Retrospective survey of candidaemia in hospitalized patients and molecular investigation of a suspected outbreak

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Episodes of candida infection at a teaching hospital were investigated. During a 3-year period from 1998 to 2000, there were 53 cases of candidaemia. Candida albicans (64.2 %) was the most common causative species, followed by Candida glabrata (17.0 %) and Candida parapsilosis (15.1 %). Molecular analysis of a cluster of eight infections from a single unit was performed using Southern blotting with Ca3 probe hybridization. This showed that the patients were each infected by unrelated strains of C. albicans. On occasion, isolates were found to be closely related within individual patients. Following Southern blot analysis, it was concluded that the infections were not part of an outbreak caused by a single, epidemic strain.

Introduction

Candida species are opportunistic pathogens that can be found living as commensals on the skin and mucous membranes of humans and other animals. In humans they can also cause superficial or deep-seated infections. Data from the Nosocomial Infection National Surveillance Service found that between 1997 and 2002, Candida species were the eighth most common cause of hospital-acquired bloodstream infection in the UK (Nosocomial Infection National Surveillance Service, 2002), and a recent UK multicentre study found an incidence rate of 3.0 per 100 000 bed days (Surveillance Service, 2002), and a recent UK multicentre study found an incidence rate of 3.0 per 100 000 bed days (Wey et al., 1988), and, in a UK study, attributable 30-day mortality was reported as 26.4 % (Kibbler et al., 2003).

Candida species are occasionally associated with clusters of nosocomial infection. Understanding the epidemiology of Candida species in the hospital environment is important in minimizing infection during hospitalization. Designing appropriate control measures will be aided by the knowledge of whether the source of infection is typically endogenous or exogenous. A range of molecular methods is available to study the epidemiology of fungal pathogens and these are useful for outbreak investigations. In this 3-year study, all cases of candidaemia affecting in-patients at the General Infirmary at Leeds were identified and Candida albicans isolates from a single ward were subjected to molecular typing to determine the degree of relatedness.

Methods

Case identification. All patients from the General Infirmary at Leeds who had a positive blood culture for Candida species in the 3-year period of 1998 to 2000 were identified from laboratory records and included in the study. Patients for whom incorrect clinical samples had been received in blood culture bottles were excluded. Data on the underlying medical conditions of the patients were taken from case notes and laboratory records. Positive blood cultures were considered as part of a single episode if they were of the same species and occurred less than 2 weeks apart. Any clusters of cases from the same ward were identified. Isolates from the clusters were retrieved from stocks in −80 °C storage and molecular typing was performed.

