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Emergence of Erythromycin- and Clindamycin-Resistant *Streptococcus pyogenes emm* 90 Strains in Hawaii[▽]

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We identified 12 erythromycin- and clindamycin-resistant *emm* 90 group A streptococcus (GAS) isolates during a retrospective invasive disease survey in Hawaii. A comparison with 20 type-matched isolates showed all resistant isolates to be e*mm* 90.4b with the constitutive or inducible macrolide-lincosamide-streptogramin B resistance phenotype ($cMLS_B$ or $iMLS_B$). All isolates had the same pulsed-field gel electrophoresis (PFGE) pattern, suggesting clonal spread.

The erythromycin resistance rate (~3%) has been low among group A streptococcus (GAS) isolates in Hawaii, where impetigo, acute rheumatic fever, and clusters of necrotizing fasciitis have been reported (5–7, 8–10; G. Erdem, G. Matsuura, G. Wheelen, C. Mizumoto, D. Esaki, and P. V. Effler, presented at the XVIIth Lancefield International Symposium on Streptococci and Streptococcal Diseases, Porto Heli, Greece, 2008). During a retrospective survey of 250 GAS cultures collected from patients with invasive GAS disease identified between the years 2005 and 2007, the reviews of the clinical information incidentally showed erythromycin and clindamycin resistance in five patients. *emm* typing of these isolates showed a single *emm* type: *emm* 90.4b. In addition to these five isolates, 15 additional isolates from individual patients with invasive disease were *emm* type 90.4b. The high percentage of invasive isolates (8%) belonging to this infrequent *emm* type and associated drug resistance prompted us to characterize the associated drug resistance patterns. We wanted to compare the resistance patterns among the isolates identified during this survey and other *emm* type 90 isolates identified from patients with noninvasive disease during our previous surveys. Of the 20 invasive GAS isolates, 12 were from blood cultures. Eight isolates were identified from wound cultures of inpatients with severe skin and soft tissue infections. Twelve additional *emm* type 90 pharyngeal isolates from our archived collection of 1,660 GAS isolates (collected from 1997 to 2007) were studied.

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emm sequencing (http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm) was done for all isolates. Etest methodology (AB Biodisk, Piscataway, NJ) was used to determine MICs for erythromycin and clindamycin (2, 3). The erythromycin-clindamycin double disk test was applied to resistant isolates. The "blunting" in the inhibition zone around the clindamycin disk next to the erythromycin disk was interpreted to be an inducible macrolide-lincosamide-streptogramin B resistance phenotype ($iMLS_B$), and susceptibility to clindamycin with no blunting indicated the M phenotype. The *mef*(A), *erm*(B), *erm*(A) (20), and *erm*(TR) resistance genes and *prtF1* were detected by PCR amplification (11, 19, 23, 24, 26, 27). Pulsed-field gel electrophoresis (PFGE) was performed with a Chef-DR III apparatus (Bio-Rad) after SmaI digestion.

All (20 isolates) invasive GAS *emm* 90 strains were of the allelic variation type 90.4b. Of the 12 noninvasive pharyngitis isolates, three *emm* 90 subtypes were identified: *emm* type 90.0 (one isolate from the year 1997), *emm* type 90.2 (6 isolates from 2004, 2005, and 2006), and *emm* type 90.4b (5 isolates: one isolate from 2005 and 4 isolates from 2007). Five of the invasive isolates had the constitutive MLS_B ($cMLS_B$) phenotype, with MICs showing resistance to both clindamycin (MIC, 3 to 4 μg/ml) and erythromycin (MIC, 12 to 64 μg/ml). Seven of them showed the $iMLS_B$ phenotype (Table 1). All of the resistant strains were isolated after July 2006. Of the 12 noninvasive isolates, only three of the *emm* 90.4 isolates showed resistance (erythromycin MIC, 12 to 24 μg/ml) with the $iMLS_B$ phenotype, and they were identified in the year 2007. Other *emm* 90 subtypes did not show resistance.

