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Isolation of *Klebsiella terrigena* from Clinical Specimens

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In a three-year survey conducted from 1988 to 1990 *Klebsiella* isolates from human clinical specimens were subjected to additional tests to identify any *Klebsiella terrigena* strains. Ten strains of *Klebsiella terrigena* (0.4 %) were found among 2355 indole-negative *Klebsiella* isolates. Most of the isolates were recovered from the respiratory tract. In the API 20EC system almost exclusively biotypes no. 1777771 and 1777671 were observed. Serotyping revealed capsule types K2, K5 and K18 in two strains each. In antibiotic susceptibility tests the strains were shown to be comparable in sensitivity to *Klebsiella pneumoniae*.

In 1981 a new species of *Klebsiella*, *Klebsiella terrigena*, was described by Izard et al. (1). *Klebsiella terrigena* closely resembles *Klebsiella pneumoniae* and can be serotyped using *Klebsiella* capsule antisera. The two species can be differentiated on the basis of tests for fermentation of melezitose, growth at 10 °C and gas production from lactose at 44.5 °C (2). *Klebsiella terrigena* has been isolated mainly from soil and water (1, 3), and in the ten years following the first description of this new species no case of isolation from humans was reported. Recently, however, we

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reported isolation of *Klebsiella terrigena* from the feces of healthy humans, with a carriage rate of 0.9 % for 5377 different stool specimens examined (4). The clinical significance of the organism is unknown as there have been no reports as yet of isolation from clinical material. We wondered whether *Klebsiella terrigena* is a nonpathogenic *Klebsiella* species or whether it has not yet been isolated from human infections as it escaped identification by standard procedures. In order to establish whether the latter is the case indole-negative *Klebsiella* isolates from clinical material were examined to identify any *Klebsiella terrigena* strains. This paper describes the first instances of isolation of *Klebsiella terrigena* from human clinical specimens.

Materials and Methods. Indole-negative *Klebsiella* strains isolated from human clinical specimens were additionally examined for their ability to ferment melezitose. Melezitose-positive isolates were subjected to further tests in the API 20EC system (API bioMérieux, Germany). Identification of *Klebsiella terrigena* was confirmed by growth at 10 °C and inability to produce gas from lactose at 44.5 °C (2). Each such strain was from a different individual. *Klebsiella terrigena* ATCC 33257, 33628, and 33629 served as reference strains. The susceptibility of the strains to 12 antimicrobial agents was determined by a microtitre broth dilution procedure according to standard German methods (5). *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 served as reference strains. Serotyping of the isolates was performed by means of the capsular swelling reaction. Polyvalent anticapsular sera were used for screening and monospecific sera for typing, as described by Ullmann (6).

Results and Discussion. In a three-year survey from 1988 to 1990 indole-negative *Klebsiella* strains isolated from clinical specimens were examined to detect any *Klebsiella terrigena* strains. Among a total of 2355 isolates examined, ten strains of *Klebsiella terrigena* (0.4 %) were observed. Data on these strains are given in Table 1.

Identification by the API 20EC system revealed almost exclusively code numbers 1777771 and 1777671 (Table 1). This system, which also has been used successfully in the identification of *Klebsiella terrigena* by numerical analysis (7), proved to be suitable for differentiation of this species. Differentiation between *Klebsiella pneumoniae* and *Klebsiella terrigena* by API 20EC is based mainly on tests for fermentation of dulcitol, melezitose and adonitol at 30 °C. Identification was confirmed by growth at 10 °C and inability to produce gas from lactose at 44.5 °C.

Most of the isolates (eight strains) were obtained from the respiratory tract (Table 1); one strain was recovered from urine and one from a wound infection. With the exception of strains T60 and T63, all strains were isolated in conjunction with other bacteria, mainly *Staphylococcus aureus* and other enterobacteria. Whether *Klebsiella terrigena* causes monomicrobial infections or participates in polymicrobial infections remains to be clarified.

