Serodiagnosis of Acute Typhoid Fever in Nigerian Pediatric Cases by Detection of Serum IgA and IgG against Hemolysin E and Lipopolysaccharide

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Abstract. Inexpensive, easy-to-use, and highly sensitive diagnostic tests are currently unavailable for typhoid fever. To identify candidate serodiagnostic markers, we have probed microarrays displaying the full *Salmonella enterica* serovar Typhi (*S. Typhi*) proteome of 4,352 different proteins + lipopolysaccharides (LPSs), with sera from Nigerian pediatric typhoid and other febrile cases, Nigerian healthy controls, and healthy U.S. adults. Nigerian antibody profiles were broad (~500 seropositive antigens) and mainly low level, with a small number of stronger “hits,” whereas the profile in U.S. adults was < 1/5 as broad, consistent with endemic exposure in Nigeria. Nigerian profiles were largely unaffected by clinical diagnosis, although the response against t1477 (hemolysin E) consistently emerged as stronger in typhoid cases. The response to LPS was also a strong discriminator of healthy controls and typhoid, although LPS did not discriminate between typhoid and nontyphoidal *Salmonella* (NTS) disease. As a first step toward the development of a point-of-care diagnostic, t1477 and LPS were evaluated on immunostrips. Both provided good discrimination between healthy controls and typhoid/NTS disease. Such a test could provide a useful screen for salmonellosis (typhoid and NTS disease) in suspected pediatric cases that present with undefined febrile disease.

INTRODUCTION

Typhoid fever (TF), caused by the human-specific pathogen *Salmonella enterica* serovar Typhi (*S. Typhi*), is recognized by the World Health Organization (WHO) as a global health problem. In 2010, there were an estimated 26.9 million TF episodes worldwide, with a case-fatality rate of ~1%.¹ In Africa and south Asia, young children represent a subgroup with the highest disease burden. The onset of the illness is insidious and clinical diagnosis is often unreliable. Definitive diagnosis is through blood or bone-marrow culture, but is labor intensive, expensive, and invasive, and has a sensitivity of 40–80%.^{2,3} WHO recommends routine TF vaccination, but currently, licensed vaccines, although moderately effective in adults (55–75% protection),^{4,5} are largely ineffective in children under 5 years⁶ of age, and are not widely used in endemic areas.

In clinical practice, the management approach in most settings consists of initiating empiric broad-spectrum antibiotics when a patient presents with a clinical syndrome that is suggestive of a bacterial infection such as TF. Relevant clinical specimens are obtained, and the antibiotic management is de-escalated when culture results are available. In most settings, however, culture results are not available and antibiotic treatment is not de-escalated. Consequently, a large proportion of the population is exposed to broad-spectrum antibiotics for a prolonged period of time, and this contributes significantly to the rise of multidrug-resistant bacteria, which are more difficult to manage when they cause an infection.

Another consequence of the absence of etiologic diagnostic is the inability to formulate appropriate preventive strategies, such as pathogen-specific vaccines or other relevant public health measures. The absence of disease burden data that is based on sensitive and specific diagnostic tools

creates complacency about disease control. This is particularly challenging in most developing countries, where malaria is endemic and most febrile clinical syndromes are often misclassified as malaria.

Thus, to stem the rising tide of multidrug-resistant infections and to provide more precise pathogen-specific disease burden data, there is an urgent need to develop rapid, sensitive, and inexpensive diagnostic methods that will inform the development of clinical practice and public health preventive strategies, such as implementation or development of appropriate vaccines.

In this study, we have used protein microarrays displaying the full *S. Typhi* proteome to screen antibody profiles in febrile pediatric cases with blood culture confirmed or nonconfirmed TF, and with sera from healthy pediatric controls and healthy adults from the United States. Despite broad antibody profiles in Nigerian children against *S. Typhi* proteins, only t1477 (hemolysin E [hlyE]) emerges as a candidate marker of acute infection. We also find that lipopolysaccharide (LPS)-specific antibody is also strongly diagnostic of acute typhoid. These antigens have excellent potential for diagnosing acute typhoid in a point-of-care test.

