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Original Article

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Development of resistance by *Enterobacter cloacae* during therapy of pulmonary infections in intensive care patients

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Abstract. The emergence of resistance during therapy and the efficacy of different antibiotic therapy regimens were studied in 38 intensive care patients suffering from pulmonary infections caused by *Enterobacter cloacae*. Every three days a fresh isolate was obtained from each patient and tested in vitro for susceptibility to 16 antibiotics by determination of the minimal inhibitory concentrations. During therapy with cefotaxime and tobramycin the *E. cloacae* strains from 47% of the patients became resistant to cefotaxime within 6 days. In all cases resistance encompassed all other broad-spectrum penicillins and cephalosporins tested, as well as aztreonam. Development of resistance regularly led to persistence of bacteria. Resistance to tobramycin, ciprofloxacin or imipenem was not observed. Treatment of 25 patients with persisting *E. cloacae* infections was successful in 17 out of 18 patients treated with imipenem and in 6 out of 7 patients receiving ciprofloxacin.

Key words: Enterobacter – Infections – Resistance – Antibiotics

The emergence of resistance during a course of antimicrobial therapy can be a serious therapeutic problem, especially if resistance encompasses antibiotics that have never been used in the therapy of the individual patient. It has been reported that some gram-negative bacterial species, such as *Enterobacter cloacae, Citrobacter freundii, Pseudomonas aeruginosa* and species of *Serratia* or *Acinetobacter*, possess an inducible chromosomally encoded β-lactamase capable of inactivating all acylureidopenicillins as well as cephalosporins, including the third-generation cephalosporins and monobactams [8, 19–21]. Many penicillins and cephalosporins act as potent inducers of this class of enzymes [15, 16]. More importantly, permanent high-level β-lactamase production often (10^{-5} to 10^{-8}) arises spontaneously by mutation to stable derepression that does not require the presence of an inducer [8]. During therapy with an antibiotic that is not stable against this β-lactamase, these multiresistant mutants are often selected [11, 17]. This possibility has to be considered during the therapy of infections caused by these pathogens.

Various reports on the development of such broad β-lactam resistance in the above-mentioned bacterial species and consequent failure of β-lactam therapy have recently been published [2, 13, 14, 18]. However, only scant information is available about the frequency of such an event. Therefore, in the last few years we have focused attention on the emergence of resistance and therapeutic failure in patients infected with *E. cloacae*. This organism causes 12–15 % of the nosocomial pulmonary infections in mechanically ventilated patients in the surgical and neurosurgical intensive care units of the University of Giessen.

Patients and methods

Patients

The present study comprises 38 mechanically ventilated patients admitted to the surgical or neurosurgical intensive care units of the University of Giessen. Patient characteristics are given in Table 1. All patients suffered from pulmonary infections caused by *E. cloacae* as defined later in this paper. For perioperative and primary therapy during the first few days of intensive care, patients with peritonitis or multitraumatized patients with suspicion of aspiration received amoxicillin/clavulanic acid

Abbreviation: MIC = Minimal inhibitory concentration

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Table 1. Characteristics of 38 patients with pulmonary infections caused by *Enterobacter cloacae*

No. of patients	38
Sex (male/female)	29/9
Age (years)[a]	46 (12–75)
Total days on ventilator[a]	21 (7–35)
Underlying illness:	
Multiple trauma/head injuries	22
Peritonitis	3
Postoperative complications/ Intracranial bleeding	13

[a] Expressed as median (range)

or a second-generation cephalosporin (cefuroxime or cefotiam). Further antibiotic therapy was directed specifically against the isolated pathogens. Patients with post-operative complications who showed no signs of infection did not receive antibiotics.

Microbiological investigations

Tracheobronchial secretions were monitored daily by microscopy and cultures [6]. Furthermore, in patients with pulmonary infections samples were obtained by bronchoscopy [1]. Microscopic examination was performed after Giemsa staining. Counts of bacteria and numbers and type of cells were determined. Microbiological cultures were performed by incubating for 24–48 h at 37°C on Columbia/sheep blood-agar, chocolate agar (Oxoid, FRG); MacConkey and Sabouraud yeast agar (Merck, FRG). Identification of bacteria was performed according to standard procedures [4].

