Review

Pseudomonas aeruginosa outbreaks in the neonatal intensive care unit – a systematic review of risk factors and environmental sources

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Pseudomonas aeruginosa is a Gram-negative bacterium commonly occurring in soil and water. It is an opportunistic pathogen and an important cause of healthcare-associated infections, particularly among infants in neonatal intensive care units (NICUs). Several reports regarding outbreaks of P. aeruginosa in NICUs have been published. MEDLINE and EMBASE databases were searched using the MeSH terms [Pseudomonas aeruginosa], [Outbreak OR Infection OR bacteremia, OR sepsis OR disease] and [Neonat* OR baby OR babies OR newborn*]. Fifteen studies describing a total of 414 infants colonized or infected with P. aeruginosa were reviewed. The mean percentage of infections occurring in the populations that had been colonized by the organism (calculated as $n_{\text{infected}}/n_{\text{infected}} + n_{\text{colonized}}$) was 22%. Environmental sampling was performed in 14 studies, nine of which detected P. aeruginosa. The risk factors identified were antimicrobial drug use and the number of days of antimicrobial therapy prescribed before positive blood culture, exposure to particular healthcare workers (HCW), transfusion of blood products, and intravenous delivery of nutrients/electrolytes. Exposure to umbilical venous catheters was associated with bloodstream infections. Increasing age and use of artificial fingernails were risk factors for colonization of hands of HCWs. Low birth weight pre-term infants were at greater risk of mortality from P. aeruginosa infection than older infants.

Background

Pseudomonas aeruginosa is a Gram-negative bacterium commonly occurring in soil and water. It is an opportunistic pathogen, causing disease in vulnerable individuals, such as the immunocompromised, those where host defences have been breached, such as burns patients, and infants in whom the immune system is not yet developed. Most commonly, P. aeruginosa infections occur in the urinary tract, in burn wounds and as chronic lung infections in cystic-fibrosis patients. However, the organism is also an important cause of healthcare-associated infections, particularly among infants in neonatal intensive care units (NICUs) (Jarvis et al., 1987) owing to their underdeveloped immune system and the fact that such infants are often intubated, catheterized with intravascular catheters/devices in-situ, and/or receiving parenteral nutrition, which may increase risk of infection (Gaynes et al., 2005). Unfortunately, such infections in neonates can be fatal, as was the case in a recent UK outbreak in which four babies died (Wise, 2012). The ubiquitous nature of P. aeruginosa in the environment makes the sources of such outbreaks difficult to identify.

Several outbreaks of P. aeruginosa colonization and infection in NICUs have been published. Simon et al. (2008) published a systematic review of confirmed outbreaks in both neonatal and paediatric patients that were identified using the PubMed database and the web-based register for nosocomial outbreaks. That review did not search for reports of outbreaks listed in the Embase database and so may have been biased by the omission of relevant data. Additionally, the review itself is not listed in PubMed/
MEDLINE and so is not readily available to a wide readership. Therefore, we set out to provide an updated review of the literature reporting on \textit{P. aeruginosa} colonization and infection in NICUs using both Embase and MEDLINE. We have performed a systematic review of \textit{P. aeruginosa} outbreak reports and prospective surveillance studies following outbreaks of \textit{P. aeruginosa} infections in neonates, specifically in the NICU setting.

**Methods**

Search terms were constructed as shown in Fig. S1 (Available in JMM Online). Searches were performed between 28th and 29th January 2012 and were initially run on the MEDLINE database followed by the Embase database. Search terms were entered as MeSH terms rather than text terms. Only articles in the English language were included in the final list of studies to review. Fig. S1 shows the number of studies retrieved for each of the search terms and the methods used to select and reject studies.