MATERIALS AND METHODS

Ethics statement. This study was conducted with informed consent and approved by the ethics committees of the Federal Capital Territory of Nigeria, Federal Medical Center Keffi, Aminu Kano Teaching Hospital, and the University of Nebraska Medical Center, Omaha, Institutional Review Board. Sera were shipped on dry ice to the University of California Irvine (UCI) for array probing and assay development without patient identifiers, and were classified as exempt status by the UCI Institutional Review Board.

Sera. We screened children aged 8 months to 13 years (median ~4 years, Table 1) who presented to primary or secondary health centers in central and northwest Nigeria with an acute febrile illness and other symptoms that were suggestive of bacteremia. After informed consent from the parent

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TABLE 1
Categories and age distributions of donors used for antibody profiling by proteome microarray

	U.S. adults	Nigerian children			
		Healthy controls	No growth	Growth, <i>Salmonella enterica</i> serovar Typhi	Growth, nontyphoidal <i>Salmonella</i>
Minimum age, months (years)	312 (26.0)	5 (0.4)	8 (0.7)	8 (0.7)	10 (0.8)
Maximum age, months (years)	648 (54.0)	36 (3.0)	153 (12.8)	158 (13.2)	97 (8.1)
Median age, months, years	456 (38.0)	10.5 (0.9)	49 (4.1)	47 (3.9)	23 (1.9)
Sample, <i>n</i>	11	10	67	47	15

or guardian, we obtained blood aseptically from a peripheral vein for blood culture, and saved an aliquot for serum separation. Blood sampling and processing were as previously described.^{7,8} In brief, only aerobic blood culture bottles were used and held in a Bactec 9050[®] incubator (Becton Dickinson, Temse, Belgium) for a maximum of 5 days. Bacteria were identified by morphology, and for Enterobacteriaceae, by using an API 20 E rapid identification system (BioMerieux, Marcy-l'Étoile, France). Bacterial isolates were stored in skim milk at -70°C, and further characterized at the Clinical Microbiology laboratory, University of Nebraska Medical Center. Healthy control children were also enrolled from immunization clinics in the same facilities, where children present for routine immunizations and typically are in a stable state of health. Only children who were asymptomatic and did not have a history of a febrile illness in the past month or had taken any antibiotic during the same period were eligible. No blood cultures were performed on the healthy controls. Sera of children for proteome microarray probing were classified based on culture results as 1) febrile positive for *S. Typhi*, 2) febrile positive for other bacteria, 3) febrile, but no bacterial growth, and 4) healthy controls. A sample of healthy U.S. adults from a nonendemic area (Orange County, CA) was also probed on arrays as a baseline control.

Protein microarrays. Full *S. Typhi* proteome microarrays (Antigen Discovery Inc., Irvine, CA) were manufactured according to published methods.^{9,10} The content of the arrays comprised an ORFeome library cloned previously with ~63% coverage of the *S. Typhi* proteome supplemented with the additional *S. Typhi* open reading frames (ORFs) to provide full coverage. In addition, LPSs from *Salmonella typhosa* (*S. Typhi*), purified by gel filtration (L2387; Sigma-Aldrich, Dorset, United Kingdom),¹¹ was printed in four 3-fold serial dilutions from 0.1 to 0.003 mg/mL. Serum samples were shipped to UCI on dry ice and were probed onto proteome arrays as described previously.^{9,10} Arrays were scanned in a GenePix 4200AL (Molecular Devices, Sunnyvale, CA), and captured TIFF files were quantified using ScanArray Express software (Perkin Elmer, Waltham, MA). Median spot pixel intensities subtracted of local background were imported into Microsoft Excel (Microsoft Corp., Redmond, WA). For graphic outputs of raw data, mean fluorescence intensity (MFI) signals for each *in vitro* transcription/translation (IVTT) spot were corrected by subtraction of the median of the sample-specific control IVTT spots (foreground minus background), to give a corrected MFI. For statistical analysis, data were normalized by first setting a floor of 100, dividing the values for each IVTT protein spot by the median of the sample-specific IVTT control spots (fold-over control [FOC]) and then taking the base-2 logarithm of the ratio (log₂ FOC). Two-tailed Student's *t* tests were performed on the log₂ FOC data and *P* values were

adjusted for false discovery using the Benjamini-Hochberg method.¹² Box and whisker plots were created in JMP (SAS Institute, Inc., Cary, NC) using Log₂ FOC data for IVTT spots, and raw data for purified LPS. Receiver operating characteristic (ROC) plots were produced using the ROC package in R (<http://www.r-project.org>).

