

ANTIBIOTIC CONSUMPTION AND DEVELOPMENT OF RESISTANCE AMONG GRAM-NEGATIVE BACILLI IN INTENSIVE CARE UNITS IN OMAN

Alya M. Al-Lawati, MBChB, MSc; Nigel D. Crouch, AIMSLS;
Kamal M. Elhag, MD, FRCPath

Since the discovery of antimicrobial agents, micro-organisms have developed virtually unlimited resistance to them.¹ Hospitals and particularly intensive care units are an important breeding ground for the development of antibiotic-resistant bacteria. This is the consequence of heavy antibiotic use. In addition, a high-density patient population in frequent contact with health care staff and the attendant risk of cross-infection contributes to the spread of antibiotic-resistant micro-organisms. This in turn increases the morbidity and mortality associated with infections, and contributes to rising costs of health care.^{2,3} The aim of this study was to investigate the prevalence of antibiotic resistance among gram-negative bacteria in relation to antibiotic use in the intensive care unit (ICU) at the Royal Hospital, the main referral hospital in Oman.

Materials and Methods

The Royal Hospital is a 630-bed tertiary care hospital consisting of major medical and surgical departments, in addition to specialized units such as intensive care, neonatal, renal, cardiac and oncology. The intensive care unit (ICU) at the Royal Hospital is a general unit admitting both medical and surgical patients, and has a capacity of 12 beds divided between adults and pediatrics.

Specimen Collection and Identification of Isolates

One hundred consecutive gram-negative bacterial isolates from different sites were collected from patients admitted to the adult ICU at the Royal Hospital. The clinical significance of the isolates was confirmed by analysis of patients' records and discussion with the treating clinician. All bacterial strains were identified by their colonial morphology, gram reaction, the oxidized and other biochemical reactions as performed by either API 20E, or API 20NE (bioMerieux, France).

From the Department of Microbiology, The Royal Hospital, Seeb, Sultanate of Oman.

Address reprint requests and correspondence to Dr. Al-Lawati: Department of Microbiology, The Royal Hospital, P.O. Box 1331, C.P.O. Seeb, PC 111, Sultanate of Oman.

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TABLE 2. Antibiotic susceptibilities of the bacterial strains.

Micro-organism	No.	IMP	CAZ	ATM	CRO
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Susceptibility Testing

The susceptibilities of the strains to 12 antibiotics (co-amoxiclav, cefuroxime, cefotaxime, ceftriaxone, ceftazidime, aztreonam, piperacillin, piperacillin/tazobactam, imipenem, gentamicin, amikacin, and ciprofloxacin) were performed by determining the minimum inhibitory concentration (MIC), using the E-test (AB BIODISK, Sweden), with *E. coli* NCTC 10418 and *P. aeruginosa* NCTC 10662 as controls. The interpretation standards for MICs of the NCCLS were used to determine antibiotic susceptibilities.⁴ To detect extended spectrum β -lactamases (ESBL), ceftazidime-resistant strains of *E. coli* and *Klebsiella* spp. were further tested against ceftazidime/clavulanic acid. Isolates with a reduction of ceftazidime MIC by >3 two-fold dilutions in the presence of clavulanic acid were considered ESBL producers, and thus resistant to other cephalosporins.

Antibiotic Consumption

The quantities of antibiotics consumed in the ICU during the period of the study were obtained from the hospital pharmacy records, and the numbers of the patients discharged were obtained from the hospital records. The estimated days of antibiotic treatment were calculated from the antibiotic daily dose, the total amount consumed and the number of patients who left ICU during the period of study. The antibiotic consumption is expressed as days of treatment per 100 patient discharges.

TABLE 1. Types of bacterial strains and sites of isolation.

Micro-organism	Total	Respiratory	Urine	Wound	Blood	Others*
<i>Pseudomonas aeruginosa</i>	21	19	1	1	0	0
<i>Klebsiella</i> spp.	20	14	2	1	3	0
<i>E. coli</i>	13	1	7	3	1	1
<i>Enterobacter</i> spp.	12	7	3	0	1	1
<i>S. maltophilia</i>	9	7	0	1	0	1
<i>Acinetobacter</i> spp.	6	4	0	2	0	0
<i>Proteus mirabilis</i>	6	3	0	3	0	0
Other gram-negative bacilli	13	10	1	0	2	0
All gram-negative bacilli	100	65	14	11	7	3

*Others=central line tip (2), abdominal drain site (1).