Fifteen studies met the inclusion criteria and were included in the systematic review. In order to analyse the combined results of the studies, the framework outlined in the ORION (Outbreak Reports and Intervention Studies of Nosocomial Infection) statement (Stone et al., 2007) was used to review the studies. Outbreak reports and subsequent investigations, including non-randomized studies, form the main body of evidence regarding risk factors and clinical importance of healthcare-associated infections. Such studies often lack details on study design and basic information such as case definitions, selection of controls and denominator information. The ORION guidelines seek to build on the CONSORT (Begg et al., 1996) definitions, selection of controls and denominator information. The ORION guidelines seek to build on the CONSORT (Begg et al., 1996) and TREND (Des Jarlais et al., 2004) guidelines and aim ‘to raise the standards of research and publication in hospital epidemiology, to facilitate synthesis of evidence and promote transparency of reporting, to enable readers to relate studies to their own experience and assess the degree to which results can be generalised’. ORION consists of a 22-item checklist and although it was designed for reporting of new outbreak and intervention studies, it provided an appropriate framework for the systematic analysis of a number of studies as presented here. Seventeen ORION criteria were used to screen studies and withdraw information. Criteria that were not used to screen studies for the present review were economic outcomes, sample size calculations, recruitment, ancillary analysis and harms. These criteria were not used as they apply only to economic evaluations or to intervention studies and not to case reports or outbreak investigations as reviewed here.

**Results**

Based on titles and abstracts, 17 studies reporting nosocomial outbreaks of \textit{P. aeruginosa} colonization or infection of infants in NICUs from 17 different hospitals were initially identified for inclusion in the present review. However, two studies were excluded after inspection of the full-text documents, both describing prospective studies; Mammina et al. (2008) described investigation of colonization by antibiotic-resistant \textit{P. aeruginosa} but did not describe an outbreak of \textit{P. aeruginosa} infection or state that this work followed such an outbreak and Mammina et al. (2008) and Hu et al. (2010) described prospective surveillance of \textit{P. aeruginosa} colonization among mechanically ventilated neonates. Therefore, 15 studies (Garcia et al., 1989; Verweij et al., 1993; Garland et al., 1996; Archibald et al., 1998; Muylermans et al., 1998; Foca et al., 2000; Moolenaar et al., 2000; Loureiro et al., 2002; Gras-Le Guen et al., 2003; Majumdar et al., 2004; Schutze et al., 2004; Zabel et al., 2004; Crivaro et al., 2009; Sánchez-Carrillo et al., 2009; and Naze et al., 2010) were not mentioned in the original review by Simon et al., (2008). The outbreaks reported in the 15 studies occurred between 1986 and 2003. A summary of the details of these studies is shown in Table 1. In total, 414 infants were colonized or infected with \textit{P. aeruginosa} (Table S1, available in JMM Online). Three studies considered cases of infection only (Verweij et al., 1993; Archibald et al., 1998; Loureiro et al., 2002), two studies did not differentiate between colonization and infection (Foca et al., 2000; Moolenaar et al., 2000) and 10 studies reported infection and colonization separately. (Garcia et al., 1989; Garland et al., 1996; Muylermans et al., 1998; Gras-Le Guen et al., 2003; Majumdar et al., 2004; Schutze et al., 2004; Zabel et al., 2004; Crivaro et al., 2009; Sánchez-Carrillo et al., 2009; Naze et al., 2010). The overall mean prevalence of colonization or infection reported from eight studies (Archibald et al., 1998; Muylermans et al., 1998; Foca et al., 2000; Moolenaar et al., 2000; Loureiro et al., 2002; Crivaro et al., 2009; Sánchez-Carrillo et al., 2009; Naze et al., 2010), which included information on the number of unaffected individuals, was 18.5% (range 10.5–42%). For the studies that reported infection and colonization separately, the mean percentage of infections occurring in populations who had been colonized by the organism, calculated as $\frac{n_{\text{infectected}}}{n_{\text{infectected}} + n_{\text{colonized}}}$ was 22% (range 4.8–75%).

Microbiological sampling of the environment was performed in all but one of the reviewed studies (Loureiro et al., 2002) (Table S2). Of these 14 studies, 12 studies (Garcia et al., 1989; Garland et al., 1996; Muylermans et al., 1998; Foca et al., 2000; Moolenaar et al., 2000; Loureiro et al., 2002; Gras-Le Guen et al., 2003; Majumdar et al., 2004; Zabel et al., 2004; Crivaro et al., 2009; Sánchez-Carrillo et al., 2009; Naze et al., 2010) reported the presence of \textit{P. aeruginosa} in one or more environmental samples. Methods for culture and identification of \textit{P. aeruginosa} were clearly described for nine studies (Garland et al., 1996; Muylermans et al., 1998; Moolenaar et al., 2000; Foca, 2002; Loureiro et al., 2002; Zabel et al., 2004; Crivaro et al., 2009; Sánchez-Carrillo et al., 2009; Naze et al., 2010). Culture methods differed between studies with various microbiological media, incubation conditions and diagnostic methods being used (Table S2).

