

Klebsiella spp. as Nosocomial Pathogens: Epidemiology, Taxonomy, Typing Methods, and Pathogenicity Factors

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INTRODUCTION	589
EPIDEMIOLOGY	589
TAXONOMY OF THE GENUS <i>KLEBSIELLA</i>	591
DIFFERENTIATION OF <i>KLEBSIELLA</i> SPECIES	591
TYPING OF <i>KLEBSIELLA</i> ISOLATES.....	592
Biotyping	592
Serotyping	592
Phage Typing	592
Bacteriocin Typing.....	592
Molecular Typing Methods	592
PATHOGENICITY FACTORS OF <i>KLEBSIELLA</i>	592
Capsular Antigens	593
Pili (Fimbriae).....	594
Type 1 (common) pili	594
Type 3 pili	595
Serum Resistance and Lipopolysaccharide.....	595
Siderophores.....	596
VACCINATION EFFORTS	597
Lipopolysaccharides.....	597
Capsular Polysaccharides.....	597
CONCLUDING REMARKS.....	597
REFERENCES	598

INTRODUCTION

Klebsiella is well known to most clinicians as a cause of community-acquired bacterial pneumonia, occurring particularly in chronic alcoholics (40) and showing characteristic radiographic abnormalities (81) due to a severe pyogenic infection which has a high fatality rate if untreated.

The vast majority of *Klebsiella* infections, however, are associated with hospitalization. As opportunistic pathogens, *Klebsiella* spp. primarily attack immunocompromised individuals who are hospitalized and suffer from severe underlying diseases such as diabetes mellitus or chronic pulmonary obstruction. Nosocomial *Klebsiella* infections are caused mainly by *Klebsiella pneumoniae*, the medically most important species of the genus. To a much lesser degree, *K. oxytoca* has been isolated from human clinical specimens. It is estimated that *Klebsiella* spp. cause 8% of all nosocomial bacterial infections in the United States and in Europe. No great geographical variations in frequency have been noted. In the United States, *Klebsiella* accounts for 3 to 7% of all nosocomial bacterial infections, placing them among the eight most important infectious pathogens in hospitals (104, 211), and data collected from the United Kingdom (26) and from Germany (242) are remarkably similar to those reported by the Centers for Disease Control and Prevention.

Table 1 lists the most frequent nosocomial infections caused

by *Klebsiella*. The urinary tract is the most common site of infection. *Klebsiella* accounts for 6 to 17% of all nosocomial urinary tract infections (UTI) and shows an even higher incidence in specific groups of patients at risk, e.g., patients with neuropathic bladders or with diabetes mellitus (25, 133). As a cause of nosocomial gram-negative bacteremia, *Klebsiella* is second only to *Escherichia coli* (35, 70, 181, 258).

In pediatric wards, nosocomial *Klebsiella* infections are especially troublesome—particularly in premature infants and intensive care units. *Klebsiella* species are often the pathogens involved in neonatal sepsis (Table 1), in both early-manifestation and late-manifestation infections (90).

Due to the extensive spread of antibiotic-resistant strains, especially of extended-spectrum β -lactamase (ESBL)-producing strains, there has been renewed interest in *Klebsiella* infections.

EPIDEMIOLOGY

Klebsiella spp. are ubiquitous in nature. Klebsiellae probably have two common habitats, one being the environment, where they are found in surface water, sewage, and soil and on plants (15, 33, 71, 140, 214), and the other being the mucosal surfaces of mammals such as humans, horses, or swine, which they colonize. In this respect, the genus *Klebsiella* is like *Enterobacter* and *Citrobacter* but unlike *Shigella* spp. or *E. coli*, which are common in humans but not in the environment.

In humans, *K. pneumoniae* is present as a saprophyte in the nasopharynx and in the intestinal tract. Carrier rates differ considerably from study to study. The detection rate in stool samples ranges from 5 to 38%, while rates in the nasopharynx

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TABLE 1. Hospital-acquired bacterial infections caused by *Klebsiella* spp.

Infection	% of infections caused by <i>Klebsiella</i>	Rank ^a	References
UTI	6-17	5-7	26, 46, 104, 242
Pneumonia	7-14	2-4	26, 44, 104
Septicemia	4-15	3-8	34, 35, 45-47, 64, 70, 135, 181, 242, 258
Wound infections	2-4	6-11	104, 142, 242
Nosocomial infections in intensive care unit patients	4-17	4-9	26, 104, 226, 242
Neonatal septicemia	3-20	2-8	24, 84, 168, 235, 240, 247

^a Ranking of *Klebsiella* compared to all other bacterial pathogens.

range from 1 to 6% (62, 206, 208, 236). Because gram-negative bacteria do not find good growth conditions on the human skin (207), *Klebsiella* spp. are rarely found there and are regarded simply as transient members of the flora (126).

These carrier rates change drastically in the hospital environment, where colonization rates increase in direct proportion to the length of stay. Even hospital personnel have elevated rates of *Klebsiella* carriage (42, 43, 52). Reported carrier rates in hospitalized patients are 77% in the stool, 19% in the pharynx, and 42% on the hands of patients (52, 62, 112, 193, 206, 215, 225). The high rate of nosocomial *Klebsiella* colonization appears to be associated with the use of antibiotics rather than with factors connected with delivery of care in the hospital (193, 206). Previous antibiotic therapy is significantly associated with acquisition of *Klebsiella* by the patient. In one study, 2 weeks after admission to the hospital, a two- to fourfold increase in the colonization rates with *Klebsiella* was observed (193); this increase occurred primarily in patients receiving antibiotics, especially in persons receiving broad-spectrum or multiple antibiotics. In the hospital setting, the local antibiotic policy is a major determinant of the colonization pattern. The significance of increased colonization was illustrated by the observation that the attack rate of *Klebsiella* nosocomial infection in patients carrying hospital-acquired intestinal *Klebsiella* was four times as high as for noncarriers (215). Furthermore, widespread use of antimicrobial therapy has often been held responsible for the occurrence of multiply resistant *Klebsiella* strains in hospitals (215, 239). Because these undesired effects may be reversed by strict control of antibiotic use, demands for strategies to avoid the overuse of antibiotics in prophylaxis and empirical therapy are increasingly being expressed.

Apart from medical equipment (contaminated due to faulty hygienic procedures) and blood products (89, 116, 198), the principal reservoirs for transmission of *Klebsiella* in the hospital setting are the gastrointestinal tract of patients and the hands of hospital personnel (156). The ability of this organism to spread rapidly (129) often leads to nosocomial outbreaks, especially in neonatal units (98). Of the 145 epidemic nosocomial infections reported in the literature published in English between 1983 and 1991, 13 were caused by *Klebsiella* (68). According to the statistics of the Centers for Disease Control and Prevention, *Klebsiella* spp. account for 8% of endemic hospital infections and 3% of epidemic outbreaks (227).

