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., *Acinetobacter*, *Moraxella*, *Oligella*, and *Psychrobacter* belong to the family *Moraxellaceae* (*Gammaproteobacteria*) (1), and *Balneatrix*, *Bergeyella*, *Chryseobacterium*, *Elizabethkingia*, *Empedobacter*, *Myroides*, *Sphingobacterium*, *Wautersiella*, and *Weeksella* belong to the family *Flavobacteriaceae* (*Bacteroidetes*) (2).

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 (3). No person-to-person spread has been documented for the species covered in this chapter, except for *Acinetobacter* and *Moraxella catarrhalis*.

CLINICAL SIGNIFICANCE

Although for almost all of the species in this chapter, case reports, e.g., of 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), *M. catarrhalis* (respiratory tract infections), and species of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex.

A. baumannii ventilator-associated pneumonia and bloodstream infections have been documented to be associated with a high degree of mortality and morbidity (4). Particular manifestations of *A. baumannii* are its implication in severely war-wounded soldiers (5), from which stems its popular designation "*Iraqibacter*," and in victims of natural disasters (6).

The clinical impact of infections with *A. baumannii* is a continuous source of debate (7, 8). 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. Still, 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 (9, 10).

The clinical role of the closely related species *Acinetobacter pittii* and *Acinetobacter nosocomialis* resembles that of *A. baumannii*, although, compared to these two species, *A. baumannii* is more often associated with multidrug resistance and epidemic spread in hospitals and possibly also with higher mortality among patients with systemic infections (11, 12).

Other *Acinetobacter* species occasionally associated with human infections are listed in Table 1. *A. johnsonii*, *A. lwoffii*, and *A. radioresistens* seem to be common inhabitants of human skin (13). *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 (14). Several species, *A. ursingii* in particular, have been found to cause bloodstream infections in hospitalized patients (15–18), while *A. junii* and *A. soli* have also been implicated in outbreaks of neonatal infections (19, 20). *A. parvus* is regularly isolated from blood cultures (18, 21), but is often overlooked because of its small colonies and misidentification by API 20NE as *A. lwoffii* (our unpublished data). Many of the infections with these species are related to intravascular catheters or have another iatrogenic

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TABLE 1 Oxidase-negative, indole-negative, nonfermentative, Gram-negative rods: the genus *Acinetobacter*[a]

Characteristic	*A. baumannii* (25)[b,c]	*A. calcoaceticus* (11)[c]	*A. nosocomialis* (20)[c]	*A. pittii* (20)[c]	*A. beijerinckii* (16)	*A. berezinae* (16)	*A. guillouiae* (17)	*A. gyllenbergii* (9)	*A. haemolyticus* (17)	*A. junii* (15)	*A. johnsonii* (20)	*A. lwoffii* (14)	*A. parvus* (10)	*A. radioresistens* (12)	*A. schindleri* (22)	*A. soli* (8)	*A. ursingii* (30)
Growth at:																	
44°C	100[d]	0	95	10	0	0	0	0	0	0	0	0	0	0	0	0	0
41°C	100	9	100	100	0	0	0	0	94	87	0	7	0	100	100	88	0
37°C	100	100	100	100	100	100[e]	0[e]	100	100	100	25[f]	100	90	100	100	100	100
Acidification of D-glucose	100	91	100	95	0	88	0	0	82	0	0	7	0	0	0	100	0
Assimilation of:																	
Adipate	88	100	95	100	0	63	100	100	0	0	0	79	0	92	38	100	100
β-Alanine	100	91	85	90	0	100	94	100	0	0	0	0	0	0	0	100	0
4-Aminobutyrate	100	100	100	100	100	100	88	/[g]	100	87	75	79	0	100	0	100	0
L-Arginine	100	100	100	100	0	0	0	100	88	93	70	0	0	83	0	100	0
L-Aspartate	100	100	100	100	100	100	100	0	18	27	75	0	0	0	0	100	97[f]
Azelate	88	100	95	100	0	63	100	100	0	0	0	100	0	100	64	100	100
Citrate (Simmons)	100	100	100	100	100	100	100	100	82	80	85	14	0	0	59	100	97
D-Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0
Glutarate	96	91	95	90	0	100	100	/[g]	0	0	0	0	0	100	95	88	97
Histamine	0	0	0	0	0	63	65	0	0	0	0	0	0	0	0	0	0
L-Histidine	96	100	100	100	100	94	94	100	100	93	0	0	0	0	0	100	0
DL-Lactate	100	100	100	100	0	100	100	100	0	93	100	93	0	100	100	100	100
Malonate	88	100	20	95	100	0	18	78	0	0	50	7	0	100	0	100	0
Phenylacetate	84	100	85	75	0	25	65	100	0	0	0	71	0	100	0	100	0
Gelatinase	0	0	0	0	13	0	0	100	88	0	0	0	0	0	0	0	0
Hemolysis of sheep blood	0	0	0	0	100	0	0	100	100	47	25[f]	0	0	0	0	75	0

[a]All data were provided by one of the authors (A. Nemec) using standardized tests described in detail previously (67).
[b]Numbers in parentheses after organism names are numbers of strains tested.
[c]Species of the A. *calcoaceticus*-A. *baumannii* complex (65).
[d]The numbers are percentages of strains positive in a given test. Carbon assimilation tests were evaluated after 6 days of incubation at 30°C.
[e]Growth tested at 38 instead of 37°C.
[f]Weak growth of most strains with positive reactions.
[g]Unreproducible or delayed growth of most strains.

origin (15, 22), and their course is generally benign. For various other named or yet-unnamed *Acinetobacter* species, although recovered from clinical specimens (23, 24), 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) (25–27), 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 (25). 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 (28). 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 (28) 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. Furthermore, M. *nonliquefaciens* (29, 30) and M. *osloensis* (31, 32) 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 (33) and from debilitated patients (27). M. *lacunata* has been involved in eye infections and in infective endocarditis (34, 35).

COLLECTION, TRANSPORT, AND STORAGE OF SPECIMENS

Standard methods for collection, transport, and storage of specimens as detailed in chapters 11 and 18 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; these should be cultured on media containing blood.

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., MacConkey agar, is variable and may be influenced by lot-to-lot variations in the composition of media. Gram-negative, nonfermentative bacteria (GNF) 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. (36) and for *Moraxella* spp. (37).

IDENTIFICATION

This chapter provides 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. This key is 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 and 3 to 6, which provide further keys to identify the species of these five groups on the basis of biochemical reactions. Results for enzymatic reactions can 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. 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.

Although several of the genera discussed in this chapter 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 in this chapter, except those of the genus *Acinetobacter*, the biochemical tests listed have been carried

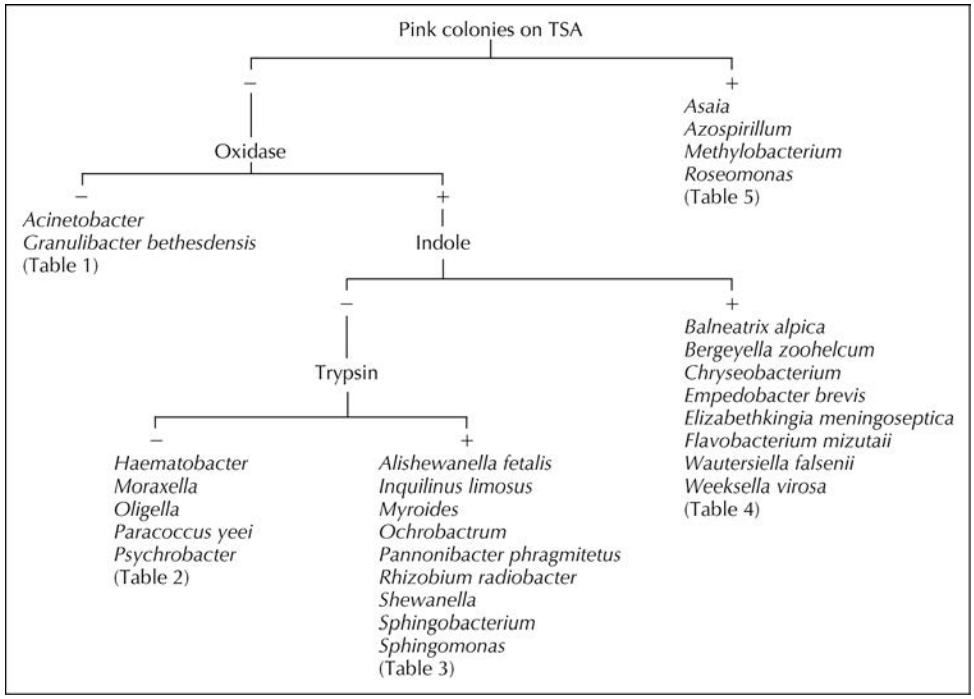

FIGURE 1 Identification of miscellaneous GNF. The organisms covered in this chapter belong to a group of taxonomically diverse, Gram-negative, nonfermentative rods and coccobacilli. They all share the common phenotypic features of failing to acidify the butt of KIA or TSI agar or of oxidative-fermentative 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 42) and those of the species of genera that previously were named as *Pseudomonas* (chapter 43). *a*, *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.
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out by one of us (G. Wauters), according to standardized protocols, described in detail in chapter 33. 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 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 oxidation-fermentation 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 GNF 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 (38), 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. (39) compared 16S rRNA gene sequencing for the identification of clinically relevant isolates of GNF (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 *Enterobacteriaceae*. However, for rare organisms the performance of these systems can be poor.

