SIGNA VITAE 2017; 13(1): 75-79

# Outbreak of Pseudomonas aeruginosa in a Neonatal Intensive Care Unit: Are Point-of-Use Filters Useful?

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## ABSTRACT

Pseudomonas aeruginosa, in intensive care units (ICUs), causes infections with high morbidity and mortality rates. Tap water outlets are often contaminated with P.aeruginosa and may represent a source of endemic infections in ICUs.
The aim of this study was to explore the role of point-of-use (POU) filters in neonatal intensive care unit (NICU) in reducing P.aeruginosa colonizations/infections.
Routine surveillance cultures, environmental cultures and samples from the hands of healthcare personnel, were taken and cultivated. P.aeruginosa isolates were identified according to standard procedures. For epidemiological purposes, antimicrobial susceptibility testing and pulsefield gel electrophoresis were performed.
Data regarding use of antibiotics, disinfectants, antiseptics, gloves and gowns from 2006 to 2012 were investigated.
In March 2008, in the NICU of the Clinical Hospital Centre Zagreb (CHC Zagreb), we observed an increase in the total number of pseudomonas infections compared to the previous months. This higher number remained the same until October, despite rigorous infection control measures.
Pseudomonas isolates were found in tap water, but not on the hands of healthcare workers. In that moment POU filters were introduced. The number of P.aeruginosa isolates in surveillance cultures dropped significantly.
The number of positive cultures of P.aeruginosa in two consecutive periods (before and after installation of POU filters) showed a statistically significant difference.
After the implementation of all infection control measures, we managed to stop the spread of pseudomonas colonization/infection. POU filters contributed only as one of these measures, resulting in a reduction of chronically endemic P.aeruginosa infection/colonisation in the NICU.

*Key words: P.aeruginosa, neonatal intensive care unit, point of use filters, infection control*

## INTRODUCTION

Pseudomonas aeruginosa is a frequently isolated gram-negative pathogen and is a common cause of infection in intensive care units (ICUs). In hospital settings, P.aeruginosa causes infections mostly in immunocompromised patients with high morbidity and mortality rates. It is well known that tap water outlets are often contaminated with P. aeruginosa and may represent a source of endemic infections in neonatal intensive care units (NICUs). (1,2)
Recently, several cases of P. aeruginosa colonization/infection in NICUs have been published. In the review by Jefferies et al., published in 2012, there is clear evidence that P. aeruginosa can be imported into the NICU from a contaminated environment, especially from water sources (water taps, sinks, water baths, etc.). The implementation of point-of-use (POU) filters (Pall-Aquasafe, Pall Medical, Switzerland) in ICUs, has been shown to be a successful infection control measure because it has led to a significant reduction in P.aeruginosa colonizations/infections. (3-5)

## METHODS

At the time of the study, the NICU consisted of 38 beds. Surveillance microbiological cultures (tracheal, gastric and nasopharyngeal aspirates; stools) have been performed for years as part of weekly routine procedures. In March 2008, we observed an increase in the number of P. aeruginosa isolates from surveillance cultures in the NICU. Standard control measures (contact isolation and strict hygiene of environmental surfaces, hand disinfection of NICU staff), were implemented immediately. At that moment, no environmental samples were taken at all.
Given the increased number of pseudomonas isolates in October 2008, the infection control team repeated the survey. Routine surveillance cultures, environ-

mental samples, including sinks and other surfaces in areas adjacent to patients, as well as fingerprints of all healthcare personnel, were taken.

Additional measures included: washing of neonates with sterile water, keeping the temperature of hot tap water over 50°C and having designated nurses for taking care of infected or colonized neonates.

Investigation of the water system was performed. An increased number of P. aeruginosa isolates in tap water outlets was found. At the end of October 2008, POU filters were installed.

Specimens were inoculated on blood agar (BioMerieux, Marcy L'Etoile, France) and MacConkey agar plates (BBL, Le Pont de Claix, France) according to standard microbiological procedures and incubated at 35°C for 48 hours, as described previously. (6) Isolates of P. aeruginosa were identified by Vitek 2 system (BioMerieux, Marcy L'Etoile, France) and frozen at -80°C for further analyses.

