# Acinetobacter, Chryseobacterium, Moraxella, and Other Nonfermentative Gram-Negative Rods\*

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# TAXONOMY

The organisms covered in this chapter belong to a group of taxonomically and phylogenetically diverse, gram-negative nonfermentative rods and coccobacilli. Still, several of the genera dealt with belong to the same family; i.e., Acineto-bacter, Moraxella, Oligella, and Psychrobacter belong to the family Moraxellaceae (Gammaproteobacteria) (182), and Balneatrix, Bergeyella, Chryseobacterium, Elizabethkingia, Empedobacter, Myroides, Sphingobacterium, Wautersiella, and Weeksella belong to the family Flavobacteriaeeae (Bacteroidetes) (11).

### **DESCRIPTION OF THE AGENTS**

The species dealt with in this chapter all share the common phenotypic features of being catalase positive and failing to acidify the butt of Kligler iron agar (KIA) or triple sugar iron (TSI) agar or of oxidative-fermentative media, indicating their inability to metabolize carbohydrates by the fermentative pathway. These organisms grow significantly better under aerobic than under anaerobic conditions, and many, i.e., those species that can use only oxygen as the final electron acceptor in the respiratory pathway, fail to grow anaerobically at all.

#### EPIDEMIOLOGY AND TRANSMISSION

Most of the organisms described in this chapter are found in the environment, i.e., soil and water. For methylobacteria, tap water has been implicated as a possible agent of transmission in hospital environments, and methods for monitoring water systems for methylobacteria have been described previously (178). No person-to-person spread has been documented for the species covered in this chapter.

# **CLINICAL SIGNIFICANCE**

Although for almost each of the species in this chapter, as for most other species in other chapters, case reports of, e.g., meningitis and endocarditis can be found, their clinical importance is mostly restricted to that of opportunistic pathogens, except, e.g., for *Elizabethkingia meningoseptica*, *Moraxella lacunata* (eye infections), or *Moraxella catarrhalis* (respiratory tract infections).

The clinical role of Acinetobacter species has been reviewed previously (58, 115, 164). These organisms are typical opportunistic pathogens that usually only form a threat to critically ill, hospitalized patients. Hospital-acquired Acinetobacter infections comprise ventilator-associated pneumonia, bloodstream infections, urinary tract infections, wound infections, skin and soft tissue infections, and secondary meningitis. Acinetobacter baumannii is the species most commonly implicated in hospital-acquired infections. The clinical role of the closely related Acinetobacter genomic species 3 and 13TU resembles that of A. baumannii (18, 22). For the purpose of this review, we consider A. baumannii to comprise these two species as well, unless stated otherwise. A. baumannii ventilator-associated pneumonia and bloodstream infections have been documented to be associated with a high degree of mortality and morbidity (41, 188). Particular manifestations of A. baumannii are its implication in severely war-wounded soldiers (32, 52), from which stems its popular designation "Iragibacter," and in victims of natural disasters (161).

The clinical impact of infections with A. *baumannii* is a continuous source of debate (58, 164). Indeed, although severe infections with A. *baumannii* have been documented, colonization is much more frequent than infection, and differentiation between these conditions can be difficult.

Although uncommon, community-acquired infections with A. *baumannii* occur. In particular, community-acquired pneumonia with A. *baumannii* is increasingly reported from tropical areas, like Southeast Asia and tropical Australia (3, 134).

Other Acinetobacter species occasionally implicated in nosocomial infections are listed in Table 1. A. *johnsonii*, A. *lwoffii*, and A. *radioresistens* seem to be natural inhabitants of human skin (186). A. *johnsonii*, which has also been found frequently in feces of nonhospitalized individuals (59), has been implicated in cases of meningitis (189). A. *lwoffii* was a frequent species in clinical specimens during an 8-year study in a university hospital, where it was isolated mainly from blood or intravascular lines (220). A. *ursingii* and A. *junii* have been found to cause bloodstream infections in hospitalized patients (63, 107, 139, 210), while A. *junii* has also been implicated in outbreaks of infection in neonates

<sup>\*</sup>This chapter contains information presented in chapter 50 by Paul C. Schreckenberger, Maryam I. Daneshvar, and Dannie G. Hollis in the ninth edition of this *Manual*.

Characteristic	A. calcoaceticus-A. baumanr complex (70)	A. beijerinckii (15)	A. bereziniae (16)	A. guillouiae (17)	A. gyllenbergü (9)	A. haemolyticus (21)	A. junii (21)	A. johnsonii (20)	A. lwoffii (26)	A. parvus (14)	A. radioresistens (22)	A. schindleri (22)	A. ursingii (29)
Growth at:													
44°C	$D^b$	_	_	_	_	_	_	_	_	_	_	_	_
41°C	$D^{c}$	_	_	_	_	D	D	_	_	_	D	+	_
37°C	+	+	$+^d$	d	+	+	+	_	D	+	+	+	+
Acidification of D-glucose	+	_	D <sup>e</sup>	_	_	D	_	_	D	_	_	_	_
Assimilation of:													
Adipate	+	_	D	+	+	_	_	_	+	_	+	D	+
β-Alanine	+	_	+	+	+	_	_	_	_	_	_	-	_
4-Aminobutyrate	+	+	+	D	D	+	D	D	D	_	+	-	_
L-Arginine	+	_	_	_	+	+	+	D	_	_	+	-	_
L-Aspartate	+	+	+	+	_	D	_	D	_	_	_	-	+
Azelate	+	-	D	+	+	-	D	D	+	_	+	D	+
Citrate (Simmons)	+	+	+	+	+	D	D	+	D	_	_	D	+
Glutarate	+	_	+	+	D	_	_	_	_	_	+	+	+
Histamine	_	_	D	D	_	_	_	_	_	_	_	—	_
L-Histidine	+	+	+	+	+	+	+	_	_	_	_	_	_
DL-Lactate	+	_	+	+	+	—	+	+	+	_	+	+	+
Malonate	D	+	-	D	D	_	_	D	_	_	+	_	_
Phenylacetate	D	_	D	D	+	_	_	_	+	_	+	_	_
Gelatinase	_	D	_	_	+	+	-	-	-	_	_	_	_
Hemolysis of sheep blood	_	+	_	_	+	+	D	_	_	_	_	_	_

 TABLE 1
 Oxidase-negative, indole-negative nonfermenters: the genus Acinetobacter<sup>a</sup>

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<sup>a</sup>Data for the A. calcoaceticus-A. baumannii complex, A. haemolyticus, A. junii, A. johnsonii, A. lwoffii, and A. radioresistens are from Gerner-Smidt et al. (75), except for the results of the assimilation of phenylacetate, L-arginine, and adipate, which were provided by one of us (A. Nemec).

Data for the other species are from Nemec et al. (149, 150, 154, 155). Numbers between parentheses are the numbers of strains tested. +, positive for 90 to 100% of strains; -, positive for 0 to 10% of strains; D, positive for 11 to 89% of strains.

<sup>b</sup>Typically positive only for A. *baumannii* and most strains of genomic species 13TU.

<sup>c</sup>Typically negative only for A. *calcoaceticus* strains.

<sup>d</sup>Growth tested at 38 instead of 37°C.

<sup>e</sup>Positivity, 88%.

(55, 121) and ocular infections (172). A. *parvus* is regularly isolated from blood cultures (150, 210) but is misidentified by API 20NE as A. *lwoffii* (M. Vaneechoutte, unpublished data). Many of the infections with these species are related to intravascular catheters or have another iatrogenic origin (9, 63, 194, 238, 248), and their course is generally benign. For various other named or yet-unnamed Acinetobacter species, although recovered from clinical specimens (21, 206), a possible role in infection has not been documented.

Moraxella species are rare agents of infections (conjunctivitis, keratitis, meningitis, septicemia, endocarditis, arthritis, and otolaryngologic infections) (54, 122, 191, 223), but M. *catarrhalis* has been reported to cause sinusitis and otitis media by contiguous spread of the organisms from a colonizing focus in the respiratory tract (122). However, isolation of M. *catarrhalis* from the upper respiratory tract (i.e., a throat culture) of children with otitis media or sinusitis does not provide evidence that the isolate is the cause of these infections, because M. *catarrhalis* is present frequently as a commensal of the upper respiratory tract in children (232). Isolates from sinus aspirates and middle ear specimens obtained by tympanocentesis should be identified and reported. Similarly, little is known about the pathogenesis of lower respiratory tract infection in adults with chronic lung diseases, although a clear pathogenic role may be assigned to this species because M. catarrhalis is not a frequent commensal of the upper respiratory tract in adults (232) and because examination of Gram-stained smears of sputum specimens from patients with exacerbations of bronchitis and pneumonia due to M. catarrhalis usually reveals an abundance of leukocytes, the presence of many gram-negative diplococci as the exclusive or predominant bacterial cell type, and the presence of intracellular gram-negative diplococci. Such specimens may yield M. catarrhalis in virtually pure culture, and the organism should be identified and reported. M. lincolnii is not frequently isolated from clinical samples. M. nonliquefaciens and M. osloensis are the two species most frequently isolated, approximately in equal numbers, from nonrespiratory clinical material, especially blood cultures from patients at risk.

M. *canis* has been isolated from dog bite wounds (111) and from debilitated patients (223). M. *lacunata* has been involved in eye infections (180).

# COLLECTION, TRANSPORT, AND STORAGE OF SPECIMENS

Standard methods for collection, transport, and storage of specimens as detailed in chapters 9 and 16 are satisfactory for this group of organisms. The only fastidious species handled in this chapter are Asaia species, Granulibacter bethesdensis, Methylobacterium species, and some Moraxella species.

# DIRECT EXAMINATION

There are no characteristics available that can help to recognize the species dealt with in this chapter by means of direct microscopic examination of the samples. On Gram stain, organisms appear as gram-negative rods, coccobacilli, or diplococci. Neither direct antigen tests nor molecular genetic tests to use directly on clinical materials have been developed.

# **ISOLATION PROCEDURES**

Initial incubation should be at 35 to 37°C, although some strains, among them many of the pink-pigmented species, grow better at or below 30°C and may be detected only on plates left at room temperature. In such cases, all tests should be carried out at room temperature. In fact, some of

the commercial kits, such as the API 20NE, are designed to be incubated at 30°C.

Growth on certain selective primary media, e.g., Mac-Conkey agar, is variable and may be influenced by lot-to-lot variations in the composition of media. Nonfermenters that grow on MacConkey agar generally form colorless colonies, although some form lavender or purple colonies due to uptake of crystal violet contained in the agar medium. Selective media have been described for *Acinetobacter* spp. (8, 113) and for *Moraxella* spp. (231), but their usefulness remains to be assessed.

# **IDENTIFICATION**

This chapter starts with an overview in Fig. 1, which provides a key to the five large groups that can be distinguished among the species described in this chapter. In the previous edition, the simplified scheme for identification of this group of organisms in the clinical laboratory was based on microscopic morphology, oxidase reaction, motility, acidification of carbohydrates, indole production, and the production of pink-pigmented colonies. The identification scheme presented here (Fig. 1) is further simplified and based only on colony color (pink or not) and the presence or absence of oxidase, of benzyl arginine aminopeptidase (trypsin) activity, and of the production of indole.

Figure 1 refers to Tables 1 to 5, which provide further keys to identify the species of these five groups on the basis of biochemical reactions. Results for enzymatic reactions can



**FIGURE 1** Identification of miscellaneous GNF. The organisms covered in this chapter belong to a group of taxonomically diverse, gram-negative nonfermentative rods and coccobacilli. They all share the common phenotypic features of failing to acidify the butt of KIA or TSI agar or of oxidative-fermentative media, indicating their inability to metabolize carbohydrates by the fermentative pathway. These characteristics are shared with those of the species of the emended genus *Pseudomonas* (chapter 40) and those of the species of genera that previously were named as *Pseudomonas* (chapter 41). G. *bethesdensis* grows slowly and poorly on SBA. A. *parvus* forms small colonies as well, but these are already visible after 24 h of incubation.

be read within hours or up to 2 days of incubation, whereas results of carbon source assimilation tests (*Acinetobacter*) and acid production from carbohydrates are read after up to 6 and 7 days, respectively.

