ANTIMICROBIAL SUSCEPTIBILITY

Natural antibiotic susceptibility of *Klebsiella pneumoniae*, *K. oxytoca*, *K. planticola*, *K. ornithinolytica* and *K. terrigena* strains

INGO STOCK and BERND WIEDEMANN

Institut für Medizinische Mikrobiologie und Immunologie, Pharmazeutische Mikrobiologie, Universität Bonn, Germany

The natural susceptibility of 221 *Klebsiella* strains to 71 antibiotics was examined. The strains were isolated from clinical specimens and the environment, and belonged to *K. pneumoniae* subsp. *pneumoniae* (n = 40), *K. pneumoniae* subsp. *ozaenae* (37), *K. pneumoniae* subsp. *rhinoscleromatis* (10), *K. oxytoca* (44), *K. planticola* (40), *K. ornithinolytica* (25) and *K. terrigena* (25). MIC values were determined by a microdilution procedure in IsoSensitest broth according to the German standard (DIN). All *Klebsiella* spp. were naturally resistant or intermediate to amoxicillin, ticarcillin and to antibiotics to which other Enterobacteriaceae are also intrinsically resistant. *Klebsiella* spp. were naturally sensitive or intermediate to several penicillins, all tested cephalosporins, aminoglycosides, quinolones, tetracyclines, trimethoprim, co-trimoxazole, chloramphenicol and nitrofurantoin. *K. pneumoniae* subsp. *ozaenae* and subsp. *rhinoscleromatis* strains were generally more susceptible to antibiotics than strains of other *Klebsiella* taxa. *K. pneumoniae* subsp. *rhinoscleromatis* was the most susceptible taxon, being highly susceptible to cefuroxime, anti-folates and naturally intermediate to erythromycin and clarithromycin. *K. pneumoniae* subsp. *ozaenae* was most susceptible to glycopeptides. *K. oxytoca* and *K. terrigena* strains were least susceptible to cefazoline, cefoperazone and fosfomycin, respectively. The results of the present study describe a database of the natural antimicrobial susceptibility of *Klebsiella* spp., which can be used for the validation of antibiotic susceptibility results of these bacteria. MIC patterns to β -lactams indicate the expression of chromosomally encoded class A β -lactamases in all the species, including the subspecies of *K. pneumoniae*. Similar natural susceptibility patterns of *K. planticola* and *K. ornithinolytica* to all tested antibiotics support the status of *K. ornithinolytica* as a biovar of *K. planticola*.

Introduction

Klebsiella spp. are opportunist pathogens that cause a wide range of infections in man. They account for 8% of all nosocomial bacterial infections in the USA and in Europe [1]. Most frequently, *Klebsiella* spp. are isolated as causative agents of urinary tract infections, pneumonia, bacteraemia, neonatal sepsis and wound infections [1]. The leading organism in these infections is *K. pneumoniae* subsp. *pneumoniae* (*K. pneumoniae*^A) [2], followed by *K. oxytoca*. In contrast to diseases caused by these taxa, infections due to *K.*

pneumoniae subsp. *ozaenae* (*K. Ozaenae*^A) and *K. pneumoniae* subsp. *rhinoscleromatis* (*K. Rhinoscleromatis*^A) are restricted to certain body sites and in most cases affect only the human nose. *K. Ozaenae* is the cause of an atrophic rhinitis called ozena, but sporadic cases of other infections due to this organism are known [3, 4]. *K. Rhinoscleromatis* is the aetiological agent of rhinoscleroma, a chronic granulomatous infection of the nose, which is endemic in several countries [5]. While originally considered to be without clinical significance and restricted to water, botanical and soil environments, *K. planticola* and *K. terrigena* have been shown to occur in clinical specimens [5–10]. *K. planticola* has been isolated from human infections with a frequency of 3.5–20% among clinical

Received 25 Feb. 2000; revised version received 16 Oct. 2000; accepted 25 Oct. 2000.

Corresponding author: Dr I. Stock (e-mail: ingostock@hotmail.com).

^Ataxonomic style according to Le Minor [2].

isolates of *Klebsiella* spp. [6–9]. Human strains have been isolated mainly from respiratory tract secretions, wounds and urine [9]. Recently, *K. planticola* was also isolated from newborns in a neonatal ward [11]. It is likely that *K. planticola* causes human disease, because isolates have been recovered from monomicrobial specimens and could have been assigned to the corresponding infections [9]. *K. planticola* has the ability to express putative virulence factors similar to *K. Pneumoniae*, such as type 1 and type 3 fimbriae [12], and possesses the high-pathogenicity island of many virulent *Yersinia* strains [13]. In contrast to *K. planticola*, *K. terrigena* strains seem to be rarely associated with human infections. Podschun and Ullmann found these bacteria in 0.4% of clinical *Klebsiella* isolates [10]. However, because most commercial identification systems do not permit a reliable identification of *K. terrigena* and *K. planticola*, the true clinical significance of these *Klebsiella* species is unknown. In 1989, Sakazaki *et al.* proposed the name '*K. ornithinolytica*' for ornithine decarboxylase- and indole-positive *Klebsiella* strains [14]. This name is well accepted in Japan but not in the USA. The distinctness of *K. ornithinolytica* from *K. planticola* needs to be confirmed, as DNA–DNA relatedness studies in the USA and Japan gave different results [14, 15]. The clinical significance of *K. ornithinolytica* remains obscure, even though clinical isolates of this species are not uncommon (unpublished data). Finally, in 1999 a sixth *Klebsiella* species was created. Based on 16S rRNA genes sequences, it was found that *Calymmatobacterium granulomatis*, the aetiological agent of a chronic granulomatous genital infection called donovanosis, is highly related to *Klebsiella* spp. [16], implying its reclassification as *K. granulomatis* [17].

Despite their occurrence in clinical specimens, there is little information about the antibiotic susceptibility and no information about the natural antibiotic sensitivity and resistance of *Klebsiella* strains which do not belong to *K. Pneumoniae* and *K. oxytoca*. Data about the natural antibiotic susceptibility of *K. Pneumoniae* and *K. oxytoca* are also rare. The aim of the present study was to create a database of the natural susceptibility of all known *Klebsiella* species and subspecies, except *K. granulomatis*, to a wide range of antibiotics. The data from 221 *Klebsiella* strains tested with 71 antibiotics could be valuable for the validation of routine susceptibility test results and their consistency with identification to species or subspecies level.

