SUPPLEMENT ARTICLE

Integration of Molecular Characterization of Microorganisms in a Global Antimicrobial Resistance Surveillance Program

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The SENTRY Antimicrobial Surveillance Program has incorporated molecular strain typing and resistance genotyping as a means of providing additional information that may be useful for understanding pathogenic microorganisms worldwide. Resistance phenotypes of interest include multidrug-resistant pathogens, extended-spectrum β-lactamase (ESBL)–producing Enterobacteriaceae, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci, and fluoroquinolone-resistant (FQR) strains of gram-negative bacilli and *Streptococcus pneumoniae.* Clusters of ⩾2 isolates within a given resistance profile that are linked temporally and by hospital location are flagged for DNA fingerprinting. Further characterization of organisms with respect to resistance genotype is accomplished with use of polymerase chain reaction and DNA sequencing. This process has been highly successful in identifying clonal spread within clusters of multiresistant pathogens. Between 50% and 90% of MRSA clusters identified by phenotypic screening contained evidence of clonal spread. Among the Enterobacteriaceae, ESBL-producing strains of *Escherichia coli* and *Klebsiella pneumoniae* are the most common pathogens causing clusters of infection, and ~50% of recognized clusters demonstrate clonal spread. Clusters of *Pseudomonas aeruginosa, Acinetobacter* species, and *Stenotrophomonas maltophilia* have been noted with clonal spread among patients with urinary tract, respiratory, and bloodstream infections. Characterization of mutations in the FQR-determining region of phenotypically susceptible isolates of *E. coli* and *S. pneumoniae* has identified first-stage mutants among as many as 40% of isolates. The ability to characterize organisms phenotypically and genotypically is extremely powerful and provides unique information that is important in a global antimicrobial surveillance program.

Antimicrobial resistance is a problem of worldwide magnitude [1]. With the increased interest and concern regarding antimicrobial resistance, there are now several major programs that have been organized to conduct surveillance for antimicrobial resistance on a national and international basis. Examples of some of the more prominent surveillance programs include the National Nosocomial Infection Surveillance system, Project ICARE (Intensive Care Antimicrobial Resistance Epidemiology), the SENTRY Antimicrobial Surveillance Program, the Surveillance and Control of Pathogens of Epidemiologic Importance project, the Alexander Project, and The Surveillance Network [2–7]. Each of these programs involves the use of conventional broth- and agar-based antimicrobial susceptibility testing methods to provide a phenotypic profile of the response of microbial pathogens to an array of antimicrobial agents. The data available from each of these programs build on those of the others and provide a compelling body of information that underscores the global nature of the resistance problem [2–6]. This information is useful for alerting the public, as well as the medical and scientific community, to the problem of antimicrobial re-

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Table 1. Genotypic methods for epidemiological typing of microorganisms in the SENTRY program.

Method	Comments	Examples
Plasmid analysis	Plasmids may be digested with restriction endonuclease enzymes; useful in tracking spread of resistance genes	Staphylococci, Enterobacteriaceae
Restriction endonuclease analysis of chromosomal DNA with conventional electrophoresis	Large number of bands; difficult to interpret; not amenable to computer analysis; a *comparative* typing method only	Enterococci, *Staphylococcus aureus, Clostridium difficile, Candida* species
Genome restriction fragment-length polymorphism analysis: ribotyping, insertion sequence probe fingerprinting	Fewer bands; amenable to automation and computer analysis; sequence-based profiles; *library* typing methods	Enterobacteriaceae, staphylococci, and *Pseudomonas aeruginosa, Candida* species
PCR-based methods: repetitive-elements PCR spacer typing, selective amplification of genome restriction fragments, multilocus allelic sequence-based typing	Crude nucleic acid extracts and small amounts of DNA may suffice; amenable to computer analysis	Enterobacteriaceae, *Acinetobacter* species, staphylococci
Pulsed-field gel electrophoresis	Fewer bands; amenable to computer analysis; very broad application; may be used as either a *comparative* or *library* typing system	Enterobacteriaceae, staphylococci, enterococci, *Candida* species

NOTE. The table contains examples of available methods and applications and is not intended to be all inclusive (adapted from [11]).

sistance and for designing empirical treatment regimens and formulating possible preventive strategies [1].

Although useful as a screen for detecting certain resistance profiles and for selecting potentially useful therapeutic agents, conventional antimicrobial susceptibility testing methods are insensitive for tracing the spread of individual strains within a hospital or region and for detecting resistance mechanisms that may be present at low levels in a population of organisms and where selection or induction of resistance is necessary before the resistant phenotype is expressed [8, 9]. The techniques of molecular biology have proven to be invaluable in the diagnosis of many infectious diseases and provide further insight into the mechanisms of antimicrobial resistance and the clonal spread of many key microbial pathogens [8–12]. Comprehensive molecular typing of organisms collected in a global surveillance program may also provide information regarding the emergence and distribution of specific pathogenic strains as well as the spread of resistance determinants [13–15].

Among the active surveillance programs, the SENTRY Antimicrobial Surveillance Program has incorporated molecular strain typing and resistance genotyping as a means of providing additional information that may be useful for understanding pathogenic microorganisms worldwide. Examples of this work are provided in other manuscripts in this supplement. The material discussed herein will provide an overview of the methods used, the process by which molecular methods are applied

Table 2. Molecular methods for detecting antimicrobial resistance in the SENTRY program.

Antimicrobial agent(s)	Organisms	Gene	Detection methods
Methicillin, oxacillin	Staphylococci	*mec A*[a]	DNA probe, branched-chain DNA probe, PCR
Vancomycin	Enterococci	*van A, B, C, D*[b]	DNA probe, PCR
β-Lactams	Enterobacteriaceae, *Haemophilus influenzae,* other nonenteric gram-negatives	bla_{TEM} and bla_{SHV}[c]	DNA probe, PCR and RFLP, PCR and sequencing, isoelectric focusing
Quinolones	Enterobacteriaceae, *H. influenzae,* gram-positive cocci	Point mutations in *gyr A, gyr B, par C,* and *par E*	PCR and sequencing

NOTE. RFLP, restriction fragment-length polymorphism. Table is adapted from [9].

[a] *mec A* encodes for the altered penicillin binding protein 2a; phenotypic methods may require ⩾48-h incubation to detect resistance and are <100% sensitive. Detection of *mec A* has potential for clinical application in specific circumstances.