Molecular typing. Molecular typing was performed using Southern blotting and hybridization with the Ca3 probe. The Ca3 probe is a complex probe specific for C. albicans that will distinguish unrelated isolates and will also reveal microevolution in related isolates (Pujol et al., 1997; Sadhu et al., 1991). DNA was isolated using yeast lytic enzyme following standard protocols and restriction endonuclease digestion was performed using EcoRI. Electrophoresis and Southern blotting were performed using standard methods. Hybridization of the labelled Ca3 probe and subsequent detection were carried out using the DIG labelling system (Roche Applied Science) according to the manufacturer’s instructions. Blots were analysed using BioNumerics software (Applied Maths) and similarity index dendograms generated using the Pearson correlation with optimization at 1 %. Two isolates sharing a similarity index of greater than 90 % were considered to represent closely related strains.
<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Underlying medical condition(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>75 years</td>
<td>Pancreatitis</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>49 years</td>
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<td>4</td>
<td>F</td>
<td>85 years</td>
<td>Pneumonia, bowel obstruction</td>
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<td>F</td>
<td>46 years</td>
<td>Pneumonia, neutropenia</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>31 years</td>
<td>Abdominal wounds from stabbing, on total parenteral nutrition</td>
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<tr>
<td>8</td>
<td>M</td>
<td>2 months</td>
<td>Prematurity, necrotizing enterocolitis</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>76 years</td>
<td>Perforated duodenal ulcer</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>58 years</td>
<td>Leukaemia, neutropenia</td>
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<tr>
<td>11</td>
<td>M</td>
<td>21 years</td>
<td>Hemicolectomy</td>
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<td>Gastroschisis</td>
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<td>13</td>
<td>M</td>
<td>82 years</td>
<td>Hepatomegaly, jaundice, confusion</td>
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<td>38 years</td>
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<td>F</td>
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<td>Not known</td>
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<tr>
<td>19</td>
<td>F</td>
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<td>Necrotizing enterocolitis on total parenteral nutrition</td>
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<td>61 years</td>
<td>Diabetes, multiple sclerosis</td>
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<td>71 years</td>
<td>Intracerebral bleed, on total parenteral nutrition</td>
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<td>25</td>
<td>F</td>
<td>33 years</td>
<td>Ulcerative colitis</td>
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<tr>
<td>26</td>
<td>F</td>
<td>37 years</td>
<td>Pelvic malignancy, diabetes, on total parenteral nutrition</td>
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<td>28</td>
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<td>Prematurity, jaundice, respiratory distress, on total parenteral nutrition</td>
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<tr>
<td>30</td>
<td>F</td>
<td>76 years</td>
<td>Lymphoma, infected aortic graft, enteric fistula, on total parenteral nutrition</td>
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<tr>
<td>31</td>
<td>F</td>
<td>10 months</td>
<td>Diabetes, hydrocephalus</td>
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<tr>
<td>32</td>
<td>M</td>
<td>Newborn</td>
<td>Prematurity, anaemia, respiratory distress, total parenteral nutrition, ventilated</td>
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<tr>
<td>33</td>
<td>F</td>
<td>54 years</td>
<td>Removal of plasmacytoma from spine</td>
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<tr>
<td>34</td>
<td>F</td>
<td>3 months</td>
<td>Prematurity, necrotizing enterocolitis on total parenteral nutrition</td>
</tr>
<tr>
<td>35</td>
<td>M</td>
<td>1 month</td>
<td>Prematurity, necrotizing enterocolitis on total parenteral nutrition</td>
</tr>
<tr>
<td>36</td>
<td>F</td>
<td>1 year</td>
<td>Fundoplication, microcephaly, failure to thrive, developmental delay</td>
</tr>
<tr>
<td>37</td>
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<td>Prematurity, pulmonary haemorrhage, ventilated, on total parenteral nutrition, intraventricular haemorrhage</td>
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<td>4 months</td>
<td>Prematurity, cardiomyopathy, cystic fibrosis, oesophageal atresia, on total parenteral nutrition</td>
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<td>70 years</td>
<td>Pancreaticoduodenectomy, on total parenteral nutrition</td>
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<td>40</td>
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<td>1 year</td>
<td>Diaphragmatic hernia repair, on total parenteral nutrition, ventilated</td>
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<td>41</td>
<td>F</td>
<td>1 month</td>
<td>Aortic repair, on total parenteral nutrition, ventilated</td>
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<tr>
<td>42</td>
<td>F</td>
<td>70 years</td>
<td>Acute aortic aneurysm repair</td>
</tr>
<tr>
<td>43</td>
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<td>6 months</td>
<td>Prematurity, gastroschisis, on total parenteral nutrition</td>
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<tr>
<td>44</td>
<td>F</td>
<td>62 years</td>
<td>Uterine abscess, diabetes, renal failure</td>
</tr>
<tr>
<td>45</td>
<td>F</td>
<td>65 years</td>
<td>Mitral valve replacement, renal failure</td>
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<tr>
<td>46</td>
<td>M</td>
<td>Newborn</td>
<td>Prematurity, renal failure</td>
</tr>
<tr>
<td>47</td>
<td>F</td>
<td>3 years</td>
<td>Multiple congenital anomalies, on gastrostomy feeds</td>
</tr>
<tr>
<td>48</td>
<td>M</td>
<td>44 years</td>
<td>Diarrhoea and vomiting, dental infection, oesophagitis, diabetes, multiple sclerosis</td>
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<tr>
<td>49</td>
<td>M</td>
<td>57 years</td>
<td>Aortic valve disease, bacterial endocarditis</td>
</tr>
<tr>
<td>50</td>
<td>M</td>
<td>10 months</td>
<td>Short bowel/Hirschsprung’s disease, gastrectomy, on total parenteral nutrition</td>
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<tr>
<td>51</td>
<td>M</td>
<td>7 months</td>
<td>Multiple congenital anomalies, bronchomalacia, fundoplication, ventililated</td>
</tr>
<tr>
<td>52</td>
<td>F</td>
<td>1 year</td>
<td>Multiple congenital anomalies, short bowel, on total parenteral nutrition</td>
</tr>
<tr>
<td>53</td>
<td>M</td>
<td>Newborn</td>
<td>Prematurity, bowel perforation, on total parenteral nutrition, ventilated</td>
</tr>
<tr>
<td>55</td>
<td>M</td>
<td>1 year</td>
<td>Mitochondrial disorder, cardiomyopathy, developmental delay, gastrostomy, on total parenteral nutrition</td>
</tr>
<tr>
<td>56</td>
<td>F</td>
<td>2 years</td>
<td>Acute lymphocytic leukaemia, neutropenia, on total parenteral nutrition, renal failure</td>
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<tr>
<td>57</td>
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<td>1 month</td>
<td>Metabolic disorder, neutropenia, on total parenteral nutrition</td>
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<tr>
<td>58</td>
<td>M</td>
<td>74 years</td>
<td>Fractured hip replacement, chest infection post-operatively</td>
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<td>61</td>
<td>F</td>
<td>53 years</td>
<td>Laparotomy/bowel resection, on total parenteral nutrition</td>
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<tr>
<td>62</td>
<td>F</td>
<td>65 years</td>
<td>Post-mitral valve repair</td>
</tr>
</tbody>
</table>
Results and Discussion