For each group of closely related species, we present their taxonomic history (explaining the use of other names in the past and the taxonomic changes introduced since the previous edition), address the clinical importance of the species, and describe the phenotypic data that are useful to differentiate this group from other groups and to differentiate the species within this group (emphasizing the major differences from the previous edition). When relevant, antibiotic susceptibility characteristics and treatment options are discussed immediately; otherwise, they are discussed at the end of each section for the five large groups in this chapter.

The chapter on miscellaneous nonfermentative gramnegative bacteria in this edition of the *Manual* differs from chapter 50 of the previous edition in several aspects. Some of the species described in this chapter in the ninth edition are dealt with in other, more appropriate chapters: *Alcaligenes faecalis* (chapter 43), *Achromobacter denitrificans* and *Achromobacter xylosoxidans* (chapter 43), *Advenella incenata* (chapter 43), *Bordetella* (chapter 43), *Herbaspirillum* (including EF-1 isolates) (chapter 41), *Kerstersia gyiorum* (chapter 43), *Neisseria weaveri* and *N. elongata* (chapter 32), and CDC groups EF-4a and EF-4b (described here as *Neisseria animaloris* and *Neisseria zoodegmatis* [218]) (chapter 32). *Halomonas venusta, Laribacter hongkongensis*, and Massilia *timonae* are no longer included.

In addition, we have included only species with validated names and no longer deal with the following groups: Achromobacter group F (87); Agrobacterium yellow group (247); CDC halophilic nonfermenter group 1 (close to Halomonos venusta) (242); CDC groups Ic (247), IIg (85), EO-3 (45), EO-4, EO-5, NO-1 (86), O-1 (close to Hydrogenophaga palleronii) (174), O-2 (close to Caulobacter vibrioides), O-3 (47), OFBA-1 (244), Pseudomonas-like group 2 (formerly included in the heterogeneous CDC group IVd and close to Herbaspirillum rubrisubalbicans), and a group of thermophilic bacteria, some classified as "Tepidimonas arfidensis" (128). Pedobacter species (199) are also no longer mentioned because they have little or no clinical relevance.

Former unnamed groups have been described as species with validated names in the meantime and are discussed under their appropriate names in this chapter: Achromobacter groups B and E have been described as Pannonibacter phragmitetus (94), CDC groups IIh and IIc as Chryseobacterium hominis (228), part of the CDC group IIe strains as Chryseobacterium anthropi (120), and CDC group EO-2 strains as Paracoccus yeei (48). Additional new species have been described in the meantime, and those that are included are Granulibacter bethesdensis (79) and Wautersiella falsenii (119). Although genera like Acinetobacter and Chryseobacterium comprise many more species than the ones addressed here, we focus on those species that can be isolated from clinical samples.

### Classical Biochemical Identification Schemes Presented in This Chapter

For all the species that remain in this chapter, except those of the genus *Acinetobacter*, the biochemical tests listed have been carried out by one of us (G. Wauters), according to standardized protocols, described in detail in chapter 31. This means that for most species the number of strains tested is smaller than the number of strains tested in the previous edition, but that the data listed are not compiled from the literature, whereby different authors may have used different media and protocols. The limited number of tests that have been used to discriminate between the species dealt with in this chapter have been selected because they can be carried out easily and quickly, because they mostly yield uniform results per group or species, and because they are highly discriminatory. For the genus *Acinetobacter*, data based on standardized physiological and nutritional tests were adapted from the literature or were provided by one of the authors (A. Nemec) (see footnotes to Table 1).

# Automated, Commercially Available Phenotypic Identification Systems

Traditional diagnostic systems, e.g., those based on oxidationfermentation media, aerobic low-peptone media, or buffered single substrates, have now been replaced in many laboratories by commercial kits or automated systems like the Vitek 2 (bioMérieux, Marcy L'Etoile, France) and the Phoenix (BD Diagnostic Systems, Sparks, MD). The ability of commercial kits to identify this group of nonfermenters is variable and often results in identification to the genus or group level only, necessitating the use of supplemental biochemical testing for species identification. O'Hara and Miller (160), using the Vitek 2 ID-GNB identification card, reported that of 103 glucose-fermenting and nonfermenting nonenteric strains, 88 (85.4%) were correctly identified at probability levels ranging from excellent to good and that 10 (9.7%) were correctly identified at a low level of discrimination, for a total of 95.1% accuracy within this group. Bosshard et al. (19) compared 16S rRNA gene sequencing for the identification of clinically relevant isolates of nonfermenting gram-negative bacteria (non-Pseudomonas aeruginosa) with two commercially available identification systems (API 20NE and Vitek 2 fluorescent card; bioMérieux). By 16S rRNA gene sequence analysis, 92% of the isolates were assigned to species level and 8% to genus level. Using API 20NE, 54% of the isolates were assigned to species level, 7% were assigned to genus level, and 39% of the isolates could not be discriminated at any taxonomic level. The respective numbers for Vitek 2 were 53, 1, and 46%. Fifteen percent and 43% of the isolates corresponded to species not included in the API 20NE and Vitek 2 databases, respectively. Altogether, commercial identification systems can be useful for identification of organisms commonly found in clinical specimens, like Enterobacteriacaeae. However, for rare organisms the performance of these systems can be poor. This is also illustrated by the performance of API 20NE and Vitek 2 for clinical isolates of Acinetobacter (reference 12 and below).

#### **Chemotaxonomic Methods**

Identification of nonfermenters by automated cellular fatty acid analysis has also been attempted (237). In view of the difficulties inherent in this approach (162), it is recommended that fatty acid profiles be used only in conjunction with traditional or commercial diagnostic systems. The fatty acid profiles for the most common species of nonfermenting bacteria have been published (247). Unless specifically relevant, we have omitted fatty acid composition data, which were presented in the tables of the previous edition.

A recently developed method of bacterial identification is matrix-assisted laser desorption ionization-time-of-flight mass spectrometry, for which commercial systems, with bacterial mass spectrum databases, have become available recently (Autoflex II mass spectrometer [Bruker Daltonics, Billerica, MA] and Axima [Shimadzu, Kyoto, Japan]). A recent evaluation showed that 84.1% of 1,660 bacterial isolates analyzed were correctly identified to the species level (190). However, few of the species dealt with in this chapter were included. Another recent application of this technology deals with the *Burkholderia cepacia* complex, indicating its applicability for gram-negative nonfermenters (GNF) (233).

#### **DNA Sequence-Based Methods**

Sequence-based methods involving rRNA (16S, 16S-23S spacer, or 23S) and housekeeping genes, such as those encoding RNA polymerase subunit B (rpoB), gyrase subunit B (gyrB), or the RecA protein (recA), have become standard techniques to identify bacteria in general (167) and have contributed to the better delineation of several of these groups and the discovery and description of new species. Because these are generally applicable methods, their application for species of this chapter is not outlined in detail. Other sequence-based methods, based on DNA array hybridization, have been used for some species of these groups (129, 201). DNA sequence-based fingerprinting methods like amplified ribosomal DNA (rDNA) restriction analysis (227, 230), amplified fragment length polymorphism (AFLP) (112), and tDNA PCR (31, 67) have been applied for the identification of species of several groups as well. These fingerprinting approaches are also generally applicable, but they require reference fingerprint libraries and are often poorly exchangeable between different electrophoresis platforms and laboratories.

# IDENTIFICATION OF THE FIVE GENOTYPIC GROUPS

#### **Oxidase-Negative GNF**

#### Acinetobacter

The taxonomy of the genus Acinetobacter (21, 23, 57, 206, 224) has recently been updated with extended descriptions and formal species names for three species previously designated with provisional designations. These include A. *venetianus* (229) and A. *bereziniae* and A. *guillouiae* (previously designated genospecies 10 and 11, respectively) (21, 154). New species, comprising strains of clinical origin, have been described as well, i.e., A. *beijerinckii* (154), A. *gyllenbergii* (154), A. *parvus* (150), A. *schindleri* (149), and A. *ursingii* (149). Some of the species that were described recently have been shown to be synonymous to already existing species:

A. grimontii (29) was shown to be synonymous to A. junii (225), and "A. septicus" is synonymous to A. ursingii (156). At present, the genus comprises 21 validly named species and 11 species with provisional names. The G+C content of the genus ranges from 38 to 47 mol%. The genomes of seven Acinetobacter strains have been sequenced (NCBI, July 2009; http://www.ncbi.nlm.nih.gov/genome?term=acinetobacter).

Members of the genus *Acinetobacter* are widespread in nature and have been cultured from soil, water, sewage, and food and from human and animal specimens. The ecology of most species is unknown. Species of clinical importance are listed in Table 1.

Bacteria belonging to the genus Acinetobacter are strictly aerobic, nonfermenting gram-negative coccobacillary microorganisms with a negative oxidase reaction and a positive catalase reaction. Tween 80 esterase activity is frequently present, hemolysis and gelatinase production vary, and nitrate reductase is mostly absent. Motility (hanging drop) is negative, but twitching motility on soft agar occurs occasionally. Individual cell sizes are 0.9 to 1.6  $\mu$ m in diameter and 1.5 to 2.5  $\mu$ m in length. In the stationary phase, the organisms are usually coccoid. Cells frequently occur in pairs, resembling Neisseria species, but this may be strain or species dependent. In the Gram stain, the organisms can be slightly gram positive. Growth temperature varies, but most species grow between 20 and 35°C. Clinically important species commonly grow well at 37°C or at higher temperatures.

The organisms can form a pellicle on the surface of fluid media. They grow well on complex media, including blood agar, nutrient agar, and MacConkey agar. Colonies are 1 to 2 mm in diameter (sometimes pinpoint), colorless to beige, domed, and smooth to mucoid (Fig. 2). Colonies on MacConkey agar can become pink. Many strains can use a wide variety of carbon sources for growth. Selective enrichment can be obtained in mineral media with acetate as the carbon source and ammonium salt as the nitrogen source with shaking incubation at 30°C (8, 56, 61). General features of *Acinetobacter* species have been reviewed previously (57, 116).

For genus level identification of Acinetobacter isolates, the following characters can be used: gram-negative coccobacilli, oxidase negative, aerobic (nonfermenting), and nonmotile. Phenotypic identification of Acinetobacter species in the clinical microbiology laboratory by commercial identification systems is problematic (12). This results from the small number of relevant characters tested in these systems and/or from the insufficient quality of reference data in the identification matrices. A. baumannii and the closely related species Acinetobacter genomic species 3 and 13TU, which are clinically the most important species, and



**FIGURE 2** Differences in the size of the colonies formed by different *Acinetobacter* species isolated from human clinical specimens. The strains were grown on TSA (Oxoid) at 30°C for 24 h. (a) A. *parvus* NIPH 384<sup>T</sup>; (b) A. *ursingii* LUH 3792<sup>T</sup>; (c) A. *schindleri* LUH 5832<sup>T</sup>; (d) A. *baumannii* ATCC 19606<sup>T</sup>.

A. calcoaceticus, an environmental species, together referred to as the A. calcoaceticus-A. baumannii complex, are generally not differentiated by these systems. Nonetheless, these systems can be useful for genus level identification and, when supplemented with aerobic acidification of glucose (oxidation-fermentation test), hemolysis, and growth at 44°C, also for presumptive identification of A. baumannii (Table 1). We compared Vitek 2 and Phoenix for the ability to identify 76 isolates of 16 clinical Acinetobacter species and found that only 19 isolates were correctly identified by Vitek 2 and 5 by Phoenix (M. Vaneechoutte, unpublished data). Phenotypic identification of Acinetobacter species can be achieved using physiological, i.e., biochemical and growth temperature, characteristics, and nutritional, i.e., assimilation, characteristics, based on the system of Bouvet and Grimont (22). Table 1 presents a recent update of this system aimed to differentiate all validly named species of clinical importance. Assimilation tests were carried out using the minimal medium of Cruze et al. (44), dispensed into tubes (12-mm inner diameter) in 3-ml volumes inoculated with a small inoculum. Growth on carbon sources was evaluated after 2, 4, 6, and 10 days by means of visual comparison between inoculated tubes containing carbon sources and control tubes containing only inoculated basal medium. Unfortunately, the species of the A. calcoaceticus-A. baumannii complex are not clearly distinguished from each other by this approach. In addition, the need for in-house preparation of most of the tests precludes the use of this identification scheme in most diagnostic laboratories.

