Antimicrobial Susceptibility of Flavobacteria as Determined by Agar Dilution and Disk Diffusion Methods

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A total of 106 clinical isolates of flavobacteria, including 41 isolates of Flavobacterium meningosepticum, 59 of Flavobacterium indologenes, and 6 of Flavobacterium odoratum were collected from January 1992 to December 1995 from patients in Taiwan. The in vitro activities of antimicrobial agents were determined concomitantly by the standard agar dilution and disk diffusion methods. More than 90% of the flavobacterial isolates were resistant to cephalothin, cefotaxime, ceftiraxone, moxalactam, aztreonam, imipenem, aminoglycosides, erythromycin, and glycopeptides. The majority of F. meningosepticum isolates were susceptible to piperacillin and to minocycline but resistant to ceftazidime, with MICs at which 90% of the isolates are inhibited being 8, 4, and >128 μg/ml, respectively. Approximately half of the F. indologenes isolates were susceptible to piperacillin, cefoperazone, ceftazidime, and minocycline, with MICs at which 50% of the isolates are inhibited being 4, 16, 8, and 4 μg/ml, respectively. The majority of F. odoratum isolates were resistant to all the antimicrobial agents tested except minocycline, to which five of six isolates were susceptible. With least-squares regression analysis and error rate-bounded analysis methods, the following resistant and susceptible zone diameter breakpoints were established: ≤12 and ≥17 mm, respectively, for piperacillin against F. meningosepticum and F. indologenes; ≤13 and ≥18 mm, respectively, for ceftazidime against F. meningosepticum and F. indologenes, ≤17 and ≥21 mm, respectively, for ofloxacin against F. indologenes; ≤16 and ≥20 mm, respectively, for ciprofloxacin against F. meningosepticum. Valid breakpoints for the disk diffusion method could not be established for cefoperazone and ofloxacin against F. meningosepticum and for minocycline against F. meningosepticum and F. indologenes due to a poor correlation coefficient for the regression line or for cefoperazone and ciprofloxacin against F. indologenes due to the presence of remarkable error rates.

Flavobacteria are commonly found in soil, plants, foodstuffs, and water sources, including those in hospitals (32). Flavobacterium meningosepticum, the best-known species of flavobacteria, has been implicated in human diseases such as neonatal meningitis, bacteremia, endocarditis, pneumonia, wound sepsis, and keratitis (5, 16, 22, 24, 28–31, 34). Flavobacterium indologenes, previously designated Flavobacterium species CDC group IIb, is the most common human isolate and has been documented to associate with a variety of clinical infections (11–13, 25, 26). Flavobacterium odoratum has been reported to cause severe soft tissue infection (10). Most of these isolates are resistant to a variety of antimicrobial agents, including β-lactam antibiotics, aztreonam, aminoglycosides, and chloramphenicol (1, 4, 6, 14, 27, 33, 35). In vitro susceptibility of commonly used antimicrobial agents against flavobacteria, especially F. meningosepticum, has been reported; however, only a limited number of antimicrobial agents and flavobacterial isolates have been tested (1, 4, 11–15, 23, 27). Moreover, several investigators have demonstrated remarkable discrepancies between the susceptibility results obtained by disk diffusion methods and the dilution MICs of several antimicrobial agents (1, 4, 33). The resistant and susceptible zone diameter breakpoints (RSZDBs) adopted by these investigators were those of Enterobacteriaceae or Pseudomonas species, which might be not adequate for Flavobacterium species (20). Unfortunately, in Taiwan, most microbiology laboratories still use the disk diffusion method for the majority of clinically significant isolates, including flavobacteria, to provide clinical guidance in the selection of the appropriate antimicrobial agent(s).

In the present study, we report the antimicrobial susceptibilities of 106 isolates of flavobacteria (F. meningosepticum, F. indologenes, and F. odoratum) to 19 antimicrobial agents and describe an effort to determine the RSZDBs of antimicrobial agents tested against flavobacteria.