Molecular typing was performed using pulse field gel electrophoresis (PFGE) in 10 studies. (Garland et al., 1996; Archibald et al., 1998; Foca et al., 2000; Moolenaar et al., 2000; Loureiro et al., 2002; Gras-Le Guen et al., 2003; Majumdar et al., 2004; Zabel et al., 2004; Crivaro et al., 2009; Sánchez-Carrillo et al., 2009). Two studies used the
random amplification of polymorphic DNA (RAPD) method (Muyldermans et al., 1998; Zabel et al., 2004), one used inter-repeat PCR (Verweij et al., 1993), one used plasmid profiling (Garcia et al., 1989) and one used variable number tandem repeat (VNTR) analysis with high resolution melting curve analysis (HRMC) (Naze et al., 2010). Molecular typing methods were fully reported or referenced in nine studies (Garcia et al., 1989; Verweij et al., 1993; Muyldermans et al., 1998; Loureiro et al., 2002; Schutze et al., 2004; Zabel et al., 2004; Crivaro et al., 2009; Sánchez-Carrillo et al., 2009; Naze et al., 2010) and partially described in two (Foca et al., 2000; Moolenaar et al., 2000). Four studies mentioned, but did not detail, the methods used for molecular typing (Table S2) (Garland et al., 1996; Archibald et al., 1998; Gras-Le Guen et al., 2003; Majumdar et al., 2004). Antibiotic-susceptibility testing was performed in seven studies (Garcia et al., 1989; Muyldermans et al., 1998; Foca et al., 2000; Loureiro et al., 2002; Majumdar et al., 2004; Zabel et al., 2004; Crivaro et al., 2009; Sánchez-Carrillo et al., 2009), five of which (Garcia et al., 1989; Muyldermans et al., 1998; Loureiro et al., 2002; Crivaro et al., 2009; Sánchez-Carrillo et al., 2009) reported the methods employed. Resistance of *P. aeruginosa* to at least one antibiotic was reported in five studies (Garcia et al., 1989; Foca et al., 2000; Loureiro et al., 2002; Majumdar et al., 2004; Crivaro et al., 2009) and intermediate susceptibility was reported in one study (Verweij et al., 1993). In studies which demonstrated clinical infection, the crude mortality rates in infants with *P. aeruginosa* infection could be calculated for eight studies and ranged from 18.2 to 100% with a mean value of 62.7% (Table S3) (Verweij et al., 1993; Garland et al., 1996; Muyldermans et al., 1998; Gras-Le Guen et al., 2003; Crivaro et al., 2009; Sánchez-Carrillo et al., 2009; Naze et al., 2010). *P. aeruginosa* isolates with identical molecular typing profiles to the outbreak strain were found in environmental samples taken from the NICU or NICU staff in eight studies (Garland et al., 1996; Muyldermans et al., 1998; Foca et al., 2000; Moolenaar et al., 2000; Majumdar et al., 2004; Zabel et al., 2004; Crivaro et al., 2009; Naze et al., 2010). Three studies (Gras-Le Guen et al., 2003; Majumdar et al., 2004; Sánchez-Carrillo et al., 2009) identified outbreak strains from environmental sources outside of, but associated with, the NICU (e.g. preparation areas).

Although none of the studies set out to test the efficacy of interventions as the primary outcome, interventions to control outbreaks were reported in 13 studies; these were wide-ranging and are described in Table S1. Of the 13 studies that reported interventions (Garcia et al., 1989; Verweij et al., 1993; Garland et al., 1996; Archibald et al., 1998; Muyldermans et al., 1998; Foca et al., 2000; Gras-Le Guen et al., 2003; Majumdar et al., 2004; Schutze et al., 2004; Zabel et al., 2004; Crivaro et al., 2009; Sánchez-Carrillo et al., 2009; Naze et al., 2010), 10 studies reported the intervention as being successful (Garcia et al., 1989; Garland et al., 1996; Muyldermans et al., 1998; Gras-Le Guen et al., 2003; Majumdar et al., 2004; Schutze et al., 2004; Zabel et al., 2004; Crivaro et al., 2009; Sánchez-Carrillo et al., 2009; Naze et al., 2010) and one study reported temporary success (Foca et al., 2000). Two studies did not report the outcome of the intervention (Archibald et al., 1998; Moolenaar et al., 2000). Table S5 details the authors’ interpretation of the results of interventions. Interestingly, only one study reported coordinated efforts of NICU staff and staff from related units as a successful approach to controlling and preventing outbreaks. One study used covert surveillance to assess the effect of an educational intervention on hand washing behaviour, reporting before and after results (Crivaro et al., 2009). The authors concluded that this intervention had been successful but full details of the intervention, which would enable it to be repeated elsewhere, were not included in the study.