Immunostrips. *S. Typhi* proteins were purified and printed on immunostrips as described previously.¹⁰ In brief, proteins were expressed from the plasmids used for protein microarrays in *Escherichia coli* BL-21 cell, and were extracted using BugBuster (Novagen, Billerica, MA). Inclusion body preparations were obtained by centrifugation, solubilized in urea, and dialyzed against phosphate-buffered saline to remove urea. The proteins were then quantified, printed at ~0.1 mg/mL onto HiFlow Plus HFB24004 membrane (Cat no. HF24004XSS; Millipore Corp., Billerica, MA) using a BioJet noncontact printer (BioDot, Irvine, CA), air dried, and cut into strips. Titrations of human IgG and IgA were printed as positive controls for the secondary antibody. For probing, sera were preincubated for 30 minutes at 1/250 dilution in Tris-buffered saline containing 0.05% Tween 20 (TTBS) containing 5% dried milk powder and *E. coli* DH5α lysate (Antigen Discovery Inc.) to a final concentration of 20 mg/mL. Immunostrips preblocked in TTBS/5% milk powder were incubated with preincubated sera for 2 hours at room temperature, washed and bound IgG was visualized using goat antihuman IgG conjugated to alkaline phosphatase (Jackson ImmunoResearch, West Grove, PA), and developed using 1-Step[™] nitro-blue tetrazolium and 5-bromo-4-chloro-3'-indolylphosphate substrate solution (ThermoFisher Scientific, Waltham, MA). Images of developed immunostrips were obtained using a document scanner (Hewlett-Packard, Palo Alto, CA), and captured TIFF files were converted to grayscale and inverted in Photoshop (Adobe Systems, San Jose, CA). Bands were quantified using Image J (<http://imagej.nih.gov/ij/>). ROC plots were produced using the ROC package in R (<http://www.r-project.org>).

RESULTS

Proteome arrays reveal broad preexisting IgG and IgA profiles in Nigerian children. The aims of the present study were to assess the prevalence of antibodies to *S. Typhi* in febrile Nigerian children, and if possible to define antigens that have utility for rapid diagnosis of acute typhoid. The protein array reported herein, designated SE2, is a full proteome array of *S. Typhi* strain Ty2. The serum samples probed on the array are listed in Table 1, and comprised healthy Nigerian control children, pediatric febrile cases negative for *Salmonella* spp. by blood culture ("No Growth"), pediatric febrile cases positive by blood culture for *S. Typhi* ("Growth, *S. Typhi*") or nontyphoidal *Salmonella* ("Growth, NTS"),

FIGURE 1. Nigerian serum samples have very broad IgG reactivity to *Salmonella enterica* serovar Typhi (*S. Typhi*) antigens. Full proteome microarrays comprising ~4,352 different *S. Typhi* in vitro-expressed gene products were probed with serum samples as described in Table 1. Each panel shows average corrected MFIs (mean fluorescence intensities) of each *S. Typhi* protein by different groups of donors. The signals from healthy Nigerian control children are overlaid onto each panel and used to rank the antigens from left to right in descending order of signal intensity. Only the top 1,000 antigens are used for the figure. Horizontal dashed line is a seropositivity cutoff defined as the mean plus two standard deviations of the reactivity to all proteins by the U.S. controls. Values in the upper right corner are the number of seroreactive antigens recognized by each group (Nigerian healthy children were reactive to 477 antigens). Several antigen “spikes” are annotated with the gene ID.

and healthy U.S. adults. An overview of the IgG reactivity is shown in the bar charts in Figure 1. The line for the Nigerian healthy controls is above that of the U.S. adults (Figure 1A), despite the former being significantly younger than the U.S. adults (Table 1). This is consistent with the notion that there is endemic *Salmonella* exposure in Nigeria, with acquisition of antibodies occurring at a young age. Thus, 477 proteins were seropositive in Nigerian healthy controls, compared with 107 proteins in the U.S. controls. In contrast, the healthy control line overlaps closely with the trend for all the febrile cases regardless of clinical diagnosis (Figure 1B–D); these groups were reactive to 554, 472, and 643 proteins, respectively.

