Development and Spread of Bacterial Resistance to Antimicrobial Agents: An Overview

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(See the comment by Williams on pages S116–7)

Resistance to antimicrobial agents is emerging in a wide variety of nosocomial and community-acquired pathogens. The emergence and spread of multiply resistant organisms represent the convergence of a variety of factors that include mutations in common resistance genes that extend their spectrum of activity, the exchange of genetic information among microorganisms, the evolution of selective pressures in hospitals and communities that facilitate the development and spread of resistant organisms, the proliferation and spread of multiply resistant clones of bacteria, and the inability of some laboratory testing methods to detect emerging resistance phenotypes. Twenty years ago, bacteria that were resistant to antimicrobial agents were easy to detect in the laboratory because the concentration of drug required to inhibit their growth was usually quite high and distinctly different from that of susceptible strains. Newer mechanisms of resistance, however, often result in much more subtle shifts in bacterial population distributions. Perhaps the most difficult phenotypes to detect, as shown in several proficiency testing surveys, are decreased susceptibility to β-lactams in pneumococci and decreased susceptibility to vancomycin in staphylococci. In summary, emerging resistance has required adaptations and modifications of laboratory diagnostic techniques, empiric anti-infective therapy for such diseases as bacterial meningitis, and infection control measures in health care facilities of all kinds. Judicious use is imperative if we are to preserve our arsenal of antimicrobial agents into the next decade.
staphylococci [11] or recognizing extended-spectrum β-lactamase (ESBL)–producing strains of Klebsiella pneumoniae and Escherichia coli [12, 13] can be difficult. The ever expanding array of additional screening tests (e.g., vancomycin agar screen plates for enterococci) or disk diffusion tests that use cefazidime with and without clavulanic acid for ESBL–producing enteric bacteria) is placing a substantial burden on the microbiology laboratory in terms of both time and expense [14]. Thus, emerging resistance has required adaptations and modifications of laboratory diagnostic techniques, anti-infective therapy, and infection control.

KEY FACTORS IN EMERGENCE OF RESISTANCE

The emergence and spread of multiply resistant organisms represent the convergence of a variety of factors that include mutations in common resistance genes that extend their spectrum of activity; the exchange of genetic information among microorganisms in which resistance genes are transmitted to new hosts; the development of environmental conditions in hospitals and communities (selective pressures) that facilitate the development and spread of resistant organisms; the proliferation and spread, in some cases globally, of multiply resistant clones of bacteria; and the inability of some laboratory testing methods to detect emerging resistance phenotypes [15–17].

The role of mutations in the genesis of bacterial resistance to antimicrobial agents. In the early days of the “antibiotic era,” it was assumed that the development of resistance to antimicrobial agents would be a function of mutational events in the bacterial chromosome and, therefore, probably would be a rare event [18]. When multidrug resistance first began to appear, particularly in gram-negative enteric bacilli, much of the resistance was plasmid mediated [18]. With the ensuing discovery of transposons and integrons, the role of mutations in the development of resistance in bacteria was often overlooked. However, for some organisms, such as Mycobacterium tuberculosis, mutations in genes that encode key metabolic pathways or housekeeping functions play a primary role in resistance to rifampin, streptomycin, ethambutol, and fluoroquinolones [19, 20]. Similarly, fluoroquinolone resistance in organisms, such as Neisseria gonorrhoeae [21], Streptococcus pneumoniae [22], and Salmonella species [23], is often a result of mutations in the gyrA, gyrB, parC, and parE loci. These types of mutations transform susceptible organisms into resistant ones.

Some mutations in preexisting resistance genes, however, serve a different function by increasing the level of resistance to specific antimicrobial agents or by expanding the spectrum of resistance. The development of high-level cefotaxime and ceftriaxone resistance in pneumococci is an example of the former, whereas the development of ESBLs is an example of the latter.

In 1992, Sloas and coworkers reported that 3 children with documented pneumococcal meningitis did not respond to adequate doses of either cefotaxime or ceftriaxone [24]. All were infected with a serotype 23F strain of S. pneumoniae that is now commonly referred to as the “Tennessee clone.” Analysis of 1 of these isolates, for which the MIC of penicillin was 0.25 µg/mL and the MIC of cefotaxime was 32 µg/mL, revealed a point mutation in the ptpX gene that encodes 1 of the key penicillin-binding proteins that is involved in β-lactam resistance [25]. This Thr-550-Ala mutation, which immediately followed the Lys-Ser-Gly motif, increased the level of cephalosporin resistance from a more typical level of 2 µg/mL to 32 µg/mL while concomitantly decreasing the level of penicillin resistance. Reversing this mutation by site-directed mutagenesis resulted in an isolate for which the MIC of penicillin was 2.0 µg/mL and the MIC of cefotaxime was 8 µg/mL.