Six studies (Archibald et al., 1998; Muyldermans et al., 1998; Foca et al., 2000; Moolenaar et al., 2000; Loureiro et al., 2002; Gras-Le Guen et al., 2003) included a statistical analysis of risk factors (Table S4). The risk factors identified were use of antimicrobial drugs before positive blood culture, the number of days of antimicrobial therapy prescribed before positive blood culture (Loureiro et al., 2002) and exposure to two particular HCWs (Moolenaar et al., 2000). In one study, infants with *P. aeruginosa* sepsis were found to have been given a mean of 3.6 different antimicrobials prior to providing a blood culture that was positive for *P. aeruginosa* compared to the mean use of 2.1 antimicrobials in non-case infants (Loureiro et al., 2002). Transfusion of fresh frozen plasma or human albumin also appeared to increase the risk for *P. aeruginosa* infection but did not reach significance (Muyldermans et al., 1998). Exposure to a particular HCW, and intravenous delivery of total parenteral nutrition simultaneously with glucose/electrolyte solution were significantly associated with *P. aeruginosa* bacteraemia. Exposure to umbilical venous catheters also showed evidence of an association with bloodstream infections (Archibald et al., 1998). Increasing age and the use of fingernail wraps (artificial fingernails) were deemed risk factors for colonization of hands of HCWs (Foca et al., 2000). In another study, infants exposed to a particular (colonized) HCW and those exposed to a second un-colonized HCW were also at increased risk of infection (Foca et al., 2000). Low birth weight pre-term infants were at greater risk of mortality from *P. aeruginosa* infection than older infants (Gras-Le Guen et al., 2003). Measures of association for all risk factors are listed in Table S4. Two studies did not include a formal risk factor analysis but used molecular typing to identify outbreak sources as commercially bottled mineral water (Crivaro et al., 2009) and contamination of a feeding-bottle preparation room (Sánchez-Carrillo et al., 2009).

In general, the studies reviewed here provide evidence that *P. aeruginosa* can be introduced to the NICU via a number of routes, including environmental contamination, transmission by HCWs, transfer of colonized patients and...
### Table 1. Summary of the 15 studies included in the current review, including study setting and design

