Clinical and microbiological characteristics of Chryseobacterium spp. isolated from neonates in Kuwait

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Introduction: Generally considered to be part of the environmental flora, Chryseobacterium spp. have been reported to cause infection in humans, albeit rarely. The clinical significance of these organisms remains to be fully established, despite being isolated from patients, especially neonates, and immunocompromised subjects.

Case presentation: We present a study of 10 isolates of Chryseobacterium spp. cultured from blood and endotracheal secretions of neonates in two hospitals, Farwaniya Hospital (FH) and Maternity Hospital (MH), Kuwait, identified using the Phoenix or Vitek 2 system from April to November 2012. The clinical features of the patients were assessed, and antimicrobial susceptibilities of the isolates were performed by disk diffusion test and Etest. Molecular identification of bacteria was done by 16S rRNA gene sequencing and fingerprinting by random amplified polymorphic DNA (RAPD). Patients suffered from sepsis, pneumonia or other clinical conditions. Two strains of Chryseobacterium indologenes and eight strains of Chryseobacterium meningosepticum (now Elizabethkingia meningosepticum) were cultured from clinical samples from FH and MH, respectively. Both C. indologenes and six of the C. meningosepticum strains were isolated from endotracheal secretions, and two of the latter were from blood. Identification of isolates was confirmed by 16S rRNA gene sequencing. All isolates were multidrug resistant and eight were metallo-β-lactamase positive. Five patterns of Chryseobacterium spp. were identified by RAPD.

Conclusion: It appears that Chryseobacterium spp. are emerging pathogens for neonates in Kuwait, causing serious systemic infections.

Keywords: bacteraemia; Chryseobacterium spp.; colonisation; infection; neonates; pneumonia.
Phoenix (Becton Dickinson) (Lee et al., 2006; Sudharani et al., 2011). The identification may be confirmed to the species level by genetic analysis (Bellaïs et al., 2002; Chiu et al., 2000). As no standard MIC breakpoints for antimicrobial agents have been described by the Clinical and Laboratory Standards Institute for these organisms, quantitative MIC results have been interpreted using breakpoints for non-Enterobacteriaceae (Chou et al., 2011) or those approved for bacteria that grow aerobically (Matsumoto et al., 2012).

There is an increasing concern over the inherent multiple antimicrobial resistance exhibited by these organisms and the possibility of disseminating plasmid-mediated genes for carbapenem hydrolysing enzymes from Chryseobacterium strains (Zeba et al., 2009). Also, resistance has been described due to Ambler class A extended-spectrum β-lactamases (ESBLs) (Bellaïs et al., 2002; Kirby et al., 2004; Matsumoto et al., 2012). Epidemics have been reported to occur, with the mortality rate reaching 55% in a nursery outbreak (Chiu et al., 2000).

In this report, we studied chryseobacteria from clinical samples of 10 neonates from two different hospitals. Clinical features of infection in the patients and microbiological characteristics of the isolates are described. To the best of our knowledge, this is the first report of chryseobacterium infection in Kuwait.

Case report

From April to November 2012, 10 strains of Chryseobacterium spp. were isolated from clinical samples (eight from endotracheal secretions and two from blood samples) from 10 patients (nine neonates and one infant of 2 months old). An episode of significant bacteraemia due to Chryseobacterium spp. was defined as one or more positive blood cultures together with signs of clinical sepsis, whereas a patient was considered colonised with Chryseobacterium spp. if the neonate had a positive endotracheal culture with no signs of sepsis or pneumonia and also patients who were infected with other known pathogens or had underlying clinical conditions not necessarily associated with infection (Ceyhan and Celik, 2011; Lin et al., 2010).

In Maternity Hospital (MH), the patients were admitted to either neonatal intensive care unit 1 (NICU1) or 2 (NICU2). These two units are widely separated and there is no inter-unit transfer of patients. In Farwaniya Hospital (FH), the patients were admitted to the paediatric intensive care unit (PICU). The index case was a 2-month-old, full-term neonate, admitted to FH in late March 2012, for treatment of respiratory distress and bronchopneumonia. Isolation of Chryseobacterium indologenes from endotracheal secretions of this patient was followed by similar findings in another baby admitted in the same room as the first patient after a period of 2 weeks. However, Chryseobacterium meningosepticum strains were isolated from clinical samples (six from endotrachaeal secretions and two from blood samples) from eight neonates admitted to MH during a period of 7 months beginning in May 2012. The clinical characteristics of all 10 patients are presented in Table 1. Male patients (70%) predominated. The mean gestational age of these neonates was 32 weeks and the mean birth weight was 1.98 kg. The organism appeared to have been acquired nosocomially by all patients, as positive chryseobacterium cultures were obtained only after the first 72 h of hospitalisation. The patients had a pre-existing infection due to other microorganisms(s), had an underlying genetic malformation or had received an array of antibiotics, including aminoglycosides, β-lactams, β-lactam/β-lactamase inhibitor and carbapenems, as shown in Table 1.

