Antibiotic susceptibility of *Kingella kingae* isolates from respiratory carriers and patients with invasive infections

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The antimicrobial drug susceptibilities of 145 isolates of *Kingella kingae* to eight antibiotics were determined by the disc diffusion method. In addition, penicillin MICs were determined by the Etest. Study isolates included 37 from blood, 34 from the skeletal system and 74 from respiratory carriers. All isolates were β-lactamase negative and susceptible to erythromycin, gentamicin, chloramphenicol, tetracycline and ciprofloxacin. A single isolate exhibited resistance to trimethoprim–sulphamethoxazole, and 56 (38.6%) were resistant to clindamycin. The penicillin MIC$_{50}$ was 0.023 mg/L and the MIC$_{90}$ was 0.047 mg/L. The distribution of MIC values did not differ according to the site of isolation.

Introduction

For most of the three decades that have elapsed since the first characterization of *Kingella kingae*, this Gram-negative coccobacillus has been considered a rare cause of human infections. In recent years, increasing familiarity of microbiology laboratories with the identification of the organism and improved isolation techniques have resulted in the emergence of *K. kingae* as a common cause of bacteraemia and skeletal infections in young children. With improved culture methods, the annual incidence of invasive *K. kingae* infections detected among children younger than 24 months living in southern Israel was 27.4 per 100,000 and represented one-quarter of that of invasive *Haemophilus influenzae* type b found in the same population before the introduction of the vaccine. In a second study, *K. kingae* constituted the most common cause of septic arthritis in children younger than 2 years and was isolated in 48% of all culture-proven cases.

Despite the increasing recognition of *K. kingae* as an important cause of invasive infections, information on the antibiotic susceptibility profiles of the organism remains limited. This study was conducted to examine the prevalence of antimicrobial drug resistance in a large collection of *K. kingae* isolates.

Materials and methods

The study included 142 *K. kingae* strains isolated in different areas of Israel since the late 1980s, and three American Type Culture Collection (ATCC) strains. The source of isolates was blood (*n* = 37, including ATCC strains 23331 and 23332), skeletal system (*n* = 34) and respiratory tract of healthy carriers (*n* = 74, including ATCC strain 23330). Organisms were isolated in the late 1980s (*n* = 3), 1991 (*n* = 6), 1992 (*n* = 7), 1993 (*n* = 8), 1994 (*n* = 57), 1995 (*n* = 6), 1996 (*n* = 25), 1997 (*n* = 13), 1998 (*n* = 8) and 1999 (*n* = 9). Isolates were kept frozen at −70°C, and susceptibility tests were performed in batches.

Identification of the organism was based on the typical morphological and physiological characteristics of the species: Gram-negative bacteria that appeared as pairs or short chains of small bacilli with tapered ends; growth and production of β-haemolysis on trypticase soy agar with added 5% sheep haemoglobin (blood-agar medium) and failure to grow on MacConkey agar; positive oxidase and negative catalase; urease production; motility; and indole reactions and production of acid from glucose and maltose, but not from other sugars. Isolates were thawed and subcultured twice on blood-agar plates. Antibiotic susceptibilities to trimethoprim–sulphamethoxazole, erythromycin, clindamycin, tetracycline,
chloramphenicol, gentamicin and ciprofloxacin were determined by the disc diffusion method of Kirby and Bauer on Mueller–Hinton plates with 5% added sheep blood (Hy Laboratories, Rehovot, Israel). Antibiotic content of the discs (manufactured by Oxoid, Hampshire, UK) was as follows: trimethoprim–sulphamethoxazole 1.25 and 23.75 μg, respectively, erythromycin 15 μg, clindamycin 2 μg, tetracycline 30 μg, chloramphenicol 30 μg, gentamicin 10 μg and ciprofloxacin 5 μg. As there are no standardized criteria for determining antibiotic susceptibility of K. kingae, disc diffusion results were interpreted according to the NCCLS guidelines for Staphylococcus aureus.7

Presence of β-lactamase was determined by the nitrocefin method. Because different penicillins and cephalosporins are widely used for the treatment of systemic infections, penicillin was selected as the β-lactam group representative. The MIC of penicillin G was determined by the Etest (AB Biodisk, Solna, Sweden) on the same medium.

In addition, the influence of prolonged incubation of plates and atmosphere on susceptibility testing results was also studied. A random subset of 10 isolates was incubated with and without added 5% CO2, and zones of inhibition around discs and Etest strips were read after 24 and 48 h of incubation. Three capnophilic isolates (including ATCC 23332) were tested in the CO2-enriched atmosphere only.

Results

Inhibition zone diameters around the trimethoprim–sulphamethoxazole, erythromycin, clindamycin, tetracycline, chloramphenicol, gentamicin and ciprofloxacin discs are summarized in the Table. A single isolate exhibited resistance to trimethoprim–sulphamethoxazole (no inhibition around the disc) and 56 (38.6%) appeared to be resistant to clindamycin (inhibition zone diameter ≤14 mm). All isolates were β-lactamase negative. The distribution of penicillin MICs is shown in the Figure. The MIC values ranged between ≤0.002 and 0.064 mg/L. The MIC50 was 0.023 mg/L and the MIC90 was 0.047 mg/L. The distribution of MIC values did not differ between blood, skeletal system and respiratory tract isolates, or according to the date of isolation (data not shown). The MICs of penicillin for ATCC strains 23330, 23331 and 23332 were ≤0.002, 0.006 and 0.012 mg/L, respectively.