Materials and methods

Bacterial strains

A total of 221 *Klebsiella* strains was examined. The vast majority of the *K. Pneumoniae* and *K. oxytoca* strains originated from a multi-centre study by the Paul

Ehrlich Society conducted in 1986 at 30 centres in Germany, Switzerland and Austria. Twenty two *K. Ozaenae* and some further strains – *K. Rhinoscleromatis* ($n = 6$), *K. ornithinolytica* (2), *K. terrigena* (2) and *K. planticola* (1) – were kindly provided by G. Stempf and H. Grimm (Weingarten, Germany). Apart from *K. Rhinoscleromatis* strains, which were gathered in the last decade, these strains were collected during 1996–1998 and originated from different hospitals and from outpatients in cities in southern Germany. Most of the remaining *K. Ozaenae*, four *K. Rhinoscleromatis*, 20 *K. planticola*, 20 *K. terrigena* and 23 *K. ornithinolytica* strains were kindly provided by R. Podschun (Kiel, Germany); these strains served as reference strains. They had also been identified and serotyped at the Hygiene-Institut of Kiel and were of clinical or environmental origin. *K. terrigena* ATCC 33257, *K. terrigena* ATCC 33629, *K. terrigena* ATCC 33630, *K. planticola* ATCC 33558, *K. planticola* CUETM 78117 and six further *K. planticola* strains were from the culture collection of Merlin-Diagnostika (Bornheim, Germany). No clinical isolates were repeat isolations from a given patient or patients on the same ward.

Identification

All strains were identified to the genus level with a commercial identification system for Enterobacteriaceae (Micronaut-E, Merlin-Diagnostika). This identification system includes biochemical key reactions for Enterobacteriaceae species with clinical significance. The inoculum for the identification tests was prepared from overnight cultures on solid media in physiological saline and was $c. 10^6$ cfu/ml. The incubation times were 24 h, the incubation temperature was 36°C ($\pm 1^\circ\text{C}$). To identify klebsiellae to species level, the Micronaut-E system and additional assimilation tests (reactions according to Podschun and Ullmann [1] and Monnet and Freney [18]), i.e., utilisation of ethanolamine (EA), histamine (HA), D-melezitose (MZ) and m-hydroxybenzoate (HB) (all chemicals obtained from Sigma, Deisenhofen, Germany) were performed in microtitration plates. In each plate assimilation patterns of four *Klebsiella* strains were tested in triplicate. Aqueous solutions of carbon source 10 g/L (EA, HA and HB) and carbon source 20 g/L (MZ and glucose – growth control) were prepared, sterilised by filtration and stored at 4°C. Lines A–D and line G of the microtitration plate were given 25 μl of the appropriate carbon sources. Sterilised water was added to lines E, F and H, which served as negative controls. Then 100 μl of AUX-Medium (bioMerieux, Marcy l'Etoile, France) were added to each well of the test plate. Finally, 50 μl of the bacterial suspensions were added to the wells in lines A–G, and 50 μl of physiological saline were added to the wells in line H. Overnight cultures of the bacteria grown on solid medium (IsoSensitest Agar, Oxoid) at 36°C were used for the preparation of the bacterial suspensions. Suspensions were made in saline

0.9% to obtain 1×10^8 cfu/ml. Inoculated microtitration plates were sealed with a perforated film, incubated at 30°C and read after 48, 72 and 96 h with a photometer for microtitration plates (LabSystems Multiskan, Multisoft, Helsinki, Finland). Test results were defined as positive if the extinction value in at least two wells with the same carbon source for the appropriate strain was >0.07 compared with the arithmetic average of the corresponding negative controls (wells containing bacteria without carbon source). Further assimilation tests were performed with all strains submitted and pre-identified as *K. Pneumoniae*, *K. oxytoca*, *K. planticola* and *K. terrigena*. *K. Ozaenae*, *K. Rhinoscleromatis* and *K. ornithinolytica* strains were not examined, because the Micronaut-E system allowed a correct identification of these taxa.

Antibiotics and antibiotic susceptibility testing

Antibiotic susceptibility was tested by a microdilution procedure in IsoSensitest Broth (Oxoid) according to the German standard (DIN). MIC values were determined with a photometer for microtitration plates (see above) after inoculation of antibiotic-containing microtitration plates (Merlin-Diagnostika) with 100 µl of appropriate bacterial suspension ($\sim 10^5$ cfu/ml) and incubation for 22 h at $36^\circ\text{C} \pm 1^\circ\text{C}$. MIC data were evaluated with EXCEL (Microsoft). All antibiotics were kindly provided for use by Merlin-Diagnostika by the manufacturers.

Evaluation of natural antibiotic susceptibility

The procedure has been described previously [19–24].

Results

Identification

An overview of the biochemical features of the *Klebsiella* strains examined is shown in Table 1. Generally, there were no significant differences between the results and those reported by Farmer [25]. Interestingly, several strains of different species were able to produce arginine dihydrolase. Citrate assimilation was seen in $>90\%$ of the *K. Ozaenae* strains tested, whereas Farmer found only 30% citrate-positive strains of this taxon [25]. Differences in biochemical features were not seen between *Klebsiella* strains of environmental and clinical origin (data not shown).

Except for *K. ornithinolytica*, *K. Ozaenae* and *K. Rhinoscleromatis*, the Micronaut E-identification system did not allow the correct discrimination of *Klebsiella* strains. Additional testing of EA, HA, MZ and HB utilisation was required for identification of *Klebsiella* strains belonging to *K. Pneumoniae*, *K. oxytoca*, *K. planticola* and *K. terrigena*. Identification results of all submitted reference strains were confirmed by testing these assimilation reactions. *K.*

Pneumoniae strains were uniformly EA-positive and HA-, MZ- and HB-negative. Nearly all *K. oxytoca* strains were also EA-positive and HA-negative, but uniformly HB-positive and in most cases able to assimilate MZ. *K. planticola* strains were uniformly HA-positive, but EA-, MZ- and HB-negative. *K. terrigena* strains were able to assimilate all additional substrates except EA (Table 1).

