ABSTRACT

Current strategies to reduce the epidemic of resistant bacterial strains focus on 2 recurring themes: selective antibiotic pressure and inadequate infection control. Selective antibiotic pressure is frequently, but inaccurately, referred to as inducing resistance. However, induction as originally described refers to a reversible phenomenon that occurs when an organism is exposed to an antibiotic, and among Gram-negative organisms, this has not been shown to be clinically significant in patients. Clinical appearance of resistant strains more often results from selection of spontaneously arising mutant strains in an antibiotic-sensitive population. It is important to understand the concept of induction versus selection, because it affects choices for reducing or preventing resistant organisms. Multiresistance (ie, resistance to several different drugs or drug classes in one organism) is becoming more common and is affecting surveillance data. Surveillance data within hospitals may provide insights into how resistance is promoted (ie, through selective pressure of antibiotics or through inadequate infection control). Molecular epidemiology is providing important information regarding the mechanisms for multidrug resistance. Among isolates of a species of bacteria, a limited number of clones suggests that management may most likely be achieved through improved infection control procedures. In contrast, polyclonal epidemiology may necessitate antibiotic restriction.

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A clinical scenario that hospital infectious disease specialists may encounter is the following: a surgical intensive care unit (SICU) notes a significant increase in the rates of multidrug resistant organisms, including Pseudomonas aeruginosa, extended-spectrum beta-lactamase (ESBL)-producing Klebsiella pneumoniae, and vancomycin-resistant enterococci (VRE). In an attempt to decide the best approach to this problem, a multidisciplinary team is assembled. What factors should this group consider in its initial discussion to address the problem of multiresistance?

CURRENT STRATEGIES TO REDUCE THE EPIDEMIC OF RESISTANT BACTERIAL STRAINS FOCUS ON 2 RECURRING THEMES: SELECTIVE ANTIBiotic PRESSURE AND INADEQUATE INFECTION CONTROL. DETERMINING WHAT INFLUENCE EITHER OF THESE CAUSES HAS IS FIRST DERIVED FROM ASKING, "DOES THE ANTIBiotic FOSTER SOME PATTERN OF RESISTANCE?"

DEFINING THE CAUSES OF ANTIBIOTIC RESISTANCE

More than a decade ago, Chow et al showed that exposure to previously administered antibiotics affected the susceptibility of Enterobacter, the factors affecting mortality, and the emergence of antibiotic resistance during therapy for Enterobacter bacteremia. This study of 129 adult patients showed a marked association of multiresistant Enterobacter in initial positive blood cultures in those with a history of third-generation cephalosporin (TGC) therapy (69% vs 20%; P < .001). The emergence of resistance to TGC during therapy was also dramatically higher in those with previous exposure to this class of drugs (19% TGC vs 0.01% aminoglycosides [P = .001] vs 0% other beta-lactams [P = .002]). These differences resulted in increased mortality in those with multiresistant Enterobacter (32% vs 15%; P = .03). Many have interpreted these data as induction of resistance, but in fact, they provide a classic example of selection of mutant strains that are resistant to antibiotics.

*Based on a presentation given by Dr Karam at a symposium held in conjunction with the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy.

1Paula Garvey Manship Professor of Medicine, Louisiana State University School of Medicine; Head, Department of Internal Medicine, Earl K. Long Medical Center, Baton Rouge, Louisiana.

Address correspondence to: George H. Karam, MD, Earl K. Long Medical Center, 5825 Airline Highway, Baton Rouge, LA 70805. E-mail: gkaram@lsuhsc.edu.
The noun “induction” was introduced in a 1986 study by Sanders and Sanders. They used an in vitro system to examine the potential of type I beta-lactamases to be produced by certain Enterobacteriaceae and Pseudomonas aeruginosa with exposure to certain beta-lactam compounds that were termed “strong inducers” (i.e., cefoxitin, imipenem, and clavulanic acid). In the Sanders study, the induction of type I beta-lactamase was reversible with removal of the antibiotic. The mechanism for this induction was later suggested by Lindberg et al. The gene coding for type I beta-lactamase (ampC) is controlled by the ampR regulatory gene in Pseudomonas and several Enterobacteriaceae, including Enterobacter species and Serratia species. Beta-lactam antibiotics do not enter the cytoplasm but are able to turn on ampR through the formation of a complex between penicillin-binding protein on the cell surface and the antibiotic (Figure 1). This complex signals the ampR to induce ampC production of type I beta-lactamase. Removal of the antibiotic dissolves the complex and thus turns off ampR and therefore ampC. To date, no studies have definitively shown that induction occurs in a clinically significant way in vivo in Gram-negative bacteria.