<table>
<thead>
<tr>
<th>Reference</th>
<th>Title/description (Orion 1)</th>
<th>Background/rationale (Orion 2)</th>
<th>Type of study (Orion 3)</th>
<th>Study dates (Orion 4)</th>
<th>Objectives (Orion 5)</th>
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<tbody>
<tr>
<td>Gras-Le Guen et al. (2003)</td>
<td>Contamination of a milkbank pasteurizer causing a <em>P. aeruginosa</em> outbreak in a neonatal intensive care unit</td>
<td>Environmental investigation and cohort study following a <em>P. aeruginosa</em> outbreak in a NICU</td>
<td>Environmental investigation and cohort study (stated)</td>
<td>June 2001</td>
<td>Not stated</td>
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<td>Loureiro et al. (2002)</td>
<td><em>P. aeruginosa</em>: study of antibiotic resistance and molecular typing in hospital infection cases in a neonatal intensive care unit from Rio de Janeiro city, Brazil</td>
<td>Analysis of demographic and bacteriological data</td>
<td>Case-control study (not stated)</td>
<td>Jul 1997–Jul 1999 (2 years)</td>
<td>&quot;To analyse the demographic and bacteriologic data of 32 hospitalized newborns ...seized by <em>P. aeruginosa</em> sepsis&quot;*</td>
</tr>
<tr>
<td>Zabel et al. (2004)</td>
<td>Surveillance of <em>P. aeruginosa</em> isolates in a neonatal intensive care unit over a one year period</td>
<td>Investigation of the colonization patterns and identification of potential outbreak sources by epidemiological investigations, environmental surveillance and typing of isolates</td>
<td>Cohort study (not stated)</td>
<td>Outbreak, August 1996–Dec 1997 (one year) surveillance, April 1996 to December 1997 (20 months)</td>
<td>Not stated</td>
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<td>Muyldermans et al. (1998)</td>
<td>Neonatal infections with <em>P. aeruginosa</em> associated with a waterbath used to thaw fresh frozen plasma</td>
<td>‘Four newborns were found to be colonised or infected with <em>P. aeruginosa</em> within a period of one week’</td>
<td>Case control study (stated in text)</td>
<td>Published 1998 ('one week')</td>
<td>Not stated</td>
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<td>Schutze et al. (2004)</td>
<td>Use of DNA fingerprinting in decision making for considering closure of neonatal intensive care units because of <em>P. aeruginosa</em> bloodstream infections</td>
<td>Utilization of molecular typing technique to identify the source of bloodstream infections in a NICU</td>
<td>Outbreak investigation and cohort study (not stated)</td>
<td>June 22nd 1998–Aug 16th 1998 (24 days)</td>
<td>Not stated</td>
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<tr>
<td>Verweij et al. (1993)</td>
<td>Cross-infection with <em>P. aeruginosa</em> in a neonatal intensive care unit characterized by PCR fingerprinting</td>
<td>Description of two cases of cross-infection and the results of PCR fingerprinting</td>
<td>Two case reports (stated)</td>
<td>Dec 1992 (11 days)</td>
<td>Not stated</td>
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<tr>
<td>Foca et al. (2000)</td>
<td>Endemic <em>P. aeruginosa</em> infection in a neonatal intensive care unit</td>
<td>Epidemiological and molecular investigation of endemic <em>P. aeruginosa</em> infection in a NICU.</td>
<td>Outbreak report and cohort study (not stated)</td>
<td>Jan 1997–Sep 1999 (33 months)</td>
<td>Epidemiological and molecular investigation</td>
</tr>
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<td>Garcia et al. (1989)</td>
<td>An outbreak of multiply resistant <em>Pseudomonas aeruginosa</em> in an neonatal unit: plasmid pattern analysis</td>
<td>Epidemiological and investigation of an outbreak of <em>P. aeruginosa</em> infection in an NICU using antibiograms and plasmid typing of outbreak and environmental <em>P. aeruginosa</em> isolates</td>
<td>Outbreak report and risk-factor analysis</td>
<td>18th Mar–10th Apr 1986 (19 days)</td>
<td>Not stated</td>
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<tr>
<td>Reference</td>
<td>Title/description (Orion 1)</td>
<td>Background/rationale (Orion 2)</td>
<td>Type of study (Orion 3)</td>
<td>Study dates (Orion 4)</td>
<td>Objectives (Orion 5)</td>
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<td>Moolenaar et al. (2000)</td>
<td>A prolonged outbreak of <em>P. aeruginosa</em> in a neonatal intensive care unit: did staff fingernails play a role in transmission?</td>
<td>Description of outbreak, determination of risk factors and recommendations</td>
<td>Cohort and embedded case control study (stated)</td>
<td>Jan 1st 1997–Mar 12th 1998 (15 months)</td>
<td>“To describe an outbreak of <em>P. aeruginosa</em> bloodstream infection and endotracheal tube colonisation in an NICU, determine risk factors and make preventative recommendations”</td>
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<td>Archibald et al. (1998)</td>
<td><em>Enterobacter cloaceae</em> and <em>Pseudomonas aeruginosa</em> polymicrobial bloodstream infections traced to extrinsic contamination of a dextrose multidose vial</td>
<td>Identification of risk factors for polymicrobial bloodstream infections following an outbreak of <em>Enterobacter cloaceae</em> and <em>P. aeruginosa</em> bloodstream infections in a NICU</td>
<td>Retrospective cohort study after outbreak (stated)</td>
<td>April 25th–May 7th 1986 (11 days)</td>
<td>“To identify risk factors for polymicrobial bloodstream infections (BSI) in a neonatal intensive care unit patients during an outbreak of BSI”</td>
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<td>Garland et al. (1996)</td>
<td><em>P. aeruginosa</em> outbreak associated with a contaminated blood-gas analyser in a neonatal intensive care unit</td>
<td>Environmental surveillance and genomic DNA fingerprinting of isolates to investigate a 10-month <em>P. aeruginosa</em> outbreak in a NICU</td>
<td>Outbreak report (not stated)</td>
<td>Published 1996 (*10 months’)</td>
<td>Not stated</td>
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<tr>
<td>Majumdar et al., (2004)</td>
<td>An outbreak of imipenem-resistant <em>P. aeruginosa</em> in an intensive care unit</td>
<td>Outbreak of imipenem-resistant <em>P. aeruginosa</em> in an intensive care unit (not neonatal ICU), possible transmission of <em>P. aeruginosa</em> via transfer of patients from high-dependency unit</td>
<td>Letter</td>
<td>December 2002–April 2003 (5 months)</td>
<td>Not stated</td>
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<tr>
<td>Crivaro et al. (2009)</td>
<td><em>Pseudomonas aeruginosa</em> in a neonatal intensive care unit: molecular epidemiology and infection control measures</td>
<td>Prospective surveillance of <em>P. aeruginosa</em> isolated during a period of increased infections in a NICU and the infection control measures taken to limit transmission</td>
<td>Prospective education intervention and cohort study (not stated)</td>
<td>July 2005–June 2007 (2 years)</td>
<td>‘(i) To analyse the molecular epidemiology and antimicrobial susceptibility patterns of <em>P. aeruginosa</em> isolates; (ii) to describe the infection control measures undertaken to limit spread of <em>P. aeruginosa</em> in the ward’</td>
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<td>Sánchez-Carrillo et al. (2009)</td>
<td>Contaminated feeding bottles: the source of an outbreak of <em>Pseudomonas aeruginosa</em> infections in a neonatal intensive care unit</td>
<td>Outbreak investigation following increased colonization and infection in the NICU (five positive surveillance samples in 1 day)</td>
<td>Outbreak investigation</td>
<td>September 2004 (1 month)</td>
<td>‘To describe an outbreak because of <em>P. aeruginosa</em> that occurred in the neonatal NICU, the way the source of this was investigated and how we managed it’</td>
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through the use of contaminated water to prepare milk or other nutrition. The studies also demonstrate that molecular typing is a valuable tool in the investigation of *P. aeruginosa* outbreaks and for the surveillance of environmental contamination. The overall evidence is shown in Table S5.

