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National Department of Defense Surveillance Data for Antibiotic Resistance and *emm* Gene Types of Clinical Group A Streptococcal Isolates from Eight Basic Training Military Sites†

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Antibiotic resistance and *emm* gene types were examined from 692 Group A streptococci isolates from eight United States military basic training sites between 1998 and 2001. Macrolide resistance was associated with geographic sites and *emm* type. These data are useful for vaccine development initiatives and antimicrobial treatment considerations.

Group A streptococci (GAS; *Streptococcus pyogenes*) are responsible for a variety of illnesses that affect humans (14). In the 1980s both United States civilian and military personnel experienced numerous outbreaks of acute rheumatic fever, the first for the United States military in over 20 years (4, 30, 31). Epidemiological data suggest that these epidemics may have been due to emergent, potentially more virulent GAS strains (3, 12, 27).

In response to these concerns, GAS surveillance was established at eight military basic training sites throughout the United States (1, 5, 26). Between January 1998 and December 2001, GAS pharyngeal culture isolates from symptomatic military recruit trainees were systematically collected along with basic demographic data (Table 1).

This research has been conducted in compliance with all applicable federal regulations governing the protection of human subjects in research, under protocol no. 31230.

Training sites preserved GAS specimens in tryptic soy broth with 15% glycerol at −70°C. Isolates were reconfirmed as GAS by colony morphology, bacitracin sensitivity, and a positive latex agglutination test (Hardy Diagnostics, Santa Maria, Calif.). MICs of penicillin, erythromycin, clindamycin, tetracycline, levofloxacin, and vancomycin were performed on confirmed isolates with E-test methodology (AB Biodisk, Piscataway, N.J.). Plates were incubated for 18 to 24 h at 36°C with 5% CO_2. National Committee for Clinical Laboratory Standards (NCCLS) breakpoints for agar dilution of streptococci were used to interpret MIC results and to define criteria for resistant, intermediate susceptible, and susceptible isolates (21, 22). For quality control, *Streptococcus pneumoniae* ATCC 49619 was tested with each batch of samples.

GAS isolates were *emm* typed by using procedures adapted from those of the Centers for Disease Control and Prevention (2, 6). Sequencing products were analyzed with an ABI Prism 3100 sequencer as described by the manufacturer (Applied Biosystems, Foster City, Calif.). DNA sequences were submitted to a BLAST search, requiring 95% or greater homology with a reference *emm* gene sequence for *emm* type assignment (2).

Univariate analyses were initially performed to assess possible associations between demographic variables and antibiotic resistance or *emm* type. Variables associated with the outcome of interest ($P \leq 0.15$) were included in subsequent exact multivariable logistic regression model analyses. Analyses and final models were conducted by using SAS and included only those variables independently associated with the outcome of interest with *P* values of ≤0.05.

From January 1998 to December 2001, 692 GAS samples were received from eight military basic training sites (Table 1). Susceptibility to penicillin, levofloxacin, and vancomycin was seen in 100% of samples. Forty-four isolates (6.4%) were resistant to erythromycin, 34 (4.9%) were resistant to tetracycline, 4 (0.58%) were resistant to clindamycin, and 10 (1.45%) were multidrug resistant. Sustained temporal trends in antibiotic resistance over the duration of the study were not seen (data not shown).

Gender and training site were associated with antibiotic resistance (Table 1). Women were more likely to be resistant to tetracycline in multivariable modeling. In addition, isolates from the Air Force site were much more likely to be resistant to erythromycin, while isolates from Army sites were more likely to be resistant to tetracycline.

Over 30 different *emm* types were identified, with the most prevalent being *emm29* (18.0%), *emm3* (15.2%), *emm6* (13.5%), *emm44/61* (9.1%), *emm2* (7.3%), *emm75* (6.4%), and *emm1* (4.8%) (Table 2). Univariate analyses demonstrated that training site was statistically associated with *emm* type. The Navy's Illinois site had proportionally more *emm6* and *emm44/61*.