Of the strains originating from a multi-centre study by the Paul Ehrlich Society conducted in 1986, 10 of 49 strains pre-identified as '*K. oxytoca*' and one of 41 strains pre-identified as '*K. Pneumoniae*' were assigned to *K. planticola*. However, five of 34 '*K. planticola*' strains had to be re-assigned as *K. oxytoca*. *K. terrigena* strains were not found among *K. Pneumoniae* and *K. oxytoca* isolates.

Antibiotic susceptibility, natural sensitivity and primary resistance

The MIC distributions for the *Klebsiella* strains tested are presented in Table 2. MICs of antibiotics are presented separately for each species for which a distinctive susceptibility pattern was demonstrated. Susceptibility patterns within one species did not differ based on the origin of the strain (data not shown). The natural antibiotic susceptibility phenotypes of *Klebsiella* spp. are summarised in Table 3.

Discussion

Little information about the clinical significance of klebsiellae not belonging to *K. pneumoniae* or *K. oxytoca* correlates with the knowledge about the antibiotic susceptibility of these taxa. In the literature there are few data about the susceptibility to antimicrobial agents and there is no information about the natural antibiotic sensitivities and resistances of these micro-organisms. With the exception of *K. granulomatis*, in the present study the natural susceptibility of all known *Klebsiella* spp., including *K. Ozaenae* and *K. Rhinoscleromatis*, to a wide range of antibiotics was examined. Consistent MIC patterns to most of the β -lactam antibiotics tested were found among the species and subspecies. All taxa were naturally resistant or intermediate to amoxicillin and ticarcillin, but sensitive to amoxicillin/clavulanate and cephalosporins. These findings are consistent with data of Perkins *et al.* [26] and Podschun and Ullmann [10]. Because of higher MIC values to all β -lactam agents it is likely that the strains included in the latter study showed an increased β -lactamase expression compared with strains in the present study. Comparison of the results of the present study with those of the study by Freney *et al.* [27], by examining the antibiotic susceptibility of *K. terrigena* and *K. planticola*, could lead to erroneous conclusions, because only MIC50 and MIC90 values were cited. Nevertheless, the MICs

Table 1. Biochemical features of *Klebsiella* spp.

Biochemical test	Positive reactions (% of strains)						
	<i>K. pneumoniae</i> subsp. <i>pneumoniae</i>	<i>K. pneumoniae</i> subsp. <i>ozaenae</i>	<i>K. pneumoniae</i> subsp. <i>rhinoscleromatis</i>	<i>K. oxytoca</i>	<i>K. planticola</i>	<i>K. ornithinolytica</i>	<i>K. terrigena</i>
1. Tryptophan deaminase	0	0	0	0	0	0	0
2. H ₂ S production	3	0	0	0	0	0	0
3. β -Glucosidase (aesculin hydrolysis)	100	84	70	100	100	100	100
4. Tryptophanase (indole production)	0	0	0	100	73	100	0
5. <u>Urease</u>	<u>98</u>	<u>35</u>	<u>0</u>	98	93	100	8
6. <u>Lysin decarboxylase</u>	<u>90</u>	<u>41</u>	<u>0</u>	95	98	100	96
7. Ornithine decarboxylase	0	0	0	0	0	100	0
8. Arginine dihydrolase	10	32	0	0	23	48	0
9. Glucose fermentation	100	100	100	100	100	100	100
10. <u>Citrate assimilation</u>	<u>98</u>	<u>92</u>	<u>0</u>	100	100	100	96
11. Malonate assimilation	93	14	100	100	100	100	96
12. <u>Voges Proskauer reaction</u>	<u>95</u>	<u>0</u>	<u>0</u>	93	93	84	92
13. Rhamnose fermentation	98	62	90	100	100	100	100
14. Sucrose fermentation	100	19	60	100	100	100	100
15. Adonitol fermentation	95	97	100	61	95	100	88
16. (Myo)-inositol fermentation	95	86	90	100	100	100	100
17. Xylose fermentation	100	100	100	100	100	96	100
18. Sorbitol fermentation	98	59	100	100	98	100	92
19. <u>β-Galactosidase (ONPG)</u>	<u>100</u>	<u>100</u>	<u>0</u>	100	98	100	100
20. <u>β-Xylosidase (ONPX)</u>	<u>90</u>	<u>43</u>	<u>0</u>	73	70	100	12
21. β -Glucuronidase (PGUR)	0	0	0	0	0	0	0
22. Ethanolamine assimilation	100*	NT	NT	95*	0	NT	0
23. Histamine assimilation	0*	NT	NT	0	100†	NT	100
24. Melezitose assimilation	0	NT	NT	75	0	NT	100
25. Hydroxybenzoate assimilation	0	NT	NT	100‡	0	NT	96

Most of the reactions (nos. 1–21) were included in the panels of the Micronaut-E system (Merlin-Diagnostika, Bornheim, Germany). The remaining tests (nos. 22–25) were additional assimilation reactions. The results were read after 24 h (nos. 1–21, T:37°C) and 48 h (nos. 22–25, T:30°C), respectively. Key discriminating reactions for *Klebsiella* spp. are shown in bold print, discriminating reactions for *K. pneumoniae* subspecies are underlined. NT, not tested.

*Two; †one; ‡three strain(s) showed positive results after 96 h.

of ampicillin for 90% of *K. planticola* strains in this study were 4 mg/L, which would correlate with the natural ampicillin sensitivity of the species. The reasons for these findings are unclear and in contrast to the present study and to studies by Liu *et al.* [28] who investigated (among others) the β -lactam susceptibility and β -lactamases of *K. oxytoca* and *K. planticola*, and found *K. planticola* strains uniformly resistant to ampicillin.

