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Surveillance of antimicrobial resistance among Gram-negative isolates from intensive care units in eight hospitals in Turkey

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With the participation of eight major reference hospitals in Turkey, 749 aerobic Gram-negative isolates obtained from 473 intensive care patients in 1997 were tested for their susceptibility to 13 commonly employed antibacterial agents. The frequency with which species were isolated and resistance rates were compared with data from the previous 2 years. Imipenem was the most active agent against the majority of isolates (75%), followed by ciprofloxacin, cefepime and amikacin. The per cent susceptibility to all antibiotics declined from 1995 to 1996. With the exception of imipenem, for which there was no change in resistance, the per cent susceptibility somewhat increased in 1997. However, it was still lower than in 1995.

Introduction

Surveillance is an essential component of infection control programmes. Awareness of antimicrobial resistance patterns is crucial not only for choosing an empirical antimicrobial treatment, but also for the implementation and evaluation of programmes to minimize resistance.

We have recently set up a programme to monitor the antimicrobial resistance of Gram-negative bacteria isolated from intensive care units (ICUs) of eight major hospitals in Turkey. Though far from being a national survey of resistance, this study has yielded valuable information on antibiotic susceptibility patterns.[1]

The frequency with which Gram-negative bacteria were isolated in the ICUs during 1997, and the prevalence of resistance to selected antibiotics, was determined and compared with data from the previous 2 years.

Materials and methods

Seven university hospitals and one large community hospital from six different cities participated in the study. Each of the hospitals collected aerobic Gram-negative isolates from patients in ICUs during 1997. Each institution identified and tested its own isolates.

For each isolate, the MICs of 12 antibiotics (imipenem, ceftazidime, ceftriaxone, cefotaxime, cefepime, cefodizime, cefuroxime, piperacillin–tazobactam, amoxycillin–clavulanate, gentamicin, amikacin and ciprofloxacin) were determined. Additionally, 99 isolates of *Escherichia coli* and 106 of *Klebsiella* spp. were tested for their susceptibility to ceftazidime–clavulanate. MICs were determined on Mueller–Hinton agar by the Etest (AB Biodisk, Solna, Sweden) method in accordance with the manufacturer's instructions. Testing procedures were validated following NCCLS guidelines by measuring the MICs of reference strains on a regular basis. For data analysis, resistance rates were reported using NCCLS breakpoints.[2]

The ceftazidime:ceftazidime–clavulanate MIC ratios have been proposed as a simple screening test for production of extended-spectrum β-lactamases (ESBLs).[3] Ratios of ≤ 4 are considered to indicate lack of ESBLs while ratios of ≥ 16 strongly suggest an ESBL-producing strain. Strains with a ratio of 8 were excluded from the analysis.

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Surveillance of Gram-negative bacteria isolated from ICUs of the participating hospitals has been carried out since 1995. Susceptibility testing has been performed with the same antibacterial agents each year, except that susceptibility to cefepime and cefodizime was not tested in 1995. Results for 1997 were compared with those of the previous years.

Results

A total of 749 isolates were obtained from 473 patients. Of these, 386 (51.6%) were single isolates and 128 (17.1%) were derived from polymicrobial growths on the same occasion. Seventy-five (10.0%) isolates were the initial growth of multiple reisolations and 160 (21.3%) were obtained from repeat cultures, indicating persistent colonization.

Body site

The majority of organisms were isolated from the respiratory tract (n = 269; 36.0%) or urinary tract (n = 150; 20.0%), from wounds, drainage fluids and abscesses (n = 164; 21.9%) or from blood (n = 129; 17.2%); the remaining 37 isolates (4.9%) were from various body sites.

Organisms

Each institution submitted 65–103 (mean 94) Gram-negative isolates. The distribution of isolate pools by species is shown in the Table. *Pseudomonas* spp. was the most frequently isolated Gram-negative species (33.4%), of which the main isolate was *Pseudomonas aeruginosa* (24.6%). *Klebsiella pneumoniae* constituted 64.3% of *Klebsiella* spp. (16.8%). *E. coli*, *Acinetobacter* spp. and *Enterobacter* spp. were also commonly encountered. Gram-negative non-fermenters and some infrequently isolated microorganisms, such as *Aeromonas* and *Salmonella* spp., were grouped as 'Others' in the Table.