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TABLE 1. Characteristics of military trainees and multivariable logistic regression modeling of antibiotic resistance among GAS isolates

Variable	Total no. of samples (%)	No. of erythromycin-resistant isolates (%)	OR (95% CI)[a]	No. of tetracycline-resistant isolates (%)	OR (95% CI)[a]
Gender[b]					
Male[c]	592 (85.9)	34 (5.7)		25 (4.2)	
Female	97 (14.1)	10 (10.3)	NSS	9 (9.3)	2.3 (1.0–5.2)
Age (yrs)[d]					
17–18[c]	196 (28.6)	13 (6.6)		11 (5.6)	
19–20	281 (41.0)	20 (7.1)	NSS	11 (3.9)	NSS
21–22	99 (14.5)	6 (6.1)	NSS	5 (5.1)	NSS
≥23	109 (15.9)	5 (4.6)	NSS	6 (5.5)	NSS
Season					
Summer (June–August)[c]	73 (10.6)	5 (6.9)		4 (5.5)	
Fall (September–November)	252 (36.4)	7 (2.8)	NSS	18 (7.1)	NSS
Winter (December–February)	161 (23.3)	10 (6.2)	1.5 (0.6–3.8)	5 (3.1)	NSS
Spring (March–May)	206 (29.7)	22 (10.7)	2.2 (1.1–4.9)	7 (3.4)	NSS
Training site					
Navy, Ill.[c]	222 (32.1)	7 (3.2)		5 (2.3)	
Marines, Calif.	3 (0.4)	0 (0.0)	NSS	0 (0.0)	NSS
Marines, S. C.	282 (40.7)	1 (0.4)	NSS	10 (3.6)	NSS
Army, S. C.	4 (0.6)	0 (0.0)	NSS	1 (25.0)	11.5 (1.1–117.8)
Army, Ky.	23 (3.3)	2 (8.7)	5.4 (1.1–27.3)	4 (17.4)	6.7 (2.0–21.9)
Army, Mo.	37 (5.4)	4 (10.8)	7.1 (2.0–25.0)	4 (10.8)	3.4 (1.1–10.9)
Army, Okla.	19 (2.8)	0 (0.0)	NSS	2 (10.5)	NSS
Air Force, Tex.	102 (14.7)	30 (29.4)	25.1 (11.0–57.1)	8 (7.8)	2.4 (1.0–5.7)

[a] OR, multivariable adjusted odds ratio; CI, confidence interval; NSS, not statistically significant.
[b] Missing gender data from three patients.
[c] Reference category for multivariable exact logistic regression.
[d] Missing age data from seven patients. Variable not statistically significant at the univariate level; not included in multivariable model.