[b] Vancomycin resistance in enterococci may be related to 1 of 4 distinct genotypes, of which *van A* and *van B* are most important. Genotypic detection of resistance is useful in validation of phenotypic methods.

[c] The genetic basis of resistance to β-lactam antimicrobials is extremely complex. The bla_{TEM} and bla_{SHV} genes are the 2 most common sets of plasmid-encoded β-lactamases. The presence of either a bla_{TEM} or bla_{SHV} gene implies ampicillin resistance. Variants of the bla_{TEM} and bla_{SHV} genes (extended-spectrum β-lactamases) may also encode for resistance to a range of third-generation cephalosporins and to monobactams.

Table 3. Comparison of ribotyping and pulsed-field gel electrophoresis (PFGE) for typing bacterial isolates.

Organism	No. of		Index of discrimination	
	Ribogroups	PFGE types	Ribotyping	PFGE
Staphylococcus aureus ($n = 57$)	30	28	0.93	0.91
Escherichia coli ($n = 97$)	38	72	0.78	0.96
Klebsiella pneumoniae ($n = 53$)	42	50	0.97	0.98
Pseudomonas aeruginosa ($n = 12$)	4	10	0.73	0.89
Enterococcus faecium ($n = 17$)	8	14	0.80	0.95
Enterobacter cloacae ($n = 43$)	42	38	0.95	0.97

NOTE. Table is adapted from [18].

in a comprehensive surveillance program, and additional examples highlighting instances in which molecular characterization has proved useful for understanding groups of resistant pathogens.

MOLECULAR METHODS USED IN THE SENTRY PROGRAM

The laboratory characterization of microorganisms to provide evidence of biological and genetic relatedness is frequently of use to physicians, microbiologists, and epidemiologists as an aid in the investigation of infectious diseases [9]. The need to determine the relatedness of organisms may arise during an outbreak investigation in which a cluster of infections due to organisms of the same species and antimicrobial resistance phenotype is identified and in which the goals are to determine clonal spread in a microenvironment and identify the source of infection [16].

Alternatively, epidemiological surveillance conducted over time requires the monitoring of clonal spread and prevalence of strains within a population as an aid in long-term evaluation of control strategies or for detection and monitoring of emerging infections [13, 14, 17]. Likewise, characterization of the molecular and biological mechanisms of antimicrobial resistance (resistance genotyping) may enhance the ability of microbiologists and epidemiologists to track the spread of antimicrobial resistance within and among health care facilities and communities [8].

Microbial strain delineation by DNA-based typing methods (DNA fingerprinting) is a very powerful tool that contributes

Table 4. Molecular analysis of organism clusters exhibiting multidrug resistance (MDR) patterns observed during 1999: SENTRY objective A (bloodstream infections).

Organism	Total isolates[a]	No. of MDR clusters (no. of isolates)	No. of sites with MDR clusters	No. of MDR clusters with possible clonal spread (no. of isolates)[b]	No. of sites with MDR clones
Staphylococcus aureus	2348	49 (250)	34	35 (143)	28
Klebsiella pneumoniae	677	14 (51)	12	8 (20)	7
Escherichia coli	1755	14 (53)	10	6 (22)	6
Enterococcus faecium	157	4 (11)	4	2 (7)	2
Acinetobacter baumanii	125	4 (12)	3	2 (6)	2
Pseudomonas aeruginosa	477	3 (12)	3	3 (7)	3
Enterobacter aerogenes	84	2 (9)	2	2 (4)	2
Enterobacter cloacae	265	1 (4)	1	1 (3)	1
Stenotrophomonas maltophilia	64	1 (3)	1	1 (2)	1
Proteus mirabilis	140	1 (2)	1	1 (2)	1
Streptococcus pneumoniae	455	1 (3)	1	1 (2)	1
Total	6547	94 (410)	44[c]	62 (218)	33[c]

[a] Total number of isolates of each species tested in objective A, 1999 (from the United States, Canada, Latin America, Europe, Israel, and Turkey; total of 72 study sites).
[b] Possible clonal spread is defined as isolates with identical ribotypes and pulsed-field gel electrophoresis profiles.
[c] Some sites had more than 1 cluster/clone.

Table 5. Molecular analysis of organism clusters exhibiting multidrug resistance (MDR) patterns observed during 1999: SENTRY objective C (pneumonia in hospitalized patients).

Organism	Total isolates[a]	No. of MDR clusters (no. of isolates)	No. of sites with MDR clusters	No. of MDR clusters with possible clonal spread (no. of isolates)[b]	No. of sites with MDR clones
Staphylococcus aureus	975	13 (70)	13	12 (53)	12
Pseudomonas aeruginosa	859	7 (32)	6	4 (18)	3
Acinetobacter baumanii	76	7 (29)	6	5 (18)	4
Stenotrophomonas maltophilia	117	6 (25)	6	5 (13)	5
Klebsiella pneumoniae	244	2 (12)	2	1 (2)	1
Klebsiella oxytoca	63	2 (9)	2	0	0
Citrobacter koseri	20	1 (2)	1	0	0
Enterobacter aerogenes	67	1 (2)	1	0	0
Escherichia coli	100	1 (3)	1	0	0
Acinetobacter calcoaceticus	20	1 (12)	1	1 (10)	1
Total	1582	41 (196)	26[c]	28 (114)	21[c]

[a] Total number of isolates of each species tested in objective C, 1999 (from the United States, Canada, Latin America, Europe, Israel, and Turkey; total of 72 study sites).
[b] Possible clonal spread is defined as isolates with identical ribotypes and pulsed-field gel electrophoresis profiles.
[c] Some sites had more than 1 cluster/clone.

to our understanding of outbreaks and recurrent infection [11]. Investigators have used a variety of DNA-based methods to genotype microbial pathogens (table 1). All of these methods depend on the generation of a distinct pattern or DNA "fingerprint" that may be visualized by ethidium bromide staining or by nucleic acid hybridization. An additional level of discrimination may be achieved by DNA sequencing to detect single base-pair changes. This approach is particularly useful in characterizing various antimicrobial resistance genes and mutations causing resistance to specific classes of antimicrobial agents [8] (table 2).

