components of the tap outlet.[15] Remedial work can be difficult to undertake and there are no standards on the correct procedure. Indeed, we found that despite remedial work on tap outlets, contamination with the same strain of *P. aeruginosa* was seen 6 months later.[2] For this reason we were interested in a tap outlet that could withstand high-level disinfection. We found in a laboratory setting that a thermal washer-disinfector cycle gave a ≥ 5 $\log_{10}$ reduction in CFU in three different components of the tap outlet, and most importantly, a tap outlet that was free from *P. aeruginosa*.

However, whilst this first phase of work gave confidence to install the new tap outles in a clinical area, the artificial system may not represent the formation of biofilm in real-life clinical settings. We found that although the test taps were clear of *P. aeruginosa,* high TVCs were detected as soon as one week after installation. This is not surprising, as water is known to harbour microorganisms, with biofilms developing quickly.[25] We were then able to establish the feasibility of disinfecting taps in a real-life setting, and also showed that the absence of *P. aeruginosa* was sustained, aside from two episodes of low level and transient contamination

A review by Loveday *et al.,* (2014) indicated that there is poor evidence for remediation.[22] However, we found that replacing half the tap outlets on an ICU area resulted in a significant reduction of *P. aeruginosa* clinical isolates based on Poisson regression models.

The frequency of water testing of tap outlets for *P. aeruginosa* was originally recommended to be six-monthly.[7] This recommendation has since been updated, and a risk assessment approach is now recommended to determine the frequency of water testing.[15] However, there is a lack of evidence in the literature as to the appropriate frequency of testing. We have previously suggested that a six-monthly sampling regimen may result in a number of positives being missed.[17] Indeed. Bayesian models predicted that monthly sampling would enhance the detection rate of *P. aeruginosa* in tap outlets and allow problems to be rectified more promptly.

A Poisson regression model identified that the two most important interventions in reducing *P. aeruginosa* were the fitting of filters to selected taps across ICU, and alteration of the disposal of waste water and cleaning protocols. However, these interventions actually appeared to be associated with a slight increase in the number of cases of *P. aeruginosa*; we believe that this was coincidental rather than

causative. The regression model used to analyse ICU A alone, suggested the only important intervention was the fitting of the new taps.

The introduction of the new taps was associated with an immediate and sustained 72% decrease in the acquisition of *P. aeruginosa* on ICU A. If these findings were confirmed in larger studies they would help hospitals decide to invest in replacing taps as a more cost-effective alternative to fitting filters. It must be noted that QEHB is unique in having such a large ICU, and the impact of changing tapes may not be generalizable. However, we believe our results should be helpful to other hospitals faced with ongoing problems with *P. aeruginosa* contamination of tap outlets.

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Conflicts of interest statement

None.

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None.

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TABLES

Table 1. Total number of ICU water outlets positive for *Pseudomonas aeruginosa* per year between 2013-2017.

ICU	Positive outlets 2013*	Positive outlets 2014*	Positive outlets 2015*	Positive outlets 2016*	Positive outlets 2017*
Area A	(20) 29%	(21) 30%	(28) 40%	(29) 41%	(20) 29%
Area B	(11) 22%	(14) 29%	(14) 28%	(10) 20%	(15) 30%
Area C	(8) 15%	(13) 28%	(15) 30%	(12) 24%	(9) 18%
Area D	(7) 10%	(14) 23%	(17) 28%	(22) 36%	(19) 31%
Total	(46) 20%	(59) 26%	(54) 24%	(73) 31%	(63) 27%

Key: *Numbers in the brackets refer to number of positive outlets

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Table 2. The predicted probabilities of (at least one) positive tap outlet over a six-month period from the different sites, depending upon whether sampling is undertaken monthly or six-monthly.

Site	Tap type	Probability of Positive Isolate	
		Monthly sample	6-Monthly sample
A2 Lobby	Control	0.386	0.083
A4	Control	1.000	0.917
A7	Control	0.639	0.167
A10	Control	1.000	0.944
A20	Control	1.000	0.889
A22	Control	0.386	0.083
A25	Control	0.916	0.361
Blood Gas	Control	0.639	0.167
A11 Lobby	Test	0.146	0.028
A12	Test	0.146	0.028
A13	Test	0.274	0.056
A14	Test	0.146	0.028
A15	Test	0.146	0.028
A16	Test	0.146	0.028
A17	Test	0.274	0.056
Blood Gas	Test	0.146	0.028

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FIGURES

Figure 1. Marwik 21, Armitage Shanks tap outlet installed onto ICU A.

