Multidrug-resistant organisms in a routine ward environment: differential propensity for environmental dissemination and implications for infection control

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Multidrug-resistant organisms (MDROs) pose significant infection-control challenges in settings with high prevalence and limited isolation facilities. This observational study in an 800-bed hospital determined the prevalence, bacterial density and genetic relatedness of MDROs isolated from ward surfaces, medical devices and the hands of healthcare professionals. The targeted MDROs were meticillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant enterococci (VRE), Escherichia coli and Klebsiella pneumoniae resistant to extended-spectrum cephalosporins, and carbapenem-resistant (CR) Acinetobacter baumannii. During a 2-month period, microbiological sampling and molecular typing were performed on environment isolates, clinical isolates and isolates recovered from the hands of healthcare professionals. The target MDROs were recovered from 79% of sampled surfaces, predominantly MRSA (74% of all tested surfaces) and CR A. baumannii (29%) but also VRE (2%) and K. pneumoniae (1%). MRSA was recovered from most tested surfaces throughout the ward, whilst CR A. baumannii was significantly more likely to be recovered from near-patient surfaces. Hand sampling demonstrated infrequent recovery of MRSA (5%), CR A. baumannii (1%) and VRE (1%). Molecular typing of the study isolates identified seven MRSA and five Acinetobacter clonal clusters, respectively, and typing identified similar strains from the environment, patients and hands. Thus, in a healthcare setting with endemic circulation of MDROs, MRSA and CR A. baumannii were the predominant organisms recovered from ward surfaces, with MRSA in particular demonstrating widespread environmental dissemination. Molecular typing demonstrated the presence of related strains in patients, in the environment and on the hands of healthcare workers.

INTRODUCTION

The widespread prevalence of multidrug-resistant organisms (MDROs) in modern healthcare settings poses a real challenge to conventional infection-control practices. Single-room isolation is not usually feasible in settings with a high prevalence of circulating MDROs. In the absence of standard screening protocols, a substantial proportion of patients may be silently colonized with MDROs that are not detected during their routine hospital stay (Harris et al., 2006; Salgado & Farr, 2006).

Environmental contamination with MDROs assumes a greater importance when patients are managed in wards with shared facilities, as bacterial contamination of near-patient surfaces, computers and medical equipment has been demonstrated in healthcare environments (Brady et al., 2009; Po et al., 2009). With increasing use of electronic healthcare, shared use of medical equipment, and imperfect hand hygiene, the role of frequently touched environmental surfaces for the potential dissemination of MDROs is now of greater importance.

There is substantial evidence that meticillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE) can be recovered from the environment, sometimes over extended periods (Dancer, 2008). There are fewer supporting data for environmental persistence of...
Gram-negative bacilli, although *Acinetobacter* spp. have been isolated from various environmental sources during episodic outbreaks (Lemmen et al., 2004). In the absence of a defined outbreak but in the context of high circulating levels of MDROs, there is little information on the contamination of near-patient surfaces and frequently touched surfaces.

This study was carried out in a hospital setting with a relatively high prevalence of MDROs, which included MRSA, *Escherichia coli* and *Klebsiella pneumoniae* with extended-spectrum β-lactamases, and carbapenem-resistant (CR) *Acinetobacter baumannii*. The aims of the study were to define the extent of environmental contamination with MDROs in the absence of a defined outbreak, to provide semi-quantitative data on the density of targets MDROs on various surface types, and to determine the genetic relatedness of environmental isolates with clinical isolates and isolates recovered from the hands of healthcare professionals.

**METHODS**

The study was carried out in an 800-bed acute-care hospital. The two wards selected for environmental sampling were open wards consisting of six to eight bays, with each bay housing six to eight beds. Ward cleaning was carried out on a daily basis by contract cleaning staff, using a phenolic-based disinfectant. This individual was responsible for cleaning all routine environmental surfaces, including near-patient surfaces. Cleaning of clinical equipment was the responsibility of ward nursing staff and was performed when surfaces were visibly soiled. Computer keyboards were routinely cleaned twice daily by ward attendants with single-use alcohol-impregnated wipes.