The conformity of MIC patterns to most β -lactam agents among the species indicates the presence of typical chromosomally encoded class A β -lactamases described for *K. Pneumoniae* [29] and *K. oxytoca* [30] in all *Klebsiella* species. The enzymes of *K. Pneumoniae* and *K. oxytoca* are usually produced in small amounts and confer a relatively low degree of resistance to ampicillin and carboxypenicillins, but no resistance to amoxicillin/clavulanate and cephalosporins [30, 31]. This antibiotic phenotype was found in all species, although slight differences in natural β -lactam susceptibilities indicate the presence of different β -lactamases (see below).

In the present study it was shown that *K. oxytoca* strains were less susceptible to cefazoline and cefoperazone than other klebsiellae, although three strains were highly susceptible to the latter. *K. oxytoca* is known to produce a set of related β -lactamases called OXY-1 and OXY-2 β -lactamases [31], expression of which depends on the individual strain [32]. At least some of them hydrolyse penicillins, narrow-spectrum cephalosporins and, to a lesser extent, cefoperazone and aztreonam. Although the resistance phenotype of *K. oxytoca* strains is usually restricted to penicillins, low expression of these enzymes might correspond to the observed phenotype, i.e., natural sensitivity but a lower susceptibility to cefoperazone.

High susceptibility to cefoperazone in *K. oxytoca* was associated with different mechanisms. In two cases susceptibility was combined with an increased susceptibility to mezlocillin. This phenotype might be due to an extremely low-level expression of a cefoperazone-hydrolysing enzyme or, more likely – because strains highly susceptible to cefoperazone and mezlocillin showed the same susceptibility patterns to other β -lactam agents as did other *K. oxytoca* strains – to an OXY enzyme without activity against cefoperazone (and mezlocillin). In one strain, high susceptibility to cefoperazone was attributed to the individual inability to produce β -lactamase, recognised by sensitivity to amoxicillin and ticarcillin. β -Lactamase-negative *Klebsiella* strains are exceptional and have been reported for *K. Pneumoniae* [33].

In contrast to *K. oxytoca*, natural populations of other *Klebsiella* spp. showed unimodal distributions to cefoperazone and other β -lactam antibiotics, indicating the continuous expression of one enzyme or the

presence of several enzymes with the same substrate affinities in each species.

K. Pneumoniae strains express a chromosomally encoded class A β -lactamase designed K2 which has a pI 7.6 variant called SHV-1 and a pI 7.1 variant called LEN-1 [34]. Both variants possess similar substrate profiles and *K. Pneumoniae* strains produce only one variant, in most cases the pI 7.6 enzyme [32]. Haegmann *et al.* also detected the K2 gene in type strains of *K. Ozaenae* and *K. Rhinoscleromatis* by PCR [34]. These results are in agreement with the MIC data from the present study. Despite being generally more susceptible to most antibiotics, strains of *K. Ozaenae* and *K. Rhinoscleromatis* showed the same MIC patterns to β -lactam agents as strains of *K. Pneumoniae* (including the same synergic effects seen after the addition of β -lactamase inhibitors to aminopenicillins), pointing to related or identical enzymes in these strains.

In *K. planticola* and *K. ornithinolytica*, the slightly weaker synergic effect seen after the addition of clavulanic acid to amoxicillin and the stronger synergic effect after the addition of sulbactam to ampicillin (compared with the synergic effects seen in *K. oxytoca* and *K. pneumoniae*) indicate chromosomally encoded class A β -lactamases that differ from the well-known klebsiella enzymes. Liu *et al.* isolated chromosomally encoded β -lactamases with different IPs in *K. planticola* and found little sequence homology between the β -lactamase genes of *K. planticola* and *K. oxytoca* strains [28]. Although only four isolates were described, from the data of this and the present study it seems likely that *K. planticola* strains express a different set of β -lactamases to *K. oxytoca* and *K. pneumoniae*. Likewise, the clearly weak synergic effect observed in *K. terrigena* after the addition of clavulanic acid to amoxicillin allows the postulation of another chromosomally encoded class A enzyme specific to *K. terrigena*. If *K. ornithinolytica* and *K. planticola* strains – which show the same susceptibility patterns to β -lactam agents – express identical β -lactamases, this would provide additional evidence to support the status of *K. ornithinolytica* as a biogroup of *K. planticola* [5, 15].

In the present study significant differences in susceptibility to non- β -lactam antibiotics among *Klebsiella* species were seen with sulphamethoxazole and fosfomycin. Whereas the resistance of *K. terrigena* to fosfomycin seems to be associated with an intrinsic resistance phenomenon, the background of the 'natural' resistance of *K. Pneumoniae* to sulphamethoxazole has to be proven. Because mechanisms contributing to natural resistance are considered to depend on the appropriate species [22, 23], it is surprising that *K. Ozaenae* and *K. Rhinoscleromatis* strains were naturally sensitive to sulphamethoxazole, whereas *K. Pneumoniae* strains were not. Furthermore, a study by

Table 2. Antibiotic susceptibility of *Klebsiella* strains

Antibiotic	Concentrations examined (mg/L)	Taxon*	Number of strains with MIC (mg/L) of																
			0.01	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
Tetracyclines																			
Tetracycline	0.03–64	1A,1B							19	39	5	1	3	1		9			
		1C					9	1											
		2,3,4,5					17	104	7	2	1					3			
Doxycycline	0.03–64	1A						11	16			6	3	4					
		1C				2	7	1											
Minocycline	0.03–64	1B,2,3,4,5						15	134	15	6		1						
		1A						1	24	5	2	6	2						
		1C			1	8	1												
		1B,2,3,4,5						7	105	50	6	3							
Aminoglycosides																			
Amikacin	0.13–256	1A,2,3,4,5						1	92	77	3	1							
		1B,1C			9	15	16	7											
Gentamicin	0.06–128	1A,2,3,4,5				1	37	112	17	1	3	3							
		1B,1C			18	13	14	2											
Netilmicin	0.06–128	1A,2,3,4,5					45	122	4	2	1								
		1B,1C			19	22	6												
Tobramycin	0.06–128	1A,2,3,4,5					29	108	30	1	4	2							
		1B,1C			22	12	10	3											
Streptomycin	0.13–256	1A,2,3,4,5								49	100	8	3	8	4	1	1		
		1B,1C			5	11	15	11	5										
Kanamycin	0.13–256	1A,2,3,4,5							23	113	24	3	2	2				7	
		1B,1C			13	6	17	10	1										
Neomycin	0.13–256	1A,2,3,4,5						73	76	17	1			5	2				
		1B,1C			19	21	7	1											
Spectinomycin	0.13–256	1A,2,3,4,5									1	86	64	7	6	3	4	3	
		1B,1C								6	29	11	1						
Apramycin	0.06–128	1A,2,3,4,5					1	24	111	35	3								
		1B,1C			4	2	8	16	12	4	1								
Ribostamycin	0.06–128	1A,2,3,4,5						1	105	49	6	2	1	3		7			
		1B,1C					7	21	15	4									
Lividomycin A	0.06–128	1A,2,3,4,5							22	111	30	2	1	1		7			
		1B,1C			4	1	12	17	11	2									
β-Lactams: penicillins																			
Benzylpenicillin	0.01–32	1A,1C,2,3,4,5										16	74	63	27				
		1B									7	13	14	3					
Oxacillin	0.03–64	1A,2,3,4,5										1	57	81	35				
		1B			3						1	8	13	10	1	1			
Amoxicillin	0.06–128	1C					1	1	1			1	2	4					
		1A,1C,2					1						4	20	44	8	17		
		1B									2	10	13	7	4	1			
		3,4										2	8	37	14	3	1		
		5									6	10	7	1	1				