In gram-negative bacilli, the progression of common β-lactamases that typically mediate resistance to ampicillin and narrow-spectrum cephalosporins into ESBLs— that is, enzymes that are capable of hydrolyzing extended-spectrum cephalosporins and monobactams— has become a classic example of mutation and selection of resistance determinants with an enhanced spectrum of activity [26, 27]. Mutations in 1 or more sites of the blaTEM or blasmX genes, primarily in K. pneumoniae, Klebsiella oxytoca, and E. coli, lead to enhanced resistance. Such strains have been recognized throughout the world [28–30]. At least 120 such ESBLs have been reported [31], and the list now includes enzymes of type blaOXA [31]. Recent reports have suggested that the selective pressure caused by high cephalosporin use in hospitals contributes to the emergence and spread of such organisms [32, 33]. As noted by Monnet and colleagues, ESBL-mediated resistance may increase as much as 50% in a single hospital during a period of 2 years [34]. Organisms that express novel enzymes that are capable of hydrolyzing carbapenems, such as imipenem and meropenem, also are beginning to appear [35].

Genetic exchange. The ability of bacteria to exchange genetic information by a variety of mechanisms has been recognized for >40 years [36, 37]. The most commonly recognized modes of exchange are transformation and transduction (among gram-positive organisms), and conjugation (among gram-negative organisms). Among gram-negative organisms, plasmid transfer among a variety of enteric bacilli led to prolonged outbreaks of multiresistant organisms in hospitals [38–40]. More recently, the acquisition of multidrug resistance plasmids by strains of Vibrio cholerae [41] and Shigella dysenteriae [42] has made control of diarrheal disease and dysentery difficult in many African countries.

The ability of gram-positive organisms to exchange DNA via
conjugation is often overlooked by microbiologists; however, it is a very effective means for transmitting antimicrobial agent resistance genes among organisms. The sharing of aminoglycoside resistance genes among several species of staphylococci and enterococci is one example of an active pathway [43–45]. Genetic exchange pathways also exist between gram-positive and gram-negative organisms in which the transfer of kanamycin resistance genes has been observed [46]. Several of the known pathways of genetic exchange among bacterial genera are shown in figure 1.

Enterococci provide an excellent example of how organisms can accumulate resistance genes by genetic exchange and develop into multidrug-resistant pathogens. The first vancomycin-resistant enterococci (VRE) were reported in the United States in 1989; most were recovered from patients in intensive care units [47]. In 1996, the percentage of VRE isolates in intensive care units approached 14% [48], and many of these were also resistant to ampicillin, gentamicin, and streptomycin, leaving few therapeutic options. Most of the resistance determinants in these multiresistant strains were borne on mobile plasmids and transposons [2, 7, 48]. The genetics of enterococcal resistance to glycopeptides is complex and involves a number of unique determinants [49, 50]. In addition to the vanA (high-level), vanB (moderate-level), and vanC (low-level, intrinsic resistance) determinants in enterococci, 2 novel determinants (i.e., vanD [51] and vanE [52]) were recently described. The vanD determinant was first recognized in New York City in 1990 [53] and has subsequently been recognized in a Boston medical center [54].

Initially, VRE appeared primarily in animals in Europe [55], but in the United States it appeared almost exclusively in hospitalized patients [48]. Infections with VRE in humans are emerging throughout the world. VRE now appear to be present in all 50 states in the United States [48, 56, 57] and in Europe [58–60], South America [61], South Africa [62], Australia [63], and Taiwan [64]. Just as the organisms have disseminated, so have the resistance genes also migrated to other species and genera. The vanA determinant has been detected in Oerskovia turbata, Arcanobacterium haemolyticum [65], and Bacillus circulans [66], and the vanB gene has been detected in Streptococcus bovis [67]. The transfer of the vanA gene from E. faecalis to S. aureus has been accomplished in the laboratory [68], but naturally occurring isolates of S. aureus with high-level vancomycin resistance have yet to be recovered from humans or animals.

Not all resistance genes that transfer among bacteria are expressed or even maintained; however, the limitations to expression are not well understood [69]. Nonetheless, the ability of bacteria to acquire resistance genes from other organisms, including those that constitute the normal bacterial flora of humans, under the selective pressure of use of antimicrobial agents should not be underestimated.