The target MDROs for the study were MRSA, *E. coli* and *K. pneumoniae* resistant to third-generation cephalosporins, CR *A. baumannii* and VRE. The frequency of these MDROs in 2011 was as follows: MRSA: incidence density, 19 per 10 000 patient days (37% of all *S. aureus*); ceftriaxone-resistant *E. coli*: incidence density, 25 per 10 000 patient days (25% of all *E. coli*); ceftriaxone-resistant *K. pneumoniae*: incidence density, 14 per 10 000 patient days (32% of all *Klebsiella spp.*); CR *A. baumannii*: incidence density, 6 per 10 000 patient days (60% of all *Acinetobacter spp.*) and VRE: incidence density, 2 per 10 000 patient days (11% of all *Enterococcus spp.*). As an indication of infection rates, the corresponding hospital-wide bacteraemia rates for the target MDROs in 2011 were as follows: MRSA: 5.3 per 10 000 patient days; ceftriaxone-resistant *E. coli*: 3.8 per 10 000 patient days; ceftriaxone-resistant *K. pneumoniae*: 1.6 per 10 000 patient days; carbapenem-resistant *A. baumannii*: 0.8 per 10 000 patient days and VRE: 0.2 per 10 000 patient days.

Bacterial sampling for the study was carried out over a 2-month period, in the absence of a known outbreak. Neither cleaning nor ward staff were informed about the environmental sampling, which was performed at random intervals during the routine working day by non-ward-based technologists. The timing of sampling was distributed equally during morning and afternoon periods. During the study period, target MDROs isolated from clinical samples in the study wards were retained for molecular typing. However, no attempt was made to distinguish between infection and colonization for these patients.

**Environmental sampling.** Seven surface locations were chosen for testing. They were classified into three categories: the immediate patient environment (bed frames and overbed tables), commonly accessed surfaces not in close physical proximity to patients (telephones and toilet door handles) and commonly used equipment (mobile computer keyboards, sphygmomanometer cuffs and stethoscopes). A sterile flocked nylon swab moistened with sterile saline solution was rotated and swabbed in a standardized pattern within the defined area for each sampling point. Computer keyboards were sampled by moving the swab over the letter keys. Swabs for each surface type were pooled in a sterile container with 3 ml sterile saline and vortexed, and 100 μl of the eluted solution was plated onto each test medium.

**Hand sampling of healthcare professionals.** Bacterial sampling was performed from the hands of healthcare professionals in both participating wards, and included equal numbers of physicians, nurses and allied health professionals. Sampling was carried out at random intervals throughout the day, with a total of 75 samples collected. Hand sampling was performed using the glove juice technique (Waterman et al., 2006), with 300 μl of the eluted solution plated onto the same culture medium used for environmental sampling. Hand-hygiene compliance rates were routinely monitored on all hospital wards during the study period, and there was no observed change in compliance rates on the targeted wards.

**Selective bacterial culture, bacterial identification and susceptibility testing.** Trypticase soy agar with 5% sheep blood (BD) was used for total viable counts, whilst selective media were used to isolate specific MDROs. These comprised MRSASelect (Bio-Rad), Enterococcosel agar containing 8 μg vancomycin (Bloxwich) ml⁻¹ and CHROMagar Orientation (BD) containing 4 μg ceftriaxone ml⁻¹ and 8 μg vancomycin ml⁻¹.

Culture media were incubated at 35 °C for 48 h. For each selective medium, semi-quantitative enumeration of target MDROs was performed. Bacterial identification and susceptibility testing of the target organisms was performed using a Vitek 2 Compact (bioMérieux).

**Molecular characterization of target organisms.** Bacterial typing was performed to identify the clonal relationships between clinical, environmental and hand-sampling isolates for the two most prevalent MDROs, namely MRSA and *A. baumannii*. *A. baumannii* was typed using a multiplex rep-PCR protocol (Grundmann et al., 1997), whilst MRSA was typed using multilocus variable-number tandem repeat analysis (MLVA) (Francois et al., 2005). The PCR products were analysed and imaged by capillary electrophoresis (Experion; Bio-Rad). Phylogenetic clustering was performed by the unweighted pair group method with arithmetic mean using the Dice coefficient and position tolerance settings of 4% (Bionumerics; Applied Maths). MRSA isolates with a similarity index of >80% were considered to be closely related, whilst a similarity cut-off of >90% was used for *A. baumannii*.

**RESULTS**

**Clinical samples**

During the study period, 28 clinical isolates of MDROs were retained for molecular typing. These consisted of MRSA (*n*=20), CR *A. baumannii* (*n*=3), ceftriaxone-resistant *K. pneumoniae* (*n*=4) and VRE (*n*=1).