Antibiotics and determination of susceptibility

Standard microdilution methods [12] were used to determine minimal inhibitory concentrations (MIC) of gentamicin (Merck, Germany; concentrations tested: 1–16 mg/l), tobramycin (Eli Lilly, Germany; 1–16 mg/l), amikacin (Bristol, Germany; 4–64 mg/l), ampicillin (Hoechst, Germany; 1–32 mg/l), ampicillin/clavulanic acid (Smith Kline Beecham, Germany; 1–32 mg/l), mezlocillin (Bayer, Germany; 4–128 mg/l), piperacillin (Lederle, Germany; 4–128 mg/l), cefazolin (Eli Lilly, Germany; 1–32 mg/l), cefuroxime (Hoechst, Germany; 1–32 mg/l), cefoxitin (Takeda, Germany; 1–32 mg/l), cefotaxime (Hoechst, Germany; 1–64 mg/l), ceftriaxone (Hoffmann-LaRoche, Germany; 1–64 mg/l), ceftazidime (Cascan, Germany; 1–64 mg/l), aztreonam (Heyden, Germany; 1–32 mg/l), imipenem (MSD, Germany; 0.5–16 mg/l) and ciprofloxacin (Bayer, Germany; 0.25–4 mg/l). The organisms were processed in microdilution trays (Microscan, Baxter Healthcare Corporation, USA) containing test compounds diluted in cation-adjusted Mueller-Hinton broth. The final inocula were 10^5 cfu/ml. The MICs were read after 18 h of incubation at 37°C as the lowest concentration of drug preventing visible growth. In cases of persistence of the organisms and continuation of the infection the persisting organisms were tested for susceptibility by estimation of the MIC every 3 days. Appropriate quality-control procedures were carried out with the following strains of the American Type Culture Collection: *E. coli 25922, E. coli 35218* and *Pseudomonas aeruginosa 27853*. With respect to cefotaxime (resistance corresponds to an MIC of >32 mg/l) susceptible and resistant breakpoints for antimicrobial agents tested were as given by the NCCLS [12].

Definitions

A pulmonary infection was assumed in patients with leukocytosis ($>12\,000/\mu l$), fever ($>38^5$°C), purulent tracheobronchial secretions (leukocytes $>10^5$/ml), isolation of $>10^5$/ml *E. cloacae* from tracheobronchial secretions, impairment of lung function and/or infiltrates in the chest X-ray film. Therapeutic failure was defined as persistence of the pathogens at the site of infection associated with clinical symptoms, such as leukocytoses $>10^5$/ml, fever $>38^5$°C and infiltrates in the chest X-ray.

Therapy

Patients with pulmonary infections caused by *E. cloacae* initially received 3×2 g cefotaxime and 3×80 mg tobramycin daily i.v. In those cases in which microorganisms persisted either to resistance against cefotaxime or in which patients showed no clinical improvement and persistence of the organisms cefotaxime was replaced by imipenem (3×1 g) or ciprofloxacin (4×200 mg for 3 days, then reduced to $2–3 \times 200$ mg). There were no set criteria for decision of imipenem or ciprofloxacin treatment. In patients with renal insufficiency the doses were adjusted.

Results

At the time of first isolation all *E. strains* were susceptible to cefotaxime and tobramycin. Ninety percent of the isolates were inhibited by 1 mg/l cefotaxime and 1 mg/l tobramycin. During therapy

Table 2. In vitro activity of 16 antibiotics against *Enterobacter cloacae* isolates that became resistant to cefotaxime during therapy. The strains were isolated from 18 patients before therapy and after 3 and 6 days of therapy with cefotaxime and tobramycin

Antibiotics	Minimal inhibitory concentration (MIC) mg/l[a]					
	Before therapy		After 3 days		After 6 days	
	MIC90	Range	MIC90	Range	MIC90	Range
Cefuroxime	16	(4–16)	>32	(32–>32)	> 32	(>32)
Cefoxitin	32	(<4–32)	>32	(32–>32)	> 32	(>32)
Cefotaxime	<1	(<1–2)	32	(<1–>64)	> 64	(16–>64)
Ceftriaxone	<1	(<1–4)	32	(<1–>64)	> 64	(16–>64)
Ceftazidime	<1	(<1–2)	32	(<1–>64)	> 64	(16–>64)
Mezlocillin	<4	(<4–8)	32	(<4–>128)	>128	(32–>128)
Piperacillin	<4	(<4–8)	32	(<4–>128)	>128	(16–>128)
Aztreonam	<1	(<1)	8	(<1–16)	16	(2–32)
Gentamicin	1	(<1–2)	1	(<1–2)	1	(<1–2)
Tobramycin	1	(<1–2)	1	(<1–2)	1	(<1–2)
Amikacin	<4	(<4–8)	<4	(<4–8	< 4	(<4–8)
Imipenem	1	(0.5–2)	1	(0.5–2)	1	(0.5–2)
Ciprofloxacin	<0.25	(<0.25)	<0.25	(<0.25)	< 0.25	(<0.25)

[a] The MICs of ampicillin, ampicillin/clavulanic acid and cefazolin were initially >32 mg/l in all cases

with cefotaxime 18 of 38 *Enterobacter* isolates (47%) became resistant to cefotaxime within 6 days. In general, these strains showed a stepwise increase of the MIC with MIC90 values of 32 mg/l after 3 days and >64 mg/l after 6 days of cefotaxime therapy. In five patients MIC values of >64 mg/l were observed already after 3 days. Without exception, resistance to cefotaxime was accompanied by resistance to all other cephalosporins and acylureidopencillins tested, as well as aztreonam. The results are shown in Table 2.