Especially feared are epidemic hospital infections caused by multiresistant strains. In the 1970s, these strains were chiefly aminoglycoside-resistant *Klebsiella* strains (48, 60, 138, 160). Since 1982, strains that produce ESBLs, which render them resistant to extended-spectrum cephalosporins, have evolved (22, 53, 63, 85, 96, 113, 144, 146, 200). The hallmark of these

strains, resistance to ceftazidime, is observed in both *K. pneumoniae* and *K. oxytoca* isolates (224). In Europe, the β -lactamases of ceftazidime-resistant *Klebsiella* strains are commonly of the SHV-5 type, whereas TEM-10 and TEM-12 are more prevalent in the United States (22, 85, 108, 130, 144, 179, 213, 243, 245). The incidence of ESBL-producing *Klebsiella* isolates in the United States has been reported to be 5% of the *K. pneumoniae* strains tested in the National Nosocomial Infection Study system (108). In Europe, the frequency of such strains seems to be even higher. A percentage of 14 to 16% ESBL producers among clinical *Klebsiella* isolates has been reported for France and England (221). In particular regions or hospitals, the incidence can reach 25 to 40% (38). However, the percentage of ceftazidime-resistant strains may be much higher, because the conventional disc diffusion criteria used in the routine laboratory underestimate the incidence of these isolates (109).

ESBLs are usually plasmid mediated. Since these plasmids are easily transmitted among different members of the *Enterobacteriaceae*, accumulation of resistance genes results in strains that contain multiresistant plasmids. For this reason, ESBL-producing isolates are resistant to a variety of classes of antibiotics. Moreover, the emergence of these multiply resistant *Klebsiella* strains is unfortunately accompanied by a relatively high stability of the plasmids encoding ESBLs. Even years after discontinuation of ceftazidime and other extended-spectrum cephalosporins, continued colonization of patients by ESBL-producing *Klebsiella* strains has been observed (101). Risk factors for acquisition of these strains seem to be the length of stay in hospital and the performance of invasive procedures (132).

Since ESBL production frequently is accompanied by multiresistance to antibiotics, therapeutic options become limited. So far, however, ESBL-producing *Klebsiella* strains have been susceptible to carbapenems such as imipenem or meropenem. Both antibiotics are the drugs of choice in the treatment of infections due to ESBL-producing organisms. In this respect, a recent observation is very disturbing. For the first time, ESBL-producing *K. pneumoniae* strains which showed an additional resistance to imipenem have been isolated (30). These strains possessed a transmissible plasmid-mediated AmpC-type β -lactamase. This development should be monitored closely, since the emergence of imipenem-resistant ESBL-producing *Klebsiella* strains will have a serious impact on remaining therapeutic options.

In the last several years, the question has arisen whether it is necessary to determine if each isolated *Klebsiella* strain is an ESBL producer. The answer depends on the epidemiologic situation of a country or a hospital, but it should definitely be positive if a high percentage of ceftazidime-resistant strains is to be expected. To date, two diagnostic tests have been most commonly used for the detection of such isolates. In the double-disc synergy test, a disc of clavulanic acid and a disc of an extended-spectrum cephalosporin such as ceftazidime are placed close together on an agar surface inoculated with the test organism (111). Enhancement of the zone of inhibition around the cephalosporin disc towards the clavulanate-containing disc indicates the presence of an ESBL-producing strain. A commercially available product is the ESBL screening E test strip (AB Biodisk, Solna, Sweden). This method is based on the evaluation of the difference between the antimicrobial activity of ceftazidime alone compared to that of ceftazidime plus clavulanic acid (119).

It should be kept in mind that a number of measures have been recommended to prevent the nosocomial spread of *Klebsiella*. Strict adherence to basic epidemiological standards for

TABLE 2. Species classification of the genus *Klebsiella* by different taxonomic systems^a

	Classification by:		
	Cowan	Bascomb	Ørskov
<i>K. aerogenes</i>	<i>K. aerogenes/oxytoca/</i>	<i>K. pneumoniae</i>	
<i>K. edwardsii</i>	<i>edwardsii</i>	<i>subsp. pneumoniae</i>	
<i>subsp. edwardsii</i>	<i>K. pneumoniae</i>	<i>subsp. ozaenae</i>	
<i>subsp. atlantae</i>	<i>sensu stricto</i>	<i>subsp. rhinoscleromatis</i>	
<i>K. pneumoniae</i>	<i>sensu lato</i>	<i>K. oxytoca</i>	
<i>K. ozaenae</i>	<i>K. ozaenae</i>	<i>K. terrigena</i>	
<i>K. rhinoscleromatis</i>	<i>K. rhinoscleromatis</i>	<i>K. planticola</i> (syn.	
	<i>K. "unnamed group"</i>	<i>K. trevisanii</i>)	
	<i>Enterobacter aerogenes</i>	<i>K. ornithinolytica</i>	

^a Data from references 18, 31, 54, 110, 174, and 228.

the management of urinary catheters, intravenous tracheostomies, and wounds, maintenance and care of equipment, and good hand-washing practices all help to prevent the spread of nosocomial *Klebsiella* infections. Detailed information on this subject is given in an excellent review by Montgomerie (156). Another measure to control *Klebsiella* infections is the regulation of antibiotic use in the hospital to prevent misuse and overuse of antibiotics. Furthermore, nosocomial infection surveillance is necessary to collect data that are used in the prevention and control of nosocomial *Klebsiella* infection rates.

TAXONOMY OF THE GENUS *KLEBSIELLA*

The taxonomy of *Klebsiella* is characterized by a nomenclature reflecting its colorful taxonomic history. Originally, the medical importance of the genus *Klebsiella* (family *Enterobacteriaceae*) led to its being subdivided into three species corresponding to the diseases they caused: *K. pneumoniae*, *K. ozaenae*, and *K. rhinoscleromatis*. As the taxonomy became increasingly refined due to the development of new methods such as numerical taxonomy, the species classification in this genus was continually revised. In time, three main classifications emerged, those of Cowan, Bascomb, and Ørskov (Table 2).

In the early 1980s, *Klebsiella* isolates from the environment, which had previously been classified as "*Klebsiella*-like organisms" (groups J, K, L, and M), were increasingly being classi-

fied into provisional taxa (87). These groups gave rise to four new species: *K. terrigena* (107), *K. ornithinolytica* (210), *K. planticola* (14), and *K. trevisanii* (82). In 1986, the last two species were combined into one species, *K. planticola*, because of their extensive DNA sequence homology (86). While originally considered to be without clinical significance and restricted to aquatic, botanical, and soil environments, *K. terrigena* and *K. planticola* have recently been reported as occurring in human clinical specimens (158, 189, 190). According to these findings, particularly *K. planticola* has been isolated from human infections with a surprisingly high frequency of 3.5 to 18.5% among clinical isolates of *Klebsiella* species. More than half of these isolates were recovered from respiratory tract secretions; wound and urine isolates were the next most common (189). However, since most of the isolates were obtained from polymicrobial specimens, it is difficult to estimate the significance of these strains as causative agents of disease. Nevertheless, 6 of the 94 isolates were recovered from monomicrobial specimens and could be assigned to corresponding infections. Thus, at present it seems possible that in addition to *K. pneumoniae* and *K. oxytoca*, a third *Klebsiella* species exists that is able to cause human infections.