Genotyping methods may be broken down into 2 broad categories: comparative methods and library typing methods [12]. Both are useful in a surveillance program. Comparative typing methods are most often applied in the setting of an outbreak investigation as an aid in addressing the short-term control of transmission in a hospital or community setting. In this situation a typing method is used to compare a limited number of isolates collected over a relatively short period (days to weeks) to identify clonally related (epidemic) strains and unrelated (sporadic) strains. The performance requirements for a comparative typing system are good reproducibility within a

Table 6. Molecular analysis of organism clusters exhibiting multidrug resistance (MDR) patterns observed during 1999; SENTRY objective D (wound infections).

Organism	Total isolates[a]	No. of MDR clusters (no. of isolates)	No. of sites with MDR clusters	No. of MDR clusters with possible clonal spread (no. of isolates)[b]	No. of sites with MDR clones
Staphylococcus aureus	217	12 (49)	12	6 (21)	6
Pseudomonas aeruginosa	90	4 (13)	4	1 (2)	1
Escherichia coli	83	3 (9)	3	2 (4)	2
Enterobacter cloacae	33	2 (6)	2	1 (4)	1
Acinetobacter baumanii	20	2 (4)	2	1 (2)	1
Klebsiella pneumoniae	25	1 (2)	1	0	0
Klebsiella oxytoca	5	1 (2)	1	0	0
Streptococcus pyogenes	17	1 (11)	1	1 (7)	1
Total	490	26 (96)	17[c]	12 (40)	9[c]

[a] Total number of isolates of each species tested in objective D, 1999 (from the United States, Canada, Latin America, Europe, Israel, and Turkey; total of 72 study sites).
[b] Possible clonal spread is defined as isolates with identical ribotypes and pulsed-field gel electrophoresis profiles.
[c] Some sites had more than one cluster/clone.

single assay, a high index of discrimination (>0.95), and the ability to provide results for each organism (full typeability) [18]. Several different DNA-based typing methods fulfill these criteria, but the most widely used method for comparative typing is pulsed-field gel electrophoresis (PFGE) [11, 12]. PFGE is used as a comparative typing method in the SENTRY program because of its excellent discriminatory power and broad applicability for most gram-negative and gram-positive organisms [18].

Library typing methods are most useful in the context of a prospective epidemiologic surveillance effort. The collection and characterization of large numbers of organisms over a prolonged period (months to years) require the use of strain markers with a standardized nomenclature and with high reproducibility over time. The patterns must be amenable to computer-based analysis and storage, and the discriminatory power of the method must be balanced against the evolutionary stability of the organism of interest to allow the recognition of clonal dispersion over more prolonged periods [12]. Library typing systems are used to aid in the monitoring of the geographic spread and prevalence shifts of epidemic and endemic clones and in the long-term evaluation of preventive strategies [12].

Because surveillance studies such as SENTRY typically involve hundreds to thousands of organisms collected over an extended period, the use of a method that is highly standardized and provides a high throughput is essential to generate meaningful results. These performance characteristics are met by the RiboPrinter Microbial Characterization System (Qualicon), which is a commercially available automated system that performs ribotyping and uses computer-assisted pattern analysis to ensure a high degree of standardization, excellent run-to-run comparability, high throughput, and reduction in labor costs [18]. Ribotyping by means of the RiboPrinter displays a high degree of typeability but generally less discrimination than PFGE [18] (table 3). The RiboPrinter is used as the primary (library) typing system in the SENTRY program [13–15, 17]. Isolates sharing the same ribotype profile are further discriminated by PFGE [13, 14, 18].

Molecular methods may be used to detect specific antimicrobial resistance genes (resistance genotyping) in a wide variety of organisms and when coupled with DNA fingerprinting have made substantial contributions to our understanding of the genetics of antimicrobial resistance and the spread of resistance determinants [8–10]. The resistance mechanisms in gram-negative and gram-positive bacteria are both complex and numerous and include enzymatic inactivation, target alteration, decreased uptake, and increased efflux. Organisms may contain several different resistance genes, and subtle variations (e.g., point mutations) in certain genes may result in the expression of extremely broad-spectrum resistance factors such as extended-spectrum β-lactamases (ESBLs) [10].

Table 7. Common pulsed-field gel electrophoresis (PFGE) patterns of multidrug-resistant/methicillin-resistant *Staphylococcus aureus* bloodstream isolates contained within the 2 most common ribotypes, including geographic distribution.

Ribotype	PFGE type[a]	No. of isolates	Location(s)
184-5	I	26	Singapore, Hong Kong, Australia
	II	9	France, Japan, South Africa
	III	13	Italy, Hong Kong, Singapore, Texas
	IV	20	France, Poland, South Africa, Italy, Portugal
	V	9	France, Taiwan
	VI	8	Michigan, Illinois, New York, Missouri, Virginia
	VII	7	New York, California, Texas
893-5	I	24	Portugal, Italy, Argentina, Brazil, Chile

NOTE. Table is adapted from [13].

[a] Each PFGE type represents strains that differ by 3 or fewer bands or share >80% similarity (DENDRON).

Despite this complexity, DNA probe– or PCR-based methods have proved extremely effective in detecting antimicrobial resistance genes, including those encoding for methicillin resistance in staphylococci *(mec A),* vancomycin resistance in enterococci *(van A, B, C, D),* β-lactam resistance in Enterobacteriaceae (bla_{TEM}, bla_{SHV}), and fluoroquinolone resistances in gram-negative bacilli and gram-positive cocci (point mutations in *gyr A, gyr B, par C,* and *par E*), to name a few (table 2) [10]. The use of isoelectric focusing to define the complement of β-lactamase enzymes produced by an organism, followed by PCR and sequencing of the amplified product, has been useful in identifying new β-lactamases and in detecting the transmission of resistance genes among different strains and species [19, 20].

Likewise, characterization of the quinolone-resistance-determining region (QRDR) of gram-negative and gram-positive bacteria may be accomplished by amplification of the *gyr A, gyr B, par C,* and *par E* genes by PCR, followed by sequencing of the amplicons to detect point mutations [21–23]. Because fluoroquinolone resistance arises in a stepwise manner dependent upon the number of QRDR mutations, molecular characterization of the QRDR may be useful, not only to confirm the mechanism of high-level fluoroquinolone resistance but also to determine the frequency of first-step mutations, which may not produce overt resistance but will create a population of organisms that will rapidly develop high-level resistance once a second mutation is acquired [21].

