POSTER ABSTRACTS
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Abstract #: 52

Use of Anti-O12 Monoclonal Antibody in Tubex Test to Identify Salmonella Bacteria from Blood Culture

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Both culture and serological methods are used to diagnose enteric fever. An anti-Salmonella lipopolysaccharide O12 monoclonal antibody could be used to detect Salmonella organism directly from routine blood culture broths. Blood from 78 young outpatients suspected of having enteric fever was incubated in enrichment broth, and after 2 or 4 days, broth samplings were examined by Tubex-TP as well as by conventional agar culture and identification. Tuber TP was performed before the culture results. Fifteen isolates of S. Typhi and 4 isolates of S. Paratyphi A were obtained by conventional method. In all instances Tubex TP was positive, thus 100% sensitive. Twelve Escherichia coli, Alkaligenes spp and 1 Enterobacter spp were isolated. All of this case including all the 36 culture negative broths, were Tubex-negative i.e. Tubex TP was 100% specific. THus Tubes Tp is a useful adjuncts to conventional culture because they can save considerable time (>2 days), costs, and manpower.
Abstract #: 53

Subgrouping of *Salmonella enterica* serovar Typhi haplotypes using rapid Multiplex Ligation-dependent Duy Pham Thanh, Nga Tran Vu Thieu, Kathryn E Holt, Martin Lodén, Kiki Tuin, Gordon Dougan, Jeremy Farrar, Stephen Baker duypt@oucru.org The Hospital for Tropical Diseases, Wellcome Trust Major Overseas Programme, Oxford University Clinical Research Unit Ho Chi Minh City, Viet Nam

Salmonella Typhi is the causative agent of typhoid fever, a systemic infection that occurs predominantly in middle and low-income countries. The organism has remarkable genetic conservation, making strain subtyping, for local and international epidemiology, challenging. We aimed to develop a simple *S.* Typhi subtyping scheme based on genomic information that would offer some relevant phylogenetic information for strains circulating in endemic locations using minimal molecular biology equipment. We designed a standardized Multiplex Ligation-dependent Probe Amplification (MLPA) genotyping scheme, targeting 11 phylogenetically relevant genomic insertions/deletions (indels) across the *S.* Typhi genome. We validated the method by conventional PCR and compared the results to Single Nucleotide Polymorphism (SNP) typing, the current gold standard used in research facilities in industrialized countries. We found that the MLPA method demonstrated approximately 90% concordance with SNP typing, with the ability to detect H58 strains and other currently relevant genotypes. Our assay additionally permitted the detection of nalidixic acid resistance inducing mutations in the DNA gyrase gene, *gyrA*, and the topoisomerase gene, *parC*, with a sensitivity of approximately 90%. The methodology we have developed is simple and reliable, providing phylogenetically and phenotypically relevant subgrouping information that has international utility. Our MLPA method can distinguish between the strains that currently dominate the global population structure of *S.* Typhi, offering a more sensitive and simple alternative to the subgrouping methodologies currently being used in low and middle-income countries.
Abstract #: 57

Inhibition of B and T cell responses by Salmonella Pathogenicity Island II

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"Background: New vaccines against NTS and typhoid are urgently required and human and mouse infection models indicate a critical role for CD4+ T cells and B cells in protective immunity. Previous studies also suggest that Salmonella can actively inhibit host protective immunity, but the mechanism of inhibition remains poorly defined. Here, we have developed new tools to visualize T and B cell responses to a live vaccine strain of Salmonella and have directly observed the inhibition of protective immune responses.

Methods: Resistant (129xB6) mice were infected IV with Salmonella typhimurium, χ4550 or ΔSPI2 χ4550, which express ovalbumin (OVA). An OVA-specific B cell tetramer and TCR transgenic mice were used to analyze B and T cell expansion and germinal center formation by flow cytometry. Serum was collected and OVA-specific antibody responses were also analyzed by ELISA.

Results: OVA-specific B expansion and germinal center formation was delayed until 45 days in Salmonella-OVA-vaccinated mice, suggesting active inhibition by bacteria. Salmonella also inhibited B cell responses to OVA vaccination and T cell responses to flagellin. This inhibition of adaptive immunity was highly dependent on the Salmonella SPI2 locus.

Conclusions: The results of this work suggest two dilemmas for Salmonella vaccination. 1. SPI2 effectors cause a sub-optimal Salmonella-specific B and T cell response during live vaccination. 2. Subunit vaccination in conjunction with active Salmonella infection, may elicit a weaker immune response to the administered vaccine. Studies are underway to identify the SPI2 effector protein responsible for dampening Salmonella-specific adaptive immune responses."
Gatifloxacin versus ofloxacin for uncomplicated enteric fever: an open-label, randomized, controlled trial

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Background: Although fluoroquinolones are widely used in the treatment of enteric fever, there are no direct comparisons of two fluoroquinolones in the treatment of this disease; in addition our hospital in Nepal which is considering its policy on which fluoroquinolone to recommend in uncomplicated enteric fever requested a direct comparison of two fluoroquinolones: ofloxacin and gatifloxacin.

Methods: An open-label randomized trial was conducted at Patan Hospital in Kathmandu, Nepal to investigate whether gatifloxacin is more effective than ofloxacin in the treatment of uncomplicated enteric fever. From July 28, 2008 to August 28, 2011 adults and children clinically diagnosed with enteric fever received either gatifloxacin (10mg per kg per day) in a single oral dose or ofloxacin (20mg per kg per day) in two divided oral doses for 7 days. Patients were randomly allocated treatment (1:1) in blocks of 50 without stratification. Masking was not possible because of the different dose schedule. The primary outcome measure was treatment failure in the nalidixic-acid resistant, culture-positive population. Treatment failure consisted of at least one of the following: persistent fever at day 10, need for rescue treatment, microbiological failure, relapse until day 31, and enteric-fever-related complications. The primary outcome was assessed in all patients randomly allocated treatment and reported separately for nalidixic acid resistant, culture-confirmed patients, for all culture-positive patients, and for the intention to treat group.

Secondary outcome measures were fever clearance time, late relapse, and faecal carriage. The trial is registered on controlled-trials.com, number ISRCTN 63006567.

Results: 627 patients with median age 16 (IQR 9-23.5) were enrolled in the trial and randomly allocated to treatment. 170 patients had nalidixic acid-resistant, culture-confirmed enteric fever. 83 were treated with ofloxacin and 87 with gatifloxacin. 6 patients had treatment failure in the ofloxacin group compared with 5 in the gatifloxacin group (hazard ratio) [HR] of time to failure 0.81, 95%CI 0.25 to 2.65, p=0.73). The median time to fever clearance was 4.70 days (2.98-5.90) in the ofloxacin group vs. 3.31 days (2.29-4.75) in the gatifloxacin group (HR= 1.59, 95% CI 1.16-2.18, p=0.004). In addition if persistent fever on day 7 would be a treatment failure event, then there would have been 21 vs. 11 treatment failures (with 16 patients in the ofloxacin arm vs. 7 patients in the gatifloxacin arm with persistent fever on day 7: HR=0.46 (CI 0.22-0.96), p=0.04. At one month only one patient in the gatifloxacin arm was stool-culture positive and none in the ofloxacin arm. There were no other positive stool cultures later on. One blood-culture positive late relapse was noted in both arms. After day 62 no culture positive relapses were noted. 215 patients (68%) experienced an adverse event (AE) in the
olfloxacin arm and 223 patients (72%) experienced an AE in the gatifloxacin group. Most of these AEs were mild as only 5 patients needed discontinuation of therapy for presumed AE of the drugs.

Conclusion: Gatifloxacin proved to be very effective in the prompt treatment of enteric fever. Olfloxacin with adequate dosing (20 mg/kg) seems to treat enteric fever slowly but successfully.
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Risk factors for typhoid & paratyphoid fever in Kathmandu: A matched case control study

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Background: Enteric fever is endemic in Nepal and remains the most common clinical and blood culture-confirmed diagnosis of patients with febrile illness at Patan Hospital, a large public facility in central Kathmandu. Although studies conducted elsewhere have indicated contaminated food, contact with enteric fever patients and poor hygiene habits as important risk factors for infection, no specific risk factors for enteric fever have been identified in Kathmandu. The aim of this study was to identify such risk factors in order to develop targeted prevention and control measures specifically for this setting.