Susceptibility

High resistance rates were observed for all antibiotics studied (Table). Imipenem was the most active agent against the majority of isolates. Ciprofloxacin, cefepime and amikacin were relatively effective, with resistance rates around 40%.

Amikacin, imipenem and piperacillin–tazobactam were the most active agents against *P. aeruginosa*. Cefepime, ceftazidime and ciprofloxacin followed, with susceptibility rates of 43%.

Klebsiella spp. were consistently susceptible to imipenem. Ceftazidime–clavulanate, ciprofloxacin and cefepime were

Table. Susceptibility (per cent) of isolated microorganisms

Organism	n	%	Antibiotic (NCCLS breakpoint values; mg/L)										
			IMP (4)	CAZ (8)	CAX (8)	CFT (8)	CPM (8)	CFD (8)	CFU (8)	PTZ (16[a])	AUG (8)	GM (4)	AMK (16)	CP (1)
Acinetobacter	164	21.9	55.5	12.2	9.8	8.5	27.4	9.8	7.3	11.0	7.9	17.1	34.8	32.9
E. coli	138	18.4	99.3	73.9	75.4	79.7	94.2	72.5	59.4	64.5	42.8	77.5	85.5	81.2
Enterobacter spp.	50	6.7	96.0	32.0	30.0	34.0	72.0	28.0	24.0	28.0	16.0	56.0	68.0	92.0
Klebsiella spp.	126	16.8	96.8	27.0	31.0	42.1	65.9	23.8	14.3	23.8	27.0	34.1	53.2	70.3
Proteus spp.	7	0.9	71.4	85.7	100	100	100	100	85.7	85.7	85.7	71.4	100	100
P. aeruginosa	184	24.6	48.4	42.9	10.3	12.0	42.9	5.4	1.6	47.3	2.2	29.9	62.5	43.5
Pseudomonas spp.	66	8.8	84.8	51.5	21.2	25.8	66.7	12.1	7.6	54.5	10.6	30.3	66.7	73.0
Serratia spp.	6	0.8	100	66.7	33.3	33.3	100	33.3	0.0	33.3	–	50.0	83.3	83.3
Others	8	1.1	62.5	12.5	12.5	12.5	37.5	12.5	12.5	12.5	–	37.5	37.5	50.0
Total	749	100.0	74.6	39.5	29.0	32.4	57.8	26.0	18.6	37.8	17.5	38.9	60.1	59.4

AMK, amikacin; AUG, amoxycillin–clavulanate; CAX, ceftriaxone; CAZ, ceftazidime; CFD, cefodizime; CFT, cefotaxime; CFU, cefuroxime; CP, ciprofloxacin; CPM, cefepime; GM, gentamicin; IMP, imipenem; PTZ, piperacillin–tazobactam.
[a]Sixty-four for *Pseudomonas* spp.

also active agents. *Klebsiella* spp. were resistant to the other antibiotics studied.

E. coli was generally susceptible to all the antibiotics studied except cefuroxime and amoxycillin–clavulanate. Imipenem and cefepime were the most effective agents.

Ceftazidime-resistant bacteria

In this study, multiresistant pathogens were commonly encountered. When ceftazidime-resistant strains were taken into account, about 30% of *P. aeruginosa* and 50% of *Acinetobacter* spp. appeared susceptible to imipenem. Imipenem was active against >90% of the other commonly encountered Gram-negative isolates that were resistant to ceftazidime. Ciprofloxacin, cefepime and amikacin were also active against ceftazidime-resistant *Enterobacter* spp., *E. coli* and *Klebsiella* spp. No antibacterial agent other than imipenem proved effective against *Acinetobacter* spp. (49.3% susceptible). Ceftazidime-resistant Gram-negative bacteria appeared uniformly resistant to other antibacterial agents studied.

