

Short Communication

Antimicrobial Resistance among Gram-Negative Bacteria Isolated from Intensive Care Units in a Cardiology Institute in Istanbul, Turkey

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SUMMARY: Antibiotic resistance among Gram-negative rods (GNRs) causing hospital-acquired infections poses a threat, particularly to intensive care unit (ICU) patients. This study was undertaken in order to achieve the following: to detect the frequency of GNRs isolated from coronary and surgical ICUs at the Institute of Cardiology, Istanbul University, between January 2000-December 2002; to compare the resistance of these GNRs to selected antibiotics; and to determine the prevalence of extended-spectrum beta-lactamases (ESBLs). A total of 367 isolates were obtained from 171 patients. Susceptibility testing and detection of ESBLs were performed using the E-test method. Ceftazidime-clavulanate was used for the detection of ESBLs. The majority of microorganisms were isolated from the respiratory tract (45.5%) and blood (36.7%). *Acinetobacter baumannii* was the most frequently isolated GNR (24.5%), followed by *Pseudomonas aeruginosa* (22%). ESBL positivity was found to be 21.1%. High rates of the ESBLs of *Escherichia coli* and *Klebsiella pneumoniae* were observed, i.e., 27.7 and 57.5%, respectively. High rates of resistance to all antibiotics studied were observed. The most active agent against the majority of the isolates was imipenem (79.2%), followed by levofloxacin (77%) and ciprofloxacin (71%).

Nosocomial infections are associated with an increase in morbidity, mortality, and significant economic costs. These infections occur 5- to 7-fold more often in intensive care unit (ICU) patients than in patients on general wards (1,2). Gram-negative rods (GNRs) are frequently associated with nosocomial infections in ICU patients, especially ventilator-associated pneumonia and catheter-associated urinary tract infections. The use of broad-spectrum antibiotics can lead to colonization with resistant GNRs and consequently to serious infections (3). Extended-spectrum β -lactamases (ESBLs) were first identified in the early 1980s; since then, ESBLs have been identified worldwide and have been found in a number of different organisms, including *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus mirabilis*, and *Salmonella* spp. (4-6). The advent of ESBL producers has represented a great threat to the use of many classes of antibiotics, particularly cephalosporins. It is well known that poor outcomes occur when patients with serious infections due to ESBL-producing organisms are treated with antibiotics to which the organism is frankly resistant. The mortality rate in such patients is significantly higher than that in patients treated with antibiotics to which the organism is susceptible (7).

The aim of this study was to determine the frequency of GNRs isolated from patients hospitalized in the coronary and surgical ICUs of a Cardiology Institute in Istanbul; furthermore, the resistance of these GNR isolates to 12 different antibiotics was compared, and ESBL positivity was determined.

Between January 2000-December 2002, a total of 367 GNRs were recovered from various clinical specimens obtained from patients who were hospitalized in the coro-

nary and surgical ICUs of the Institute of Cardiology at Istanbul University. For the blood cultures, the Bactec 9050 blood culture instrument (Becton Dickinson, Baltimore, Md., USA) was used. All specimens were inoculated on blood agar, endo agar (Oxoid, Unipath Ltd., Basingstoke, UK) and Mueller Hinton broth (MHB) (Oxoid); all plates were incubated for 24 h at 35°C aerobically. Gram staining was performed on positive cultures in order to identify the organism likely to be present. All GNRs were identified in terms of motility, citrate and urease tests, glucose and lactose fermentation and oxidation, indole and oxidase production (8), and by using the API ID 32E and 32GN system (BioMérieux, Lyon, France). After identification, all isolates were maintained in tryptic soy broth with 10% glycerol at -70°C until further testing. Before testing, the bacteria were subcultured twice on Mueller Hinton agar (MHA) (Oxoid).

Each isolate was tested for in vitro susceptibility to imipenem, ceftazidime, ceftriaxone, cefotaxime, cefodizime, cefepime, piperacillin-tazobactam, amoxicillin-clavulanate, gentamicin, amikacin, ciprofloxacin, and levofloxacin. Susceptibility testing was performed on MHA by E-test (AB Biodisk, Solna, Sweden) methodology in accordance with the manufacturer's instructions. After overnight growth on brain heart infusion agar, the organisms were suspended in saline to a turbidity equal to that of a 0.5 McFarland turbidity standard. The suspension was used to inoculate the MHA plates, which were swabbed with a cotton swab soaked with isolates. After the plates were left to dry for 15 min, E-test strips were placed on the plates, which were then incubated for 18 h at 35°C. The MIC was interpreted as the point at which the inhibition ellipse intersected with the E-test strip edge. The testing procedures were validated in accord with the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (9), i.e., by measuring the MICs of the reference strains on a regular basis. For each isolate, the date of sampling, the name of the ICU, the source

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of the isolate, a patient identifier, and reports of the recurrent use of antibiotics were recorded. The ESBL-test was performed using the E-test to identify all isolates. Ceftazidime-clavulanate was used for the ESBL test. ESBL production was defined by a ceftazidime MIC of $\geq 2 \mu\text{g/mL}$, which decreased more than 4-fold in the presence of clavulanate (10).