The adoption of a consistent nomenclature has been further complicated by the fact that Great Britain and the former Commonwealth countries adhere to the classification of Cowan while the USA prefers Ørskov's classification. Consequently, the same bacterium may be called *K. pneumoniae* in one country and *K. aerogenes* in another. Most European countries follow the American example and recognize the worldwide predominant classification of Ørskov.

DIFFERENTIATION OF *KLEBSIELLA* SPECIES

Klebsiella species are usually identified and differentiated according to their biochemical reactions. The genus is defined as containing gram-negative, nonmotile, usually encapsulated rod-shaped bacteria of the family *Enterobacteriaceae*, which produce lysine decarboxylase but not ornithine decarboxylase and are generally positive in the Voges-Proskauer test (75). Within the genus *Klebsiella*, the individual species can be differentiated on the basis of the features listed in Table 3. Whereas most *Klebsiella* species can be identified by standard microbiological laboratory tests, the species *K. terrigena* and

TABLE 3. Biochemical reactions of *Klebsiella* species^a

Characteristic	<i>Klebsiella pneumoniae</i>			<i>K. oxytoca</i>	<i>K. terrigena</i>	<i>K. planticola</i>	<i>K. ornithinolytica</i>
	<i>subsp. pneumoniae</i>	<i>subsp. ozaenae</i>	<i>subsp. rhinoscleromatis</i>				
Indole	—	—	—	+	—	√ ^b	+
Ornithine decarboxylase	—	—	—	—	—	—	+
Lysine decarboxylase	+	v	—	+	+	+	+
Pectate degradation	—	—	—	+	—	—	—
Gas from lactose at 44.5°C	+	—	—	—	—	—	—
Growth at 10°C	—	—	—	+	+	+	+
Acid from:							
D-Melezitose	—	—	—	v	+	—	—
L-Sorbose	v	—	—	+	+	+	—
Utilization of:							
<i>m</i> -Hydroxybenzoate	—	—	—	+	+	—	—
Hydroxy-L-proline	v	—	—	v	v	+	—
Malonate	—	—	+	+	+	+	+
Methyl red test	—	+	+	—	+	v	+
Voges-Proskauer reaction	+	—	—	+	+	+	+

^a Data summarized from references 14, 79, 107, 153, 154, 174, 210, and 228.

^b v, variable reaction.

K. planticola require special, nonconventional reactions (such as utilization of *m*-hydroxybenzoate or hydroxy-L-proline, pectate degradation, acid from melezitose, or growth at 10°C).

TYPING OF *KLEBSIELLA* ISOLATES

From an epidemiological point of view, it is often necessary to determine the clonality of the strains. This is particularly important in endemic and epidemic nosocomial outbreaks of *Klebsiella* infections to improve the management of such outbreaks. A variety of methods have been used with various degrees of success in *Klebsiella* typing and are discussed below.

Biotyping

Biotyping based on an extended panel of biochemical and culture tests is certainly the most practicable method of typing for smaller laboratories that are epidemiologically not optimally equipped. Biotyping can be carried out by using macrotube tests alone (100, 202) or by combining a commercially available miniaturized system such as the API 20E system with additional macrotube tests (185, 217). However, because of the large number of reactions to be tested and the often long cultivation times—up to 90 days for demonstration of gelatinase (228)—biotyping of *Klebsiella* spp. is not very suitable as an epidemiological tool.

Serotyping

Serotyping is currently the most widely used technique for typing *Klebsiella* spp. It is based mainly on a division according to the capsule antigens (177). *Klebsiellae* usually have well-developed polysaccharide capsules, which give their colonies their characteristic mucoid appearance. Of 82 capsule antigens described, 77 types form the basis for an internationally recognized capsule antigen scheme (176). Although 12 different O-antigen types of *Klebsiella* have also been described, they are difficult to classify because their determination is hampered by the heat-stable capsules (175, 177). Capsule typing, by contrast, shows good reproducibility and is capable of differentiating most clinical isolates (12). The drawback of this method is the large number of serological cross-reactions that occur among the 77 capsule types. Thus, individual sera have to be absorbed with the cross-reacting K-antigens. Moreover, the typing procedure is cumbersome because of the time needed to perform the test and is susceptible to subjective interpretations because of weak reactions that are not always easy to interpret. Since anti-capsule antisera are not commercially available, this technique is practiced mostly in specialized laboratories. However, in contrast to capsule typing, neither biochemical typing, bacteriocin typing, nor phage typing alone is sufficiently discriminative and reproducible for epidemiological purposes except under certain conditions (177). The combined use of biotyping and capsule typing enables the differentiation of a large number of bioserotypes (202).

Phage Typing

Phage typing of *Klebsiella* was first developed in the 1960s (196, 223). Although the phage reaction is easily read and the reproducibility of the method is acceptable, this technique shows a relatively poor typing rate of 19 to 67% (209, 222). Since it is not an alternative to capsule typing, this procedure has never become widespread and is useful mainly as a secondary method in combination with serologic testing (48, 53, 113). It should be stressed, however, that it is possible to develop capsule- and O-antigen-specific phage typing if appro-

priate efforts are made, as a number of reports have demonstrated (143, 180, 237).

Bacteriocin Typing

Although capsule typing is the preferred method for *Klebsiella*, it has been advised to include an additional feature independent of the capsule type to enable more precise epidemiological analysis. Many authors recommend typing *Klebsiella* via bacteriocins (20, 36, 73, 97, 223). Bacteriocins are bactericidal substances, usually proteins, produced by bacteria to inhibit the growth of other bacteria, usually members of the same species. An isolate can be characterized either by its ability to inhibit specific indicator strains or by its sensitivity to bacteriocins synthesized by a set of producer strains. Since the synthesis of bacteriocins is not frequent enough in *Klebsiella*, the latter technique has become the method of choice for bacteriocin typing of organisms belonging to this genus. However, the two principal early methods—the growth-in-broth method and the cross-streak method—both show considerable disadvantages. Because of the instability of bacteriocin preparations, the reproducibility of the growth-in-broth method is low. In addition, the conventional cross-streak method results in low typability of strains (36, 73, 97). The limitations of both of these methods have been surmounted by a modification of the “scrape-and-point” procedure (20), which avoids the use of potentially unstable preproduced and stored bacteriocins. Instead, the bacteriocins are synthesized on an agar medium immediately before the strains to be typed are inoculated by a multipoint inoculator. This method has proven superior for bacteriocin typing of clinical and environmental *Klebsiella* strains (21, 187, 188) as well as of nosocomial outbreaks of *Klebsiella* (22).

Molecular Typing Methods

Molecular typing methods, as applied to the genus *Klebsiella*, are still in their infancy. Preliminary descriptions have been presented on plasmid profiles (22, 28, 53, 99, 157, 185, 250), ribotypes (8, 27, 28), multilocus enzyme analyses (51, 161), and applications of pulsed-field gel electrophoresis (8, 91, 192). The procedures vary from laboratory to laboratory and lack standardization, making it difficult to compare them.