For the 10 random isolates in which the influence of different susceptibility test incubation conditions was investigated, neither incubation of plates for an additional 24 h nor addition of 5% CO2 were found to modify the results.

Discussion

Most of the literature on invasive K. kingae infection consists of single case reports or short series of patients with joint or bone infections or endocarditis. Information on the susceptibility of the organism to antimicrobial drugs is, therefore, fragmentary and the laboratory techniques used for testing are not described in detail in these reports.2,3 In a recently published study, Kugler et al.8 examined the susceptibility of Gram-negative organisms of the HACEK group to a wide array of β-lactam drugs, fluoroquinolones,

Table. Results of susceptibility testing of 145 K. kingae isolates by the disc diffusion method

<table>
<thead>
<tr>
<th>Drug</th>
<th>cut-off (mm)</th>
<th>mean ± S.D. (mm)</th>
<th>median (mm)</th>
<th>range (mm)</th>
<th>n (%) below cut-off value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimethoprim–sulphamethoxazole</td>
<td>≤10</td>
<td>48.3 ± 8.2</td>
<td>48</td>
<td>6–66</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>≤13</td>
<td>34.0 ± 5.1</td>
<td>34</td>
<td>26–50</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>≤14</td>
<td>15.7 ± 3.8</td>
<td>16</td>
<td>6–28</td>
<td>56 (38.6)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>≤14</td>
<td>31.0 ± 3.5</td>
<td>30</td>
<td>26–42</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>≤12</td>
<td>44.7 ± 5.0</td>
<td>44</td>
<td>34–58</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≤12</td>
<td>25.1 ± 3.2</td>
<td>26</td>
<td>16–42</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≤12</td>
<td>39.2 ± 4.5</td>
<td>40</td>
<td>30–52</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>
rifampicin and trimethoprim–sulphamethoxazole using the Etest. Unfortunately only three *K. kingae* isolates were included in the study and results of susceptibility testing of these organisms were combined with those obtained with *Kingella denitrificans*. Only three studies have systematically examined the antibiotic susceptibilities of *K. kingae*. In the 1980s, Claesson et al. tested 13 isolates from Sweden, Norway and Australia by the disc diffusion method, and Prère et al. studied three isolates of *K. kingae* from French children with septic arthritis using the agar-diffusion method. More recently, Jensen et al. studied 46 clinical isolates from Scandinavian countries by disc diffusion and a macrodilution MIC method. Sixteen isolates were derived from bone or joint exudates, 15 from blood, 11 from respiratory cultures and the remaining isolates were from cerebrospinal fluid, a corneal ulcer, peritoneal fluid and an unknown source. In these studies, all *K. kingae* isolates were found to be susceptible to penicillin and to a wide array of antimicrobial drugs, and resistant to trimethoprim.

Other reports of *K. kingae* isolates from patients with invasive infections, however, suggest that susceptibility of the organism to antibiotics is not uniform. Production of β-lactamase has been detected in an isolate from an adult AIDS patient with bacteraemia, and in three of five *K. kingae* isolates from children with bacteraemia or skeletal infections in Iceland. In addition, sporadic resistance to the trimethoprim–sulphamethoxazole combination and to ciprofloxacin has also been reported. The results of the present study, which comprises a large number of organisms isolated from individuals living in different geographical areas of Israel and collected over a 12 year period, show that antimicrobial susceptibility patterns of *K. kingae* are quite uniform and predictable. All test organisms were β-lactamase negative, exhibited low penicillin MICs and were susceptible to erythromycin, gentamicin, chloramphenicol, tetracycline and ciprofloxacin, and all but one were susceptible to trimethoprim–sulphamethoxazole. This finding is consistent with the clinical observation that invasive *K. kingae* infections respond promptly to antimicrobial therapy and especially to drugs such as β-lactams, macrolides or trimethoprim–sulphamethoxazole, which are often administered empirically to young children. On the other hand, a large fraction of isolates were found to be resistant to clindamycin. This observation is consistent with the resistance of *K. kingae* isolates to lincomycin, a related antimicrobial drug, reported in other studies.

Susceptibility of isolates to glycopeptides was not tested because this class of antimicrobial drugs is ineffective against Gram-negative organisms due to the large size of the molecule, which cannot pass through the outer membrane to reach the peptidoglycan target site. In fact, in a previous study we have used a vancomycin-containing selective medium to inhibit growth of Gram-positive flora and facilitate the isolation of *K. kingae* from pharyngeal cultures. In recent years, important paediatric pathogens such as *Streptococcus pneumoniae*, *H. influenzae* and *Moraxella catarrhalis* have developed resistance to β-lactams, macrolides or trimethoprim–sulphamethoxazole. These organisms are commonly carried in the respiratory tract of young children and are therefore frequently exposed to selective antibiotic pressure. A few years ago, it was demonstrated that *K. kingae* is also a component of the normal respiratory flora of young children. In a prospective study conducted among young attendees at a day-care centre in southern Israel, 109 of 624 (27.5%) throat cultures yielded *K. kingae* and 35 of 48 (72.9%) children yielded the organism at least once over an 11 month period. The results of the present study show that despite the frequent respiratory carriage of the organism, *K. kingae* remains susceptible to antimicrobial drugs that are commonly prescribed to young children with bacteraemia or skeletal infections.

**References**