Nine of the ten isolates could be typed with *Klebsiella* anticapsular sera (Table 1). Two strains each were positive for capsules types K2, K5 and K18. These K antigens have likewise been found in fecal isolates of *Klebsiella terrigena* (4). Interestingly, capsule types K2 and K5 have been demonstrated to be associated with virulence in mice (8, 9). K2 is one of the capsule types most often encountered in clinical *Klebsiella pneumoniae* isolates (10). K antigens K70 and K14, which have been reported to be most frequent in fecal *Klebsiella terrigena,* were not observed, however, among our clinical isolates of *Klebsiella terrigena.* The antibiotic susceptibility of the isolates to 12 antimicrobial agents is shown in Table 2. All strains were susceptible to cefotaxime, imipenem, amoxicillin/clavulanic acid, doxycycline, ofloxacin and ciprofloxacin. Six of the ten isolates were resistant to cotrimoxazole, which represents a higher frequency than that found in fecal *Klebsiella terrigena* (0 %) (4) or in clinical *Klebsiella pneumoniae* strains (15 %) (10). The majority of the isolates showed intermediate sensitivity to mezlocillin, piperacillin, amoxicillin/clavulanic acid and doxycycline. Freney et al. (11) reported environmental *Klebsiella terrigena* strains to be highly sensitive to doxycycline, as demonstrated by a MIC90 of 1 µg/ml. In contrast to this finding, we observed MICs of doxycycline to be exclusively 2–4 µg/ml. On average the clinical isolates of *Klebsiella terrigena* proved to be slightly more resistant than fecal isolates. For meaningful comparisons, however, greater numbers of this species need to be examined. Nevertheless, our study demonstrates that *Klebsiella terrigena* can be found in human clinical material. The incidence of this species appears to be very low, however, and we do not suggest that extending the range of standard tests for *Klebsiella* identification is warranted in the routine microbiological laboratory.

Table 1: Source, biotype and capsular type of ten *Klebsiella terrigena* strains isolated from clinical material.

Strain no.	API 20EC code no.	Capsule type	Source	Diagnosis
T56	1777771	2	tracheal secretion	tracheostoma infection
T57	1777671	–	wound	wound infection
T58	1777771	18	tonsil swab	tonsillitis
T59	1777771	18	tracheal secretion	thoracic aortic aneurysm
T60	1777671	71	sputum	fever of unknown origin
T61	1777671	33	throat swab	scarlet fever
T62	1777771	5	tonsil swab	tonsillitis
T63	1777631	2	sputum	chronic bronchitis
T64	1777671	5	midstream urine	cystitis
T65	1777671	32	tracheal secretion	pneumonia

Table 2: Susceptibility to 12 antimicrobial agents of *Klebsiella terrigena* (n = 10) isolated from clinical material.

Antimicrobial agent	Cumulative numbers of strains susceptible at following concentrations (µg/ml)											
	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256
Ampicillin	–	0	0	0	0	0	0	0	–	–	–	–
Mezlocillin	–	–	0	0	0	2	6	7	8	–	–	–
Piperacillin	–	–	–	0	0	1	5	7	7	10	–	–
Amoxicillin/CA	–	0	0	0	2	8	10	10	–	–	–	–
Cefazolin	–	–	0	0	0	7	8	8	10	–	–	–
Cefotaxime	–	9	10	10	10	10	10	10	–	–	–	–
Imipenem	–	–	3	6	9	10	10	10	10	–	–	–
Doxycycline	0	0	0	0	8	10	10	–	–	–	–	–
Gentamicin	0	0	1	3	6	9	10	–	–	–	–	–
Cotrimoxazole	–	–	–	–	–	0	0	0	1	3	4	4
Ofloxacin	2	7	9	10	10	10	10	–	–	–	–	–
Ciprofloxacin	9	10	10	10	10	10	10	–	–	–	–	–

–: concentration not tested.