PATHOGENICITY FACTORS OF *KLEBSIELLA*

The terms “pathogenicity factor” and “virulence factor” are used synonymously by some authors (212), while others lay emphasis on a clear-cut distinction between them. In this review, the term “pathogenicity” defines the ability of a bacterium to cause disease while “virulence” is the measurement or degree of pathogenicity of any bacterial species.

Nosocomial *Klebsiella* infections most commonly involve the urinary and respiratory tracts. Since these two body sites differ considerably with respect to the host defense mechanisms, it should be expected that the pattern of virulence factors found in UTI-causing strains of *Klebsiella* will differ from that observed in strains isolated from pulmonary sources of patients with pneumonia.

The search for the pathogenic mechanisms of *Klebsiella* infections has identified a number of bacterial factors that contribute to the pathogenesis of these bacteria. Both in vitro and in vivo models have been established to investigate the interaction of bacterial cells and the host. The use of animal models has been a critical element in the study of *Klebsiella* pathogenicity by providing vital information that cannot be obtained from in vitro studies. In particular, animal models have been

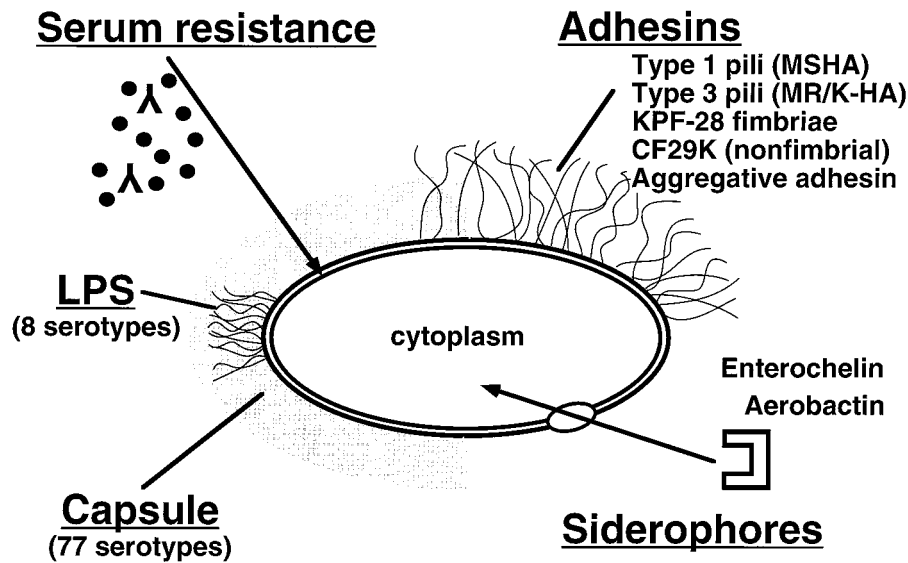


FIG. 1. Schematic representation of *Klebsiella* pathogenicity factors.

established to study *Klebsiella* virulence factors in UTIs; mice and rats seem to be appropriate animal types. Lower UTIs have been investigated in the diuresis mouse or rat model of cystitis by intravesicular injection of organisms (77, 134). To study *Klebsiella*-mediated upper UTI, a rat model of experimental retrograde pyelitis has been established (78). Frequently, both models include scanning electron microscopy of the surface of the bladder or renal pelvis.

The current research into the pathogenicity of *Klebsiella* focuses on the group of five factors shown in Fig. 1.

Capsular Antigens

As mentioned above, klebsiellae usually develop prominent capsules composed of complex acidic polysaccharides. The capsular repeating subunits, consisting of four to six sugars and, very often, uronic acids (as negatively charged components), can be classified into 77 serological types (177). Capsules are essential to the virulence of *Klebsiella* (56, 69, 76, 103). The capsular material forms thick bundles of fibrillous structures covering the bacterial surface in massive layers (Fig. 2) (7). This protects the bacterium from phagocytosis by polymorphonuclear granulocytes, on the one hand (186, 191, 218, 219), and prevents killing of the bacteria by bactericidal serum factors, on the other (252). The molecular mechanism presumably consists of inhibiting the activation or uptake of complement components, especially C3b (254). Apart from their antiphagocytic function, *Klebsiella* capsule polysaccharides have been reported to inhibit the differentiation and functional capacity of macrophages in vitro (260, 261). Moreover, injection of large doses of *Klebsiella* capsular polysaccharide (CPS) may even produce immunological paralysis, as has been demonstrated in mice that showed a dose-dependent decrease in the production of antibodies to the specific capsular antigen (19).

While *Klebsiella* CPS were generally considered to mediate virulence properties, this consideration has recently been abandoned because of the great differences in virulence observed among different capsular types: strains expressing the capsule antigens K1 and K2 were found to be especially virulent in a mouse peritonitis model, whereas isolates of other serotypes showed little or no virulence (120, 151). In experi-

mentally induced skin lesions in mice, *Klebsiella* strains of serotypes K1, K2, K4, and K5 were more virulent than were those expressing other capsule types (220). At present, strains expressing capsule types K1 and K2 are considered especially likely to be virulent, although only a few of the 77 different K antigens have been systematically studied in this regard.

The degree of virulence conferred by a particular K antigen might be connected to the mannose content of the CPS. Capsular types with low virulence, such as the K7 or K21a antigen (166, 191), contain repetitive sequences of mannose- α -2/3-mannose or L-rhamnose- α -2/3-L-rhamnose. These sequences are recognized by a surface lectin of macrophages, which mediates opsonin-independent (i.e., complement- and antibody-independent) phagocytosis, known as lectinophagocytosis (9). Lectinophagocytosis has been defined as nonopsonic phagocytosis that is based on recognition between surface lectins on one cell and surface carbohydrates on the opposing cell (164). Lectinophagocytosis may be mediated either by bacterial surface lectins such as fimbriae or by phagocyte lectins that act as receptors. Macrophages with the mannose- α -2/3-mannose-specific lectin or mannose receptor recognize, ingest, and subsequently kill *Klebsiella* serotypes containing the CPS repeating sequences Man α 2/3Man or L-Rha α 2/3L-Rha. In contrast, strains that lack these repeating sequences are not recognized by macrophages and hence phagocytosis does not take place. This model is consistent with the marked virulence of K2, which completely lacks mannose- α -2/3-mannose structures (117, 166). Thus, *Klebsiella* strains bearing capsule types devoid of these mannose or rhamnose sequences should be more closely associated with infectious diseases.

