Moellerella wisconsensis: identification, natural antibiotic susceptibility and its dependency on the medium applied

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Abstract

The present study establishes a data compilation on biochemical features and natural antibiotic susceptibilities of Moellerella wisconsensis strains. 17 moellerellae isolated from humans (n = 11), food (n = 5) and water (n = 1) were tested. Identification was carried out using two commercially available systems and conventional tests. MIC determinations of 74 antibiotics were performed applying a microdilution procedure in Cation-adjusted Mueller Hinton broth and IsoSensitest broth. M. wisconsensis was naturally sensitive to doxycycline, minocycline, all tested aminoglycosides, numerous β-lactams, all fluoroquinolones, folate-pathway inhibitors, chloramphenicol and nitrofurantoin. Natural resistance was found with oxacillin, penicillin G, all tested macrolides, lincomycin, streptogramins, ketolides, glycopeptides, fusidic acid, linezolid and rifampicin. Medium-dependent differences in susceptibility affecting clinical assessment criteria were seen with tetracycline, clindamycin and fosfomycin. From the data of the present study it is possible that some moellerellae are misidentified as Klebsiella pneumoniae subsp. ozaenae. © 2003 Elsevier Science Inc. All rights reserved.

1. Introduction

Within the Enterobacteriaceae, strains of the genus Moellerella represent a monophyletic species, named M. wisconsensis, which was first recognized as Enteric Group 46 in 1980 from cultures that had been sent to the Centers for Disease Control (CDC, Atlanta, GA) in the United States. In 1984, the name M. wisconsensis was proposed for Enteric Group 46 (Hickman-Brenner et al., 1984); the specific epithet 'wisconsensis' was coined because the majority of the strains examined had been isolated from clinical specimens in the Wisconsin area (Hickman-Brenner et al., 1984).

M. wisconsensis strains resemble in all respects well-established Enterobacteriaceae, representing Gram-negative, facultative anaerobic, nitrate-reducing and Oxidase-negative rods producing enterobacterial common antigen (Hickman-Brenner, 1984; Ramia et al., 1982). Taxonomically, M. wisconsensis is 23–32% related to Providencia spp. and 18–26% related to Proteus spp.; to other Enterobacteriaceae, its relatedness is lower than 19% (Hickman-Brenner et al., 1984).

After its initial isolation in the United States, strains of M. wisconsensis have been isolated in several European countries, i.e., the Czech Republic and Slovakia (Cabadjova & Kudrna, 1988), France (Ohanessian, 1987; Richard, 1989; Wallet et al., 1994), Germany (Wittke et al., 1988), and the UK (Marshall et al., 1986). M. wisconsensis seems to be distributed widely in nature and several strains have been isolated from animals (Bangert et al., 1988), water (Aldová, 1992; Hickman-Brenner et al., 1984) and human food (Aldová, 1992). However, the majority of moellerellae has been isolated from human clinical specimens, in particular feces (Hickman-Brenner et al., 1984; Marshall et al., 1986), and there is evidence that M. wisconsensis is associated with human diarrhea (Brenner, 1992). Unfortunately, there is only one study on the prevalence of Moellerella in stool specimens (Marshall et al., 1986) and research in virulence factors of Moellerella was never performed. Apart from human stools, M. wisconsensis has also been isolated from non-fecal specimens, i.e., gall bladders (Ohanessian et al., 1987; Wittke et al., 1985), bronchial aspirates (Aldová, 1992; Wallet et al., 1994) and one peritoneum exsudate (Aldová, 1992), indicating clinical significance.