The erythromycin resistance rates increased each subsequent year (0% in 2005, 56% in 2006, and 86% in 2007). All of these MLS_B isolates had the *erm*(A) and *erm*(TR) genes and tested negative for the presence of *mef*(A) and *erm*(B) genes. All noninvasive isolates tested positive for the presence of the *prtF1* gene. Four PFGE patterns were identified among *emm* 90.4 isolates, and all drug-resistant isolates ($cMLS_B$ and $iMLS_B$ phenotypes) had one identical PFGE pattern. Among the *emm* type 90.4b isolates, there was an approximately 250-kb-size band difference between the drug-resistant and nonresistant isolates.

Erythromycin or clindamycin is recommended as an alternative treatment of streptococcal pharyngitis in case of allergy to beta-lactams, and clindamycin could be used in combination with penicillin for treatment of severe streptococcal infections (12, 31). Resistance of *Sreptococcus pyogenes* to macrolides and lincosamides has been reported, and overenthusiastic use of several macrolides may further increase this problem (14, 15, 19, 27, 28). A few *emm* types, such as *emm* 4, 6, 12, 58, 75, 77,

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TABLE 1. Isolation years, sources, *emm* typing, drug MICs, phenotypes, and resistance genes identified in invasive GAS isolates listed according to original culture and identification dates

Isolate	*emm* type	Yr	MIC (μg/ml) of drug[a]		Drug resistance phenotype	Resistance genes	Source
			EM	CM			
1	90.4b	2005	0.19	0.125			Blood
2	90.4b	2006	24	0.125	$iMLS_B$	*erm*(A), *erm*(TR)	Blood
3	90.4b	2006	0.25	0.19			Blood
4	90.4b	2006	32	0.19	$iMLS_B$	*erm*(A), *erm*(TR)	Blood
5	90.4b	2006	0.38	0.25			Blood
6	90.4b	2006	0.25	0.125			Wound
7	90.4b	2006	48	0.094	$iMLS_B$	*erm*(A), *erm*(TR)	Wound
8	90.4b	2006	24	0.19	$iMLS_B$	*erm*(A), *erm*(TR)	Wound
9	90.4b	2006	24	0.125	$iMLS_B$	*erm*(A), *erm*(TR)	Wound
10	90.4b	2006	24	4	$cMLS_B$	*erm*(A), *erm*(TR)	Wound
11	90.4b	2006	12	0.125	$iMLS_B$	*erm*(a), *erm*(TR)	Wound
12	90.4b	2006	0.125	0.125			Blood
13	90.4b	2006	24	0.047	$iMLS_B$	*erm*(A), *erm*(TR)	Foot
14	90.4b	2006	0.19	0.047			Blood
15	90.4b	2006	0.125	0.125			Blood
16	90.4b	2006	0.25	0.25			Wound
17	90.4b	2006	48	3	$cMLS_B$	*erm*(A), *erm*(TR)	Blood
18	90.4b	2007	24	3	$cMLS_B$	*erm*(A), *erm*(TR)	Blood
19	90.4b	2007	48	3	$cMLS_B$	*erm*(A), *erm*(TR)	Blood
20	90.4b	2007	64	3	$cMLS_B$	*erm*(A), *erm*(TR)	Blood

[a] EM, erythromycin; CM, clindamycin.

89 and 114, have been associated with resistance (1, 4, 14, 17, 19, 24, 26, 28). The *emm* type 90 isolates have not been associated with invasive GAS disease or with macrolide resistance (1, 4, 22, 25, 27, 30). This *emm* type appeared to be common after 2006 among invasive GAS strains, although it has been infrequently identified during our previous studies (5–7, 8–10; Erdem et al., XVIIth Lancefield International Symposium on Streptococci and Streptococcal Diseases). Considering that we have previously showed a wide variety of rather infrequent continental U.S. *emm* types and low levels of resistance, the emergence of these clonal, drug-resistant and invasive strains in Hawaii is concerning (29).