The vernacular in the medical community has now evolved to include the term “inducing resistance” to describe the appearance of resistant bacterial isolates among patients who have been exposed to antibiotics. However, these clinical appearances of resistant strains result from a mechanism very different from “induction,” i.e., that of selecting mutant strains. Mutant strains arise spontaneously (e.g., 1 in every 10⁶ or 10⁷ in Gram-negative organisms that produce type I beta-lactamase) in an antibiotic-sensitive population in the absence of drug selection. In the presence of antibiotics, a resistant mutant is selected by drug treatment as the sensitive strains die off, and resistance becomes clinically manifest during therapy. It is not yet possible to predict which strains are resistant, so clinical surveillance is required.

Although the mechanism of selection is widely known, it is often incorrectly referred to as induction of resistance. In fact, induction, as it was originally described by Sanders and Sanders, refers only to the in vitro phenomenon of reversibly activating the gene responsible for beta-lactamase production. Selection results in clinical consequences, namely treatment failures during antibiotic therapy. The consequences can be costly, as demonstrated by Cosgrove et al in a study of 477 hospitalized patients with TGC-susceptible Enterobacter species, of whom 46 had subsequent cultures of TGC-resistant Enterobacter spp. Those with resistant species had higher mortality rates (26% vs 13%; P = .06), longer median lengths of stay (29.5 days vs 19 days; P < .001), and twice the hospital charges ($79,323 vs $40,406; P < .001). Overall, emergence of resistance resulted in a median increased length of stay of 9 days and $29,379 in additional hospital charges, above those seen with treating susceptible strains. After adjusting for comorbidities, severity of illness, intensive care unit (ICU) admission, surgery, transfer from another hospital, sex, and age, emergence of resistant strains was associated with a 5-fold increase in risk of mortality and 1.5 fold-increases in length of stay and hospital charges.

Distinguishing the Role of Antibiotic Use versus Infection Control in Emerging Resistance

Epidemiologic resistance data are often collected as aggregates from many hospitals because their intent is to collect and report resistance patterns across multiple institutions. However, these data are limited in their ability to capture the true impact of antibiotic use and its contribution to the emergence of resistant strains. Determining the role of antibiotic use versus infection control in emerging resistance requires a more nuanced approach, considering not only the selection pressures created by antibiotic use but also the effectiveness of infection control measures in preventing the spread of resistant strains. It is important to recognize that while antibiotics are vital tools in the treatment of infections, their judicious use is crucial to prevent the emergence and spread of resistance. By understanding and implementing effective infection control strategies, healthcare providers can mitigate the risks associated with antibiotic overuse and contribute to the overall health of the communities they serve.
to observe changes in resistance over time. However, it is worthwhile to look at resistance trends within a particular hospital and within areas of a hospital, to assess differences in populations served (e.g., Veterans Affairs vs inner city hospital) and hospital department (e.g., ICU vs non-ICU vs outpatient areas). The Intensive Care Antimicrobial Resistance Epidemiology (ICARE) Project is a joint venture between the Division of Healthcare Quality Promotion of the Centers for Disease Control and Prevention and the Rollins School of Public Health at Emory University, both in Atlanta, Georgia. During Project ICARE studies, resistance patterns were measured in 23 hospitals participating in the ICU surveillance component of the National Nosocomial Infections Surveillance (NNIS) System from 1996 through 1999. Susceptibility reports were obtained for all clinical isolates each month, whether associated with hospital- or community-acquired infections or colonization. The study showed significant, consistent increases in resistance in only 4 types of bacteria (oxacillin-resistant Staphylococcus aureus, ciprofloxacin-resistant Pseudomonas aeruginosa, ciprofloxacin-resistant Escherichia coli, and vancomycin-resistant enterococci), and only in patients outside the ICU. The authors concluded that the differences in factors present outside ICUs, such as excessive quinolone use or inadequate infection-control practices, may explain the observed trends. Although a small study, these results validate the emerging concept that resistance results from either certain patterns of antibiotic use or inadequate infection control measures.