Methods: Outpatients between 2 and 65 years of age who were blood-culture positive for S. Typhi or S. Paratyphi A at Patan Hospital in Lalipur Submetropolitan City in Kathmandu were considered for enrolment between April – October 2011. Community controls matched for age, sex and residential ward were enrolled at a ratio of 3:1. Any potential controls with a recent history of fever, gastrointestinal disturbance or enteric fever were excluded. Questionnaires detailing clinical information as well as familial hygienic practices, eating habits and environmental and household characteristics were administered. Conditional logistic regression was used to evaluate the associations between exposures of interest and risk of enteric fever infection.

Results: Of the 103 identified blood-culture positive patients, the proportion positive for S. Typhi (48%; 49) and S. Paratyphi A (52%; 54) were roughly equivalent. When comparing risk factor exposures between S. Typhi and S. Paratyphi A cases, those with S. Typhi were more likely to use poor-quality water sources and report presence of overflowing sewage pipes in their neighborhood. When comparing cases to their respectively matched controls through multivariate conditional logistic regression, having had a typhoid contact in the 8 weeks prior to interview and having a metal covering on household water storage remained protective for both S. Typhi and S. Paratyphi A cases. Low household income (Odds Ratio [OR]: 4.1, 95% Confidence Interval [CI]: 1.1-14.9) was a significant risk factor for S. Typhi infection, whereas sourcing water from a well (OR: 0.3; 95%CI: 0.1-0.9) when the normal household source was unavailable remained protective against infection. Renting a house (OR: 5.2; 95%CI: 1.1-24.3), compared to owning it, and reported streetfood consumption in the two weeks prior to interview (OR:3.9; 95%CI:1.2-12.5) were significant risk factors for S. Paratyphi A infection.

Conclusions: It has been previously suggested that S. Typhi and S. Paratyphi A follow different transmission routes and our study provides some evidence for this theory. It is thought that S. Paratyphi A requires a higher infectious dose for clinical disease and thus it may be possible that S. Typhi is more likely to be contracted through contaminated water supply and S. Paratyphi A through contaminated food, where poor hygiene-standards allow for lengthy incubation periods for the bacteria. Additionally
we were able to identify protective behavioural differences between our cases and controls which provides palatable and feasible public health measures to reduce overall disease burden in Kathmandu.
Abstract #: 66

The Human Gut Mucosal Cognate Cellular Response to Live Oral Typhoid Ty21a Vaccination

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Background: The human gut mucosal cellular response to oral vaccination has never been directly assessed. We studied the cognate cellular immune response to the live oral typhoid Ty21a vaccine in the gut mucosa of human volunteers, and compared it with that seen in peripheral blood.

Methods: 27 healthy volunteers were randomly assigned to a vaccinated (n=14) or a control (n=13) group for Ty21a typhoid vaccine (3 doses). Peripheral blood was collected from all volunteers prior to vaccination and 18 days following immunisation or recruitment. Mucosal samples (15 jumbo biopsies from duodenum (n=25) ± colon (n=18)) were collected from all volunteers at gastroscopy +/- sigmoidoscopy on day 18. Mononuclear cells were isolated from mucosal tissue by mechanical disruption and collagenase digestion, and from blood by differential centrifugation. Cells were stimulated with heat-killed Ty21a or control antigens, and stained for surface phenotype and intracellular cytokine production. Antigen-specific IFNγ, TNFα, and IL-2 production was determined by flow cytometric analysis for CD3+/CD8+ and CD3+/CD8- (CD4+) lymphocytes.

Results: Oral immunisation with Ty21a significantly increased the proportion of antigen-specific cytokine-producing CD8 positive (p<0.05) and CD8 negative (p<0.05) lymphocytes within the duodenal mucosa, but no antigen-specific response was seen in colon. The cellular responses observed demonstrated wide functionality, with antigen-specific responses involving IFNγ, IL-2 and TNFα production, and single, double and triple cytokine-producing T-cells.

In blood, anti-LPS humoral IgG responses were seen, and antigen-specific proliferative and cytokine-producing cellular responses were seen among CD4+ cells, but not CD8+ cells.

There was clear evidence of regional compartmentalisation of the gut mucosal response, with a lack of cellular responses seen in sigmoid colon samples.

Conclusion: This is the first direct demonstration of show an antigen-specific cellular response in human gut mucosal lymphocytes after live oral vaccination, and shows broad functionality of the cellular response following oral vaccination with Ty21a.

The absence of a detectable cognate response from the colon may indicate compartmentalisation of the gut mucosal response to the embryological mid-gut, where typhoid antigen is likely presented at immune inductive sites in Peyers patches.
Further planned studies using the typhoid human challenge model would permit prospective evaluation of these and other mucosal cellular responses in relation to protection against disease, and would provide important surrogate markers for development of live oral or conjugate vaccines, and a better understanding of how to optimise mucosal responses to vaccination.
Abstract #: 70

New combination of gyrA mutation with reduced susceptibility to ciprofloxacin in Salmonella Typhi

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Background: The emergence of Salmonella enterica serovar Typhi with multi drug resistances especially resistance to ciprofloxacin and cephalosporins has been increasing day by day resulting in complicated treatment strategy in Bangladesh. Objective: The present study aims at characterization of multi-drug resistant Salmonella Typhi strains isolated from the blood culture of clinically diagnosed typhoid patients in Bangladesh during 2009–2010 by different typing methods. Method: A total of 35 serologically confirmed S. Typhi isolates were subjected to antibiotic susceptibility test and ciprofloxacin MIC, analysis of integrons and other gene cassettes, sequencing of gyrA, gyrB and parC genes and pulsed-field gel electrophoresis (PFGE). Result: 49% of the S. Typhi isolates were found to be multi-drug resistant (MDR). Three of the isolates, which showed reduced ciprofloxacin susceptibility (MIC 2 µg/ml), harbored a double mutation in the codon 83 (TCC to TAC) and 133 (GGA to GAA) in QRDR of gyrA, resulting in the amino acid substitution from Serine to Tyrosine and from Glycine to Glutamate, respectively. Two of the same isolates harbored a point mutation at codon 492 (AGC to AAC) in gyrB gene resulting in the amino acid substitution from Serine to Asparagine. Class 1 integron was found in 13 S. Typhi isolates where only one isolates contained class 2 integron. Gene cassette analysis of class 1 integron revealed the presence of dhfrA7 gene conferring resistance to trimethoprim. Neither SXT element nor class 3 or 4 integron were present. Pulsed-field gel electrophoresis (PFGE) yielded 11 different pulsotypes and cluster analysis suggested the clonal expansion of MDR S. Typhi. Conclusion: In conclusion, isolates with reduced susceptibility to ciprofloxacin might be important in clinical development of complete ciprofloxacin resistance. Continuous monitoring of ciprofloxacin MIC, the incidence of integron and clonal expansion is required for the launch of better treatment policies.

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In most cases, the cause of a febrile illness is a self limiting and presumed viral disease. However, 5–10% of febrile illnesses have serious bacterial infections such as pneumonia, urinary tract infection, meningitis, bacteraemia or typhoid infection (which usually present with fever). These bacterial conditions can be difficult to distinguish from viral infections and benefit from early antibiotic therapy. The consequences of a delayed or missed diagnosis can be serious and, occasionally, fatal for the patient in the outbreak setting.

When a patient presents with typhoid fever, the decision to commence antibiotics is based on a thorough clinical evaluation by the treating physician and often supplemented by serological testing. The reference standard for most serious bacterial diseases is a positive microbiological culture from a sterile sample such as blood, urine, stool or bone marrow. However, cultivation methods are time-consuming and furthermore demonstrate relatively low sensitivities and specificities. Consequently, culture methods cannot be used to guide the management in the acute setting.

A number of serological tests have been developed for proper diagnosis of typhoid infection, but most of them are not validated and thus not widely used in clinical practice. Furthermore, the most applied serological tests have not shown acceptable sensitivity and specificity to be applied for routine use and may seldom correlate with the clinical features of typhoid fever. Several studies have been reported comparing the use of TUBEX TF with variable results (sensitivity 60-94% and specificity 70-94%) mostly due to selection of patients for assessment of an acute illness and interpretation of Tubex results.

This literature study emphasized how TUBEX TF was evaluated with regard to different factors affecting its accuracy the appropriate use and interpretation of TUBEX TF test results. The assessment showed the importance of presenting the clinical evaluation of typhoid fever, including the use of control subjects that is regarded as the cornerstone of clinical practice, but has rarely been evaluated. Recently, the problems of missed or delayed diagnosis have been discussed, but no clear solutions provided unless an improved diagnostic tests has been developed such as TUBEX TF that is proven to be sensitive and specific for early diagnosis of typhoid infection.
Abstract #: 74

Bactericidal potential of S. Typhimurium LPS-specific antibodies from HIV-infected African Adults

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Background: Nontyphoidal Salmonellae (NTS) are a major cause of bacteraemia in sub-Saharan Africa and are often associated with HIV infection. Sera from some HIV-infected African adults have impaired ability to kill Salmonella and this is associated with high levels of anti-LPS O-antigen (OAg) IgG.

