Antimicrobial Susceptibility and Epidemiology of a Worldwide Collection of Chryseobacterium spp.: Report from the SENTRY Antimicrobial Surveillance Program (1997–2001)

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Limited data are available on Chryseobacterium spp. leading to an evaluation of the patient demographics and susceptibility patterns for Chryseobacterium spp. collected in the first 5 years of the SENTRY Antimicrobial Surveillance Program (1997 to 2001). Fifty isolates (24 Chryseobacterium meningosepticum, 20 Chryseobacterium indologenes, two Chryseobacterium gleum, and 4 Chryseobacterium spp. isolates) were collected. The highest Chryseobacterium prevalence was detected among the elderly. The most active antimicrobials were the newer quinolones (garenoxacin, gatifloxacin, and levofloxacin, each with a MIC at which 90 percent of the isolates are inhibited [MIC90] of 1 µg/ml and 98.0% susceptibility) followed by rifampin (MIC90, 2 µg/ml and 85.7% susceptibility). Trimethoprim-sulfamethoxazole, ciprofloxacin, and piperacillin-tazobactam also showed reasonable activity; vancomycin showed poor potency.

Ubiquitous in nature, Chryseobacterium species are found primarily in soil and water. Environmental studies have revealed that these organisms can survive in chlorine-treated municipal water supplies, often colonizing sink basins and taps and creating potential reservoirs for infections inside hospital environments. Colonization of patients via contaminated medical devices involving fluids (respirators, intubation tubes, mist tents, humidifiers, incubators for newborns, ice chests, syringes, etc.) has been documented (8, 12). Contaminated surgically implanted devices such as intravascular catheters and prosthetic valves have also been reported (18). In other clinical settings, chryseobacteria have been described as etiological agents of meningitis, bacteremia, pneumonia, endocarditis, infections of skin and soft tissue, ocular infections, and other infections (6). Primarily opportunistic pathogens, they infect mainly newborns and immunocompromised hosts from all age groups.

Chryseobacterium meningosepticum is the most pathogenic member of the genus. As an agent of neonatal meningitis, it reportedly demonstrates mortality rates of up to 57% and produces severe postinfection sequelae including hydrocephalus, deafness, and developmental delay. C. meningosepticum is involved to a lesser extent in cases of pneumonia and bacterial sepsis in neonates and adults (6, 23).

Antimicrobial susceptibility data on Chryseobacterium spp. remain very limited, since this pathogen has been rarely isolated from clinical specimens. In addition, results of susceptibility testing vary when different methods are used. Results from disk diffusion methods may not be reliable, so broth reference quality microdilution tests should be performed when possible (1, 9).

Increasing concerns over the possible dissemination of plasmid-mediated carbapenem-hydrolyzing enzymes has led researchers to characterize the natural reservoir of chromosomally linked metallo-β-lactamases (MβLs) found in Chryseobacterium strains (3, 5). Multiple heterogeneous carbapenem-hydrolyzing enzymes have been reported in a single strain of C. meningosepticum. Investigations have also detected synergistic effects between cephalosporins and clavulanic acid, adding Ambler class A extended-spectrum β-lactamases to the growing list of resistance mechanisms found in chryseobacteria (4, 21).

Chryseobacterium spp. are known to exhibit resistance to aminoglycosides, tetracyclines, chloramphenicol, erythromycin, clindamycin, and teicoplanin (1, 6, 14). However, some fluoroquinolones have shown favorable results (9, 25). Minocycline has also shown good in vitro activity, while susceptibility to doxycycline and trimethoprim-sulfamethoxazole appears variable. Rifampin is usually active in vitro and has been used as part of a combination therapy to clear persistent infection (11). Vancomycin alone or in combination with other agents, including rifampin, has been used successfully in the treatment of meningitis in infants (20). However, the usefulness of vancomycin against Chryseobacterium spp. infections has more recently been questioned (6, 9). Thus, there is no optimal regimen for the treatment of Chryseobacterium spp. infections and antimicrobial therapy should be based on MIC from properly performed susceptibility tests.

The SENTRY Antimicrobial Surveillance Program is a world-wide study monitoring the susceptibility and resistance patterns of bacterial and fungal pathogens. This investigation was conducted by using results from over 119 sentinel hospitals and laboratories in North America, Latin America, Europe, and the Asia-Pacific region from the initial 5 years of the program (1997 to 2001). During this time period, over 155,811 clinical isolates were collected from several sites of infections, including bloodstream, the lower respiratory tract, skin and...
soft tissue, and the urinary tract. All *Chryseobacterium* spp. isolates collected during this period were evaluated. Fifty isolates unique to a given patient were identified and selected for detailed characterization and additional antimicrobial susceptibility testing.