TABLE 2. Factors associated with *emm* gene type of GAS isolates

Variable	Total no. of isolates	% of isolates of type:								Univariate χ^2 *P* value[b]
		emm1 (*n* = 33)	*emm2* (*n* = 50)	*emm29* (*n* = 123)	*emm3* (*n* = 104)	*emm44/61* (*n* = 62)	*emm6* (*n* = 92)	*emm75* (*n* = 44)	Other[a] (*n* = 175)	
Gender										
Male	587	4.94	8.01	18.6	16.2	8.86	13.8	6.47	23.17	0.018
Female	96	4.17	3.13	14.6	9.38	10.4	11.5	6.25	40.63	
Age (yrs)										
17–18	196	1.02	8.16	20.9	20.4	5.61	10.2	5.61	28.06	0.002
19–20	278	4.68	6.83	17.99	16.19	8.99	15.83	7.91	21.58	
21–22	98	4.08	6.12	18.37	11.22	13.3	13.27	6.12	27.55	
≥23	107	11.2	8.41	12.2	7.48	12.2	14.02	4.67	29.91	
Season										
Summer (June–August)	73	4.11	2.74	27.4	6.85	2.74	8.22	6.85	41.10	<0.001
Fall (September–November)	250	3.6	12.4	25.6	23.6	5.20	2.80	0.80	26.00	
Winter (December–February)	159	6.92	3.77	16.4	10.1	13.8	21.4	9.43	18.24	
Spring (March–May)	204	5.88	5.39	6.37	11.8	12.3	22.1	10.8	25.49	
Training site										
Navy, Ill.	222	2.70	8.56	0.0	8.11	27.0	32.9	0.90	19.82	<0.001
Marines, Calif.	3	66.7	33.3	0.0	0.0	0.0	0.0	0.0	0.0	
Marines, S. C.	280	3.57	8.21	43.2	25.7	0.0	1.43	0.36	17.50	
Army, S. C.	4	0.0	0.0	0.0	0.0	0.0	25.0	0.0	75.0	
Army, Ky.	22	0.0	4.6	0.0	4.6	0.0	4.6	36.4	50.0	
Army, Mo.	36	2.78	8.33	5.56	30.6	5.56	2.78	5.56	38.89	
Army, Okla.	18	33.3	11.1	0.0	5.6	0.0	0.0	0.0	50.00	
Air Force, Tex.	101	9.90	0.99	0.0	0.99	0.0	11.9	30.7	45.54	
Antibiotic resistance										
Erythromycin	43	4.65	0.0	0.0	0.0	0.0	0.0	65.12	30.23	<0.001
Tetracycline	31	3.23	0.0	3.23	0.0	0.0	0.0	0.0	93.54	<0.001

[a] Other category includes the following *emm* types: *emm12* (*n* = 22), *emm22* (*n* = 13), *emm11* (*n* = 19), *emm5* (*n* = 14), *emm89* (*n* = 15), *emm28* (*n* = 19), *emm18* (*n* = 10), *emm27L/77* (*n* = 14), *emm4* (*n* = 9), *emm58* (*n* = 3), *emm73* (*n* = 6), *emm82* (*n* = 2), *emm96* (*n* = 3), *emm9* (*n* = 6), and 20 additional types with *n* = 1 each.
[b] *P* values are based on the Monte Carlo estimate for the Pearson chi-square exact test.