Antimicrobial resistance genotyping is used selectively in the SENTRY program to further characterize groups of organisms

with specific resistance phenotypes and to identify subpopulations of apparently susceptible organisms that may be at risk of becoming highly resistant (e.g., first-step QRDR mutants). In addition, the combination of resistance genotyping with DNA fingerprinting has proved to be a powerful means of characterizing the epidemiology of antimicrobial resistance among nosocomial and community-acquired pathogens [21, 22, 24–27].

APPLICATION OF MOLECULAR METHODS IN A GLOBAL SURVEILLANCE SYSTEM

Rationale. In any surveillance program the phenotypic characterization of microorganisms and identification of clusters of certain species and resistance phenotypes is the primary role of the microbiology laboratory. In this way, the surveillance laboratory serves as an "early warning system," alerting the submitting institution of a potential problem with resistant organisms in the patient population [28, 29]. For most of the existing antimicrobial resistance surveillance programs, whether they are local, regional, national, or international in scope, the process does not go much beyond phenotypic characterization and reporting. However, to be of maximum service to individual hospitals, regions, or countries, the surveillance laboratory must go one step further and provide a rapid assessment of microbial clonality by molecular typing [11, 29, 30].

The rapid emergence and dissemination of drug resistance among bacteria has raised the call for control of these pathogens to be a strategic priority for hospitals on a global scale [31], and determination of clonality within a phenotypically identical cluster may have a direct impact on the method of intervention [16, 30]. If the clustered isolates are distinct genotypically, then the clustering may be due to chance alone, to grouping of several highly susceptible patients together, or to excessive drug pressure resulting in the selection of a resistant phenotype within a group of unrelated isolates. Clonal dissemination of a resistant strain illustrates the need for more extensive investigation to identify the mechanism of spread and for renewed attention to infection control efforts.

The identification of an endemic resistance problem that is due to the occurrence of multiple small clusters of organisms requires a composite approach of antimicrobial restriction and focused use of barrier infection-control precautions [30]. Additional determination of the resistance genotype provides insight into the mechanism of resistance and may be necessary to understand whether a resistance problem is due to the transmission of resistance genes from organism to organism (control by antimicrobial restriction) or transmission of a single resistant strain from person to person (control by infection-control precautions). Identification of a subpopulation of organisms that are first-step mutants in a multistep pathway leading to high-level resistance (e.g., fluoroquinolone resistance) may trigger strategies that could both prevent the development of high-level resistance and improve therapy overall [21].

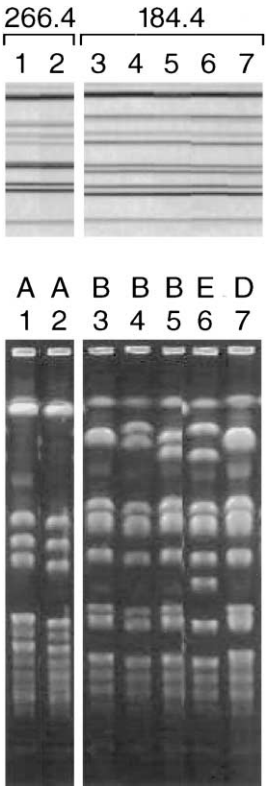

Figure 1. Ribotype and pulsed-field gel electrophoresis (PFGE) profiles of 7 methicillin-resistant *Staphylococcus aureus* isolates. The 2 isolates with ribotype 266-4 demonstrate indistinguishable PFGE profiles (*A*). The 5 isolates encompassed by ribotype designation 184-4 represent 3 different strains by PFGE analysis: *B* (3 isolates), *E* (1 isolate), and *D* (1 isolate). This example demonstrates the usefulness of employing both molecular typing methods.

On a broader scale, identification of resistant clones with extensive geographic distribution may provide insight into strain virulence and pathogenesis and also may result in public health interventions such as vaccination and antimicrobial restrictions aimed at reducing the spread of the pathogen and the resistance problem [13, 14, 32, 33].

All of the above considerations provide the rationale for the comprehensive molecular typing program that is integral to the SENTRY Antimicrobial Surveillance Program. Each year hundreds of organisms are molecularly characterized (tables 4–6), the information is reported to the individual participating centers, and the aggregated data are used to help describe the epidemiology of resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) [13, 14], vancomycin-resistant enterococci [34], and ESBL-producing gram-negative bacilli [15].

Process for selecting organisms for molecular characterization in the SENTRY program. Each year (1997–1999)

Table 8. Molecular characterization of 6 *Escherichia coli* and 13 *Klebsiella pneumoniae* strains having ESBL enzymes isolated from BSIs and UTIs at medical centers in 5 Latin American countries.

Species, medical center	Country	Infection	Molecular typing		IEF results
			Ribotype	PFGE pattern	
E. coli					
040	Argentina	BSI	252-1	ND	5.4, 7.0, 7.6
042	Chile	BSI	241-4	ND	5.4, 7.0
046	Brazil	BSI	241-4	M	5.4, 8.0
048	Brazil	BSI	182-2	G	5.4, 7.0, 7.6
048	Brazil	BSI	182-2	G	5.4, 7.0, 8.2
048	Brazil	BSI	182-2	G	5.4, 7.0, 8.2
K. pneumoniae					
039	Argentina	BSI	614-1	G	5.4, 6.8, 7.6
039	Argentina	BSI	614-1	G	5.4, 6.8, 7.6
039	Argentina	BSI	621-1	C	5.4, 6.5, 6.8, 7.6, 8.0
039	Argentina	BSI	621-1	C	5.4, 6.5, 6.8, 7.6, 8.0
041	Brazil	BSI	204-1	G	5.4, 7.0, 7.6
041	Brazil	BSI	204-1	G	5.4, 7.0, 8.2
041	Brazil	BSI	204-1	G	7.6, 8.2
041	Brazil	UTI	204-1	B	5.4, 7.6, 8.2
041	Brazil	UTI	622-3	E	ND
044	Colombia	BSI	746-3	ND	5.4, 7.6, 8.2
044	Colombia	BSI	746-6	ND	5.4, 7.6, 8.2
044	Colombia	BSI	324-2	ND	7.0, 8.2
045	Mexico	BSI	756-7	ND	5.4, 7.0

NOTE. BSI, bloodstream infection; ESBL, extended-spectrum β-lactamase; IEF, isoelectric focus; ND, not done; UTI, urinary tract infection. Table is adapted from [25–27].