Selective pressures in health care and community settings. The development and spread of VRE in health care institutions in the United States in the early 1990s is, to a certain degree, a result of the increased use of vancomycin during that era [70, 71]. Vancomycin was used frequently for infections caused by methicillin-resistant S. aureus and Clostridium difficile and for line-related bacteremia caused by coagulase-negative staphylococci. This increase in vancomycin use contributed to the selective pressure that encouraged the development and spread of VRE.

Selective pressure refers to the environmental conditions that enhance the ability of bacteria to develop resistance to antimicrobial agents and to proliferate. This ability to survive may be the result of acquisition of new DNA (as is often the case with VRE) or it may be due to spontaneous mutation, as is often the case for rifampin-resistant organisms. Expanded use of antimicrobial agents in hospitals and in sites outside the hospital (e.g., long-term care facilities [72], day care centers [73], animal feedlots [74], and other agricultural sites [75]) increases the selective pressure for resistant organisms to emerge in these settings. The intensity of use of antimicrobial agents appears to be proportional to the resistance levels in organisms in hospital settings. Recent studies have shown that, among staphylococci, enterococci, and pseudomonads, levels of resistance are highest in organisms from patients in intensive care units (where use of antimicrobial agents is highest) but are lower in patients from other wards in the hospital and are even lower in outpatient settings [76].

Selection of resistance in bacteria can occur in a variety of ways. In a study reported by Rasheed et al. [77], one strain of E. coli that was isolated, on multiple occasions, from the blood samples of a young girl with aplastic anemia was originally
noted to carry a TEM-1 β-lactamase. During therapy with extended-spectrum cephalosporins, the organism acquired an SHV-1–type β-lactamase that, because of hyperproduction, began to manifest resistance to ceftazidime and other extended-spectrum cephalosporins. A spontaneous mutation in the SHV-1 β-lactamase led to the development of a novel SHV-8 variant with enhanced ceftazidimase activity, increasing the ceftazidime MICs from 16 µg/mL to >64 µg/mL. This was the result of a single amino acid change from aspartate to asparagine at position 179. Simultaneously, the organism lost 1 of its porins (outer-membrane channels), thus becoming resistant to cephamycins (i.e., cefoxitin and cefotetan). All these changes occurred within 3 months while the child was undergoing multiple courses of anti-infective chemotherapy.

*S. pneumoniae* is an example of a community-acquired organism that has become increasingly resistant to a wide variety of antimicrobial agents [78]. In 1994, 25% of pneumococcal strains from the metropolitan Atlanta area were no longer susceptible to penicillin and 9% were no longer susceptible to extended-spectrum cephalosporins [79]. Rates have continued to increase in metropolitan Atlanta and are similar in many US cities [80, 81]. Fluoroquinolone resistance is also beginning to emerge in pneumococci, as revealed by recent Canadian surveillance studies [82]. The widespread use of antimicrobial agents, particularly those used prophylactically in children, is an issue that must be reassessed [83, 84].

The influence of the selective pressure exerted by vancomycin has also been noted in staphylococci. Isolates with vancomycin MICs of 8 µg/mL have been reported from Japan [1], several US cities [85, 86], and Hong Kong [87]. Additional strains of *S. aureus*, for which the vancomycin MICs are only in the range of 1–4 µg/mL, have been shown to contain subpopulations of cells with vancomycin MICs of 4–8 µg/mL [88–92]. The clinical significance of the latter strains has yet to be established, but their isolation is described with increasing frequency in the literature. While the mechanism of decreased susceptibility to vancomycin in *S. aureus* and other species of staphylococci remains elusive, the role of cell wall turnover, increased binding of vancomycin to cell wall monomers, and changes in cell metabolism have been proposed [93, 94]. *S. aureus* strains with decreased susceptibility to vancomycin have been recognized with increasing frequency in Japan [95]. Recent data have suggested that some strains of *S. aureus* with decreased vancomycin susceptibility are not stable and that their susceptibility to vancomycin increases with nonselected passage in the laboratory [96]. Data from Pfeltz et al. [97] also suggest that not all strains of *S. aureus* are capable of achieving higher levels of resistance to vancomycin, whereas other data suggest that vancomycin and β-lactam drugs work synergistically against strains with reduced vancomycin susceptibility [98].

**DETECTION OF RESISTANCE TO ANTIMICROBIAL AGENTS IN THE CLINICAL LABORATORY**

Decreased susceptibility to vancomycin in *S. aureus* can be difficult to detect in the laboratory [11]. Disk diffusion does not differentiate vancomycin-susceptible strains from those with increasing resistance; however, use of vancomycin agar screening plates made with either Brain Heart Infusion agar [11] or Mueller-Hinton agar [99] works well. This is one of several examples of novel resistance phenotypes that are difficult to detect using traditional antimicrobial agent susceptibility testing methods.