Despite the similarities between the Nigerian profiles, a small number of antigen “spikes” were revealed in this analysis, including t1477 (hlyE), t2787 (pathogenicity island 1 effector protein, sipB), and t0391 (ethanolamine utilization protein, EutQ). None appeared to be entirely specific

to one particular group, and may represent antibodies that the healthy control group (which were younger overall than the febrile cases) were yet to acquire. The t1477 antigen is more strongly recognized by Nigerian typhoid cases. Antigen t1266 (pathogenicity island effector protein, sseB) may represent another potential diagnostic marker, although the MFI is low. In general, however, the serology of Nigerian children is characterized by broad, largely overlapping antibody profiles that are very similar between groups.

Evaluation of IgG and IgA responses to *S. Typhi* t1477 (hlyE) as a diagnostic for typhoid. To better identify antigens for diagnosing typhoid, *t* tests were performed on normalized (Log2 FOC) data. In Figure 2, different comparisons are shown, with the Benjamini–Hochberg corrected *P* values (*p*_{BH}) overlaid onto a bar chart of normalized data. With the normalized data, a value of 0.0 means the intensity is no different than background, whereas a value of 1.0

FIGURE 2. IgG and IgA profiles reveal few antigens able to discriminate between Nigerian pediatric groups. To identify candidate serodiagnostic antigens for typhoid, array data were normalized using the Log2 FOC method. Two-tailed t tests were performed between groups, and P values were corrected for false discovery by the Benjamini-Hochberg method (p_{BH}). In each panel, the top 30 significant antigens ($p_{BH} < 0.05$) are ranked left to right independently in each panel by average signal intensity of the typhoid group and plotted as a bar chart ($\pm$ SD), with the corrected P values (secondary y-axis) overlaid. (A) IgG: healthy Nigerian children vs. Nigerian typhoid, (B) IgG: Nigerian “nontyphoidal *Salmonella* (NTS) disease” vs. Nigerian typhoid (note only three antigens were significant), (C) IgA: healthy Nigerian children vs. Nigerian typhoid, and (D) IgA: Nigerian “NTS” disease vs. Nigerian typhoid.

indicates a doubling with respect to background, and provides a useful cutoff for seropositivity. In the IgG profiles, only three protein antigens (t1477, t2787, and t0391) emerge as strong candidates for discriminating between

Nigerian pediatric “Healthy” controls and “Growth, *S. Typhi*” groups (Figure 2A), being both significant ($p_{BH} < 0.05$) and seropositive ($\text{Log}_2 \text{FOC} > 1$). This paucity of antigens for discriminating acute typhoid from healthy controls is

remarkable, given that the whole *S. Typhi* proteome of > 4,300 proteins was screened, but is caused by the relatively broad, but low-level preexisting background of *S. Typhi*-specific IgG antibodies in healthy Nigerian children. Since the potential to discriminate between typhoid and NTS disease has relevance for patient management, we also compared IgG profiles between the “Growth, *S. Typhi*” and “Growth, NTS” groups. Only three antigens reached significance, of which only t1477 was seropositive (Figure 2B). Antigens t2787 and t0918 (flagellin) were also seropositive, but corrected *P* values failed to reach significance.

We hypothesized that IgA antibody profiles might reveal more discriminatory antigens, based on the assumption that *S. Typhi* infections are acquired enterically. As with IgG, we observed broad, low-level IgA profiles in all Nigerian groups tested. Comparison of “Healthy” and “Growth, *S. Typhi*” groups revealed ~7 antigens that were both significant and seropositive, with t1477 again the dominant antigen (Figure 2C). Other potential discriminatory antigens included t0391 and t2919, although average signals were low. Comparison between NTS and *S. Typhi* revealed several significant antigens, although none were above the seropositivity threshold (Figure 2D).