MATERIALS AND METHODS

Microorganisms. A total of 106 clinical isolates of flavobacteria recovered from 106 patients were collected from two major microbiological laboratories in Taiwan from January 1992 to December 1995. Forty-one isolates of F. meningosepticum were isolated from sputa (n = 26), blood (n = 9), pus (n = 2), urine (n = 1), ascites fluid (n = 1), pleural effusion (n = 1), and bronchial washing material (n = 1). Fifty-nine isolates of F. indologenes were isolated from blood (n = 22), sputa (n = 20), urine (n = 9), pus (n = 4), ascites fluid (n = 2), a central venous catheter tip (n = 1), and eye discharge (n = 1). Six isolates of F. odoratum were isolated from blood (n = 3), urine (n = 2), and pus (n = 1). All isolates were identified by conventional methods as described previously (25) and confirmed by means of the API 20NE system (BioMerieux, Marcy l’Etoile, France), and the GNI System (Vitek Systems, BioMerieux Vitek, Hazelwood, Mo.). All isolates were stored at −70°C in tryptic soy broth (Difco Laboratories, Detroit, Mich.) with 15% glycerol until testing.

Antimicrobial susceptibility testing. Antimicrobial susceptibilities of all isolates were determined concomitantly by the agar dilution and disk diffusion methods described in the National Committee for Clinical Laboratory Standards (NCCLS) documents (19, 20). For susceptibility testing by the agar dilution method, the following antimicrobial agents were obtained as standard reference powders of known potency for laboratory use: piperacillin and minocycline from...
TABLE 1. Susceptibility of 106 isolates of flavobacteria to 19 antimicrobial agents

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>F. meningosepticum&lt;sup&gt;a&lt;/sup&gt;</th>
<th>F. indologenes&lt;sup&gt;b&lt;/sup&gt;</th>
<th>F. odoratum&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC (μg/ml) Range</td>
<td>50%</td>
<td>90%</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>1–32</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>16–&gt;128</td>
<td>32</td>
<td>128</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>16–&gt;128</td>
<td>32</td>
<td>64</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>2–&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Moxalactam</td>
<td>8–&gt;128</td>
<td>64</td>
<td>128</td>
</tr>
<tr>
<td>Imipenem</td>
<td>1–128</td>
<td>64</td>
<td>128</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>8–&gt;128</td>
<td>64</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>1–8</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.5–16</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>8–&gt;128</td>
<td>16</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Minocycline</td>
<td>1–8</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>4–32</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>8–64</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>1–&gt;16</td>
<td>8</td>
<td>&gt;16</td>
</tr>
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<sup>a</sup> n = 41.  
<sup>b</sup> n = 59.  
<sup>c</sup> n = 6.

RESULTS

The correlations between the reference and experimental MICs for the quality control strains were acceptable for all of the antimicrobial agents tested, with a difference of not more than 1 dilution. Comparison of expected and observed zone diameters of inhibition for quality control strains revealed differences of only 1 to 2 mm for all antimicrobial agents tested. The MIC ranges and the MICs at which 50% (MIC<sub>50</sub>) and 90% (MIC<sub>90</sub>) of the isolates of flavobacteria were inhibited are given in Table 1. Cephalothin, cefotaxime, ceftriaxone, moxalactam, aztreonam, imipenem, aminoglycosides, erythromycin, and glycopeptides all had poor activities against most flavobacterial isolates tested. The majority of F. meningosepticum isolates were susceptible to piperacillin (MIC<sub>90</sub> 8 μg/ml) and minocycline (MIC<sub>90</sub> 4 μg/ml). Only half of the F. meningosepticum isolates were susceptible to ofloxacin (MIC<sub>50</sub> 2 μg/ml) and ciprofloxacin (MIC<sub>50</sub> 1 μg/ml). Piperacillin (MIC<sub>50</sub> 4 μg/ml; MIC<sub>90</sub> 128 μg/ml), cefoperazone (MIC<sub>50</sub> 16 μg/ml; MIC<sub>90</sub> >128 μg/ml), ceftazidime (MIC<sub>50</sub> 8 μg/ml; MIC<sub>90</sub> 32 μg/ml), minocycline (MIC<sub>50</sub> 4 μg/ml; MIC<sub>90</sub> 8 μg/ml), and trimethoprim (MIC<sub>50</sub> 4 μg/ml; MIC<sub>90</sub> 16 μg/ml) were shown to be active against approximately half of the F. indologenes isolates. Except for minocycline, all antimicrobial agents tested had poor activities against F. odoratum isolates.