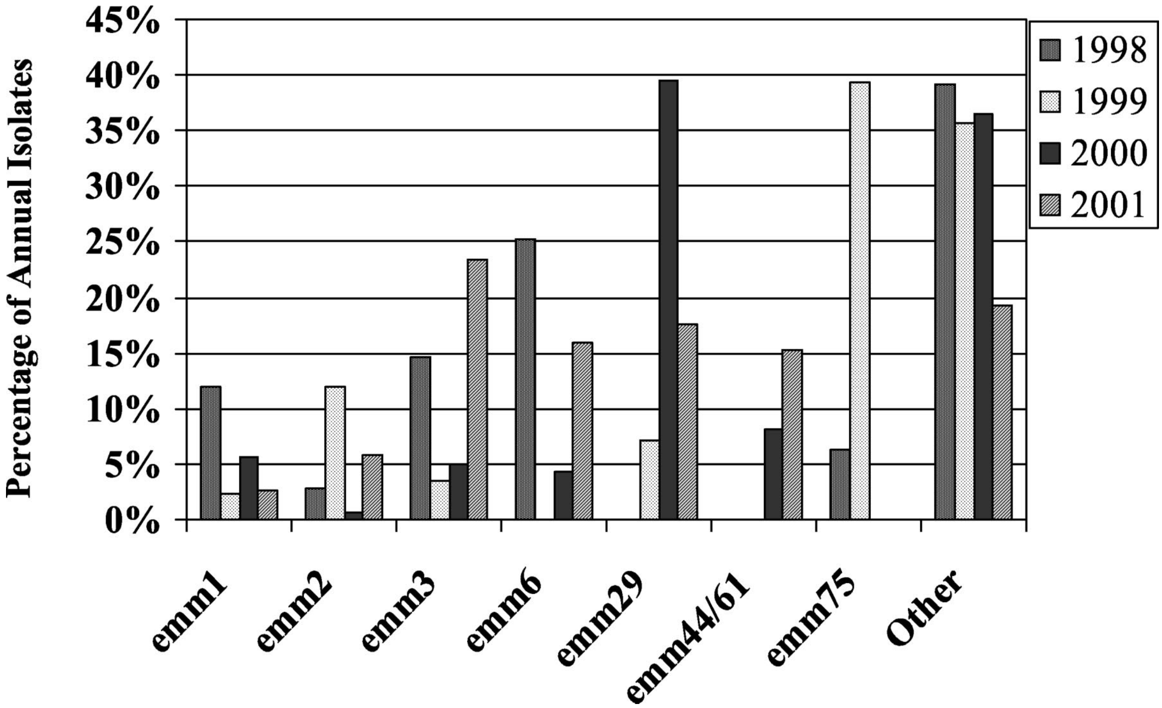

FIG. 1. Most common *emm* types of GAS isolates identified in military basic training surveillance, 1998-2001.

Both *emm29* and *emm*3 types were more prevalent at the Marine's site in South Carolina. The Air Force's site had a predominance of *emm75* among its GAS isolates. Additionally, the prevalence of *emm* types varied from year to year (Fig. 1). An association between *emm* types and antibiotic resistance was also observed. Erythromycin resistance was strongly associated with *emm75* isolates ($P < 0.001$). Tetracycline resistance was associated with *emm29* and *emm1* isolates.

Discussion. The growth of antibiotic resistance in GAS is a major concern for both military and civilian populations (1, 5, 19). We noted a prevalence of 6.4% erythromycin resistance and 4.9% tetracycline resistance among our population. Mass penicillin prophylaxis among recruits has proven effective in decreasing the frequency of outbreaks (11, 28). This practice is seasonal and/or dynamic and is modified depending upon local surveillance indicators. Prior research has demonstrated that prophylaxis of the penicillin-allergic individuals with erythromycin is useful in further reducing outbreak occurrences (9). However, this practice is site specific and variable. Due to the observed erythromycin resistance rate, antibiotic prophylaxis among the recruits at the training sites was examined. No apparent correlation with erythromycin resistance and a history of erythromycin use at the sites was noted; however, other researchers have shown the reality of this concern (10, 25).

From the molecular perspective, several GAS virulence factors are suggested to be associated with disease prevalence (13, 20, 24). The M protein is one of these virulence factors that offers GAS several mechanisms of defense against the host system (7, 8, 23, 29). Many common *emm* types reported by the Centers for Disease Control and Prevention's Active Bacterial Core Surveillance for invasive GAS were found in our surveillance of noninvasive illnesses: *emm1*, *emm3*, *emm12*, *emm28*, and *emm89* (see Table 2). These *emm* types from noninvasive illnesses are comparable to those seen in civilian populations and reaffirm the concern that virulent strains of GAS are circulating in more benign presentations and potentially could escalate into invasive disease trends and mortality if not properly managed (16, 18). Furthermore, the heterogeneity and shifts of *emm* types observed reflects the importance of considering multiple *emm* types in designing GAS vaccines (17). Since the M protein is presently described as a major virulence factor in GAS infections, a number of present vaccine constructs are composed of multivalent M protein sequences specific to particular diseases or geographic regions (15). Our surveillance data may help to guide these constructs.

In conclusion, surveillance for GAS isolates among United States military trainees has revealed a significant prevalence of macrolide resistance. The *emm* type distribution varied across military training sites, with *emm75* strongly correlated with erythromycin resistance at the Air Force site. Use of alternative antibiotics in penicillin-allergic individuals should be considered at sites with a background of increased macrolide resistance. Continued laboratory-based monitoring of GAS infections is important for the appropriate use of antibiotics and development of vaccines, thus minimizing streptococcal disease morbidity in both civilian and military populations.

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