~30,000 bacterial isolates are identified and tested for susceptibility and resistance to up to 70 different antimicrobial agents by the SENTRY coordinating laboratories in Iowa City, Iowa, and Adelaide, Australia. The antimicrobial susceptibility test (AST) data and identification data for each organism are reviewed on a weekly basis by the laboratory directors. When unusual resistance profiles and identifications that are inconsistent with the AST patterns are detected, the AST and identification are repeated to ensure a high degree of accuracy.

The phenotypic data are grouped according to the submitting center, and demographic data (age, sex, hospital location, date of admission, date of infection, underlying disease) are examined concurrently with the AST and identification information. Multidrug-resistant (MDR) pathogens are defined as those organisms that are resistant to at least 4 different classes of antimicrobial agents. Other resistance phenotypes that are of interest in the review process include potential ESBL-producing Enterobacteriaceae, fluoroquinolone-resistant (FQR) strains of gram-negative bacilli and *Streptococcus pneumoniae,* MRSA, and vancomycin-resistant enterococci. As the data are reviewed, clusters of 2 or more isolates with a given resistance phenotype that are also linked in time and space (hospital location) are flagged for immediate confirmation of AST profile and identification and for DNA fingerprinting.

The high throughput offered by the automated RiboPrinter allows the generation of ribotype profiles for most organisms within 1 day. The profile for each organism is matched against all profiles in the RiboPrinter database, and each organism is assigned to a ribogroup according to standardized criteria [18]. The RiboPrinter data are then reviewed, and if PFGE is required for further discrimination, the organism is scheduled for PFGE analysis. Once all work has been completed, the data are entered into the SENTRY database and a report is generated and sent to the submitting center. This process has been highly successful in identifying clonal spread within clusters of MDR pathogens (tables 4–6). Characterization of organisms with respect to antimicrobial resistance genotype is usually performed later as strains accumulate.

Hierarchical typing. The goals of molecular typing in a surveillance program are often multiple. On the one hand, there is a need to use a library typing system to monitor geographic spread and prevalence shifts of epidemic and endemic clones

Table 9. Epidemic cluster of extended-spectrum β-lactamase–producing strains of *Proteus mirabilis* causing pneumonia and bloodstream infection at a single medical center in 1998.

Organism no.	Date of culture	Molecular typing	
		Ribotype	PFGE pattern
1	Apr 8	637-8[a]	C
2	Apr 8	637-8	Z
3	Apr 30	637-8	Z
4	May 15	637-8	Z
5	Jun 16	637-8	Z
6	Oct 16	637-8	Z
7	Oct 16	637-8	Z
8	Oct 19	637-8	Z
9	Sep 18	1019-4[b]	W
10	Oct 19	1019-4	W
11	Nov 5	1019-4	W

NOTE. The isoelectric focus results for each organism were 5.4, 7.6, and 7.9. PFGE, pulsed-field gel electrophoresis. Table is adapted from [19].

[a] Six strains from bloodstream infections at the same medical center were observed with this same ribotype (637-8).

[b] One strain from a bloodstream infection at the same medical center was observed with this same ribotype (1019-4).

over time [13, 14]. On the other hand, a comparative typing method is useful to study smaller clusters of infection in greater detail and with greater discriminatory power [18]. Finally, when isolates with unusual or unique resistance phenotypes are detected, it is necessary to determine the resistance genotype. Thus, the flow of work in a large surveillance project such as SENTRY proceeds in a hierarchical fashion, from the detailed review of phenotypic data to the use of a library system such as ribotyping, in order to provide a secondary level of discrimination, followed by the use of a comparative typing system such as PFGE to further delineate strains within a cluster sharing the same phenotypic characteristics and ribotype. Resistance genotyping may be employed at any stage along this continuum to provide further information regarding resistance mechanisms.

CLUSTERS OF RESISTANT ORGANISMS IN SENTRY

In a review of the number of MDR clusters of organisms identified and molecularly characterized for SENTRY objective A (bloodstream infections), objective C (pneumonia in hospitalized patients), and objective D (skin and soft-tissue infections), it is clear that *S. aureus* is both the most prominent pathogen in terms of total numbers of infections and an important nosocomial pathogen with a high degree of nosocomial transmission (tables 4–6). Between 50% and 92% of MDR-MRSA clusters identified by phenotypic screening (same antibiogram, isolated in same hospital during the same time frame) contained evidence of clonal spread, based on a combination of ribotype and PFGE profiles (tables 4–6).

Further analysis of MDR-MRSA isolates from bloodstream infections has demonstrated a very broad spread of certain clones, not only within a hospital or city but among geographic regions (continents) as well [13, 14] (table 7; figure 1). For example, the same MDR-MRSA clone (defined by ribotype and PFGE profiles) has been observed in Brazil, Italy, and Portugal (table 7). In individual institutions within these geographic areas, local spread within hospitals was also observed [13]. A number of additional examples of MDR-MRSA spread are delineated by Diekema et al. [13, 14], and all serve to underscore the importance of *S. aureus,* especially MDR-MRSA, as a global pathogen. Persistent, comprehensive surveillance systems such as SENTRY provide the opportunity to trace not only the MDR phenotype but the major MDR genotypes as well [13].

Real-time analysis and reporting of these strains to individual hospitals is an additional service of the SENTRY program and may aid in the control of this important global pathogen.

Among the Enterobacteriaceae collected in the SENTRY program, *Escherichia coli* and *Klebsiella pneumoniae* are the most common MDR bloodstream infection pathogens causing clusters of infections (tables 4–6); ~50% of the recognized clusters represent clonal spread. The instances of clonal spread are due almost entirely to ESBL-producing strains, and most often the infections originate in the urinary tract, bloodstream, or respiratory tract [25–27] (table 8). In most instances, clonal spread of ESBL-producing strains is confined to a single institution or to 1 city, but interinstitutional spread within a country or region has also been observed. Notably, the spectrum of β-lactamase enzymes identified by isoelectric focusing within each cluster

Table 10. Molecular evaluation of vancomycin-resistant *Enterococcus faecium* producing urinary tract infections in 3 monitored medical centers.

Medical center	Molecular typing	
	Ribotype	PFGE pattern
014	702-3	A
014	702-3	A
014	274-1	B
017	187-4	C
017	187-4	D
017	187-4	E
008	187-4	F
008	187-4	G

NOTE. PFGE, pulsed-field gel electrophoresis. Table is adapted from [34].