The patterns of IgG and IgA reactivity for t1477 are given in more detail in the box plots in Figure 3A and B, respectively. The difference between the “Healthy” and “Growth, *S. Typhi*” groups is highly significant (IgG, $P < 0.0001$; IgA, $P < 0.0001$). Accordingly, the area under the ROC curve (AUC) comparing the “Healthy” and “Growth, *S. Typhi*” groups (Figure 3C and E) is 0.898 for IgG, with improved discrimination by IgA (AUC = 0.956). The IgG response to t1477 also shows good discrimination between “Growth, *S. Typhi*” and “Growth, NTS” (Figure 3D and E).

Evaluation of IgG and IgA responses to LPS as a diagnostic for typhoid. Also printed on the proteome array was purified LPS from *S. Typhosa* (*S. Typhi*). This antigen is widely used in existing diagnostic tests, and was printed on the SE2 array to facilitate the comparison of IVTT array data with conventional serological assays. Figure 4A and B shows that the strongest IgG and IgA responses were seen in individuals in the “Growth, *S. Typhi*” group. Discrimination by LPS of “Healthy” and “Growth, *S. Typhi*” was very strong, with a sensitivity and specificity of 91.5% and 80.0%, respectively, for IgG, and 100% and 100% for IgA (Figure 4C and E). Accordingly, the AUC values for IgG and IgA recognition of LPS by the “Healthy” and “Growth, *S. Typhi*” groups

FIGURE 3. Hemolysin E (t1477) as a potential serodiagnostic target antigen. Box plots of t1477 protein array data; (A) IgG data, (B) IgA data. Boxplots were generated from normalized (Log2 fold-over control [FOC]) protein array data, with each dot representing a single serum sample. Boxes show interquartile range with median; whiskers are third quartile + 1.5*(interquartile range) and first quartile - 1.5*(interquartile range). The tables to the left in each plot show paired group comparisons that gave significant *P* values using the Wilcoxon nonparametric *t* test. (C) Receiver operating characteristic (ROC) plot of IgG and IgA responses to t1477 comparing “Growth, *S. Typhi*” vs. “Healthy” Nigerian controls; (D) “Growth, *S. Typhi*” vs. “Growth, nontyphoidal *Salmonella*”; (E) Tabulated % sensitivity, % specificity, and area under the curve (AUC) for the two ROC plots shown in panels C and D.

FIGURE 4. Lipopolysaccharide (LPS) is a potential serodiagnostic target antigen. Boxplots and receiver operating characteristic (ROC) curves were generated for LPS array data as described in Figure 3.

were 0.955 and 1.000, respectively (Figure 4E). For discriminating typhoid and NTS disease, LPS-specific IgG was very sensitive, but had essentially no specificity (97.9% and 53.3%, respectively). IgA is less sensitive than IgG (83.3% versus 97.9%), although specificity is improved (86.7% versus 53.3%; Figure 4E).

Development of t1477 and LPS immunostrips as potential deployable diagnostic tests for typhoid. Overall, the array data described above led to LPS and t1477 as lead candidates for further development. We therefore printed purified t1477 and four different sources of purified LPS in parallel lines on nitrocellulose membrane using a noncontact jet printer, and the membrane was dried and cut into immunostrips. Antigens t3710 and t1459 described in a previous study¹⁰ were also printed, but were unreactive (not shown). Immunostrips were then probed with 56 randomized serum samples from the U.S. control, Nigerian healthy control, “No Growth,” “Growth, S. Typhi,” and “Growth, NTS” groups.

The heat map in Figure 5A shows LPS from *S. Typhosa* (*S. Typhi*) and *Salmonella* Typhimurium were reactive with both IgG and IgA from the majority of the “Growth, S. Typhi” and “Growth, NTS” samples, less reactive with “No Growth” samples, and unreactive with healthy Nigerian controls or U.S. controls. ROC analyses are shown in Figure 5B–E. For clarity, data for the *S. Typhi* LPS is shown only, although *S. Typhimurium* LPS behaved in an

identical fashion. On the immunostrip format, discrimination of “Healthy” controls and typhoid by t1477-specific IgG was improved compared with the array format (Figure 5B and E). This is because, reactivity by the control group was negative on the immunostrips, whereas there was modest IgG reactivity by some samples to IVTT-expressed protein on the array (Figure 3A). In contrast, discrimination between typhoid and NTS disease by t1477-specific IgG was greatly reduced on the immunostrip format (Figure 5B and E), despite showing good discrimination in the array (Figure 3A). The performance of LPS for discriminating between healthy controls and typhoid was maintained on the immunostrip for both IgG and IgA, although sensitivity was reduced slightly. In contrast, discrimination between typhoid and NTS disease by LPS-specific IgG (Figure 5C) and IgA (Figure 5D) was reduced compared with the array format (Figure 4A and B).