ESBL-producing Klebsiella spp. and E. coli

As judged by ceftazidime:ceftazidime–clavulanate MIC ratios, 121 strains did not produce ESBLs while 73 did. Eleven isolates with a ceftazidime:ceftazidime–clavulanate MIC ratio of 8 were excluded from the analysis. Amikacin, ciprofloxacin, cefepime and imipenem were effective against 43.8, 69.9, 69.9 and 98.6%, respectively, of the ESBL producers. However, only 19.2% of these were susceptible to piperacillin–tazobactam. Piperacillin–tazobactam, amikacin, ciprofloxacin, cefepime and imipenem were effective against 58.7, 85.1, 75.2, 88.4 and 97.5%, respectively, of the non-producers.

Comparison with previous years' data

The species distribution of isolates in 1997 was similar to that in 1995 and 1996 except for *Klebsiella* spp., which declined from 25–26% to 17% in 1997 ($P < 0.001$) and *Acinetobacter* spp., which showed a steady rise from 8% in 1995 to 11% in 1996 and then to 22% in 1997 ($P < 0.001$).

As shown in the Figure, the proportion of isolates that was susceptible to each antibiotic declined from 1995 to 1996 ($P < 0.0001$). With the exception of susceptibility to imipenem, which remained stable, rates somewhat increased in 1997 ($P < 0.001$). However, 1997 susceptibility rates were still lower than those for 1995 ($P < 0.001$).

Discussion

In order to deal with ever-increasing prevalence of antimicrobial resistance, it is prudent to monitor resistance patterns carefully. Though surveillance studies have long been carried out in Turkey at individual institutions, it is not until recently that efforts towards a national surveillance programme have been initiated.

Nosocomial infections in the ICU are predominantly pneumonia and urinary tract infections and, in accordance with this, most of the isolates were obtained from respiratory or urinary tracts.[4] *Pseudomonas* spp. were the most frequently isolated Gram-negative species (33.4%), followed by *Acinetobacter* spp. (21.9%), a finding absent in our studies of the previous years and European surveys.[1,4,5]

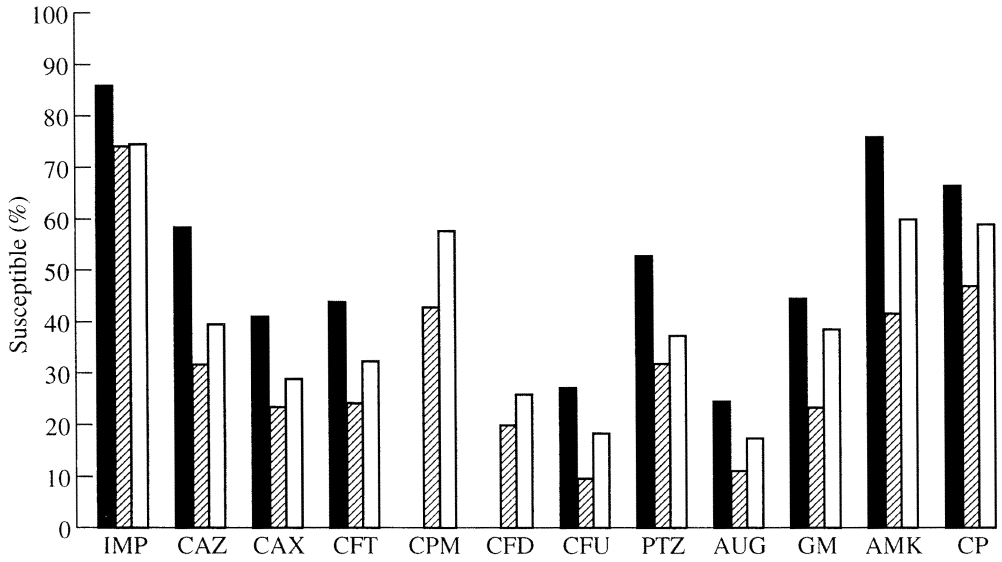

Figure. Susceptibility of isolated bacteria in 1995 (■; $n = 1010$), 1996 (▨; $n = 748$) and 1997 (□; $n = 749$). AMK, amikacin; AUG, amoxycillin–clavulanate; CAX, ceftriaxone; CAZ, ceftazidime; CFD, cefodizime; CFT, cefotaxime; CFU, cefuroxime; CP, ciprofloxacin; CPM, cefepime; GM, gentamicin; IMP, imipenem; PTZ, piperacillin–tazobactam.

