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 emm 90.4b with the constitutive or inducible macrolide-lincosamide-streptogramin B resistance phenotype (cMLSBL or iMLSB). 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. Matuura, 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/streptblast.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 (iMLSBL), 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 prfF1 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 90.0 (one isolate from the year 1997), emm 90.2 (6 isolates from 2004, 2005, and 2006), and emm 90.4b (5 isolates: one isolate from 2005 and 4 isolates from 2007). Five of the invasive isolates had the constitutive MLSBL (cMLSBL) 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 iMLSBL 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 iMLSBL 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 MLSBL isolates had the erm(A) and erm(TR) genes and tested negative for the presence of mef(A) and mef(B) genes. All noninvasive isolates tested positive for the presence of the prfF1 gene. Four PFGE patterns were identified among emm 90.4 isolates, and all drug-resistant isolates (iMLSBL and MLSBL 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 Streptococcus 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,
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 and invasive strains in our study. Facinelli et al. also 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 *emm* 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