Amoxicillin/ clavulanic acid	0.06–128	1A,2,3,4 1B 1C 5	13	12 1	4 8 7	99 3 2 11	23 1 14	8	1 1	1 1	4 4	3 1	1 1	
Ampicillin/ sulbactam	0.06–128	1A,1C 1B,3,4,5 2				4 1	67 53 6	29 53 6	10 4 28	2 4 4	1 2 1	1 2 1	2 2 2	
Piperacillin	0.13–256	1A,2,3,4,5 1B,1C		3	7	14	36 17	54 5	52 1	5 1	3 3	1 1	2 3	7
Piperacillin/ tazobactam	0.13–256	1A,2,3,4,5 1B,1C		2 32	42 12	72 2	34 1	10	3	2	1 3	1 1	1 1	2
Ticarcillin	0.13–256	1A,1C,2,3,4,5 1B					1			1	2 11	39 14	62 8	45 2 13 1
Mezlocillin	0.13–256	1A,2,3,4,5 1B,1C		2		8	7 21	63 10	71 5	12 1	3 2	2 2	2 2	1 1 9
Azlocillin	0.25–512	1A,2,3,4,5 1B,1C					2 3	2 10	18 20	67 10	62 3	5 1	3	3 2 9
β-Lactams: cephalosporins														
Cefaclor	0.13–256	1A,2,3,4,5 1B,1C		11 23	124 16	23 6	6 1		1 3	1		1 4		
Cefazoline	0.13–256	1A,3,4,5 1B,1C 2		1	15	21 1	6 8	5 15	2 13	3 2	1 1	1 1	1 2	2
Loracarbef	0.13–256	All strains		65	101	37	7	2	4		1		1	2
Cefuroxime	0.03–64	1A,2,3,4,5 1B 1C			5 3	34 16	64 7	51 5	9 2	7 1	2 1	3	4	
Cefotiam	0.03–64	1A,2,3,4,5 1B,1C	1 15	1 71	5 60	3 10	6 4	3 3	1		2 1	1 1		
Cefetamet	0.03–64	All strains	51	121	31	12	1	3	2					
Cefoxitin	0.03–64	1A,1C,2,3,4,5 1B			2 1	12 12	12 12	70 6	12 2	7 1	2 1	1 1	1	
Cefixim	0.03–64	All strains	182	21	6	8	2							
Cefpodoxime	0.03–64	All strains	82	98	22	8	5	3	1	1	1			
Cefdinir	0.03–64	All strains	64	104	34	7	5	2	3	2				
Cefoperazone	0.03–64	1A 1B, 1C 3,4,5 2		7 11 44 1	17 9 22	3 6 8	5 3 1		3		1 1	1 2	2 1	1 3
Cefotaxime	0.03–64	All strains	191	11	7	4	4	1					2	3
Ceftibutene	0.03–64	All strains	171	31	13	3	2	1						
Ceftriaxone	0.03–64	All strains	189	14	9	3	2		2	2				
Ceftazidime	0.03–64	1A,1C,3,4,5 1B	21 28	83 8	55	11	6 1	4 1	1 1	1 1		1		
Cefepime	0.03–64	All strains	198	9	6	4	3	1						
β-Lactams: carbapenems														
Imipenem	0.03–64	1A 1B, 1C 2,3,4,5			12 11 25	15 1 7	10 3		1		1 1	1		
Meropenem	0.03–64	All strains	230	8	1	1				1	1			

(continued)

Antibiotic	Concentrations examined (mg/L)	Taxon*	Number of strains with MIC (mg/L) of																
			0.01	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
Biapenem	0.03–64	1A,1C,2,3,4,5 1B		46 15	74 5	28 3	23 7	10 6	1	2	1								
<i>β</i>-Lactams: monobactams																			
Aztreonam	0.03–64	1A,1B,1C,3,4,5 2		145 16	18 15	10 8	2 1	1		1	1			1					
Quinolones																			
Ciprofloxacin	0.01–32	1A 2,3,4,5,1B,1C	9 142	19 26	3 9	2 2	4 2	2	1										
Sparfloxacin	0.01–32	1A 2 3,4,5,1B,1C	1 4 86	12 24 36	17 13 8	3 1 5	3 1 2	2 1 2	1	1									
Norfloxacin	0.03–64	1A 2,3,4,5,1B,1C			1 102	18 57	12 13	2 2	4 3	1	2								
Ofloxacin	0.01–32	1A 2,3,4,5,1B,1C			5 113	24 49	2 11	4 3	2	1	2								
Enoxacin	0.01–32	1A 2,3,4,5,1B,1C				1 122	28 38	4 17	1 2	2 1	3 1	1							
Fleroxacin	0.01–32	1A 2,3,4,5,1B,1C			1 105	28 61	4 8	1 6	3 1	1 1	2								
Pefloxacin	0.01–32	All strains			68	119	18	7	6	1	2								
Pipemidic acid	0.06–128	1A 2,3,4,5,1B,1C							112	20 55	11 14	1 3	5 3	2	1				
Macrolides																			
Erythromycin	0.03–64	1A,2 3,4,5 1B 1C												5	28 71 6	51 19 3			
Roxithromycin	0.03–64	1A,2,3,4,5 1B 1C								2	8	3	4	21	1	173			
Clarithromycin	0.03–64	1A,2,3,4,5 1B 1C									5	4	1	3	15	17			
Azithromycin	0.03–64	1A,2 3,4,5 1B 1C									2 11 10	27 70 3	47 7 1	7 2		1			
Lincosamides																			
Lincomycin	0.01–32	All strains											1		220				
Clindamycin	0.01–32	1A,2,3,4,5 1B 1C										1	6 2	19 3	154 17 5				