Therefore, genotypic methods are indispensable for unambiguous identification of *Acinetobacter* species. Wellvalidated methods are amplified rDNA restriction analysis (60, 227) and whole genomic fingerprinting by AFLP, based on the selective amplification of chromosomal restriction fragments (57, 112). Currently, sequence-based species identification is becoming more and more the standard. Targets for this purpose are the 16S rDNA sequence (224), the *rpoB* gene sequence (81), and the 16S-23S rRNA gene spacer region (34), which has also been used for oligonucleotide array-based identification of species of the *A. calcoaceticus-A. baumannii* complex (129). PCR detection of the *bla*<sub>oxa-51-like</sub> gene has been shown to be a rapid method for identification of *A. baumannii* isolates (212).

The ecology of most Acinetobacter species is still poorly resolved. A. baumannii and Acinetobacter genomic species 3 and 13TU have been mainly recovered from clinical specimens in hospitals. Human skin carrier rates of A. baumannii outside hospitals have been shown to be as low as 0.5 to 3% (10, 186), but higher rates (also for Acinetobacter genomic species 3 and 13TU) have been found in tropical areas (40). A. baumannii has been isolated from sick animals (15, 226), but an animal or environmental reservoir has not been found. A. baumannii is, due to its role as a prominent nosocomial pathogen, the species for which the epidemiology has been studied most intensively. Epidemic strains of this species can survive well in the environment, as they have been found on equipment and on environmental surfaces and materials (219), usually in the vicinity of colonized patients. Multiple sites of the skin and mucosae of patients can be colonized, and colonization may last days to weeks (61, 142).

#### Genotyping and Epidemiology

A variety of genotyping methods have been described for differentiation between isolates of the same species and study of the epidemiology of acinetobacters, in particular that of A. *baumannii*. Standardized random amplification PCR-fingerprinting was useful for local typing, but its (interlaboratory) reproducibility was limited (80). Macrorestriction analysis with pulsed-field gel electrophoresis allowed for 95% intra- and 89% interlaboratory reproducibility (187). AFLP fingerprinting also enables genotyping of strains (57, 62, 153), and its robustness makes it suited for setting up a local database for longitudinal studies. Genotyping based on the variable number of tandem repeat loci has allowed for additional subtyping in conjunction with pulsed-field gel electrophoresis analysis (209).

With the introduction of sequence-based methods, it has become possible to set up Internet-based databases to study the global epidemiology of organisms. Three multilocus sequence typing systems, mainly aimed at studying the population biology of *A. baumannii*, have been developed (6, 65; S. Brisse et al., unpublished data [http://www.pasteur.fr/ mlst]). Further to typing in the strict sense, specific antibiotic resistance genes like the OXA genes, which confer resistance to carbapenems, are frequently used for additional characterization of *Acinetobacter* isolates (39, 130).

Various methods, often in combination, of genotyping A. baumannii isolates from different institutes and countries have identified three major groups of genetically highly related strains, the so-called European clones I to III (151, 221). Many of the strains allocated to these clones are multidrug resistant and have been implicated in outbreaks. Clone I prevailed in the 1980s, but recent studies indicate that subclones of clone II have emerged in the United Kingdom, the Czech Republic, and Portugal (50, 152, 208). Identification of isolates of these clones can be obtained by comparing them to reference sets of the three clones by AFLP analysis (62, 151). Comparative typing of isolates to only one reference strain of each clone may lead to underidentification of the clones, since one reference strain does not cover the intraclonal variation. Multilocus sequence typing with seven genes (http://www.pasteur.fr/mlst) is expected to be the most reliable method for identification of strains of clones I to III (Brisse et al., unpublished). Rapid assignment to the clones by a multiplex PCR targeting the ompA, csuE, and blaOXA-51-like gene sequences is promising (208).

#### Antimicrobial Susceptibilities

Acinetobacter species are increasingly resistant to multiple antibiotics (108, 134). With the emergence of carbapenem resistance, a last option for treatment of infections with these organisms is disappearing. Multidrug resistance is mainly confined to A. baumannii, but strains of the closely related species Acinetobacter genomic species 3 can also be multidrug resistant (18, 234). The rates of resistance to different antibiotics can vary among hospitals and regions, depending on the endemic or epidemic presence of multidrug-resistant A. baumannii. Resistance mechanisms in A. baumannii comprise all currently known mechanisms, including enzymatic breakdown, modification of target sites, active efflux, and decreased influx of antibiotics. The known mechanisms have been reviewed previously (58), and new mechanisms have been discovered since (2, 171).

Recent genomic studies have shed new light on the genetic organization of resistance determinants and their transmission. For example, a resistance island integrated within the ATPase gene has been found in different *A. baumannii* strains for which the genome has been sequenced (1, 71, 109). Among these strains, a variable composition

of resistance determinants interspersed with transposons, integrons, and other mobile elements has been identified. Other elements, like insertion sequence elements (211), distributed throughout the genome, are also important for the overall resistance (1).

In vitro determination of antimicrobial susceptibility can be achieved by disk diffusion, agar dilution, or broth microdilution, as recommended by the Clinical and Laboratory Standards Institute (CLSI) (203), or by Etest. The panel of tested antibiotics should cover the spectrum of agents with potential action against A. baumannii, including third- or fourth-generation cephalosporins, sulbactam, ureidopenicillins, carbapenems, aminoglycosides, fluoroquinolones, and tetracyclines. Of note, susceptibility to polymyxins, a current last option for treating pandrugresistant A. baumannii, should not be tested by disk diffusion due to poor diffusion of these compounds in agar. Etest and broth microdilution for determination of the MIC for colistin have been compared and showed a good concordance in the MIC range of 0.25 to 1 mg/liter (4). In case of carbapenem resistance, the genes encoding beta-lactamases with carbapenemase activity can be determined by specific PCR (68), to provide better insight into the epidemiology of the resistance.

#### Granulibacter bethesdensis

Granulibacter bethesdensis (Acetobacteraceae, Alphaproteobacteria) (79) is a gram-negative, aerobic, coccobacillary to rod-shaped bacterium, the only species of a new sublineage within the acetic acid bacteria in the family Acetobacteraceae. This fastidious organism grows poorly and slowly on sheep blood agar (SBA) at an optimum temperature of 35 to 37°C and an optimum pH of 5.0 to 6.5. It produces a yellow pigment, oxidizes lactate and weakly acetate to carbon dioxide and water, acidifies ethanol, and can use methanol as a sole carbon source, all characteristics that distinguish it from other acetic acid bacteria. The two major fatty acids are C18:1w7c and C16:0. The DNA base composition is 59.1 mol% G+C. It was first isolated from three patients with chronic granulomatous disease (79) and from an additional patient with chronic granulomatous disease more recently (138).

### Oxidase-Positive, Indole-Negative, Trypsin-Negative Nonfermenters

#### Haematobacter

Three Haematobacter species (Rhodobacteraceae, Alphaproteobacteria) have been described, i.e., H. massiliensis (former Rhodobacter massiliensis), H. missouriensis, and Haematobacter genomospecies 1 (Table 2) (84). These species cannot easily be differentiated phenotypically, and even the 16S rRNA gene sequences are closely related. Haematobacter species were described as asaccharolytic, but using low-peptone phenol red agar (see chapter 31), H. missouriensis is clearly saccharolytic, producing acid from glucose and xylose and sometimes from mannitol, whereas H. massiliensis strains do not acidify carbohydrates. Acid is produced from ethylene glycol by all species. All the species are strongly urease and phenylalanine deaminase positive. Arginine dihydrolase is also positive but sometimes delayed. Asaccharolytic Haematobacter strains resemble Psychrobacter phenylpyruvicus but can be differentiated by the lack of tributyrine esterase, the lack of growth improvement by Tween 80, and the presence of arginine dihydrolase. Differences from Psychrobacter faecalis, Psychrobacter pulmonis, and related species are the lack of tributyrine and Tween 80 esterase, the lack of nitrate reductase, and a positive arginine dihydrolase test.

Strains received at the CDC have been mainly from patients with septicemia.

Haematobacter strains have low MICs for amoxicillin, fluoroquinolones, aminoglycosides, and carbapenems but variable MICs for cephalosporins, monobactams, and piperacillin.

#### Moraxella

The genus *Moraxella* comprises approximately 20 species that have been validly named. M. *catarrhalis*, M. *osloensis*, M. *nonliquefaciens*, and M. *lincolnii* are part of the normal microbiota of the human respiratory tract. Animal species include M. *bovis*, isolated from healthy cattle and other animals, including horses; M. *boevrei* and M. *caprae* (goats); M. *canis* (dogs, cats, and camels); M. *caviae* (guinea pigs); M. *cuniculi* (rabbits); and M. *ovis* and M. *oblonga* (sheep). The clinical importance of the different species is addressed below.

Both M. catarrhalis and M. canis grow well on sheep blood agar (SBA) and even on tryptic soy agar (TSA), and their colonies may reach more than 1 mm in diameter after 24 h of incubation. Colonies of M. catarrhalis grow well on both blood and chocolate agars, and some strains also grow well on modified Thaver-Martin and other selective media. Colonies are generally gray to white, opaque, and smooth and measure about 1 to 3 mm after 24 h of incubation. Characteristically, the colonies may be nudged intact across the plate with a bacteriological loop like a "hockey puck" and can be removed from the agar entirely, being very consistent. Most M. canis colonies resemble those of the Enterobacteriaceae (large, smooth colonies) and may produce a brown pigment when grown on starch-containing Mueller-Hinton agar (111). Some strains may also produce very slimy colonies resembling colonies of Klebsiella pneumoniae (111). M. nonliquefaciens forms smooth, translucent to semiopaque colonies 0.1 to 0.5 mm in diameter after 24 h and 1 mm in diameter after 48 h of growth on SBA plates. Occasionally, these colonies spread and pit the agar. The colonial morphologies of M. lincolnii (217), M. osloensis, and Psychrobacter phenylpyruvicus (formerly M. phenylpyruvica) are similar, but pitting is rare. On the other hand, pitting is common with M. lacunata, whose colonies are smaller and form dark haloes on chocolate agar. Rod-shaped Moraxella species, especially M. atlantae and M. lincolnii, are more fastidious and display smaller colonies on SBA, less than 1 mm in diameter after 24 h. Colonies of M. atlantae are small (usually 0.5 mm in diameter) and show pitting and spreading (24). The growth of M. atlantae is stimulated by bile salts, which explains its growth on MacConkey agar. M. nonliquefaciens and M. osloensis produce colonies that are somewhat larger than those of M. atlantae and that are rarely pitting. Colonies of M. nonliquefaciens may be mucoid. A selective medium, acetazolamide agar, inhibiting growth of neisseriae when incubated in ambient atmosphere, has been described for M. catarrhalis (231).