Very recently, Paterson et al reported the association between previous administration of beta-lactam antibiotics containing an oxyimino group (cefuroxime, cefotaxime, ceftriaxone, ceftazidime, and aztreonam) and bacteremia due to ESBL-producing strains with a risk ratio of 3.9, after adjusting for confounding variables. In this prospective, observational study of 440 patients with 455 consecutive episodes of Klebsiella pneumoniae, 30.8% of episodes of bacteremia and 43.5% of episodes acquired in the ICU were due to ESBL-producing strains. The authors noted that “the infection control implications of ESBL-producing K pneumoniae are underrecognized.”

The rates of multiresistance among ESBL-producing K pneumoniae are increasing. A prospective study of consecutive patients with community-acquired and nosocomially acquired K pneumoniae bacteremia was performed in 12 hospitals throughout 7 countries. Of the 452 episodes of K pneumoniae bacteremia that were studied, 5.5% (n = 25) were caused by a strain that was resistant to ciprofloxacin in vitro. Multivariate analysis revealed that one of the risk factors for ciprofloxacin resistance was prior exposure to a quinolone (P = .0065) and presence in an ESBL-producing strain. In fact, 18% of the ESBL-producing strains of K pneumoniae were ciprofloxacin resistant, and ESBL production was observed in 60% of the ciprofloxacin-resistant strains. The investigators recognized the importance of the association between ciprofloxacin resistance and ESBL production: “This association is of grave concern since ESBL-producing isolates are usually resistant to penicillins, cephalosporins, aminoglycosides, and trimethoprim-sulfamethoxazole. Therefore, ciprofloxacin resistance severely limits already restricted treatment options. . . There was significant global diversity in the association. It was most likely in Turkey and the United States, where at least 33% of ESBL producers were ciprofloxacin-resistant.”

A case-control study of all patients infected with ESBL-producing K pneumoniae or E coli in 2 Pennsylvania hospitals over a 16-month period showed that 43 of 77 infections studied (55.8%) were resistant to fluoroquinolones. Independent risk factors for fluoroquinolones included worsening of respiratory failure, severity of underlying disease, type of ICU hospitalization, presence of shock, age >45 or 50 years, corticosteroid therapy, prior antibiotic use, inadequate initial antibiotic therapy, infection with a resistant or high-risk organism (e.g., Pseudomonas, Acinetobacter, Stenotrophomonas, and MRSA), use of H2 blockers, >9 hospital days for VAP, >3 days of mechanical ventilation prior to development of VAP.

### Risk Factors for Mortality in Ventilator-Associated Pneumonia

- Worsening of respiratory failure
- Severity of underlying disease
- Type of ICU hospitalization
- Presence of shock
- Age >45 or 50 years
- Corticosteroid therapy
- Prior antibiotic use
- Inadequate initial antibiotic therapy
- Infection with a resistant or high-risk organism (e.g., Pseudomonas, Acinetobacter, Stenotrophomonas, and MRSA)
- Use of H2 blockers
- >9 hospital days for VAP
- >3 days of mechanical ventilation prior to development of VAP

ICU = intensive care unit; MRSA = methicillin-resistant Staphylococcus aureus; VAP = ventilator-associated pneumonia.
quinolone resistance were prior exposure to fluoroquinolone (odds ratio [OR], 11.20) or aminoglycosides (OR, 5.83) and long-term care facility residence (OR, 3.39). Thus, resistance prevalence increased through both selective pressure from antibiotic use and human contact (inadequate infection control).