Streptogramins																
Dalfopristin	0.03–64	All strains												2	219	
Quinupristin	0.03–64	All strains												1	220	
Dalfopristin/quinupristin (Synercid)	0.03–64	1A,2,3,4,5												5	169	
		1B											5	4	28	
		1C											3	6	1	
Anti-folates																
Sulphamethoxazole	0.25–512	1A														40
		1B							1	7	7	8	6	2	1	5
		1C			4	3	3									
		2							1	1	2	6	9	7	5	13
		3							1	1	4	7	3	1	9	14
		4							1	6	5	2	2	3	5	1
Trimethoprim	0.03–64	5									4	8	4	2	2	5
		1A,1B,2,3,4,5		27	87	60	19	7			3		6			
Trimethoprim/sulphamethoxazole (co-trimoxazole)	0.13–256	1C	6	4												
		1A,1B					12	34	19	1	2	5	1		2	
		1C			10											
		2,3,4,5				10	79	32	6	1	1				5	
Glycopeptides																
Teicoplanin	0.06–128	1A,1C,2,3,4,5														184
		1B											1	2	8	26
Vancomycin	0.03–64	1A,1C,2,3,4,5													184	
		1B											6	13	18	
Other antibiotics																
Chloramphenicol	0.06–128	All strains		8	109		54	26	3	4	3	1		4	9	
Nitrofurantoin	0.13–256	All strains					3	26	99	54	25	10		3	1	
Rifampicin	0.01–32	1A,2,3,4,5							20	125	29					
		1C,5						1	24	10						
Fosfomycin	0.13–256	1B	1	1		6	20	6	2	1						
		1A								15	14		3	4	1	3
		1B					3	3	3	5	16		7			
		1C								1	2		2	4	1	
		2						2	13	16	11					2
		3, 4								8	24		19	8		3
Fusidic acid	0.01–32	5									2	3	3	3	14	
		1A,1B,2,3,4,5							1	1	213					
		1C						1	7	2						

The number of strains with the corresponding MIC value is cited. Strains in the column for the lowest concentration of the antibiotic ($c_{\min}$) have MICs less than or equal to this lowest concentration ($\text{MIC} = c_{\min} \rightarrow \text{MIC} \leq c_{\min}$). MICs higher than the highest concentration tested were assigned to two times the highest concentration that was tested. MIC values in shaded areas indicate the clinically intermediate area according to the German standard (DIN). A black thick line indicates the breakpoint between clinically sensitive and clinically resistant strains, if the ‘intermediate’ does not apply. If the DIN criteria for an antibiotic are not applicable, other standards were employed. US breakpoints were used for spectinomycin, cefdinir, dalfopristin, quinupristin, dalfopristin/quinupristin, sulphamethoxazole and teicoplanin; French standards were used for streptomycin, kanamycin, neomycin, pefloxacin, lincomycin and fosfomycin and Swedish criteria for roxithromycin, clarithromycin, rifampicin and fusidic acid. UK breakpoints were used for trimethoprim. No established breakpoints exist for apramycin, ribostamycin, lividomycin A and biapenem.

*Abbreviations for taxa were used as follows: 1A, *K. pneumoniae* subsp. *pneumoniae*; 1B, *K. pneumoniae* subsp. *ozaenae*; 1C, *K. pneumoniae* subsp. *rhinoscleromatis*; 2, *K. oxytoca*; 3, *K. planticola*; 4, *K. ornithinolytica*; 5, *K. terrigena*.

Table 3. Grouping of the natural populations of *Klebsiella* taxa into the categories sensitive (S), intermediate (I) and resistant (R)*

Taxon	Benzylpenicillin, oxacillin	Amoxicillin	Ticarcillin	Azlocillin	Mezlocillin, ampicillin/sulbactam	Other penicillins	All tested cephalosporins	All tested carbapenems	Aztreonam	Erythromycin, clarithromycin	Roxithromycin	Azithromycin	Fosfomycin	All tested tetracyclines	Sulphamethoxazole	Trimethoprim, co-trimoxazole	All tested quinolones	All tested aminoglycosides	Chloramphenicol	Nitrofurantoin	Rifampicin	Fusidic acid	All lincosamides tested	All glycopeptides tested	All streptogramins tested
K. Pneumoniae	R	R	I-R	S-I	S-I	S	S	S	S	R	R	R	S-R	S-I	R [†]	S	S	S	S	S	S	R	R	R	R
K. Ozaenae	R	I-R	I-R	S	S	S	S	S	S	R	R	S-I	S-R	S-I	S	S	S	S	S	S	S	R	R	R	R
K. Rhinoscleromatis	R	R	I-R	S	S	S	S	S	S	I	I-R	S	S-R	S	S	S	S	S	S	S	S	R	R	R	R
K. oxytoca	R	R	I-R	S-I	S-I	S	S	S	S	R	R	R	S	S-I	S	S	S	S	S	S	S	R	R	R	R
K. planticola/K. ornithinolytica	R	R	I-R	S-I	S	S	S	S	S	R	R	I-R	S-R	S-I	S	S	S	S	S	S	S	R	R	R	R
K. terrigena	R	I-R	I-R	S-I	S	S	S	S	S	R	R	I-R	R	S-I	S	S	S	S	S	S	S	R	R	R	R

*According to the standards described in Table 2.