Methods: Sera were from HIV-uninfected (HIV- control serum) and HIV-infected African adults that could (HIV+ non-inhibitory serum) and could not kill Salmonella Typhimurium (HIV+ inhibitory serum). Anti-OAg antibody avidity, complement deposition on Salmonella and complement consumption were examined. The effect of antibody concentration and antibody isotype on serum killing by anti-LPS antibodies purified from the three groups of sera was explored.

Results: Impaired killing of Salmonella by some HIV-infected adult sera was not due to differences in anti-Salmonella antibody avidity or complement consumption. Impaired killing was primarily concentration-dependent and could be removed by diluting these sera while providing an exogenous source of complement. LPS-specific IgG and IgM, but not IgA, purified from African HIV-uninfected and HIV-infected adult sera were able to effect cell-free complement-mediated killing of Salmonella. Despite their bactericidal potential, both LPS-specific IgG and IgM inhibited serum killing of bacteria at high concentration, as did LPS-specific IgA.

Conclusion: Anti-Salmonella Typhimurium LPS antibodies extracted from sera of HIV-infected and –uninfected African adults have bactericidal potential, indicating the potential of anti-LPS antibodies to mediate protective immunity against NTS. The isotype-dependency of this bactericidal activity, which is restricted to IgG and IgM, suggests that vaccine design, formulation and regimes that induce antibody responses consisting predominantly of the IgG and IgM isotypes are more likely to elicit protection. However, despite their inherent bactericidal potential, LPS-specific IgG and IgM inhibited serum killing of Salmonella at high concentrations, as did LPS-specific IgA. While the antibody isotype of LPS-specific antibodies affects ability to kill Salmonella, impaired serum killing observed in HIV-infected African adults is not isotype-dependent, but concentration-dependent.
Antibody-dependent cell-mediated killing of nontyphoidal Salmonella in HIV-infected Malawian adults

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Abstract

Background

Nontyphoidal Salmonellae (NTS) are a major cause of fatal invasive disease in HIV-infected individuals in Africa. Impaired serum killing of NTS has been associated with high levels of antibodies to Salmonella LPS O-antigen (OAg) in some HIV-infected African adults. We investigated the effect of opsonisation with serum and anti-OAg antibodies from Africans on blood phagocyte-mediated killing of NTS.

Methods

Sera were from HIV-uninfected and HIV-infected Malawian adults with varying levels of anti-LPS OAg antibodies. Killing of invasive African S. Typhimurium D23580 opsonised with serum or affinity-purified anti-OAg antibodies from these sera, by washed peripheral HIV-uninfected adult blood cells was measured using in vitro blood cell killing, phagocytosis and oxidative burst assays.

Results

Blood cell killing and phagocytosis of Salmonellae opsonised with serum from some HIV-infected adults with high levels of anti-OAg antibodies was impaired, although oxidative burst induced by these opsonised Salmonellae was not affected. All sera were able to kill a galE- mutant of S. Typhimurium lacking LPS OAg. Absorption of antibodies from the HIV-infected sera with associated impaired killing using LPS prior to opsonisation increased cell-dependent killing of the bacteria. Opsonisation of Salmonellae with high titres of purified anti-OAg antibodies inhibited killing of the bacteria by blood cells. However, this inhibition was concentration-dependent and phagocyte killing of NTS was induced following opsonisation with lower concentrations of anti-OAg antibodies.

Conclusions

Antibodies to Salmonella OAg can inhibit phagocyte killing of NTS at high levels found in sera from some HIV-infected African adults. At lower concentrations, the same antibodies are opsonic and induce cell-mediated killing of NTS.
Abstract #: 76

East London Experience with Enteric Fever 2010-2012

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Background

Current policy at the Royal London Hospital and Newham University Hospital in East London is to treat suspected enteric fever empirically with ceftriaxone and subsequently determine the antibiotic susceptibility of any isolates of Salmonella enterica serovar Typhi and Salmonella enterica serovar Paratyphi. Treatment is changed as indicated by antibiotic susceptibility results. Clinical recurrence and antibiotic resistance to chloramphenicol, azithromycin and ciprofloxacin are increasingly reported in travellers returning from South East Asia with enteric fever. We report the clinical presentation and treatment of typhoid presenting at two East London Hospitals. We also determined how many UK resident patients received vaccination against typhoid prior to travel.

Methods

Microbiological records were reviewed for patients admitted with enteric fever at the Royal London and Newham hospitals over the period 2005-2012. In addition we have reviewed the clinical case notes from patients admitted during January 2010—December 2012 with a microbiologically confirmed diagnosis of enteric fever. S. typhi and S. paratyphi systemic infection was defined by at least one positive blood culture. Further information collected included demographic data, clinical presentation, laboratory parameters, travel history, treatment and outcome profiles including relapses and eradication. Enhanced surveillance was used to determine the vaccination status.

Results

For the time period 2005-2012, we identified 155 blood cultures with S. typhi from 115 patients and 82 blood cultures from 59 patients with S. paratyphi.

Review of clinical case notes (2010-2012) identified 56 patients with enteric fever; of these, 37 cases were associated with S. typhi and 19 were caused by S. paratyphi.

The median age of the patients was 23 years, (range 2-60). For the 44 patients where data was available, the median duration prior to hospital admission from date of entry to the UK was 14 days (range 1–74). Forty four out of 56 patients had recently travelled abroad and the remaining 12 were visitors or new entrants who developed symptoms in the UK. Of the 44 returning travellers, 23 were from Bangladesh, 10 from India, five from Pakistan, one from Nepal, five were from an unknown location. Of the 12 patients who were visitors or new entrants to the UK, six were from India, four from Bangladesh and two from Pakistan. Median time to clinical resolution following the onset of treatment...
was five days (range 1-14) and median time to resolution of pyrexia was five days (range 1-17). Three patients continued to have pyrexia post discharge. Forty one out of 56 patients were given ceftriaxone empiric treatment, three received other antibiotics as well as ceftriaxone empirically, one patient received azithromycin, eight received other antibiotics given empirically including gentamicin, coamoxiclav, tazocin and benzyl penicillin. In eight cases a diagnosis of enteric fever was not considered on admission and 12 patients were sent home from Accident & Emergency without a diagnosis of typhoid and only subsequently readmitted when blood cultures were positive.

Five patients were readmitted with positive blood cultures for S. typhi or S. paratyphi following a full course of treatment. In 2007, only two out of 18 isolates showed decreased susceptibility to ciprofloxacin. However in 2011, out of 14 isolates tested, 10 isolates were ciprofloxacin resistant, one was ciprofloxacin intermediate and only three isolates were fully susceptible. In 2007 only two isolates were tested for susceptibility to azithromycin and both were susceptible whereas in 2011, only one out of eight isolates tested appeared susceptible to azithromycin. Data on vaccination status from the enhanced surveillance data for 2010-2012 for patients in East London will be presented in the poster.

Conclusion
- Typhoid fever is a significant problem amongst returning travelers, visitors and new entrants to the UK from the sub-continent.
- In East London, most patients receive empirical treatment using ceftriaxone, subsequently modified according to microbiological findings.
- Clinical relapse requiring re-admission is not uncommon.
- Preliminary investigations show evidence of increasing antibiotic resistance.
Abstract #: 77

**Vi capsular polysaccharide increases resistance to complement-mediated killing while being a target of bactericidal antibody.**

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Background

The roles played both by Vi polysaccharide capsule and anti-Vi antibodies in the pathogenesis of and immunity to typhoid fever are not fully understood. Effective vaccines against typhoid fever consisting of Vi polysaccharide indicate that anti-Vi antibodies can be protective. However the mechanism of protection afforded by anti-Vi antibodies remains unclear. Vi expression has been previously shown to reduce C3 deposition and a role for Vi capsule in promoting serum resistance has been proposed.

Methods

Serum bactericidal assays (SBA) using isogenic Vi-expressing and non-expressing S. Typhi and S. Typhimurium were used to characterise the role of Vi capsule in resistance to serum killing. Antibody and complement component deposition onto the isogenic strains was measured by flow cytometry. Antibody depleted sera and sera from animals immunised with Vi polysaccharide were used in SBA to examine the role of Salmonella specific antibody in serum killing.