**Using Selection to Affect Risk Factors**

With increasing prevalence of resistant strains, the mortality risk increases. Yet, there are many risk factors for mortality from bacterial infections, some of which are modifiable. Using ventilator-associated pneumonia (VAP) as an example, numerous risk factors have been identified (see Sidebar, page S264), of which 3 are potentially modifiable: prior antibiotic use, inadequate initial antibiotic therapy, and infection with a resistant or high-risk organism. While the ability to impact prior or inappropriate antibiotic use may be arguable, the ability to prevent resistance may result from decisions of where to place a patient within the hospital setting. Might we be able to impact the pathogenesis of infection with resistant organisms such as *Pseudomonas* or *Acinetobacter* within the hospital?

A study of 8 patients in the SICU of 1 hospital, who had developed imipenem-resistant *K pneumoniae* (IRKP), shows the importance of studying molecular epidemiology and the way it can affect infection control strategies. Isolates from the 8 patients were initially resistant to all cephalosporins, aminoglycosides, and beta-lactam inhibitor combinations. After treatment with imipenem for 5 to 36 days, IRKP was recovered from each patient. Laboratory analysis revealed 3 different clones suggesting the development of stepwise clonal resistance through incremental decreases in imipenem penetration of the cell wall. Because this pathway to resistance does not suggest a plasmid-mediated mechanism, but rather the appearance of a limited number of clones, the authors noted that this type of resistance would be best controlled through strict infection control procedures.

Similarly, surveillance of 15 Brooklyn, New York, hospitals revealed the features of carbapenem-resistant *Acinetobacter baumannii*. In the initial report, a single clone accounted for 62% of the samples and was isolated from patients at all 15 hospitals. A subsequent analysis showed that 2 strains accounted for 82% of the resistant isolates, and the isolates had reduced expression of 3 outer-membrane proteins as well as increased expression of a class C cephalosporinase. Again, the limited number of clones suggests that a plasmid mechanism was not involved, so this type of resistance would be attenuated by more effective infection control measures.

The concept of cross-resistance was recently emphasized using a study of mutant strains of *P aeruginosa*. Livermore et al described strains that had acquired multiresistance to fluoroquinolones and imipenem and reduced susceptibility to meropenem through mutation. These strains were selected by fluoroquinolones, but not carbapenems and had 2 important features: upregulated efflux pumps (which can remove antibiotics from the cytoplasm) and closed porin channels (which render the membrane impermeable to drugs). The classic teaching about resistance in *P aeruginosa* has focused on beta-lactamase derepression, but it appears that porin closure may play a larger role with certain classes of antibiotics. The OprD porin is accessible to carbapenems but not other beta-lactams. Efflux pumps remove certain beta-lactams, chloramphenicol, fluoroquinolones, macrolides, novobiocin, sulfonamides, tetracycline, and trimethoprim, thus rendering the strain resistant to virtually all classes of antimicrobials. Not all antibiotics are subject to efflux; for example, meropenem is subject to efflux, whereas imipenem is not. However, efflux may be co-regulated with porin closure. Resistance to quinolones may lead to resistance to completely different classes of drug.

Similarly, a study of 393 consecutive patients who developed at least 1 episode of VAP showed that 3 significant independent factors were associated with increased risk of piperacillin-resistant *P aeruginosa*: presence of an underlying fatal condition (OR, 5.6), previous fluoroquinolone use (OR, 4.6), and initial disease severity (OR, 0.8). Importantly, a significant relationship was found between resistance to piperacillin and cross-resistance to other antimicrobial agents. Piperacillin-resistant strains in this study were at least 50% resistant to each drug tested compared with less than 22% of piperacillin-sensitive strains. A total of 21 strains were resistant to at least 3 antibiotics. The authors note that only fluoroquinolone use can be controlled to reduce risk of developing these resistant strains.

Isogenic mutants of *P aeruginosa* were constructed to determine the substrates for each efflux pump. Each mutant was designed to constitutively overproduce 1 of
the 3 efflux systems and lack the other 2. A schematic diagram of the efflux pump is shown in Figure 1 in Dr Quinn's article (see page S257). The results show that all of the efflux systems extrude a wide variety of antibiotic groups (Table 1), suggesting that development of resistance to 1 drug has a strong chance of including resistance to at least 1 other antimicrobial agent or class of drug (ie, cross-resistance).