For epidemiological purposes, phenotypic characteristics (antimicrobial susceptibility testing) and genotyping methods (pulse-field gel electrophoresis - PFGE), were used. Antimicrobial susceptibility testing was performed according to CLSI standards as described previously. (7)

Data regarding consumption of antibiotics, disinfectants, antiseptics, gloves and gowns for the period from 2006 to 2012 were also investigated.

PFGE was performed on genomic DNA which was exposed to digestion of restriction enzyme SpeI. The products were electrophoreticaly separated and the resulting PFGE profiles were analyzed regarding the clonality of P. aeruginosa strains isolated from the patients' clinical samples, surveillance cultures and environmental surfaces, as described previously. (8)

The results were interpreted according to the manufacturer recommendations.

The similarity of isolates was analyzed using the computer program Gel Compar, in which the similarity of each pair of isolates was assessed using a dendrogram (Applied Maths, Ghent, Belgium). All isolates with a genetic similarity of 80% or higher were considered to be genetically related, according to criteria by Tenover et al. (9,10)

## STATISTICAL ANALYSIS

Statistical analysis was performed using STATISTICA v. 12 (StatSoft, Inc., OK, USA). Time trends of a monthly number of positive isolates for two time periods (before and after installation of POU filters) were compared using partial linear regression analysis. The yearly data for the number of positive isolates, positive isolates per 100 hospital days and an index of change (calculated as the yearly change in positive isolates per 100 hospital days; index >1 means an increase in the number of positive isolates per 100 hospital days and index <1 means a decrease in the number of positive isolates per 100 hospital days) were presented. An association was calculated with the variables representing the consumption of antibiotics and the application of sanitary and prevention methods using both univariate and multivariate regression analysis. Multivariate regression was done using a forward stepwise method. For all tests p<0.05 was used as statistically significant. (11)

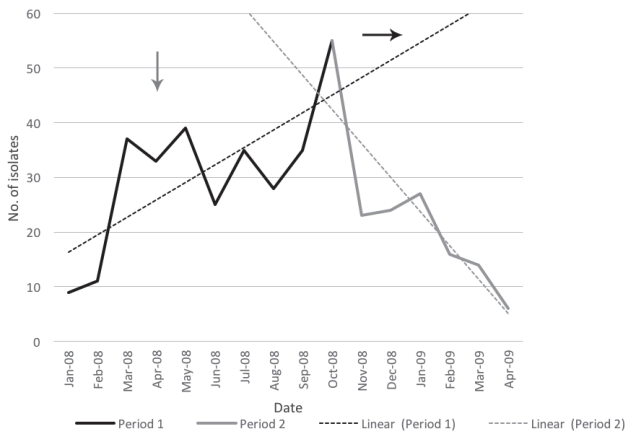


*Figure 1. Number of isolates (positive cultures of Pseudomonas aeruginosa) in two consecutive time periods (before and after installation of point water filters) with linear regression curves; linear regression curves show a statistically significant difference (p<0.001).*

→ *Implementation of standard control measures*
→ *Implementation of filters*

## RESULTS

In October 2008, increased numbers of positive P. aeruginosa isolates in tap water outlet were found. P. aeruginosa was isolated in four (4) out of nine (9) samples.

In addition to standard measures, POU filters were installed. From this point onwards, the number of pseudomonas isolates fell constantly (Table 1).

In December 2008 there were five (5) P. aeruginosa positive blood cultures from three (3) neonates taken on three consecutive days.

Environmental samples were taken again. We found one (1) P. aeruginosa isolate out of seven (7) samples, on a sink in the NICU. The number of positive surveillance cultures was not increased compared to the previous months (23 P. aeruginosa isolates in November, 24 in December). However, there were no P. aeruginosa isolates found on the hands of healthcare workers.

Standard control measures were revised and tightened. From that moment, the number of P. aeruginosa positive isolates in the surveillance cultures had constantly decreased, and there were no more positive blood cultures (Table 1 and Figure 1).

Partial linear regression analysis for the two time periods (before and after installation of POU filters) showed that there was a statistically significant difference (figure 1) between the linear regression curves of these two time periods (p<0.001). The first period showed a significant positive regression for the number of positive isolates (positive cultures of P. aeruginosa) showing an increase in the number of positive isolates till October 2008 (r=0.719, p=0.019). The second period (after installation of POU filters) showed a significant inverse linear regression (r=-0.856, p=0.014).