Results

Using the isogenic S. Typhimurium and S. Typhi strains we found that Vi expression is associated with a significant increase in resistance to antibody-mediated cell-free killing. Vi-expressing S. Typhi had significantly lower IgM binding to its surface compared with isogenic Vi-non-expressing S. Typhi. Vi expression was also associated with a significant reduction in C3 and MAC deposition. Sera from animals immunised with Vi polysaccharide were able to kill Vi-expressing Salmonellae and sera absorbed with Vi non-expressing Salmonellae were able to kill Vi-expressing isogenic strains.

Conclusions

Our findings support a protective role for Vi capsule in preventing serum killing of Salmonella that can be overcome by specific anti-Vi antibodies. This provides insights into the mechanisms by which Vi-based vaccines protect against typhoid.
Abstract #: 81


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Invasive non-typhoidal Salmonella (iNTS) is the leading cause of bloodstream infections in Sub-Saharan Africa. These infections are severe and can be life-threatening. Although extended spectrum cephalosporins (ESC) are important for the treatment of iNTS infections, the increased detection of iNTS displaying resistance to these antimicrobials is of particular concern. Amongst the NTS serovars, Salmonella enterica serotype Isangi (Salmonella Isangi) is less frequently recognised, but has the propensity to cause bacteraemia in immune suppressed individuals in South Africa. This pathogen may be characterized by increased resistance to ESC due to the production of extended spectrum β-lactamases (ESBLs), thus limiting treatment options.

In this study, ESBLs were characterized in 246 South African isolates of invasive Salmonella Isangi, from 2004 to 2009.

All Salmonella isolates received at the Centre for Enteric Diseases (CED) were serotyped and antimicrobial minimum inhibitory concentrations were determined using Etests®. The presence of ESBLs was established by the double disc diffusion screening method. ESBL genotypes (blaTEM, blaSHV and blaCTX-M) and AmpC β-lactamase genotype (blaCMY) were determined using PCR and nucleotide sequencing. Pulsed-field gel electrophoresis (PFGE) analysis was used to investigate the genetic diversity of the isolates.

For the years 2004 -2009, 259 invasive Salmonella Isangi isolates were received at the CED for surveillance purposes. Of these, 246 (95%) were confirmed ESBL producers. Susceptibility testing revealed that all of the ESBL-producing invasive Salmonella Isangi isolates showed high resistance to ampicillin, chloramphenicol, streptomycin and sulphamethoxazole; All isolates were susceptible imipenem; 99% (n=244) were resistant to tetracycline; 88% (n=217) were resistant to nalidixic acid and 95% (n=234) showed intermediate susceptibility to ciprofloxacin. BlaTEM-63 detected in 86% of the isolates was the most common ESBL genotype and blaCMY-2 detected in 49% of the isolates was the most common AmpC β-lactamase genotype. Other ESBL genotypes included blaCTX-M-15 (n=8), blaCTX-
M-22 (n=1), blaSHV-12 (n=2), blaSHV-5 (n=2), blaSHV-165 (n=1) and blaSHV-18 (n=1). Carriage of multiple ESBL genotypes was also detected and included: blaTEM and blaCMY (n=119), blaTEM and blaCTX-M (n=9), blaTEM and blaSHV (n=3), and blaTEM, blaSHV and blaCMY (n=1). PFGE analysis showed a diversity of 26 patterns amongst the isolates.

The production of ESBL by the greater number of invasive Salmonella Isangi isolates is a concerning public health problem in South Africa. This emphasizes the need for continued surveillance and promotion of correct usage of antimicrobials to halt further spread of these bacteria.
Salmonellosis: Magnitude of problem in and around Kolkata, India

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Background:
Salmonellosis, the infections caused by Salmonella spp is broadly categorized into four groups: 1) Gastroenteritis (most frequent manifestation) 2) Enteric fever 3) Bacteraemia with or without extra-intestinal manifestation 4) Asymptomatic carrier state. The association of various Salmonella serovars with disease manifestations and global regions is rather interesting. Salmonellae associated with human diseases are generally divided into human-restricted invasive typhoidal serotypes (eg, S. Typhi [ST] and S. Paratyphi A [SPA]) and non-typhoidal Salmonella (NTS) serotypes with wide vertebrate host range usually causing gastroenteritis. The overall data, however, suggests that NTS is a far less significant problem than typhoid fever in Asian countries including India. This study was undertaken to determine the magnitude of problem of salmonellosis in and around Kolkata representing the Eastern parts of India.

Methods:
Blood and stool samples were systematically collected from fever and diarrhea patients respectively attending Govt hospitals in Kolkata during 2009-2012. Environmental samples comprising of feces and meat from local live stocks (poultry, goat, cattle) were also included in the study. All samples were processed for isolation and identification of Salmonella serovars at the Bacteriology laboratory of NICED following standard procedures. Antimicrobial susceptibility profiles of the Salmonella isolates were determined following standard guidelines. Mechanisms of resistance and transferability of resistance were also explored. Data were entered and results were analysed using suitable software programs.

Results:
In blood samples, isolation rate of S. typhi (6%, 24/310) was significantly higher than that of S. Paratyphi A (0.96%, 3/310). NTS were isolated from 1.5% (5/334) of clinical (stool) samples and 15% (52/337) of environmental samples, S. Typhimurium and Kentucky being the predominant serovars among NTS, isolated predominantly from poultry samples.

Antimicrobial resistance (AMR) profiles of ST showed multidrug resistance (MDR, resistance to ampicillin, chloramphenicol, cotrimoxazole) in 14% of isolates, which were encoded by bla-tem, catA and sul2 genes respectively. All isolates except one was resistant (96%) to nalidixic acid (NAR) and decreased susceptibility to ciprofloxacin (DSC, MICCIP≥0.125µg/ml) was observed in 76% ST isolates leading to treatment failure cases. High level of resistance (MICCIP≥4µg/ml) to ciprofloxacin (CIP) was obtained in 17% of ST isolates. All (100%) SPA isolates showed NAR and DSC. Mutations in QRDRs of
gyrA and ParC genes were shown in CIP resistant isolates. A heavy plasmid (212kb) of IncHI type was responsible for transfer of drug resistance in ST. Among NTS, drug resistance was higher in clinical isolates than the environmental strains. Not all serovars were equally prone to develop AMR. S. Worthigton, Seftenberg, Kentucky isolates developed more AMR than S. Weltevreden, Bareilly etc. Resistance to third generation cephalosporins and fluoroquinolones was shown to be transferable in NTS by IncFIA-FIB plasmid types and by many other mobile genetic elements like integrons, insertion sequences and transposons etc. Occasional food associated outbreaks of diarrhoeal diseases caused by NTS has been reported from India.

Conclusions:

Rapid development and spread of AMR in Salmonella spp. is a major threat and cause of concern in India. Uncontrolled and widespread use of antimicrobial agents in animal feed and clinical practice may be responsible for such outcome. More controlled use of antimicrobials for human health and animal industries are recommended. Additionally, continued surveillance of AMR of Salmonella spp is mandatory for controlling the spread of resistant organisms.
Abstract #: 84

O-antigen of invasive African nontyphoidal Salmonella is a major target of bactericidal antibodies

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Background: A key role for antibodies has been identified in cell-free complement-mediated bactericidal activity against nontyphoidal Salmonellae in Africans. Determining the antigen targets of these bactericidal antibodies is a significant consideration in developing a vaccine against Salmonellae. The O-antigen of lipopolysaccharide is an obvious candidate for the development of a glycoconjugate vaccine, but the discovery of inhibitory anti-O-antigen antibodies in HIV-infected Africans has raised questions about the suitability of O-antigen as a vaccine target.

Methods: We used a range of invasive African Salmonella isolates, laboratory Salmonella strains and constructed Salmonella lipopolysaccharide mutants to adsorb specific-antibodies from human serum in order to better understand the targets of bactericidal antibodies. The effects of adsorption on specific-antibodies and complement deposition were assessed by flow cytometry, ELISA, and hemolytic and serum bactericidal assays.

Results: Adsorption of serum with Salmonellae produced an effective serovar-specific removal of both antibodies against surface elements of Salmonella and bactericidal activity. Adsorption did not adversely affect the activity of complement. Salmonellae lacking O-antigen failed to remove Salmonella serovar-specific antibodies and killing of their parent strains. S. Typhimurium and S. Enteritidis O-antigen chimeric strains, expressing either native or heterologous O-antigen (O:4 or O:9), demonstrated that removal of antibodies and bactericidal activity against O:9-expressing strains was entirely dependent on the O-antigen of the adsorbing strain rather than other serovar-specific antigens. O:4-expressing strains showed a similar, but less pronounced effect.