FUTURE DIRECTIONS

The second phase of Project ICARE provided a summary of important relationships between antibiotic use and appearance of resistant strains. Twelve sentinel combinations of antimicrobial agents and resistant organisms were acknowledged (see Sidebar, below). They provided a first step in creating a guide for participating hospitals to identify those antibiotic agents most likely to be associated with a pattern of resistance in a specific organism.

Project ICARE has continued to identify evolving associations of resistance with patterns of antibiotic use. A prospective ecological study of 126 adult ICUs from 60 US hospitals (January 1996-July 1999) showed that the rates of vancomycin use (P < .001) as well as TGC (P = .002) use were independently associated with VRE prevalence (in a weighted linear regression model controlling for type of ICU and rates of VRE among non-ICU inpatient areas). In the largest series to date (233 cases of VRE and 647 matched controls), the main predictors for VRE (after being matched for hospital location, calendar time, and duration of hospitalization) were main admitting diagnosis, a coexisting condition, and infection or colonization with methicillin-resistant S aureus or Clostridium difficile in the past year. After controlling for these variables, the antibiotics associated with VRE were TGC and parenteral metronidazole. A significant linear relationship existed between intensity (duration) of exposure to fluoroquinolones and risk for VRE. Interestingly, vancomycin use was not associated with VRE.

These data were corroborated in a recent review of 74 published studies to explore risk factors for nosocomial infections with methicillin-resistant S aureus, VRE, C difficile, ESBL-producing Gram-negative bacilli, and Candida. The analysis shows “impressive commonality of risk factors across these diverse multiresistant organisms’ including

Table 1. Substrate Specificities of Efflux Systems in Pseudomonas aeruginosa

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Linker</th>
<th>Channel</th>
<th>Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>MexB</td>
<td>MexA</td>
<td>OprM</td>
<td>Quinolones, macrolides, tetracyclines, lincomycin, chloramphenicol, novobiocin, beta-lactams except imipenem</td>
</tr>
<tr>
<td>MexD</td>
<td>MexC</td>
<td>OprJ</td>
<td>Quinolones, macrolides, tetracyclines, lincomycin, chloramphenicol, novobiocin penicillins except carbenicillin and sulbenicillin, cephems except ceftazidime, flomoxef, meropenem, S-4661</td>
</tr>
<tr>
<td>MexY</td>
<td>MexX</td>
<td>OprM</td>
<td>Quinolones, macrolides, tetracyclines, lincomycin, chloramphenicol, aminoglycosides, penicillins except carbenicillin and sulbenicillin, cephems except cefsulodin and ceftazidime, meropenem, S-4661</td>
</tr>
</tbody>
</table>

Sentinel Antimicrobial-Resistant Organisms: Phase 2 of Project ICARE, January 1996 to December 1997

- Methicillin-resistant CNS
- Methicillin-resistant Staphylococcus aureus
- Vancomycin-resistant enterococci
- Penicillin-resistant Streptococcus pneumoniae
- Ceftazidime-resistant Pseudomonas aeruginosa
- TGC-resistant Enterobacter spp
- TGC-resistant Klebsiella pneumoniae
- TGC-resistant Escherichia coli
- Ofloxacin- or ciprofloxacin-resistant E coli
- Ofloxacin- or ciprofloxacin-resistant P aeruginosa
- Piperacillin-resistant P aeruginosa
- Imipenem-resistant P aeruginosa

ICARE = Intensive Care Antimicrobial Resistance Epidemiology; CNS = coagulase-negative staphylococci; TGC = third-generation cephalosporin.

Data from Fridkin et al.
commonly used antibiotics (vancomycin, cephalosporins, and fluoroquinolones), as shown in Table 2. As the authors suggested, hospital programs to prevent or control these types of organisms “that focus on only one organism or one antimicrobial drug are unlikely to succeed.” Murray reviewed VRE infections several years ago and noted that the use of vancomycin does not cause enterococci to become vancomycin resistant but rather selects for VRE strains that may already be colonizing. This may also occur in the presence of TGC.