**Discussion**

**Water as a reservoir of *P. aeruginosa***

The 15 studies reviewed here represent outbreaks of *P. aeruginosa* infection and/or colonization in NICUs from a wide range of developed and middle-income countries. Water as an environmental source of infection was a common factor in among studies which identified point source outbreaks of *P. aeruginosa* infections. Two studies identified contaminated water baths as reservoirs (Muyldermans *et al.*, 1998; Gras-Le Guen *et al.*, 2003); other ‘wet’ reservoirs were a pasteurizer used to sterilize milk (Gras-Le Guen *et al.*, 2003), humidifying equipment for ventilators (Zabel *et al.*, 2004) and a tap in a neighbouring high-dependency unit (Majumdar *et al.*, 2004). One study also found *P. aeruginosa* unrelated to the outbreak strain in a sink drain (Moolenaar *et al.*, 2000). One study identified multi-dose vials of parenteral nutrition and a further study found that feeding bottles were likely to have become contaminated as a result of water-contamination in a preparation area whilst an additional study traced the outbreak to commercially bottled mineral water used to prepare multidose milk. *P. aeruginosa* is primarily an environmental organism that is adapted to survive in numerous conditions and is particularly well-adapted to wet or damp conditions. Water systems are well-recognized as potential reservoirs of this organism for healthcare-associated infections (Trautmann *et al.*, 2005). The World Health Organization has published guidelines for safe water quality that state that all healthcare facilities should have specific water safety plans to control waterborne healthcare-associated infections including *P. aeruginosa* as part of their infection control programme (WHO, 2006).