Investigations

All strains grew well on routine culture media (blood, chocolate and MacConkey agar plates). Whilst the two isolates from FH were identified using the Phoenix Automated Microbiological System (software version 4.01; Becton Dickinson) as C. indologenes, all eight strains isolated at MH were identified as C. meningosepticum using the Vitek ID-GNB (Vitek 2; bioMérieux) identification system. The isolates were further confirmed by 16S rRNA gene sequencing using primers S-D-Bact-0008-a-S-20 (forward) and S-Univ-1492-b-A-21 (reverse) as described by Suau et al. (1999). The 1.5 bp amplicon was sequenced using a GeneAmp PCR system 9700 by cycle sequencing with BigDye termination (Applied Biosystems). Sequences were analysed using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Susceptibility to the antimicrobial agents amikacin, gentamicin, imipenem, meropenem, ciprofloxacin, levofloxacina, pipercillin, pipercillin/tazobactam (TZP), rifampicin, ceftriaxone, ceftazidime, cefepime and trimethoprim/sulfamethoxazole (SXT) was determined by disk diffusion using the Kirby–Bauer method (Kirby et al., 2004). In addition, a selected number of antimicrobial agents, based on availability, were tested using Etest strips (AB Biodisk). Results were interpreted by applying Clinical and Laboratory Standards Institute breakpoints recommended for non-Enterobacteriaceae (Chou et al., 2011). All isolates were screened phenotypically for metallo-β-lactamase (MBL) production using an imipenem/EDTA double-disk synergy test (Lee et al., 2001), a modified Hodge test (Lee et al., 2010) and an Etest according to the manufacturer’s instructions.

The antimicrobial susceptibility test performed by disk diffusion revealed that the C. indologenes and C. meningosepticum strains were all resistant to most of the antibiotics tested, showing susceptibility to TZP, SXT and rifampicin. However, both strains of C. indologenes in addition demonstrated susceptibility to cefazidime. The Etest confirmed the findings of the disk diffusion test. The
Table 1. Clinical data of patients with *Chryseobacterium* spp. infection

<table>
<thead>
<tr>
<th>Patient*</th>
<th>Gestational age (weeks)</th>
<th>Gender</th>
<th>Birth weight (kg)</th>
<th>Location</th>
<th>Diagnosis on admission†</th>
<th>Specimen‡</th>
<th>Antibiotics received§</th>
<th>Days in hospital</th>
<th>C/I‖</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27</td>
<td>M</td>
<td>1.235</td>
<td>NICU1</td>
<td><em>E. coli</em> sepsis, meningitis</td>
<td>ES</td>
<td>AMP, AMK, TZP, MEM</td>
<td>70</td>
<td>I</td>
<td>Expired</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>F</td>
<td>1.640</td>
<td>NICU1</td>
<td><em>K. pneumoniae</em> sepsis and ARDS</td>
<td>Blood</td>
<td>MEM</td>
<td>117</td>
<td>I</td>
<td>Survived</td>
</tr>
<tr>
<td>3</td>
<td>NA</td>
<td>M</td>
<td>2.090</td>
<td>NICU2</td>
<td>Trisomy 18, Klienfilter’s syndrome</td>
<td>ES</td>
<td>AMP, AMK</td>
<td>19</td>
<td>C</td>
<td>Expired</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>F</td>
<td>1.140</td>
<td>NICU2</td>
<td>Sepsis with ESBL+ <em>K. pneumoniae</em></td>
<td>ES</td>
<td>MEM, AMK</td>
<td>44</td>
<td>I</td>
<td>Expired</td>
</tr>
<tr>
<td>5</td>
<td>31</td>
<td>M</td>
<td>2.300</td>
<td>NICU1</td>
<td><em>E. cloacae</em>+<em>E. coli</em> sepsis</td>
<td>ES</td>
<td>TZP, MEM, AK</td>
<td>134</td>
<td>C</td>
<td>Survived</td>
</tr>
<tr>
<td>6</td>
<td>37</td>
<td>M</td>
<td>3.190</td>
<td>NICU1</td>
<td>Down’s syndrome with imperforate anus</td>
<td>ES</td>
<td>TZP, MEM</td>
<td>108</td>
<td>C</td>
<td>Survived</td>
</tr>
<tr>
<td>7</td>
<td>32</td>
<td>M</td>
<td>1.625</td>
<td>NICU1</td>
<td>RDS, bilateral pneumothorax, NEC, BPD</td>
<td>ES</td>
<td>TZP</td>
<td>30</td>
<td>C</td>
<td>Survived</td>
</tr>
<tr>
<td>8</td>
<td>34</td>
<td>M</td>
<td>2.640</td>
<td>NICU2</td>
<td>Sepsis, meningitis (?), IEM</td>
<td>Blood</td>
<td>AM, AK, TZP, MEM</td>
<td>3</td>
<td>I</td>
<td>Expired</td>
</tr>
<tr>
<td>9</td>
<td>38</td>
<td>M</td>
<td>NA</td>
<td>PICU</td>
<td>Bronchopneumonia</td>
<td>ES</td>
<td>CTX, TZP, AK</td>
<td>73</td>
<td>C</td>
<td>Survived</td>
</tr>
<tr>
<td>10</td>
<td>38</td>
<td>F</td>
<td>NA</td>
<td>PICU</td>
<td>GBS sepsis</td>
<td>ES</td>
<td>CTX</td>
<td>21</td>
<td>C</td>
<td>Survived</td>
</tr>
</tbody>
</table>