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Figure 2. Timeline of interventions during the study.

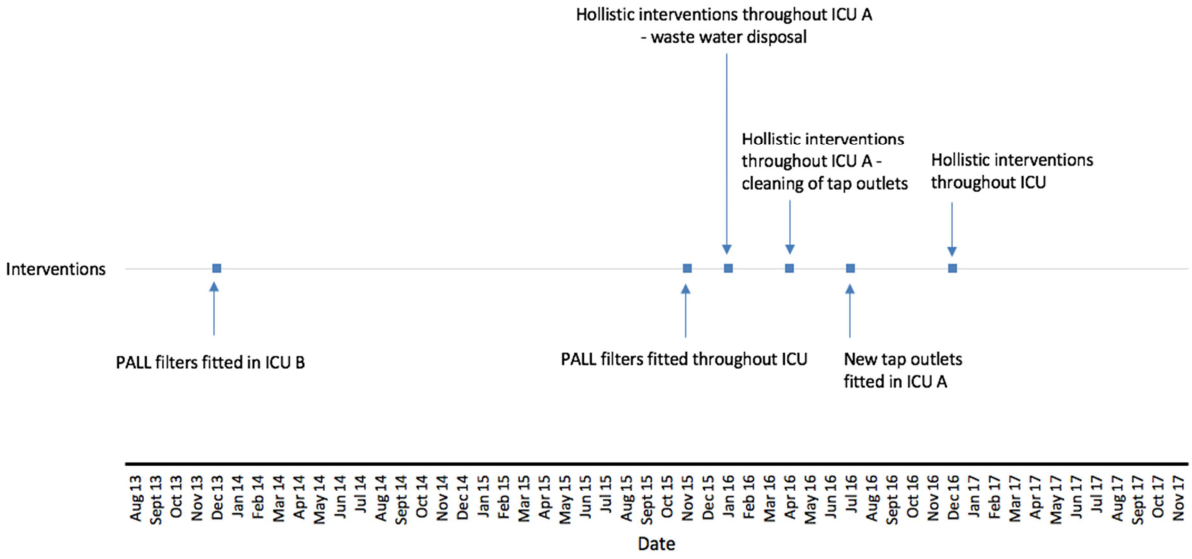

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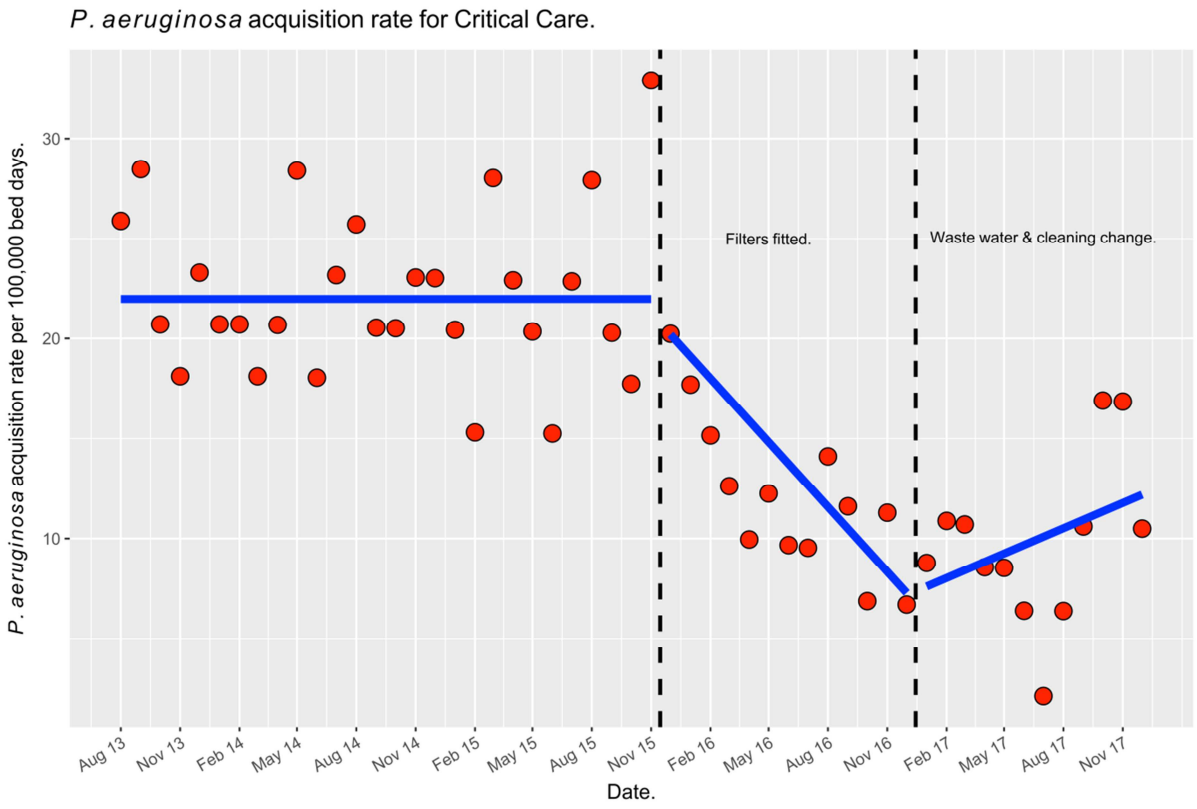
Figure 3A. Rate of *P. aeruginosa* clinical isolates per 100,000 bed days in the entire critical care.