Moraxella species are coccoid or coccobacillary organisms (plump rods), occurring predominantly in pairs and sometimes in short chains, that tend to resist decolorization in the Gram stain (49). M. canis and M. catarrhalis are Neisseria-like diplococci, and they can easily be distinguished from other moraxellae or other coccoid species by performing a Gram stain on cells cultured in the vicinity of a penicillin disk: cells of M. canis and M. catarrhalis remain

Characteristic	Haematobacter massiliensis (5)	Haematobacter missouriensis (4)	Haematobacter genomospecies 1 (1)	Moraxella atlantae (5)	Moraxella canis (5)	Moraxella catarrhalis (7)	Moraxella lacunata (4)	Moraxella lincolnii (2)	Moraxella nonliquefaciens (15)	Moraxella osloensis (21)	Oligella ureolytica (3)	Oligella urethralis (6)	Paracoccus yeei (11)	Psychrobacter faecalis (10)	Psychrobacter immobilis ATCC $43116^{T}$	Psychrobacter phenyl pyruvicus (7) <sup>b</sup>	Psychrobacter pulmonis (4)
Motility (flagella)	0	0	0	0	0	0	0	0	0	0	67 (pt)	0	0	0	-	0	0
MacConkey agar	00	100	100	100	00	0	0	0	0	50	0	50	100	100	T	100	(2)
Alkalinization of	100	100	100	0	100	0	75	0	0	100	100	100	100	100	+	71	100
Susceptibility to desferrioxamine	0	0	0	60	100	100	100	0	100	0	100	100	0	0	_	0	0
Acidification of:																	
Glucose	0	100	0	0	0	0	0	0	0	0	0	0	100	100	+	0	0
Mannitol	0	(50)	(100)	0	0	0	0	0	0	0	0	0	(45)	0	-	0	
Xylose	0	100	0	0	0	0	0	0	0	0	0	0	100	100	+	0	0
Ethylene glycol	100	100	100	0	100	0	25	0	0	100	100	100	100	100	+	57	0
Gelatinase	0	0	0	0	0	0	100	0	0	0	0	0	0	0	—	0	0
Tween 80 esterase	0	0	0	0	0	0	100	0	0	5	0	0	0	100	+	100	100
Tributyrate esterase	0	0	0	0	100	100	100	0	100	100	0	0	100	100	+	100	100
Alkaline phosphatase	0	0	0	100	0	0	75	0	0	75	0	0	0	0	_	0	0
Phenylalanine deaminase	100	100	100	0	0	0	0	0	0	0	0	100 <sup>W</sup>	0	10	_	86	0
Pyrrolidonyl aminopeptidase	0	0	0	100	0	0	0	0	0	0	0	0	0	0	_	0	0
Urease	100	100	100	0	0	0	0	0	0	0	100	0	100	0	+	100	0
Nitrate reductase	0	0	0	0	80	100	100	0	100	24	100	0	100	100	+	28	100
Nitrite reductase	0	0	0	0	20	100	0	0	0	0	100	100	0	100	_	0	100
Arginine dihydrolase	100	100	100	0	0	0	0	0	0	0	0	0	0	0	_	0	0

**TABLE 2** Oxidase-positive, indole-negative, trypsin-negative, coccoid nonfermenters<sup>a</sup>

<sup>a</sup>Numbers in parentheses after organism names are numbers of strains tested. Values are percentages; those in parentheses represent delayed positivity. W, weakly positive; pt, peritrichous.

<sup>b</sup>Growth markedly promoted by Tween 80.

spherical diplococci of 0.5 to  $1.5 \,\mu\text{m}$  in diameter, although of irregular size, whereas coccobacilli show obviously rod-shaped and filamentous cells.

Moraxella species are asaccharolytic and strongly oxidase positive. M. catarrhalis and M. canis are also strongly catalase positive, and most strains reduce nitrate and nitrite. M. catarrhalis and M. canis may be easily distinguished from the commensal Neisseria species, which are also frequently isolated from respiratory clinical specimens, by the ability of the former to produce DNase and butyrate esterase (tributyrine test). Rapid butyrate esterase tests have been described (198), and the indoxyl-butyrate hydrolysis spot test is commercially available (Remel, Inc., Lenexa, KS). Butyrate esterase is, however, also present in some other Moraxella species. M. canis acidifies ethylene glycol and alkalinizes acetate, in contrast to M. catarrhalis. There are few biochemical differences between M. catarrhalis and M. nonliquefaciens, which are differentiated from each other mainly on the basis of morphological characteristics and by nitrite reductase and DNase activity of *M. catarrhalis*.

*M. atlantae* is the only *Moraxella* species to be pyrrolidonyl aminopeptidase (17) positive. *M. lacunata* is the only proteolytic species with gelatinase activity. Using the plate method (see chapter 31), gelatin hydrolysis occurs usually within 2 to 4 days. A more rapid and almost equally specific test to differentiate *M. lacunata* from other moraxellae is the detection of Tween 80 esterase activity, which is often positive within 2 days, whereas all other species, except for very rare *M. osloensis* strains, remain negative. This species should also be distinguished from *Psychrobacter* species, which are also Tween 80 esterase positive, but *P. phenylpyruvicus* is urease positive and *P. immobilis* and related species exhibit luxuriant growth on plain agar, like TSA, even at 25°C.

M. *lincolnii* is biochemically quite inactive.

M. osloensis is acetate alkalinization positive, acidifies ethylene glycol, and is resistant to desferrioxamine (250-µg disk). *M. nonliquefaciens* has opposite properties to those of *M. osloensis* and is, in addition, always nitrate positive.

#### Antimicrobial Susceptibilities

Most Moraxella species are susceptible to penicillin and its derivatives, cephalosporins, tetracyclines, quinolones, and aminoglycosides (70, 197). Production of beta-lactamase has been only rarely reported for Moraxella species other than M. catarrhalis, of which most isolates produce an inducible, cell-associated beta-lactamase (231). Isolates of M. catarrhalis are generally susceptible to amoxicillin-clavulanate, expanded-spectrum and broad-spectrum cephalosporins (i.e., cefuroxime, cefotaxime, ceftriaxone, cefpodoxime, ceftibuten, and the oral agents cefixime and cefaclor), macrolides (e.g., azithromycin, clarithromycin, and erythromycin), tetracyclines, rifampin, and fluoroquinolones.

#### Oligella urethralis and O. ureolytica

The genus Oligella comprises two species, O. *ureolytica* (formerly CDC group IVe) and O. *urethralis* (formerly *Moraxella urethralis* and CDC group M-4) (181), which have both been isolated chiefly from the human urinary tract and have been reported to cause urosepsis (173). A case of septic arthritis due to O. *urethralis* has also been reported (144).

Colonies of O. *urethralis* are smaller than those of M. *osloensis* and are opaque to whitish. Colonies of O. *ureolytica* are slow growing on blood agar, appearing as pinpoint colonies after 24 h but large colonies after 3 days of incubation. Colonies are white, opaque, entire, and nonhemolytic.

O. *ureolytica* and O. *urethralis* are small asaccharolytic coccobacilli that rapidly acidify ethylene glycol and are susceptible to desferrioxamine. Most strains of O. *ureolytica* are motile by peritrichous flagella; all are strongly urease positive (with the urease reaction often turning positive within minutes after inoculation) and reduce nitrate. Oligella *urethralis* strains are nonmotile and urease and nitrate reductase negative, but they reduce nitrite and are weakly phenylalanine deaminase positive. Bordetella bronchiseptica and Cupriavidus pauculus are also rapidly urease positive but are desferrioxamine resistant.

O. *urethralis* and M. *osloensis* have biochemical similarities, e.g., accumulation of poly- $\beta$ -hydroxybutyric acid and failure to hydrolyze urea, but can be differentiated on the basis of nitrite reduction and alkalinization of formate, itaconate, proline, and threonine, all positive for O. *urethralis* (169). Moreover, O. *urethralis* is susceptible to desferrioxamine and tributyrate esterase is negative, in contrast to M. *osloensis*.

O. *urethralis* is generally susceptible to most antibiotics, including penicillin, while O. *ureolytica* exhibits variable susceptibility patterns (70).

#### Paracoccus yeei

The genus *Paracoccus* (*Rhodobacteraceae*, *Alphaproteobacteria*) comprises approximately 25 species, of which only *P*. *yeei* is of some clinical importance. Daneshvar et al. (48) proposed the name *Paracoccus yeeii*, later changed to *P*. *yeei*, for the former CDC group EO-2.

Colonies are large and mucoid, with a pale yellow pigmentation. *Paracoccus yeei* organisms are coccoid cells, showing many diplococci and a few very short rods. Microscopically, *P. yeei* is characterized by distinctive O-shaped cells (Fig. 3) upon Gram stain examination due to the presence of vacuolated or peripherally stained cells. The species is saccharolytic and urease positive.



FIGURE 3 Gram stain of *Paracoccus yeei*, showing characteristic doughnut-shaped morphology.

*P. yeei* has been isolated from various human wound infections (48).

#### Psychrobacter

The genus *Psychrobacter* (117) comprises more than 30 species, of which only a few are clinically important. Apart from *Psychrobacter phenylpyruvicus*, the *Psychrobacter* strains isolated from clinical material were considered until recently as belonging to the species *Psychrobacter immobilis*. In a recent study, 16 *Psychrobacter* isolates of clinical origin were analyzed. Ten were identified as *P. faecalis*, four were identified as *P. pulmonis*, and two could not be identified but clustered close to *Psychrobacter* when the 16S rRNA gene sequence was determined (G. Wauters, unpublished data). These findings suggest that the majority of the clinical isolates belong to *P. faecalis* and *P. pulmonis*, both first described to occur in animals (pigeons [118] and lambs [235], respectively). *P. immobilis* itself is apparently rarely isolated, if at all, from humans.

*P. faecalis* and *P. pulmonis* are coccoid gram-negative rods growing on TSA with large, creamy colonies. *P. faecalis* is saccharolytic and acidifies glucose and xylose, while *P. pulmonis* is asaccharolytic. Both species produce acid from ethylene glycol. They are Tween 80 esterase and tributyrate esterase positive. They are nitrate reductase positive and, unlike the type strain of *P. immobilis*, are urease negative and nitrite reductase positive. Colonies may resemble those of *Haematobacter*, but the latter lack nitrate reductase, Tween 80 esterase, and tributyrin esterase and are strongly urease positive, arginine dihydrolase positive, and phenylalanine deaminase positive.

One case of ocular infection (76) and one case of infant meningitis (137) have been reported to be caused by *P. immobilis*, but in light of the data reported here, this might concern infection with one of the other *Psychrobacter* species.

*P. phenylpyruvicus*, formerly Moraxella phenylpyruvica (25), has the morphological and cultural appearance of moraxellae but is urease and phenylalanine deaminase positive. A unique feature of the species is its marked growth improvement by Tween 80. Colonies on TSA with 1% Tween 80 have a size two to three times larger than on SBA. The other *Psychrobacter* species, in contrast to *P. phenylpyruvicus*, grow abundantly on ordinary media such as TSA, and their growth is not promoted by Tween 80. They resemble *Haematobacter* species. *Psychrobacter* species are resistant to penicillin but susceptible to most other antibiotics (76, 137).

# Oxidase-Positive, Indole-Negative, Trypsin-Positive Nonfermenters

#### Alishewanella fetalis

Alishewanella fetalis (Alteromonadaceae, Gammaproteobacteria) (Table 3) is a gram-negative rod that grows at temperatures between 25 and 42°C, with optimum growth at 37°C. A. fetalis can withstand NaCl concentrations of up to 8% but not 10%, which helps differentiate this species from Shewanella algae, which can grow in 10% NaCl (240). Also, in contrast to Shewanella species, it does not produce H<sub>2</sub>S in the butt of TSI and KIA. The type strain tested by us acidifies glucose and does not hydrolyze esculin.

A. *fetalis* has been isolated from a human fetus at autopsy, although its association with clinical infection is unknown (240).