NA, Not available.

*Patients 1–8 were in MH from whom *C. meningosepticum* was isolated; patients 9 and 10 were in FH from whom *C. indologenes* was isolated.

†ARDS, acute respiratory distress syndrome; BPD, bronchopulmonary dysplasia; IEM, inborn error of metabolism; GBS, group B streptococcus; NEC, necrotizing enterocolitis; RDS, respiratory distress syndrome.

‡ES, endotracheal secretion.

§AMP, ampicillin; AMK, amikacin; MEM, meropenem; CTX, cefotaxime.

‖C/I, colonisation/infection due to *Chryseobacterium* spp.

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<table>
<thead>
<tr>
<th>Isolate</th>
<th>Etest (MIC, mg l⁻¹)</th>
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<tbody>
<tr>
<td></td>
<td>CTX</td>
</tr>
<tr>
<td><em>C. meningosepticum</em> (n=8)</td>
<td>8–24 (50 % R)</td>
</tr>
<tr>
<td><em>C. indologenes</em> (n=2)</td>
<td>32 (R)</td>
</tr>
</tbody>
</table>
MICs for all the strains are presented in Table 2. MIC\textsubscript{90} values for TZP, gentamicin, amikacin, cefotaxime and ceftazidime were 2.0, 32, 96, 16 and $>256\ \mu$g ml\textsuperscript{-1}, respectively, for the \textit{C. meningosepticum} strains. Although TZP was the only antibiotic to which all 10 strains showed susceptibility, four strains (50 \%) of \textit{C. meningosepticum} also showed susceptibility to cefotaxime. All except two isolates (isolates 1 and 4) were MBL producers. The \textit{chryseobacterium} isolates were typed by random amplified polymorphic DNA (RAPD) fingerprinting with the primer 5'-GTCGATGTCG-3', as described previously (Chiu \textit{et al}., 2000). The amplified products were separated by 1.5 \% agarose gel electrophoresis and, after staining with ethidium bromide, were visualised and photographed under UV illumination.

The identification of all isolates to the species level was confirmed by 16S rRNA gene sequence analysis. The fingerprinting patterns obtained by RAPD from the two \textit{C. indologenes} and eight \textit{C. meningosepticum} isolates are presented in Fig. 1. In repeated experiments, some of the high-molecular-mass bands were not clearly visible. However, there appeared to be five distinct patterns. These were formed by isolates 1, 4, 5 and 6 (\textit{C. meningosepticum}); isolates 2 and 7 (\textit{C. meningosepticum}); isolates 3 and 8 (\textit{C. meningosepticum}); isolate 9 (\textit{C. indologenes}); and isolate 10 (\textit{C. indologenes}). Isolates 1, 4, 5 and 6 (same RAPD profile) and isolate 2 with a different RAPD profile presented similar antibiograms with MICs of gentamicin 12–16 mg l\textsuperscript{-1}, ceftazidime $>256\ \mu$g l\textsuperscript{-1} and amikacin 32–48 mg l\textsuperscript{-1}. The antimicrobial susceptibility pattern was similar for both \textit{C. indologenes} isolates. The fingerprinting data suggested that different clones were causing the infections.

**Treatment and outcomes**

A total of four patients died. Of the six neonates who were treated with TZP, five survived. The two patients who expired (1 and 8) despite receiving appropriate antibiotic therapy were both diagnosed with sepsis and either confirmed or unconfirmed meningitis, although \textit{C. meningosepticum} was isolated only from endotracheal secretions in one and from blood in the other. The death in patient 1 was due to \textit{Escherichia coli} and in patient 8 was due to \textit{C. meningosepticum}. Of the remaining two patients who died and did not receive TZP (patients 3 and 4), one was diagnosed with sepsis with ESBL-positive \textit{Klebsiella pneumoniae} and was treated with meropenem and amikacin, whilst the other was diagnosed with trisomy (Klinefelter’s syndrome) and the baby succumbed to other non-infectious causes.

