Real-time Molecular Epidemiologic Analysis of an Outbreak of *Streptococcus pyogenes* Invasive Disease in US Air Force Trainees

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• ***Objective.*—To determine if molecular epidemiologic techniques, including comparative automated DNA sequencing of polymorphic virulence genes, could be used in the course of a bacterial disease outbreak to unambiguously determine clonal relationships among implicated strains.**

***Design.*—Strains recovered from all patients with invasive infections and a sample of carriers were analyzed by multilocus enzyme electrophoresis and automated DNA sequencing of a gene encoding an extracellular protease and a highly polymorphic part of the streptokinase gene.**

***Setting.*—A US Air Force training facility in San Antonio, Tex.**

***Patients.*—A squadron with about 800 Air Force trainees, including three recruits with invasive *Streptococcus pyogenes* infections.**

***Results.*—Multilocus enzyme electrophoresis and automated DNA sequencing of polymorphic virulence genes unambiguously defined person-to-person spread of an otherwise rare *S pyogenes* clone in the course of the disease outbreak and clarified strain relationships in real time.**

***Conclusions.*—Molecular strain characterization techniques can be employed rapidly in a disease outbreak to definitively resolve complex relationships among pathogenic bacteria, infer patterns of clone spread, and help formulate rational public health control measures. The approach has broad applicability to other infectious agents.**

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Application of molecular typing techniques to the study of microbial pathogens has provided significant new insights about the epidemiology of many infectious agents. Several new typing approaches have been formulated in recent years, and those employed to investigate bacterial transmission patterns include plasmid and chromosomal restriction fragment length polymorphism profiling, multilocus enzyme electrophoresis, and, recently, arbitrarily primed polymerase chain reaction profiling and related techniques.[1] Although the strain-resolving power of these methods has enhanced the precision of fine-structure molecular epidemiologic studies, they have rarely been exploited to study an infection cluster in real time. We here report the use of multilocus enzyme electrophoresis and automated DNA sequencing of polymorphic virulence genes to unambiguously define person-to-person spread of an otherwise rare *Streptococcus pyogenes* (group A *Streptococcus*) clone causing episodes of invasive disease in military recruits. The molecular epidemiologic study clarified strain relationships in the course of the streptococcal outbreak, that is, in real time. The approach employed has broad applicability to other infectious agents.

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DESCRIPTION OF OUTBREAK

In early February 1993, a cluster of three invasive *S pyogenes* infections (including one fatal case of meningitis) occurred in a 6-day period in military trainees assigned to a single squadron (arbitrarily designated squadron A) at Lackland Air Force Base (LAFB), San Antonio, Tex.

REPORT OF CASES

Case 1

On February 2, 1993, a 20-year-old black man presented with fever, myalgias, and sinus congestion. He had a temperature of 40°C, appeared moderately ill, and was admitted for observation. Results of a physical examination were unremarkable, and chest and sinus roentgenograms were normal. Approximately 12 hours after admission, four of four blood culture bottles obtained on entry yielded gram-positive cocci in chains (identified the next day as *S pyogenes*), and intravenous aqueous penicillin G therapy was initiated. On the second hospital day, the patient complained of severe left thigh pain.

The examination was notable for a continued temperature of 40°C, and warmth and mild erythema over the medial aspect of the left thigh, accompanied by severe pain on passive and active movement of the left hip joint. Thorough evaluation for deep soft-tissue or joint involvement identified only a small left hip effusion, but the fluid quantity was insufficient to perform diagnostic arthrocentesis. The patient defervesced, and he was observed for the development of necrotizing fasciitis or progressive septic arthritis. Parenteral antimicrobial therapy for 4 weeks (4 weeks of penicillin and 2 weeks of gentamicin) resulted in resolution of the fever and hip pain.

Case 2

On February 4, 1993, a 22-year-old white man presented to the emergency department with a severe bitemporal headache accompanied by frontal throbbing, visual scotoma, and hand paresthesias. The white blood cell count was 13.1×10^9/L, with 0.77 neutrophils. The cerebrospinal fluid was clear and colorless, with the following laboratory values: red blood cells, 9×10^6/L; white blood cells, 2×10^6/L (0.84 lymphocytes and 0.03 neutrophils); glucose, 3.5 mmol/L (63 mg/dL); and protein, 0.33 g/L. No organisms were seen by acridine orange or india ink staining. The patient was given a working diagnosis of migraine headache and treated with ergotamine.