Point-of-use filtration using 0.2 μm filtration units was associated with a significant reduction in chronically endemic *P. aeruginosa* colonizations/infections on a surgical ICU where *P. aeruginosa* was cultured from 113/117 (97 %) of tap water samples and was also shown to be cost-effective when changed weekly by designated personnel (Trautmann *et al.*, 2008). However, the filtration units do not eradicate the organism from the system, but only prevent discharge to the environment from the filtered outlet. In the Health Technical Memorandum 04-0 (Department of Health, 2006), continuous long-term use of point-of-use filtration is not recommended, except where there is no effective alternative to eradicate the organism from the water system. Such an intervention may also be useful in cases of endemic *P. aeruginosa* in NICU settings. Whilst filtration has proved to be useful in situations where tap water has been shown to harbour *P. aeruginosa*, contamination of such filters from environmental *P. aeruginosa* in the sink below the filter unit is a possibility. It is recognized that strains of *P. aeruginosa*
within sink traps may not be the same as those that cause infection. In the present systematic review, 11 of the 15 studies identified the outbreak strain in the environment. Nevertheless, environmental reservoirs such as sinks have the potential to lead to outbreaks. An outbreak due to splash-back from contaminated sink drains was reported from the ICU and transplant unit of a Canadian hospital in 2009 (Hota et al., 2009). In the UK, the EPIC2 guidelines of the Hospital Infection Society were published in February 2007 (Pratt et al., 2007). These guidelines are wide-ranging and cover many aspects of infection control; however, they do not refer to design of sinks and other water systems. NHS guidelines for water system specifications state that ‘swan-neck outlets should be avoided’ and recommend that ‘in existing facilities, when such an outlet has become damaged and is due for repair that it be completely replaced by an appropriate, alternative component as described’ (Ayliffe et al., 2000). Other guidelines also state that ‘taps discharging into a shallow sink or directly into a drain hole can cause splashing which disperses contaminated aerosols. Thus, the tap outlet flow should not point directly into the sink outlet’ (SHFN, 2007). Oxidising biocides such as chlorine dioxide or ionisation systems that release copper and silver ions can be used as control measures. These methods have been shown to be effective in the control of organisms in water systems (Department of Health, 2006).

Biofilm formation and antibiotic resistance

The potential of \textit{P. aeruginosa} to form biofilms on environmental surfaces is well known and causes difficulties in eradicating this organism. Biofilms can be defined as structured microbial communities of single or multiple species attached to a surface and surrounded by a matrix of extracellular polymers or ‘slime’. Bacteria grown as biofilms display tolerance to antibiotics in the absence of known resistance determinants (Costerton et al., 1987) and may also be hard to eradicate using environmental antibacterial agents due to their complex structure and protective matrix. Recently, it has been demonstrated that biofilms generate extensive genetic diversity, both among resident bacteria and among cells that detach and disperse from biofilms (Molin & Tolker-Nielsen, 2003; Webb et al., 2003). This phenomenon is thought to enhance the ability of biofilm cells to persist and spread under diverse environmental stresses, including antibiotic use. Antibiotic-resistant strains of \textit{P. aeruginosa} were associated with five of the outbreaks reported here and multiple resistance was reported in three studies. Emergence of antibiotic-resistant strains is occurring in Europe and elsewhere (Souli et al., 2008; Lautenbach & Polk, 2007). The use of antibiotics and antimicrobial compounds may provide a driving mechanism for the emergence of antibiotic resistant \textit{P. aeruginosa}. This hypothesis is strengthened by the study of Loureiro et al. (2002), which demonstrated that prior antibiotic use was associated with bloodstream infections in the NICU. Recently, a case-control study showed that multi-drug resistant (MDR) \textit{P. aeruginosa} caused significantly higher mortality rates among patients with chronic obstructive pulmonary disease than did infection with non-MDR strains (Montero et al., 2009). MDR strains are also of particular concern in vulnerable infants, such as those being treated in the NICU; Crivaro et al. (2009) reported that both deaths associated with \textit{P. aeruginosa} infections in the NICU outbreak reviewed here were associated with MDR isolates of \textit{P. aeruginosa}.