Conclusions: These data indicate that anti-O-antigen antibodies are key effectors of complement-mediated killing of invasive African nontyphoidal Salmonellae. This supports the development of an O-antigen based vaccine against Salmonella."
Abstract #: 86

Host and clinical characteristics of Salmonella typhi and non-typhi among children in urban Bangladesh

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Background: Diarrhoea due to Salmonella typhi and non-typhi among children has been less often reported. However, both of them are responsible for diarrhoea especially among individual with compromised immunity. Available comparative information regarding socio-demographic as well as host and clinical characteristic are lacking. Thus, we aimed to describe comparative socio-demographic, host and clinical features between Salmonella typhi and non-typhi gastroenteritis among 0-19 years old individuals urban individuals in urban Bangladesh.

Methods: In total, 27,373 under-5 children and 7,224 aged 5-19 years were enrolled into the Diarrhoeal Disease Surveillance System during 1993 to 2011. Of them, 70 (0.3%) typhi and 337 (1.2%) non-typhi cases belonged to under-5 years old, and 51 (0.7%) and 97 (1.3%) were aged 5-19 years and confirmed by stool culture. Salmonella typhi constituted as cases; whereas, non-typhi served as comparison group.

Results: Significant difference in mean age [22 vs. 13 months; mean difference-9.0 months (95% CI- 5.24, 11.96) p<0.001] was observed among under-5 children between Salmonella typhi and non-typhi. Children with Salmonella gastroenteritis were more underweight [73% vs. 58%; 1.87 (1.02, 3.45) 0.041] presented with fever (≥38.0 degree centigrade) [23% vs. 13%; 2.08 (1.04, 4.14) 0.037]. On the other hand, individuals aged 5-19 years also presented with fever [22% vs. 7%; 3.54 (1.16, 11.03) 0.023], less vomiting [71% vs. 90%; 0.28 (0.10, 0.73) 0.007], duration of diarrhoea more than 24 hours [61% vs. 41%; 2.21 (1.05, 4.69) 0.037] and less often had macrophage in stool [31% vs. 40%; 0.27 (0.15, 0.50) <0.001] compared to non-typhi group. Two seasonal peaks of Salmonella typhi were observed during the months of April to June and September to November; however only one peak was observed in June-August for non-typhi.

Conclusions: Significant differences in host and clinical characteristics were observed between Salmonella typhi and non-typhi individuals.
Abstract #: 87

"Non-typhoid Salmonella (NTS) isolated from stool of rural under-5 children presented with moderate-to-severe disease" (MSD)

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Background: Non-typhoid Salmonella (NTS) is responsible for gastroenteritis. It is also associated with systemically invasive disease and bacteremia particularly in immunocompromised patients. There is lack of evidence-based information about NTS infection in under-five children with moderate-to-severe disease (MSD) in rural Bangladesh. We aimed to describe the presenting characteristics of children with MSD whose stool specimen yielded growth of NTS.

Methods: From December 2007 to December 2010, a total of 3,859 under-5 children were studied. Of them, 1,394 presented with MSD and NTS was isolated from 42 (3%) of them. 2,465 were age-sex-community matched healthy controls while NTS was isolated from 29 (1%) of them. Of the two comparison groups, one had 165 (12%) MSD children without presence of any enteric pathogen in the stool, and another group contained 740 (30%) healthy controls with sterile stool specimen.

Results: Nearly eighty percent of the MSD children with NTS had dysentery, fever, abdominal pain, and rectal straining. Children aged 0-11 months were 3 times more likely to develop diarrhea with NTS than their sterile healthy controls [OR=3.23, (95% CI; 1.62, 6.49)]. Such infection significantly decreases with increases age [0.40, (0.17, 0.92)]. MSD children with caretakers washing hands with soap were less likely to be infected with NTS compared to MSD children [0.33, (0.14, 0.78)] and healthy controls but had sterile stool [0.39, (0.19, 0.82)]. Children whose caretakers used only water for cleaning their bottom were 5 times more likely to have NTS infection compared to those MSD children whose stool was free from any enteric pathogen [5.33, (1.34, 21.60)].

Conclusions: NTS was often detected in stool specimens of infants, clinical features of dysentery were common among NTS infected children, and optimal hygienic practices may prevent spread of the disease.
Salmonella Blood-Stream Infection in Malawi: A fall in Nontyphoidal Salmonella and an outbreak of Typhoid

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Background: Blood-stream invasive Nontyphoidal Salmonellae (iNTS) have, until recently, been the most common cause of blood stream infection (BSI) in Malawi and throughout Sub-Saharan Africa. Risk factors include malaria, malnutrition and HIV in children and HIV in adults. Mortality is high and HIV infected survivors experience frequent relapse. In other African settings, successful malaria control has been associated with dramatic falls in iNTS. It is hoped that the rollout of anti-retroviral therapy (ART), malaria control interventions and improved food security will contribute to a reduction in the incidence of iNTS. In contrast, Typhoid fever has been uncommon in Malawi, with an average of 13 cases per year between 2000 and 2009.

Methods: Queen Elizabeth Central Hospital (QECH) is the largest hospital in Malawi. Routine surveillance for BSI has been conducted here through a quality-controlled blood culture service for over 15 years. From 2009 – 2012 a cohort of HIV infected adults with iNTS disease was recruited and followed up for 1 year and clinical outcomes were compared to those of a 2001 cohort that pre-dates the rollout of ART.

Results: Isolation of iNTS at QECH has declined by 85% (p=<0.001) since a peak in 2003, despite no significant decline in malaria, but during which time over 500,000 Malawians have initiated ART. Adult in-patient mortality from iNTS disease has declined from 47% in 2001 to 8% in 2010-11 and 1-year survival has also markedly improved (from 23% to 70%). This is part of a continual fall in mortality also observed in a cohort between 2001 and 2008. Relapse becomes infrequent following early initiation of ART, dropping from 43% of survivors to 5%. Since 2011 there has been a sustained outbreak of Typhoid, with 68 cases in 2011 and 191 in 2012.

Conclusions: There has been a significant fall in the isolation of NTS at QECH, Malawi. This has occurred in the absence of a comparable fall in malaria in children and against the background of highly effective ART rollout in adults. In the last 2 years there has been a sustained outbreak of Typhoid in Blantyre and, in 2012, S. Typhi was more commonly isolated at QECH than iNTS Typhimurium for the first time since surveillance began.
Abstract #: 89

Mechanism of serum killing of Salmonella and inhibition of killing by sera of HIV-infected Africans

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Background: Antibodies are important for protection against invasive nontyphoidal Salmonella disease in Africa. Roles for complement-fixing bactericidal and opsonising antibodies have been identified, although high-titer antibodies specific for Salmonella O-antigen are associated with inhibition of Salmonella-killing in HIV-infected Africans. Elucidating the mechanisms of antibody-dependent killing and inhibition is important for the design of a safe and effective Salmonella vaccine for Africa.

Methods: To investigate this inhibition and visualize serum-mediated Salmonella-killing, we exposed invasive African Salmonellae to HIV-uninfected bactericidal (bactericidal) and HIV-infected inhibitory (inhibitory) serum. We used electron microscopy, immuno-gold labeling, and flow cytometry to image locations and quantities of antibody binding and complement deposition on both serum-damaged and intact Salmonellae.

Results: Consistent with viable counts from serum bactericidal assays, inhibitory serum had substantially fewer damaged cells than bactericidal serum. Hallmarks of serum damage were: widening of the periplasmic space, contraction and disruption of cytoplasm integrity, breaks in both outer and inner membranes and an associated release of cytoplasm and formation of ghost cells. Binding of IgG, IgM and IgA, and deposition of C3 and C5b-9 membrane attack complex (MAC) were associated with outer and inner membranes, as well as sites distal from the bacterial membrane. Damaged Salmonellae all had C3 and MAC deposition on the outer and inner membranes, whereas many healthy Salmonellae did not. Surprisingly, electron microscopy revealed inhibitory serum deposited significantly less MAC on Salmonellae than bactericidal serum, despite normal complement function.

Conclusions: Our findings provide insight into the mechanism of killing of Salmonella by serum antibodies in vitro and indicate that the lack of killing observed with serum from some HIV-infected Africans is associated with impaired antibody-dependent MAC deposition.
Abstract #: 91

Is there a scope for developing a useful tool for diagnosing Typhoid fever in endemic countries?

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Background:

Typhoid fever remains a common enteric illnesses in tropical countries, including Bangladesh. Although blood culture remains an ideal tool for confirmation of the pathogen, access to laboratory and affordability of blood culture are limited in many impoverish settings. Use of a valid bed side diagnostic tool can assist clinicians treating typhoid fever at an early stage. We compared performance of two rapid diagnostic tests for typhoid fever in an urban slum in Dhaka city Bangladesh.