Three days later (February 7), he returned to the emergency department with similar symptoms. His temperature was 41°C, he was alert and oriented, there was no nuchal rigidity or sinus tenderness, and the lungs were clear to auscultation. The peripheral white blood cell count was 14.8×10^9/L, with 0.87 neutrophils. Lumbar puncture revealed clear and colorless spinal fluid with the following laboratory values: red blood cells, 28×10^6/L; white blood cells, 6×10^6/L (0.32 neutrophils); glucose, 4.7 mmol/L (85 mg/dL); and protein, 0.37g/L. No organisms were seen after acridine orange staining. The patient was diagnosed with acute viral syndrome and migraine headache, admitted for observation, and blood cultures were obtained. Approximately 15 hours later, he was noted to be confused, and was oriented only to person and place. Physical examination identified nuchal rigidity but no skin lesions. Parenteral antimicrobial therapy was initiated immediately with aqueous penicillin G, ceftriaxone, and acyclovir. A repeated lumbar puncture showed cloudy fluid and disclosed the following laboratory values or findings: white blood cells, 15×10^6/L (0.76 neutrophils); glucose, 0.2 mmol/L (4 mg/dL); protein, 3.90 g/L; and gram-positive cocci in chains. Shortly thereafter apnea developed and he was intubated. A computed tomographic scan of the head showed obliteration of the lateral ventricles, minimal enhancement of the temporal lobe meninges, and no parenchymal or parameningeal abnormalities. The next day, a neurologic evaluation revealed absence of brain-stem function, and life support measures were withdrawn.

Streptococcus pyogenes grew from blood cultures obtained on admission, and cerebrospinal fluid specimens collected on February 8 when the patient's condition worsened, but not from cerebrospinal fluid cultures obtained on February 4, or on entry.

Case 3

On February 2, 1993, a 20-year-old white man suffered a thermal burn to his hand. He presented to the ambulatory troop clinic on February 4 with erythema and pain at the burn site, was diagnosed with cellulitis and treated with oral dicloxacillin. A subcutaneous abscess was aspirated and yielded *S pyogenes*. The patient became unavailable to follow up due to transfer to a new assignment after completion of training.

All US Air Force enlistees undergo 6 weeks of basic training at LAFB. Trainees are assigned to a squadron composed of about 800 individuals who are housed in a single building. The squadron is divided into units called flights, each with 40 to 60 members of the same gender. Members of the squadron share a dining hall, classrooms, laundry room, bathroom, and shower facilities, and sleep in two large open bays with single beds spaced 3 feet apart. Two flights share a common television room for recreation. Trainees arrive at LAFB daily from geographically diverse localities, and training begins when two flights (80 to 100 individuals) are assembled. Recruits are not given antimicrobial prophylaxis when they arrive at LAFB, or in the course of training.

Colonies of the *S pyogenes* isolates cultured from patients 1 and 3 were noted by clinical microbiology laboratory technologists to be unusually mucoid, and reexamination of the organisms recovered from patient 2 also identified the same mucoid colony phenotype. The patients were from three different flights (arbitrarily designated flight A, B, and C, for patients 1, 2, and 3, respectively) in squadron A.

Because it was not known if the three cases were related as a consequence of person-to-person transmission of a single clone or represented temporal clustering of unrelated infections, one of us (J.M.M.) was contacted on February 9 with a request for molecular strain typing to differentiate between these two possibilities. Single isolates from patients 1 and 3, the blood and cerebrospinal fluid isolates from patient 2, and a mucoid strain recovered in the outbreak period from the throat of a 54-year-old spouse of a retired military member (patient 4) with no known association with LAFB trainees were forwarded to the laboratory of one of us (J.M.M.).