Table 11. Molecular epidemiological analysis of multiply resistant isolates of *Acinetobacter* species (10 isolates), *Pseudomonas aeruginosa* (15 isolates), and *Stenotrophomonas maltophilia* (6 isolates) from patients hospitalized with pneumonia at 4 medical centers in 3 different countries.

Organism, medical center	Country	Specimen type	Patient in ICU	Molecular typing	
				Ribotype	PFGE pattern
Acinetobacter species					
039	Argentina	SP	Yes	815-2	Z
039	Argentina	SP	Yes	815-2	Z
039	Argentina	IP	Yes	815-2	Z
039	Argentina	IP	Yes	815-2	Z
039	Argentina	SP	Yes	815-2	Z
048	Brazil	SP	Yes	693-4	ND
048	Brazil	SP	Yes	693-4	Y
048	Brazil	SP	Yes	693-4	Y
048	Brazil	SP	Yes	693-4	Y
048	Brazil	SP	Yes	1008-2	ND
P. aeruginosa					
039	Argentina	SP	Yes	1033-2	ND
041	Brazil	IP	No	1033-2	H
041	Brazil	IP	No	1033-3	H
041	Brazil	IP	No	1033-3	H
041	Brazil	IP	No	1034-2	ND
044	Colombia	IP	Yes	1003-2	ND
048	Brazil	SP	Yes	1004-2	ND
048	Brazil	SP	Yes	1005-7	ND
048	Brazil	IP	Yes	1005-1	G
048	Brazil	SP	Yes	1005-1	G
048	Brazil	SP	Yes	1005-1	G
048	Brazil	SP	Yes	1004-2	ND
048	Brazil	SP	Yes	559-4	G
048	Brazil	SP	Yes	559-4	G
048	Brazil	SP	Yes	559-4	G
S. maltophilia					
041	Brazil	IP	No	1016-7	ND
041	Brazil	IP	No	191-6	ND
041	Brazil	IP	Yes	191-6	ND
041	Brazil	IP	NA	1019-5	ND
041	Brazil	IP	Yes	1019-7	ND
041	Brazil	IP	No	280-8	ND

NOTE. ICU, intensive care unit; IP, invasive pulmonary sample (bronchoalveolar lavage, protected brush, or tracheal aspiration specimen); NA, not available; ND, not done; SP, sputum. Table is adapted from [19].

is similar but may show some geographic variation, even within a single clone (table 8).

Clusters of ESBL-producing Enterobacteriaceae are not limited to *E. coli* and *K. pneumoniae* [19]. An extensive cluster of respiratory tract and bloodstream infections due to a single strain of ESBL-producing *Proteus mirabilis* was identified in a hospital in Argentina as a result of SENTRY surveillance efforts (table 9). Such ongoing efforts are exactly the role that a global surveillance program should play in assisting and augmenting local infection-control efforts.

It is interesting that vancomycin-resistant enterococci have not proved to be much of a problem with respect to clonal

Table 12. Mutations in the quinolone-resistance-determining region of the *gyr A* gene among ciprofloxacin-susceptible *Escherichia coli* strains isolated from patients with urinary tract infections in Latin America.

Nation	MIC of ciprofloxacin, μg/mL[a]	*gyr A* position	Amino acid substitution
Brazil	≤0.015	NA	None
Argentina	≤0.015	NA	None
Argentina	≤0.15	NA	None
Brazil	0.12	87	Asp→Gly
Chile	0.12	83	Ser→Leu
Chile	0.12	87	Asp→Gly
Brazil	0.25	87	Asp→Asn
Brazil	0.25	83	Ser→Leu
Colombia	0.25	83	Ser→Leu
Brazil	0.5	83	Ser→Leu
Mexico	0.5	83	Ser→Leu
Mexico	0.5	83	Ser→Leu

NOTE. Susceptibility breakpoints determined by National Committee for Clinical Laboratory Standards criteria (≤1 μg/mL). Asn, asparagine; Asp, aspartic acid; Gly, glycine; Leu, leucine; NA, not applicable; Ser, serine. Table is adapted from [21].

[a] MICs for 12 isolates selected from a total of 203 isolates susceptible to ciprofloxacin.

spread within the SENTRY program. Only 2 instances of probable clonal spread in 2 different institutions were recognized among isolates from bloodstream infections (table 4). Additional clonal and sporadic spread of vancomycin-resistant enterococci among patients with urinary tract infections have also been noted [34] (table 10).

A particularly diverse and highly resistant group of gram-negative nosocomial pathogens includes the nonenteric gram-negative bacilli, *Pseudomonas aeruginosa, Acinetobacter* species, and *Stenotrophomonas maltophilia.* These organisms cause primarily bloodstream and respiratory tract infections and are often resistant to virtually every class of antimicrobial agents [17, 36]. Clusters of MDR strains of *P. aeruginosa* causing bloodstream infection, pneumonia, and urinary tract infection have been recognized in the SENTRY system [17, 26, 34] (tables 4–6 and 11). These organisms are often resistant to carbapenems as well as β-lactams and aminoglycosides, and transmission of epidemic strains within individual medical centers has been well documented [19] (table 11).

Similar patterns of infection with MDR clusters of *Acinetobacter* species and *S. maltophilia* have been noted, with clonal spread—especially of *Acinetobacter* species—documented in patients with pneumonia and urinary tract infection [19, 34, 35] (table 11). These organisms are ubiquitous in the hospital environment, and with the advent of more effective gram-positive spectrum agents [37], they must be kept under careful scrutiny, as they constitute a major infectious threat without adequate current antimicrobial coverage [17, 36].

An additional means of providing molecular characterization of resistant organisms in a surveillance program is by defining the mechanism of resistance [21–23, 38]. In the SENTRY program, in addition to providing phenotypic evidence of ESBL production, the entire complement of β-lactamase enzymes possessed by a given organism is determined with use of isoelectric focusing [15] (tables 8 and 9). We have found that in most instances ESBL-producing strains of Enterobacteriaceae have at least 4 different β-lactamases [15] and that most but not all of the time the β-lactamase profile is consistent with the DNA fingerprint profile for a given cluster of ESBL-producing gram-negative bacilli (tables 8 and 9). In some instances differences in the β-lactamase profile may provide additional insights into the molecular epidemiology of the MDR cluster (table 8).