Data on the total number of P. aeruginosa isolates from primary sterile sites, surveillance cultures and environmental samples from 2006 till 2012 are presented in table 2.

Yearly data, from 2006 to 2012, on positive isolates were also analysed for associations with the variables representing the use of antipseudomonas antibiotics (per 100 hospital days and an index of change), sanitation and prevention methods (disinfectants, antiseptics, gloves and gowns consumption) using both univariate and multivariate regression analysis.

According to univariate regression analysis there were statistically significant associations in consumption of cefepime and meropenem with the number of positive isolates (p<0.05). Consumption of

*Table 1. The monthly number of Pseudomonas aeruginosa isolates in surveillance cultures from January 2008 till April 2009.*

| No.of Ps | Jan 08 | Feb 08 | Mar 08 | Apr 08 | May 08 | Jun 08 | Jul 08 | Aug 08 | Sep 08 | Oct 08 | Nov 08 | Dec 08 | Jan 09 | Feb 09 | Mar 09 | Apr 09 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 9 | 11 | 37* | 33 | 39 | 25 | 35 | 28 | 35 | 55** | 23 | 24 | 27 | 16 | 14 | 6 |

*Implementation of standard control measures
**Implementation of filters

*Table 2. Number of Pseudomonas isolates in NICU in different specimens from 2006 to 2012*

| Year | Surveillance cultures | Blood cultures, iv catheters | Environmental cultures | Total number |
|---|---|---|---|---|
| 2006 | 106 | 4 | 7 | 117 |
| 2007 | 188 | 1 | 8 | 197 |
| 2008 | 353 | 5 | 6 | 364 |
| 2009 | 156 | 4 | 0 | 160 |
| 2010 | 132 | 1 | 2 | 135 |
| 2011 | 10 | 0 | 0 | 10 |
| 2012 | 11 | 0 | 0 | 11 |

cefepime was very high till 2010 because of the high number of P. aeruginosa isolates, but significantly decreased in 2011 and 2012 (table 2). On the contrary, the consumption of meropenem significantly increased in 2010 and 2011.

Using multivariate regression analysis, a statistically significant association with dependent variable 'surveillance/100 hospital days' was found for the consumption of penicillin (DDD), chlorhexidine, gloves, glucoprotamine and cefepime (R2 for the model=0.999, p<0.001). A statistically significant association with the 'index of change for positive isolates' was found for the use of chlorhexidine and glucoprotamine (R2 for the model=0.965, p=0.011).

## PFGE RESULTS

PFGE was used for molecular typing of pseudomonas isolates taken from clinical, surveillance, and environmental samples in the period from October to December 2008. In total, we analyzed 26 isolates: five (5) from blood cultures, five (5) from environmental cultures and sixteen (16) from surveillance cultures. Several different PFGE profiles were identified; the PFGE profiles from blood cultures were identical to PFGE profiles in tracheal aspirates; in environmental samples different PFGE profiles were found.

## DISCUSSION

Several outbreaks of P. aeruginosa infections in high risk departments (haematologic, surgical, paediatric, NICU) have been described. (4,11-16)

P. aeruginosa is a ubiquitous bacterium in the environment, as well as part of endogenous flora, and therefore it is difficult to find the source of healthcare associated infections. (2,4,12)

In the NICU of the CHC Zagreb, P. aeruginosa isolates have been found periodically in the past in surveillance cultures of neonates, but in March 2008 an increase in the total number of pseudomonas infections occurred, in comparison to the previous months.

Introducing stringent standard control measures, we managed to maintain the number of pseudomonas isolates at a stable level till October.