In determining the RSZDIs, the regression lines were not established for cephalothin, cefotaxime, ceftriaxone, moxalactam, aztreonam, imipenem, gentamicin, netilmicin, amikacin, erythromycin, and vancomycin against any flavobacteria be-
cause a considerable majority (>90%) of the isolates were resistant or intermediate to these agents, and they were not established for any antimicrobial agents against *F. odoratum* isolates because only a small number of isolates were tested. Regarding the above-mentioned 11 agents for which new RSZDBs could not be established, when we applied the RSZDBs for *Pseudomonas* species or enterococci (vancomycin only) to *Flavobacterium* species (20), there were some VMEs for moxalactam against *F. indologenes* (two isolates) and for gentamicin against *F. meningosepticum* (five isolates). A significant number of errors were found for vancomycin against *F. meningosepticum* (32 isolates) and *F. indologenes* (47 isolates). The scatter plots, correlation coefficients, and regression lines for piperacillin, cefoperazone, ceftazidime, ofloxacin, ciprofloxacin, and minocycline against *F. meningosepticum* and *F. indologenes* are shown in Fig. 1 to 6, respectively.

**Piperacillin.** For piperacillin against *F. meningosepticum* and *F. indologenes* (Fig. 1), valid RSZDBs of ≤12 (resistant) and ≥17 mm (susceptible) were established, with 2% MaE rates and 3% MiE rates. Three *F. odoratum* isolates were susceptible to piperacillin, with inhibition zone diameters (IZDs) of 15, 16, and 19 mm. The remaining three isolates had no inhibition zones.

**Cefoperazone.** The correlation coefficients for regression lines from cefoperazone against *F. meningosepticum* (Fig. 2A) and *F. indologenes* (Fig. 2B) were −0.32 and −0.88, respectively. However, valid RSZDBs for cefoperazone against *F. indologenes* could not be established because there were high error rates (5% VME rates and 12% MiE rates for RSZDBs of ≤15 and ≥20 mm, respectively). Of the five resistant *F. odoratum* isolates, three had no inhibition zone and two had IZDs of 10 and 12 mm. One *F. odoratum* isolate susceptible to cefoperazone had an IZD of 16 mm.

**Ceftazidime.** Valid RSZDBs of ≤13 and ≥18 mm were established for ceftazidime against *F. meningosepticum* and *F. indologenes* (Fig. 3), with 9% MiE rates. Three resistant *F. odoratum* isolates for which MICs were >128 μg/ml had no inhibition zones; two for which MICs were 64 μg/ml had IZDs of 9 and 12 mm, respectively; and one susceptible isolate for which the MIC was 4 μg/ml had an IZD of 19 mm.

**Ofloxacin.** The correlation coefficients for regression lines from ofloxacin against *F. meningosepticum* (Fig. 4A) and *F. indologenes* (Fig. 4B) were −0.67 and −0.88, respectively. Valid RSZDBs of ≤17 and ≥21 mm were established for ofloxacin against *F. indologenes*, with 2% MaE rates and 12% MiE rates. Four *F. odoratum* isolates fell into the resistant category and had no inhibition zone, and two isolates in the susceptible category had IZDs of 16 mm.

**Ciprofloxacin.** For ciprofloxacin against *F. meningosepticum* (Fig. 5A), valid RSZDBs of ≤16 and ≥20 mm were estab-
lished, with 15% MiE rates. As for *F. indologenes* isolates (Fig. 5B), valid RSZDBs could not be established, because there were high error rates (2% MaE rates and 22% MiE rates for RSZDBs of $\leq$18 and $\geq$23 mm, respectively). No inhibition zone was found for four resistant *F. odoratum* isolates, and two isolates (one in the susceptible and one in the intermediate category) had IZDs of 19 mm.

**Minocycline.** For minocycline against *F. meningosepticum* and *F. indologenes* isolates, a regression line was calculated, with poor correlation (Fig. 6). For *F. odoratum* isolates, five susceptible strains had IZDs of 19 to 25 mm and one in the intermediate category had an IZD of 18 mm.

**DISCUSSION**

Although *Flavobacterium* species have been reported to be recovered from only $<2\%$ of clinical materials, clinical diseases caused by these organisms have been documented (32). Besides *F. meningosepticum*, the best-known human pathogen, strains of *F. indologenes* and *F. odoratum* have also been reported to be associated with invasive diseases in humans (5, 10–13, 24–26). Clinical experience in choosing optimal therapeutic regimens for treating flavobacterial infections is limited due not only to the multiresistant nature of these organisms but also to the small number of infected patients reported, especially patients infected with *Flavobacterium* species other than *F. meningosepticum* (8, 10–13, 25). Vancomycin has been previously recommended as the drug of choice for the treatment of infantile meningitis due to *F. meningosepticum* (7, 8, 21). However, our results as well as those of others show that the vancomycin MICs for nearly all of the *F. meningosepticum* isolates were lower than those of *F. indologenes*.
isolates and other Flavobacterium species are high (≥8 μg/ml). At least, this finding indicates that vancomycin should not be considered the drug of choice for treating flavobacterial infections, especially meningitis (1, 23). Moreover, the discrepancies between the in vivo and in vitro susceptibilities of vancomycin may be due to limited clinical experience as well as to inappropriate MIC breakpoints (the RSZDBs of this drug recommended by the NCCLS are originally derived from gram-positive organisms and may not be appropriate for flavobacteria). Teicoplanin, a new glycopeptide, had MICs two- to fourfold higher than those of vancomycin for the isolates of flavobacteria tested, causing it to be contraindicated clinically for the treatment of these infections.