Likewise, although it is not difficult to identify phenotypically strains of organisms with high-level resistance to fluoroquinolones, for unusual species such as *Moraxella catarrhalis* and *Haemophilus influenzae* it is quite useful to characterize the mutations in the QRDR that are responsible for resistance in these organisms [23, 38]. Similarly, as part of a comprehensive surveillance program, molecular characterization of first-stage QRDR mutants may provide useful information (not readily apparent on the basis of phenotypic methods) that allows one to track the progress of first-stage and second-stage QRDR mutants throughout specific microbial populations [21] (table 12). In the SENTRY program, we have done this for both *E. coli* and *S. pneumoniae* (tables 12 and 13). The results are of great concern and indicate rather extensive subpopulations of apparently susceptible organisms (by phenotypic methods) that may rapidly move into the resistant category, given the acquisition of a second mutation [21].

Table 13. Molecular characterization of fluoroquinolone-susceptible *Streptococcus pneumoniae.*

	No. of strains with indicated trovafloxacin MIC, μg/mL			
Years	0.12	0.25	0.5	1
Strains tested				
1994/95	37	12	1	0
1997/98	32	15	2	1
	No. with mutations in			
	par C	*par E*	*gyr A*	*par C/gyr A*
Results				
1994/95	1	0	0	0
1997/98[a]	19	0	1	1

[a] Eighteen of 21 strains from 3 of 34 institutions.

SUMMARY AND CONCLUSIONS

This overview demonstrates the very important role of molecular characterization of organisms obtained during the course of an antimicrobial resistance surveillance program. In order to provide a comprehensive molecular characterization service, the organisms themselves must be readily available. This illustrates the need for a central reference laboratory to serve as a repository of organisms as well as a standardized source for all microbial characterizations. We believe that the ability to have organisms characterized both phenotypically and genotypically provides levels of standardization, quality control, flexibility, and responsiveness that are unequaled by any other surveillance program design. The ability to provide molecular epidemiology services to individual submitting centers is a significant "value-added" aspect of the SENTRY program.

Likewise, the ability to utilize various methods to follow the geographic spread of specific clones provides new information and offers additional opportunities for research that may clarify issues of pathogenesis and epidemiology. Finally, application of molecular testing to define resistance mechanisms among the different strains of bacteria may be useful in the evaluation of resistance phenotypes, in the design of alternative agents, and in predicting the emergence of groups of organisms with the potential of high-level resistance to an entire class of antimicrobial agents. Perhaps with the use of such information to alter prescribing practices, the progression to high-level resistance may be prevented, thus saving an entire class of highly effective antimicrobial agents.

References

1. Jones RN, Pfaller MA. Bacterial resistance: a worldwide problem. Diagn Microbiol Infect Dis **1998**; 31:379–88.
2. Jarvis WR, Edwards JR, Culver DH, et al. Nosocomial infection rates in adult and pediatric intensive care units in the United States. Am J Med **1991**; 91(Suppl 3B):185S–91S.
3. Edmond MB, Wallace SE, McClish DK, Pfaller MA, Jones RN, Wenzel RP. Nosocomial bloodstream infections in United States hospitals: a 3-year analysis. Clin Infect Dis **1999**; 29:239–44.
4. Fridkin SK, Steward CD, Edwards JR, et al. Surveillance of antimicrobial use and antimicrobial resistance in United States hospitals: Project ICARE, phase 2. Clin Infect Dis **1999**; 29:245–52.
5. Diekema DJ, Pfaller MA, Jones RN, et al. Survey of blood stream infections due to gram-negative bacilli: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, and Latin America for the SENTRY Antimicrobial Surveillance Program, 1997. Clin Infect Dis **1999**; 29:595–607.
6. Sahm DF, Marsilio MK, Piazza G. Antimicrobial resistance in key bloodstream bacterial isolates: electronic surveillance with The Surveillance Network database—United States. Clin Infect Dis **1999**; 29: 259–63.
7. Felmingham D, Gruneberg RN, the Alexander Project Group. The Alexander Project 1996–1997: latest susceptibility data from this international study of bacterial pathogens from community-acquired lower respiratory tract infections. J Antimicrob Chemother **2000**; 45: 191–203.
8. Bergeron MG, Ouellette M. Preventing antibiotic resistance using rapid DNA-based diagnostic tests. Infect Control Hosp Epidemiol **1998**; 19: 560–4.
9. Pfaller MA. Molecular approaches to diagnosing and managing infectious diseases: practicality and costs. Emerg Infect Dis **2001**; 7:312–8.
10. Cockerill FR III. Genetic methods for assessing antimicrobial resistance. Antimicrob Agents Chemother **1999**; 43:199–212.
11. Pfaller MA. Molecular epidemiology in the care of patients. Arch Pathol Lab Med **1999**; 123:1007–10.
12. Struelens MJ, DeGheldre Y, Deplano A. Comparative and library epidemiological typing systems: outbreak investigations versus surveillance systems. Infect Control Hosp Epidemiol **1998**; 19:565–9.
13. Diekema DJ, Pfaller MA, Turnidge J, et al. Genetic relatedness of multidrug-resistant, methicillin (oxacillin)-resistant *Staphylococcus aureus* bloodstream isolates from SENTRY Antimicrobial Resistance Surveillance Centers worldwide, 1998. SENTRY Participants Group. Microb Drug Resist **2000**; 6:213–21.
14. Diekema DJ, Pfaller MA, Schmitz FJ, et al. Survey of infections due to *Staphylococcus* species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific for the SENTRY Antimicrobial Surveillance Program, 1997–1999. Clin Infect Dis **2001**; 32(Suppl 2):S114–32 (in this issue).
15. Winokur PL, Canton R, Casellas J-M, Legakis N. Variations in the prevalence of strains expressing an extended-spectrum β-lactamase phenotype and characterization of isolates from Europe, the Americas, and the Western Pacific. Clin Infect Dis **2001**; 32(Suppl 2):S94–103 (in this issue).
16. Back NA, Linnemann CC, Pfaller MA, Staneck JL, Morthland V. Recurrent epidemics caused by a single strain of erythromycin-resistant *Staphylococcus aureus:* the importance of molecular epidemiology. JAMA **1993**; 270:1329–33.
17. Gales AC, Jones RN, Turnidge J, Rennie R, Ramphal R. Characterization of *Pseudomonas aeruginosa* isolates: occurrence rates, antimicrobial susceptibility patterns, and molecular typing in the SENTRY Antimicrobial Surveillance Program, 1997–1999. Clin Infect Dis **2001**; 32(Suppl 2):S146–55 (in this issue).
18. Hollis RJ, Bruce JL, Fritschel SJ, Pfaller MA. Comparative evaluation of an automated ribotyping instrument versus pulsed-field gel electrophoresis for epidemiological investigation of clinical isolates of bacteria. Diagn Microbiol Infect Dis **1999**; 34:263–8.
19. Lewis MT, Gales AC, Sader HS, Pfaller MA, Jones RN. Frequency of occurrence and antimicrobial susceptibility patterns for pathogens isolated from Latin American patients with a diagnosis of pneumonia: results from the SENTRY Antimicrobial Surveillance Program (1998). Diagn Microbiol Infect Dis **2000**; 37:63–74.
20. Neuwirth C, Siebor E, Lopez J, Pechinot A, Kazmierczak A. Outbreak of TEM-24-producing *Enterobacter aerogenes* in an intensive care unit and dissemination of the extended spectrum β-lactamase to other members of the family Enterobacteriaceae. J Clin Microbiol **1996**; 34: 76–9.
21. Gales AC, Gordon KA, Wilke WW, Pfaller MA, Jones RN. Occurrence of single-point *gyr A* mutations among ciprofloxacin-susceptible *Escherichia coli* isolates causing urinary tract infections in Latin America. Diagn Microbiol Infect Dis **2000**; 36:61–4.
22. Jones RN, Pfaller MA, Doern GV. Comparative antimicrobial activity of trovafloxacin tested against 3049 *Streptococcus pneumoniae* isolates from the 1997–1998 respiratory infection season. Diagn Microbiol Infect Dis **1998**; 32:119–26.
23. Biedenbach DJ, Jones RN. Fluoroquinolone-resistant *Haemophilus influenzae:* frequency of occurrence and analysis of confirmed strains in the SENTRY Antimicrobial Surveillance Program (North and Latin America). Diagn Microbiol Infect Dis **2000**; 36:255–9.
24. Gales AC, Jones RN, Pfaller MA, Gordon KA, Sader HS. Two-year assessment of the pathogen frequency and antimicrobial resistance patterns among organisms isolated from skin and soft tissue infections in