[†]See Discussion.

Bauernfeind showing MIC50 values of 32 mg/L for sulphamethoxazole to *K. Pneumoniae* brings the natural resistance phenomenon of sulphamethoxazole into question in this subspecies [35]. It might be possible that all *K. Pneumoniae* strains investigated in the present study form a population with acquired sulphamethoxazole resistance. Within the Enterobacteriaceae, *sulI* and *sulII* genes encoding a sulphonamide-insensitive dihydropteroate synthetase are widely distributed and have been found (among others) in *Escherichia coli*, *Citrobacter* spp. and also in *K. Pneumoniae* [36, 37]. On the other hand, sulphamethoxazole resistance determinants in *K. Pneumoniae* might be located on transposons integrated into the bacterial chromosome.

The molecular background for the natural resistance of *K. terrigena* to fosfomycin is unknown. Although in previous studies some species of Enterobacteriaceae like *Morganella morganii* [20], *Providencia stuartii* [21], *Escherichia vulneris* [24] and *Rahnella aquatilis* [38] were found to be naturally resistant to fosfomycin, there are no data about the underlying resistance mechanisms. Resistance to fosfomycin in *K. Pneumoniae* has been attributed to the expression of fosfomycin:glutathione-S-transferase and to a reduced permeability of the cell membrane to this antibiotic [39]. However, these data on resistance mechanisms acquired by *K. Pneumoniae* do not permit any statement about the mechanism(s) conferring intrinsic fosfomycin resistance in *K. terrigena*.

Further questions about the factors affecting differences in antibiotic susceptibility arise from the MIC data of *K. Ozaenae* and *K. Rhinoscleromatis* strains. Both taxa were generally more susceptible to most antibiotics than *K. Pneumoniae* and other *Klebsiella* strains. Interestingly, these strains also showed MIC patterns that are not seen in most other Enterobacteriaceae, e.g., naturally intermediately resistant to erythromycin and clarithromycin (*K. Rhinoscleromatis*), and an increased susceptibility to glycopeptides (*K. Ozaenae*) and fusidic acid (*K. Rhinoscleromatis*). Because natural resistance to these antibiotics in Enterobacteriaceae is mainly due to the impermeability of the outer membrane [40–42], it seems likely that the increased susceptibility of *K. Ozaenae* and *K. Rhinoscleromatis* strains to these and to the other antibiotics tested is at least in part attributed to a different cell envelope of these bacteria, allowing an increased entry of certain compounds into the cell. The increased susceptibilities might be due to a different peptidoglycan architecture, but could also be influenced by different components of the polysaccharide capsules.

We are very grateful to Rainer Podschun, Kiel, Germany, and Gerda Stempfel, Weingarten, Germany, for providing numerous isolates. We thank Barbara Körber for her excellent technical assistance in the identification of several strains. The generous support from Merlin-Diagnostika, Bornheim, Germany is gratefully acknowledged.

