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Molecular Epidemiology of Group A Streptococcus Infections in Cambodian Children, 2007–2012

To the Editors:

Group A *Streptococcus* (GAS) is responsible for significant morbidity and mortality globally. Two strong vaccine candidates are currently under evaluation: a 30-valent type–specific M protein–based vaccine and a vaccine targeting the conserved J8 region of M protein.[1] The 30-valent vaccine covers the most frequent serotypes circulating in high-income countries, but coverage may be suboptimal in low-income settings with greater GAS *emm*-type diversity.[2] Sixty-eight allelic variants have been described for J8, and the relation between allelic diversity and vaccine efficacy is unclear.[1] Limited epidemiologic data are available from many regions making vaccine coverage estimates imprecise. There are no previous data for low-income countries in South East Asia.[2]

The clinical microbiology database at Angkor Hospital for Children, a nongovernmental pediatric hospital serving the population of northern Cambodia, was searched to identify clinical GAS isolates cultured between January 1, 2007 and December 31, 2012. These isolates underwent molecular *emm*-typing. *emm*-clusters, and the J8 vaccine antigen content, were deduced from the *emm*-typing result as previously described.[3] The 30-valent vaccine coverage was estimated using currently available cross-opsonization data.[3,4] Strain diversity was assessed by Simpson Reciprocal Index.

One hundred fifty GAS isolates from 149 patients were characterized. The median patient age was 3.8 years (range: 0–18.6). One hundred eighteen (78.6%) isolates were from skin and soft tissue infections, 16 (10.7%) from bloodstream infections, 7 (4.7%) from bone/joint infections, 7 (4.7%) from pharyngitis and 2 (1.3%) from infections at other sites. Fifty *emm*-types were identified from 13 *emm*-clusters and 2 isolates were considered nontypeable (see Table, Supplemental Digital Content, http://links.lww.com/INF/C234). No novel *emm*-types were identified. The Simpson Reciprocal Index was 28.5 (95% confidence interval: 23.1–37.3) indicating considerable diversity, similar to that seen in other low-income countries.[2]

Potential coverage of the J8 vaccine was predicted to be excellent with 43 (28.7%) and 104 (69.3%) isolates predicted to have the J8 and J8.1 allele, respectively. Therefore, J8 vaccine coverage could be expected to be 98.0% (95% confidence interval: 94.2%–99.6%). Fifty isolates (33.3%) were of *emm*-types covered by the 30-valent vaccine, and an additional 42 isolates (28.0%) have been shown to be potentially covered by the vaccine as a result of cross-opsonization. Therefore, the potential coverage could be expected to be 61.3% (95% confidence interval: 53.0%–69.2%) but may be higher because 26.0% of the isolates belong to 16 *emm*-types, which have not yet been examined for evidence of cross-opsonization.

Comparison with the only other available regional data set revealed considerably greater *emm*-type diversity in the Cambodian isolates compared with those isolated in Thailand between 1985 and 2004.[5] Fifty-nine *emm*-types from 13 *emm*-clusters were represented in the combined data set. Only 8 of the 13 *emm*-clusters were found in Thailand. There were 10 shared *emm*-types, comprising 64.2% of the Thailand isolates but only 26.4% of the Cambodia isolates.

Our study had several limitations. Pharyngeal isolates were not well represented because of clinician sampling practices and also there was an absence of adult sampling in the data set. Also, the population studied may not be representative of Cambodia as a whole. Finally, comparisons between the current data and the Thailand data set are limited by the differences between the study populations and nonoverlapping study periods.

Overall, these data indicate a high diversity of circulating GAS strains in Cambodia, the potential high coverage of the J8 vaccine candidate and the need for complementary studies to assess the potential coverage of the 30-valent vaccine candidate. These results highlight the need for robust regional and country-level data for vaccine planning purposes.

