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A surveillance study of antimicrobial resistance of Gram-negative bacteria isolated from intensive care units in eight hospitals in Turkey

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This study was carried out with the participation of eight hospitals in Turkey to determine the frequency of Gram-negative bacteria isolated in intensive care units (ICU) and to compare their resistance rates to selected antibiotics. Aerobic Gram-negative bacteria isolated from ICUs during 1996 were studied. Antibiotic susceptibilities to imipenem, ceftazidime, ceftazidime–clavulanate, ceftriaxone, cefotaxime, cefepime, cefodizime, cefuroxime, piperacillin/tazobactam, amoxycillin–clavulanate, gentamicin, amikacin and ciprofloxacin were determined by Etest. A total of 748 isolates were obtained from 547 patients. The majority of organisms were isolated from the respiratory (38.8%) and urinary tracts (30.9%). *Pseudomonas* spp. were the most frequently isolated Gram-negative species (26.8%), followed by *Klebsiella* spp. (26.2%). *Escherichia coli*, *Acinetobacter* spp. and *Enterobacter* spp. were the other commonly isolated organisms. High resistance rates were observed for all antibiotics studied. Imipenem appeared to be the most active agent against the majority of isolates. Although resistance rates exceeded 50%, ciprofloxacin, cefepime and amikacin were found to be relatively effective. Extended-spectrum β-lactamase (ESBL) production appeared to be a major mechanism of resistance to β-lactam antibiotics. In contrast to ceftazidime–clavulanate, piperacillin/tazobactam showed poor activity against organisms thought to produce ESBL, suggesting the presence of an enzyme resistant to tazobactam action. This study has yielded high rates of resistance in aerobic Gram-negative isolates from ICUs in Turkey. High resistance rates to all the other antibacterials studied leave imipenem as the only reliable agent for the empirical treatment of ICU infections in Turkey.

Introduction

Intensive care units (ICUs), despite their apparent impact on patient outcome, have become high-risk areas for nosocomial infections. The patient in the ICU has a 5- to 7-fold higher risk of a nosocomial infection compared with the average patient and 20–25% of all nosocomial infections develop in ICUs.[1,2] Infections due to Gram-negative organisms continue to be one of the leading causes of morbidity and mortality. This is a consequence of a complex interaction between the patient's underlying disease, the severity of illness, the type of ICU, the duration of stay, and the number, type and duration of invasive devices and procedures.[1] Medical care of these patients involves closer and more frequent contact with nurses, physicians, or technicians. Hand washing and asepsis may be overlooked in urgent conditions, which may further promote horizontal transmission.[3] Empirical and frequent use of broad-spectrum antibiotics results in the selection of resistant strains. Thus the ICU patient frequently experiences

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colonization and infections by resistant pathogens, which pose major clinical problems despite the introduction of new and potent antibiotics.[3,4]

Limited data are available concerning infection and mortality rates and antibiotic susceptibility of pathogens from ICUs in Turkey, although studies have yielded higher infection and mortality rates with greater antimicrobial resistance in southern Europe than in northern Europe.[1,5]

This study was undertaken with the participation of eight hospitals in Turkey to determine the frequency of Gram-negative bacteria isolated in the ICUs and to compare their rates of resistance to selected antibiotics.

Materials and methods

Seven university hospitals and one large community hospital from seven different cities participated in the study. Each of the hospitals collected aerobic Gram-negative isolates from patients in ICUs during 1996. Each institution identified and tested its own isolates.

For each isolate, MICs were determined for 13 different antibiotics: imipenem, ceftazidime, ceftazidime–clavulanate, ceftriaxone, cefotaxime, cefepime, cefodizime, cefuroxime, piperacillin/tazobactam, amoxycillin–clavulanate, gentamicin, amikacin and ciprofloxacin. Susceptibility testing was performed on Mueller–Hinton agar by Etest (AB Biodisk, Solna, Sweden) in accordance with the manufacturer's instructions. Testing procedures were validated following the National Committee for Clinical Laboratory Standards (NCCLS) guidelines,[6] by measuring the MICs of reference strains on a regular basis. For each isolate, the date of sampling, the name of the ICU, a patient identifier and the source of material were recorded. For the data analysis, resistance rates were reported using the NCCLS breakpoints for the susceptible category in which moderately susceptible isolates were classified as resistant.[6]

Ceftazidime:ceftazidime–clavulanate MIC ratios have been proposed as a simple screening test for ESBL production.[7] Ratios ≤ 4 are considered to lack ESBLs and ratios ≥ 16 strongly suggest an ESBL-producing strain. Strains with a ratio of 8 were excluded from the analysis.