Macrolide resistance has been linked to invasiveness with possible introduction of drug resistance and virulence genes via lateral gene transfer (11). This may explain the presence of drug-resistant and invasive strains in our study. Facinelli et al. described the drug-resistant intracellular streptococci escaping the effects of macrolides (11). They found a high proportion of drug-resistant isolates to be PrtF1 positive, suggesting PrtF1 as a virulence factor (11). In contrast, almost none of the invasive and drug-resistant strains in our study had this gene. This finding may be more in-line with PrtF1 association with persistent infection or carriage (11, 21).

We do not know whether our strains have a higher invasion efficiency, since we have not done similar internalization and cell invasion assays. However, we have shown isolates harboring the rarely reported, constitutively expressed *erm*(TR) gene sharing the same PFGE pattern with the inducible resistant phenotypes after 2006 (17, 18, 20). Expression of the *erm* genes can be constitutive and inducible, and the selective pressure of clindamycin may lead to selection of constitutively resistant derivatives with alterations in the attenuator of the *erm*(TR) gene (12, 15). Point mutations in the *erm* regulatory region leading to constitutive methylase expression and high-level resistance due to the destabilization of the stem-loop structure have also been reported (17). In pneumococci, constitutive expression of resistance was found to be due to deletions, duplications, or point mutations in the attenuator sequence, leading to the derepressed production of the methylase (15, 16).

The single PFGE band difference between most of the drug-resistant and -sensitive isolates was an interesting finding of our study. Macrolide resistance is more likely to occur from a horizontal gene transfer than a spontaneous chromosomal mutation when observed in a rapid spread of a single *emm* type (28). In one study, the acquisition of the resistance phenotype was associated with the appearance of a new ~30- to 40-kb band in the PFGE pattern of a recipient macrolide-susceptible strain, suggesting a transposable element (13). Further studies need to be done on the 250-kb genomic fragment of our drug-resistant isolates to understand whether e*mm* 90.4b developed drug resistance from a genomic insertion. Clonal spread of erythromycin-resistant strains belonging to a single *emm* type has been reported (14, 19, 24, 26). Our study shows similar concerns of clonal emergence in Hawaii with this multidrug-resistant *emm* type.

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REFERENCES

1. **Barrozo, C. P., K. L. Russell, T. C. Smith, A. W. Hawksworth, M. A. Ryan, and G. C. Gray.** 2003. 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. J. Clin. Microbiol. **41:**4808–4811.
2. **CLSI.** 2008. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically for streptococcus spp. beta-hemolytic group: approved standard, 7th ed. CLSI, Wayne, PA.
3. **CLSI.** 2008. Performance standards for antimicrobial disk susceptibility tests for streptococcus spp. beta-hemolytic group: approved standard, 9th ed. CLSI, Wayne, PA.