Pseudomonas aeruginosa ATCC 27853, *E. coli* ATCC 25922, and *K. pneumoniae* BCC 1395 were used as control strains.

A total of 367 nosocomial isolates were obtained from 171 ICU patients. The following isolates were found to be the most common: 90 *Acinetobacter baumannii* (24.5%), 81 *P. aeruginosa* (22%) and 73 *K. pneumoniae* (19.9%). The following isolates were found to be common: 37 *Enterobacter* spp. (10.1%), 36 *E. coli* (9.8%), 23 *Serratia* spp. (6.3%), and 16 *Stenotrophomonas maltophilia* (4.4%). The following isolates were found to be the most infrequent: 5 *P. mirabilis* (1.4%), 3 *Citrobacter* spp. (0.8%), and 3 *Pseudomonas* spp. (0.8%).

High resistance rates were observed against the antibiotics studied (Table 1). In the case of all isolates, ESBL positivity was found to be 21.1%. High rates of ESBLs of *K. pneumoniae* and *E. coli* were found (57.5 and 27.7%, respectively).

The widespread use of broad-spectrum antibiotics, especially in ICUs has led to the emergence of antibiotic-resistant strains of many Gram-negative bacteria. Carriage of bacteria on the hands of healthcare workers also contributes to the spread of Gram-negative microorganisms. Nosocomial lower respiratory tract infections, especially pneumonia, was the most common type of resistant infection among these ICU patients. In accordance with this finding, most of the isolates were obtained from the respiratory tract in previous studies (3,11). The species profile of isolated Gram-negative bacteria differed in several ways from those reported based on European surveys and those conducted in the USA (11-14). In our unit, *A. baumannii* was the most frequently isolated Gram-negative bacteria (24.5%), followed by *P. aeruginosa* (22%) and *K. pneumoniae* (19.9%). I found high resistance rates to all of the major antibiotics used to treat serious infections (Table 1). The lowest resistance rate was observed with imipenem (20.8%), followed by levofloxacin (23%) and

ciprofloxacin (29%). However, high rates of resistance to third-generation cephalosporins (46.9-67.1%) were observed. In our study, multiresistant pathogens were more prevalent than has been reported in surveys conducted in Europe and the USA (11-14). However, I found lower antibiotic resistance rates than those of previous studies carried out in Turkey (15-17). Antibiotics are extensively used in Turkey, and this situation remains uncontrolled at both the community and hospital levels. High resistance rates may be due to the extensive use of antibiotics. The rate of *K. pneumoniae* resistance against either cefotaxime or ceftazidime was 56.2%, and in *E. coli*, the resistance rate was 25% against these agents. The results of three surveillance studies from ICUs in Turkey published in different years revealed that the rates of resistance of *E. coli* and *Klebsiella* spp. to cefotaxime and ceftazidime were 30.4, 28.6%; 96.7, 85.5% (15), 20.3, 26.1%; 58, 73% (16) and 11, 12%; 57, 65% (17). In studies conducted in the USA and in certain European countries, the rates of resistance of *K. pneumoniae* and *E. coli* to cefotaxime and ceftazidime were lower than in Turkey (11-14). In the present study, high resistance to ceftazidime (60.5%) was found in strains of *P. aeruginosa*. Gunseren et al. (15), Aksaray et al. (16), and Leblebicioglu et al. (17) also found high levels of ceftazidime resistance in *P. aeruginosa* (62.6, 57.1 and 51%). In other studies conducted in European countries and the USA, the resistance rates of *P. aeruginosa* to ceftazidime were lower than in Turkey (14,18).

ESBL-producing microorganisms are an increasing problem in ICUs worldwide. ESBLs were first described in the USA, and have resulted in the significant resistance of *Enterobacteriaceae* to cephalosporins (2,5,6). ESBLs hydrolyze oxyimino cephalosporins and monobactams and are commonly inhibited by β -lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam. Many of these enzymes have evolved from the TEM-1, TEM-2, and SAV-1 β -lactamases, which are widely distributed among the *Enterobacteriaceae* (6). Many patients infected with ESBLs are found in ICUs. The specific risk factors that apply to ICU patients include the length of hospital stay, the severity of illness, the length of time spent in the ICU, as well as mechanical ventilation, urinary or arterial catheterization, and previous exposure to antibiotics (19). Metallo- β -lactamases (MBLs) are known to be responsible for resistance to