A carrier study was conducted on February 9, 11, and 12 to determine the frequency of pharyngeal colonization with *S pyogenes* among personnel in the 17 flights of squadron A. A total of 823 trainees and instructors were cultured over a 4-day period. All individuals were given either 1.2 million U of intramuscular benzathine penicillin G or oral erythromycin, 250 mg twice daily for 30 days, at the time of culture.

METHODS

Microbiologic Procedures

Organisms were recovered and identified as *S pyogenes* by standard clinical microbiology laboratory procedures. Strains were subcultured to blood agar plates, sent by overnight courier to the laboratory of J.M.M., and received on February 11. On arrival, chromosomal DNA was prepared from each strain from the growth on one-half of each plate, and the remaining bacteria were used to inoculate a broth culture.

For analysis by multilocus enzyme electrophoresis[2,3] bacteria grown overnight in 150 mL of Todd-Hewitt broth supplemented with glucose were harvested by centrifugation, resuspended in 1.5 mL of 50 mmol/L of TRIS hydrochloride, 5 mmol/L of EDTA (pH, 8.0), and sonicated to release soluble metabolic enzymes. We examined electrophoretically demonstrable allelic variation at 12 metabolic enzyme loci by techniques described elsewhere.[2,3] This characterization method determines genetic relationships between strains by indexing polymorphisms in a sample of chromosomally encoded metabolic "housekeeping" enzymes. Isolates with identical allele profiles are classified as a clone and are considered to share lineal descent from a common precursor cell.[4]

Automated DNA Sequencing of Polymorphic Virulence Genes

Two polymorphic genes (*speB* and *ska*) were chosen for characterization by automated DNA sequencing (model 373A, Applied Biosystems Inc, Foster City, Calif). The *speB* gene[5] encodes streptococcal pyrogenic exotoxin B, an extracellular cysteine protease that cleaves interleukin-1 beta (IL-1β) precursor to form mature, biologically active IL-1β.[6] The *speB* DNA fragment studied (1437 base pairs [bp]) represents the entire coding region (1197 bp), and 160 bp of upstream and 80 bp of downstream sequence. Thirty-nine distinct alleles have been identified in *S pyogenes* based on sequence analysis of 67 strains from intercontinental sources.[7] In general, strains assigned to distinct multilocus enzyme genotypes have different *speB* alleles. The *ska* gene (1320 bp) encodes streptokinase, a

molecule that is thought to act as a virulence factor by enhancing bacterial spread.[8] This gene contains two variable regions, designated V1 and V2, that occur in a DNA segment of approximately 400 bp located in the middle of the coding region.[9] The nucleotide sequence of a 387-bp segment encompassing variable domain V1 (nucleotides 504 through 730) and V2 (nucleotides 809 through 882) was characterized.

Amplification of *speB* and contiguous noncoding regions was performed by the polymerase chain reaction[10] with the oligonucleotide primers 5×[mN]-GTTGTCAGTGTCAACTAACCGT-3×[mN] and 5×[mN]-ATCTGTGTCTGATGGATAGCTT-3×[mN]. Polymerase chain reaction amplification of a 513-bp segment that includes the two variable domains of *ska* was performed with the primers 5×[mN]-AACCTTGCCGACCCAACCTGT-3×[mN] and 5×[mN]-GTGAACAGTTTCAAGTGACTGCGAT-3×[mN]. Unincorporated nucleotides and oligonucleotide primers were removed by filtration through microconcentrators (Microcon 100, Amicon Inc, Beverly, Mass). Sequencing reactions with the *Taq* DyeDeoxy terminator cycle sequencing kit (Applied Biosystems Inc) were performed with PCR-amplified DNA as template and appropriate primers. The unincorporated dye terminators and primers were separated from the extension products by spin column purification (Centri-Sep, Princeton Separations Inc, Adelphia, NJ). Samples were dried in a vacuum centrifuge. Prior to gel loading, the samples were resuspended in loading buffer (5:1 deionized formamide: 50-mmol/L EDTA; pH, 8.0) and heat denatured for 2 minutes at 90°C. Both strands of the genes were sequenced.