**Environmental sampling**

Pooled swabs were obtained from 82 environmental sites, of which 65 samples (79%) yielded a total of 97 isolates.
### Table 1. Environmental recovery of MDROs from sampled surfaces

<table>
<thead>
<tr>
<th>Area</th>
<th>Surface</th>
<th>No. samples</th>
<th>MRSA</th>
<th>CR A. baumannii</th>
<th>VRE</th>
<th>Ceph-R Klebsiella spp.*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Positive (%)</td>
<td>Positive (c.f.u. cm(^{-2}))</td>
<td>Positive (%)</td>
<td>Positive (c.f.u. cm(^{-2}))</td>
</tr>
<tr>
<td>Immediate patient</td>
<td>All sampled areas</td>
<td>50</td>
<td>82</td>
<td>0.42</td>
<td>40</td>
<td>0.47</td>
</tr>
<tr>
<td>environment</td>
<td>Bed frame</td>
<td>25</td>
<td>88</td>
<td>0.41</td>
<td>48</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>Overbed table</td>
<td>25</td>
<td>76</td>
<td>0.44</td>
<td>32</td>
<td>0.46</td>
</tr>
<tr>
<td>Commonly used equipment</td>
<td>All sampled areas</td>
<td>13</td>
<td>62</td>
<td>0.83</td>
<td>15</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Glucometer</td>
<td>2</td>
<td>50</td>
<td>1.54</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Stethoscope</td>
<td>6</td>
<td>67</td>
<td>0.64</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>BP cuff</td>
<td>5</td>
<td>60</td>
<td>0.84</td>
<td>40</td>
<td>0.31</td>
</tr>
<tr>
<td>Commonly touched surfaces</td>
<td>All sampled areas</td>
<td>19</td>
<td>63</td>
<td>0.59</td>
<td>10</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>Bedside medical computer</td>
<td>6</td>
<td>100</td>
<td>0.37</td>
<td>17</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>Door handle</td>
<td>7</td>
<td>43</td>
<td>0.87</td>
<td>14</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>Telephone</td>
<td>6</td>
<td>50</td>
<td>0.76</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>All sites</td>
<td>82</td>
<td>74</td>
<td>0.51</td>
<td>29</td>
<td>0.42</td>
</tr>
</tbody>
</table>

*Ceph-R, third-generation cephalosporin resistant.
Sixty samples (73%) were positive for two MDROs, with one sample positive for three MDROs. MRSA was the most frequently recovered MDRO (74% of all samples), whilst CR A. baumannii was recovered from 29% of samples (Table 1). Ceftriaxone-resistant K. pneumoniae and VRE were recovered much less frequently, whilst no ceftriaxone-resistant E. coli was recovered from the environment. Surfaces in the immediate environment of the patient were more likely to be contaminated with MRSA (risk ratio 1.3, 95% confidence interval 0.97–1.77), but MRSA was also widely recovered from most other sampled surfaces including computer keyboards, medical equipment and door handles. In contrast, the recovery of CR A. baumannii was much more likely to be confined to the immediate surroundings of the patient (risk ratio 3.2, 95% confidence interval 1.2–8.5). The relative surface density of MDROs was highest for MRSA (mean 0.5 c.f.u. cm\(^{-2}\), range 0.4–1.5 c.f.u. cm\(^{-2}\)), followed by CR A. baumannii (mean 0.4 c.f.u. cm\(^{-2}\), range 0.1–0.5 c.f.u. cm\(^{-2}\)) (Table 1).

**MDRO isolates from hand samples**

Glove juice hand samples collected from the hands of 75 healthcare professionals showed higher contamination rates with Gram-positive (7%) than with Gram-negative (1%) organisms. MRSA was recovered from four samples (5%), whilst CR A. baumannii and VRE were each recovered from one sample (1%). Ceftriaxone-resistant E. coli and K. pneumoniae were not recovered from any hand samples.

**Molecular typing data**

Six predominant MRSA clonal clusters were identified by MLVA typing, with four large clusters accounting for 64 (79%) of 81 MRSA isolates (Fig. 1). Clusters A and C clearly demonstrated dissemination of closely related MRSA strains between patients and their immediate surrounding environment, which included bed frames and overbed tables. In addition, the strains from cluster C were also recovered from commonly touched surfaces and medical equipment. Clusters E and F showed the recovery of closely related MRSA strains from patients, their immediate environment and the hands of healthcare professionals on the same ward. Clusters B and D consisted only of strains recovered from environmental sources, with no identified clinical source.