Key: The dotted lines represent the infection prevention and control interventions, which had a significant impact upon the segmented Poisson regression models. The blue lines represent the mean values predicted by the Poisson regression model.

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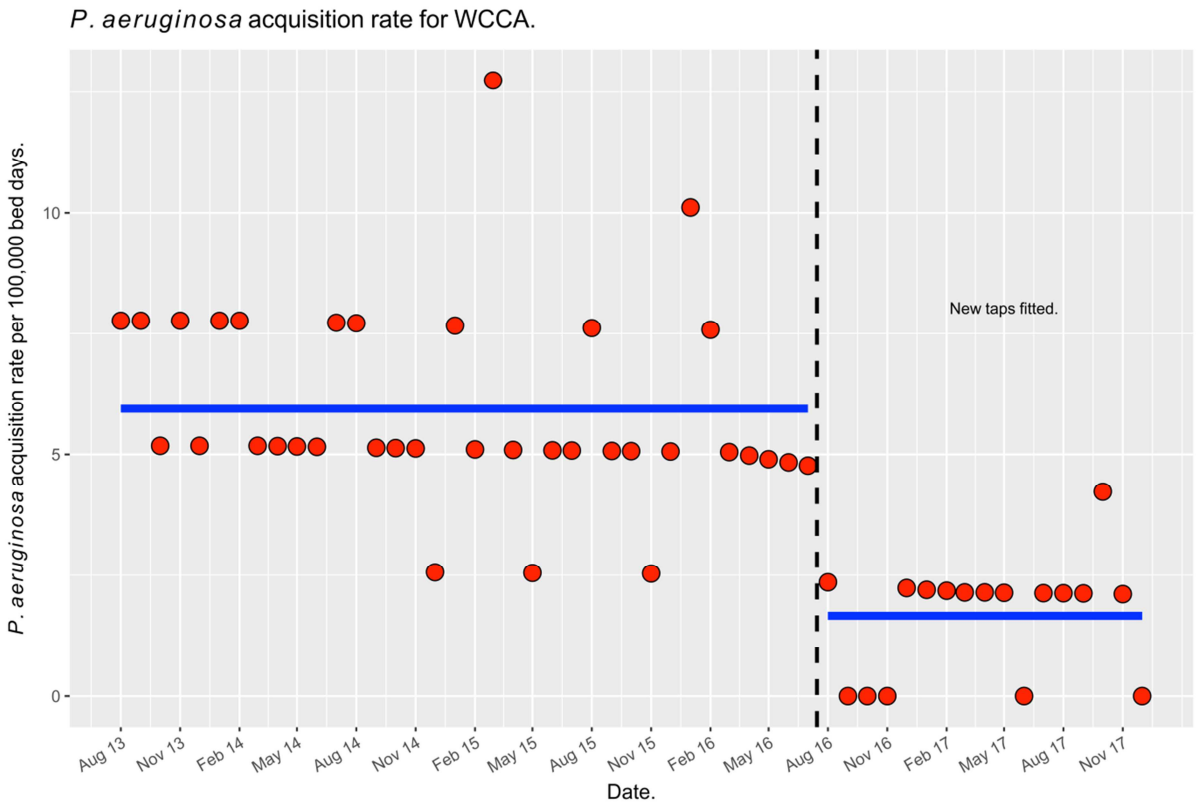
Figure 3B. Rate of Pseudomonas aeruginosa clinical isolates per 100,000 bed days in the critical care unit A.



Key: The dotted line represents the infection prevention and control intervention, which had a significant impact upon the segmented Poisson regression models. The blue lines represent the mean values predicted by the Poisson regression model.

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