The data were assembled and edited with EDITSEQ, ALIGN, and SEQMAN programs (DNASTAR, Madison, Wis), and the sequences were compared with *speB*[5,7] and *ska* alleles (manuscript in preparation) previously identified. Phylogenetic analysis of *ska* sequences was performed by parsimony with the computer program PAUP 3.0s.[11]

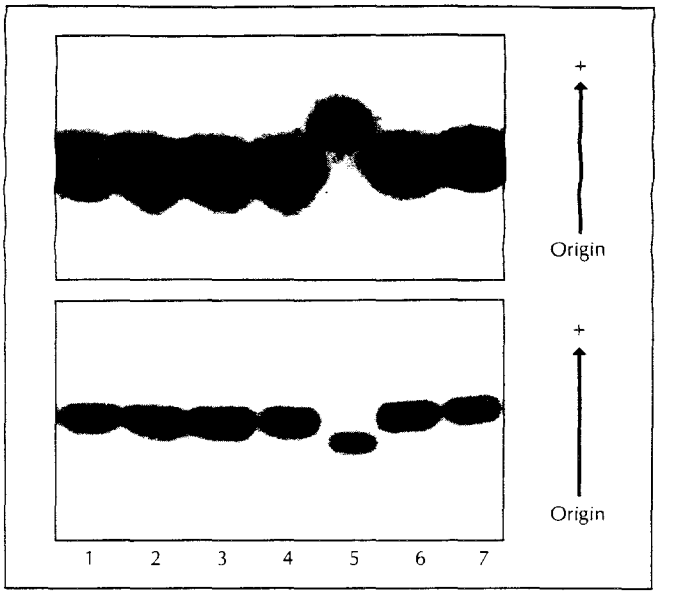

Fig 1.—*Starch gel electrophoresis and histochemical staining of two representative polymorphic enzymes from strains of* Streptococcus pyogenes. *Aqueous cytoplasmic extracts were prepared as described in the text and were electrophoresed in a TRIS citrate (pH, 8.0) buffer system. Enzyme activity was detected as described elsewhere.[2,3] Lanes 1 through 5 contained extracts from strains recovered from the following sources: lane 1, patient 1; lane 2, patient 2 (blood); lane 3, patient 2, (cerebrospinal fluid); lane 4, patient 3; lane 5, pharyngitis patient; lane 6, M75 type strain (MGAS 1911); lane 7, acute rheumatic fever patient isolate (MGAS 758 expressing opacity factor type 75). Top, leucylalanine peptidase; bottom, nucleoside phosphorylase.*

RESULTS

Initial Laboratory Investigations: Multilocus Enzyme Electrophoresis and Nucleotide Diversity in *speB* and *ska*

Four mucoid strains recovered in the invasive disease outbreak period from patients 1, 2, and 3, and a fifth mucoid organism cultured from the patient with pharyngitis were analyzed initially, one day after receipt of strains. Two multilocus enzyme genotypes were identified among the five strains (Fig 1). The four strains cultured from the three patients with invasive episodes were identical in allele profile at all 12 of the metabolic enzyme loci assayed for electrophoretic mobility. This multilocus enzyme genotype was arbitrarily designated electrophoretic type (ET) 59. In contrast, the organism grown from the individual with pharyngitis (patient 4) differed at five of the 12 loci examined, and was assigned to ET 68. We interpreted the occurrence of the identical allele profile in all invasive isolates as evidence that patients 1, 2, and 3, but not patient 4, had been infected by the same clone, referred to as the case clone. This information was reported to the LAFB medical team on February 13.

The four ET 59 strains recovered from the three individuals with invasive disease, and the ET 68 strain from the patient with pharyngitis, were also characterized for the nucleotide sequence of the 387-bp *ska* segment representing variable domain V1 and V2. The four ET 59 organisms were identical to one another in the *ska* gene fragment analyzed, but differed from ET 68 at 118 nucleotide sites, and were also distinct from all other *S pyogenes* analyzed in this study and examined previously in our laboratory (Musser et al[3] and unpublished data, 1993) (Fig 2).