Chemotaxonomic Methods

The fatty acid profiles for the most common species of GNF have been published (40).

Matrix-Assisted Laser Desorption Ionization–Time-of-Flight Mass Spectrometry

Few developments in clinical bacteriology have had as rapid and profound an impact on identification of microorganisms as matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) (41, 42). Conventional identification methods rely on biochemical properties and require sometimes lengthy incubation procedures, whereas MALDI-TOF MS can identify bacteria within minutes directly from colonies grown on culture plates. Two commercial platforms, Bruker Biotyper (Bruker Daltonics, Billerica, MA) and VITEK MS (bioMérieux), are available, and although the initial cost of mass spectrometry equipment and maintenance costs are relatively high, the additional identification costs per isolate are minimal.

Studies on the reliability and accuracy of MALDI-TOF MS in identifying species dealt with in this chapter are summarized in Table 2. MALDI-TOF MS appears to be a promising tool for identification of these species, with a reported 80.4% correct species identifications and 92.3% correct genus identifications (43, 44), with most difficulties in obtaining a correct identification due to a missing spectrum in the database at that time. These problems can be overcome by creating new databases or by adding missing spectra (43, 44).

The accuracy of both MALDI-TOF MS systems was found to be similar for identifying GNF (45), and both systems demonstrated performance superior to that of conventional methods (45). Mellmann and coworkers (46) reported 98.75% interlaboratory reproducibility among eight laboratories in a study of MALDI-TOF MS for identifying 480 GNF isolates. Sedo et al. (47) successfully applied MALDI-TOF MS for identification of selected *Acinetobacter* species, but could not differentiate between different strains belonging to the same species. Besides bacterial identification, MALDI-TOF MS is also being explored for the phenotypic detection of certain antibiotic resistance mechanisms, e.g., the detection of carbapenemase enzymatic activity in *A. baumannii* strains (48), and for typing.

Schaller and coworkers (49) found MALDI-TOF MS to be a rapid and robust tool for typing the two subpopulations and three 16S rRNA types of *M. catarrhalis* described previously. Mencacci and coworkers (50) explored the potential utility of MALDI-TOF MS to detect nosocomial spread of multidrug-resistant *A. baumannii* outbreaks in comparison with the repetitive sequence-based PCR DiversiLab system (bioMérieux) and suggested that it shows promise in routine clinical microbiology.

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 recombination A protein (*recA*), have become standard techniques to identify bacteria in general (51) 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 (52). DNA sequence-based fingerprinting methods like amplified ribosomal DNA (rRNA gene) restriction analysis (ARDRA) (53, 54) and tDNA PCR (55, 56) 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

Members of the genus *Acinetobacter* are strictly aerobic, oxidase-negative, catalase-positive, coccobacillary bacteria.

TABLE 2 Accuracy of MALDI-TOF MS compared to other identification methods[a]

Species	MALDI-TOF MS result	Reference(s)
Acinetobacter baumannii (141)[b,c,d,e,f]	*A. baumannii* (135),[g,h] *Acinetobacter* sp. (6)[g,i]	42, 126, 201–203
A. beijerinckii (3)[e,j]	*A. beijerinckii* (2), *A. tjernbergiae* (1)	47, 201
A. berezinae (18)[e,j]	*A. berezinae* (11),[k] A. genomic species 3 (7)	47, 201
A. calcoaceticus (11)[b,d,e]	*A. calcoaceticus*, *Acinetobacter* sp. (8),[g] A. genomic species 3 (2)	42, 201, 202
A. genomic species 3 (8)[e,j]	A. genomic species 3 (8)	201
A. genomic species 13TU (3)[e,j]	*Acinetobacter* sp., *A.baumannii* (2)	201
A. genomic species 14BJ (2)[d,e,j]	A. genomic species 3, *A. pittii*[l]	42, 201
A. genomic species 17[d,j]	*Acinetobacter* sp.	42
A. guillouiae (11)[e]	*A. guillouiae* (11)	47
A. haemolyticus (3)[d]	*A. haemolyticus* (3)	42, 47
A. johnsonii (5)[b,f]	*A. johnsonii* (2),[g,h] *A. junii*,[g] *Acinetobacter* sp.[g,h]	126, 202–203
A. junii (7)[d,e,f]	*A. junii* (7)[g,h]	42, 126, 201
A. lwoffii (8)[c,d,f]	*A. lwoffii* (6),[g,h,i] *Acinetobacter* sp.,[g] *Ochrobactrum anthropi*[g]	42, 126
A. nosocomialis (18)[d]	*A. nosocomialis* (18)[m]	42
A. pittii (16)[d]	*A. pittii* (16)	42
A. radioresistens (4)[b,d,f,j]	*A. radioresistens* (3),[g,h] misidentification[n]	42, 126, 202
A. schindleri[f]	*A. schindleri*[g,h]	126
A. soli[e,j]	*A. baylyi*	201
A. ursingii (2)[f]	*A. ursingii*,[g,h] *Acinetobacter* sp.[g,h]	126
Acinetobacter sp.[b]	*A. junii*/*A. haemolyticus*[g]	202
Chryseobacterium gleum[b]	No identification[g,o]	203
C. indologenes (6)[b,f]	*C. indologenes* (5),[g] no identification (1)[k,o]	44, 45, 203
Chryseobacterium spp. (1)[b]	*C. indologenes* (1)[g]	203
Elizabethkingia meningoseptica (3)[b,f]	*E. meningoseptica* (2),[g,h] *E. miricola* (1)[k]	44, 45, 126
Moraxella catarrhalis (18)[b]	*M. catarrhalis* (18)[g]	202
Ochrobactrum anthropi (29)[b,f]	*O. anthropi* (6),[g,k] *Ochrobactrum* sp. (21),[g,k] *O. tritici* (2)[g,k]	203; Wauters (unpublished)
O. intermedium (9)[b,f]	*O. intermedium* (9)[g,k]	Wauters (unpublished)
Ochrobactrum spp.[b]	*Ochrobactrum anthropi*[g]	203
Oligella spp.[b]	*Oligella urethralis*[g]	203
Psychrobacter phenylpyruvicus[f,j]	No identification[g]	126
Rhizobium radiobacter (3)[b,f]	*R. radiobacter* (3)[g,h]	126, 203
Roseomonas mucosa[f,j]	Misidentification[g,h]	126
Shewanella algae[f,j]	No identification[g,h]	126
Sphingobacterium spiritivorum[b]	*S. spiritivorum*[g]	203
No identification (5)[b,f]	*Acinetobacter* sp. (2),[k] *Wohlfahrtiimonas chitiniclastica* (3)[g]	44; Wauters (unpublished)

[a]All identifications obtained with the Bruker MALDI-TOF MS system, except for those of reference 45, which compared the Bruker and VITEK systems. Numbers in parentheses after organism names are numbers of strains tested.
[b]Identification by conventional methods, with discrepant results resolved by 16S rRNA gene sequencing.
[c]Conventional biochemical identification.
[d]Identification by ARDRA (61), rRNA intergenic spacer (ITS), *recA* sequencing, and/or bla_{OXA-51} PCR.
[e]Identification by bla_{OXA-51} PCR and/or *rpoB* gene sequencing.
[f]Identification by 16S rRNA gene sequencing.
[g]Direct transfer method.
[h]Identification also taking into account the rule of thumb that the difference between the first two species listed is larger than 0.2 if the first log score is <1.7.
[i]Ethanol/formic acid extraction.
[j]Not present in current database or in database at time of research.
[k]Using own MALDI-TOF MS database.
[l]*Acinetobacter* gen. sp. 14BJ by ARDRA and *recA* sequencing, but *A. pittii* by ITS sequencing and MALDI-TOF MS.
[m]After including *A. nosocomialis* in database.
[n]Identified to the wrong genus level.
[o]Log score of <1.7.

They are Gram negative but may be difficult to destain. Most strains do not reduce nitrate to nitrite in a laboratory test, owing to the lack of a dissimilative nitrate reductase. Tween 80 esterase activity is frequently present, while hemolysis and gelatinase production varies. Swimming motility is negative, but "twitching motility" on soft agar may occur. Individual cell sizes are 0.9 to 1.6 μm in diameter and 1.5 to 2.5 μm in length. In the stationary phase, the cells are usually coccoid and occur in pairs. Most strains grow at between 20 and 35°C. Medically relevant species commonly grow well at 37°C, although some of them (*A. johnsonii* and *A. guillouiae*) show reduced or no *in vitro* growth at this temperature.