The role of quinolones, especially ciprofloxacin, in the treatment of flavobacterial infections has been heralded due to their low MICs for flavobacterial isolates as well as to their reported successes in clinical treatment (10, 14, 15, 23). However, this study demonstrates that the activities of ofloxacin and ciprofloxacin against flavobacteria, especially isolates of F. indologenes and F. odoratum, are limited. Respecting MIC, piperacillin appears to be promising for the treatment of infections caused by F. meningosepticum and F. indologenes. However, our findings are not consistent with those reported by other investigators (4, 15). The activity of erythromycin against F. meningosepticum has been controversial (1, 2, 17). In this study, erythromycin had poor activity against all flavobacteria tested. This resistance is thought to have evolved in response to differing uses of antibiotics at the community and institutional levels (1). In Taiwan, the widespread use of erythromycin and other macrolides may contribute to poor activity against isolates of flavobacteria as well as other bacteria (9). Among the antimicrobial agents tested, minocycline was the most active against all three Flavobacterium species. Nevertheless, the use of minocycline to treat invasive infections caused by flavobacteria needs further evaluation.

The lack of in vitro activity of a variety of antimicrobial agents commonly prescribed for the treatment of infections caused by gram-negative bacteria against isolates of Flavobacterium species necessitates routine susceptibility testing for all clinically significant strains of these organisms. Most clinical microbiology laboratories, including those in Taiwan, still perform susceptibility testing by disk diffusion rather than by dilution to provide clinical guidance on the optimal choices of antimicrobial agents. However, several investigators have found that the disk diffusion test does not reliably predict antimicrobial susceptibility of flavobacteria (1, 33). Despite the fact that these investigators tested only a limited number of flavobacterium isolates, they demonstrated a remarkably poor correlation between the results of the two methods for clindamycin, erythromycin, cefoxitin, and vancomycin. However, the majority of the antimicrobial agents that were tested are no longer the drugs of choice for treating invasive infections due to multidrug-resistant gram-negative bacteria. To our knowledge, the present study is the first study on determining the RSZDBs of antimicrobial agents against Flavobacterium species.

In the present study, the regression lines were not calculated for the agents with poor activities, including cephalothin, cefotaxime, ceftriaxone, moxalactam, aztreonam, imipenem, gentamicin, netilmicin, amikacin, erythromycin, and vancomycin. Inadequate correlation coefficients were found for cefoperazone and ofloxacin against F. meningosepticum (r = 0.32 and –0.67, respectively) and for minocycline against F. meningosepticum and F. indologenes (r = –0.74). Although the correlation coefficients for cefoperazone and ciprofloxacin against F. indologenes were adequate (r = –0.88 and –0.90, respectively), the existence of considerable error rates made the establishment of valid RSZDBs impossible. Thus, the poor correlation between the two methods extends to cefoperazone and ofloxacin against F. meningosepticum, to minocycline against F. meningosepticum and F. indologenes, and to cefoperazone and ciprofloxacin against F. indologenes. Therefore, for these drug-microorganism combinations, the dilution method is preferable to the disk diffusion method for susceptibility testing. Good correlation and acceptable error rates were observed for piperacillin and ceftazidime against F. meningosepticum and F. indologenes, for ofloxacin against F. indologenes, and for ciprofloxacin against F. meningosepticum.

In summary, we recommend RSZDBs of ≤12 and ≤17 mm for piperacillin against F. meningosepticum and F. indologenes, ≤15 and ≥18 mm for ceftazidime against F. meningosepticum and F. indologenes, ≤17 and ≥21 mm for ofloxacin against F. indologenes, and ≤16 and ≥20 mm for ciprofloxacin against F. meningosepticum. For antimicrobial agents for which valid RSZDBs have not been established, direct measurement of MICs rather than use of the disk diffusion method is mandatory.

REFERENCES