CR A. baumannii isolates were differentiated into four predominant clusters by repetitive PCR (Fig. 2). Cluster A demonstrated limited dissemination of related strains between patients with clinical isolates and the immediate environment. The other three clusters consisted only of environmental isolates, but cluster D demonstrated that closely related strains on the same ward were present on patient bed frames, the hands of a healthcare professional and sphygmomanometer cuffs.

**DISCUSSION**

The distribution of MDROs in this study reflected both the ability of the organisms to survive on inanimate surfaces and the prevalence of MDROs in our patient population, with nearly eight out of every ten samples surfaces yielding an MDRO. In line with other studies, our findings showed that S. aureus and Acinetobacter spp. survive in the environment and on most commonly accessed surfaces. The widespread detection of MRSA in our study suggests that environmental contamination of wards and medical equipment by undetected carriers may be more prevalent than previously suspected. Although near-patient sites were highly likely to be contaminated with MRSA, the prevalence of MRSA recovered from other more distant sites represents a significant infection-control challenge. The proportion of environmental sites positive for MRSA was higher in our study than in other studies, which report a mean positivity rate of one-third of sampled surfaces (Dancer, 2008). Despite a lower incidence density in clinical specimens, CR A. baumannii isolates were recovered from nearly one-third of surfaces. When the environment is surveyed in the setting of an outbreak, Acinetobacter spp. are readily recovered from multiple surfaces (Aygün et al., 2002; Das et al., 2002; Getchell-White et al., 1989). However, routine surveillance of surfaces in the absence of a defined outbreak usually reports lower rates of detection (Lemmen et al., 2004; Paavilainen et al., 2001). Our results suggest that, in a healthcare setting with a relatively high prevalence, CR A. baumannii has the potential to persist in the immediate patient environment, with less dissemination to other areas. This finding emphasizes the importance of thorough disinfection and cleaning of beds and furniture in the immediate patient environment, especially in periods of high patient turnover. Despite the relatively high prevalence of ceftriaxone-resistant E. coli and Klebsiella spp. in clinical isolates, these MDROs were very infrequently recovered from the environment. One final important finding was that the relative bacterial density of most MDROs was low, with most surfaces showing <1 c.f.u. per cm\(^2\). There are not yet sufficient data to determine what level of bacterial contamination with MDROs increases the risk of patient acquisition from the environment, although some authors have suggested a standard of <1 c.f.u. cm\(^{-2}\) for any potential pathogen or an aerobic colony count of <2.5 c.f.u. cm\(^{-2}\) from high-touch surfaces (Dancer, 2004; Malik et al., 2003).

The molecular typing data for both MRSA and Acinetobacter spp. demonstrated that clonally related strains circulated between patients, the environment and the hands of healthcare professionals. The link between clinical and environmental strains of MRSA has been demonstrated by numerous studies (Lemmen et al., 2004; Sexton et al., 2006), but there are limited data showing a link between isolates from the environment, patients and the hands of healthcare workers (Boyce et al., 1997). In a study by Creamer et al. (2010), MRSA was recovered from...
10% of sampled hands following contact with the patient’s environment. To our knowledge, there are no equivalent data for the circulation of multidrug-resistant A. bauman-
nii between these various potential sources.

There are some limitations to our study. We did not perform admission screening for the target MDROs on patients admitted to, or present in, the study wards. Given the relatively high prevalence of MDROs in the patient population, it is likely that a substantial number of patients were silently colonized with one or more of the targeted MDROs. Because sampling of environmental surfaces was performed using swabs without an enrichment phase, it is possible that the number of contaminated sites was underestimated. However, our study method allowed a semi-quantitative enumeration of the extent of bacterial contamination for each sampled surface.

In a high-prevalence setting, our study findings suggest that commonly accessed surfaces, commonly touched equipment and patient areas might serve as potential reservoirs for cross-transmission within the hospital setting (Boyce et al., 1997). Whilst enhanced cleaning has been demonstrated to reduce new MRSA infections in a high-prevalence setting (Dancer et al., 2009), no such data exist for Acinetobacter spp. The concern about environment contamination with MDROs and increased risk of transmission to patients has resulted in recommendations for systematic cleaning programmes to evaluate hygiene standards in healthcare environments (Carling & Bartley, 2002).

Fig. 1. Molecular typing of MRSA isolates. E, Environmental isolate; P, patient/clinical isolate. VNTR, variable number of tandem repeats.

Fig. 2. Molecular typing of CR A. baumannii isolates.
2010). In the interim, further studies are recommended to both define the sequence of transmission of MDROs between patients, healthcare workers and the environment, and to document the effect of environmental interventions on subsequent acquisition.

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