#### Inquilinus limosus

Inquilinus limosus is a rod-shaped gram-negative bacterium that measures 1.5 to 2  $\mu$ m in width by 3.5  $\mu$ m in length; it grows at 35 and 42°C but poorly at 25°C. Colonies are non-pigmented and very mucoid and grow on ordinary media such as TSA. Some strains are motile by one or two polar flagella, but motility is difficult to demonstrate due to the mucoid character of the colonies. In opposition to the original description (42), the species is saccharolytic, acidifying glucose, mannitol, xylose, and other carbohydrates. In contrast, ethylene glycol is not acidified. Beta-galactosidase, pyrrolidonyl aminopeptidase, and trypsin are positive, but alkaline phosphatase is negative. Esculin is hydrolyzed. All strains are positive for catalase, beta-glucosidase, proline aminopeptidase, and pyrrolidonyl aminopeptidase and negative for lysine, arginine, ornithine, denitrification, and indole production (42).

Characteristic	Alishewanella fetalis (1)	Inquilinus limosus (3)	Myroides odoratimimus (20)	Myroides odoratus (4)	Ochrobactrum anthropi (39)/ O. intermedium (3)	Pannonibacter phragmitetus (7) <sup>c</sup>	Rhizobium radiobacter (19) <sup>c</sup>	Shewanella algae (6) <sup>d</sup>	Shewanella putrefaciens (2) <sup>d</sup>	Sphingobacterium multivorum (6)	Sphingobacterium spiritivorum (4)	Sphingobacterium thalpophilum (3)	Sphingomonas spp. (15)
Motility	0	.33	0	0	100	100	100	100	100	0	0	0	66
Flagella	0	P. 1	0	0	Pt. L	Pt. L	Pt. L	P. 1	P. 1	0	0	0	P. 1
Growth on MacConkey agar	100	(100)	100	75	100	100	100	100	100	100	(50)	100	0
Acidification of:													
Glucose	100	100	0	0	100	100	100	0	50	100	100	100	87 (13)
Mannitol	0	100	0	0	55	28	100	0	0	0	100	0	0
Xylose	0	100	0	0	100	100	100	0	0	100	100	100	87
Ethylene glycol	0	0	0	0	100	86	100	100	100	0	100	0	93
Urease	100	(33)	100	100	98	100	95	0	0	100	100	(100)	0
Nitrate reductase	100	Ó	0	0	98	86	89	100	100	0	0	100	7
Nitrite reductase	0	0	100	100	90	100	58	0	0	0	0	0	0
Production of H <sub>2</sub> S (on KIA)	0	0	0	0	0	0	0	100	100	0	0	0	0
Esculin hydrolysis	0	100	0	0	12	86	100	0	0	100	100	100	100
Gelatinase	100	0	100	100	0	0	0	100	100	0	0	0	0
Pyrrolidonyl aminopeptidase	0	100	100	100	100	100	100	100	100	100	100	100	27
Alkaline phosphatase	100	0	100	100	0	0	0	100	100	100	100	100	100
Beta-galactosidase (ONPG) <sup>e</sup>	0	100	0	0	0	100	100	0	0	100	75	100	100
Requirement for NaCl Susceptibility to:	0	0	0	0	0	0	0	100	0	0	0	0	0
Desferrioxamine	100	0	0	100	0	100	21	0	0	0	0	0	7
Colistin	100	0	0	0	100/0 <sup>b</sup>	0	68	66	100	0	0	0	7
Production of flexirubin pigments	0	0	100	100	0	0	0	0	0	0	0	0	0

TABLE 3.	Oxidase-positive, indo	le-negative, trypsin	-positive nonfermenters <sup>a</sup>
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<sup>a</sup>Numbers in parentheses after organism names are numbers of strains tested. Values are percentages; those in parentheses represent delayed positivity. Pt, peritrichous; P, polar; L, lateral.

<sup>b</sup>Ochrobactrum anthropi strains are colistin susceptible, whereas O. intermedium strains are colistin resistant (236).

"Pannonibacter organisms are tributyrate esterase positive within 30 mins. Rhizobium organisms are positive only after several hours or remain negative.

<sup>d</sup>Both Shewanella species are ornithine decarboxylase positive.

<sup>e</sup>ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

I. limosus can be distinguished from Sphingobacterium spp. by its lack of alkaline phosphatase activity and by its acidification of mannitol. Sphingobacterium spiritivorum also produces acid from mannitol but acidifies ethylene glycol, unlike Inquilinus. It differs from Rhizobium radiobacter and Pannonibacter phragmitetus by the absence of acid production from ethylene glycol (also negative in a quarter of the P. phragmitetus strains) and by the lack of nitrate reductase activity.

Identifying the species is difficult because it is not contained in the databases of commercial identification kits and its mucoid appearance may lead to confusion with mucoid *P. aeruginosa* strains (170, 246). Isolates can be recovered on colistin-containing *B. cepacia* selective media but are inhibited on *B. cepacia* selective agar, which also contains gentamicin (37).

All isolates are reported to be resistant to penicillins and cephalosporins, kanamycin, tobramycin, colistin, doxycycline, and trimethoprim-sulfamethoxazole and susceptible to imipenem and ciprofloxacin (37, 170, 246).

All strains have been recovered from respiratory secretions of cystic fibrosis patients. The natural habitat of *I. limosus* is unknown to date, and the clinical impact of chronic colonization with *I. limosus* remains unclear. Chiron et al. (37) reported that for one patient, *I. limosus* was the only potential pathogen recovered from the sputum; *Inquilinus* acquisition was followed by a worsening of his lung function.

#### Myroides odoratimimus and M. odoratus

The genus Myroides includes two species, M. odoratimimus and M. odoratus, formerly Flavobacterium odoratum (215), which can be isolated from clinical samples. Cells are thin, middle-sized (0.5  $\mu$ m in diameter and 1 to 2  $\mu$ m long) nonmotile rods, but both species display a gliding motility. The spreading colonies develop a typical fruity smell, similar to the odor of Alcaligenes faecalis. The yellow pigment, although less pronounced than that of some Chryseobacterium species, is of the flexirubin type.

Myroides species grow on most media, including MacConkey agar. Growth occurs at 18 to 37°C but usually not at 42°C. Myroides species are asaccharolytic, urease positive, and nitrate reductase negative, but nitrite is reduced. M. odoratus can routinely be differentiated by its susceptibility to desferriox-amine, while M. odoratimimus is resistant. They also differ by their cellular fatty acid pattern, with M. odoratimimus having significant amounts of C13:0i and C15:0 (215).

Organisms identified as *M. odoratus* have been reported mostly from urine but have also been found in wound, sputum, blood, and ear specimens (95). Clinical infection with *Myroides* species is exceedingly rare. However, cases of rapidly progressive necrotizing fasciitis and bacteremia (104) and recurrent cellulitis with bacteremia (5) have been reported. In our experience, based on 24 clinical isolates, *M. odoratimimus* is four to five times more frequently isolated from clinical material than *M. odoratus*.

Most strains are resistant to penicillins, cephalosporins, aminoglycosides, aztreonam, and carbapenems (95).

#### Ochrobactrum anthropi and O. intermedium

The genus Ochrobactrum comprises at present 13 species, of which two are of some clinical importance. Ochrobactrum anthropi (93) comprises the so-called urease-positive Achromobacter species, formerly designated CDC group Vd (biotypes 1 and 2), and Achromobacter groups A, C, and D, described by Holmes et al. (92). Subsequent studies showed that biogroup C and some strains belonging to biogroup A constitute a homogeneous DNA-DNA hybridization group separate from O. *anthropi*, named O. *intermedium* (236).

Colonies on SBA are small, about 1 mm after overnight incubation, but grow large and creamy after 2 days and appear smooth, circular, and clearly delineated. *Ochrobactrum* species are medium-length gram-negative rods and motile by peritrichous flagella, although most cells have only one or two very long lateral flagella.

Ochrobactrum anthropi and O. intermedium have similar phenotypic properties. These species are strongly trypsin and pyrrolidonyl aminopeptidase positive and are saccharolytic, with rapid acidification of glucose and xylose. Acidification of mannitol is irregular and often delayed positive. Ethylene glycol is acidified. Urease is positive. Nitrate is reduced, and the vast majority of the strains also reduce nitrites.

Both Ochrobactrum species (Brucellaceae, Alphaproteobacteria) are closely related to Brucella species, with O. intermedium occupying a phylogenetic position that is intermediate between O. anthropi and Brucella (236).

O. anthropi has been isolated from various environmental and human sources, predominantly from patients with catheter-related bacteremias (93, 125, 183) and rarely with other infections (141). One hospital outbreak in transplant patients has been described (69). One case of pyogenic liver infection due to O. intermedium has been reported (145), but because of the close phenotypic similarity of O. anthropi and O. intermedium, it is possible that certain infections thought to be caused by O. anthropi were actually caused by O. intermedium.

O. anthropi strains are usually resistant to beta-lactams, such as broad-spectrum penicillins, broad-spectrum cephalosporins, aztreonam, and amoxicillin-clavulanate, but are usually susceptible to aminoglycosides, fluoroquinolones, imipenem, tetracycline, and trimethoprim-sulfamethoxazole (13, 125, 183). O. intermedium is resistant to colistin, while O. anthropi is susceptible.

#### Pannonibacter phragmitetus

*P. phragmitetus*, of the family *Rhodobacteraceae* (Alphaproteobacteria), has been shown to be identical to the strains formerly designated Achromobacter groups B and E (94). The species resembles most strongly *Rhizobium radiobacter*, but saccharolytic activity is somewhat weaker and not as extended; i.e., raffinose is not acidified and mannitol acidification is positive only for the strains belonging to the former Achromobacter group B. An easy and reliable differential test is the hydrolysis of tributyrin (diagnostic tablets; Rosco Diagnostica A/S, Taastrup, Denmark), which is positive within 30 min for *P. phragmitetus*, whereas *R. radiobacter* strains are positive only after several hours' to overnight incubation or remain negative. Ochrobactrum species do not hydrolyze tributyrin.

Cases of septicemia due to Achromobacter group B have been reported (88, 114).

#### Rhizobium radiobacter

The former genus Agrobacterium contained several species of plant pathogens occurring worldwide in soils. Four distinct species of Agrobacterium were recognized: A. radiobacter (formerly A. tumefaciens and CDC group Vd-3), A. rhizogenes (subsequently transferred to the genus Sphingomonas as Sphingomona rosa), A. vitis, and A. rubi (185). More recently, an emended description of the genus Rhizobium (Rhizobiaceae, Alphaproteobacteria) was proposed to include all species of Agrobacterium (252). Following this proposal the new combinations are *Rhizobium radiobacter*, *R. rhizogenes*, *R. rubi*, and *R. vitis* (252). Only *R. radiobacter* is clinically important.

Colonies of *R. radiobacter* are circular, convex, smooth, and nonpigmented to light beige on SBA, with a diameter of 2 mm at 48 h. Colonies may appear wet and become extremely mucoid and pink on MacConkey agar with prolonged incubation. *R. radiobacter* cells measure 0.6 to 1.0 by 1.5 to 3.0 µm and occur singly and in pairs.

*R. radiobacter* grows optimally at 25 to 28°C but grows at 35°C as well. *R. radiobacter* is phenotypically very similar to the *Ochrobactrum* species, although phylogenetically separate. *R. radiobacter* differs clearly from *Ochrobactrum* species by a positive beta-galactosidase test and by the production of ketolactonate, which is, however, not routinely tested. *R. radiobacter* has a broad saccharolytic activity, including mannitol and raffinose.

*R. radiobacter* has been most frequently isolated from blood, followed by peritoneal dialysate, urine, and ascitic fluid (64, 105). The majority of cases have occurred in patients with transcutaneous catheters or implanted biomedical prostheses, and effective treatment often requires removal of the device (66). Most strains are susceptible to broad-spectrum cephalosporins, carbapenems, tetracyclines, and gentamicin, but not to tobramycin (64, 241). Testing of individual isolates is recommended for clinically significant cases.

#### Shewanella algae and S. putrefaciens

The organisms formerly called *Pseudomonas putrefaciens*, *Alteromonas putrefaciens*, *Achromobacter putrefaciens*, and CDC group Ib have been placed in the genus *Shewanella* (140), which comprises over 50 species. *S. putrefaciens* was described with two CDC biotypes. CDC biotype 1 was later described as *S. putrefaciens* sensu stricto, whereas CDC biotype 2 was subsequently assigned to a new species, *S. alga* (158), later corrected to *S. algae*.