Computer comparison of ET 59 with the multilocus enzyme genotypes of a sample of about 1500 strains previously analyzed in the laboratory of J.M.M., including strains representing all M protein serotypes and many strains not typeable for this antigen, identified a close genetic affinity with certain strains expressing M49 serotype. Importantly, we found that the multilocus enzyme genotype of the ET 59 case clone was identical to an organism (strain MGAS 758) recovered in 1986 from a patient in Utah with acute rheumatic fever, and identified as opacity factor type 75, and an M75 strain (MGAS 1911) used by the National Streptococcus Reference Laboratory, Colindale, United Kingdom, to make antisera against M75 protein.

To further substantiate the genetic relationship between the ET 59 case clone and strains MGAS 758 and MGAS 1911, a randomly selected ET 59 case organism (MGAS 1991), and the ET 68 strain MGAS 1990, were characterized for nucleotide variation at the *speB* locus. The *speB* allele in all three ET 59 organisms was identical and differed from the *speB* allele in the ET 68 strain at a total of six nucleotides (positions 483, 631, 765, 915, 1023, and 1116).[7] Strains MGAS 758, MGAS 1911, MGAS 1991, and MGAS 1990 were also studied for *ska* allele sequence. The *ska* segments in MGAS 758 and MGAS 1911 were identical to the sequence found in the ET 59 case clone strains, but were distinct from the *ska* allele in strain MGAS 1990.

In summary, based on data provided by multilocus enzyme electrophoresis and automated DNA sequencing of two polymorphic virulence genes, within 3 days

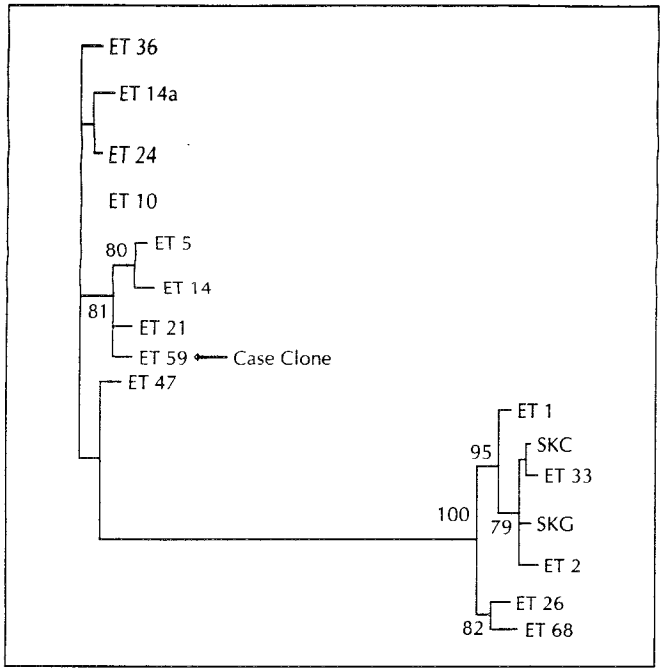

Fig 2.—*Phylogenetic tree indicating relationships among 16 alleles of the streptokinase gene based on nucleotide sequence polymorphism in a 387–base pair variable fragment. The tree was constructed with the computer program PAUP 3.0s.*[11] *The numbers adjacent to the nodes represent the proportion of 2000 replicate bootstrap trees that contained the same grouping to the right of the node. The bootstrap statistical method involves repeated sampling of the dataset and construction of a grove of bootstrap trees, followed by construction of a consensus tree. The frequency of occurrence of a group of operational taxonomic units, in this case* ska *alleles, as a single cluster (indicated to the right of the nodes) is interpreted to be the confidence level of the group. For additional information, see Felsenstein.*[28] *ET indicates electrophoretic type.*

we had generated irrefutable evidence that the three recruits with invasive disease episodes had been infected by the same virulent streptococcal clone, and we successfully differentiated the case clone organism from a temporally associated strain sharing the unusual mucoid phenotype.