Table 1. Susceptibility rates of isolated microorganisms

Organism	n	%	Antibiotic NCCLS breakpoint MIC value (mg/ml)												
			AMK (16)	GM (4)	IMP (4)	AUG (8)	CFT (8)	CAZ (8)	CAX (8)	CFD (8)	CPM (8)	PTZ (16)	CP (1)	LV (2)	CAZ/CLV (16)
<i>A. baumannii</i>	90	24.5	55.5	21.1	66.6	5.5	2.2	61.1	8.8	4.4	71.1	82.2	75.5	73.3	10.0
<i>P. aeruginosa</i>	81	22.0	58.0	72.8	83.9	27.1	19.7	39.5	23.4	13.6	59.2	34.5	49.3	75.3	13.5
<i>K. pneumoniae</i>	73	19.9	52.0	53.4	89.0	34.2	43.8	43.8	42.4	41.0	63.0	61.6	82.2	82.2	57.5
<i>Enterobacter</i> spp.	37	10.1	83.8	62.2	91.9	16.2	59.2	62.2	59.5	62.2	64.9	67.5	81.0	81.0	18.9
<i>E. coli</i>	36	9.8	83.3	58.3	91.6	55.5	75.0	75.0	75.0	75.0	75.0	75.0	55.5	55.5	27.7
<i>Serratia</i> spp.	23	6.3	86.9	73.9	91.3	0	65.2	82.6	69.5	65.2	95.6	95.6	100	100	0
<i>Stenotrophomonas maltophilia</i>	16	4.4	0	0	0	0	18.7	50.0	6.2	18.7	31.2	43.7	62.5	87.5	0
<i>P. mirabilis</i>	5	1.4	100	100	100	60.0	100	100	100	100	100	100	100	100	0
<i>Citrobacter</i> spp.	3	0.8	100	100	100	66.6	100	100	100	100	100	100	100	66.6	0
<i>Pseudomonas</i> spp.	3	0.8	100	66.6	100	0	0	33.3	0	0	66.6	66.6	66.6	66.6	0
Total	367	100	61.8	51.2	79.2	22.5	34.0	53.1	35.9	32.9	67.0	64.8	71.0	77.0	21.1

AMK, amikacin; GM, gentamicin; IMP, imipenem; AUG, amoxicillin-clavulanate; CFT, cefotaxime; CAZ, ceftazidime; CAX, ceftriaxone; CFD, cefodizime; CPM, cefepime; PTZ, piperacillin-tazobactam; CP, ciprofloxacin; LV, levofloxacin; CAZ/CLV, ceftazidime-clavulanate.

carbapenems. MBLs have only one zinc ion on their active side. MBLs were first identified in many species of GNRs in Japan (20-22). Widespread dissemination of MBLs has not yet occurred in Japan (23). However, in Italy, serious problems have emerged with respect to the proliferation of GNRs (24,25). Numerous methods have been proposed for the detection of ESBLs in clinical isolates such as disk diffusion, three-dimensional testing, and broth-dilution and E-tests. Molecular approaches have also been utilized (e.g., pulsed-field gel electrophoresis [PFGE]) to examine the epidemiology associated with strains involved in outbreaks of infections caused by ESBLs. Other methods of studying the epidemiology of these strains include plasmid profiles, ribotyping, random amplified polymorphic DNA (RAPD) and arbitrarily primed PCR (19).

I detected high rates of resistance of *K. pneumoniae* (57.5%) and *E. coli* (27.7%) to ceftazidime-clavulanate, as determined by E-test. The ESBL rates of *Klebsiella* spp. and *E. coli* in the Turkish ICU studies were found to be 61.8 and 12.1% (15); 56.6 and 13.1% (16), and 51 and 51% (17). In studies performed in European countries and in the USA, the rates of ESBL positivity in *K. pneumoniae* and *E. coli* isolates were lower than those of our study (6,14). Third-generation cephalosporins such as ceftriaxone, cefodizime, cefotaxime, and ceftazidime were extensively used in our unit before 2000. Therefore, the resistance observed here may be due to ESBLs, which may have appeared under the selective influence of the extensive usage of these antibiotics.

Tazobactam is expected to inhibit ESBLs. Piperacillin-tazobactam should be a good choice for inhibiting ESBL-producing microorganisms. However, in this study, only 26.6% of the ESBL-producing isolates were susceptible to piperacillin-tazobactam. This is probably a result of the widespread distribution in Turkey of non-TEM/SHV ESBLs, such as PER-1, which is known to be resistant to tazobactam (26,27).

In conclusion, the present study revealed high rates of resistance of GNRs to a number of antibiotic agents. In addition, high rates of the ESBLs of *K. pneumoniae* and *E. coli* were found among the ICU patients admitted to a Cardiology Institute in Istanbul. The present results may be used as a guide to choosing an appropriate therapy, particularly when treating suspected ESBL infections in our ICU patients.

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