Colonies of *Shewanella* species on SBA are convex, circular, smooth, and occasionally mucoid, produce a brown to tan soluble pigment, and cause green discoloration of the medium. Cells are long, short, or filamentous, reminiscent of *Myroides*. Motility is due to a single polar flagellum.

Most strains of both *Shewanella* species produce  $H_2S$  in KIA and TSI agar, a unique feature among clinically relevant nonfermenters. Both are also ornithine decarboxy-lase positive and have strong alkaline phosphatase, strong trypsin, and strong pyrrolidonyl aminopeptidase activities. S. *algae* is halophilic, asaccharolytic, and requires NaCl for growth, with growth occurring already on TSA plus 0.5% NaCl. S. *putrefaciens* does not require NaCl for growth and is saccharolytic, producing acid from maltose and sucrose, and irregularly and weakly from glucose.

Khashe and Janda (126) have reported that *S. algae* is the predominant human clinical isolate (77%), while *S. putrefaciens* represents the majority of nonhuman isolates (89%). Although infrequently isolated in the clinical laboratory, *S. putrefaciens* and *S. algae* have been recovered from a wide variety of clinical specimens and are associated with a broad range of human infections, including skin and soft tissue infections (36), otitis media (99), ocular infection (28), osteomyelitis (20), peritonitis (46), and septicemia (110). The habitat for *S. algae* is saline, whereas *S. putrefaciens* has been isolated mostly from fish, poultry, and meats as well as from freshwater and marine samples.

Shewanella species are generally susceptible to most antimicrobial agents effective against gram-negative rods, except penicillin and cephalothin (70, 241). The mean MICs of *S. algae* for penicillin, ampicillin, and tetracycline are higher than the corresponding MICs of *S. putrefaciens* (126, 239).

#### Sphingobacterium

A total of 15 species have been described as belonging to the genus *Sphingobacterium*. Based on 16S rRNA gene sequence data, the indole-producing *Flavobacterium mizutaii* belongs to the genus *Sphingobacterium* (G. Wauters and M. Vaneechoutte, unpublished observation) and should be transferred to the genus *Sphingobacterium* as *Sphingobacterium mizutaii*. As a consequence, the description of the genus *Sphingobacterium* as indole negative will have to be emended.

The species of the genus *Sphingobacterium* encountered in clinical material include S. *multivorum* (formerly *Flavobacterium multivorum* and CDC group IIk-2), S. *spiritivorum* (including the species formerly designated as *Flavobacterium spiritivorum*, *F. yabuuchiae*, and CDC group IIk-3), S. *thalpophilum*, and *Flavobacterium mizutaii* (205, 249).

Colonies are yellowish. Sphingobacterium species are middle-sized, nonmotile gram-negative rods. Species of this genus do not produce indole, but *Flavobacterium mizutaii* is indole positive and is therefore dealt with among the indole-positive nonfermenters in Table 4. All species are strongly saccharolytic; i.e., glucose, xylose, and other sugars are acidified. No acid is formed from mannitol, except by S. *spiritivorum*, which is also the only species to produce acid from ethylene glycol. S. *thalpophilum* can be distinguished from other *Sphingobacterium* species by its nitrate reductase and its growth at 42°C.

S. *multivorum* and S. *spiritivorum* can be distinguished from *Sphingomonas paucimobilis* (formerly CDC group IIk-1) because they are nonmotile, urease positive, and resistant to polymyxin. Many strains of other *Sphingomonas* species are also colistin resistant.

S. multivorum is the most common human species. It has been isolated from various clinical specimens but has only rarely been associated with serious infections (peritonitis and sepsis) (73, 91). Blood and urine have been the most common sources for the isolation of S. spiritivorum (90). F. mizutaii has been isolated from blood, cerebrospinal fluid (CSF), and wound specimens (247). S. thalpophilum has been recovered from wounds, blood, eyes, abscesses, and an abdominal incision (247).

Sphingobacterium species are generally resistant to aminoglycosides and polymyxin B while susceptible in vitro to the quinolones and trimethoprim-sulfamethoxazole. Susceptibility to beta-lactam antibiotics is variable, requiring testing of individual isolates (197).

#### Sphingomonas Species

On the basis of 16S rRNA gene sequence and the presence of unique sphingoglycolipid and ubiquinone types, the genus Sphingomonas (Sphingomoadaceae, Alphaproteobacteria) was created for organisms formerly known as *Pseudomonas paucimobilis* and CDC group IIk-1 (89, 250). Since the original proposal, a total of almost 60 novel species, originating from various environments, have been added to the genus Sphingomonas. The former genus Sphingomonas can be divided into four phylogenetic groups, each representing a different genus (204), whereby the emended genus Sphingomonas contains at least 12 species, of which only S. *paucimobilis* and S. *parapaucimobilis* are thought to be clinically important. However, recent 16S rRNA gene sequencing

Characteristic	Balneatrix alpica (1)	Bergeyella zoohelcum (6)	Chryseobacterium anthropi (9)	Chryseobacterium gleum (11)	Chryseobacterium hominis (12)	Chryseobacterium indologenes (14)	Elizabethkingia meningoseptica (16)	Empedobacter brevis (7)	Flavobacterium mizutaii (4)	Wautersiella falsenii (45)	Weeksella virosa (10)
Motility	100	0	0	0	0	0	0	0	0	0	0
Beta-hemolysis (after 3 days on SBA)	0	0	0	0	0	100	0	0	0	0	0
Production of flexirubin pigments	0	0	0	100	0	72	0	0	0	0	0
Production of other pigments	PY	_	-/PS	_	-/PY	_	-/PY/PS	PY	PY	-/PY	_
Growth on MacConkey agar	0	0	0	100	0	66	88	100	0	100	0
Growth at 42°C	0	0	0	100	0	0	50	0	0	0	100
Acidification of:											
Glucose	100	0	100	100	100	100	100	100	100	100	0
Mannitol	100	0	0	0	0	0	100	0	0	0	0
Xylose	0	0	0	27	0	0	0	0	100	0	0
l-Arabinose	0	0	0	100	0	0	0	0	75	0	0
Maltose	100	0	100	100	100	100	100	100	100	100	0
Sucrose	0	0	0	0	33	21	0	0	100	0	0
Ethylene glycol	0	0	0	100	100	0	100	0	0	44	0
Esculin hydrolysis	0	0	0	100	100	100	100	0	100	55	0
Gelatinase	0	100	100	100	100	100	100	100	0	55/(45)	100
Urease	0	100	0	73	0	0	06	0	0	100	0
Nitrate reductase	100	0	0	73	75	28	0	0	0	0	0
Nitrite reductase	0	0	0	54	75	14	83	0	75	15	0
Beta-galactosidase (ONPG <sup>c</sup> )	0	0	0	0	0	0	100	0	100	31	0
Benzyl-arginine aminopeptidase (trypsin)	0	100	100	100	100	100	100	100	100	100	100
Pyrrolidonyl aminopeptidase Susceptibility to:	0	0	100	100	100	100	100	100	100	100	100
Desferrioxamine	100	100	78	0	0	0	0	0	0	12	100
Colistin/polymyxin	100	0	0	0	17	0	0	0	0	0	100

**TABLE 4** Oxidase-positive, indole-positive nonfermenters<sup>a</sup>

<sup>a</sup>Numbers in parentheses after organism names are numbers of strains tested. Values are percentages; those in parentheses represent delayed positivity. -, none; PY, pale yellow; PS, pale salmon-pink.

<sup>b</sup>Elizabethkingia miricola is urease positive (data from reference 127).

'ONPG, o-nitrophenyl-β-D-galactopyranoside.

of 12 strains of clinical origin (Wauters, unpublished) revealed that several named and unnamed *Sphingomonas* species were present, but no *S. paucimobilis* and only two *S. parapaucimobilis* isolates. Because many phenotypic characteristics are shared by these species, routine laboratories best report them as *Sphingomonas* species.

Sphingomonas colonies are slow growing on blood agar medium, with small colonies appearing after 24 h of incubation. Growth occurs at 37°C but not at 42°C, with optimum growth at 30°C. Almost all strains produce a yellow insoluble pigment, different from flexirubin pigments, as can be established by the KOH test (11). Few strains are nonpigmented or develop a pale yellow color after several days. Older colonies demonstrate a deep yellow (mustard color) pigment.

Sphingomonas species are medium to long motile rods with a single polar flagellum. Motility occurs at 18 to 22°C but not at 37°C. However, few cells are actively motile in broth culture, thus making motility a difficult characteristic to demonstrate. Oxidase is only weakly positive or even absent. All the strains are saccharolytic, but some acidify glucose only weakly and slowly. Urease is always negative, and nitrate reduction is only very rarely positive. Esculin is hydrolyzed, and beta-galactosidase and alkaline phosphatase are positive. The yellow pigment of some strains may hamper a correct reading of the yellow color shift when nitrophenyl compounds of the latter substrates are used.

Members of this genus are known as decomposers of aromatic compounds and are being developed for use in bioremediation.

Sphingomonas species are widely distributed in the environment, including water, and have been isolated from a variety of clinical specimens, including blood, CSF, peritoneal fluid, urine, wounds, the vagina, and the cervix, as well as from the hospital environment (103, 148, 175). S. *parapaucimobilis* clinical isolates have been obtained from sputum, urine, and the vagina (250).

Most strains are resistant to colistin, but all are susceptible to vancomycin, which is exceptional for gram-negative nonfermenting rods. This is elsewhere only found in *Chryseobacterium* and related genera like *Elizabethkingia* and *Empedobacter*. Most *Sphingomonas* strains are susceptible to tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole, and aminoglycosides. Susceptibility to other antimicrobial agents, including fluoroquinolones, varies (70, 103, 175).

#### **Oxidase-Positive, Indole-Positive Nonfermenters**

The natural habitats of most oxidase-positive, indole-positive nonfermenters (Table 4) are soil, plants, and food and water sources, including those in hospitals. Clinically relevant species include *Chryseobacterium* species, *Elizabethkingia meningoseptica*, *Empedobacter brevis*, *Wautersiella falsenii*, *Flavobacterium mizutaii*, *Weeksella virosa*, *Bergeyella zoohelcum*, and *Balneatrix alpica*. All are indole, trypsin, pyrrolidonyl aminopeptidase, and alkaline phosphatase positive, except for *B. zoohelcum*, which is pyrrolidonyl aminopeptidase negative, and *B. alpica*, which is both trypsin and pyrrolidonyl aminopeptidase negative. Table 4 presents an overview of the characteristics useful to differentiate among these species.

#### Balneatrix alpica

*B. alpica* was first isolated in 1987 during an outbreak of pneumonia and meningitis among persons who attended a hot (37°C) spring spa in southern France (51). Isolates from eight patients were recovered from blood, CSF, and sputum, and one was recovered from water. This species is only rarely isolated from human clinical specimens.

*B. alpica* produces colonies that are 2 to 3 mm in diameter, convex, and smooth. The center of the colonies is pale yellow after 2 to 3 days and pale brown after 4 days. *B. alpica* is a straight or curved gram-negative rod. It is the only motile species among the clinically relevant indole-positive nonfermenters. Cells have one or two polar flagella.

The species is strictly aerobic and saccharolytic. Both trypsin and pyrrolidonyl aminopeptidase are negative, unlike with other indole-positive nonfermenters. Growth occurs at 20 to 46°C on ordinary media such as TSA but not on MacConkey agar. It acidifies glucose, mannose, fructose, maltose, sorbitol, mannitol, glycerol, inositol, and xylose. *B. alpica* is nitrate reductase and weakly gelatinase positive. It is similar to *E. meningoseptica* but can be differentiated from this species by its motility and nitrate reductase and by the absence of beta-galactosidase.