During the past several years, various proficiency testing studies, including those conducted by the Centers for Disease Control and Prevention (CDC) [12, 100] and the College of American Pathologists [101], have highlighted the difficulties that laboratories experience in detecting several of the newer bacterial resistance mechanisms with current laboratory methods. Data from proficiency testing studies conducted by the CDC suggest that laboratories not only have difficulty detecting ESBL-producing strains, but often do not follow National Committee for Clinical Laboratory Standards (NCCLS) guidelines regarding the appropriate antimicrobial agents to test and report [12, 102, 103]. For example, 6 of 38 laboratories did not test an extended-spectrum cephalosporin or aztreonam against gram-negative bacilli reported to be isolates from blood cultures.

Perhaps the most difficult phenotypes to detect are decreased susceptibility to β-lactams in pneumococci and decreased susceptibility to vancomycin in staphylococci [104]. In one survey of laboratories participating in the World Health Organization’s External Quality Assurance System for Antimicrobial Susceptibility Testing, 40.5% of laboratories failed to detect decreased susceptibility to penicillin in a pneumococcal isolate with a modal penicillin MIC of 0.06 µg/mL. For those laboratories that assessed penicillin MIC, the range of values reported was 0.007–2 µg/mL. The frequency of reported penicillin MICs is shown in figure 2. Although the laboratories reported using NCCLS guidelines, several laboratories reported results for 10-unit penicillin disks and 30-µg cefotaxime and ceftriaxone disks, although no interpretive criteria are listed for these agents in the NCCLS guidelines. With regard to decreased susceptibility to vancomycin in staphylococci, almost 90% of laboratories reported vancomycin-susceptible results for a *Staphylococcus epidermidis* strain for which the vancomycin MIC was 8 µg/mL. Clinical laboratories must be constantly aware of changes that occur in the susceptibility patterns of pathogenic microorganisms. However, growing restraints on the personnel and supply budgets in hospital-based microbiology laboratories may hinder the widespread implementation of the newer tests.
that improve detection of these novel phenotypes. In fact, newer MIC testing systems often use only 1–3 dilutions of a drug to determine resistance. Often the highest dilution tested on commercially prepared test panels is only in the intermediate range. These breakpoint panels eliminate important quantitative information, such as the actual MICs of antimicrobial agents, and result in a report that lists only the interpretive categories of susceptible, intermediate, and resistant. Thus, the ability to monitor the gradual increase in MICs of a particularly species over time is lost.

CIRCULATION OF MULTIPLY RESISTANT BACTERIAL CLONES

The final issue of importance is the development and global spread of multiply resistant bacterial pathogens. In the past, multidrug resistance was often equated with decreased virulence; however, loss of virulence clearly has not occurred with several globally disseminated strains of pneumococci and S. aureus. The serotype 23F Spanish clone of S. pneumoniae, which is resistant to penicillin, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole [105], and the serotype 6B isolate, which originated in Spain and spread throughout Iceland in the early 1990s [106], are 2 examples of multiresistant organisms that maintained their virulence. In the United States, the Spanish 23F clone was noted as a cause of infections among children who attended a day care center [73]. Some isolates of this clone have been reported to have acquired resistance to cefotaxime and erythromycin in addition to its already established multidrug resistance profile [107].

Another example of a highly virulent, multidrug resistant clone was the trans-Canadian spread of a single clone of methicillin-resistant S. aureus that was introduced into a small hospital in Canada via a patient who arrived from India with dermatitis [108]. Although the patient spent only 4 h in the hospital, 4 cases of nosocomial methicillin-resistant S. aureus were linked to the patient in that institution. An additional 21 cases were observed in a Vancouver hospital to which the patient was transferred, and 26 cases were observed in a Manitoba hospital where the patient also was admitted.

SUMMARY

Resistance to antimicrobial agents is an emerging problem that has required clinicians to alter empiric therapy for such diseases as bacterial meningitis and has prompted laboratory researchers to rethink testing strategies. The rate at which resistant organisms develop is related to their exposure to antimicrobial agents. Control strategies must include guidelines for prudent use of antimicrobial agents. The CDC has developed recommendations to help clinicians use these agents wisely to prevent the development and spread of resistant organisms [109, 110]. Judicious use is imperative if we are to preserve our arsenal of antimicrobial agents into the next decade.

References