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Table 1  
Moellerella strains of this study

<table>
<thead>
<tr>
<th>Strain (Additional designations)</th>
<th>Origin</th>
<th>Country</th>
</tr>
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<tr>
<td>CCUG 18042¹</td>
<td>Human gallbladder, 71-year-old male with acute cholecystitis</td>
<td>Germany</td>
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<tr>
<td>CCUG 18768 (ATCC 35618, CDC 1826-79)²</td>
<td>Human feces, patient with diarrhea</td>
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<td>CCUG 18773 (ATCC 35619, CDC 2552-77)²</td>
<td>Human feces, 62-year-old male with gastroenteritis</td>
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<td>CCUG 18774 (ATCC 35620, CDC 2897-78)²</td>
<td>Human feces, 38-year-old female</td>
<td>Wisconsin, USA</td>
</tr>
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<td>CCUG 18775 (ATCC 35621, CDC 3065-75)²</td>
<td>Human feces, 5-year-old female with diarrhea</td>
<td>Wisconsin, USA</td>
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<tr>
<td>CCUG 29901 (Aldová 27223)</td>
<td>Foam of ham</td>
<td>Czech Republic</td>
</tr>
<tr>
<td>CCUG 29902 (Aldová 27224)</td>
<td>Sausage filling</td>
<td>Czech Republic</td>
</tr>
<tr>
<td>CCUG 29903 (Aldová 27472)</td>
<td>Sausage, fowl</td>
<td>Czech Republic</td>
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<tr>
<td>CCUG 29904 (Aldová 27625)</td>
<td>Smear</td>
<td>Czech Republic</td>
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<td>CCUG 29905 (Aldová 28037)</td>
<td>Human peritoneum, vesicular exsudate</td>
<td>Czech Republic</td>
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<tr>
<td>CCUG 29906 (Aldová 28518)</td>
<td>Water</td>
<td>Czech Republic</td>
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<tr>
<td>CCUG 29907 (Aldová 28536)</td>
<td>Sausage</td>
<td>Czech Republic</td>
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<td>CCUG 29908 (Aldová 28663)</td>
<td>Human sputum, chronic bronchitis</td>
<td>Czech Republic</td>
</tr>
<tr>
<td>CCUG 29958 (Aleksic H1297/84)</td>
<td>Human wound</td>
<td>Germany</td>
</tr>
<tr>
<td>ATCC 35017T</td>
<td>Human feces, 16-year-old female</td>
<td>Wisconsin, USA</td>
</tr>
<tr>
<td>66-8 (Sr 1 21969/81)</td>
<td>Human clinical specimen</td>
<td>Switzerland</td>
</tr>
<tr>
<td>M-1</td>
<td>Human clinical specimen</td>
<td>Germany</td>
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</tbody>
</table>

¹ Strain published by Wittke et al. (Wittke et al., 1985)  
² Strain published by Hickmann et al. (Hickmann et al., 1984)

There is evidence that in the last decades strains of Moellerella have been perceived or misidentified. M. wisconsensis is indistinguishable from Escherichia coli on MacConkey and eosin-methylene blue agars (Hickman-Brenner et al., 1984) and may therefore be missed in clinical specimens. In addition, several strains of Moellerella resemble phenotypically some klebsiellae, in particular certain strains of Klebsiella pneumoniae subsp. ozaenae (see below).

The main scope of this research was to create a data compilation of the natural susceptibility of M. wisconsensis strains originating from different sources to a wide range of antibiotics. Antibiotic susceptibility data on Moellerella include sole strains and/or few antimicrobial agents. Natural antibiotic susceptibility patterns of Moellerella have not been published. In addition, we were interested in probable medium-dependencies in antibiotic susceptibility testing. Finally, this study was performed to evaluate two commercially available identification systems that contain M. wisconsensis in their data bank.

2. Materials and methods

2.1. Bacterial strains

A total of 17 moellerellae isolated from humans (n = 11), food (n = 5) and water (n = 1) was examined. The majority of the strains tested derived from the Culture Collection of the University of Göteborg in Sweden. M. wisconsensis 66-8 (Sr 1 21969/81), isolated from a clinical sample in 1997, was obtained from Alexander von Graevenitz (University of Zürich, Switzerland). M. wisconsensis M-1, isolated from a specimen of a outpatient in South Germany in 1998, was sent as ‘Klebsiella pneumoniae subsp. ozaenae’ by G. Stempfel (Gärtner Laboratories, Weingarten, Germany). M. wisconsensis ATCC 35017T and Escherichia coli ATCC 25922 derived from the German culture collection of microorganisms and cell cultures in Braunschweig (DSMZ). These strains and M. wisconsensis ATCC 35618 served as controls for antibiotic susceptibility testing. An overview on the origin of the strains examined is shown in Table 1.

