ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1987, p. 829–830
0066-4804/87/050829-02$02.00/0

Vol. 31, No. 5

In Vitro Susceptibility of *Citrobacter* Species to Various Antimicrobial Agents

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Received 4 December 1986/Accepted 26 February 1987

The in vitro activities of 16 antimicrobial agents against 14 clinical isolates of *Citrobacter diversus* and 27 isolates of *Citrobacter freundii* were studied. *C. freundii* isolates were more resistant, being susceptible only to amikacin, netilmicin, gentamicin, imipenem, ciprofloxacin, and enoxacin. *C. diversus* isolates were susceptible to many more of the agents tested.

Patients with cancer are at high risk of developing infectious complications, especially during episodes of severe neutropenia (2). We have recently noted an increase in the incidence of infections caused by *Citrobacter* spp. in patients with cancer admitted to our hospital. Between 1972 and 1985, the incidence of bacteremias caused by *Citrobacter* spp. has shown an eightfold increase.

In this study, we evaluated the in vitro activity of 16 antibiotics, including two new quinolones, against isolates of *Citrobacter* spp. obtained from blood culture specimens of patients treated at our institution between 1977 and 1985. The organisms were identified to species level by the scheme of Kelly et al. (11).

Susceptibility testing was performed by using a previously described microtiter broth dilution method (10). Briefly, the organisms were incubated overnight in Mueller-Hinton broth, and appropriate dilutions were made so that the final inoculum was 10^5 CFU/ml. The 16 antimicrobial agents were obtained from their respective manufacturers in the form of standard powders for laboratory use. Antibiotic concentrations were performed manually in Mueller-Hinton broth with serial twofold dilutions ranging from 128 to 0.06 μg/ml and dispensed automatically with an MIC 2000 dispenser (Dynatech Laboratories, Inc., Alexandria, Va.). Amikacin, gentamicin, and netilmicin were prepared in cation-supplemented Mueller-Hinton broth (Ca^{2+}, 50 mg/liter, and Mg^{2+}, 25 mg/liter). Timentin was prepared with stock ticarcillin which was serially diluted and mixed with diluted stock clavulanic acid to have a starting concentration of ticarcillin of 128 μg/ml and to have clavulanic acid in a constant concentration of 2 μg/ml. The MIC was defined as the lowest concentration of drug that prevented visible growth after 18 h of incubation at 35°C. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923 were used in each experiment to ensure the validity of the results obtained. Two susceptibility test runs were performed, one with freshly prepared panels and the other with panels frozen immediately after preparation and stored at −70°C for 3 to 4 days. No differences in results were noticed.

The activities of the various agents against the two species of *Citrobacter* are presented in Table 1. *C. freundii* isolates were susceptible only to amikacin, netilmicin, imipenem, and the two quinolones ciprofloxacin and enoxacin. Gentamicin was active against 89% of the *C. freundii* isolates. *C. diversus* isolates were susceptible to a larger number of agents. Among the agents that are currently used at our institution, only amikacin and imipenem inhibited all isolates of *C. freundii* and *C. diversus* at clinically achievable levels.

TABLE 1. In vitro susceptibilities of 27 *C. freundii* and 14 *C. diversus* strains to 16 antibiotics

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)[a]			% Susceptible
		50%	90%	Range	
Citrobacter freundii (27)	Amikacin	1	2	0.25–4	100
	Ampicillin	16	64	1–>128	26
	Aztreonam	8	32	≤0.06–64	52
	Cephalothin	>128	>128	8–128	4
	Cefamandole	64	>128	0.25–>128	41
	Cefoperazone	32	64	≤0.06–128	45
	Ceftriaxone	16	64	≤0.06–128	41
	Ceftizoxime	4	32	≤0.06–128	48
	Ceftazidime	32	128	≤0.06–128	41
	Ciprofloxacin	≤0.06	≤0.06	≤0.06	100
	Enoxacin	≤0.06	0.25	≤0.06–0.25	100
	Netilmicin	0.5	2	≤0.06–32	96
	Gentamicin	0.5	8	≤0.06–16	89
	Imipenem	0.25	0.5	0.125–1	100
	Piperacillin	32	128	0.25–>128	41
	Timentin	64	128	0.5–>128	37
Citrobacter diversus (14)	Amikacin	1	2	0.3–2	100
	Ampicillin	16	32	2–32	28
	Aztreonam	≤0.06	16	≤0.06–16	79
	Cephalothin	4	>128	2–>128	50
	Cefamandole	1	128	0.25–>128	64
	Cefoperazone	0.25	32	≤0.06–128	71
	Ceftriaxone	≤0.06	32	≤0.06–32	71
	Ceftizoxime	≤0.06	32	≤0.06–32	71
	Ceftazidime	0.25	32	≤0.06–64	71
	Ciprofloxacin	≤0.06	≤0.06	≤0.06	100
	Enoxacin	≤0.06	≤0.06	≤0.06–0.125	100
	Netilmicin	0.25	1	0.25–2	100
	Gentamicin	0.25	1	0.25–64	92
	Imipenem	0.25	0.5	0.25–1	100
	Piperacillin	4	64	0.5–>128	78
	Timentin	2	>128	0.5–>128	71

[a] 50% and 90%, MICs for 50 and 90% of isolates, respectively. Susceptibility breakpoints (micrograms per milliliter): 2.0, ciprofloxacin and enoxacin; 4.0, imipenem, netilmicin, and gentamicin; 8.0, ampicillin, amikacin, cephalothin, cefamandole, aztreonam, ceftazidime, ceftizoxime, and ceftriaxone; 16.0, cefoperazone; 32.0, piperacillin and timentin. Source of data, reference 14.

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The two quinolones tested, which are not currently used at our institution, were also very active against both species.

From 1977 to 1985, *C. diversus* did not acquire resistance to any of the antibiotics tested. On the other hand, whereas only 33% of *C. freundii* isolates were resistant to cefamandole, ceftriaxone, and ceftazidime in 1980, 100, 63, and 88%, respectively, were resistant in 1985.

Differences in susceptibility between *C. freundii* and *C. diversus*, especially for penicillins and cephalosporins, have been reported previously (3, 9, 12). Both species have been found to be susceptible to aminoglycosides and imipenem and most susceptible to the quinolones (1, 3, 4, 7, 8, 10, 13, 16). Of 22 *Citrobacter* spp. tested at our institution during a previous study, 90% were susceptible to aztreonam (5). Resistance of *C. freundii* to aztreonam, ceftazidime, and piperacillin has also been observed by other investigators (1, 4, 6, 15).

Our study shows that there is a difference in susceptibility to various antibiotics between the two *Citrobacter* species. Overall, *C. diversus* was often more susceptible to antibiotics than *C. freundii* was. *C. diversus* isolates may show a bimodal distribution, with one subpopulation being extremely susceptible and the other being multidrug resistant. This was not so among our isolates. The most active drugs currently available against *Citrobacter* spp. are amikacin and imipenem. The unique susceptibilities of *Citrobacter* spp. need to be recognized in those institutions where these organisms have become frequent pathogens.

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