4. **Dicuonzo, G., E. Fiscarelli, G. Gherardi, G. Lorino, F. Battistoni, S. Landi, M. De Cesaris, T. Petitti, and B. Beall.** 2002. Erythromycin-resistant pharyngeal isolates of *Streptococcus pyogenes* recovered in Italy. Antimicrob. Agents Chemother. **46:**3987–3990.
5. **Erdem, G., L. Abe, R. Y. Kanenaka, L. Pang, K. Mills, C. Mizumoto, K. Yamaga, and P. V. Effler.** 2004. Characterization of a community cluster of group A streptococcal invasive disease in Maui, Hawaii. Pediatr. Infect. Dis. J. **23:**677–679.
6. **Erdem, G., J. Ford, D. Johnson, L. Abe, K. Yamaga, and E. Kaplan.** 2005. Erythromycin-resistant group A streptococcal isolates collected between 2000 and 2005 in Oahu, Hawaii, and their *emm* types. J. Clin. Microbiol. **43:**2497–2499.
7. **Erdem, G., J. M. Ford, R. Y. Kanenaka, L. Abe, K. Yamaga, and P. V. Effler.** 2005. Molecular epidemiologic comparison of 2 unusual clusters of group A streptococcal necrotizing fasciitis in Hawaii. Clin. Infect. Dis. **40:**1851–1854.
8. **Erdem, G., C. Mizumoto, D. Esaki, L. Abe, V. Reddy, and P. V. Effler.** 2009. Streptococcal *emm* types in Hawaii: a region with high incidence of acute rheumatic fever. Pediatr. Infect. Dis. J. **28:**13–16.
9. **Erdem, G., C. Mizumoto, D. Esaki, V. Reddy, D. Kurahara, K. Yamaga, L. Abe, D. Johnson, K. Yamamoto, and E. L. Kaplan.** 2007. Group A streptococcal isolates temporally associated with acute rheumatic fever in Hawaii: differences from the continental United States. Clin. Infect. Dis. **45:**e20–e24.
10. **Erdem, G., S. Sinclair, J. R. Marrone, T. F. l'Atala, A. Tuua, B. Tuua, F. Tuumua, A. Dodd, C. Mizumoto, and L. Medina.** 2009. Higher rates of streptococcal colonization among children in the Pacific Rim Region correlates with higher rates of group A streptococcal disease and sequelae. Clin. Microbiol. Infect. **16:**452–455.
11. **Facinelli, B., C. Spinaci, G. Magi, E. Giovanetti, and E. P. Varaldo.** 2001. Association between erythromycin resistance and ability to enter human respiratory cells in group A streptococci. Lancet **358:**30–33.
12. **Fines, M., M. Gueudin, A. Ramon, and R. Leclercq.** 2001. In vitro selection of resistance to clindamycin related to alterations in the attenuator of the erm(TR) gene of *Streptococcus pyogenes* UCN1 inducibly resistant to erythromycin. J. Antimicrob. Chemother. **48:**411–416.
13. **Giovanetti, E., G. Magi, A. Brenciani, C. Spinaci, R. Lupidi, B. Facinelli, and P. E. Varaldo.** 2002. Conjugative transfer of the erm(A) gene from erythromycin-resistant *Streptococcus pyogenes* to macrolide-susceptible *S. pyogene*s, *Enterococcus faecalis* and *Listeria innocua*. J. Antimicrob. Chemother. **50:** 249–252.
14. **Green, M., J. M. Martin, K. A. Barbadora, B. Beall, and E. R. Wald.** 2004. Reemergence of macrolide resistance in pharyngeal isolates of group a streptococci in southwestern Pennsylvania. Antimicrob. Agents Chemother. **48:**473–476.
15. **Leclercq, R.** 2002. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Clin. Infect. Dis. **34:**482–492.
16. **Leclercq, R., and P. Courvalin.** 2002. Resistance to macrolides and related antibiotics in *Streptococcus pneumoniae.* Antimicrob. Agents Chemother. **46:**2727–2734.
17. **Malhotra-Kumar, S., A. Mazzariol, L. Van Heirstraeten, C. Lammens, P. de Rijk, G. Cornaglia, and H. Goossens.** 2009. Unusual resistance patterns in macrolide-resistant *Streptococcus pyogenes* harbouring erm(A). J. Antimicrob. Chemother. **63:**42–46.