Previous attempts to establish a correlation between individual *Klebsiella* serotypes and the site of infection or clinical symptoms have produced a profusion of contradictory results. Each study reports different capsular types as predominant (41, 59, 185, 202, 203, 217, 241). Geographical differences in serotypes may have contributed to this confusion. Most reports do agree, however, that the K2 serotype is among the most common capsule types isolated from patients with UTI, pneumonia, or bacteremia. It can be assumed, therefore, that K2 is the predominant serotype of human clinical isolates worldwide whereas K2 strains are very rarely encountered in the environ-

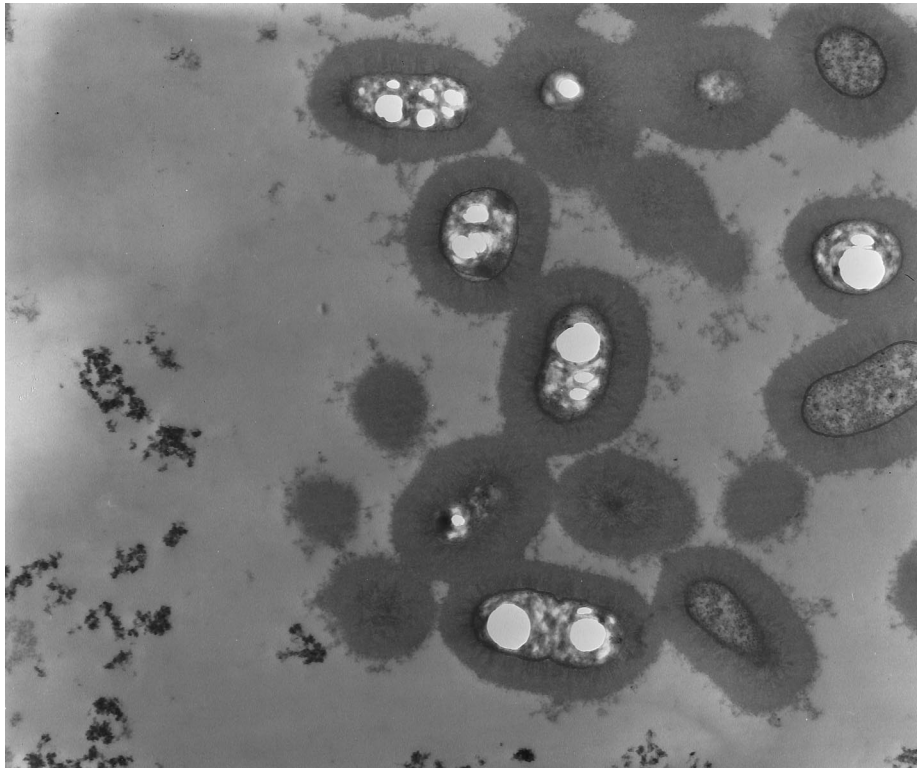


FIG. 2. Transmission electron micrograph of *K. pneumoniae* cells surrounded by thick layers of fibrillous capsular material. Courtesy of I. Ofek, Tel Aviv University, Israel. Reprinted from reference 163 with permission of the publisher.

ment (33, 74, 140, 182). Thus, the observed predominance of the K2 serotype in *Klebsiella* infections is quite consistent with the concept of lectinophagocytosis. Since the host innate immune mechanisms interact with structures commonly found on microorganisms, particular *Klebsiella* serotypes, such as the K2 type, which do not bear such structures, become selected.

The significance of capsular mannose- α -2/3-mannose sequences to the clearance of *Klebsiella* in the host is further illustrated by recent findings of Ofek's group (118). They observed that surfactant protein A (SP-A), the main protein component of lung surfactant, enhances the phagocytosis by alveolar macrophages of *Klebsiella* K21a strains (which bear a mannose- α -2/3-mannose containing capsule) but not of K2 isolates. Since the reaction was inhibited by mannan, the authors suggested that bacterial binding is mediated by the macrophage mannose receptor.

Pili (Fimbriae)

As a critical first step in the infectious process, microorganisms must come as close as possible to host mucosal surfaces and maintain this proximity by attaching to the host cell (adherence). The adhesive properties in the *Enterobacteriaceae* are generally mediated by different types of pili. Pili (otherwise known as fimbriae) are nonflagellar, filamentous projections on the bacterial surface. These structures are up to 10 μ m long and have a diameter of 1 to 11 nm (163); they consist of polymeric globular protein subunits (pilin) with a molecular mass of 15 to 26 kDa (115).

Pili are demonstrated mainly on the basis of their ability to agglutinate erythrocytes of different animal species. Depending on whether the reaction is inhibited by D-mannose, these adhesins are designated as mannose-sensitive or mannose-re-

sistant hemagglutinins (MSHA and MRHA), respectively (178). Of the different types of pili described in enterobacteria, there are two predominant types in *Klebsiella* spp. (171, 184, 195).

Type 1 (common) pili. Type 1 pili are the best investigated of the bacterial adhesins. They are MSHA which agglutinate guinea pig erythrocytes. The adhesion protein in this pilus type is located on the fimbrial shaft and is capable of binding to mannose-containing trisaccharides of the host glycoproteins (13, 83). The sugar structures presumably consist of short oligomannose chains bound via N-glycosidic linkages to the glycoproteins (216). The relevance of these pili to bacterial virulence is thought to arise mainly from binding of the bacteria to mucus or to epithelial cells of the urogenital, respiratory, and intestinal tracts (16, 162, 244). Their role in the pathogenesis of UTI was clarified mostly in studies on *E. coli* but has also been described for *K. pneumoniae* in animal models (77, 78, 134). Although associated primarily with the pathogenesis of lower UTI (106), type 1 pili may also be involved in the pathogenesis of pyelonephritis (78, 141). In this setting, these structures have been shown to bind effectively to proximal tubulus cells (248). Type 1 fimbriae are also capable of binding to soluble, mannosyl-containing glycoproteins in urine, such as the Tamm-Horsfall protein (199), or in saliva (13). These findings provide an explanation for the fact that type 1 pili mediate bacterial colonization of the urogenital and respiratory tracts (50). Adherence of bacteria to cells of the respiratory tract (11) leads to impairment of colonization resistance in the upper airways, with a subsequent proliferation of facultative pathogenic bacteria. This impairment may result in the development of pneumonia, especially in patients undergoing long-term mechanical ventilation (254).

In the appraisal of the pathogenic role of type 1 pili, however, the phenomenon of "phase variation" has to be taken into account. As mentioned above, this type of adhesin mediates bacterial colonization of the host mucosal surfaces via a rather nonspecific binding. In pathogenic microorganisms, colonization of the mucous membrane is followed by invasion of the underlying tissue, with all of the subsequent events of infectious pathogenesis. Once in the host tissue, however, the type 1 pili are no longer of use to the bacteria, since they trigger an opsonin-independent leukocyte activity known as lectinophagocytosis (167). The repulsion forces separating bacterium and leukocyte are weakened by the hydrophilic character of these pili (169), thus enabling the adhesins to bind to specific mannose-containing receptors on the leukocyte surface (205). Adhesin-binding triggers stimulation of the leukocyte (137), which ultimately leads to phagocytosis and intracellular killing of the bacterium (131). The bacterium counters this form of host defense by switching off the expression of type 1 pili in tissue (134). Thus, while type 1 pili are important for host colonization, their contribution to subsequent steps of pathogenesis is less clear.