E. coli, *Klebsiella* and *Enterobacter* spp. were also commonly isolated.

We have noted very high rates of resistance to the antibacterial agents studied, all of which are commonly and effectively used to treat nosocomial infections. The prevalence of resistance to imipenem was lowest, but this is still higher than desired. The addition of clavulanate to ceftazidime appeared to reduce resistance rates dramatically in all centres. Ciprofloxacin, cefepime and amikacin appeared relatively effective (Table).

An encouraging finding from the 1997 survey is the partial reversal of the alarming decline in susceptibility observed in 1996 (Figure). During 1997, the proportion of isolates that were susceptible to each antibacterial agent has significantly increased, except for imipenem. The stabilization of resistance to imipenem, which is at present the antibacterial agent with the widest spectrum, is also reassuring. These favourable results may in part result from the implementation of the surveillance programme and from understanding the magnitude of the resistance problem.

Pseudomonas, *Enterobacter*, *Serratia* spp. and *Proteus vulgaris* are known to produce inducible class I β-lactamase.[6] Cefepime has low affinity for β-lactamases and is highly resistant to hydrolysis,[7] which may explain why rates of susceptibility to it were relatively high compared with those for the other cephalosporins studied. These resistant pathogens, except for *P. aeruginosa*, maintain their susceptibility to imipenem.

Taking into account the high incidence of resistance to ceftazidime, which is stable against class I β-lactamases, ESBL production appears to be a major mechanism of β-lactam resistance in *Klebsiella* spp. and, less commonly, *E. coli*.[8] Ceftazidime:ceftazidime–clavulanate MIC ratios of $\geqslant$16 have been considered indicative of ESBL production.[3] Of the ceftazidime-resistant strains, 56.6% of *Klebsiella* and 13.1% of *E. coli* were found to match this criterion. As expected, 98.6% and 69.9% of these strains maintained susceptibility to imipenem and cefepime, respectively. Tazobactam is expected to inhibit ESBL, so piperacillin–tazobactam should be a good choice for ESBL-producing microorganisms. However, only 19.2% of the putative ESBL producers isolated in this study were susceptible to piperacillin–tazobactam. This is probably a result of the widespread distribution of non-TEM/SHV ESBLs, such as PER-1, which is resistant to tazobactam, in Turkey.[9]

Probably because ESBL genes occur predominantly on large plasmids carrying multiple resistance genes,[10] putative producers had rates of amikacin resistance as high as 56.2%, while non-producers had resistance rates of 14.9% ($P < 0.001$). Surprisingly, quinolone resistance is known to co-exist with ESBL production, but the association is poorly understood because quinolone resistance is chromosomally mediated. As is the case for amikacin, we detected 30.1% ciprofloxacin resistance in putative ESBL producers in contrast to 24.8% resistance in non-producers ($P > 0.05$).

Although the mechanism of resistance is different, generally resulting from changes in membrane permeability, ceftazidime-resistant *Pseudomonas* spp. tend to also be resistant to imipenem.[11] In our study, only 30.5% of ceftazidime-resistant *P. aeruginosa* isolates were susceptible to imipenem.

Conclusion

This study has shown that there are high rates of resistance in aerobic Gram-negative isolates from ICUs in Turkey. Overall resistance rates were lowest with imipenem, followed by ciprofloxacin, amikacin and cefepime. ESBL production appeared to be a major mechanism of resistance, probably by an enzyme resistant to tazobactam action.

These high rates of resistance leave imipenem as the only reliable agent for the empirical treatment of ICU infections in Turkey. However, the current condition is the result of ineffective hospital infection control and antibiotic policies, which will probably result in increasing rates of resistance to all antibiotics, including imipenem.

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