The prevalence of antibiotic resistance among gram negative bacilli from Intensive Care Units in Oman

Kamal M. Elhag, MD, FRCPath, Martina Reed, AIMLS, Hassan M. Al-Lawaty, MBBS Dip Bact.

ABSTRACT

Objectives: This study aims to investigate antibiotic consumption and the prevalence of antibiotic resistance among gram-negative bacteria in Intensive Care Units.

Methods: Antibiotic consumption during the study period was recorded. Ninety six gram-negative bacteria from patients in the intensive care units were identified and tested for susceptibilities to 12 antibiotics. Ceftazidime-resistant Escherichia coli and Klebsiella spp. were further tested for production of extended spectrum β-lactamases.

Results: Antibiotic consumption, mostly penicillins and cephalosporins was equivalent to 605 treatment days per 100 hospital discharges. The commonest isolates were Pseudomonas aeruginosa, followed by Klebsiella spp. and Escherichia coli, mostly recovered from sputum and blood. Ciprofloxacin and imipenem, showed the highest in-vitro activity, inhibiting 94% and 92% of the strains. Thirty six percent of the strains were susceptible to ceftriaxone, 40% to cefotaxime and 78% to ceftazidime. Piperacillin and piperacillin/tazobactam inhibited 73% and 82% of strains. Aminoglycosides' activity was similar to that of piperacillin/tazobactam. Forty one percent of Klebsiella spp. were resistant to all cephalosporins and aztreonam. Imipenem resistance occurred with Pseudomonas spp., Acinetobacter spp. and Stenotrophomonas maltophilia.

Conclusion: Overuse of antibiotics could be behind this high rate of drug resistance. Rational prescribing of antibiotics should be encouraged through educational programs, surveillance and audit.

Keywords: Gram negative bacteria, β-lactamase, resistance, intensive care units.


Since the introduction of antibiotics, the emergence of resistance has become an ever-evolving problem. The spread of drug-resistant organisms in hospital environments has been related to the widespread use of antimicrobial agents. Jacobson and his colleagues demonstrated a linear relationship between the number of days of antibiotic therapy and risk of developing resistance.1,2 Resistance among gram-negative bacilli emerged in the 1970s and, since the mid 1980s, strains resistant to 3rd-generation cephalosporins by the stable overproduction of chromosomally mediated class 1 β-lactamases have become more widespread. Klebsiella pneumoniae and Escherichia coli producing plasmid-mediated extended spectrum β-lactamases (ESBLs) were first described in 19833 and now occurs in many hospitals worldwide, particularly in intensive care units (ICU’s).4,5 Aminoglycoside resistance has evolved by several mechanisms and surveillance studies in some countries have demonstrated modest increases in aminoglycoside resistance among Pseudomonas
aeruginosa, Enterobacter spp. or Citrobacter spp.\(^6\) Resistance to fluoroquinolones has developed among P. aeruginosa, K. pneumoniae, Serratia spp. and Acinetobacter spp.\(^7\) Antibiotic resistance surveillance studies are necessary to make the hospital staff aware of the problem. A survey carried out at the Royal Hospital in 1996 showed a high rate of antibiotic resistance, particularly among gram negative bacilli.\(^8\) The aim of this study was to investigate the prevalence of antibiotic resistance among Gram-negative bacteria in the ICU's and the Special Care Baby Unit (SCBU) at the Royal Hospital, Oman.

**Methods.** The Royal Hospital is a 630 bedded tertiary care hospital, consisting of major medical and surgical departments, in addition to specialized units, such as intensive care, neonatal, renal, cardiac care and oncology. The capacity of the ICUs is 12 beds divided between adults and pediatrics. It is a general ICU, catering for all surgical and medical patients. The SCBU has 30 beds and caters for newborn infants, who need medical or surgical attention.

**Antibiotic consumption.** The types of antimicrobial agents and quantities consumed by patients in the above units during the period of study were obtained from the Hospital Pharmacy records and the number of patients discharged from these units during the same period were obtained from the Hospital Records. The daily dose of each antibiotic was worked out and referred to as day treatment. The antibiotic consumption, in this study is expressed as days of treatment per 100 patient discharges.