*B. alpica* has been reported to be susceptible to penicillin G and all other beta-lactam antibiotics and to all aminoglycosides, chloramphenicol, tetracycline, erythromycin, sulfonamides, trimethoprim, ofloxacin, and nalidixic acid. It is resistant to clindamycin and vancomycin (30).

#### Bergeyella zoohelcum

Bergeyella zoohelcum and Weeksella virosa are morphologically and biochemically similar organisms with cells measuring 0.6 by 2 to 3  $\mu$ m, with parallel sides and rounded ends. *B. zoohelcum* colonies are sticky and tan to yellow.

Both species fail to grow on MacConkey agar and are nonsaccharolytic. Both species are susceptible to desferrioxamine and have the unusual feature of being susceptible to penicillin, a feature that allows them to be easily differentiated from the related genera *Chryseobacterium* and *Sphingobacterium*. B. zoohelcum can be differentiated from W. virosa because it is pyrrolidonyl aminopeptidase negative, strongly urease positive, and resistant to colistin. B. zoohelcum comprises formerly CDC group IIj strains (97).

B. zoohelcum is isolated mainly from wounds caused by animal (mostly dog) bites (97, 176). Meningitis or septicemia

due to *B. zoohelcum* has occurred in patients either bitten by a dog (146) or with continuous contact with cats (157).

Both *B. zoohelcum* and *W. virosa* are susceptible to most antibiotics. However, at present no specific antibiotic treatment is recommended, and antimicrobial susceptibility testing should be performed on significant clinical isolates.

#### Chryseobacterium

CDC group IIb comprises the species *Chryseobacterium indologenes*, C. *gleum*, and other strains, which probably represent several unnamed taxa.

Strains included in CDC group IIb are nonmotile rods. Cells of C. *indologenes* are similar to those of E. *meningoseptica*, C. *anthropi*, C. *hominis*, and F. *mizutaii*; i.e., they are thinner in their central than in their peripheral portions and include filamentous forms.

CDC group IIb strains are oxidase and catalase positive, produce flexirubin pigments (11, 168), are moderately saccharolytic, and are esculin and gelatin hydrolysis positive. *C. indologenes* and *C. gleum* can easily be differentiated from each other by four characteristics: *C. indologenes* displays a broad beta-hemolysis area within 3 days of incubation at 37°C on SBA, is always arabinose negative, does not acidify ethylene glycol, and does not grow at 42°C (214). *C. gleum* exhibits pronounced alpha-hemolysis, resembling viridans discoloration; always acidifies ethylene glycol; is arabinose positive or delayed positive; and grows at 42°C.

Beta-hemolysis is absent or very rare in other strains of CDC group IIb and is therefore almost specific for the identification of *C. indologenes*, while the profile of *C. gleum* may be shared by other strains of this group. It should be noted that some *C. indologenes* strains do not produce flexirubin.

Among CDC group IIb species, C. *indologenes* is usually considered most frequently isolated from clinical samples, although it rarely has clinical significance (241). It causes bacteremia in hospitalized patients with severe underlying disease, although the mortality rate is relatively low even among patients who were administered antibiotics without activity against C. *indologenes* (195). Nosocomial infections due to C. *indologenes* have been linked to the use of indwelling devices during hospital stays (7, 102, 159).

Still, the frequency of C. *indologenes* as reported in the literature should be interpreted with caution, because until recently and without molecular biology, C. *indologenes* could almost not be distinguished routinely from other CDC group IIb strains. We have recently examined 21 CDC group IIb strains both phenotypically and by 16S rDNA sequencing and found 9 C. *indologenes* isolates, 5 C. *gleum* isolates, and 7 isolates belonging to unnamed Chryseobacterium species.

The production of novel types of metallo-beta-lactamases from *C. indologenes* has been studied in detail (136, 166).

Chryseobacterium anthropi represents part of the strains formerly designated as CDC group IIe (120). Most strains display very sticky colonies, which are nonpigmented but may develop a slightly salmon-pinkish, rarely yellowish color after a few days. In contrast to C. hominis, the species is negative for esculin hydrolysis and acidification of ethylene glycol. In addition, many strains are susceptible to desferrioxamine. One case of meningitis caused by CDC group IIe has been reported (245). Most clinical isolates used for the description of the species were from wounds and blood cultures (120).

*Chryseobacterium hominis* includes the strains formerly included in CDC group IIc and most of the strains of CDC

group IIh (228). This species does not produce flexirubin pigments, but some strains exhibit a slightly yellowish pigmentation. Colonies are often mucoid. C. *hominis* can be differentiated from C. *gleum* by the absence of flexirubin pigments and the lack of acid production from arabinose. C. *indologenes* strains lacking flexirubin pigments may resemble C. *hominis*, but the latter is never beta-hemolytic and always acidifies ethylene glycol.

Many strains have been isolated from blood. Others have been isolated from dialysis fluid, pus, the eye, infraorbital drain, and aortic valve, but their clinical significance remains to be assessed (228).

#### Elizabethkingia meningoseptica and E. miricola

Colonies of *Elizabethkingia meningoseptica*, formerly *Chryseobacterium meningosepticum* (127), are smooth and fairly large, either nonpigmented or producing a pale yellow or slightly salmon-pinkish pigment after 2 or 3 days. Characteristic features are acid production from mannitol and beta-galactosidase activity. Gelatin and esculin hydrolysis are positive. *Elizabethkingia* and *Chryseobacterium* species can be differentiated as well on the basis of 16S rRNA sequence analysis (120, 127).

*E. meningoseptica* has been reported to be associated with (neonatal) meningitis and nosocomial outbreaks (14, 33, 38, 106, 195, 207), endocarditis (16), cystic fibrosis airway infections (131), retroperitoneal hematoma (133), community-acquired osteomyelitis (132), adult pneumonia and septicemia (14, 135, 192, 241), respiratory colonization and infection following aerosolized polymyxin B treatment (26), and infections reported in dialysis units (135, 165). A clinical case of *E. miricola* was reported only once, in a case of sepsis (77).

#### Empedobacter brevis

*Empedobacter brevis* (216) colonies are yellowish pigmented but do not produce flexirubin. *E. brevis* can be differentiated from *C. indologenes*, *C. gleum*, other CDC group IIb strains, and *C. hominis* by its lack of esculin hydrolysis. Growth on MacConkey agar and a stronger gelatinase activity are useful to distinguish it from *C. anthropi*. The species is rarely recovered from clinical material.

#### Flavobacterium mizutaii

*F. mizutaii* is saccharolytic, producing acid from a large number of carbohydrates, including xylose, similar to *Sphingobacterium* species, from which it can be distinguished by its indole production and by its failure to grow on MacConkey agar and its usual lack of urease activity (247).

*F. mizutaii* can be distinguished from *Chryseobacterium* and *Empedobacter* species by its lack of gelatin hydrolysis and of flexirubin production. *F. mizutaii* produces acid from xylose but not from ethylene glycol, allowing differentiation from other indole-positive species. The phenotypic profile of *F. mizutaii* is similar to that of the strains described as *Chryseobacterium* CDC group IIi. Furthermore, 16S rRNA gene sequencing confirms that most CDC group IIi strains actually belong to the species *F. mizutaii*.

*F. mizutaii* has been described as an indole-negative species (249), but in our hands all strains tested, including the type strain, produce as much indole as the *Chryseobacterium* strains. According to 16S rRNA gene sequencing, this species is closely related to *Sphingobacterium* species, indicating that *F. mizutaii*—formerly *Sphingobacterium mizutae* (98)—should be transferred back to the genus *Sphingobacterium* as *S. mizutaii*. *F. mizutaii* has been isolated from blood, CSF, and wound specimens (247).

#### Wautersiella falsenii

*Wautersiella falsenii* is closely related to *E. brevis*, from which it differs by its urease activity. Two genomovars have been described (119): genomovar 1 is always esculin positive and beta-galactosidase negative, whereas 90% of the genomovar 2 strains are esculin negative and 63% are beta-galactosidase positive.

W. falsenii was described as belonging to a separate genus from *Empedobacter*, based on comparison of its 16S rRNA gene sequence with an *E. brevis* EMBL sequence of poor quality. A high-quality sequence of the rRNA gene of the type strain of *E. brevis* indicates that W. falsenii probably has to be renamed as *Empedobacter falsenii*.

W. *falsenii* is much more frequently isolated from clinical samples than *E. brevis* (119). Its clinical significance remains to be assessed.

#### Weeksella virosa

W. virosa colonies are mucoid and adherent to the agar, reminiscent of the sticky colonies of B. zoohelcum. Colonies are not pigmented after 24 h of incubation but may become yellowish, tan to brown, after 2 or 3 days. The cellular morphology of Weeksella virosa is dealt with above in the discussion of Bergeyella zoohelcum. W. virosa can be differentiated from B. zoohelcum because it is urease negative and polymyxin B and colistin susceptible, whereas B. zoohelcum is rapid urease positive and polymyxin B and colistin resistant. W. virosa comprises formerly CDC group IIf strains (96). W. virosa is isolated mainly from urine and vaginal samples (96, 177), in contrast to B. zoohelcum, which is isolated mostly from animal bites.

The appropriate choice of effective antimicrobial agents for the treatment of chryseobacterial infections is difficult (106). Chryseobacterium species and E. meningoseptica are inherently resistant to many antimicrobial agents commonly used to treated infections caused by gram-negative bacteria (aminoglycosides, beta-lactam antibiotics, tetracyclines, and chloramphenicol) but are often susceptible to agents generally used for treating infections caused by grampositive bacteria (rifampin, clindamycin, erythromycin, trimethoprim-sulfamethoxazole, and vancomycin) (70, 197, 241). Although early investigators recommended vancomycin for treating serious infection with E. meningoseptica (83), subsequent studies showed greater in vitro activity of minocycline, rifampin, trimethoprim-sulfamethoxazole, and quinolones (14, 72, 197). Among the quinolones, levofloxacin is more active than ciprofloxacin and ofloxacin (197). C. *indologenes* is reported to be uniformly resistant to cephalothin, cefotaxime, ceftriaxone, aztreonam, aminoglycosides, erythromycin, clindamycin, vancomycin, and teicoplanin, while susceptibility to piperacillin, cefoperazone, ceftazidime, imipenem, quinolones, minocycline, and trimethoprim-sulfamethoxazole is variable, requiring testing of individual isolates (133, 197, 243). Several studies reported that administration of quinolone, minocycline, trimethoprim-sulfamethoxazole, or rifampin, and treatment of local infection improve the clinical outcome of patients with E. meningoseptica infections. The choice of appropriate antimicrobial therapy is further complicated by the fact that MIC breakpoints for resistance and susceptibility of chryseobacteria have not been established by the CLSI and the results of disk diffusion testing are unreliable in predicting antimicrobial susceptibility of Chryseobacterium species (35, 72, 243). The Etest is a possible alternative to the standard agar dilution method for testing cefotaxime, ceftazidime, amikacin, minocycline, ofloxacin, and ciprofloxacin but not piperacillin (101). Definitive therapy for clinically significant isolates should be guided by individual susceptibility patterns determined by an MIC method.

#### **Pink-Pigmented Nonfermenters**

It should be noted that colonies of *C. anthropi* and *E. meningoseptica* can be lightly salmon colored on some media after several days of incubation, but this is not to be confused with the clearly pink colonies of the taxa discussed here (Table 5).

#### Asaia

Asaia is a genus of the family Acetobacteraceae (Alphaproteobacteria), with some clinically relevant members, such as A. bogorensis (251) and A. siamensis (123). The natural habitats of Asaia species are reported to be the flowers of the orchid tree, plumbago, and fermented glutinous rice, all originating in hot tropical climates, particularly in Indonesia and Thailand.