DISCUSSION

TF is endemic in Nigeria although estimates of TF incidence vary substantially.^{3,13–16} In large part, this is due to poor access to reliable diagnostics in this setting. Measured by its burden and influence on antibiotic use, TF is perhaps one of the leading causes of invasive bacterial infection for which a rapid diagnostic does not exist. To address this, we used the bacteremia surveillance platform that was established a few years ago for young Nigerian children to

FIGURE 5. Discriminatory properties of t1477 and LPS using immunostrip format. Purified *Salmonella enterica* serovar Typhi (*S. Typhi*) antigens t1477 and LPS from *S. "Typhosa"* (Typhi), *Salmonella* Typhimurium and *Escherichia coli* were printed on nitrocellulose in three concentrations (0.1, 0.03, and 0.01 mg/mL) and cut into strips. Strips were probed in duplicate with randomized sera from U.S. controls, healthy Nigerian children, "No Growth," "Growth, *S. Typhi*," and "Growth, NTS" ($N = 5, 10, 17, 22$, and 10 , respectively), and IgG and IgA were visualized on separate strips for each sample. Data were normalized against the IgG and IgA controls by median scaling, and the ability to discriminate between Nigerian "Healthy" control and "Growth, *S. Typhi*" groups, and "Growth, *S. Typhi*" and "Growth, NTS" determined by receiver operating characteristic (ROC) analysis. (A) Heat map of deconvoluted immunostrip data, with darker shades corresponding to stronger signals. (B) ROC plot for t1477-specific IgG data. (C) ROC plot for LPS-specific IgG data. (D) ROC plot for LPS-specific IgA data. (E) Tabulated % sensitivity, % specificity, and AUC for the three ROC plots shown in panels B, C and D. AUC = area under the ROC curve; LPS = lipopolysaccharide; NTS = nontyphoidal *Salmonella*.

define the epidemiology of bacteremia, and to further understand host immune response to *S. Typhi*, which we identified as the leading cause of bacteremia in these children.^{7,8} TF in this setting is often diagnosed without laboratory confirmation by culture, and patients are offered broad-spectrum empiric antibiotics. Thus, access to this population in a TF endemic area and with diagnosis confirmed by blood culture provides an unprecedented opportunity for dissecting host immune response.

In many countries, the Widal's agglutination test, which is based on formalin-inactivated *S. Typhi* organisms, is still widely used. Use of the test is still controversial, and it is of limited use in endemic countries.¹⁷ Blood culture is the test of choice, but is not rapid and requires specialized equipment and training. A simple and rapid point-of-care test is urgently needed.

Herein, we have used a novel protein microarray approach displaying the full *S. Typhi* proteome plus LPS, in an effort to identify antigens suitable for development of rapid serodiagnostics and potential vaccine candidates. In previous

studies, we described the production and use of a *S. Typhi* partial proteome microarray (~63%) for profiling antibodies in human adult typhoid cases from Malawi,⁹ Vietnam,¹⁰ and Bangladesh,¹⁸ and in the mouse *S. Typhimurium* model.⁹ The study reported herein is the first to describe results obtained from the full *S. Typhi* proteome array of > 4,300 proteins. We used well-characterized sera from pediatric cases in Nigeria with infections confirmed initially by culture, and subsequently by molecular means. All of the Nigerian pediatric groups were more seroreactive than U.S. adults, consistent with endemic exposure to *Salmonella* spp. at an early age in Nigeria. Reactivity in U.S. adults (where *S. Typhi* is not endemic) to *S. Typhi* antigens on the array is likely due to cross-reactivity of antibodies to conserved proteins found in the different *S. enterica* serovars (such as those associated with gastroenteritis) to which U.S. adults are more likely to have been exposed.