The organisms can form a pellicle (biofilm) on the surface of fluid media. They grow well on complex media, including blood agar, nutrient agar, and mostly on MacConkey agar. After 24 h of incubation, colonies are 1 to 2 mm in diameter (except for *A. parvus*, with markedly smaller colonies), 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 organic compounds as single sources of carbon and energy. Selective enrichment can be

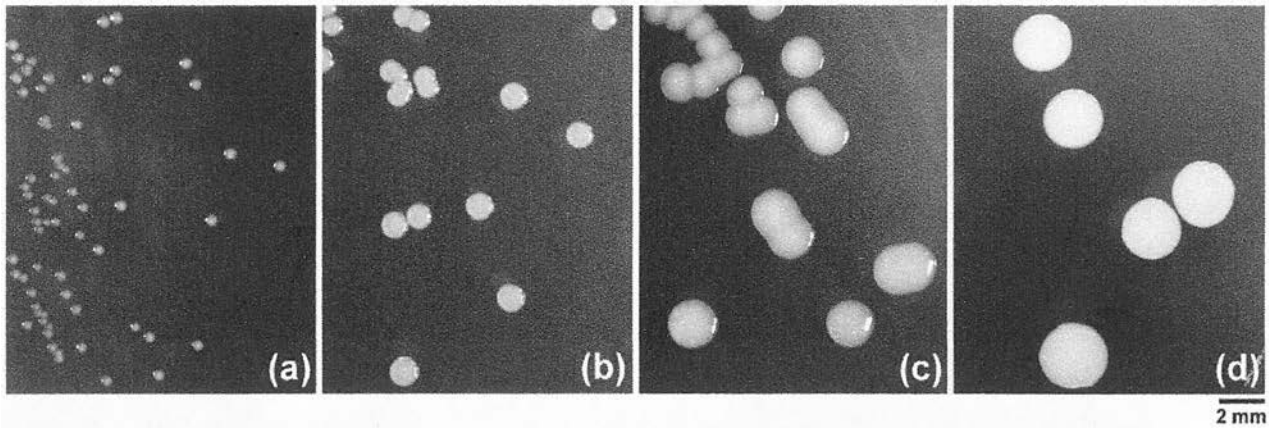

FIGURE 2 Differences in the size of the colonies formed by different *Acinetobacter* species isolated from human clinical specimens. The strains were grown on TSA (Oxoid) at 30°C for 24 h. (a) *A. parvus* NIPH 384^T; (b) *A. ursingii* NIPH 137^T; (c) *A. schindleri* NIPH 1034^T; (d) *A. baumannii* ATCC 19606^T. doi:10.1128/9781555817381.ch44.f2

obtained in mineral media with acetate as the carbon source and ammonium salt as the nitrogen source with shaking incubation at 30°C (36). General features of *Acinetobacter* have been reviewed previously (57, 58).

Members of the genus *Acinetobacter* are widespread in nature and have been found in soil, water, sewage, and food, as well as in human and animal specimens. Some of the *Acinetobacter* species have been predominantly cultured from humans (e.g., *A. baumannii*, *A. nosocomialis*, and *A. ursingii*), while others inhabit diverse ecosystems (*A. johnsonii* and *A. guillouiae*) or are confined to specific niches (*A. nectaris* and *A. boissieri*) (59).

The ecology of most clinically relevant *Acinetobacter* species is still poorly resolved. *A. baumannii*, *A. pittii*, *A. nosocomialis*, and *A. ursingii* have been mainly recovered from clinical specimens in hospitals. Human skin carrier rates of *A. baumannii* outside hospitals are low (13), but higher rates (also for *A. pittii* and *A. nosocomialis*) have been found in tropical areas (60). *A. baumannii*, including multidrug-resistant strains, has been isolated from sick animals (61) and from hospitalized horses (62), 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 (63), 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 (64).

Species Diversity and Identification

The taxonomy of the genus *Acinetobacter* has recently been updated with valid names for several medically relevant genomic species known previously under provisional designations, i.e., *A. pittii* (65), *A. bereziniae* (66), *A. guillouiae* (66), and *A. nosocomialis* (65) (former genomic species 3, 10, 11, and 13TU, respectively), and with new medically relevant species, i.e., *A. beijerinckii* (67), *A. gyllenbergii* (67), *A. parvus* (21), and *A. soli* (20, 68). Some of the recently proposed species names have been shown to be synonymous with already existing ones: *A. grimontii* with *A. junii* (69) and "*A. septicus*" with *A. ursingii* (70).

At present, the genus *Acinetobacter* comprises 31 distinct species with valid names (http://www.bacterio.net/acinetobacter.html) and a number of taxa that include either genomic species delineated by DNA-DNA hybridization (7, 57) or species with effectively (but not validly) published names. Recent studies have shown that nearly all *Acinetobacter* isolates from colonized or infected patients belong to one of these species (14, 17, 18). This indicates that the species diversity of acinetobacters found in the human body environment is well covered by the current classification. Validly named species found in human clinical specimens are listed in Table 1.

Surprisingly, and in spite of its name (derived from the Greek *akineto*, meaning "motionless" or "nonmotile") and its lack of flagella, *A. baumannii* spreads rapidly over certain surfaces. This is likely the result of twitching motility, a form of surface translocation described before for the genus *Acinetobacter* (71). As shown for *A. baumannii* ATCC 17978, cultured at 24°C, white (low-intensity) light, and in particular its blue component, is a stimulus governing several metabolic processes, inhibiting motility and the formation of biofilms and pellicles (72). Light regulation is lost at 37°C in *A. baumannii* (72).

Further studies showed that light regulation is not restricted to *A. baumannii*, but widespread within the genus *Acinetobacter* (73). In fact, blue light modulates motility and biofilm formation in many species of the genus. In the *Acinetobacter* species studied thus far, except *A. baumannii*, blue light contributes to the decision between motility and sessility and also may facilitate acclimation to different environments. For *A. baumannii*, both motility and sessility (biofilm formation) were inhibited by blue light and only at low temperature (72).

Identification of *Acinetobacter* species by commercial identification systems based on biochemical and physiological properties is problematic. This stems from the small number of relevant characters included in these systems and/or from the insufficient quality of reference data in the identification matrices. *A. baumannii* and the other species of the *A. calcoaceticus-A. baumannii* complex (i.e., *A. pittii*, *A. nosocomialis*, and *A. calcoaceticus*, the latter a primarily soil-dwelling organism) are generally not differentiated by these systems, while non-glucose-oxidizing species such as *A. ursingii* and *A. schindleri* can be misidentified (15). 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). Nonetheless, the diagnostic systems can be useful for genus-level identification and, when supplemented with testing growth ability at 44°C, also for presumptive differentiation between *A. baumannii* and *A. nosocomialis* (Table 1).

More reliable phenotypic identification of *Acinetobacter* species can be achieved using physiological and nutritional (mainly carbon source assimilation) tests based on the modified system of Bouvet and Grimont (67). Table 1 presents an update of this system aimed to differentiate validly named species of clinical relevance. Assimilation tests were carried out using a basal liquid medium supplemented with 0.1% (wt/vol) carbon source (67). Growth was evaluated after 4, 6, and 10 days of culture at 30°C by means of visual comparison between inoculated tubes containing carbon sources and control tubes containing only inoculated basal medium. However, 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 routine diagnostics.

The most precise identification of *Acinetobacter* species can be achieved by a number of genotypic methods (57), with those based on sequencing particular genes being the current standard. Common targets for this purpose are the *rpoB* gene (67), currently the best-studied single-gene taxonomic marker for *Acinetobacter*; the 16S rRNA gene sequence (74); and the 16S-23S rRNA gene spacer region (75). PCR detection of the $bla_{\text{OXA-51}}$-like gene, which is intrinsic to *A. baumannii*, enables rapid identification of this species (76). The whole-genome sequences of >100 *Acinetobacter* strains reflecting the currently known breadth of *Acinetobacter* diversity have been recently published at the NCBI website (http://www.ncbi.nlm.nih.gov/bioproject/183623), and the availability of such a comprehensive set of genomic data may further facilitate efficacious identification.

Genotyping and Epidemiology

A number of molecular 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 typing within one laboratory, and its (interlaboratory) reproducibility has been evaluated (77). Macrorestriction analysis with pulsed-field gel electrophoresis allowed for 95% intra- and 89% interlaboratory reproducibility (78). Amplified fragment length polymorphism fingerprinting also enables genotyping of strains (57, 63), and its robustness makes it suited for setting up a local database for longitudinal studies. Typing based on the variable number of tandem repeats has allowed for discrimination between genotypically related but epidemiologically distinct strains, i.e., those belonging to a single type based on several typing methods including macrorestriction analysis and PCR fingerprinting (79, 80). Sequence analysis of the OXA-51-like gene has been proposed as a useful typing method, based on the correlation between particular OXA-51-like enzymes and epidemic clones of *A. baumannii* (81).