Initial Characterization of Carrier Isolates and Carrier Clones

To determine the prevalence of ET 59 organisms and other *S pyogenes* clones in trainees and instructors of squadron A, 823 individuals were studied for oropharyngeal carriage. Sixteen of the 17 flights had at least one individual who carried *S pyogenes*, and the overall carriage rate was 6.9% (n=57 isolates). Six flights had only one carrier, four flights had two carriers, three flights had three carriers, and one flight each had 10, 11, and 13 carriers. Fourteen carrier strains had a mucoid phenotype; 11 of these organisms were cultured from recruits in a single flight (arbitrarily designated D), and the other three isolates were recovered from members of three flights other than flight A through D. Five nonmucoid *S pyogenes* recovered from 207 individuals were inadvertently discarded and were not available for analysis. Of the remaining 52 organisms cultured from 616 individuals, 14 had a mucoid phenotype characteristic of the ET 59 case clone. No instructor was an oropharyngeal carrier of *S pyogenes*.

The 14 mucoid strains and a random sample of 10 of the other 38 carriage organisms were analyzed by multilocus enzyme electrophoresis on February 25, 1 day after receipt. All 14 mucoid strains were ET 59. There were five distinct multilocus enzyme genotypes represented in the 10 nonmucoid strains, and none was ET 59.

Subsequent Laboratory Investigations: Multilocus Enzyme Electrophoretic Analysis of Additional Invasive and Carrier Isolates

On February 27, a fourth invasive *S pyogenes* isolate was recovered from a 60-year-old armed forces veteran living in San Antonio. The organism was cultured from blood and bullous lesions on the left shoulder. Because this strain also exhibited a mucoid phenotype, there was considerable concern that the virulent case clone had entered the civilian community. Molecular strain typing of these two organisms by multilocus enzyme electrophoresis was therefore conducted 1 day after receipt. The data showed that the strains had the same multilocus enzyme genotype, but were distinct from all other invasive or carrier strains (data not shown). The results ruled out the possibility that the patient was infected by the ET 59 case clone, and considerably reduced concern that the case clone had spread into the civilian community.

In the middle of March, the additional 28 nonmucoid strains recovered in the carrier study of squadron A were examined by multilocus enzyme electrophoresis. Of the total of 52 carrier isolates analyzed, 11 distinct genotypes were identified and included organisms assigned to case clone ET 59 (n=14 isolates), ET 24 (n=12 isolates), and ET 76 (n=13 isolates).[3,7] Surprisingly, none of the trainees carrying case clone ET 59 were in flights A, B, and C, with invasive episodes.

There was strong association of streptococcal clones and flights. For example, all 11 carriers in flight D were colonized with mucoid strains of ET 59, nine of 13 carriers in flight E had ET 24 organisms, and eight of 10 strains recovered from flight F were ET 76. The *S pyogenes* carriage rate in flights D, E, and F was about 20%.

Analysis of Carrier Isolates From New Recruits Assigned to Squadron A

To determine the prevalence of carriage of *S pyogenes* among trainees in a non–outbreak setting, we then cultured 366 new recruits assigned to eight flights in squadron A immediately on arrival at LAFB. *Streptococcus pyogenes* was recovered from 13 airmen, for an overall carriage rate of 3.6%. No flight had more than three trainees who carried *S pyogenes*. Analysis of the organisms by multilocus enzyme electrophoresis identified 11 distinct ETs. The sample had no ET 59 case clone organisms.

COMMENT

Our investigation demonstrates the feasibility of employing multilocus enzyme electrophoresis and automated DNA sequencing of polymorphic genes to delineate the basic molecular epidemiologic features of an outbreak of invasive *S pyogenes* disease in real time. The multilocus enzyme genotypes of the case strains were available 1 day after receipt of the organisms and 4 days after initial recognition of the disease cluster, and virtually all of the automated DNA sequencing data were generated by the end of the third day. Taken

together, the data were compelling and unambiguous evidence that the case isolates were related as a consequence of lineal descent from a common precursor cell, which indicates epidemiologic association and, hence, defines an outbreak.