Consistent with our previous studies of adults in other endemic countries, we identified t1477 hlyE as the lead candidate antigen for the discrimination of Nigerian

pediatric typhoid cases and controls. Although several additional potential diagnostic antigens were discovered in Vietnamese adults with typhoid ($N = 15$ IgG reactive and > 70 IgM reactive), on immunostrips, only the t1477 antigen was reported to retain any discriminatory utility.¹⁰ In Bangladesh also, the same array revealed t1477 as the immunodominant antigen, able to discriminate between acute typhoid cases and uninfected controls in both serum and in plasmablast culture supernatants.¹⁸ The t1477 antigen was not revealed as dominant in Malawian children,⁹ although only two cases of typhoid were examined.

In marked contrast, LPS was not revealed in these published studies as a potential diagnostic antigen, possibly because IgA to LPS was not investigated. We speculate that the IgA response to LPS in typhoid is short-lived, so that it is more readily detected in acute infections and absent from the broad serological background seen in Nigerian children from endemic exposure. Further studies will be needed to determine the half-life of anti-LPS IgA in acute typhoid to test this hypothesis.

Although t1477 and LPS are good discriminators of typhoid and healthy controls, the ability to discriminate between typhoid and NTS disease is less convincing in the immunostrip format. This is consistent with our previous studies.^{9,10} Presumably, this is because of the high level of antigenic conservation of these antigens between *S. Typhi* and NTS serovars. Nevertheless, we did see some *S. Typhi*/NTS discrimination using the array format, particularly by t1477-specific IgG, and LPS-specific IgA. The reduced ability of both antigens to discriminate when used on the immunostrip compared with the array may be related to the reduced dynamic range of the colorimetric readout of the immunostrip format compared with that of the fluorescence readout of the array.

We saw roughly equivalent diagnostic properties of LPS from either *S. Typhi* or *S. Typhimurium*, as exemplified by the immunostrip studies reported here (Figure 5), which is presumably also due to high antigenic conservation. However, LPS per se is not inherently cross-reactive, as typhoid and NTS cases with reactivity to *S. Typhi* and *S. Typhimurium* LPS do not react with LPS from *E. coli* for example (Figure 5). The use of LPS as a serodiagnostic marker of salmonellosis is not new; several studies have reported its utility for detection of acute typhoid in serum and saliva.^{19–22} However, uptake and wide clinical evaluation has been slow, possibly because of perceived problem with a lack of specificity. More detailed studies are underway in this laboratory to evaluate the issue of LPS specificity and cross-reactivity, and to evaluate the diagnostic properties of LPS from several different bacterial species to detect specifically the antibodies of the corresponding infection.

For diagnostic purposes, it is of interest whether a serological test might offer improved sensitivity compared with the culture test. Sensitivity of the culture test is dependent on the presence of organisms in the blood sample taken, and so, is less sensitive when there are lower numbers of organisms in the blood. For example, 50–60% individuals in the “No Growth” group, show modest IgG signals against t1477 and LPS on both immunostrips (Figure 5A) and arrays (Figures 3A and 4A). Similarly, IgA reactivity to both antigens was also seen within the “No Growth” group on arrays (Figures 3B and 4B), and to LPS (only) on the immunostrip

(Figure 5). It is possible that these were blood culture false negatives. All febrile cases are treated with antibiotics, so there is no clinical data to confirm whether these were undiscovered typhoid cases. Nevertheless, the potential of a serological test, particularly one based on LPS-specific IgA, warrants further evaluation as a diagnostic for acute typhoid and NTS disease.

In summary, we have screened a *S. Typhi* full proteome array with sera from well-characterized pediatric typhoid cases and controls from Nigeria. As seen in our other studies of typhoid in endemic countries, t1477 hlyE is the immunodominant protein antigen. Together with *Salmonella* LPS, these antigens have excellent potential for diagnosing febrile cases with acute typhoid in a point-of-care test.

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Conflict of interest: D. Huw Davies and Philip L. Felgner disclose a financial interest in Antigen Discovery Inc., which has licensed the protein microarray technology. D. Huw Davies, Philip L. Felgner, and the University of California Irvine may financially benefit from this interest if the company is successful in marketing its products that are related to this research. The terms of this arrangement have been reviewed and approved by the University of California Irvine, in accordance with its conflict of interest policies.

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