Individual strains came from hospitalized patients in the Asia-Pacific region (16 strains from 7 centers), Europe (5 strains from 5 centers), Latin America (14 strains from 6 centers), and North America (15 strains from 15 centers). Each strain was tested against 47 antimicrobials, of which only selected agents were used for comparison of activity. Manufacturers for each agent supplied standard compounds that were placed in dry-form, validated microdilution panels (Trek Diagnosti Systems Inc., Cleveland, Ohio). All organisms were tested by broth microdilution methodology as specified by the NCCLS (16). Interpretation of quantitative MIC results was in accordance with NCCLS (16, 17) criteria. Selected gram-positive-active drugs were tested against *Chryseobacterium* spp. isolates, and the results were interpreted according to breakpoints approved for *Staphylococcus* or *Enterococcus* species by the NCCLS (17).

Multiple isolates of the same species isolated by the same medical center were selected for molecular typing. These isolates were characterized by macrorestriction analysis of chromosomal DNA by using pulsed-field gel electrophoresis (PFGE) as previously described (19, 24). Meropenem hydrolysis was evaluated for 21 randomly selected strains (11 *C. indologenes* and 10 *C. meningosepticum* isolates) by UV spectrophotometric assays (Pharmacia LKB Ultraphase II) in 1-cm light path cuvettes with readings recorded at 2-s intervals for 2 min at a wavelength of optimal absorbance (loss of substrate at 298 nm). The ability to induce MβLs was assessed by challenging mid-log growth cells with cefoxitin at 0.25 μg/ml for 2 h (26). The cells were harvested by centrifugation (12,000 × g), washed in the aforementioned buffer, andribolysed. The ribolysed cells were centrifuged at 12,000 × g to remove cellular debris. The activities of the enzymes were converted to a specific activity (micromolar substrate hydrolyzed/minute/milligram of protein) using ε2,500 as the extinction coefficient for meropenem as previously described (26).

The 50 isolates were collected from 33 medical centers in 16 countries. Only three medical centers sent multiple isolates of the same species. *Chryseobacterium* spp. represented only 0.27% (50 of 18,569) of the processed nonfermentative gram-negative bacilli and 0.03% (50 of 155,811) of all bacterial isolates collected by the SENTRY Program during the 5-year period evaluated (1997 to 2001). In addition, *Chryseobacterium* spp. represented only 0.10% of respiratory tract isolates and 0.03% of bloodstream infection isolates. The low frequency of this pathogen as a cause of infection is probably related to its reduced degree of pathogenicity. Some studies have shown that *Chryseobacterium* can be rapidly cleared by the immune system when introduced into the bloodstream or respiratory tract of a healthy animal or human host (6, 8, 22).

The most frequently isolated species was *C. meningosepticum* (24 isolates [48.0%]), followed by *C. indologenes* (20 isolates [40%]) and *C. gleum* (2 isolates [4.0%]). Four isolates (8.0%) could not be identified to the species level. All isolates were from hospitalized patients, and the vast majority was recovered from either lower respiratory tract (26 isolates [52.0%]) or blood cultures (23 isolates [46.0%]). The frequency of *Chryseobacterium* among respiratory tract specimens (0.10%; 26 of 25,657 specimens evaluated) was threefold higher than among positive blood cultures (0.03%; 23 of 74,236). Among isolates from bloodstream infections, 52.2% were *C. meningosepticum* and 30.4% were *C. indologenes*. Conversely, one-half of the isolates from the respiratory tract were *C. indologenes* and 42.3% were *C. meningosepticum*. One isolate (*C. meningosepticum*) was recovered from skin and/or soft tissue infection. The highest frequency of *Chryseobacterium* spp. infection occurred among the elderly (≥65 years old; 0.045%) and the lowest frequency occurred among children ≤5 years of age (0.016%).

The quinolones showed the highest potency and spectrum of activity against the overall collection of *Chryseobacterium* spp. Garenoxacin was the most active quinolone (MIC₅₀, 0.12 μg/ml; MIC₉₀, 1 μg/ml), and this new desfluoro compound inhibited 98.0% of isolates at the proposed susceptible breakpoint for other nonfermentative gram-negative bacilli (=2 μg/ml) (13). Gatifloxacin (MIC₅₀, 0.25 μg/ml) and levofloxacin (MIC₅₀, 0.5 μg/ml) also inhibited 98.0% of the isolates at susceptible breakpoints (17), and the rate of susceptibility to ciprofloxacin (MIC₅₀ 0.5 μg/ml) was significantly lower (80.0% overall). Trimethoprim-sulfamethoxazole showed reasonable activity (87.8% susceptibility overall). Among the β-lactams, the most active agents overall were piperacillin-tazobactam (MIC₅₀, 4 μg/ml; 80.0% susceptibility), piperacillin (MIC₅₀, 8 μg/ml; 74.0% susceptibility), and cefepime (MIC₅₀ 8 μg/ml; 62.0% susceptibility). The carbapenems (6 to 12% susceptible) and the aminoglycosides (8 to 14% susceptible) exhibited poor activity against these pathogens. MβL activity was demonstrated for all isolates evaluated. Activity ranged from 381 to 788 (average 529) μmol/min/mg of protein.