18. **Malhotra-Kumar, S., L. Van Heirstraeten, C. Lammens, S. Chapelle, and H. Goossens.** 2009. Emergence of high-level fluoroquinolone resistance in emm6 *Streptococcus pyogenes* and in vitro resistance selection with ciprofloxacin, levofloxacin and moxifloxacin. J. Antimicrob. Chemother. **63:**886–894.
19. **Martin, J. M., M. Green, K. A. Barbadora, and E. R. Wald.** 2002. Erythromycin-resistant group A streptococci in schoolchildren in Pittsburgh. N. Engl. J. Med. **346:**1200–1206.
20. **Morosini, M. I., R. Canton, E. Loza, R. del Campo, F. Almaraz, and F. Baquero.** 2003. *Streptococcus pyogenes* isolates with characterized macrolide resistance mechanisms in Spain: in vitro activities of telithromycin and cethromycin. J. Antimicrob. Chemother. **52:**50–55.
21. **Neeman, R., N. Keller, A. Barzilai, Z. Korenman, and S. Sela.** 1998. Prevalence of internalisation-associated gene, prtF1, among persisting group-A streptococcus strains isolated from asymptomatic carriers. Lancet **352:**1974–1977.
22. **O'Brien, K. L., B. Beall, N. L. Barrett, P. R. Cieslak, A. Reingold, M. M. Farley, R. Danila, E. R. Zell, R. Facklam, B. Schwartz, and A. Schuchat.** 2002. Epidemiology of invasive group A streptococcus disease in the United States, 1995–1999. Clin. Infect. Dis. **35:**268–276.
23. **Oster, P., A. Zanchi, S. Cresti, M. Lattanzi, F. Montagnani, C. Cellesi, and G. M. Rossolini.** 1999. Patterns of macrolide resistance determinants among community-acquired *Streptococcus pneumoniae* isolates over a 5-year period of decreased macrolide susceptibility rates. Antimicrob. Agents Chemother. **43:**2510–2512.
24. **Perez-Trallero, E., J. M. Marimon, M. Montes, B. Orden, and M. de Pablos.** 1999. Clonal differences among erythromycin-resistant *Streptococcus pyogenes* in Spain. Emerg. Infect. Dis. **5:**235–240.
25. **Pournaras, S., A. Efstratiou, J. Douboyas, R. C. George, and A. Tsakris.** 2000. Genetic relatedness of group A streptococci of the newly designated serotype M90 causing a food-borne outbreak and sporadic infections. Epidemiol. Infect. **125:**299–301.
26. **Reinert, R. R., R. Lutticken, J. A. Sutcliffe, A. Tait-Kamradt, M. Y. Cil, H. M. Schorn, A. Bryskier, and A. Al-Lahham.** 2004. Clonal relatedness of erythromycin-resistant *Streptococcus pyogenes* isolates in Germany. Antimicrob. Agents Chemother. **48:**1369–1373.
27. **Richter, S. S., K. P. Heilmann, S. E. Beekmann, N. J. Miller, A. L. Miller, C. L. Rice, C. D. Doern, S. D. Reid, and G. V. Doern.** 2005. Macrolide-resistant *Streptococcus pyogenes* in the United States, 2002–2003. Clin. Infect. Dis. **41:**599–608.
28. **Robinson, D. A., J. A. Sutcliffe, W. Tewodros, A. Manoharan, and D. E. Bessen.** 2006. Evolution and global dissemination of macrolide-resistant group A streptococci. Antimicrob. Agents Chemother. **50:**2903–2911.
29. **Steer, A. C., I. Law, L. Matatolu, B. W. Beall, and J. R. Carapetis.** 2009. Global *emm* type distribution of group A streptococci: systematic review and implications for vaccine development. Lancet Infect. Dis. **9:**611–616.
30. **Tanz, R. R., S. T. Shulman, V. D. Shortridge, W. Kabat, K. Kabat, E. Cederlund, J. Rippe, J. Beyer, S. Doktor, and B. W. Beall.** 2004. Community-based surveillance in the United States of macrolide-resistant pediatric pharyngeal group A streptococci during 3 respiratory disease seasons. Clin. Infect. Dis. **39:**1794–1801.
31. **Zimbelman, J., A. Palmer, and J. Todd.** 1999. Improved outcome of clindamycin compared with beta-lactam antibiotic treatment for invasive *Streptococcus pyogenes* infection. Pediatr. Infect. Dis. J. **18:**1096–1100.