Type 3 pili. Unlike other fimbriae, type 3 pili agglutinate only erythrocytes that have been treated with tannin. Although its name, mannose-resistant, *Klebsiella*-like hemagglutination (MR/K-HA), implies that this fimbrial type is synthesized only by *Klebsiella*, later studies demonstrated that type 3 pili occur in many enteric genera (50). Moreover, type 3 pili apparently are not identical in all genera of enterobacteria, since serological studies showed considerable antigenic diversity (170). Originally described as the adhesion organelles of *Klebsiella* inhabiting plant roots (128), these pili were later found to be capable of binding to various human cells. Strains of *K. pneumoniae* expressing type 3 pili adhere to endothelial cells, epithelia of the respiratory tract, and uroepithelial cells (105, 232, 256). In the kidneys, these pili mediate bacterial adhesion to tubular basement membranes, Bowman's capsules, and renal vessels (231). Binding to tannic acid-treated erythrocytes is inhibited by spermidine, a polyamine that is also secreted in urine (88). Since spermidine is exposed on the cell surface of damaged erythrocytes, it has been suggested that MR/K hemagglutination is mediated by spermidine (88). This might explain why type 3 pili bind to tannic acid- or heat-treated erythrocytes but not to untreated erythrocytes.

The role of this fimbrial type in the pathogenetic process is largely unknown. So far, the only evidence of a correlation between the type 3 MrkD hemagglutinin and disease has been the observation of expression of type 3 pili in *Providencia stuartii* in catheter-associated bacteriuria (152). This species is not a common cause of UTI in short-term-catheterized or noncatheterized persons but has a much higher prevalence in the urine of patients with long-term indwelling catheters. In the above-mentioned study, it was demonstrated that the higher prevalence of *P. stuartii* in catheter-associated bacteriuria was due to its ability to adhere and persist to the catheter in the catheterized urinary tract by expression of the MR/K hemagglutinin. Unfortunately, so far, no experimental animal model has been established to investigate the role of these pili in infection. The structure of the corresponding host receptors is unknown.

Three new types of *Klebsiella* adhesins have been recently reported. The R-plasmid-encoded CF29K adhesin of *K. pneumoniae* has been demonstrated to mediate adherence to the human intestinal cell lines Intestine-407 and CaCo-2 (61). This adhesin type seems to be identical to the CS31-A adhesive protein of human diarrheal *E. coli* strains (66) and belongs to the K88 adhesin family. The available data suggest that CF29K

probably is a product of the transfer of CS31A genetic determinants from *E. coli* to *K. pneumoniae* strains in the human intestine. A particular adherence pattern characterized by aggregative adhesion to intestinal cell lines is mediated by another new *Klebsiella* adhesin that seems to be composed of capsule-like extracellular material (80). While the two adhesins mentioned above are nonfimbrial, a third putative colonization factor of the human gut is a new fimbria that has been termed KPF-28 (67). Interestingly, this fimbrial type has been found in the majority of *K. pneumoniae* strains producing CAZ-5/SHV-4 type ESBL.

To date, however, little is known about the frequency and distribution of these newly described adhesins, their geographical variations, their expression by different species of *Klebsiella*, their site of isolation from the host, or their significance in pathogenicity.

Serum Resistance and Lipopolysaccharide

The first line of defense by the host against invading microorganisms includes, in addition to phagocytosis by polymorphonuclear granulocytes, the bactericidal effect of serum. The serum bactericidal activity is mediated primarily by complement proteins. After their cascade-like activation, these proteins accumulate as membrane attack complex on the surface of the microorganism (233). This complex consists of the terminal complement proteins C5b-C9, which produce a transmembranous pore in the outer membrane of gram-negative bacteria (197), leading to an influx of Na⁺ and subsequent osmotic lysis of the bacteria (234). The complement cascade can be activated by two different mechanisms: the classic complement pathway, which typically requires specific antibodies to be activated, and the alternative complement pathway, which can be activated even in the absence of antibodies. The alternative pathway is also regarded as an early defense system of innate immunity, which enables the host to react to invading microorganisms even before specific antibodies are formed (114). Both complement pathways lead, via the activation of C3, to the formation of the opsonin C3b, which ultimately results in formation of the terminal C5b-C9 complex and thus plays a key role in this defense system.

In response to this host defense, pathogenic microorganisms have developed strategies to counter the serum bactericidal effect. Most commensal gram-negative bacteria are sensitive to the bactericidal effect of human serum, whereas pathogenic strains often exhibit serum resistance properties (172). Thus, clinical isolates of enterobacteria often show resistance to serum (249), and the feature "serum resistance" has been correlated with the onset of infection (172, 204) and severity of symptoms (29, 92). Since the main role of the serum bactericidal system is thought to prevent microorganisms from invading and persisting in the blood, even differences in the degree of bacterial serum susceptibility may determine whether a strain is able to infect as well as the length of time it takes the organisms to establish the infection.

To date, the exact mechanism underlying bacterial serum resistance is unknown. Aside from various proteins of the outer membrane, such as the TraT lipoprotein or porins (3, 155), primarily CPS and O antigens (lipopolysaccharides [LPS]) have been implicated (49, 173, 194, 230, 237, 246, 252). For *Klebsiella*, two hypotheses have been propounded (145). First, capsule polysaccharides may cover and mask the underlying LPS and exhibit a surface structure that does not activate complement. On the other hand, the O side chains of the LPS may reach through the capsule layer and be exposed to the

exterior milieu in certain *Klebsiella* capsule types (238). Since LPS is generally able to activate complement, C3b is subsequently deposited onto the LPS molecules. However, since it is fixed preferentially to the longest O-polysaccharide side chains, C3b is far away from the bacterial cell membrane. Thus, the formation of the lytic membrane attack complex (C5b–C9) is prevented, and subsequent membrane damage and cell death do not take place.

In addition to this steric hindrance of the lytic complement action by LPS, the quantity of deposited C3b also determines the degree of serum resistance (2). While serum-sensitive strains activate both the classical and the alternative complement pathways, the smooth LPS of serum-resistant strains activates only the alternative pathway. The activation of both complement pathways by serum-sensitive strains leads to higher levels of deposited C3b, resulting in greater damage and bacterial killing.

It should be borne in mind, however, that all previous studies in this field were done with strains expressing the O1 serotype. Even though O1 is the most common O antigen found among clinical *Klebsiella* isolates, a number of different O serotypes, many of them neutral polysaccharides, are known. Originally there were 12 chemically different O types, but the results of structural investigations later reduced the number of *Klebsiella* O antigens to 8 (151). To date, it is unclear whether serum resistance is mediated solely by the O1 antigen or whether this feature is generally conferred by *Klebsiella* LPS.

Nevertheless, even within a given O serotype, serum resistance does not seem to be a stable characteristic; environmental factors affect the composition and effect of LPS. Recently, the influence of different osmolarity conditions on LPS was demonstrated in *Aeromonas hydrophila* serotype O:34 (1); cells grown at high osmolarity showed smooth LPS, whereas growth at low osmolarity resulted in rough LPS. Correspondingly, cells cultivated at high osmolarity were resistant to normal human serum while bacteria grown at low osmolarity proved to be serum sensitive. Thus, the same bacterial strain may be serum resistant at host body sites with a high-osmolarity milieu, such as the urinary tract, and serum sensitive at low-osmolarity body locations like the respiratory tract.