Results

Composition of isolate pool by patients

A total of 748 isolates were obtained from 547 patients. Of these, 433 (57.9%) were single isolates, whereas 175 (23.4%) were mixed. Fifty-nine (7.9%) isolates were the initial growth of multiple re-isolations and 81 (10.8%) were obtained from repeat cultures.

Composition of isolate pool by body site

The great majority of organisms were isolated from the respiratory ($n = 290$; 38.8%) and urinary ($n = 231$; 30.9%) tracts; 80 (10.7%) were cultured from wounds, drainage fluids and abscesses and 81 (10.8%) from blood.

Composition of isolate pool by species

Each institution submitted 70–102 Gram-negative isolates (mean 94). The distribution of isolate pools by species is shown in Table I. *Pseudomonas* spp. were the most frequently isolated Gram-negative species (26.8%), of which the main isolate was *Pseudomonas aeruginosa* (22.1%). *Klebsiella pneumoniae* constituted 66% of *Klebsiella* spp. (26.2%). *Escherichia coli*, *Acinetobacter* spp. and *Enterobacter* spp. were the other commonly encountered pathogens. Gram-negative non-fermenters and some infrequently isolated microorganisms such as *Aeromonas* spp. and *Salmonella* spp. are grouped as 'others' in Table I.

In all but one centre (where *E. coli* predominated) *Klebsiella* spp. or *Pseudomonas* spp. were the leading isolated pathogens. However, great variations in distribution of isolated pathogens were observed among individual hospitals. This was particularly apparent in the cases of *E. coli* and *Acinetobacter* spp., the isolation rates of which varied as much as six-fold among the participating centres.

Antibiotic susceptibility rates of isolates

High resistance rates were observed for all antibiotics studied (Table I). Imipenem appeared to be the most active agent against the majority of isolates. Although resistance rates exceeded 50%, ciprofloxacin, cefepime and amikacin were found to be relatively effective. Ceftazidime–clavulanate appeared quite effective against the limited number of pathogens studied, which were mainly *Klebsiella* spp.

The antibiotic susceptibility of the most commonly isolated pathogens, namely *P. aeruginosa*, *Klebsiella* spp. and *E. coli*, varied considerably among the institutions. As the most active agent against *P. aeruginosa*, imipenem had resistance rates as low as 14.3% and as high as 80.0% in different ICUs. Piperacillin/tazobactam and ceftazidime were moderately effective in some of the hospitals, with resistance rates of 35.7–85.7% and 17.5–85.7%, respectively. *P. aeruginosa* had resistance rates of $\geq 40\%$ to the other antibacterials studied.

Imipenem was consistently effective against *Klebsiella* spp. However, in one of the centres the rate of resistance was 35.0%, in contrast to rates $>12\%$ in the other hospitals. *Klebsiella* spp. was found to be resistant to the other antibiotics studied, excluding ceftazidime–clavulanate.

E. coli was generally susceptible to all the antibiotics studied except for cefuroxime and amoxycillin–clavulanate in all centres. Imipenem was again the most effective agent.

The resistance rates of initial and repeat isolates were comparable, except for two instances: imipenem resistance appeared to be higher in repeat *Acinetobacter* spp. (23.9%