**Microbiological methods.** One hundred consecutive Gram-negative bacterial isolates were collected from 76 patients admitted to ICU's and SCBU at the Royal Hospital from February to November, 1996. All bacterial strains were identified according to standard microbiological procedures. The bacterial strains were tested for their susceptibilities to co-amoxiclav, cefuroxime, ceftriaxone, cefotaxime, ceftazidime, aztreonam, piperacillin, piperacillin/tazobactam, imipenem, gentamicin, amikacin and ciprofloxacin, using E-test (AB Biodisk, Sweden). Ceftazidime-resistant strains of E. coli and K. pneumoniae were further tested against ceftazidime/clavulanic acid (ceftazid) for production of ESBLs. Susceptibility testing was performed according to manufacturers' instructions and the interpretation standards for MIC’s of the NCCLS were used to determine antibiotic susceptibilities.\(^9\) Strains of Klebsiella spp. resistant to ceftazidime, but susceptible to ceftazidiv were identified as ESBL producers and thus resistant to other cephalosporins.\(^10\)

**Results.** The consumption of antibiotics in ICU's and SCBU is shown in Table 1. The total amount of antibiotics consumed by these units during 1996 was 10,494 grams, equivalent to 605 days of treatment per 100 hospital discharges. Penicillins were the most frequently used antibiotics, followed by cephalosporins and aminoglycosides. Among the individual drugs, cloxacillin was the most commonly used, followed by ceftriaxone, gentamicin and penicillin. Among the least consumed drugs were chloramphenicol, fusidic acid, cefuroxime and aztreonam.

Four strains were excluded due to incomplete data and 96 gram negative bacterial strains, obtained from 76 patients were thus analyzed. The types and number of bacterial strains and the sites of isolation are shown in Table 2. The bacterial strains were P. aeruginosa (41), Klebsiella spp. (22), E. coli (9), and other Gram-negative bacilli (24). The latter comprised of Serratia spp. (5), Acinetobacter spp. (4), Enterobacter cloacae (4); two each of Stenotrophomonas maltophilia, Pseudomonas spp., Kluvera spp., and one each of Escherichia spp., Salmonella spp., E. aerogenes and Morganella morganii; while one was unidentifiable. Sputum and blood specimens gave the highest yield of bacterial isolates. The most common isolate from sputum was P. aeruginosa and from blood was Klebsiella spp. All strains of Acinetobacter spp and S. maltophilia were recovered from sputum.
Antibiotic susceptibilities of the bacterial strains are shown in Table 3. Most isolates showed in-viro susceptibility to ciprofloxacin and imipenem (94% and 92%), and least to cefuroxime and co-amoxiclav (20% and 24%). The susceptibility of the isolates to cephalosporins ranged from 20% for cefuroxime to 78% for ceftazidime. Only 36% of the strains were susceptible to ceftriaxone and 40% to cefotaxime. Ceftazidime showed high activity against *Pseudomonas aeruginosa*, while ceftazidime, cefotaxime and ceftriaxone were highly active against *E. coli*. Resistance to cephalosporins was mostly encountered with *Klebsiella spp.*, *E. cloacae* and *Acinetobacter spp.* Aztroenam showed similar activity to ceftazidime against all strains. Piperacillin and piperacillin/tazobactam inhibited 73% and 82% of strains. Piperacillin/tazobactam was more effective than piperacillin against *Klebsiella spp.* and *E. coli*. Aminoglycosides showed similar activity to Piperacillin/tazobactam against all strains. They were particularly active against *Pseudomonas aeruginosa* and *E. coli*, but less so against *Klebsiella spp.* and *Acinetobacter spp.* Two strains of *E. cloacae*, one *Serratia spp.* and one *Acinetobacter spp.* were resistant to cephalosporins, aztreonam and piperacillin/tazobactam. Nine strains of *Klebsiella spp.* and one *E. coli* were resistant to ceftazidime and aztreonam, but sensitive to ceftazidime. A strain of *Pseudomonas aeruginosa* was resistant to imipenem, but sensitive to ceftazidime and piperacillin. Two strains of *Acinetobacter spp.* and all *S. maltophilia* were resistant to imipenem. *S. maltophilia* strains were also resistant to all other β-lactams.

**Discussion.** It appears from this study that every hundred patients treated in the ICU’s and SCBU during this period had received an average of 605 days of an antibiotic treatment. This massive antibiotic pressure should, no doubt cast its shadow over the microbial ecology. Indeed, the predominant isolates from ICU’s and SCBU were *Pseudomonas spp.*, *Klebsiella spp.*, *E. coli*, *Acinetobacter spp.* and *Enterobacter spp.*, all of which are either naturally resistant to antimicrobial agents or capable of acquiring resistance by various mechanisms. It has been shown that resistance was associated with prior use of cefotaxime, ceftazidime and piperacillin. This was further confirmed by Chow and co-
workers, who showed that previous administration of 3rd generation cephalosporins could be associated with infection by multi-resistant Enterobacter spp. Cross infection could also contribute to the spread of multi-resistant bacterial clusters in the ICU environment. Several bacterial strains in our series showed an identical antibiotic sensitivity pattern, which strongly suggests the possibility of cross infection as an additional factor to the spread of resistance.