<i>Pseudomonas aeruginosa</i>	21	48	81	90	38	5	0	95	100	0	71	71	80
<i>Klebsiella spp.</i>	20	100	85	85	90	90	80	45	65	20	85	90	80
<i>E. coli</i>	13	100	77	85	69	69	69	30	85	23	85	92	69
<i>Enterobacter spp.</i>	12	67	17	17	25	25	8	17	42	8	33	58	50
<i>S. maltophilia</i>	9	11	78	56	44	67	11	67	89	66	56	44	80
<i>Acinetobacter spp.</i>	6	100	50	33	17	17	0	0	50	0	83	67	83
<i>Proteus mirabilis</i>	6	67	100	100	100	100	100	100	100	83	83	100	100
Other gram-negative bacilli*	13	54	69	54	46	62	8	69	85	8	69	58	69
All gram-negative bacilli	100	69	71	69	55	52	34	56	78	20	71	73	75

IMP=imipenem; CAZ=ceftazidime; ATM=aztreonam; CRO=ceftriaxone; CTX=cefotaxime; CXM=cefuroxime; PIP=piperacillin; PTZ=piperacillin/tazobactam; AUG=co-amoxiclav; GM=gentamicin; AMK=amikacin; CIP=ciprofloxacin. *Other gram-negative bacilli= *Pseudomonas spp.* (5), *Morganella morganii* (3), *Citrobacter spp.* (1), *Weesella zoohelcum* (1).

TABLE 3. Antibiotic consumption in intensive care units in Oman.

Antibiotic	Estimated days of treatment per 100 discharges
Piperacillin	7
Piperacillin/Tazobactam	13
Cefuroxime	8
Cefotaxime	15
Ceftriaxone	218
Ceftazidime	142
Aztreonam	0
Meropenem	3
Imipenem	76
Gentamicin	60
Amikacin	49
Ciprofloxacin	33
Co-amoxiclav	0
Others*	292
Total	916

*Others=penicillin, cloxacillin, ampicillin, cephradine, co-trimoxazole, chloramphenicol, vancomycin, teicoplanin, erythromycin, fusidic acid and metronidazole.

Results

Bacterial Isolates

The frequency of the bacterial isolates and their sites of isolation are shown in Table 1. The most common isolates were *P. aeruginosa* (21), *Klebsiella spp.* (20), *E. coli* (13), *Enterobacter spp.* (12), and *Stenotrophomonas maltophilia* (9). The most common sites of isolation were the respiratory tract (65%), urine (14%), wounds (11%) and blood (7%). *P. aeruginosa* was the most frequent isolate from the respiratory specimens, *E. coli* was the most from urine and *P. mirabilis* from wounds.

Susceptibility Patterns

Antibiotic susceptibilities of the bacterial strains are shown in Table 2. The highest *in vitro* susceptibility was to piperacillin/tazobactam and ciprofloxacin (78% and 75%), and the lowest was to cefuroxime and coamoxiclav (34%, 20%). The susceptibility of the isolates to cephalosporins ranged from 71% for ceftazidime to 34% for cefuroxime. Only 55% of strains were susceptible to ceftriaxone and 52% to cefotaxime. Ceftazidime showed good activity against *P. aeruginosa*, and *P. mirabilis*, inhibiting 81% and 100%, respectively. Resistance to cephalosporins was

encountered with *Enterobacter spp.* and *Acinetobacter spp.* Aztreonam showed similar activity to ceftazidime against all strains. The aminoglycosides gentamicin and amikacin were active against 71% and 73% of the isolates, respectively. Four strains, two *Klebsiella spp.*, one *P. aeruginosa*, and one *S. maltophilia* were resistant to amikacin but sensitive to gentamicin. Imipenem inhibited 69% of the isolates, but only 11% of *S. maltophilia* were inhibited with imipenem. Two of the six *P. mirabilis* isolates were resistant to imipenem but sensitive to cefuroxime and co-amoxiclav. Ciprofloxacin was active against 75% of the isolates but only 50% of *Enterobacter spp.* were sensitive to it.

Extended Spectrum b-Lactamases (ESBL)

Two of a total of 13 *E. coli* isolates and three of 20 *Klebsiella spp.* were resistant to ceftazidime and aztreonam but sensitive to ceftazclav, indicating ESBL production.

Rates of Antibiotic Consumption

The consumption of antibiotics in the ICU is shown in Table 3. The total amount of antibiotics consumed was equivalent to 916 estimated days of treatment/100 hospital discharges. The most frequently used antibiotics were the third-generation cephalosporins ceftriaxone and ceftazidime, followed by imipenem and amikacin. Among the least consumed were co-amoxiclav and piperacillin.