Many studies have shown that vancomycin has marginal in vitro activity against *Chryseobacterium* spp. isolates. In addition, some reports have documented the successful use of vancomycin to treat *C. meningosepticum* infections, and this antimicrobial agent has been recommended as a therapeutic choice (7, 8, 10). We tested vancomycin and several other antimicrobial agents used to treat gram-positive infections, and all of these compounds showed poor activity against the contemporary *Chryseobacterium* spp. isolates tested. For the vast majority of strains (87.8%) the vancomycin MIC was intermediate (8 to 16 μg/ml). Rifampin was active against the majority of strains (85.7% susceptibility overall) and can be used in combination to treat severe invasive infections (10, 11).

*C. meningosepticum* showed slightly lower susceptibility rates to the β-lactams (71.0% susceptibility to piperacillin-tazobactam) and to trimethoprim-sulfamethoxazole (79.2%) when compared to *C. indologenes* (90.0 and 95.0% susceptibility to piperacillin-tazobactam and trimethoprim-sulfamethoxazole, respectively). On the other hand, these two species had similar rates of susceptibility to fluoroquinolones and other antimicrobial agents (Table 1).

Interestingly, isolates from the Asia-Pacific region showed higher rates of resistance to the β-lactams (Table 2). The rate of susceptibility to piperacillin-tazobactam was only 50.0% in the Asia-Pacific region (16 isolates) compared to 100% in North America (15 isolates) and Europe (5 isolates) and 85.7% in Latin America (14 isolates). This higher rate of resistance...
may be related to the higher proportion of *C. meningosepticum* isolated in this region, since this species has shown higher rates of resistance than *C. indologenes*, especially for β-lactams and trimethoprim-sulfamethoxazole (Table 1). Susceptibility rates did not vary greatly among regions for other classes of antimicrobial agents (Table 2).

TABLE 1. Spectrum of selected antimicrobial agents against the most frequently isolated species

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th><em>C. meningosepticum (n = 24)</em></th>
<th><em>C. indologenes (n = 20)</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
</tr>
<tr>
<td>Gatifloxacin</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>Garenoxacin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.12</td>
<td>1</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1</td>
<td>&gt;2</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>2</td>
<td>&gt;2</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>8</td>
<td>64</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>8</td>
<td>128</td>
</tr>
<tr>
<td>Ticarcillin-clavulanate</td>
<td>128</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Cefepime</td>
<td>16</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>&gt;16</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Ceftiraxone</td>
<td>32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Imipenem</td>
<td>&gt;8</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Meropenem</td>
<td>&gt;8</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Amikacin</td>
<td>&gt;32</td>
<td>&gt;32</td>
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<tr>
<td>Gentamicin</td>
<td>&gt;8</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>&gt;16</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>&gt;16</td>
<td>&gt;16</td>
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<tr>
<td>Linezolid&lt;sup&gt;2&lt;/sup&gt;</td>
<td>8</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Teicoplanin&lt;sup&gt;e&lt;/sup&gt;</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Vancomycin&lt;sup&gt;e&lt;/sup&gt;</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Rifampin&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.5</td>
<td>2</td>
</tr>
</tbody>
</table>

<sup>a</sup> NCCLS MIC breakpoints for non-Enterobacteriaceae were categorically applied to Chryseobacterium spp.

<sup>b</sup> A susceptibility breakpoint of ≤2 μg/ml was applied (13).

<sup>c</sup> The NCCLS MIC breakpoint for Staphylococcus spp. was applied (17).

In intensive care units (ICU). All other tested isolates had distinct chromosomal DNA profiles. The finding of two small epidemic clusters involving elderly patients hospitalized in the ICU with lower respiratory tract infections raises concern for the possible occurrence of outbreaks in this patient population. *Chryseobacterium* spp. colonization in patients admitted to a respiratory-surgical ICU was reported more than two decades ago, and it was linked to the municipal water supply (8). In addition, its unusual resistance to antimicrobial agents directed to gram-negative bacteria allows for favorable environmental competition and subsequent colonization. Once patients become colonized, organisms can be transmitted to noncolonized patients primarily by hand carriage (8, 15).

In summary, the results of the evaluation of a world-wide
collection of unique Chryseobacterium strains indicate that (i) the newer quinolones (garenoxacin, gatifloxacin, and levofloxa-
cin) may represent the most appropriate antimicrobial agents
to treat infections caused by this pathogen, (ii) vancomycin and
other anti-graam-positive antimicrobial agents may not repre-
sent satisfactory therapeutic options for Chryseobacterium in-
fecions, (iii) the production of metallo-β-lactamase seems to be
constitutive among Chryseobacterium spp., and (iv) epi-
demic clusters may occur among elderly patients hospitalized
in ICUs. Extensive world-wide surveillance programs, such as
the SENTRY Program, are extremely important to guide em-
pirical antimicrobial therapy and clinical context of rarely iso-
lated pathogens.

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