References

- Podschun R, Ullmann U. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin Microbiol Rev* 1998; **11**: 589–603.
- Le Minor. The Genus *Salmonella*. In: Balows A, Trüper GG, Dworkin M, Harder W, Schleifer KH (eds) *The Prokaryotes: a handbook on the biology of bacteria: ecophysiology, isolation, identification, applications*. New York, Springer-Verlag. 1992: 2760–2774.
- Goldstein EJ, Lewis RP, Martin WJ, Edelstein PH. Infections caused by *Klebsiella ozaenae*: a changing disease spectrum. *J Clin Microbiol* 1978; **8**: 413–418.
- Tang L-M, Chen S-T. *Klebsiella ozaenae* meningitis: report of two cases and review of the literature. *Infection* 1994; **22**: 58–61.
- Grimont F, Grimont PAD, Richard C. The Genus *Klebsiella*. In: Balows A, Trüper GG, Dworkin M, Harder W, Schleifer KH (eds) *The Prokaryotes: a handbook on the biology of bacteria: ecophysiology, isolation, identification, applications*. New York, Springer-Verlag. 1992: 2775–2796.
- Arakawa Y, Ohta M, Kido N *et al.* Chromosomal β -lactamase of *Klebsiella oxytoca*, a new class A enzyme that hydrolyzes broad-spectrum β -lactam antibiotics. *Antimicrob Agents Chemother* 1989; **33**: 63–70.
- Monnet D, Freney J, Brun Y, Boeufgras JM, Fleurette J. Difficulties in identifying *Klebsiella* strains of clinical origin. *Zentralbl Bakteriol* 1991; **274**: 456–464.
- Mori M, Ohta M, Agata N *et al.* Identification of species and capsular types of *Klebsiella* clinical isolates, with special reference to *Klebsiella planticola*. *Microbiol Immunol* 1989; **33**: 887–895.
- Podschun R, Ullmann U. Incidence of *Klebsiella planticola* among clinical isolates. *Med Microbiol Lett* 1994; **3**: 90–95.
- Podschun R, Ullmann U. Isolation of *Klebsiella terrigena* from clinical specimens. *Eur J Clin Microbiol Infect Dis* 1992; **11**: 349–352.
- Podschun R, Acktun H, Okpara J, Linderkamp O, Ullmann U, Borneff-Lipp M. Isolation of *Klebsiella planticola* from newborns in a neonatal ward. *J Clin Microbiol* 1998; **36**: 2331–2332.
- Podschun R, Fischer A, Ullmann U. Expression of putative virulence factors by clinical isolates of *Klebsiella planticola*. *J Med Microbiol* 2000; **49**: 115–119.
- Bach S, de Almeida A, Carniel E. The *Yersinia* high-pathogenicity island is present in different members of the family Enterobacteriaceae. *FEMS Microbiol Lett* 2000; **183**: 289–294.
- Sakazaki R, Tamura K, Kosako Y, Yoshizaki E. *Klebsiella ornithinolytica* sp. nov., formerly known as ornithine-positive *Klebsiella oxytoca*. *Curr Microbiol* 1989; **18**: 201–206.
- Farmer JJ, Davis BR, Hickmann-Brenner FW *et al.* Biochemical identification of new species and biogroups of Enterobacteriaceae isolated from clinical specimens. *J Clin Microbiol* 1985; **21**: 46–76.
- Kharsany ABM, Hoosen AA, Kiepiela P, Kirby R, Sturm AW. Phylogenetic analysis of *Calymmatobacterium granulomatis* based on 16S rRNA gene sequences. *J Med Microbiol* 1999; **48**: 841–847.
- Carter JS, Bowden FJ, Bastian I, Myers GM, Sriprakash KS, Kemp DJ. Phylogenetic evidence for reclassification of *Calymmatobacterium granulomatis* as *Klebsiella granulomatis* comb. nov. *Int J Syst Bacteriol* 1999; **49**: 1695–1700.
- Monnet D, Freney J. Method for differentiating *Klebsiella planticola* and *Klebsiella terrigena* from other *Klebsiella* species. *J Clin Microbiol* 1994; **32**: 1121–1122.
- Stock I, Wiedemann B. [Natürliche Antibiotika-Empfindlichkeit von *Proteus*-Spezies im *Proteus-vulgaris*-Komplex]. *Chemother J* 1997; **6**: 76–84.
- Stock I, Wiedemann B. Identification and natural antibiotic susceptibility of *Morganella morganii*. *Diagn Microbiol Infect Dis* 1998; **30**: 153–165.
- Stock I, Wiedemann B. Natural antibiotic susceptibility of *Providencia stuartii*, *P. rettgeri*, *P. alcalifaciens* and *P. rustigianii* strains. *J Med Microbiol* 1998; **47**: 629–642.
- Stock I, Wiedemann B. The determination of natural antibiotic susceptibility [Die Bestimmung der natürlichen Antibiotika-Empfindlichkeit]. *Chemother J* 1998; **7**: 127–135.
- Stock I, Wiedemann B. An in-vitro study of the antimicrobial susceptibilities of *Yersinia enterocolitica* and the definition of a database. *J Antimicrob Chemother* 1999; **43**: 37–45.
- Stock I, Wiedemann B. Natural antibiotic susceptibility of *Escherichia coli*, *Shigella*, *E. vulneris*, and *E. hermannii* strains. *Diagn Microbiol Infect Dis* 1999; **33**: 187–199.
- Farmer JJ. Enterobacteriaceae: introduction and identification. In: Murray PR, Baron EJ, Pfaller M, Tenover F, Tenover R (eds) *Manual of clinical microbiology*, 6th edn. Washington, DC, ASM Press. 1995: 438–449.
- Perkins BA, Hamill RJ, Musher DM, O'Hara C. In vitro activities of streptomycin and 11 oral antimicrobial agents against clinical isolates of *Klebsiella rhinoscleromatis*. *Antimicrob Agents Chemother* 1992; **36**: 1785–1787.
- Freney J, Husson MO, Gavini F *et al.* Susceptibilities to antibiotics and antiseptics of new species of the family Enterobacteriaceae. *Antimicrob Agents Chemother* 1988; **32**: 873–876.
- Liu Y, Mee BJ, Mulgrave L. Identification of clinical isolates of indole-positive *Klebsiella* spp., including *Klebsiella planticola*, and a genetic and molecular analysis of their β -lactamases. *J Clin Microbiol* 1997; **35**: 2365–2369.
- Labia R, Fabre C, Masson J-M, Barthelemy M, Heitz M, Pitton J-S. *Klebsiella pneumoniae* strains moderately resistant to ampicillin and carbenicillin: characterization of a new β -lactamase. *J Antimicrob Chemother* 1979; **5**: 375–382.
- Sykes RB, Matthew M. The β -lactamases of Gram-negative bacteria and their role in resistance to β -lactam antibiotics. *J Antimicrob Chemother* 1976; **2**: 115–157.
- Fournier B, Roy PH, Lagrange PH, Philippon A. Chromosomal β -lactamase genes of *Klebsiella oxytoca* are divided into two main groups, bla_{OXY-1} and bla_{OXY-2}. *Antimicrob Agents Chemother* 1996; **40**: 454–459.
- Leung M, Shannon K, French G. Rarity of transferable β -lactamase production by *Klebsiella* species. *J Antimicrob Chemother* 1997; **39**: 737–745.
- Livermore DM. β -Lactamases in laboratory and clinical resistance. *Clin Microbiol Rev* 1995; **8**: 557–584.
- Haegman S, Löfdahl S, Burman LG. An allelic variant of the chromosomal gene for class A beta-lactamase K2, specific for *Klebsiella pneumoniae*, is the ancestor of SHV-1. *Antimicrob Agents Chemother* 1997; **41**: 2705–2709.
- Bauernfeind A, Hörl G, Przyklenk B. Microbiological perspectives of co-trimoxazole. *Infection* 1987; **15** Suppl 5: 232–235.
- Williams Smith H, Parsell Z. A transmissible plasmid determining lactose fermentation and multiple antibiotic resistance in a strain of *Klebsiella pneumoniae*. *J Med Microbiol* 1976; **9**: 359–362.
- Wise EM, Abou-Donia MM. Sulfonamide resistance mechanism in *Escherichia coli*: R plasmids can determine sulfonamide-resistant dihydropteroate synthases. *Proc Natl Acad Sci USA* 1975; **72**: 2621–2625.
- Stock I, Gröger T, Wiedemann B. Natural antibiotic susceptibility of *Rahnella aquatilis* and *R. aquatilis*-related strains. *J Chemother* 2000; **12**: 30–39.
- O'Hara K. Two different types of fosfomycin resistance in clinical isolates of *Klebsiella pneumoniae*. *FEMS Microbiol Lett* 1993; **114**: 9–16.
- Vaara M, Vaara T. Polycations sensitize enteric bacteria to antibiotics. *Antimicrob Agents Chemother* 1983; **24**: 107–113.
- Nikaido H, Vaara M. Molecular basis of bacterial outer membrane permeability. *Microbiol Rev* 1985; **49**: 1–32.
- Vaara M. Outer membrane permeability barrier to azithromycin, clarithromycin, and roxithromycin in gram-negative enteric bacteria. *Antimicrob Agents Chemother* 1993; **37**: 354–356.