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Changes in the Susceptibility of *Bacteroides fragilis* Group Organisms to Various Antimicrobial Agents 1979–1989

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The in vitro activity of metronidazole, chloramphenicol, clindamycin and 11 β-lactam antibiotics against 135 clinical isolates of the *Bacteroides fragilis* group was compared. In addition, changes in the resistance patterns of members of the *Bacteroides fragilis* group isolated at the Hospital Universitario San Carlos in Madrid, Spain, between 1979 and 1989 were documented. The most active β-lactam drugs were imipenem and β-lactamase inhibitor combinations. In 1989, however, two strains were found to be resistant to imipenem and to all other β-lactam agents tested. There was no emergence of resistance to metronidazole. Chloramphenicol was very effective: only one resistant strain was detected in 1979 and no chloramphenicol-resistant isolates were found during the rest of the study period. An outbreak of clindamycin resistance was noted in 1982, and the first cefoxitin resistant strains were recovered in 1985. The changing patterns of susceptibility of anaerobic bacteria to antimicrobial agents and the emergence of *Bacteroides fragilis* strains resistant to new β-lactam agents suggest that periodic antimicrobial susceptibility tests should be performed in order to guide the selection of antimicrobial agents for therapy.

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Members of the *Bacteroides fragilis* group are the anaerobes most frequently isolated in clinical infections, and resistance of these organisms to several of the traditionally used antimicrobial agents has been increasingly reported. Resistance to new β-lactam agents, such as imipenem, or β-lactamase inhibitor/β-lactam drug combinations has also recently been reported (1–5). However, as several authors have pointed out (2, 6, 7) there are differences from one medical centre to another or from one country to another in the susceptibility patterns of anaerobic bacteria. In 1989 we therefore determined the MICs of several new β-lactam drugs for organisms of the *Bacteroides fragilis* group isolated from clinical specimens in comparison to other antimicrobial agents frequently used to treat anaerobic infections in order to ascertain the current susceptibility patterns of these organisms in our hospital (Hospital Universitario San Carlos, Madrid, Spain). In addition, we performed a ten-year study (1979–1989) of the susceptibility of members of the *Bacteroides fragilis* group to different antibiotics in order to determine whether there was also emergence of resistance in these organisms in our hospital.

Materials and Methods. A total of 451 clinical strains of species of the *Bacteroides fragilis* group isolated during a ten-year period were tested as follows: 68 isolates in 1979 (36 *B. fragilis,* 17 *B. thetaiotaomicron,* 4 *B. ovatus,* 10 *B. distasonis* and 1 *B. vulgatus),* 31 isolates in 1982 (15 *B. fragilis,* 6 *B. thetaiotaomicron,* 4 *B. ovatus,* 5 *B. distasonis* and 1 *B. vulgatus),* 60 isolates in 1985 (25 *B. fragilis,* 10 *B. thetaiotaomicron,* 14 *B. ovatus,* 10 *B. distasonis* and 1 *B. vulgatus),* 63 isolates in 1987 (30 *B. fragilis,* 9 *B. thetaiotaomicron,* 9 *B. ovatus,* 10 *B. distasonis* and 5 *B. vulgatus),* 94 isolates in 1988 (58 *B. fragilis,* 16 *B. thetaiotaomicron,* 10 *B. ovatus,* 6 *B. distasonis* and 4 *B. vulgatus)* and 135 strains in 1989 (58 *B. fragilis,* 39 *B. thetaiotaomicron,* 26 *B. ovatus,* 8 *B. distasonis* and 4 *B. vulgatus).* Each organism was identified by means of the AN-Ident System (bioMérieux, France).

The antibiotics tested in 1989 were metronidazole, chloramphenicol, clindamycin, cefoxitin, cefotetan, cefmetazole, ceftizoxime, moxalactam, piperacillin, mezlocillin, imipenem, amoxicillin-clavulanic acid (2:1), ampicillin-sulbactam (1:1) and ticarcillin-clavulanic acid (2 μg/ml). We compared results obtained in 1989 with results of prior surveys conducted in 1979, 1982, 1985, 1987 and 1988. Drugs evaluated in those surveys included chloramphenicol, clindamycin,