2.2. Identification

The strains were identified with two commercial identification systems for Enterobacteriaceae and related bacteria, api20E (BioMérieux, Marcyl’Etoile, France) and Micro-Naut-E (Merlin-Diagnostika, Bornheim, Germany), according to the instructions of the manufacturers. M. wisconsensis is included in the data bank of both systems. To secure a reliable identification (see results), conventional sugar fermentation tests were performed in tubes with 0.5% salicin in Salcin broth (Fluka Chemie, Buchs, Switzerland) and in plates on bromcresol-purple-agar (Difco Laboratories, Detroit, MI, USA), supplemented to 0.5% with raffinose, L-rhamnose, trehalose and D-xylose (all Fluka Chemie). In addition, assimilation of citrate was tested using Simmons citrate agar (Oxoid, Basingstoke, UK); motility tests were performed in tubed media according to the instructions of the manufacturer (BD Biosciences, Le Pont de Claix, France). All tests were incubated at 37°C and read after 24 h. Tube and plate tests were also read after 48 h (all tests) and 7 days (fermentation of rhamnose and xylose). Oxidase tests were performed with cytochrome-oxidase test strips (Merck, Darmstadt, Germany).
2.3. Antibiotics and antibiotic susceptibility testing

Antibiotic susceptibility was tested with a microdilution procedure in cation-adjusted Mueller Hinton broth (CAMHB; Difco Laboratories) and in IsoSensitest broth (Oxoid, Basingstoke, UK). After inoculation of antibiotic-containing microtiter plates (Merlin-Diagnostika) with 100 µL of bacterial suspension, 3 × 10^8 – 7 × 10^5 cfu/mL, and incubation for 20 h at 37°C, MIC values were determined with a photometer for microtiter plates (Lab-systems Multiscan Multisoft, Helsinki, Finland). MICs were evaluated with EXCEL (Microsoft). All antibiotics were kindly provided by the manufacturers to Merlin-Diagnostika who produced the antibiotic-containing plates.

2.4. Evaluation of natural antibiotic susceptibility

Plotting the MIC of a particular antibiotic for one species against the number of strains found with the respective MIC usually results in a bimodal distribution. Generally, one peak with relatively low MICs represents the natural population and one peak with higher MICs represents the strains with acquired (secondary) resistance. Analysis of the MIC distribution of all strains of one species for each antibiotic permitted determination of the biological thresholds, which limit the natural population at high MICs but not those strains with secondary resistance. Whether the MIC values of the natural population were above or below the breakpoints of the standards, which assess the clinical susceptibility, was investigated. When the natural population was sensitive or intermediate according to the cited standard, it was described as naturally sensitive or naturally intermediate, respectively. When the natural population was clinically resistant, it was described as naturally resistant (intrinsically) resistant. The method has been described in detail previously (Stock & Wiedemann, 1998b, 1999a, 2000). In the present study, breakpoints according to the German standard (Deutsches Institut für Normung, DIN) were applied (DIN, 1998). For antibiotics for which DIN clinical assessment criteria do not exist, breakpoints according to French (Comité de l’Antibiogramme de la Société Française de Microbiologie Communiqué, 1998), Swedish (Olsson-Liljequist et al., 1997) or American standards (National Committee for Clinical Laboratory Standards, NCCLS), valid for Enterobacteriaceae (NCCLS, 2000a), Neisseria gonorrhoeae (NCCLS, 2000d) and staphylococci (NCCLS, 2000c), were employed. Breakpoints for ketolides were applied as proposed by Stone et al. (for ABT-773; Stone et al., 2000) and Soussy et al. (for telithromycin; Soussy et al., 2000). Linezolid breakpoints were applied according to the proposal of the European Committee on Antimicrobial Susceptibility Testing, EUCAST (EUCAST, 2001). Breakpoints for apramycin, ribostamycin and lvidomycin A were used as published recently (Troxler et al., 2000). The data obtained were compared with the respective data applying NCCLS assessment criteria (NCCLS, 2000a, 2000b, 2000c, 2000d).