There were 53 patients with candidaemia, each with a single episode, during the study. Of these cases, 34 were caused by *C. albicans* (64.2%), nine by *Candida glabrata* (17.0%), eight by *Candida parapsilosis* (15.1%) and two (3.8%) infections were caused by other *Candida* species: one *Candida tropicalis* and one unknown. This distribution of the three predominant species reflected that found in other studies (Pfaller et al., 1999).

The age, sex and underlying medical conditions of the patients are shown in Table 1. There were 22 patients aged less than 2 years old, including 10 premature neonates (Table 1; Fig. 1). The predominance of young children with candidaemia is noteworthy as it is consistent with reports that infants are at particular risk of candidaemia, the reasons for which are multiple. For instance, critically ill and premature babies have immature immune systems, they are often on total parenteral nutrition, they may be intubated and prematurity itself is a risk factor for candida infection (Saiman et al., 2000). Neonates and young babies are an important patient group to monitor, and it has been suggested that it is one of the few patient groups in which rates of candida infection may be rising (Hobson, 2003).

There were seven patients with a haematological malignancy (Table 1) and seven patients were staying on intensive care units (data not shown). Ten patients had problems involving the gastrointestinal tract, many of whom underwent surgery. These are all patients that fall into commonly accepted risk factor categories for candidaemia, due to either their underlying problems or the hospital treatment they have received (Bross et al., 1989; Wey et al., 1989).

It has been shown that *C. albicans* can cause outbreaks in the hospital setting (Boccia et al., 2002). It is not clear whether clusters of infection are due to epidemic strains, possibly with increased virulence, or whether infections are caused largely by unrelated strains in groups of patients with increased risk of infection. This study identified eight patients on the paediatric intensive care unit with candidaemia over a period of 13 months: seven were *C. albicans* infections and were considered to form a cluster requiring further investigation. A further patient had been on the unit immediately before their positive blood culture with *C. albicans* and was included in the analysis. Eleven isolates from these patients were typed. There were 10 blood culture isolates, and one isolate cultured from a urine sample was included in the analysis. There were two isolates each from three patients and one from the other five patients. Typing using the Ca3 probe revealed that each patient was infected by one or more isolates with a unique genotype (Fig. 2). The two isolates with greatest similarity recovered from two different patients showed less than 89% identity. The two isolates recovered from patient 2 were also only distantly related, sharing 55% identity. In contrast, the pairs of strains from patients 3 and 5 were closely related, sharing 92 and 95% identity, respectively, even though the isolates recovered from patient 3 were taken from different anatomical sites (blood and urine). From the molecular analysis of these isolates, it was concluded that the isolates from different patients were unrelated, rather than belonging to a common ‘epidemic’ strain.

In conclusion, *C. albicans* remains the most frequent cause of candidaemia, but *C. glabrata* and *C. parapsilosis* are also significant pathogens. The cluster of cases of candidaemia in the paediatric intensive care unit during the study period did not represent an outbreak caused by an epidemic strain of *C. albicans*. This suggests that the infections were caused by endogenous yeasts and that host factors have a much greater impact on the epidemiology of systemic candida infections.

References