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A.C.S. and P.R.S. contributed equally to this article.
C.T., P.T., and N.P.J.D. are supported by the Wellcome Trust of Great Britain. The authors have no other funding or conflicts of interest to disclose.
These data were presented in part at the XIX Lancefield International Symposium on Streptococci and Streptococcal Diseases, Buenos Aires, Argentina.
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Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website (www.pidj.com).

ISSN: 0891-3668/15/3412-1414
DOI: 10.1097/INF.0000000000000878

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Reimmunization May Not Be Necessary in EIA Measles Seronegative HIV-infected Children

To the Editors:

Rowson et al[1] report on their findings of measles reimmunization in human immunodeficiency virus–infected children who are "not immune against measles," despite previous measles immunizations. They identified 141 (among 224 tested) such "nonimmune" children based on negative measles immunoglobulin G (IgG) antibody measured by enzyme-linked immunoassay (EIA). However, their conclusion on the lack of immunity against measles based on negative EIA values in immunized individuals is not valid and should be challenged.[2] Of note, neither age of the children nor time intervals between measles immunization(s) and blood draws are reported. In this context, it is important to note that even in immunocompetent individuals anti-measles IgG-EIA values may turn negative with increasing time after immunization, and this does not translate to waning immunity.

It is a widely held belief that enzyme-linked immunoassays are designed to detect vaccine-induced immunity against diseases such as measles. In fact, their purpose is to detect antibodies induced by wild-type virus infection that results in higher serum antibody levels than those induced by immunization. When we analyzed anti-measles serum IgG antibody levels by EIA in adolescents 13–15 years of age, we noted negative values in 40 (42%) of 96 previously measles-immunized individuals. Specifically, 11 (52%) of 21 with 1 previous dose and 29 (39%) of 75 with 2 previous doses were EIA-IgG negative.[2] However, when we reanalyzed these serum specimens by a plaque neutralization test, the standard test to measure protective measles antibodies, all EIA-negative specimens from measles-immunized adolescents were positive. This clearly indicates measles immunity, despite (false-)negative EIA results.

The fact that Rowson et al[1] observed "response" to measles reimmunization in EIA-negative human immunodeficiency virus–infected children is not a proof of previous lack of immunity: memory B-cells will be stimulated by antigen rechallenge leading to measurable serum IgG-EIA values. Therefore, I would like to modify the authors' title to propose that measles reimmunization may not be necessary in enzyme-linked immunoassay seronegative human immunodeficiency virus–infected children. Rather, if measuring measles immunity is the goal as suggested by Rowson et al[1] in accordance with published guidelines, an appropriate assay must be used and this is not EIA but the sensitive and specific plaque neutralization test.

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Reply to Reimmunization May Not Be Necessary in EIA Measles Seronegative HIV-infected Children

Professor Heininger highlights an important finding from a study he previously conducted: enzyme-linked immunosorbent assay methods for detecting immunoglobulin G against measles may not be sensitive enough to show that immunized children are protected against measles. However, the enzyme-linked immunosorbent assay method has been used to assess immunity to measles in children with human immunodeficiency virus in a number of studies.[1–4] Enzyme-linked immunosorbent assay has also been used to monitor response to measles vaccine in human immunodeficiency virus–infected children in many studies.[5–10] Heininger therefore calls into question the validity of all these studies (as well as our own audit).

The data he presents are derived from healthy children, not those who are immunocompromised, and thus, the relevance of which test is used in children with human immunodeficiency virus is therefore not known. Clearly, this is a vulnerable group in whom protection against measles should be optimized. Until a standardized approach to this issue is agreed, use of a "conservative" test may therefore be reasonable in this population.

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The authors have no funding or conflicts of interest to disclose.
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ISSN: 0891-3668/15/3412-1415
DOI: 10.1097/INF.0000000000000838

The author has no funding or conflicts of interest to disclose.
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ISSN: 0891-3668/15/3412-1415
DOI: 10.1097/INF.0000000000000839