Multilocus sequence typing (MLST) is the standard for global and long-term epidemiological and population studies. Two MLST schemes are currently available for *A. baumannii*: the scheme proposed by Bartual et al. (82) (http://pubmlst.org/abaumannii/) and that proposed by Diancourt et al. (83) (http://www.pasteur.fr/recherche/genopole/PF8/mlst/). Both schemes are based on the internal fragments of seven housekeeping genes, and they share three genes. The two schemes yield compatible results, although a higher resolution has been reported for the scheme of Bartual et al. (82) by Karah et al. (84).

Resistance to multiple antimicrobials and hospital outbreaks have been associated with several international lineages of *A. baumannii*, particularly with the so-called European (or international) clones I and II (7, 83, 85). The earliest known strain of clone I was isolated in 1977 (86), and this lineage prevailed among outbreak and multidrug-resistant *Acinetobacter* strains in some European countries in the 1980s and 1990s (87). Currently, strains belonging to or related to clone II seem to dominate in the current global population of multidrug-resistant *A. baumannii*, although clone I and other lineages can be common or even prevail in some regions (84, 88–90). Allocation of isolates to international lineages or the identification of novel clones can be achieved by both MLST schemes. European clones I and II correspond, respectively, to MLST-based clonal complexes CC1 and CC2 in the scheme of Diancourt et al. (83) and to CC109 and CC92 in that of Bartual et al. (82), according to Karah et al. (84). Multiplex PCR, targeting the *ompA*, *csuE*, and $bla_{\text{OXA-51-like}}$ gene sequences, provides rapid assignment to the main clonal lineages (91).

Antimicrobial Susceptibilities

A. baumannii has an extraordinary capacity to acquire or develop resistance to virtually all antibiotics used in the treatment of *Acinetobacter* infections (7, 8). With the growing proportion of *A. baumannii* strains resistant to carbapenems, a last therapeutic option is disappearing, although these strains mostly remain susceptible to colistin. Even though multidrug resistance is mainly confined to *A. baumannii*, strains of other clinically relevant species, such as *A. pittii*, *A. nosocomialis*, and *A. ursingii*, can also be resistant to multiple antibiotics, including carbapenems (92, 93). 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 resistance mechanisms have been reviewed previously (94). Recent genomic studies have also shed light on the genetic organization of resistance determinants in multidrug-resistant *A. baumannii*. Large clusters of horizontally transferred genes, conferring resistance to multiple antibiotics and interspersed with mobile genetic elements (resistance islands), were found to be integrated at a specific genomic site in a number of strains (87, 95). Despite the high intraclonal variability of the resistance islands, these structures seem to have evolved independently in two main *A. baumannii* clones (87, 96).

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) (97), or by Etest. The panel of tested antibiotics should cover the spectrum of agents with potential against *A. baumannii*, including third- or fourth-generation cephalosporins, sulbactam, antipseudomonal penicillins combined with β-lactamase inhibitors, carbapenems, aminoglycosides, fluoroquinolones, polymyxins, and tetracyclines (98). Of note, susceptibility to colistin should not be tested by disk diffusion due to poor diffusion of this compound in agar. Etest and broth microdilution for determination of the MIC for this drug have been compared and showed a good concordance in the MIC range of 0.25 to 1 mg/liter (99).

Granulibacter bethesdensis

G. bethesdensis (*Acetobacteraceae*, *Alphaproteobacteria*) (100) 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 DNA base composition is 59.1 mol% G+C. It was isolated first from three patients with chronic granulomatous disease (100) and more recently from an additional patient with chronic granulomatous disease (101).

Oxidase-Positive, Indole-Negative, Trypsin-Negative GNF

Haematobacter

Three *Haematobacter* species (*Rhodobacteraceae*, *Alphaproteobacteria*) have been described, i.e., *H. massiliensis* (formerly *Rhodobacter massiliensis*), *H. missouriensis*, and *Haematobacter* genomic species 1 (Table 3) (102). These species cannot easily be differentiated phenotypically, and even the 16S rRNA gene sequences are closely related. *Haematobacter* species were described as asaccharolytic, but when low-peptone phenol red agar is used (see chapter 33), *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 Centers for Disease Control and Prevention (CDC) have been mainly isolated 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 ~20 species that have been validly named. M. *catarrhalis*, M. *osloensis*, M. *nonliquefaciens*, and M. *lincolnii* are part of the normal microbiome 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,

TABLE 3 Oxidase-positive, indole-negative, trypsin-negative, coccoid GNF[a]

Characteristic	*Haematobacter massiliensis* (10)	*Haematobacter missouriensis* (3)	*Moraxella atlantae* (7)	*Moraxella canis* (5)	*Moraxella catarrhalis* (7)	*Moraxella lacunata* (5)	*Moraxella lincolnii* (2)	*Moraxella nonliquefaciens* (16)	*Moraxella osloensis* (28)	*Oligella ureolytica* (3)	*Oligella urethralis* (7)	*Paracoccus yeei* (14)	*Psychrobacter faecalis* (19)	*Psychrobacter phenylpyruvicus*[b] (5)	*Psychrobacter pulmonis* (8)	*Psychrobacter sanguinis*[b] (15)	*Wohlfahrtiimonas chitiniclastica* (3)
Motility (flagella)	0	0	0	0	0	0	0	0	0	67 (Pt)	0	0	0	0	0	0	0
Growth on MacConkey agar	90	100	100	60	0	0	0	0	50	0	57	100	100	0	(50)	0	100
Alkalinizaton of acetate	100	100	0	100	0	60	0	0	100	100	100	100	100	100	100	87	(100[w])
Susceptibility to desferrioxamine	0	0	71	100	100	100	0	100	0	100	100	0	0	0	0	0	100
Acidification of:																	
Glucose	0	100	0	0	0	0	0	0	0	0	0	100	100	0	0	0	100
Mannitol	0	66	0	0	0	0	0	0	0	0	0	(50)	0	0	0	0	0
Xylose	0	100	0	0	0	0	0	0	0	0	0	100	100	0	0	0	(100[w])
Ethylene glycol	100	100	0	100	0	20	0	0	100	100	100	100	100	100	100	87	100
Gelatinase	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0
Tween 80 esterase	0	0	0	0	0	100	0	0	4	0	0	0	100	100	100	100	0
Tributyrate esterase	0	0	0	100	100	100	0	100	100	0	0	100	100	100	100	100	0
Alkaline phosphatase	0	0	100	0	0	80	0	0	79	0	0	0	0	0	0	0	0
Phenylalanine deaminase	100	100	0	0	0	0	0	0	0	0	100[w]	0	10	100	0	33	100
Pyrrolidonyl aminopeptidase	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urease	100	100	0	0	0	0	0	0	0	100	0	100	0	100	0	100	0
Nitrate reductase	0	0	0	80	100	100	0	100	18	100	0	100	100	20	100	33	0
Nitrite reductase	0	0	0	20	100	0	0	0	0	100	100	0	100	0	100	0	0
Arginine dihydrolase	100	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a]Numbers in parentheses after organism names are numbers of strains tested. Values are percentages; those in parentheses represent delayed positivity. Abbreviations: Pt, peritrichous; W, weakly positive.

[b]Growth markedly promoted by Tween 80. *P. phenylpyruvicus* grows in 12% NaCl tryptic soy broth supplemented with 0.1% Tween 80, in contrast to *P. sanguinis*.

cats, and camels); M. *caviae* (guinea pigs); M. *cuniculi* (rabbits); and M. *ovis* and M. *oblonga* (sheep).

Both M. *catarrhalis* and M. *canis* grow well on SBA and even on tryptic soy agar (TSA), and their colonies may reach >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 Thayer-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 (33). Some strains may also produce very slimy colonies resembling colonies of *Klebsiella pneumoniae* (33). 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* (103), M. *osloensis*, and *P. 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, <1 mm in diameter after 24 h. Colonies of M. *atlantae* are small (usually 0.5 mm in diameter) and show pitting and spreading (104). 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* (37).

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 (104). 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 spherical diplococci of 0.5 to 1.5 μm 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 (105), 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 positive for pyrrolidonyl aminopeptidase (106). M. *lacunata* is the only proteolytic species with gelatinase activity. Using the plate method (see chapter 33), 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* and *P. sanguinis* are urease positive and other *Psychrobacter* species exhibit luxuriant growth on plain agar, like TSA, even at 25°C.