The resistance among our strains could be attributed to several mechanisms, mostly related to antibiotic overuse. Resistance of E. cloacae, Serratia spp. and Acinetobacter spp. to cephalosporins, aztreonam and penicillins is probably due to the production of high levels of class 1 β-lactamases by these strains, which causes resistance to 2nd and 3rd generation cephalosporins, as well as penicillins, including piperacillin/tazobactam.13,14 The β-lactamase inhibitor tazobactam in piperacillin/tazobactam is not stable to the class 1 β-lactamases.15 The resistance of Klebsiella spp. and E.coli to ceftazidime and aztreonam is probably due to the production of ESBLs by these organisms as confirmed by their sensitivity to ceftazidime. It seems that we have various clusters of Klebsiella spp., producing variable ESBLs, as shown by the different susceptibilities of Klebsiella spp. to piperacillin/tazobactam. In a study in ICU's in Western and Southern Europe, the incidence of ESBL among Klebsiella spp. was 49% in Portugal and 59% in Turkey,16 incidences much similar to ours. Aztreonam resistance among ICU strains was similar to that of 3rd generation cephalosporins, in spite of its infrequent use. Resistance to aztreonam follows that of cephalosporins, as both drugs are inactivated by ESBLs and class 1 β-lactamases. Klebsiella spp., E.cloacae and Acinetobacter spp., have shown high resistance to aminoglycosides and cross-resistance with ceftazidime has also been observed among these strains. Ceftazidime-resistant Enterobacter spp. and Klebsiella spp. have associated resistance to aminoglycosides17 and most strains of Acinetobacter spp. were reported to be resistant to aminoglycosides.18 P.aeruginosa, Acinetobacter spp., Serratia spp. and Enterobacter spp. often evade the lethal action of carbapenems by altering their outer membrane porins, rendering them impermeable to carbapenems. The resistance among our strains of Acinetobacter spp. and P.aeruginosa to imipenem is most probably due to this mechanism. A less common mechanism of resistance to carbapenems is the production zinc-metallo β-lactamases by S. maltophilia.19 These enzymes rapidly hydrolyze drugs generally stable to other enzyme classes, such as carbapenems and confer high level resistance to imipenem, meropenem and other β-lactam compounds. The 2 strains of S.maltophilia in this study were resistant to imipenem, as well as all other β-lactam drugs, suggesting the probability of metallo-enzyme production. Patients receiving carbapenems, particularly those on mechanical ventilation are at increased risk of colonization or infection with metallo-enzyme producers such as S.maltophilia.20 Indeed, in this study the 2 strains of S.maltophilia were recovered from sputum specimens.

Apparently, 3rd generation cephalosporins are very popular drugs among our clinicians. Although cephalosporins are essential drugs in the treatment of a variety of infections, their overuse can result in widespread resistance. Indeed, when ceftazidime was used in excess in the hospital environment, a resistant sub-population of β-lactamase overproducing mutants was selected. Complete removal or diminished use of this compound resulted in a decline in resistance rates.21 It appears from our results that imipenem and ciprofloxacin are the most suitable antimicrobial agents for empirical treatment of serious gram-negative infections in our settings, being the most effective agents against the ICU isolates. However, overuse of these drugs is not without risk. Carbapenems have the highest induction potential of class 1 chromosomal β-lactamase,22 leading to high resistance to cephalosporins and penicillins. Furthermore, overuse of carbapenems may select metallo-enzyme producing S.maltophilia, as well as resistant mutants of Acinetobacter spp., E.cloacae, P.aeruginosa, Serratia spp. and Citrobacter freundii. Vura-Papp et al23 reported the development of imipenem resistance during treatment of Pseudomonas infections. Imipenem-resistant Acinetobacter infections emerged following increased use of imipenem for the treatment of ceftazidime-resistant K.pneumoniae infections.24 Overuse of fluoroquinolones, also has been associated with development of resistance. Cross resistance of ciprofloxacin and imipenem was reported to occur during treatment.25 Nevertheless, imipenem and ciprofloxacin remain to be the most suitable agents for empirical therapy of seriously ill patients in our ICU's. However, they should be used judiciously. They should not be administered for prolonged periods and should be combined with an aminoglycoside, if infection with Pseudomonas spp. is suspected. Cefuroxime and co-amoxiclav, on the other hand are not suitable for empirical treatment of ICU acquired infection in our setting and should be reserved for treating community-acquired infections and hospital infections with known susceptible pathogens. We also cannot recommend empirical use of ceftaxime and ceftriaxone in treating serious infections in our ICU setting, as these were ineffective against 60% of the ICU isolates.

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 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. Also proper infection control procedures must be practiced to prevent horizontal transfer of drug-resistant organisms.

**Acknowledgment.** We would like to thank MSD for sponsoring this project.

**References**