**Discussion**

\textit{Chryseobacterium} spp. have been reported primarily to infect premature newborns and other immunocompromised hosts (Bloch \textit{et al}., 1997; Güngör \textit{et al}., 2003). \textit{C. meningosepticum} is the most pathogenic member of the genus, responsible for causing neonatal meningitis (Calderon \textit{et al}., 2011; Ceyhan \textit{et al}., 2008; Güngör \textit{et al}., 2003; Hazuka \textit{et al}., 1977; Maraki \textit{et al}., 2009; Tekerekoglu \textit{et al}., 2003). Excluding one infant who was 2 months old, all other patients were premature newborns with a low birth weight, which is regarded as a primary risk factor. However, none of the eight neonates in MH had documented meningitis due to \textit{C. meningosepticum} and only two presented with sepsis due to primary bacteraemia. The remaining six patients from MH yielded growth of \textit{C. meningosepticum}, and both patients from FH yielded growth of \textit{C. indologenes} from endotracheal secretions. Even though colonisation of the organism in the respiratory tract of a susceptible host may not necessarily lead to clinical signs and symptoms of infection, it remains as a potential source of spread to other patients (Ceyhan and Celik, 2011). Both the patients from FH showed signs of bronchopulmonary infection and were treated successfully with TZP. Four of the six patients in MH with positive endotracheal cultures were found to be colonised with \textit{C. meningosepticum}, and three of them were later discharged home. One of the colonised patients who died did not receive specific therapy and probably died of other causes. A dominance of male patients acquiring infections due to these organisms was seen in our study, which is

![Fig. 1. RAPD fingerprinting of \textit{C. meningosepticum} (lanes 1–7 and 9 from MH hospital) and \textit{C. indologenes} (lanes 8 and 10 from FH hospital) by PCR. The PCR products were separated by agarose gel electrophoresis and photographed under UV illumination. Lanes 1–7 correspond to isolates from patients with the same serial numbers, lane 8 corresponds to the isolate from patient 9, lane 9 corresponds to the isolate from patient 8, and lane 10 corresponds to the isolate from patient 10 in Table 1. Lane 11 has the 1 kb DNA ladder.](image-url)
similar to observations made in other studies (Chen et al., 2006; Tekerekoglu et al., 2003).

Infections due to *Chryseobacterium* spp. can be community or hospital acquired and occur as sporadic cases or outbreaks (Ceyhan and Celik, 2011; Sudharani et al., 2011). All infections in our patients were hospital acquired, and appeared between March and November 2012. In previous studies, between March and July 1975 and during July 2006 and January 2007, two and three outbreaks, respectively, were reported in paediatric patients from the USA and Turkey (Ceyhan et al., 2008; Hazuka et al., 2017). Not enough studies in the literature exist to conclude any association of seasonality with the occurrence of infections due to *Chryseobacterium* spp.

*Chryseobacterium* exhibit resistance to multiple antibiotics, especially β-lactams, due to the presence of class A ESBLs (Bellais et al., 2002; Chou et al., 2011). In addition, these organisms possess class B MBLs, rendering them inherently resistant to carbapenems (Chen et al., 2006; Matsumoto et al., 2012; Yum et al., 2010). The SENTRY Antimicrobial Surveillance Program showed that these organisms are variably resistant to other antibiotics (Kirby et al., 2004). Different resistant patterns against aminoglycosides, glycopeptides and quinolones have been reported from outbreaks (Di Pentima et al., 1998; Hsu et al., 2011). Because of the multidrug-resistant nature of the organisms, it is difficult to select optimal antibiotic regimens for treating infections. All 10 clinical isolates in our study were resistant to most of the antimicrobial agents generally prescribed for neonatal infections, especially β-lactams and aminoglycosides. The effectiveness of fluoroquinolones in the treatment of *C. meningosepticum* infections has been documented (Fraser and Jorgensen, 1997), as well as a successful response to therapy with SXT, vancomycin, rifampicin, clindamycin and erythromycin (Bloch et al., 1997; Chiu et al., 2000). Although all our strains were susceptible to TZP, variable susceptibility to this drug has been reported from other regions (Kirby et al., 2004). The majority of the published data are from the Asia-Pacific region, although chryseobacteria have also been isolated from North America, Latin America and Europe (Kirby et al., 2004). To the best of our knowledge, this is the first report of *Chryseobacterium* spp. infections from Kuwait and they appear to be emerging pathogens in this country. Extensive worldwide surveillance programmes are vital to formulate an appropriate antibiotic policy for infections caused by *Chryseobacterium* spp. (Marchaim et al., 2008).

**References**