3. Results

3.1. Identification

All strains examined were unambiguously identified as M. wisconsensis by the Api20E system. Five strains (including M. wisconsensis M-1, originally labelled as K. pneumoniae subsp. ozaenae) showed unusual biochemical properties in the MCN-E system and were rhamnose- and xylose-positive (these strains gave rhamnose-negative reactions with Api20E). They were identified as K. pneumoniae subsp. ozaenae with sufficient or questionable suitability by the MCN-E data base. Applying conventional plate tests, the respective strains were rhamnose- and xylose-negative after an 24 h- and 48 h-incubation, but weakly positive after 7 days (Table 2).

In contrast to the MCN-E system, moellerellae able to ferment rhamnose and xylose can be separated from K. pneumoniae subsp. ozaenae with the Api20E system by arabinose and mannitol fermentation tests (Table 2). Additional discriminating reactions between M. wisconsensis and K. pneumoniae subsp. ozaenae were fermentation tests of cellobiose, salicin and trehalose (Table 2). The latter tests are not included in the pannels of Api20E and MCN-E.

3.2. Natural antibiotic susceptibility

The antibiotic susceptibility patterns of M. wisconsensis are shown in Table 3; its natural sensitivities and resistances are summarized in Table 4.

Strains of Moellerella were naturally sensitive to doxycycline, minocycline, all tested aminoglycosides, all β-lactams except oxacillin and benzylpenicillin, all fluoroquinolones, folate-pathway inhibitors, chloramphenicol and nitrofurantoin. Natural resistance was found with oxacillin, benzylpenicillin (to the latter some strains were of intermediate susceptibility), all tested macrolides including azithromycin, lincomycin, streptogramins, ketolides, glycopeptides, fusidic acid, linezolid and rifampicin.

3.3. Medium dependency

Medium dependencies in susceptibility testing results were seen with several antibiotics, i.e., tetracyclines, aminoglycosides, quinolones, macrolides, lincosamides and fosfomycin, but the medium-associated influence on the MICs of most antibiotics was generally small. Major medium dependencies were seen with fosfomycin, macrolides, clindamycin and tetracycline: The MICs of these antibiotics in IsoSensitest broth were three (fosfomycin) or two (macrolides, clindamycin and tetracycline) doubling dilution steps higher than in CAMHB (Table 3). In the case of fosfomycin, tetracycline and clindamycin, these differences affected the respective clinical assessment criteria (Tables 3 and 4).
3.4. Quality assurance

Within the permissible error, the MIC data of all antibiotics in both media were reproducible for *M. wisconsensis* ATCC 35017T, *M. wisconsensis* ATCC 35618 and *E. coli* ATCC 25922 (threecold determinations). Although there were medium-dependent differences in the MICs, the MIC values for *E. coli* ATCC 25922 in IsoSensitest broth and CAMHB were within the control limits for susceptibility testing according to DIN and NCCLS criteria (data not shown).