Table I. Susceptibility rates of isolated microorganisms

Organism	*n*	%	Antibiotic (NCCLS breakpoint MIC value (mg/L))												
			IMP (4)	CAZ (8)	CCL (8)	CAX (8)	CFT (8)	CPM (8)	CFD (8)	CFU (8)	PTZ (16[a])	AUG (8)	GM (4)	AMK (16)	CP (1)
Acinetobacter spp.	80	10.7	71.2	7.5	ND	5.0	5.0	11.2	2.5	2.5	6.2	2.5	8.7	29.1[b]	26.4
E. coli	112	15.0	92.0	71.4	79.1[b]	73.6[b]	69.6	79.5	63.4	25.0	50.0	25.0	67.9	67.0	67.0
Enterobacter spp.	76	10.2	82.9	22.4	ND	22.4	28.0[b]	56.6	22.4	10.5	28.9	10.5	26.3	48.7	56.6
Klebsiella spp.	193	25.8	90.2	14.5	80.2[b]	20.0[b]	3.3	46.9[b]	16.6	8.8	20.2	12.5[b]	18.2[b]	44.8[b]	53.4[b]
P. vulgaris	5	0.7	60.0	60.0	ND	40.0	40.0	40.0	40.0	20.0	60.0	40.0	40.0	60.0	40.0
Proteus spp.	19	2.5	89.5	73.7	ND	78.9	84.2	84.2	84.2	73.7	89.5	1.0	68.4	78.9	68.4
P. aeruginosa	165	22.1	44.2	37.4[b]	ND	2.4	1.8	24.8	0.6	0.0	42.4	0.0	5.5	25.9[b]	30.5[b]
Pseudomonas spp.	35	4.7	68.6	29.4[b]	ND	11.4	8.6	31.4	2.9	2.9	57.1	2.9	14.3	38.2[b]	40.0
Serratia spp.	16	2.1	87.5	43.7	ND	18.7	12.5	62.5	6.2	0.0	6.2	0.0	6.2	43.7	68.7
Stenotrophomonas maltophilia	7	0.9	14.3	28.6	ND	0.0	0.0	14.3	0.0	0.0	5.6	14.3	0.0	0.0	33.4[b]
Others	40	5.3	62.5	85.0	0.0[b]	12.5	12.5	22.5	10.0	0.0	12.5	10.0	15.0	27.5	43.6[b]
Overall[c]	748	100.0	74.1	31.4	78.8	23.3	24.0	43.0	19.7	9.5	32.0	11.3	23.3	41.9	47.2

AMK, amikacin; AUG, amoxycillin–clavulanate; CAX, ceftriaxone; CAZ, ceftazidime; CCL, ceftazidime–clavulanate; CFD, cefodizime; CFT, cefotaxime; CFU, cefuroxime; CP, ciprofloxacin; CPM, cefepime; GM, gentamicin; IMP, imipenem; PTZ, piperacillin/tazobactam.

[a]64 for *Pseudomonas* spp.

[b]Some of the isolates were not studied.

[c]Derived from the isolates studied.

ND, not done.

vs 66.7%; $P < 0.01$) and *P. aeruginosa* (52.4% vs 83.3%; $P < 0.05$) isolates.

Cross-susceptibility patterns of isolates

In this study, multiresistant pathogens were commonly encountered. When ceftazidime-resistant strains were considered, about one-third of *P. aeruginosa* and more than two-thirds of the other commonly encountered Gram-negative isolates were susceptible to imipenem (Table II). Thirty-four to 52% of ceftazidime-resistant *Klebsiella* spp., *Enterobacter* spp. and *E. coli* were sensitive to cefepime, amikacin, or ciprofloxacin. The piperacillin/tazobactam susceptibility of these strains was <20%. Amikacin, ciprofloxacin and piperacillin/tazobactam were active against 17–22% of resistant *P. aeruginosa* strains, whereas cefepime had little activity. No antibacterial other than imipenem proved effective against *Acinetobacter* spp. Ceftazidime-resistant Gram-negative bacteria were uniformly resistant to cefodizime and other cephalosporins.

Extended-spectrum β-lactamase (ESBL) producing Klebsiella spp. and E. coli

According to ceftazidime:ceftazidime–clavulanate MIC ratios, 81 strains were found to lack ESBL production and 112 were determined to be ESBL-producing strains. Ten strains with a ratio of 8 were excluded from the analysis. Amikacin, ciprofloxacin and imipenem were effective against, respectively, 41.3, 48.2 and 92.0% of the ESBL producers. However, only 12.5% of these were susceptible to piperacillin/tazobactam. Cefepime was found to be active against 35.5% of these problem pathogens. Amikacin, ciprofloxacin, cefepime and imipenem were effective against, respectively, 65.4, 71.6, 72.8 and 87.6% of the non-ESBL producers.

Discussion

This study was carried out with the participation of eight hospitals, which represent the major reference centres of Turkey. In accordance with the predominance of pneumonia and urinary tract infections among nosocomial infections in ICUs, most of the isolates were obtained from respiratory or urinary tracts.[1]

The species profile of isolated Gram-negative bacteria differed in several ways from European surveys.[1,5,8] *Pseudomonas* spp. and *Klebsiella* spp. were the leading pathogens, with somewhat higher frequencies, *E. coli* are not as predominant, and *Acinetobacter* spp. were unexpectedly common in four of the centres (10–23%).