Latin American hospitals: results from the SENTRY Antimicrobial Surveillance Program, 1997–98. SENTRY Study Group. Emerg Infect Dis **2000**; 4:75–84.
25. Sader HS, Jones RN, Gales AC, et al. Antimicrobial susceptibility patterns for pathogens isolated from patients in Latin American Medical Centers with a diagnosis of pneumonia: analysis of results from the SENTRY Antimicrobial Surveillance Program (1997). Diagn Microbiol Infect Dis **1998**; 32:289–301.
26. Sader HS, Jones RN, Winokur PL, et al. Antimicrobial susceptibility of bacteria causing urinary tract infections in Latin American hospitals: results from the SENTRY Antimicrobial Surveillance Program (1997). Clin Microbiol Infect **1999**; 5:478–87.
27. Sader HS, Pfaller MA, Jones RN, et al. Bacterial pathogens isolated from patients with blood stream infections in Latin America, 1997: frequency of occurrence and antimicrobial susceptibility patterns from the SENTRY Antimicrobial Surveillance Program. Braz J Infect Dis **1999**; 3:97–110.
28. McGowan JE Jr, Metchock BG. Basic microbiologic support for hospital epidemiology. Infect Control Hosp Epidemiol **1996**; 17:298–303.
29. Pfaller MA, Herwaldt LA. The clinical microbiology laboratory and infection control: emerging pathogens, antimicrobial resistance and new technology. Clin Infect Dis **1997**; 25:858–70.
30. Hacek DM, Suriano T, Noskin GA, Kruszynski J, Reisberg B, Peterson LR. Medical and economic benefit of a comprehensive infection control program that includes routine determination of microbial clonality. Am J Clin Pathol **1999**; 111:647–54.
31. Goldmann DA, Haskins WC. Control of nosocomial antimicrobial-resistant bacteria: a strategic priority for hospitals worldwide. Clin Infect Dis **1997**; 24(Suppl 1):S139–45.
32. Chen DK, McGeer A, de Azavedo JL, Low DE. Decreased susceptibility of *Streptococcus pneumoniae* to fluoroquinolones in Canada. N Engl J Med **1999**; 341:233–9.
33. Monnet DL, Biddle JW, Edwards JR, et al. Evidence of interhospital transmission of extended-spectrum β-lactam resistant *Klebsiella pneumoniae* in the United States, 1986 to 1993. The National Nosocomial Infection Surveillance System. Infect Control Hosp Epidemiol **1997**; 18:492–8.
34. Jones RN, Kugler KC, Pfaller MA, Winokur PL, The SENTRY Surveillance Group, North America. Characteristics of pathogens causing urinary tract infections in hospitals in North America: results from the SENTRY Antimicrobial Surveillance Program, 1997. Diagn Microbiol Infect Dis **1999**; 35:55–63.
35. Sader HS, Sampaio JLM, Zoccoli L, Jones RN. Results of the 1997 SENTRY Antimicrobial Surveillance Program in three Brazilian medical centers. Braz J Infect Dis **1999**; 3:63–79.
36. Gales AC, Jones RN, Forward KR, Liñares J, Sader HS, Verhoef J. Emerging importance of multidrug-resistant *Acinetobacter* species and *Stenotrophomonas maltophilia* as pathogens in seriously ill patients: geographic patterns, epidemiological features, and trends in the SENTRY Antimicrobial Surveillance Program (1997–1999). Clin Infect Dis **2001**; 32(Suppl 2):S104–13 (in this issue).
37. Jones RN, Low DE, Pfaller MA. Epidemiologic trends in nosocomial and community-acquired infections due to antibiotic-resistant gram-positive bacteria: the role of streptogramins and other new compounds. Diagn Microbiol Infect Dis **1999**; 33:101–12.
38. DiPersio JR, Jones RN, Barrett T, Doern GV, Pfaller MA. Fluoroquinolone-resistant *Moraxella catarrhalis* in a patient with pneumonia: report from the SENTRY Antimicrobial Surveillance Program (1998). Diagn Microbiol Infect Dis **1998**; 32:131–5.