Development of resistance was regularly associated with persistence of the microorganisms and in 16 of the 18 patients signs of infection continued. In only two patients did the clinical situation improve despite persistence of the pathogens.

In 13 patients treatment with cefotaxime and tobramycin led to elimination of the organisms and clinical improvement within 3 days. In seven patients with persisting microorganisms and signs of infection the therapy was changed despite effective in vitro activity of cefotaxime. Thus, from 38 patients included in this study, 25 were treated with imipenem or ciprofloxacin, 18 of them due to development of resistance, and 7 due to lack of clinical improvement despite retention of susceptibility to cefotaxime in the infecting strains of *E. cloacae*.

Therapy with imipenem was successful in 17 out of 18 patients (94%), and in 6 out of 7 patients (86%) treatment with ciprofloxacin led to an eradication of the pathogens within 2–3 days. During therapy with imipenem, ciprofloxacin or tobramycin, resistance against these antibiotics was never observed. MIC values for imipenem ranged from 0.5 to 2 mg/l, for tobramycin from <1 to 2 mg/l; for ciprofloxacin MICs were < 0.25 mg/l.

Discussion

The development of resistance against third-generation cephalosporins in gram-negative bacteria such as E. cloacae is an increasing problem in therapy of infections in intensive care patients. Since development of resistance parallels the use of these substances, widespread use of these therapeutics should be avoided.

The introduction of third-generation cephalosporins has improved the treatment of gram-negative bacterial infections. In general, third-generation cephalosporins, like cefotaxime, ceftriaxone, moxalactam or ceftazidime, are stable against most β-lactamases produced by gram-negative bacteria. However, certain non-fastidious, gram-negative bacilli, such as *E. cloacae*, can rapidly develop resistance against these drugs by mutationally derepressed production of a chromosomally encoded β-lactamase, which is able to inactivate even these relatively resistant β-lactams [15, 19, 20]. Under continued therapy with such antibiotics multiresistant mutants were selected [3]. This fact has to be considered in any therapy of infections caused by *E. cloacae* [3, 7, 10].

Enterobacter species have emerged in recent years as a major cause of nosocomial gram-negative infections [2].

Enterobacter often belong to the patients' endogenous flora. They are found in low numbers within the colonic flora of 40–80% of healthy individuals. When selected and amplified by antibiotics these endogenous bacteria become important nosocomial pathogens. Thus, *Enterobacter ssp.* colonizing and infecting patients normally come from an endogenous source, while horizontal transmission appears to be rare [5]. Our study confirms an endogenous infection insofar as the primarily isolated strains often varied with respect to pigmentation, biochemical characteristics and resistance patterns, whereas only resistance changed during therapy. Furthermore, the daily microbiological monitoring of tracheal secretions revealed persistence of the individual strains, which became resistant in a stepwise fashion. Superinfection possibly by horizontal transmission of a multiresistant strain was suspected when multiresistant strains were isolated from patients who had never before shown *E. cloacae* colonization and had not received antibiotics. Patients with such an infection were excluded from the study. This event was rare and observed only two times within the 2-year period of this study.

In our patient groups emergence of resistance during therapy with cefotaxime was observed in nearly half of all *E. cloacae* strains. Resistance against cefotaxime was accompanied by resistance against all cephalosporins and acylureidopenicillins and was directly responsible for persistence of infection; this finding is in agreement with other clinical reports [2, 9].

Previously administered antibiotics may affect the susceptibility profile of *Enterobacter* [2]. According to Chow et al. (1991) emergence of resistance in *E. cloacae* seems more likely to be associated with the administration of third-generation cephalosporins than with other antibiotics [2, 7]. We are not able to assess these observations because our study focused on cephalosporins. In our patient groups the emergence of resistance was equal in groups receiving perioperative cefuroxime, amoxicillin/clavulanic acid or no antibiotics.

In most cases the emergence of resistance by *E. cloacae* was associated with therapeutic failure. However, due to the daily microbiological monitoring and early change to other antibiotics, no clinical impairment or septicemia was observed. Because resistance occurs rapidly, patients with infections caused by *E. cloacae* should be monitored by bacteriological surveillance, and resistance patterns of persisting microorganisms must be screened at short intervals.

In contrast to the resistance to cephalosporins and broad-spectrum penicillins that emerged, resistance of *E. cloacae* to ciprofloxacin or imipenem is extremely rare [2, 9, 10] and was never observed in the present study.

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