Siderophores

The growth of bacteria in host tissue is limited not only by the host defense mechanisms but also by its supply of available iron. Iron is an essential factor in bacterial growth, functioning mainly as a redox catalyst in proteins participating in oxygen and electron transport processes (93). The supply of free iron available to bacteria in the host milieu is extremely low, since this element is bound intracellularly to proteins such as hemoglobin, ferritin, hemosiderin, and myoglobin and extracellularly to high-affinity iron-binding proteins such as lactoferrin and transferrin. The level of free, bioavailable iron (10^{-18} M) is several thousandfold too low for normal bacterial growth (37). The marked effect of the iron supply in the host body on the pathogenesis of infections has been demonstrated for *Klebsiella*. After parenteral administration of iron in a guinea pig model, the susceptibility to *K. pneumoniae* infections increased dramatically (121).

Many bacteria attempt to secure their supply of iron in the host by secreting high-affinity, low-molecular-weight iron chelators, called siderophores, that are capable of competitively taking up iron bound to host proteins (94). Under iron-deficient conditions, e.g., in the host milieu, enterobacteria synthesize a variety of siderophores, which belong to two different chemical groups, one consisting of the phenolate-type sidero-

phores and one consisting of the hydroxamate-type siderophores.

The more common group consists of the phenolate-type siderophores. Their best-known representative, enterobactin (also known as enterochelin), is a cyclic trimer of 2,3-dihydroxy-benzoyl-serine. This siderophore appears to comprise the main iron uptake system of enterobacteria and is synthesized by almost all clinical isolates of *E. coli* and *Salmonella* spp. (93). Studies on the contribution of enterobactin to virulence have produced conflicting results. For example, Yancey et al. (257) reported that *Salmonella typhimurium* mutants unable to produce this siderophore were less virulent in mice, while Benjamin et al. (23) found no association between virulence and the ability to synthesize enterobactin. To date, the role of enterobactin in virulence remains uncertain.

Among the hydroxamate-type siderophores, the ferri-chromes (which are synthesized only by fungi), the ferrioxamines, and aerobactin are the most important. In contrast to enterobactin, the contribution of aerobactin to bacterial virulence has been clearly demonstrated (65). While the thermodynamic stability constant of ferric enterobactin indicates a much higher affinity for Fe(III) than that of ferric aerobactin (K_s 10^{52} and 10^{23} , respectively), aerobactin still seems to be far more effective than enterobactin because of a number of physical advantages such as greater stability and better solubility (255). Moreover, while enterobactin becomes hydrolyzed by an esterase after delivery of iron, aerobactin can be recycled after each turn of iron transport.

The observations of Martinez et al. (139) indicate that enterobacterial genera can be divided into two groups according to their incidence of aerobactin synthesis. The group with a low rate of aerobactin-producing strains (<20%) comprises genera such as *Serratia*, *Proteus*, and *Salmonella*. The second group, which includes the genus *Escherichia*, shows a high incidence of aerobactin synthesis (>40%).

In the genus *Klebsiella*, the production of both enterobactin and aerobactin has been demonstrated. However, while enterobactin is synthesized by almost all strains (183, 201, 251), aerobactin-positive *Klebsiella* isolates, irrespective of the species or source of isolation, have been observed rarely (139, 183, 253). Enterobactin-positive *Klebsiella* isolates in animal models were no more virulent than enterobactin-negative strains (147). In contrast, an association between aerobactin synthesis and the virulence of *Klebsiella* strains was unequivocally demonstrated by Nassif and Sansonetti (159). In this study, the aerobactin gene was cloned from the plasmids of some *K. pneumoniae* strains of serotypes K1 and K2 and transferred to a nonvirulent (siderophore-negative) strain. The transformant then exhibited markedly enhanced virulence in a mouse peritonitis model.

Because of the common ability of strains to produce enterobactin, it has been speculated for a long time what additional advantage a bacterium, which already synthesizes enterobactin, might derive from aerobactin. The great advantage of enterobactin over aerobactin is its high iron affinity, the highest ever recorded for a ferric iron chelator. At pH 7.4, the formation constant for ferric enterobactin is 10^{52} , which is magnitudes higher than that of transferrin (93). In contrast to enterobactin, aerobactin is not an effective competitor for transferrin-bound iron because of its much lower affinity for ferric iron. Indeed, investigations have demonstrated that enterobactin sequesters iron predominantly from transferrin, while the iron source of aerobactin is host cells (32). Thus, production of these two siderophores may give access to both sources of iron, resulting in enhanced growth in the host.

Data on the incidence of aerobactin-producing *Klebsiella*

~ndicates that this siderophore does not play a central role in the pathogenicity of the genus *Klebsiella*. It should be pointed out, however, that clinical *K. pneumoniae* isolates, which do not synthesize aerobactin themselves, are entirely capable of using exogenously introduced aerobactin as their sole source of iron (253). By synthesizing only the intrinsically expressed aerobactin receptor, such strains could derive an advantage over other aerobactin-synthesizing bacteria in mixed infections. The aerobactin-mediated iron uptake system would thus be an indirect contributor to the pathogenicity of the genus *Klebsiella*.

As in many enterobacteria, and as has been especially well studied in *E. coli*, other factors have also been demonstrated in *Klebsiella* spp. Although the production of cytotoxins (102, 127, 149, 150, 229), enterotoxins (95, 122–125, 148) and hemolysin (4–6, 17) has been sporadically described, these features probably play a rather minor role in *Klebsiella*.

VACCINATION EFFORTS

As stated above, most *Klebsiella* infections are acquired during hospital stays and account for 5 to 7.5% of all nosocomial infections. The morbidity and mortality of severe systemic infections, such as bacteremia and pneumonia, remain high despite the use of appropriate antibiotic therapy. Fatality rates of 20 to 50% in *Klebsiella* bacteremia and of more than 50% in *Klebsiella* pneumonia have been reported (35, 55, 64). Moreover, *Klebsiella* infections in pediatric wards have become a major concern. In neonatal intensive care units, *Klebsiella* is one of the three or four most common pathogens (98). This is apparently related to the observation that premature neonates, especially those in neonatal intensive care units are more likely than other neonates to develop an intestinal flora in which *Klebsiella* spp. are highly prevalent. These findings, taken together with the emergence of ESBL-producing multiresistant strains, indicate the need for a means of immunological control of *Klebsiella* infections. Such measures may include immunoprophylaxis (active vaccination of patients who are at risk) as well as immunotherapy (passive immunization by hyperimmune sera).

Among the different cell constituents, two surface components are mainly being discussed as candidates for an anti-*Klebsiella* vaccine: LPS and CPS.