Method:

Blood samples were obtained for culture from 867 febrile patients (axillary temperature >38°C) who participated in an active febrile surveillance. Two different versions of Typhidot kits (Typhidot and TYPHIRapid) developed by Malaysian Bio-Diagnostics Research Sdn. Bhd, Malaysia and two different versions of Tubex kits (TubexTM and TUBEX®TF) developed by IDL Biotech AB, Sweden were applied to patient sera in the laboratory in order to detect the presence of anti-O-9 antibodies (IgM). Blood culture-confirmed cases of typhoid fever were consider as typhoid-positive, and blood culture-negative patients along with other bacteremias were considered typhoid-negative. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were compared between TubexTM and TUBEX®TF, and between Typhidot and TYPHIRapid in comparison with positive and negative blood cultures.

Results:

TubexTM test was 60% sensitive and 64% specific, with 14% PPV and 98% NPV. TubexTF test was 50% sensitive and 85% specific with 16% PPV and 97% NPV. Typhidot was 67% sensitive and 55% specific, with 13% PPV and 99% NPV. TYPHIRapid test was 42% sensitive and 87% specific with 16% PPV and 96% NPV. When the analysis was restricted to specimens obtained from patients with duration of fever for more than 3 days or ≥7 days at the time of presentation with fever, the PPV of all four tests was slightly higher than previous calculations, but other values were similar. According to the technicians all four tests were easy to perform and results obtained within a short time.

Conclusion:

None of the Tubex and Typhidot tests was found useful for the diagnosis of typhoid fever in a community clinic in urban Bangladesh where typhoid fever is endemic. However, specificity of TubexTF and TYPHIRAPID potentially improved compared to TubexTM and Typhidot, and both tests were easy to perform, indicating scopes of improvement. Industries need to invest more in developing a better
test that are useful for bed side use for the early detection of typhoid fever in the typhoid endemic countries.
Will introduction of a typhoid vaccine in the EPI program be cost effective in Bangladesh?

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The incidence of typhoid fever is twelve times higher among children <5 years of age than the incidence in older people in urban areas in Bangladesh, and causes frequent hospitalization in children. Considering multiple risk factors associated with typhoid fever including water, sanitation, food hygiene, hand washing behaviors etc. a typhoid vaccine may be a feasible prevention measure for reducing the economic burden of typhoid fever in health facilities in Bangladesh. We evaluated incremental cost effectiveness ratio (ICER) of Ty21 or Vi Polysaccharide vaccines compared to no typhoid vaccine strategy in the expanded program of immunization (EPI) from the health systems perspective in Bangladesh.

Method

The incidence of typhoid fever at health facilities was estimated from a population based surveillance conducted among children<5 years of age in urban and rural areas between 2004 and 2008. The cost of health facility for treating children with suspected typhoid fever illnesses, cost of EPI, and disability adjusted life years (DALY) due to typhoid fever were estimated with a typhoid vaccine (BCG+ OPV+ hepatitis B +Hib + measles vaccines + Ty21 or Vi polysaccharide) and without a typhoid vaccine (BCG+ OPV+ hepatitis B +Hib + measles vaccine) strategy for year 2011 in a cohort of 1000,000 children <5 years (base case). A vaccine was considered cost effective if the ICER was less than three times the gross domestic product (GDP= US$755.00) per capita of Bangladesh or (3x$755.00) $2265.00.

Results

The estimated incidence of typhoid fever per 100,000 in children <5 years of age was 834.61 without a typhoid vaccine strategy, 598.38 with a Ty21 vaccine and 464.05 with a Vi polysaccharide vaccine strategy. The total cost of treating suspected typhoid fever at health facilities was US$4,642,560 (at $74.88/episode) without a typhoid vaccine, US$4,609,126 (at $74.63/episode) with a Ty21 vaccine and US$4,584,401 (at $74.39) with a Vi polysaccharide vaccine. Cost of EPI was estimated US$485,278 without a typhoid vaccine strategy, US$751,409 with a Ty21 vaccine strategy and US$574,723 with a Vi polysaccharide vaccine strategy. Typhoid fever would attribute to 155 DALYs without a typhoid vaccine, 83 DALYs with Ty21 vaccine and 70 DALYs with Vi polysaccharide vaccine. The ICER of Ty21 vaccine was US$3,448.50 and of Vi polysaccharide vaccine was US$723.17 per DALY averted compared to no typhoid vaccine strategy.

Conclusion

Introduction of Vi polysaccharide vaccine in the current EPI program would be cost effective in Bangladesh.
Abstract #: 95

Computational analysis of the Typhoid holotoxin CdtB, pltA and pltB

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Background: Typhoid fever and other invasive salmonelloses occur due to the invasion of the pathogenic bacteria Salmonella typhi. It results millions of death annually in regions where infections are endemic. Salmonella typhi unlike other Salmonella species only infect humans and found persistent, often life time colonization by establishing residence most commonly within gall bladder. Scientists and health professionals are trying their best to combat with this virulent infection. Multi-drug resistant Salmonella typhi strains are making the treatment process more critical. Despite its public health importance, relatively little is known about the virulence factors that confer the bacterium its unique pathogenicity. Earlier it was reported that Cytolethal distending toxin B (CdtB) is the key virulence factor which causes cell cycle arrest and cellular distention conferring DNA damage. Unlike other bacteria coding CDT, Salmonella typhi does not code for A and C (i.e., CdtA, CdtC) subunits homolog of CdtB. Spano et al, 2008) reported that pltA and pltB which are present in the same pathogenic island encoding CdtB are also essential for the incorporation of typhoid toxin into host cell. CdtB, PltA, and PltB assemble to form a multipartite toxin.

Methods: In this experiment we generated the three dimensional structure of the typhoid toxin components by homology modeling approach. Evaluation, improvement and validation of the 3D model done by analyzing Ramachandran field plot, RMSD value and GROMOCS energy minimization procedure. Functional site detection and disulphide bond position was also predicted.

Result and Conclusion: 3D structure and Phylogenetic analysis of the holotoxin showed their unique characteristics compared to a broad range of microorganisms. Activity of the typhoid toxin for pore formation and invasion into cells will be more obvious from their 3D structures. 
Comparison between Conventional Culture and PCR Based Method for Isolation and Identification of Salmonella

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Background: Salmonella species is one of the dominant etiological agents in diarrheal patients in Bangladesh. To determine the disease burden due to Salmonella infection, conventional culture method is used in Bangladesh. It has been established that only conventional culture method is not sufficient to detect Salmonella infection. We have established round the clock diarrheal disease surveillance system in Kumudini Hospital, Mirzapur to determine the etiology of moderate-to-severe disease (including dysentery) and milder diarrhea in all age group in general. Comparative studies on detection of Salmonella spp. from stool samples of diarrhoeal patients was performed using PCR based method as well as conventional culture method to understand the actual burden of Salmonella infection. We hypothesized that culture method is not enough to report the actual disease burden.

Method: Eighteen hundred sixty three stool samples were collected from diarrhoeal patients attending Kumudini Hospital, Mirzapur between July, 2011 and November, 2012. All these samples were screened for the presence of Salmonella species using culture method and PCR for invA (invasion gene) specific for all serovars of Salmonella. Drug susceptibility was determined by disk diffusion following CLSI guidelines. Plasmid profiling is done following Kado and Liu method with some modifications.

Result: Of 1863 samples, Salmonella spp. was isolated from 65 (3.5%) samples by culture method whereas 80 (4.3%) samples were positive for invA gene. Of 65 culture positive isolates, 25 (38.5%) was identified as Salmonella Group B, 32 (49.2%) were identified as Salmonella Group C1, 4 (6.2%) was identified as Salmonella Group C2 and 1(1.5%) was identified as Salmonella Group D and 3 (4.6%) were other Salmonella spp. The additional 15 (~1.0%), invA positive but culture negative samples were under reported due to the limitation of traditional culture method. Antibiotic resistance profile showed that only 5 (7.7%) strains were multi drug resistance (resistant to Ampicillin, Cotrimoxazole or/and Nalidixic acid) but none were resistant to Ceftriaxone and Ciprofloxacin. Of 65 isolates, 12(18.5%) strains contain single middle range plasmid except one which contains two small plasmids. Plasmid analysis showed that 5 resistant strains contain plasmid, 2 resistant strains contain no plasmid and 6 plasmid containing strains showed no resistance to commonly used antibiotics. Which indicate that the drug resistance is not only associated with plasmid also chromosomal involvement is responsible for emergence of Multi drug resistance (MDR) strains.