Growth of Asaia species is scant to moderate on SBA. Colonies are pale pink. In opposition to Methylobacterium, colonies are not dark under UV light. Asaia species are small to middle-sized gram-negative rods, usually motile by one or two polar or lateral flagella. The species are oxidase negative and strongly saccharolytic; i.e., glucose, mannitol, xylose, and L-arabinose are acidified very rapidly, often within 1 h on low-peptone phenol red agar. Acid is also produced from ethylene glycol. Furthermore, Asaia species are biochemically rather inert, except for benzyl arginine aminopeptidase (trypsin) activity. Asaia species can be distinguished from Methylobacterium species by cell morphology, a stronger saccharolytic activity, and acid production from mannitol. Asaia bogorensis has been reported as a cause of peritonitis in a patient on automated peritoneal dialysis (196). Asaia species have been reported to be resistant to ceftazidime, meropenem, imipenem, trimethoprim, amikacin, vancomycin, aztreonam, penicillin, and ampicillin by disk diffusion (147). The *A. bogorensis* strain reported by Snyder et al. (196) was susceptible to aminoglycosides (amikacin, tobramycin, and gentamicin) and resistant to ceftazidime and meropenem by disk diffusion (196).

#### Azospirillum

The former Roseomonas genomospecies 3 (Roseomonas fauriae) and genomospecies 6 have been transferred to the genus Azospirillum (Rhodospirillaceae, Alphaproteobacteria), a genus of nitrogen-fixing plant symbionts that is in a different order of bacteria (43, 82). Some strains of this genus may occasionally be isolated from clinical material (179).

Colonies are pale pink and resemble those of *Roseomonas*. Cells are somewhat more rod shaped than *Roseomonas* and are motile by one or two polar flagella. Oxidase is positive and urea is strongly positive, as in *Roseomonas* species. A positive beta-galactosidase test and esculin hydrolysis allow differentiation of *Azospirillum* from other pink-pigmented species.

#### Methylobacterium

The genus *Methylobacterium*, of the family *Methylobacteriaceae* (*Alphaproteobacteria*), currently consists of 20 named species plus additional unassigned biovars, recognized on the basis of carbon assimilation type, electrophoretic type, and DNA-DNA homology grouping (74, 78, 213). *Methylobacterium* species are isolated mostly from vegetation but may also occasionally be found in the hospital

THE Pigmented no	mermenters							
Characteristic	Asaia spp. (2)	Azospirillum spp. (3)	Methylobacterium spp. (4)	Roseomonas cervicalis (2)	Roseomonas gilardii subsp. gilardii and Roseomonas gilardii subsp. rosea (5)	Roseomonas mucosa (8)	Roseomonas genomospecies 4 (1)	Roseomonas genomospecies 5 (3)
Oxidase	0	100	0	100	100 <sup>w</sup>	0	100	100 <sup>w</sup>
Acidification of:								
Glucose	100	0	(25)	0	(20)	100	(100)	0
Fructose	100	(100) <sup>w</sup>	0	50	60/(40)	100	100	(100)
Mannitol	100	0	0	0	(80)	100	0	0
Xylose	100	100	100	(100)	0	0	0	0
Arabinose	100	100	100	100	100	100	0	0
Ethylene glycol	100	100	100	100	100	100	0	0
Urease	0	100	100	100	100	100	100	100
Nitrate reductase	0	100	0	0	0	0	100	0
Esculin hydrolysis	0	100	0	0	0	0	0	0
Trypsin	100	0	100	0	0	0	0	0
Pyrrolidonyl aminopeptidase	0	100	0	0	100	100	0	0
Beta-galactosidase (ONPG) <sup>b</sup>	0	100	0	0	0	0	0	0
Colistin	0	0	0	0	40	50	0	33
Desferrioxamine	0	0	0	0	0	0	100	0

**TABLE 5** Pink-pigmented nonfermenters<sup>a</sup>

<sup>a</sup>Numbers in parentheses after organism names are numbers of strains tested. Values are percentages; those in parentheses represent delayed positivity. w, weak positive reaction.

<sup>b</sup>ONPG, o-nitrophenyl-β-D-galactopyranoside.



**FIGURE 4** Gram stain of *Methylobacterium* showing pleomorphic gram-negative rods with vacuoles.

environment. Methylobacterium mesophilicum (formerly Pseudomonas mesophilica, Pseudomonas extorquens, and Vibrio extorquens) and M. zatmanii have been the two most commonly reported species isolated from clinical samples.

Colonies of Methylobacterium species are small, dry, and coral pink. Under UV light, Methylobacterium colonies appear dark due to absorption of UV light (179). Growth is fastidious on ordinary media such as TSA, producing 1-mm-diameter colonies after 4 to 5 days on SBA, modified Thayer-Martin, buffered charcoal-yeast extract, and Middlebrook 7H11 agars, with the best growth occurring on Sabouraud agar and usually no growth on MacConkey agar. Optimum growth occurs between 25 and 30°C. They are able to utilize methanol as a sole source of carbon and energy, although this characteristic may be lost on subculture. Cells are pleomorphic, vacuolated rods that stain poorly and may resist decolorization (Fig. 4). Motility by one polar flagellum is difficult to demonstrate. In the description of the genus (163), methylobacteria were reported to be oxidase positive, but the strains tested by us were all oxidase negative with the dimethyl-paraphenylenediamine reagent. Saccharolytic activity is weaker than in Asaia spp., and no acid is produced from mannitol and acid is produced irregularly from glucose. Arabinose, xylose, and ethylene glycol are acidified. Urea and starch are hydrolyzed.

Methylobacterium species have been reported to cause septicemia, continuous ambulatory peritoneal dialysis-related peritonitis, skin ulcers, synovitis, and other infections often in immunocompromised patients, as well as pseudoinfections (100, 124, 184). Tap water has been implicated as a possible agent of transmission in hospital environments, and methods for monitoring water systems for methylobacteria have been described previously (178).

Active drugs include aminoglycosides and trimethoprimsulfamethoxazole, whereas beta-lactam drugs show variable patterns (27). They are best tested for susceptibility by agar or broth dilution at 30°C for 48 h (27).

#### Roseomonas

The original description of the genus Roseomonas (Acetobacteraceae, Alphaproteobacteria) included three named species, R. gilardii (genomospecies 1), R. cervicalis (genomospecies 2), and R. fauriae (genomospecies 3), and three unnamed species, Roseomonas genomospecies 4, 5, and 6 (179). Roseomonas genomospecies 3 and 6 have been transferred to the genus Azospirillum. More recently, Han et al. (82) proposed a new species, *Roseomonas mucosa*, and a new subspecies, *Roseomonas gilardii* subsp. *rosea* (to differentiate from *Roseomonas gilardii* subsp. *gilardii*).

The following Roseomonas species can be isolated from clinical samples: R. gilardii subsp. gilardii, R. gilardii subsp. rosea, R. mucosa, R. cervicalis, Roseomonas genomospecies 4, and Roseomonas genomospecies 5.

Colonies are mucoid and runny (Fig. 5) and grow larger than those of Asaia and Methylobacterium. Pigmentation varies from pale pink to coral pink. Roseomonas cells are nonvacuolated, coccoid, plump rods, mostly in pairs and short chains and usually motile by one or two polar flagella, but motility is often difficult to demonstrate. Genomospecies 5 is nonmotile. Growth occurs at 37°C on ordinary media like SBA, and mostly on MacConkey agar, but the best growth is observed on Sabouraud agar. Oxidase is dependent on the species and often weak. Saccharolytic activity is also species dependent. All Roseomonas species strongly hydrolyze urea but not esculin. They are trypsin and beta-galactosidase negative. Phenotypic distinction among the different species is based on oxidase, acid production from carbohydrates, and pyrrolidonyl aminopeptidase and nitrate reductase activities.

R. mucosa acidifies rapidly arabinose, mannitol, and fructose. Glucose is acidified within 1 to 3 days. Oxidase is negative, and pyrrolidonyl aminopeptidase is positive. R. gilardii subsp. gilardii and R. gilardii subsp. rosea exhibit a weak oxidase reaction and are pyrrolidonyl aminopeptidase positive. The two subspecies cannot be differentiated by current phenotypic tests. They are less saccharolytic than R. mucosa and produce acid from arabinose and fructose but only irregularly and slowly from mannitol and rarely from glucose. R. cervicalis is strongly oxidase positive but has no pyrrolidonyl aminopeptidase activity. Only arabinose and fructose are positive within 2 days and xylose within 3 or 4 days. The single strain of genomospecies 4 examined is also oxidase positive and pyrrolidonyl aminopeptidase negative, and its saccharolytic activity is limited to acid production from glucose and fructose. It is the only species displaying nitrate reduction. Genomospecies 5 is weakly oxidase positive and pyrrolidonyl aminopeptidase negative. It is the least saccharolytic species, with only a delayed acid production from fructose. Ethylene glycol is acidified by all species except by genomospecies 4 and 5.

Roseomonas species are uncommon isolates from humans, but they are nevertheless the most frequently isolated pink



FIGURE 5 Roseomonas on Sabouraud dextrose agar showing pink mucoid colonies.

nonfermenters. Clinical isolates have been recovered from blood, wounds, exudates, abscesses, genitourinary sites, continuous ambulatory peritoneal dialysis fluid, and bone (53, 143, 193, 200, 202). A total of 35 cases of opportunistic infections with *Roseomonas* species were reviewed (200). In multiple-case reports, about 60% of the isolates recovered have been from blood, with about 20% from wounds, exudates, and abscesses and about 10% from genitourinary sites (53, 200).

De et al. (53) summarized susceptibility data from three published reports on a combined 80 strains of *Roseomonas*. All strains were susceptible to amikacin (100%); frequently susceptible to imipenem (99%), ciprofloxacin (90%), and ticarcillin (83%); less susceptible to ceftriaxone (38%), trimethoprim-sulfamethoxazole (30%), and ampicillin (13%); and rarely susceptible to ceftazidime (5%). All strains were resistant to cefepime (53). In catheter-related infections, eradication of the organism has proven difficult unless the infected catheter is removed.

### ANTIMICROBIAL SUSCEPTIBILITIES

Decisions about performing susceptibility testing are complicated by the fact that the CLSI interpretive guidelines for disk diffusion testing of the nonfermenting gram-negative bacteria are limited to *Pseudomonas* species, *Burkholderia cepacia*, *Stenotrophomonas* maltophilia, and *Acinetobacter* species and therefore, except for *Acinetobacter* species, do not include the organisms covered in this chapter. Furthermore, results obtained with, e.g., *Acinetobacter* species by using disk diffusion do not correlate with results obtained by conventional MIC methods. In general, laboratories should try to avoid performing susceptibility testing on the organisms included in this chapter. When clinical necessity dictates that susceptibility testing be performed, an overnight MIC method, e.g., Etest (bioMérieux) (101), is recommended.

## EVALUATION, INTERPRETATION, AND REPORTING OF RESULTS

Although certain nonfermenting bacteria can on occasion be frank pathogens, e.g., Pseudomonas aeruginosa, Burkholderia pseudomallei, and Elizabethkingia meningoseptica, they are generally considered to be of low virulence and often occur in mixed cultures, making it difficult to determine when to work up cultures and when to perform susceptibility studies. Elizabethkingia meningoseptica in neonatal meningitis, Moraxella lacunata in eye infections, and M. *catarrhalis* in respiratory tract infections should be reported as significant pathogens. Direct Gram stain interpretation of clinical specimens may be of limited importance, because these organisms often occur in mixed infections and because their clinical importance has to be interpreted taking into account the considerations discussed below. Decisions regarding the significance of GNF in a clinical specimen must take into account the clinical condition of the patient and the source of the specimen submitted for culture. In general, the recovery of a GNF in pure culture from a normally sterile site warrants identification and susceptibility testing, whereas predominant growth of a GNF from a nonsterile specimen, such as an endotracheal culture from a patient with no clinical signs or symptoms of pneumonia, would not be worked up further. Because many GNF exhibit multiple-antibiotic resistance, patients who are on antibiotics often become colonized with GNF. GNF species isolated in mixed cultures can usually be reported by descriptive identification, e.g., "growth of *P. aeruginosa* and two varieties of nonfermenting gram-negative rods not further identified."

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