M. *lincolnii* is biochemically quite inactive.

M. *osloensis* alkalinizes acetate, 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 reductase positive.

Most *Moraxella* species are susceptible to penicillin and its derivatives, cephalosporins, tetracyclines, quinolones, and aminoglycosides (107, 108). Production of β-lactamase has been only rarely reported for *Moraxella* species other than M. *catarrhalis*, of which most isolates produce an inducible, cell-associated β-lactamase (37). 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 Oligella ureolytica

The genus *Oligella* comprises two species, O. *ureolytica* (formerly CDC group IVe) and O. *urethralis* (formerly *Moraxella urethralis* and CDC group M-4) (109), which have both been isolated chiefly from the human urinary tract and have been reported to cause urosepsis (110).

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 and opaque, with entire borders, and are 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, and all are strongly urease positive (with the urease reaction often turning positive within minutes after inoculation) and reduce nitrate. O. *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-β-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* (111). 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 (107).

Paracoccus yeei

The genus *Paracoccus* (*Rhodobacteraceae*, *Alphaproteobacteria*) comprises ~25 species, of which only *P. yeei* is of some clinical importance. Daneshvar et al. (112) proposed the name *P. yeeii*, later changed to *P. yeei*, for the former CDC group EO-2.

Colonies are large and mucoid, with a pale yellow pigmentation. *P. 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.

P. yeei has been isolated from various human wound infections (112).

Psychrobacter

The genus *Psychrobacter* (113) comprises 34 species, of which only a few are clinically important. Apart from *P. phenylpyruvicus*, the *Psychrobacter* strains isolated from clinical material were considered until recently as belonging to the species *Psychrobacter immobilis*. In a recent study (114), it was shown that almost all the strains formerly identified as *P. immobilis* belong in fact to the species *P. faecalis* and *P. pulmonis*, isolated first from pigeons and lambs, respectively (115, 116). *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, tributyrate esterase, and nitrate reductase positive and, unlike the type strain of *P. immobilis*, 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.

Few cases of infection have been reported to be caused by *P. immobilis*, but in light of the data reported here, these might concern infections with one of the other *Psychrobacter* species.

P. phenylpyruvicus and *P. sanguinis* are phenotypically very similar, although genetically clearly distinct. *P. sanguinis* (117) seems to occur more frequently in clinical samples than *P. phenylpyruvicus*, and the majority of older *P. phenylpyruvicus* collection strains are in fact *P. sanguinis*. A salient feature is the dramatic effect of Tween 80 in promoting growth of both species, resulting in colonies 2 to 3 times larger on Tween 80 agar compared to blood agar. Since these species grow very poorly or not at all on usual media, like TSA, supplementation with 0.1% Tween 80 is recommended when blood agar cannot be used, as for susceptibility testing to desferrioxamine or for testing growth abilities in broth at different temperatures.

P. phenylpyruvicus grows in 12% NaCl tryptic soy broth supplemented with 0.1% Tween 80, while *P. sanguinis* does not. Phenylalanine deaminase is always positive in *P. phenylpyruvicus* but only in one-third of *P. sanguinis* strains.

Most *Psychrobacter* species are resistant to penicillin but susceptible to most other antibiotics (107).

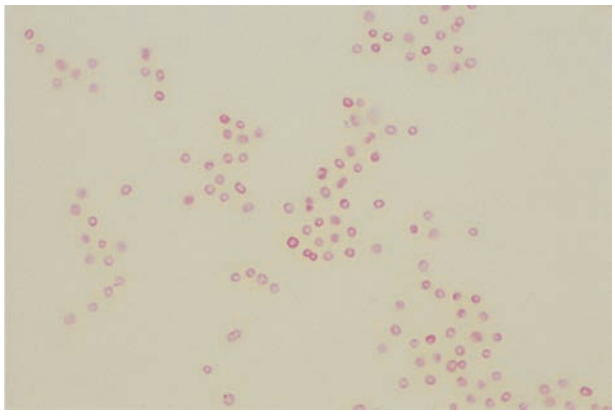

FIGURE 3 Gram stain of *P. yeei*, showing characteristic doughnut-shaped morphology.
doi:10.1128/9781555817381.ch44.f3

Wohlfahrtiimonas chitiniclastica

W. chitiniclastica is a Gram-negative, nonfermentative rod living in the larvae of some parasitic flies. It is closely related to the "Gilardi rod group 1" and to *Ignatzschineria larvae* (118, 119). The type strain of *W. chitiniclastica* was first classified as "Gilardi rod group 1."

W. chitiniclastica may be associated with human myiasis, sometimes resulting in bacteremia (120) or sepsis (118). Fifteen human strains of "Gilardi rod group 1" were isolated from wounds and blood (121).

The organisms are short, coccoid rods, growing on current media with flat, slightly spreading colonies. Some carbohydrates, like glucose, xylose, and fructose, are weakly and slowly acidified. This species exhibits a very strong phenylalanine deaminase reaction. Chitinase activity is present as in *I. larvae*, which is, however, urease positive. *W. chitiniclastica* is resistant to penicillin and susceptible to many other antibiotics.

Oxidase-Positive, Indole-Negative, Trypsin-Positive GNF

Alishewanella fetalis

A. fetalis (*Alteromonadaceae*, *Gammaproteobacteria*) (Table 4) is a Gram-negative rod that grows at temperatures between 25 and 41°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 (122). Also, in contrast to *Shewanella* species, it does not produce H_2S 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 (122).

Inquilinus limosus

I. limosus is a rod-shaped, Gram-negative bacterium that measures 1.5 to 2 μm in width by 3.5 μm in length and grows at 35 and 41°C but poorly at 25°C. Colonies are nonpigmented 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 (123), the species is saccharolytic, acidifying glucose, mannitol, xylose, and other carbohydrates. In contrast, ethylene glycol is not acidified. β-Galactosidase, pyrrolidonyl aminopeptidase, and trypsin are positive, but alkaline phosphatase is negative. Esculin is hydrolyzed. All strains are positive for catalase, β-glucosidase, proline

TABLE 4 Oxidase-positive, indole-negative, trypsin-positive GNF[a]

Characteristic	*Alishewanella fetalis* (1)	*Inquilinus limosus* (3)	*Myroides odoratimimus* (21)	*Myroides odoratus* (4)	*Ochrobactrum anthropi* (29)	*Ochrobactrum grignonense* (2)	*Ochrobactrum haematophilum* (3)	*Ochrobactrum intermedium* (9)	*Ochrobactrum pseudintermedium* (1)	*Ochrobactrum pseudogrignonense* (2)	*Pannonibacter phragmitetus* (7)	*Pseudochrobactrum asaccharolyticum* (3)	*Rhizobium radiobacter* (22)	*Shewanella algae*[b] (10)	*Shewanella putrefaciens*[b] (2)	*Sphingobacterium multivorum* (7)	*Sphingobacterium spiritivorum* (4)	*Sphingobacterium thalpophilum* (3)	*Sphingomonas* spp. (15)
Motility	0	33	0	0	100	100	100	100	100	100	100	0	100	100	100	0	0	0	66
Flagella	0	P, 1	0	0	Pt, L	Pt, L	Pt, L	Pt, L	Pt, L	Pt, L	Pt, L	0	Pt, L	P, 1	P, 1	0	0	0	P, 1
Production of flexirubin pigments	0	0	95	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Growth on MacConkey agar	100	(100)	100	75	100	100	100	100	100	100	100	100	100	100	100	100	(50)	100	0
Growth at 41°C	ND	ND	ND	ND	0	0	100	100	100	0	100	0	0	ND	ND	ND	ND	ND	ND
Growth at 45°C	ND	ND	ND	ND	0	0	0	0	100	0	0	0	0	ND	ND	ND	ND	ND	ND
Acidification of:																			
Glucose	100	100	0	0	100	100	100	100	100	100	100	(100)	100	0	50	100	100	100	87 (13)
Mannitol	0	100	0	0	59	50	33	33	0	50	28	0	100	0	0	0	100	0	0
Xylose	0	100	0	0	100	100	100	100	100	100	100	(100)	100	0	0	100	100	100	87
Ethylene glycol	0	0	0	0	100	100	100	100	100	100	86	86	100	100	100	0	100	0	93
Esculin hydrolysis	0	100	0	0	0	0	0	0	0	0	100	0	100	0	0	100	100	100	100
Gelatinase	100	0	100	100	0	0	0	0	0	0	0	0	0	100	100	0	0	0	0
Urease	100	(33)	100	100	100	0	100	(44)	0	(100)	100	0	100	0	0	100	100	(100)	0
Nitrate reductase	100	0	0	0	100	100	0	100	100	100	100	0	100	100	100	0	0	100	7
Nitrite reductase	0	0	100	100	100	100	0	100	100	100	100	0	64	100	100	0	0	0	0
β-Galactosidase (ONPG[c])	0	100	0	0	0	0	0	0	0	0	100	0	100	0	0	100	75	100	100
Pyrrolinodyl aminopeptidase	0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	27
Production of H_2S (on KIA)	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100	0	0	0	0
Alkaline phosphatase	100	0	100	100	0	0	0	0	0	0	0	0	0	100	100	100	100	100	100
Requirement for NaCl	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0
Phenylalanine deaminase	ND	ND	ND	ND	100	100	100	100	100	100	0	100	100	ND	ND	ND	ND	ND	ND
Tributyrine esterase (<1 h)	ND	ND	ND	ND	0	0	0	0	0	0	100	0	0	ND	ND	ND	ND	ND	ND
Susceptibility to:																			
Colistin	100	0	0	0	100	50	100	0	0	0	0	0	73	60	100	0	0	0	7
Desferrioxamine	100	0	0	100	0	100	33	0	0	100	100	100	18	0	0	0	0	0	7
Netilmicin	ND	ND	ND	ND	100	100	100	0	100	100	100	66	0	ND	ND	ND	ND	ND	ND
Tetracyline	ND	ND	ND	ND	100	100	100	100	0	100	100	100	100	ND	ND	ND	ND	ND	ND