Discussion

During the study period, every 100 patients treated in the ICU received an average of 916 days of antibiotic treatment, mostly third-generation cephalosporins, imipenem, ciprofloxacin and amikacin. This was 50% higher than the consumption in 1996.⁵ As for the individual antibiotics, there was a four-fold rise in the consumption of ciprofloxacin and three-fold rise in the consumption of imipenem and ceftazidime. Given this substantial use of antibiotics, it is not surprising to note the change in the microbial ecology, with predominance of multiresistant strains of *P. aeruginosa*, *Klebsiella spp.*, *Enterobacter spp.* and *S. maltophilia*. It is well documented that the indiscriminate use of antibiotics has led to the selection and dissemination of antibiotic-resistant organisms.⁶ Several

authors have reported the association of resistance to β -lactam antibiotics with prior use of third-generation cephalosporins.^{7,8} A common mechanism of cephalosporin resistance among *Klebsiella* spp. and *E. coli* is the production of ESBL.⁹ In this study, three *Klebsiella* spp. (15%) and two *E. coli* (15%) were resistant to third-generation cephalosporins and aztreonam, suggesting production of ESBL by these strains. This was confirmed by their susceptibility to ceftazclav. However, with this test alone inhibitor-resistant TEM (IRT) mutants may not be detected. Nevertheless, we believe that IRT mutants are probably prevalent in our hospitals, since 62% of *E. coli* in this study were resistant to co-amoxiclav, suggesting the possibility of IRT production. IRT-producing mutants have been reported in both general practice and hospitals.^{10,11} Nosocomial outbreaks of *Klebsiella* spp. resistant to the third-generation cephalosporins due to the production of ESBL have been reported worldwide.⁹ Although there was an increase in the consumption of cephalosporins in 1998 when this study was conducted, the incidence of probable ESBL producers was much lower than that in 1996,⁵ a fact which we are unable to explain. Carbapenems, being strong inducers of class C β -lactamases, could also have contributed to the resistance to β -lactams, including third-generation cephalosporins. Furthermore, it has been shown that treatment with imipenem, but not with other β -lactam drugs, is a major risk factor for the development of imipenem-resistant *P. aeruginosa* in hospitalized patients.¹² Imipenem resistance in this study was high, particularly among *P. aeruginosa* and *Enterobacter* spp., compared to the study done in 1996, when fewer carbapenems were used.⁵ Furthermore, patients receiving carbapenems, particularly those on mechanical ventilation, are at an increased risk of colonization or infection with class B metallo-enzyme producers such as *S. maltophilia*.^{13,14} Indeed, with the increased use of carbapenems, more strains of *S. maltophilia* were isolated compared to the earlier study. About 70% of these strains were resistant to imipenem, and most were isolated from the respiratory tract of mechanically ventilated patients.

Overuse of carbapenems in our ICUs has also provoked a unique type of resistance among *P. mirabilis*. Two strains (33%) of *P. mirabilis* were resistant to imipenem but sensitive to cefuroxime and co-amoxiclav. Medeiros attributed this resistance to an altered penicillin-binding protein to which imipenem cannot bind, but other β -lactams can.¹⁵

Resistance to gentamicin and amikacin in our study was relatively high. Four isolates were resistant to amikacin but sensitive to gentamicin. This is probably due to selective pressure associated with the high consumption of amikacin in our ICU. This phenomenon has been reported in similar situations, due to aminoglycoside-modifying enzyme N-acetyl transferase (ACC6'-[I]) that hydrolyses amikacin, tobramycin, and netilmicin, but not gentamicin.¹⁶ There was an alarming increase in the level of resistance to

ciprofloxacin in our hospital, as only 75% of the isolates were inhibited, compared to 94% in 1996.⁵ This is probably a result of the increased consumption of ciprofloxacin in 1998, leading to the selection of resistant mutants. The emergence of resistance to fluoroquinolones in virtually all species of bacteria was recognized after the introduction of these compounds for clinical use.¹⁷ Now increase in the levels of resistance to the fluoroquinolones among nosocomial isolates, like *P. aeruginosa*, *Serratia* spp. and *Klebsiella* spp., has been reported worldwide.¹⁷

The gravity of the problem of antimicrobial resistance continues to receive global attention, as evidenced by the pan-European meeting in Copenhagen.¹⁸ Given this escalation in resistance and the overwhelming evidence of overuse of antibiotics, the pragmatic and essential approach to control antibiotic resistance is control of antibiotic use. Apparently, there are reasons for optimism, as studies in various centers showed rapid reversal of resistance.¹⁹⁻²¹ National guidelines on this topic and good diagnostic and therapeutic protocols are important. Continual surveillance of prevalent strains and their resistance patterns is fundamental as a means of establishing the significance of resistance in clinical infection, and in the determination of hospital-prescribing policies. Antibiotic resistance surveillance programs associated with registration of antibiotic consumption are necessary to promote optimal use of antibiotics. Rational prescribing of antibiotics should be encouraged through educational programs, surveillance and audit. Proper infection control procedures must also be practiced to prevent horizontal transfer of drug-resistant organisms.

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