Colonization with this bacterium is often proceeded by infection, but the original source and the precise mode of transmission often remain unclear. (4)

Several outbreaks of P. aeruginosa infection in the NICU have been described. (11,12,15) In most studies, the major reservoir of P .aeruginosa was patient endogenous flora. (2,11) However, in some other studies, the environment as an exogenous source of pseudomonas infection (tap water, sinks, faucets, showers and hands of healthcare workers), has been confirmed. (1,2,5,10,12,13)

Given that environmental sources, as well as the hands of healthcare workers, can play an important role in the colonization/infection of patients, environmental samples (tap water, sinks, faucets, showers) were taken. (13,14)

Pseudomonas isolates, except in surveillance cultures, were found in tap water, but not on the hands of healthcare workers. That is when the infection control team decided to implement POU filters and in the following months, the number of P. aeruginosa isolates in surveillance cultures dropped significantly. (3,4,5,16,17)

Surprisingly, in December 2008, P. aeruginosa was isolated in blood cultures of three (3) neonates. Besides POU filters, standard control measures were tightened and the number of positive surveillance cultures began to decline, with no more positive blood cultures.

In an attempt to find the reservoir of the epidemic, we performed PFGE typing of 26 P. aeruginosa isolates from blood and surveillance cultures, as well as from environmental samples.

The same clones were found in blood cultures and in surveillance cultures of neonates, suggesting an endogenous origin of sepsis. In environmental samples, several different clones were found. In the literature, multi-clonal outbreaks with different PFGE profiles of pseudomonas are often described, indicating that P. aeruginosa in the environment is genetically diverse. (5,12)

Some authors have demonstrated that POU filters were effective in eradication of P. aeruginosa and other water borne bacteria. (3,4,17)

Regarding the results of PFGE typing and susceptibility testing, we consider that our outbreak did not originate from tap water. However, the current study was retrospective and the number of environmental samples was too small for excluding exog-

enous sources. Besides that, the number of isolates was significantly and constantly decreased only after implementation of all measures (stringent hygiene measures, POU filters). The number of positive cultures of P. aeruginosa during the two consecutive time periods (before and after installation of POU filters) showed a statistically significant difference.

Apart from introducing POU filters, we tried to detect some other parameters which contributed to the reduction of P. aeruginosa isolates. For the following parameters (use of antipseudomonas antibiotics; sanitation and prevention methods such as disinfectants, antiseptics, gloves and gowns consumption) both, univariate and multivariate regression analyses were used.

According to univariate regression analysis there were statistically significant differences in consumption of cefepime and meropenem between the two periods ($p<0.05$).

Consumption of cefepime was very high till 2010 because of the high number of P. aeruginosa isolates, but was significantly decreased in 2011 and 2012. There were two reasons for this: the number of P.aeruginosa isolates in the NICU was significantly decreased, and isolated strains became resistant to cefepime.

On the contrary, the consumption of meropenem was significantly increased in 2010 and 2011. The reason for such a dramatic increase of meropenem consumption was the multiresistance of remaining isolates of P. aeruginosa, and simultaneous appearance of extended spectrum beta-lactamase (ESBL) producing strains of Enterobacteriacae in surveillance neonatal cultures. (16)

Surprisingly, multivariate regression analysis showed that there were statistically significant differences only for the use of chlorhexidine and glucoprotamine ($p<0.05$).

## CONCLUSION

Before the implementation of infection control measures, the possible main source of P. aeruginosa was exogenous, leading to a heavy burden of pseudomonas in the environment of neonates, leading to their colonization and finally infection.

After the implementation of all infection control measures together with POU filters, we managed to stop the spread of pseudomonas colonization/infection. POU filters contributed only as one of these measures, resulting in a reduction of chronically endemic P.aeruginosa infection/colonisation in the NICU.

According to multivariate regression analysis, stringent control measures (use of chlorhexidine and glucoprotamine) were the most important factors in interruption of the epidemic.

Acknowledgments: The authors gratefully acknowledge the contribution of Jasminka Horvatic, RN, mag.med.techn, MNS for collecting data that were used in this article.

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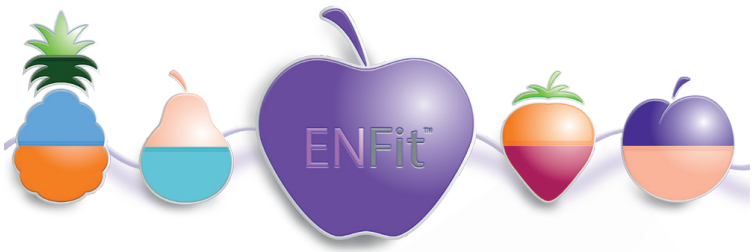


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