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### Table 2

Biochemical features of the *M. wisconsensis* strains tested

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<tr>
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<tr>
<td></td>
<td></td>
<td>ATCC 35017T</td>
<td>All strains (n=17)</td>
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<tr>
<td>Amino acid deaminase</td>
<td>Api20E, MCN-E</td>
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<td>0</td>
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<td>Arginine dihydrose</td>
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<td>Cytochromoxidase</td>
<td>Test strip</td>
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<td>β-Galactosidase</td>
<td>Api20E, MCN-E</td>
<td>+ 100</td>
<td>78 (24h) 100 (48h)</td>
<td>90</td>
<td>80</td>
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<td>Gelatinase</td>
<td>Api20E</td>
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<td>NT</td>
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<td>β-Glucuronidase</td>
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<td>NT</td>
<td>NT</td>
<td>NT</td>
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<tr>
<td>β-Glucosidase</td>
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<td>H₂S production</td>
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<td>0</td>
<td>0</td>
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<td>Lysine decarboxylase</td>
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<td>NO₃ → NO₂⁻</td>
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<td>MCN-E</td>
<td>89 (24h)</td>
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<td>80</td>
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<td>Ornithine decarboxylase</td>
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<td>Urease</td>
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<td>Voges Proskauer Test</td>
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<td>NT</td>
<td>NT</td>
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<td>β-Xylosidase</td>
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<td>− 0</td>
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<td>Assimilation of</td>
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<tr>
<td>- citrate</td>
<td>Api20E, MCN-E</td>
<td>+ 88</td>
<td>67 (24h) 100 (48h)</td>
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<td>30</td>
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<td>Fermentation of</td>
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<td>- adonitol</td>
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<td>NT</td>
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<td>100 (24h)</td>
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<td>- sucrose</td>
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<td>- trehalose</td>
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<td>- D-xylose</td>
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</table>
| a Percentages of positive reactions are given. The results for *Moellerella* are contrasted to the respective data of Hickmann-Brenner et al. (Hickmann-Brenner et al. 1984) and Farmer (Farmer, 1995) and to the data for *K. pneumoniae* subsp. *ozaenae* (Farmer, 1995). Key discriminating reactions between *M. wisconsensis* and *K. pneumoniae* subsp. *ozaenae* are shown in bold print. It should be noted that the results obtained by Farmer were read after an 48h incubation. Cleavage of 1 ortho-nitrophenyl-β-galactopyranoside (ONPG); 2 para-nitrophenyl-β-glucuronide (PGUR); 3 ortho-nitrophenyl-β-D-xylopyranoside (ONPX); 4 hydrolysis of esculin; 5 indole production; 6 weakly positive; 7 Simmons citrate; NT, not tested.

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Table 3

Antibiotic susceptibility of *M. wisconsensis*

<table>
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<tr>
<th>Antibiotic (Standard-Reference)</th>
<th>Concentrations examined [mg/L]</th>
<th>Medium</th>
<th>Number of Strains with MIC [mg/L] of</th>
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<th>0.03</th>
<th>0.06</th>
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</tr>
<tr>
<td>Tetracycline (DIN, 1998)</td>
<td>0.03–64</td>
<td>CAMHB</td>
<td>7</td>
<td>4</td>
<td></td>
<td></td>
<td>6</td>
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<tr>
<td>Doxycycline (DIN, 1998)</td>
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<td>CAMHB</td>
<td>3</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>1</td>
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<tr>
<td>Minocycline (DIN, 1998)</td>
<td>0.03–64</td>
<td>CAMHB</td>
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<td>2</td>
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4. Discussion

Within the variety of Enterobacteriaceae, there are numerous species found predominantly or exclusively in human clinical specimens, without or with only poor knowledge of their incidence and clinical significance. Such taxa include *Cedecea* spp., *Leminorella* spp., *M. wisconsensis*, *Tatumella ptyseos* and *Yokenella regensburgei* (Brenner et al., 1992). In 2000, it was shown that *Leminorella* spp. that had been formerly recovered from stool and urine only, with no clinical correlates, should be considered as *emerging nosocomial pathogens*, capable of causing numerous clinical syndromes, e.g., urinary tract and surgical site infections (Blekher et al., 2000). Although there is no evidence that such a classification might be justified with regard to *M. wisconsensis*, it is likely that it is involved in the pathogenesis of human disease. To obtain more profound information on the incidence of obviously unusual pathogens, it is