Recently, studies regarding control of biofilms in water systems have been undertaken. Such work includes studies of the materials making up the water system itself, as well as studies regarding the use of various anti-biofilm agents. Such agents include various electrochemically activated solutions (Coleman et al., 2009), sublethal concentrations of the anti-bacterial agents piperacillin–tazobactam (Fonseca et al., 2004) and low (sublethal) concentrations of nitric oxide (NO), allowing antibacterial agents to attack \textit{Pseudomonas} bacteria in the planktonic (non-biofilm) mode of growth. Barraud et al. (2006) showed that treatments using low concentrations of NO together with antimicrobials against \textit{P. aeruginosa} were highly effective in removing \textit{P. aeruginosa} biofilms. Such novel methods may be important in the control and prevention of biofilm-associated infections. However, use of chemicals directly in the water system could have significant COSHH implications especially when the water is used in highly vulnerable infants in a NICU setting.

Hand hygiene and aseptic technique

Exposure to particular HCWs and umbilical catheterization were the most important risk factors identified here. This review confirms that \textit{P. aeruginosa} can be transmitted from HCWs to patients via contaminated hands (Foca et al., 2000; Moolenaar et al., 2000). Accordingly, improved hand hygiene measures at the point of care, re-enforcement of infection control guidelines and education of healthcare staff were reported as part of successful infection control interventions for the majority of studies evaluated. The importance of HCWs keeping short clean nails, in order to prevent them acting as a reservoir of infection, was also highlighted. A recent study has reported that observing correct hand hygiene, using a protocol based on WHO recommendations (Pittet et al., 2009), minimizes the risk of contamination of hands with \textit{P. aeruginosa} even when hands are washed in water heavily contaminated with the organism (Jones, 2011).

Molecular typing

This review considered studies that had used molecular typing to provide evidence that outbreak \textit{P. aeruginosa} strains were genetically related to each other and to demonstrate an association between \textit{P. aeruginosa} infection/colonization and environmental isolates. Such techniques are now commonplace and can be performed routinely. Of the five different typing methods, namely PFGE, RAPD, inter-repeat PCR plasmid profiling and
VNTR–HRMA, reported in 12 of the studies, PFGE appears to be the most discriminatory and widely used method for typing of P. aeruginosa at present. VNTR–HRMA is a promising method that requires further validation with larger numbers of P. aeruginosa isolates in order to confirm discriminatory power. Whilst it is not currently available for small-scale outbreaks, whole genome sequencing using high-throughput ‘next generation’ sequencing technology is fast becoming available and affordable and has been used successfully to track large outbreaks of food poisoning (Mellmann et al., 2011; Rohde et al., 2011). Such technologies are likely to become the tool of choice in the future, even for local outbreak investigations.

Limitations of the studies

The studies reviewed here are outbreak reports. Seven studies (Garcia et al., 1989; Verweij et al., 1993; Garland et al., 1996; Archibald et al., 1998; Loureiro et al., 2002; Gras-Le Guen et al., 2003; Majumdar et al., 2004) did not include denominator data, meaning that the impact of the outbreak was unclear. Three studies mentioned data in the text that could be used to estimate the denominator; one as the number of beds in the NICU (Schutze et al., 2004) and two as the number of admissions per year (Muyldermans et al., 1998; Zabel et al., 2004). Other limitations were the omission of methodological data or case definitions. The small numbers of cases in a several of the studies prevent a robust analysis of risk factors. Lack of a standardized approach in reporting is a barrier to meta-analysis for the identification of risk factors.

Conclusion

The studies reviewed here have shown that P. aeruginosa, especially antibiotic-resistant strains, are a well-known cause of outbreaks of invasive and non-invasive disease in the NICU setting. Such outbreaks occur in both high- and middle-income countries and can result in both morbidity and mortality. Outbreaks can be long-lasting but can also be effectively brought under control by the use of various interventions, the most important being effective infection prevention practices, especially hand hygiene for staff and visitors; such practices are key to the prevention of P. aeruginosa outbreaks. Studies outside the scope of this review have described a number of environmental modifications as preventative measures, such as those relating to the design of sinks, taps and water-systems, which may help to prevent or reduce environmental contamination with P. aeruginosa. The reviewed studies also highlight the usefulness of molecular typing techniques and demonstrate the importance of environmental sampling for outbreak investigations. The ubiquity of P. aeruginosa and its ability to colonize environmental surfaces and water systems presents a particular challenge, as environmental strains unrelated to the outbreak strain may be present. The data presented in some studies were subject to certain limitations and future studies in this area would benefit from adherence to ORION guidelines in order to provide improved evidence.

References