Lipopolysaccharides

Due to their endotoxic properties, LPS are considered important in the pathology of septicemia. Until recently, *Klebsiella* LPS O antigens were generally considered to be masked by the capsule polysaccharides and thus not to be exposed on surface, leaving them inappropriate as vaccine candidates. Recent studies, however, demonstrated surface exposure of O-antigens in strains expressing particular capsular serotypes (238). The small number of different *Klebsiella* O-types is a great advantage with respect to their applicability as vaccines. In contrast to the K antigens, only eight O types are known, O1 being the most commonly found O type in clinical isolates. A multivalent LPS vaccine composed of these eight O antigens or at least the inclusion of the common O1 antigen in a broad spectrum capsular polysaccharide vaccine might be a promising approach. Recently, the administration of monoclonal antibodies to the *Klebsiella* O1 antigen has been reported to be protective in a mouse model of lethal endotoxemia (136). Moreover, the inclusion of O antigens in a multivalent *Klebsiella* vaccine formulation might be of additional benefit due to strong adjuvant action, as has been demonstrated for the *Klebsiella* O3 lipopolysaccharide (259). A great drawback of active

immunization with LPS-containing vaccines, however, is adverse toxic reactions, which must be expected because of the endotoxin content. Thus, each *Klebsiella* vaccine composed of O antigens has to be rendered safe by sufficient detoxification of the LPS.

Capsular Polysaccharides

CPS have been the obvious vaccine candidates for several reasons. Capsules are produced by almost all *Klebsiella* strains; they represent the outermost layer of surface structures in contact with the host milieu, and they have been proven to be highly immunogenic and nontoxic (57). A serious disadvantage of a *Klebsiella* CPS vaccine is the great number of K antigens (77 different antigens). However, in a study of the incidence of the capsule types among bacteremic *Klebsiella* isolates, Cryz et al. observed that only 25 serotypes made up 70% of all bacteremic strains (59). Based on their seroepidemiological findings, they formulated a 24-valent *Klebsiella* CPS vaccine that subsequently was proven to be safe and immunogenic (58). To date, this vaccine seems to be the most promising approach for preventing sepsis caused by *Klebsiella* and has already passed phase I human trials (72). The most recent study of the 24-valent *Klebsiella* CPS vaccine demonstrated an excellent antibody response after active immunization in patients with acute blunt or penetrating trauma (39).

CONCLUDING REMARKS

Klebsiellae are opportunistic pathogens and can give rise to severe diseases such as septicemia, pneumonia, UTI, and soft tissue infection. Typically, *Klebsiella* infections are nosocomial. The hospitalized, immunocompromised patient with underlying diseases is the main target of these bacteria. Thus, *Klebsiella* infections may serve as a paradigm of hospital-acquired infections. Their incidence of 5 to 7% of all hospital-acquired infections ranks them among the most important nosocomial pathogens.

In this context, some new trends have been observed in the past several years.

(i) An increasing number of endemic and epidemic outbreaks in pediatric wards has been reported. Especially common are *Klebsiella* infections causing septicemia and meningitis in newborns in neonatal intensive care units. Since more and more of these outbreaks have been caused by multidrug-resistant strains, *Klebsiella* neonatal infections are becoming a major concern of the pediatrician. Especially peculiar has been the repeated frequent isolation of multidrug-resistant *Klebsiella* isolates expressing serotype K55. It remains to be seen whether this observation reflects the spread of a particular "neonatal" *Klebsiella* clone.

(ii) Hospital outbreaks of multidrug-resistant *Klebsiella* spp. are often caused by a new type of strain, the ESBL producers. The incidence of ESBL-producing strains among clinical *Klebsiella* isolates has been steadily increasing over the past several years. Frequencies of up to 40% have been reported in certain regions. Currently, the available data suggest a further increase in the incidence of ESBL-producing isolates. As a result, the therapeutic options are becoming limited, so that in the near future there will be an urgent need for hospital infection control measures that counter the spread of ESBL-producing bacteria.

(iii) Until recently, *K. pneumoniae* and *K. oxytoca* have been considered to be the only pathogenic *Klebsiella* species. However, the newer species *K. terrigena* and *K. planticola*, formerly regarded as "environmental" *Klebsiella* species, have been dem-

onstrated to occur in human clinical specimens. *K. planticola*, in particular, has been isolated with astonishing frequency from human infectious processes. So far, it is unclear what kind of pathogenicity factors *K. planticola* might possess or whether this species expresses the same factors that have been described for *K. pneumoniae*. *K. planticola* can, however, be expected to be of clinical significance, and the question remains whether the identification of this species by standard laboratory procedures should be recommended.

Nosocomial *Klebsiella* infections continue to be a heavy burden on the economy and on the life expectancy of patients in developed countries. Hospital infection prevention and control programs led in the past to considerable improvements in the management and control of these infections. However, there is a general agreement that further progress in prevention of hospital-acquired infections will require new approaches to infection control. As a future challenge for the advancement of preventive measures against nosocomial *Klebsiella* infections, the usefulness of new concepts needs to be evaluated. A number of different approaches are conceivable.

One possible measure is the vaccination of persons at risk. Regardless of whether active or passive immunization is performed (the latter preferentially by a cocktail of monoclonal anti-*Klebsiella* antibodies), the question has to be raised about whom to vaccinate. While little controversy is to be expected for vaccinating immunocompromised hospitalized patients to prevent fatal *Klebsiella* pneumonia and septicemia, the justification of immunological measures in other patient groups is being debated. A good example in this respect is *Klebsiella* UTI in elderly individuals. Most cases of bacterial pyelonephritis are not caused by *Klebsiella* but by *E. coli* strains. However, although *Klebsiella* species are not a predominant cause of UTI, they can cause significant renal scarring even after a single episode of infection. Moreover, infections with these uropathogens are more likely to lead to death than are infections with most *E. coli* strains. The question whether a *Klebsiella* vaccine should be recommended for persons older than 60 years has to be clarified by cost-benefit analyses.

Another point of interest is the possible eradication of klebsiellae in patients during their hospital stay. One of the new approaches is the use of cranberry juice. This juice shows a pronounced anti-adhesive effect on enterobacteria and therefore might prevent colonization of hospitalized patients or even eradicate these bacteria in colonized persons. Cranberry juice might be of benefit both because of its high content of fructose as an inhibitor of type 1 pili and because of the presence of a high-molecular-weight constituent that blocks mannose-resistant adhesion (165). It has been postulated that the juice acts on the gastrointestinal organisms to eliminate the source of infection rather than on the bladder because recurrent but not acute UTIs were prevented (10). Daily consumption of cranberry juice cocktail has been demonstrated to cause bacteriuric elderly persons to become and remain abacteriuric. The use of cranberry juice cocktail in the hospital setting might be a paradigm for cheap and simple preventive intervention without the use of antibiotics or vaccines.

Fascinating future aspects are also raised by new findings on so far unknown host defense mechanisms. As mentioned above, lung SP-A increases phagocytosis of *Klebsiella* by acting as an opsonin and by activating alveolar macrophages. Another surfactant protein (SP-D) has been reported to interact with bacterial LPS. It has been suggested that SP-D plays an important role in the defense of the lungs against gram-negative bacteria. Investigations in the near future will show whether surfactant proteins may be useful in new therapeutic approaches and

whether they are a meaningful addition to the management of nosocomial *Klebsiella* infections.

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