Collectively, these data suggest that the way in which antimicrobial drugs are used may influence the types of resistant organisms appearing in hospitals. Cross-resistance greatly complicates efforts at infection control because very often the drugs that are now viewed as first line because of their perceived safety profile are the ones associated with the largest patterns of resistance. Although these drugs have very important roles, we might be better able to use them in the context of hospital-wide surveillance of resistance patterns and as we gain greater understanding of resistance and cross-resistance mechanisms.

**CONCLUSION**

A summary of these findings is found in Table 3. Clearly, selection plays a crucial role in the emergence of resistant strains of leading bacterial pathogens in hospitals. Antibiotics appear to impose a selective pressure that changes the flora in the hospital environment, leading to some of the current resistance epidemics; however, inadequate infection control helps to propagate those resistant strains once they appear. Understanding the difference between induction and selection becomes very important. Incorrectly assuming induction as the cause of resistance may lead one to eliminate the use of antibiotics known to be strong inducers, only to use a selector, which may have greater potential for leading to clinical resistance. Both avenues (reducing selection and improving infection control measures) must be pursued. To revisit the clinical situation discussed earlier, to address an ICU outbreak of multiresistant Pseudomonas aeruginosa, ESBL-producing organisms, and VRE, it is the pattern of drug use in that institution and the lack of adequate infection control that might provide the answer to this ever-increasing clinical challenge.

### Table 2. Risk (Odds Ratio) of Colonization or Infection Based on Anti-infective Therapy: A Review of 74 Published Studies

<table>
<thead>
<tr>
<th>Anti-infective Therapy</th>
<th>MRSA</th>
<th>VRE</th>
<th>ESBL-producing GNB</th>
<th>C. difficile</th>
<th>C. albida</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephalosporins</td>
<td>3.1</td>
<td>1.6</td>
<td>13.8</td>
<td>1.4-28.6</td>
<td>NS/</td>
</tr>
<tr>
<td>Penicillins</td>
<td>N S/*</td>
<td>N S/*</td>
<td></td>
<td>3.4-4.9</td>
<td>NS/</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>*</td>
<td>†</td>
<td></td>
<td>15.6-42</td>
<td>N S/</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>N S/*</td>
<td>2.3-10</td>
<td></td>
<td>3.1</td>
<td>275</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>38</td>
<td>1.4-8.77</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple antibiotics</td>
<td>1.7-11.3</td>
<td>1.6-14.5</td>
<td>†</td>
<td>1.6-22.6</td>
<td>1.7-25.1</td>
</tr>
</tbody>
</table>

* not evaluated
† Found significant in a multivariable model but magnitude of increased risk not quantified.

MRSA = methicillin-resistant Staphylococcus aureus; VRE = vancomycin-resistant enterococci; ESBL = extended-spectrum beta-lactamase; GNB = Gram-negative bacilli; NS = not significant.


### Table 3. Noteworthy Processes in Resistance to Antimicrobial Agents

<table>
<thead>
<tr>
<th>Pattern of Resistance</th>
<th>Class of Antibiotics</th>
<th>Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacter with production of type I beta-lactamase1</td>
<td>TGC</td>
<td>Selection</td>
</tr>
<tr>
<td>Klebsiella and Escherichia coli with ESBL production4</td>
<td>TGC</td>
<td>Selection</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa with primary resistance1,2,18</td>
<td>Multiple classes*</td>
<td>Selection</td>
</tr>
<tr>
<td>P aeruginosa with secondary resistance14</td>
<td>TGC</td>
<td>Selection</td>
</tr>
<tr>
<td>Carbapenem-resistant Acinetobacter14</td>
<td>TGC</td>
<td>Selection</td>
</tr>
<tr>
<td>Vancomycin-resistant enterococci20,21</td>
<td>TGC</td>
<td>Selection</td>
</tr>
</tbody>
</table>

* Examples of primary resistance of Pseudomonas to multiple classes of antibiotics: beta-lactamase production—extended-spectrum penicillins and cephalosporins; porin closure—arabapenems; efflux—fluoroquinolones.

TGC = third-generation cephalosporins; ESBL = extended-spectrum beta-lactamase.
REFERENCES