[a]Numbers in parentheses after organism names are numbers of strains tested. Values are percentages; those in parentheses represent delayed positivity. Abbreviations: L, lateral; ND: no data; P, polar; Pt, peritrichous.
[b]Both *Shewanella* species are ornithine decarboxylase positive.
[c]ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

aminopeptidase, and pyrrolidonyl aminopeptidase and negative for lysine, arginine, ornithine, denitrification, and indole production (123).

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*. *I. limosus* differs from *Rhizobium radiobacter* and *Pannonibacter phragmitetus* by the absence of acid production from ethylene glycol (positive in most 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 (124). Isolates can be recovered on colistin-containing *Burkholderia cepacia* selective media but are inhibited on *B. cepacia* selective agar, which also contains gentamicin (125).

All isolates are reported to be resistant to penicillins and cephalosporins, kanamycin, tobramycin, colistin, doxycycline, and trimethoprim-sulfamethoxazole and susceptible to imipenem and ciprofloxacin (124, 125).

I. limosus has been isolated from soil samples and from respiratory secretions of cystic fibrosis patients (124, 125, 127, 128). The clinical impact of chronic colonization with *I. limosus* remains unclear. Chiron et al. (125) reported that for one patient, *I. limosus* was the only potential pathogen recovered from the sputum, whereby acquisition of this bacterium was followed by a worsening of his lung function.

Myroides odoratimimus and *Myroides odoratus*

The genus *Myroides* includes two species, *M. odoratimimus* and *M. odoratus*, formerly *Flavobacterium odoratum* (129), which can be isolated from clinical samples. Cells are thin, middle-sized (0.5 μm in diameter and 1 to 2 μm 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 41°C. *Myroides* species are asaccharolytic, urease positive, and nitrate reductase negative, but nitrite is reduced. *M. odoratus* can be differentiated by its susceptibility to desferrioxamine, while *M. odoratimimus* is resistant. The species also differ by their cellular fatty acid patterns, with *M. odoratimimus* having significant amounts of C13:0*i* and C15:0 (129).

Organisms identified as *M. odoratus* have been reported mostly from urine but have also been found in wound, sputum, blood, and ear specimens (130, 131). Clinical infection with *Myroides* species is exceedingly rare. In our experience, based on 25 clinical isolates, *M. odoratimimus* is 4 to 5 times more frequently isolated from clinical material than *M. odoratus*.

Most strains are resistant to penicillins, cephalosporins, aminoglycosides, aztreonam, and carbapenems (131).

Ochrobactrum anthropi and *Ochrobactrum intermedium*

Ochrobactrum, *Pseudochrobactrum*, *Rhizobium*, and *Pannonibacter* have many morphological and biochemical characteristics in common, and therefore additional characteristics for these taxa are provided in Table 4.

The genus *Ochrobactrum* comprises at present 17 species. Two species, i.e., *O. anthropi* (132) and *O. intermedium* (133), are currently isolated from humans, but recently new species, i.e., *O. haematophilum* (134), *O. pseudogrignonense* (134), and *O. pseudintermedium* (135), have been recovered from clinical samples. *O. anthropi* (132) comprises the so-called urease-positive *Achromobacter* species, formerly designated CDC group Vd (biotypes 1 and 2), and *Achromobacter* groups A, C, and D.

Colonies on SBA are small, ~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 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 in most species; phenylalanine deaminase is always positive. Nitrate reduction and nitrite reduction are positive, except in *O. haematophilum*. *O. intermedium* can be distinguished from *O. anthropi* by colistin resistance and growth at 41°C. Urease is often negative or delayed in this species. *O. pseudintermedium* is the only species growing at 45°C. Resistance to colistin, tetracycline, and aminoglycosides may have diagnostic value for distinction between *O. intermedium* and *O. pseudintermedium*. Among aminoglycosides, netilmicin provides the most clear-cut results.

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* (133).

O. anthropi has been isolated from various environmental and human sources, predominantly from patients with catheter-related bacteremia (132, 136) and rarely with other infections (137). One hospital outbreak in transplant patients has been described (138). A few cases of *O. intermedium* infections have been reported (see, e.g., reference 139), but because of the close phenotypic similarity of *O. anthropi* and *O. intermedium*, it is possible that certain infections considered to be caused by *O. anthropi* were actually caused by *O. intermedium*.

O. anthropi strains are usually resistant to β-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 (136).

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 (140). The species resembles most strongly *R. radiobacter*, but saccharolytic activity is somewhat weaker and not as extended. Unlike *Rhizobium*, *Ochrobactrum*, and *Pseudochrobactrum*, which all have a strong phenylalanine deaminase activity, *P. phragmitetus* does not produce phenylpyruvic acid from phenylalanine. Another easy and reliable differential test is the hydrolysis of tributyrin, 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 and *Pseudochrobactrum* species do not hydrolyze tributyrin. Moreover, *P. phragmitetus* grows at 41°C while *R. radiobacter* does not.

Cases of septicemia due to *Achromobacter* group B have been reported (141).

Pseudochrobactrum

The genus *Pseudochrobactrum* was described in 2006 (142) and comprises two species, i.e., *P. saccharolyticum* and *P. asaccharolyticum*. The type strain of *P. asaccharolyticum* was isolated from a knee aspirate. Two more human strains were isolated from Belgian clinical samples: a wound and an eye swab (our unpublished data). Both species are very similar to *Ochrobactrum* species, but cells are nonmotile. *P. asaccharolyticum* slowly acidifies glucose and xylose, while *P. saccharolyticum* is more saccharolytic. Both species are susceptible to desferrioxamine and do not produce urease. Nitrate reduction and nitrite reduction are negative. The clinical significance of *Pseudochrobactrum* species remains to be assessed.

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*. More recently, an emended description of the genus *Rhizobium* (*Rhizobiaceae*, *Alphaproteobacteria*) was proposed to include all species of *Agrobacterium* (143), of which 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. It grows at 35°C as well, but not at 41°C. *R. radiobacter* is phenotypically very similar to the *Ochrobactrum* species, although phylogenetically separate. *R. radiobacter* differs clearly from *Ochrobactrum* species by a positive β-galactosidase test and by the production of ketolactonate, which is, however, not routinely tested. *R. radiobacter* has broad saccharolytic activity, including mannitol and raffinose.

R. radiobacter has been most frequently isolated from blood, followed by peritoneal dialysate, urine, and ascitic

fluid (144). The species has also been isolated from the airways of patients with cystic fibrosis (123). The few cases are from patients with transcutaneous catheters or implanted biomedical prostheses, and effective treatment often requires removal of the device. Most strains are susceptible to broad-spectrum cephalosporins, carbapenems, tetracyclines, and gentamicin but not to tobramycin (144, 145). Testing of individual isolates is recommended for clinically significant cases.

Shewanella algae and *Shewanella putrefaciens*

The organisms formerly called *Pseudomonas putrefaciens*, *Alteromonas putrefaciens*, *Achromobacter putrefaciens*, and CDC group Ib have been placed in the genus *Shewanella*, which comprises >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* (146), 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 GNF. Both are also ornithine decarboxylase 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 (147) 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 (148), including skin and soft tissue infections, otitis media, ocular infection, osteomyelitis, peritonitis, and septicemia. 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 (107, 145). The mean MICs of *S. algae* for penicillin, ampicillin, and tetracycline are higher than the corresponding MICs of *S. putrefaciens* (147).

Sphingobacterium, Indole-Negative Species

A total of 15 species have been described as belonging to the genus *Sphingobacterium*. Based on 16S rRNA gene sequence data, the indole-producing *Sphingobacterium mizutaii* also belongs to this genus, and as a consequence, the description of the genus *Sphingobacterium* as indole negative was emended to indole variable (149).