Conclusion: Characterization of the invA gene positive isolates is going on. These may be known serotype of Salmonella or new variants of Salmonella spp. which surely help to estimate the actual disease burden due to Salmonella infection.
Abstract #: 97

**Structural variability of Salmonella Typhimurium and Salmonella Enteritidis O-antigens.**

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"Background. Salmonella Typhimurium and Enteritidis are a major cause of invasive nontyphoidal Salmonella (NTS) infections in Africa, with high mortality among children and HIV infected adults. Currently, no vaccine is available against NTS disease in humans. Antibodies directed against the O-antigen of the lipopolysaccharide (LPS) molecule of Salmonella are protective and conjugation of the O-antigen to a carrier protein represents a possible strategy for vaccine development.

Methods. O-antigens were purified from different S. Enteritidis and S. Typhimurium strains and fully characterized, using analytical methods including HPLC-SEC, HPAEC-PAD, GC, GC-MS, mono- and bi-dimensional NMR. All purified O-antigens were covalently linked to CRM197 as carrier protein, using a selective conjugation chemistry through the terminal KDO sugar.

Results. S. Enteritidis O-antigens had similar molecular weight distributions and O-antigen chain structure. S. Typhimurium O-antigens had similar average chain length distributions, but differed with respect to glucosylation and O-acetylation levels and positions. Corresponding conjugate vaccines were synthesized retaining the O-antigen chain structure in an unaltered form.

Conclusions. Salmonella LPS molecules demonstrate heterogeneity within individual serovars and an improved understanding of the impact of O-antigen structural features on the immunogenicity of corresponding conjugate vaccines could help determine the design the optimal candidate vaccines."
Abstract #: 98

Non-typhoidal Salmonella gastroenteritis at a diarrheal hospital in Dhaka, Bangladesh, 1996-2011

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Non-typhoidal Salmonella (NTS) is a cause of gastroenteritis worldwide, with high rates of invasive disease in sub-Saharan Africa. Data on non-typhoidal Salmonella (NTS) infection in South Asia are sparse. We used data gathered prospectively from 1996 to 2011 as part of a hospital surveillance system in Dhaka, Bangladesh, to identify diarrheal patients with NTS isolated from stool. NTS was isolated in 1.3% (468 of 37,439) of diarrheal patients. 47% of total cases of NTS were in children <5 years of age, though older adults (≥60 years) had the highest isolation rates. NTS isolation peaked in the monsoon months of July and August. Over the study period, rates of resistance to ampicillin, chloramphenicol, and ceftrixone decreased, while rates of decreased susceptibility to ciprofloxacin increased. Compared to control patients, NTS patients were older and wealthier; however, no differences in type of housing or exposure to animals were found. NTS patients also had increased inflammatory cells in stool and required more fluid resuscitation. Routine evaluation for bacteremia is not part of the surveillance system, but NTS is not a common cause of bacteremia at the icddr,b (0.6% of all positive blood cultures in 2009-2011). In conclusion, NTS is isolated in stool cultures of cases of gastroenteritis requiring hospital care in Dhaka, Bangladesh, with a high burden in young children and the elderly, but is not a common cause of bacteremia.
Abstract #: 99

*Immune response-driven design of glycoconjugate vaccines against NTS*

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Background. Nontyphoidal Salmonella (NTS) serovars Typhimurium and Enteritidis are a major cause of fatal invasive disease in Africa, affecting mainly children and HIV-positive adults in developing countries. Conjugate vaccines provide a safe and reliable strategy against invasive polysaccharide-encapsulated pathogens and lipopolysaccharide has been indicated as possible target of protective immune response.

Methods. We have synthesised different glycoconjugate vaccines against S. Typhimurium and Enteritidis, by covalently linking O-antigen and core sugars (OAg) to the non-toxic mutant of diphtheria toxin (CRM197). OAg-CRM197 conjugates varied for: 1. OAg source, by employing different NTS strains producing OAg with specific structural differences; 2. OAg chain length; 3. OAg to CRM197 ratio. All conjugate preparations were used to immunize mice and were compared for immunogenicity and induced serum bactericidal activity.

Results. We obtained results from S. Typhimurium glyconjugates (six conjugates were OAg strain-specific; four contained OAg from the same strain but varied for OAg chain length and OAg/CRM197 ratio) and S. Enteritidis glyconjugates (four OAg strain-specific conjugates). NTS glycoconjugates were immunogenic and able to elicit bactericidal antibodies. Immunogenicity was determined by glycoconjugates characteristics and fine specificities of anti-OAg antibodies were determined by NTS strain used as OAg source. NTS glycoconjugates elicited cross-reactive bactericidal antibodies against different S. Typhimurium endemic strains, while S. Enteritidis strains were more resistant to bactericidal killing by vaccine-induced antibodies.

Conclusions. OAg fine specificities can influence glycoconjugate immunogenicity and bactericidal activity. To design the most appropriate conjugate vaccine against NTS, important factors such as the OAg strain source need to be taken into consideration.
Abstract #: 100

Mechanism of Fluoroquinolone Resistance in Salmonella Typhi

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Background:

Enteric fever, comprising both typhoid and paratyphoid fevers, appears to be a major public health problem even after the development and advent of newer antimicrobial drugs. Ciprofloxacin, a fluoroquinolone antimicrobial agent is generally highly effective in treating enteric fever. We report the emergence and characterization of ciprofloxacin resistance Salmonella Typhi in Bangladesh. The aim of this study was to characterize the molecular mechanism of fluoroquinolone resistance in Salmonella Typhi strains recently isolated in Bangladesh.

Methods:

In our study, 765 S. Typhi strains isolated at the Clinical Microbiology Laboratory of ICDDR,B between 2006 and 2010 were screened for susceptibility against commonly used antibiotics such as ampicillin, chloramphenicol, sulfamethoxazole-trimethoprim, nalidixic acid and ciprofloxacin by disk diffusion method. MIC was determined by E-test following the recommendations of Clinical and Laboratory Standards Institute (CLSI). Plasmid profiling, PFGE and sequencing analysis were performed in order to determine the clonal relationships and mutations in quinolone resistance-determining region (QRDR) to investigate the resistance mechanism against fluoroquinolone antibiotics.

Results:

Of these 765 S. Typhi strains, 474 (62%) strains were resistant to nalidixic acid. Of the 474 nalidixic acid resistance strains, 402 (85%) were classified as intermediate susceptible to ciprofloxacin. The isolates classified as intermediate susceptible to ciprofloxacin had MICs ranging from 0.064-0.25 μg/ml as opposed to those classified as ciprofloxacin resistant who had MICs ranging from 6-32 μg/ml. Antimicrobial susceptibility of S. Typhi showed that 60%, 61%, and 60% strains were resistant to ampicillin, chloramphenicol, and sulfamethoxazole-trimethoprim respectively. Plasmid analysis showed that most of the multi drug resistance strains contain plasmid and susceptible strains were plasmid less. Conjugation experiment suggested that the middle ranged plasmid (35 MDa) were self transferable but resistance to ciprofloxacin was not plasmid mediated. Sequence analysis of QRDR of resistant strains revealed that all had mutations in gyrA (Ser83 ® Phe) and/or (Asp87 ® Asn or Gly) and a single mutation in parC (Ser80 ® Ile) whereas none of the susceptible strain had the mutation in their QRDR region. The multi drug resistant (Amp, Chl, Sxt, Nal)R strains appeared to be clonally related as only a single pattern A was observed in these strains by PFGE.
Conclusions:

The present study reports the mutation in the QRDR of fluoroquinolone resistant S. Typhi from Bangladesh. The decreasing susceptibility of S. Typhi for ciprofloxacin is a worrying phenomenon that has great impact on the empirical treatment of typhoid fever.
Abstract #: 101

Molecular Characterization of Multidrug Resistant Salmonella Typhi Strains Isolated in Dhaka, Bangladesh

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Background: Typhoid fever occurs in more than 20 million people and causes approximately 200,000 deaths annually. More than 80% of typhoid fever cases are estimated to occur in Asia. The challenges of reliably diagnosing typhoid fever have led to varying estimates of the disease burden and epidemiology in Asia. The recent increase in fluoroquinolone resistance of Salmonella enterica serotype Typhi raises concerns due to limited treatment options available in typhoid endemic countries.

Methodology: A total of 11,364 strains of Salmonella specie isolated in the Clinical Microbiology Laboratory of icddr,b between January 1997 to December 2011 using standard microbiological and biochemical methods. Drug susceptibility was determined by disk diffusion and E-test, following CLSI guidelines. Representative strains were characterized using plasmid profiling, PCR, PFGE, hybridization, and sequencing analysis in order to determine the clonal relationships and mutations in quinolone resistance-determining region (QRDR). Extended-spectrum β-lactamase (ESBL) producers are extensively characterized using double disc diffusion synergy test and conjugation experiment.