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* The number of strains for the corresponding MIC value is cited. A number in the lowest concentration of the antibiotic represents the maximal MIC value at this concentration (MIC = cmin MIC ≤ cmax). An MIC value higher than the highest concentration tested is cited in the subsequent higher concentration step. MIC values in shaded areas indicate the clinically intermediate area according to the standard applied. A black thick line indicates the breakpoint between the clinically sensitive and clinically resistant strains, if the interpretation ‘intermediate’ does not exist. Abbreviations: DIN, Deutsches Institut für Normung e.V.; EUCAST, European Committee on Antimicrobial Susceptibility Testing; NCCLS, National Committee for Clinical Laboratory Standards; SFM, Comité de l’Antibiogramme de la Société Française de Microbiologie Communiqué; O.-L., Olsson-Liljequist et al.; CAMHB, cation-adjusted Mueller Hinton broth; ISOB, IsoSensitest broth.
important that an easy and reliable identification of respective strains is applicable. Commercially available identification systems are mainly addressed to the identification of well-known organisms with high medical importance and consist of a relative limited number of key tests useful to identify significant representatives within a particular group. Incomplete or sparse databases will tend to give wrong identification results. In regard to Moellerella, the data bases of both commercial identification systems applied in the present study contained M. wisconsensis, but 5 of 17 strains were misidentified with the Micronaut-E system (see results and Table 2). Interestingly, misidentification of weakly rhamnose- and xylose-positive moellerellae was obviously due to the high sensitivity of the Micronaut-E system, combined with a sparse data base (Table 2). In contrast to Micronaut-E, the api20E system was able to separate M. wisconsensis from K. pneumoniae subsp. ozaenae, mainly based on fermentation tests of arabinose and mannitol. However, because several moellerellae are known to produce acid from mannitol at prolonged incubation times (Hickman-Brenner et al., 1984, Farmer, 1995), it would be advisable to perform additional sugar fermentation tests to secure a reliable identification (Table 2).

According to the data, it is possible that in the past some moellerellae may have been identified as K. pneumoniae subsp. ozaenae. Unfortunately, the exact origin and the former identification procedure of M. wisconsensis M-1, sent to our laboratory as K. pneumoniae subsp. ozaenae, is unknown. K. pneumoniae subsp. ozaenae is known to be a colonizer of the oral and nasopharyngeal mucosa and is the classic agent of an atrophic rhinitis called ozena, but has been implicated in several other respiratory and non-respiratory diseases (Goldstein et al., 1978; Tang & Chen, 1994; Tang et al., 1997). Interestingly, M. wisconsensis has also been isolated from bronchial aspirates (Aldová, 1992; Wалlet et al., 1994).

Apart from the characterization of metabolic features leading to reliable identifications, the main scope of the present study was to create a data compilation on the natural antibiotic susceptibility of M. wisconsensis. Antibiotic susceptibility patterns showed that moellerellae share several natural phenotypes with other Enterobacteriaceae and there were no phenotypic features characteristic for the species. The natural resistance of M. wisconsensis to benzylpenicillin, oxacillin, macrolides, lincosamides, streptogramins, glycopeptides, rifampicin and fusidic acid can be found in several Enterobacteiraceae and is predominantly due to drug exclusion by the cell envelope (for an overview see Nikaido,