In summary, most species of this genus do not produce indole, but *S. mizutaii* is indole positive and is therefore dealt with among the indole-positive GNF in Table 5.

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*, *Flavobacterium yabuuchiae*, and CDC group IIk-3), *S. thalpophilum*, and *S. mizutaii* (150, 151).

Colonies are yellowish. *Sphingobacterium* species are middle-sized, nonmotile, Gram-negative rods. All species are strongly saccharolytic; i.e., glucose, xylose, and other sugars are acidified. No acid is produced 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 41°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) (152, 153). Blood and urine have been the most common sources for the isolation of *S. spiritivorum* (154). *S. thalpophilum* has been recovered from wounds, blood, eyes, abscesses, and an abdominal incision (40).

Sphingobacterium species are generally resistant to aminoglycosides and polymyxin B while susceptible *in vitro* to the quinolones and trimethoprim-sulfamethoxazole. Susceptibility to β-lactam antibiotics is variable, requiring testing of individual isolates (108).

Sphingomonas Species

On the basis of 16S rRNA gene sequence and the presence of unique sphingoglycolipid and ubiquinone types, the genus *Sphingomonas* (*Sphingomonadaceae*, *Alphaproteobacteria*) was created for organisms formerly known as *Pseudomonas paucimobilis* and CDC group IIk-1 (155, 156). The original genus *Sphingomonas* can be divided into four phylogenetic groups, each representing a different genus (157), 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 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 41°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 (2). 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 β-galactosidase and alkaline phosphatase are positive. The yellow pigment of some strains may hamper a correct

TABLE 5 Oxidase-positive, indole-positive GNF[a]

Characteristic	*Balneatrix alpica* (1)	*Bergeyella zoohelcum* (10)	*Chryseobacterium anthropi*[b] (11)	*Chryseobacterium gleum* (11)	*Chryseobacterium hominis*[b] (14)	*Chryseobacterium indologenes* (15)	*Chryseobacterium treverense*[b] (5)	*Elizabethkingia meningoseptica* (17)	*Empedobacter brevis* (7)	*Sphingobacterium mizutaii* (5)	*Wautersiella falsenii* (53)	*Weeksella virosa* (10)
Motility	100	0	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	0
Production of flexirubin pigments	0	0	0	100	0	74	0	0	0	0	0	0
Production of other pigments[c]	PY	–	–/PS	–	–/PY	–	PY	–/PY/PS	PY	PY	–/PY	–
Growth on MacConkey agar	0	0	0	100	0	67	0	83	100	0	100	0
Growth at 41°C	0	0	0	100	0	0	0	47	0	0	0	100
Acidification of:												
Glucose	100	0	100	100	100	100	100	100	100	100	100	0
Mannitol	100	0	0	0	0	0	0	100	0	0	0	0
Xylose	0	0	0	27	0	0	0	0	0	100	0	0
L-Arabinose	0	0	0	100	0	0	0	0	0	80	0	0
Maltose	100	0	100	100	100	100	100	100	100	100	100	0
Sucrose	0	0	0	0	28	20	0	0	0	100	0	0
Ethylene glycol	0	0	0	100	100	0	0	100	0	0	47	0
Esculin hydrolysis	0	0	0	100	100	100	20	100	0	100	53	0
Gelatinase	0	100	100	100	100	100	0	100	100	0	53/(47)	100
Urease	0	100	0	73	0	0	0	0[d]	0	0	100	0
Nitrate reductase	100	0	0	73	65	26	60	0	0	0	0	0
Nitrite reductase	0	0	0	54	65	13	100	88	0	80	34	0
β-Galactosidase (ONPG[e])	0	0	0	0	0	0	0	100	0	100	19	0
Benzyl-arginine aminopeptidase (trypsin)	0	100	100	100	100	100	80	100	100	100	100	100
Pyrrolidonyl aminopeptidase	0	0	91	100	100	100	80	100	100	100	100	100
Susceptibility to:												
Colistin/polymyxin	100	0	0	0	14	0	0	0	0	0	0	100
Desferrioxamine	100	100	73	0	0	0	100	0	0	0	15	100

[a]Numbers in parentheses after organism names are numbers of strains tested. Values are percentages; those in parentheses represent delayed positivity.
[b]Alkalinization of acetate is positive for *C. anthropi* and *C. hominis* and negative for *C. treverense*.
[c]Abbreviations and symbols: –, none; PS, pale salmon-pink; PY, pale yellow.
[d]*E. miricola* is urease positive (data from reference 170).
[e]ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

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. *S. paucimobilis* has been isolated from a variety of clinical specimens, including blood, cerebrospinal fluid (CSF), peritoneal fluid, urine, wounds, the vagina, and the cervix, as well as from the hospital environment (158, 159). *S. parapaucimobilis* clinical isolates have been obtained from sputum, urine, and the vagina (156).

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 (107, 158).

Oxidase-Positive, Indole-Positive GNF

The natural habitats of most oxidase-positive, indole-positive GNF (Table 5) are soil, plants, and food and water sources, including those in hospitals. Clinically relevant species include *Balneatrix alpica*, *Bergeyella zoohelcum*, *Chryseobacterium* species, *E. meningoseptica*, *Empedobacter brevis*, *S. mizutaii*, *Wautersiella falsenii*, and *Weeksella virosa*. 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 5 presents an overview of the characteristics useful to differentiate among these species.

Balneatrix alpica

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 GNF. 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 GNF. Growth occurs at 20 to 46°C on ordinary media such as TSA but not on MacConkey agar. It acidifies fructose, glucose, glycerol, inositol, maltose, mannitol, mannose, sorbitol, 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 β-galactosidase.

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 (160). 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.

Bergeyella zoohelcum

B. zoohelcum comprises former CDC group IIj strains (161).

B. zoohelcum and *W. virosa* are morphologically and biochemically similar organisms with cells measuring 0.6 by 2 to 3 μm, 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 is isolated mainly from wounds caused by animal (mostly dog) bites and can lead to meningitis and septicemia (161, 162). 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*, *Chryseobacterium 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 *Chryseobacterium anthropi*, *Chryseobacterium hominis*, *E. meningoseptica*, and *S. 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 (2), 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 41°C. *C. gleum* exhibits pronounced alpha-hemolysis, resembling viridans discoloration; always acidifies ethylene glycol; is arabinose positive or delayed positive; and grows at 41°C.

Beta-hemolysis is absent or very rare in other strains of CDC group IIb and is therefore almost specific for the

TABLE 6 Pink-pigmented GNF[a]

Characteristic	*Asaia* spp. (2)	*Azopsirillum* spp. (3)	*Methylobacterium* spp. (4)	*Roseomonas cervicalis* (2)	*Roseomonas gilardii* subsp. *gilardii* and *Roseomonas gilardii* subsp. *rosea* (5)	*Roseomonas mucosa* (9)	*Roseomonas* genomic species 4 (1)	*Roseomonas* genomic species 5 (4)
Oxidase	0	100	0	100	100[w]	11[w]	100	100[w]
Acidification of:								
Glucose	100	0	(25)	0	(20)	100	(100)	0
Fructose	100	(100)[w]	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
β-Galactosidase (ONPG[b])	0	100	0	0	0	0	0	0
Susceptibility to:								
Colistin	0	0	0	0	40	45	0	25
Desferrioxamine	0	0	0	0	0	0	100	0

[a]Numbers in parentheses after organism names are numbers of strains tested. Values are percentages; those in parentheses represent delayed positivity. Abbreviation: W, weakly positive reaction.

[b]ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

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 (145). Nosocomial infections due to C. *indologenes* have been linked to the use of indwelling devices during hospital stays (163). More recently, C. *indologenes* has also been associated with the cause of neonatal meningitis (164, 165).

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 rRNA gene 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-β-lactamases from C. *indologenes* has been studied in detail (166).

C. *anthropi* represents part of the strains formerly designated as CDC group IIe (167). 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. Most clinical isolates used for the description of the species were from wounds and blood cultures (167).

C. *hominis* includes the strains formerly included in CDC group IIc and most of the strains of CDC group IIh (168). 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 (168).

Chryseobacterium treverense has been described recently (169). Part of the strains formerly designated CDC group IIe belong to this species. Although described as indole negative, C. *treverense* strains produce indole that should be detected using the method recommended for GNF (see chapter 33). The species is phenotypically related to C. *anthropi* and C. *hominis* but can be distinguished by a negative (or very weak) gelatin hydrolysis and the absence of acetate alkalinization.

Elizabethkingia meningoseptica and *Elizabethkingia miricola*

Colonies of *E. meningoseptica*, formerly *Chryseobacterium meningosepticum* (170), 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 β-galactosidase activity. Gelatin and esculin hydrolysis are positive. *Elizabethkingia* and *Chryseobacterium* species can be differentiated as well on the basis of 16S rRNA gene sequence analysis (167, 170).