As shown by our study, the availability in real time of highly discriminating and unambiguous microbial molecular epidemiologic data facilitates investigation of infection clusters and permits rapid decisions to be made to minimize additional health risk. The identification of a close genetic affinity between the outbreak clone and streptococcal phylogenetic lineages associated with production of serotypes M75 and M49 proteins was especially important. Serotype M49 strains are historically associated with outbreaks of poststreptococcal glomerulonephritis, frequently among patients with pyoderma in relatively closed populations.[12-14] Similarly, although M75-expressing organisms are rarely reported to cause disease, including pharyngitis,[15] a strain identified as opacity factor type 75 was recovered in 1986 from an individual with acute rheumatic fever in a widely publicized disease outbreak in Utah.[16,17] Based on the genetic relationships of strains identified by our molecular approach, we alerted all Air Force physicians to be especially aware of the possibility that episodes of acute rheumatic fever or nephritis may occur in personnel assigned to LAFB in February 1993. This strategy was necessary because trainees are dispersed to many different Air Force facilities after leaving LAFB.

One unexpected discovery was that none of the three flights with an invasive episode had individuals who carried the ET 59 case clone. Inasmuch as the 17 flights are composed of an apparently random sample of new recruits drawn from throughout the United States, and relatively little fraternization occurs between flights in training, we favor the following hypothesis to explain the data. Flight D contained an index carrier of ET 59 who spread the case clone to one or more individuals in his flight. The clone was transmitted throughout the flight by the index carrier and probably by other airmen he infected. The carriers in this flight then occasionally spread clone ET 59 to other recruits in squadron A. To account for the lack of ET 59 carriers among flights with cases, we believe the three individuals who developed invasive episodes became ill soon after acquisition of ET 59, sought medical attention relatively rapidly, and therefore had little opportunity to transmit the case clone to other members of their flights. We note that a recent study by Gray et al[18] examining the effectiveness of prophylaxis with penicillin G benzathine at a military camp also suggested that recruits colonized with *S pyogenes* served as a reservoir to disseminate bacteria to other trainees in the same training group.

Although a marked decrease in the incidence of *S pyogenes* disease and sequelae in military recruits occurred from 1965 to 1985,[19] several outbreaks of severe streptococcal disease among armed forces personnel have been described in recent years.[20-22] An outbreak of acute rheumatic fever and pneumonia among US Navy trainees in San Diego, Calif, occurred between December 1986 and July 1987 and resulted in reinstitution of mass antibiotic prophylaxis with penicillin G benzathine.[20] A rheumatic fever outbreak also was reported among Army trainees at Fort Leonard Wood, Mo, and affected at least 14 individuals between February 1987 and February 1988.[21] Mucoid *S pyogenes* strains expressing M3 and M18 serotype were implicated. Interestingly, an outbreak of *S pyogenes* pharyngitis also occurred among LAFB trainees in December 1988 and January 1989.[22] The outbreak resulted in the administration of penicillin prophylaxis to about 6000 trainees and was the first time in at least 15 years that mass antibiotic prophylaxis for basic trainees was implemented at the base. Strains from the pharyngitis outbreak were not typed and were not saved for later analysis.

There are situations in which the strain typing approach employed herein, or an analogous one using any of several rapid molecular genetic techniques[23,24] in conjunction with automated DNA sequencing of polymorphic genes, would be particularly advantageous compared with typing systems based on phenotypic traits that may be difficult or impossible to readily assay for variation or are unstable. For example, as shown here, the strategy can be used in any setting in which it is necessary to rapidly differentiate between involvement of a single clone or multiple clones in temporally clustered infections. Rapid identification of a single clone may trigger particular control strategies (eg, institution of antibiotic prophylaxis, recall of a consumer product, intensive contact tracing) that may not otherwise occur. This approach may be particularly relevant to bacterial outbreaks in which the public is at unusually severe risk, such as recent disease clusters in several western states associated with ingestion of fast food contaminated with *Escherichia coli*.[25] We note that this type of DNA sequence analysis costs on the order of $100 per strain, a sum that might be considered modest in circumstances that may represent a clear and present danger to society. In addition, if future investigations of microbial pathogens demonstrate significant differences in therapeutic outcome based on infection with certain strains, the molecular approach may be used to identify patients who would preferentially benefit from a particular treatment modality. Finally, the automated DNA sequencing strategy has potential application particularly in forensic medicine and medical jurisprudence settings involving microbial pathogens and for rapid identification of mutations generating resistant organisms such as *Mycobacterium tuberculosis*[26] or the human immunodeficiency virus.[27]

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