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Medium</th>
<th>Naturally sensitive</th>
<th>Naturally intermediate</th>
<th>Naturally resistant</th>
<th>Assessment according to NCCLS Breakpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracyclines</td>
<td>Tetracycline</td>
<td>CAMHB</td>
<td></td>
<td></td>
<td>Naturally sensitive in both media</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Doxycycline, Minocycline</td>
<td>CAMHB, ISOB</td>
<td></td>
<td></td>
<td>Identical assessment</td>
</tr>
<tr>
<td>β-Lactams</td>
<td>All tested</td>
<td>CAMHB, ISOB</td>
<td></td>
<td></td>
<td>Identical assessment</td>
</tr>
<tr>
<td>Quinolones</td>
<td>All tested</td>
<td>CAMHB, ISOB</td>
<td></td>
<td></td>
<td>Identical assessment</td>
</tr>
<tr>
<td>Macrolides</td>
<td>All tested</td>
<td>CAMHB, ISOB</td>
<td></td>
<td></td>
<td>Identical assessment</td>
</tr>
<tr>
<td>Lincosamides</td>
<td>Lincomycin</td>
<td>CAMHB, ISOB</td>
<td></td>
<td></td>
<td>Naturally resistant</td>
</tr>
<tr>
<td>Ketolides</td>
<td>Teicoplanin, Vancomycin</td>
<td>CAMHB, ISOB</td>
<td></td>
<td></td>
<td>Identical assessment</td>
</tr>
<tr>
<td>Glycopeptides</td>
<td>All tested Antifolates</td>
<td>CAMHB, ISOB</td>
<td></td>
<td></td>
<td>Naturally resistant</td>
</tr>
<tr>
<td>Other antibiotics</td>
<td>Fusidic acid</td>
<td>CAMHB, ISOB</td>
<td></td>
<td></td>
<td>No breakpoints</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>CAMHB</td>
<td>CAMHB, ISOB</td>
<td></td>
<td></td>
<td>Identification</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>CAMHB</td>
<td>CAMHB, ISOB</td>
<td></td>
<td></td>
<td>Identification</td>
</tr>
<tr>
<td>Chlormphenicol</td>
<td>CAMHB</td>
<td>CAMHB, ISOB</td>
<td></td>
<td></td>
<td>Identification</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>CAMHB</td>
<td>CAMHB, ISOB</td>
<td></td>
<td></td>
<td>Identification</td>
</tr>
</tbody>
</table>

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*a Please note that IsoSensitest broth is not recommended for antimicrobial susceptibility tests according to NCCLS criteria.

1 NCCLS criteria valid for Enterobacteriaceae (NCCLS 2000a), Neisseria gonorrhoeae (NCCLS 2000b), staphylococci (NCCLS 2000c) and Pseudomonas aeruginosa and Non-Enterobacteriaceae (NCCLS 2000d) were applied. There are no NCCLS breakpoints for: streptomycin, neomycin, apramycin, ribostamycin and lividomycin A; ceftiofur; sparflloxacin and pefloxacin; oxastromycin. Abbreviations: CAMHB, cation-adjusted Mueller Hinton broth; ISOB, IsoSensitest broth.
In contrast to some Enterobacteriaceae, \textit{M. wisconsinensis} was also naturally resistant to azithromycin, indicating specific ionic interactions on the cell surface, preventing the entry of this extended-spectrum azalide. Uniform natural azithromycin resistance has been found in all close neighbors of \textit{Moellerella}, i.e., \textit{Proteus} (Stock & Wiedemann, 1997) and \textit{Providencia} species (Stock & Wiedemann, 1998), but also in \textit{Morganella morgani} (Stock & Wiedemann, 1998) and other enterobacteria (for an overview see Stock, 1999). The natural rifampicin resistance was in contrast to the results of one previous study (Richard, 1989), in which all strains examined were sensitive to rifampicin, implying either other methods (which were not stated) and/or other clinical assessment criteria (not stated).

It was shown that \textit{M. wisconsinensis} was naturally sensitive to all \(\beta\)-lactams except benzylpenicillin and oxacillin. Natural \(\beta\)-lactam susceptibility patterns were similar to those of natural populations of \textit{E. coli} and \textit{Shigella} spp. (Stock & Wiedemann, 1999b), \textit{Edwardsiella tarda} (Stock & Wiedemann, 2001) and \textit{Proteus mirabilis}, which are known to produce small amounts of naturally occurring (chromosomally encoded) \(\beta\)-lactamases (Clark et al., 1991; Livermore, 1995; Normark et al., 1980; Reger et al., 1993; Stock & Wiedemann, 2001). Although there are no reports on the \(\beta\)-lactamase of \textit{Moellerella}, it is likely that at least some strains produce small amounts of enzyme (unpublished data). The natural resistance of \textit{Moellerella} to benzylpenicillin and oxacillin is regarded to be connected to the limited permeability of the outer membrane for these penicillins (Curtis et al., 1979; Livermore, 1996). Because the interior channel size of the porins of several enterobacteria is broader than the molecular size of these \(\beta\)-lactams, it seems likely that their hydrophobicity is responsible for the failure to cross the outer membrane. Unfortunately, studies on the cell envelope of \textit{Moellerella} have not been performed.