E. meningoseptica has been reported to be associated with (neonatal) meningitis (171), nosocomial outbreaks (see, e.g., references 172 and 173), and different types of infection (e.g., adult pneumonia and septicemia [145, 172] and infections reported in dialysis units [174]). A clinical case (sepsis) of *E. miricola* was reported only once.

Empedobacter brevis

E. brevis (175) 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 stronger gelatinase activity are useful to distinguish it from C. *anthropi*. The species is rarely recovered from clinical material.

Sphingobacterium mizutaii

S. mizutaii, previously known as *Flavobacterium mizutaii* (151) but originally described as *Sphingobacterum mizutae* (176), is the only indole-positive *Sphingobacterium* species (149). It is saccharolytic—producing acid from a large number of carbohydrates, including xylose—similar to other *Sphingobacterium* species, from which it can be distinguished by its indole production, its failure to grow on MacConkey agar, and its usual lack of urease activity (40).

S. mizutaii can be distinguished from *Chryseobacterium* and *Empedobacter* species by its lack of gelatin hydrolysis and of flexirubin production. *S. mizutaii* produces acid from xylose but not from ethylene glycol, allowing differentiation from other indole-positive species. The phenotypic profile of *S. 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 *S. mizutaii* (149).

S. mizutaii has been described as an indole-negative species (151), but in our hands all strains tested, including the type strain, produce as much indole as the *Chryseobacterium* strains, using the method recommended for GNF (see chapter 33). *S. mizutaii* has been isolated from blood, CSF, and wound specimens (40).

Wautersiella falsenii

W. falsenii is closely related to *E. brevis*, from which it differs by its urease activity. Two genomovars have been described (177): genomovar 1 is always esculin positive and β-galactosidase negative, whereas 90% of the genomovar 2 strains are esculin negative and 63% are β-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 (our unpublished data). 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* (177, 178). 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 *W. virosa* is dealt with above in the discussion of *B. 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 former CDC group IIf strains (179). *W. virosa* is isolated mainly from urine and vaginal samples (179), 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 (173). *Chryseobacterium* species and *E. meningoseptica* are inherently resistant to many antimicrobial agents commonly used to treated infections caused by Gram-negative bacteria (aminoglycosides, β-lactam antibiotics, tetracyclines, and chloramphenicol) but are often susceptible to agents generally used for treating infections caused by Gram-positive bacteria (rifampin, clindamycin, erythromycin, trimethoprim-sulfamethoxazole, and vancomycin) (107, 108, 145). Although early investigators recommended vancomycin for treating serious infection with *E. meningoseptica*, subsequent studies showed greater *in vitro* activity of minocycline, rifampin, trimethoprim-sulfamethoxazole, and quinolones (108, 172). Among the quinolones, levofloxacin is more active than ciprofloxacin and ofloxacin (108). *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 (108). 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 (180). The Etest is a possible alternative to the standard agar dilution method for testing cefotaxime, ceftazidime, amikacin, minocycline, ofloxacin, and ciprofloxacin but not piperacillin (181). Definitive therapy for clinically significant isolates should be guided by individual susceptibility patterns determined by an MIC method.

Pink-Pigmented GNF

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 6).

Asaia

Asaia is a genus of the family *Acetobacteraceae* (*Alphaproteobacteria*), with some clinically relevant members, such as *A. bogorensis* (182), *A. lannensis* (183), and *A. siamensis* (184). 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.

A. bogorensis has been reported as a cause of peritonitis in a patient on automated peritoneal dialysis (185) and as a cause of bacteremia after intravenous drug abuse (186). *A. lannensis* has been identified as the cause of nosocomial infections in pediatric patients with idiopathic dilated cardiomyopathy (183).

Asaia species have been reported to be resistant to ceftazidime, meropenem, imipenem, trimethoprim, amikacin, vancomycin, aztreonam, penicillin, and ampicillin by disk diffusion (187). The *A. bogorensis* strain reported by Snyder et al. (185) was susceptible to aminoglycosides (amikacin, tobramycin, and gentamicin) and resistant to ceftazidime and meropenem by disk diffusion.

Azospirillum

The former *Roseomonas* genomic species 3 (*Roseomonas fauriae*) and genomic species 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 (188). Some strains of this genus may occasionally be isolated from clinical material (189).

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. β-Galactosidase activity 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 (190). *Methylobacterium* species are isolated mostly from vegetation but may also occasionally be found in the hospital environment. *M. 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 (189). 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 (191), methylobacteria were reported to be oxidase positive, but the strains tested by us with the dimethyl-paraphenylenediamine reagent were all oxidase negative. Saccharolytic activity is weaker than in *Asaia* spp., no acid is produced from mannitol, and acid is produced irregularly from glucose.

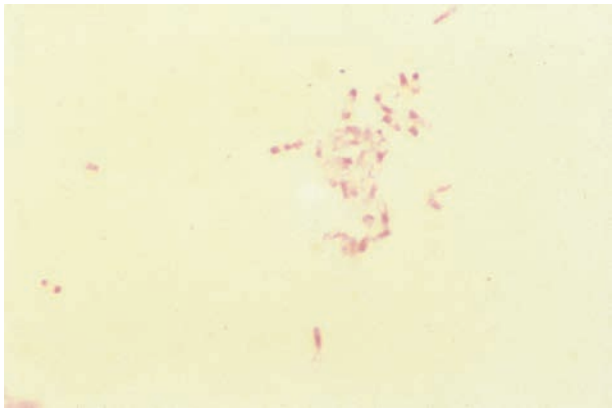

FIGURE 4 Gram stain of *Methylobacterium*, showing pleomorphic, Gram-negative rods with vacuoles.
doi:10.1128/9781555817381.ch44.f4

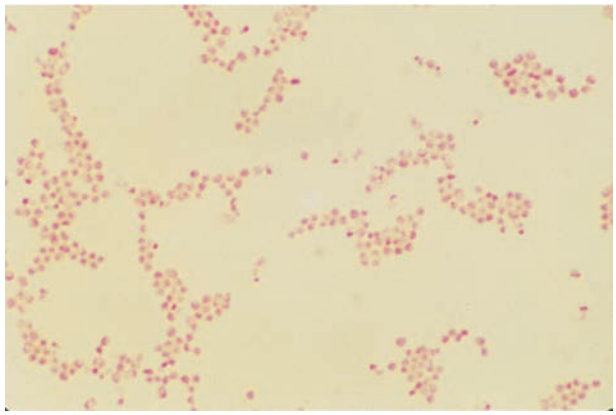

FIGURE 5 Gram stain of *Roseomonas*, showing Gram-negative, coccoid organisms. doi:10.1128/9781555817381.ch44.f5

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 (192–195). 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 (3).

Active drugs include aminoglycosides and trimethoprim-sulfamethoxazole, whereas β-lactam drugs show variable activity (196). These species are best tested for susceptibility by agar or broth dilution at 30°C for 48 h (30).

Roseomonas

The original description of the genus *Roseomonas* (*Acetobacteraceae*, *Alphaproteobacteria*) included three named species, *R. gilardii* (genomic species 1), *R. cervicalis* (genomic species 2), and *R. fauriae* (genomic species 3), and three unnamed species, *Roseomonas* genomic species 4, 5, and 6 (189). *Roseomonas* genomic species 3 and 6 have been transferred to the genus *Azospirillum*. More recently, Han et al. (188) proposed a new species, *R. mucosa*, and a new subspecies, *R. gilardii* subsp. *rosea* (to differentiate from *R. 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* genomic species 4, and *Roseomonas* genomic species 5.

Colonies are mucoid and runny 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 (Fig. 5) and usually motile by one or two polar flagella, but motility is often difficult to demonstrate. Genomic species 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 β-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 genomic species 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. Genomic species 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 genomic species 4 and 5.

Roseomonas species are uncommon isolates from humans, but they are nevertheless the most frequently isolated pink GNF. Clinical isolates have been recovered from blood, wounds, exudates, abscesses, genitourinary sites, continuous ambulatory peritoneal dialysis fluid, and bone (197–200). A total of 35 cases of opportunistic infections with *Roseomonas* species were reviewed (199). 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 (197, 199).

Dé et al. (197) 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. 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 GNF are limited to *Pseudomonas*

species, *B. 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) on Mueller-Hinton agar (181), is recommended.

EVALUATION, INTERPRETATION, AND REPORTING OF RESULTS

Although certain GNF can on occasion be frank pathogens, e.g., *Burkholderia pseudomallei*, *E. meningoseptica*, and *P. aeruginosa*, they are generally considered to be of low virulence and often occur in mixed infections, making it difficult to determine when to work up cultures and when to perform susceptibility studies. *E. meningoseptica* in neonatal meningitis, *M. 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. Identification of GNF may also be important to document nosocomial outbreaks linked to hospital environment or medical devices. 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 GNF not further identified."

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