Our findings demonstrate very high resistance rates to selected antibacterials including cephalosporins, carbapenems, aminoglycosides and β-lactam/β-lactamase inhibitor combinations, all of which are commonly and effectively used to treat nosocomial infections. Although imipenem stands out as the agent with the lowest resistance rates, rates were still higher than ideal. The addition of clavulanate to ceftazidime appeared to reduce resistance rates dramatically in all centres. Ciprofloxacin, cefepime and amikacin resistance rates exceeding 50% (Table I).

In this study, multiresistant pathogens were more prevalent than in European and USA ICU surveys.[1,5,8,9] Approximately 40% of the isolated microorganisms belong to taxa known to produce inducible Class I β-lactamase, namely *Pseudomonas*, *Enterobacter*, *Serratia* and *Citrobacter* spp. and *Proteus vulgaris*.[10] Cefepime has low β-lactamase affinity and high resistance to hydrolysis,[11] which may explain its relatively favourable susceptibility rates among cephalosporins in this study. These resistant pathogens, particularly *Enterobacter* spp.and *Serratia* spp., maintain their susceptibility to imipenem, for which their resistance rates are <20%.

Taking into account the high resistance rates for ceftazidime, which is stable against Class I β-lactamase, ESBL production appears to be a major mechanism of β-lactam resistance. *Klebsiella* spp. and less commonly *E. coli* are well-known producers.[12] Ceftazidime is known to be the best single test antibiotic for the detection of this mechanism.[13] Ceftazidime:ceftazidime–clavulanate MIC ratios ⩾16 have been considered significant for ESBL production.[7] Of the ceftazidime-resistant strains, 61.8% of *Klebsiella* spp. and 12.1% of *E. coli* were found to match this criterion. As expected, 92.0% and 35.5% of these strains maintained susceptibility to imipenem and cefepime,

Table II. Susceptibility rates of ceftazidime-resistant microorganisms

Organism	*n*	% Sensitive to each antibiotic					
		imipenem	cefepime	cefodizime	piperacillin/tazobactam	amikacin	ciprofloxacin
P. aeruginosa	102	33	7	0	22	17	18
Klebsiella spp.	165	89	38	1	12	36	47
E. coli	33	73	48	9	18	45	52
Enterobacter spp.	59	81	44	7	12	34	47
Acinetobacter spp.	74	74	7	0	3	24	23

respectively. Tazobactam is expected to inhibit ESBL; therefore piperacillin/tazobactam should be a good choice for ESBL-producing microorganisms. However, only 12.5% of the putative producers isolated in this study were susceptible to piperacillin/tazobactam. A recent report on ESBL production of *Klebsiella* spp. from ICUs in Europe, including two centres in Turkey, stated that the piperacillin/tazobactam combination inhibited 70% of all putative producers.[7] In this report, 66% of ESBL-producing *Klebsiella* strains from Turkey were found to be resistant to piperacillin/tazobactam, which is concordant with our findings. This is probably due to the widespread distribution in Turkey of non-TEM/SHV ESBLs, such as PER-1, which is resistant to tazobactam.[14,15]

Probably because ESBL genes occur predominantly on large plasmids carrying multiple resistances,[16] putative producers had amikacin resistance rates as high as 58.7%, while non-producers had resistance rates of 35.4% ($P > 0.05$). Surprisingly, quinolone resistance is known to coexist with ESBL production, but the association is poorly understood because quinolone resistance is chromosome mediated. As is the case for amikacin, we detected 51.8% ciprofloxacin resistance in putative ESBL producers, in contrast to 29.3% resistance in non-producers ($P > 0.05$).

Although the mechanism of resistance is different and generally due to changes in membrane permeability, ceftazidime-resistant *Pseudomonas* spp. tend to exhibit cross-resistance with imipenem.[17] In our study, only 33% of ceftazidime-resistant *P. aeruginosa* isolates were sensitive to imipenem.

Of the 426 cefepime-resistant strains, 79 appear to be susceptible to amikacin. Therefore, when these two agents are administered together, the probability that the responsible pathogen will be susceptible to at least one of the antibiotics becomes 53.6%, compared with 43.0% for cefepime alone. Further activity by synergy may be anticipated.[3] These figures become 47.2% and 58.7% for ciprofloxacin alone and ciprofloxacin plus amikacin, respectively. These findings suggest that the addition of an aminoglycoside may not be a solution to high resistance rates, a consequence of the high prevalence of multi-drug-resistant organisms.

In conclusion, this study has yielded 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. The ESBLs involved may be different from those commonly encountered in Europe and the USA, because piperacillin/tazobactam has only a weak effect against these organisms, indicating the presence of 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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