Apart from the antibiotics mentioned above, natural resistance of \textit{M. wisconsinensis} was found to fosfomycin in \textit{Isonisensitest} broth. Since there was no naturally occurring high-level resistance, it is likely that a reduced permeability of the cell envelope to this antibiotic, rather than a specific fosfomycin:glutathione-S-transferase contributed to the low-level resistance of several strains. Alternatively, a transferase with low affinity to fosfomycin or a low-level enzyme expression could have been responsible for the phenotype observed (O’Hara, 1993). Results in fosfomycin susceptibility testing can be highly dependent on the constituents of media and several further factors (Barry & Fuchs, 1991; Patel et al., 1997). In contrast to one previous study dealing with natural antibiotic susceptibilities of \textit{Enterobacter} spp. (Stock et al., 2001), it was shown that different media have major influence on the fosfomycin MICs for \textit{M. wisconsinensis}, resulting in the absence of strains with natural resistance, applying CAMHB (Table 3). This is in agreement with respective MIC data for \textit{Staphylococcus} species: Kresken et al. showed that MIC values of fosfomycin for staphylococci were significant higher in \textit{Isonisensitest} broth than in Mueller Hinton media (Kresken et al., 2000). Thus, whether or not (and to which extent) a medium-dependency for fosfomycin susceptibility testing exists, seems to be highly related to the species examined.

The biochemical basis of the described phenomenon is poorly understood. In contrast to Mueller Hinton media, \textit{Isonisensitest} broth contains glucose that inhibits the fosfomycin uptake into the bacterial cell (resulting in higher MICs) (Kresken et al., 2000). To enhance its uptake, the addition of glucose-6-phosphate (G-6-P) is recommended for fosfomycin susceptibility testing. However, in the present study and in the study of Kresken et al. (Kresken et al., 2000) fosfomycin susceptibility testing was performed in the presence of G-6-P, indicating that a large amount of constituents acting inhibitory on the uptake of fosfomycin, not compensated by G-6-P, remain, when \textit{Isonisensitest} broth in fosfomycin susceptibility testing for moellerellae and staphylococci is applied. Medium-dependencies in susceptibility testing affecting clinical assessment criteria were also found with tetracycline, pipemidic acid and clindamycin, although the medium-associated differences of the respective MICs were smaller than with fosfomycin (Table 3). It is likely that at least some of these differences can be attributed to variations in cation concentrations. In studies with tetracyclines it was shown that in tryptic soy solution, tetracycline MICs for \textit{S. aureus}, \textit{E. coli} and \textit{Klebsiella} species were three or more doubling dilution steps higher than in Mueller Hinton broth, attributed to medium-dependent differences in cation concentrations (Amsterdam, 1996).

In conclusion, the natural resistance of \textit{M. wisconsinensis} to azithromycin and its decreased susceptibility to fosfomycin should give rise to attention for antibiotic therapy since both drugs are in use for treatment of diarrheagenic diseases. Although the present study is not suitable to draw conclusions on the incidence of acquired resistance, it is conspicuous that some \textit{Moellerella} strains were resistant to tetracyclines, trimethoprim and sulfamethoxazole (Table 3). The natural susceptibility patterns described, the presence of strains with acquired resistance, and the probable occurrence of moellerellae misidentified as \textit{Klebsiella} should direct the interest in bacterial pathogens also to \textit{M. wisconsinensis}.

Acknowledgments

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References