Results: Of 11,364 strains of Salmonella specie isolated at icddr,b, S. typhi was the most predominant except the years 1997-99 when it was replaced by Salmonella group B, followed by Salmonella Group C1, others, and S. enteritidis. The isolation rate of S. Typhi was increased from 21.7% in 1997 to 70% in 2009 but it decreases from 2010. Recently in 2011 the isolation rate of S. Typhi was 21%. Whereas the isolation rate of Salmonella Group B was decreased from 69% in 1997 to 10% in 2010. Resistance to all first line drugs (ampicillin, chloramphenicol, sulfamethoxazole-trimethoprim) of S. Typhi isolated between 1997 and 2011 increased from 31% in 1997 to 65% in 2008 but after that the isolation of MDR S. Typhi were decreased 25-30% in 2011. Of Salmonella strains, 4% strains were found as ESBL producers. The prevalence of ESBL producers was very high in Salmonella Group B (2%) and Salmonella Group G (2%). Only one strain isolated in 2011 of the S. Typhi strain was positive for ESBLs. All strains isolated before 1999 were susceptible to nalidixic acid (Nal), but resistance to Nal increased from 4% in 1999 to 98% in 2011. The MIC to ampicillin, trimethoprim and streptomycin was >512 mg/L, whereas for chloramphenicol, tetracycline and nalidixic acid it was >256 mg/L, >128 mg/L and >256 mg/L, respectively. Of the Nal resistance strains 90-95% showed reduced susceptibility to Ciprofloxacin (MIC 0.25-0.5 mg/L) and only few strain (n=12) showed complete resistance to ciprofloxacin. All MDR strains except five harbored 120 MDa and/or 90 MDa plasmid whereas 90% susceptible strains contained no plasmid. Only the strains harboring 120 MDa plasmid belonged to the IncHI1 group. Conjugation suggested that 120 MDa plasmid harbored a self-transmissible multiple antibiotic resistance marker. All
MDR strains were positive for TEM-1, strAB, tetB, chloramphenicol acetyltransferase type I, dihydrofolate reductase type VII genes encoded resistance to ampicillin, streptomycin, tetracycline, chloramphenicol and trimethoprim respectively. TEM-1 probe hybridized with the chromosomal DNA of the plasmid less MDR strains whereas it hybridized only with plasmid DNA (120 MDa) in case of plasmid bearing strains. Thus MDR is not always necessary to be plasmid-mediated. All MDR strains had indistinguishable PFGE patterns whereas susceptible strains were heterogeneous. Sequence analysis of QRDR of resistant strains of S. Typhi and par Typhi A revealed that all had mutations in gyrA (Ser83 → Phe) and/or (Asp87 → Asn or Gly) and a single mutation in parC (Ser80 → Ile) whereas none of the susceptible strain had the mutation in their QRDR region.

Conclusion: Continuous monitoring and better understanding on the molecular epidemiology and mechanisms of multidrug resistance especially fluroquinolones and third generation cephalosporin will help for the development of new antimicrobial strategies, to stop or reduce the amount of horizontal transfer of antibiotic resistance markers at the intra and inter species level among the enteric pathogens, as well as to resolve health care problem.
Abstract #: 102

Incidence of Salmonella in street vended juice samples and a comparative characterization with clinical strains

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Background: Salmonella are recognized as one of the main causes of food poisoning worldwide. As Bangladesh is the endemic zone for Salmonella, we hypothesized that there might be a possible correlation between the pathogen and their transmission in the food.

Objective: The study was undertaken with a view to isolate Salmonella species from food samples and to characterize these strains using phenotypic and genotypic traits to understand the molecular epidemiology.

Methodology: A total of 103 fruit juice samples were collected over a period of eight months (from June 2006 to February 2007) from different vendors located in different areas in central of the Dhaka city. These vendors were selected because they operated under perceived high-risk conditions with respect to juice preparation, holding and serving practices and exhibited a noticeable lack of personal hygiene. Such juices have shown to be potential sources of bacterial pathogens notably E. coli, species of Salmonella, Shigella and Proteus. Standard methods were used to determine the presence of these pathogens in the samples. Thirty eight strains of Salmonella isolated from patients attending the Dhaka treatment center of the ICDDR,B, between 2006 and 2007, were included for comparative study. All the strains were characterized extensively using serotyping, antibiotic resistance analysis, plasmid profile analysis, and pulsed-field gel electrophoresis (PFGE) for a comparative study to determine the clonal relationship with the clinical strains.

Result: The extent of Salmonella detected in fruit juice is of significance. Eleven strains (10.6%) of Salmonella species were isolated from street vendor juice samples. Salmonella isolated from fruit juice belong to 4 different serovars, 36% (4/11) strains were identified as Salmonella paratyphi B, 9% (1/11) as Salmonella Group B, 27% (3/11) as Salmonella Group C1, 18% (2/11) as Salmonella Group C2 and 9% (1/11) as Salmonella Group H. Antibiotic susceptibility test revealed that most (72.7%) of the strains isolated from juice samples were sensitive to all the antibiotics tested. Only three strains (27.3%) were resistant to Ampicillin whereas around 13.2% clinical isolates were multidrug resistant. Heterogeneous plasmid patterns were observed among the strain isolated both from juice and clinical samples. PFGE showed that Salmonella paratyphi B isolated from juice sample and patients had an identical PFGE pattern, which suggests close genetic relatedness, indicating possible route of transmission of the strain from juice to patients. Heterogeneous PFGE pattern were yielded in case of other serovers.
Conclusion: Overall, the study reflects the prevalence of Salmonella in street juice samples and the possible route of transmission of these diseases causing organism to human individuals.
Abstract # 103:

Extended-Spectrum l-lactamase (ESBL) Producing Salmonella Species Isolated from Diarrheal Patients

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Background: Resistance of Salmonella to extended-spectrum cephalosporins (ESCs) especially third-generation cephalosporins (ceftaxone) are being reported with increasing frequency worldwide. Infections with Salmonella resistance to ESCs threaten the efficacy of ceftriaxone, the drug of choice for treating samonellosis in children. No systematic study has done yet on molecular characterization of ESBL-producing Salmonella species in Bangladesh.

Objectives: The aim of the study was to analyze the ESBL producers at phenotypic and molecular level especially to find the relevance of plasmids to the dissemination of antimicrobial resistance in Salmonella species.

Methodology: Of 2502 Salmonella species were isolated and identified from children and adults with diarrhea and typhoid cases at ICDDR,B between 1999 and 2007 using standard microbial and serological method. Among these 200 strains, of which S. Typhi was 60, S. Paratyphi A was 42, Salmonella Group C1 was 36 (18%), Salmonella Group B was 32(16%), and Salmonella Group G was 30(15%), were randomly selected for detailed ESBL-screening. ESBL producers are extensively characterized using antibiogram, double disc diffuision synergy test, plasmid profiling, PCR, conjugation experiment and PFGE.

Results: Of 200 Salmonella strains, 4% (n=8) strains were found as ESBL producers. The prevalence of ESBL producers was very high in Salmonella Group B (2%, n=4) and Salmonella Group G (2%, n=4). Only one Salmonella Typhi strain isolated in 2011 was positive for ESBLs. Most of the ESBL-positive strains were resistance to 3rd and 4th generation cephalosporins and monobactan. Plasmid profiling showed that 75% (n=6) strains harbored 62 MDa and 25% (n=2) harbored 90 MDa plasmid. PCR analysis revealed that blaTEM (n=6, 75%) was most prevalent followed by blaOXA (n=4, 50%), blaSHV (n=2, 25%) and blaCTX-M-1 (n=2, 25%) genes. Fifty percent (n=4) strains were positive for int1 gene. Conjugation study revealed that 62 MDa plasmid was transferable which contained blaTEM and blaCTX-M-1 genes as detected by PCR. PFGE analysis revealed that same clone was disseminated within ESBL producer and non producer.

Conclusion: Emergence of ESBL-producing Salmonella is of great concern. Horizontal gene transfer played an important role in the spread of ESBL. Appropriate monitoring in the usage of these drugs for treating the patients should be handed carefully.
Abstract #: 112

Clinical Profile of Typhoid Patients attending an Urban Hospital, Dhaka, Bangladesh in 2012

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Typhoid fever is a major cause of illness in most developing countries including Bangladesh. We reviewed the hospital patients records of culture positive (Salmonella typhi or Salmonella paratyphi) admitted in Dhaka hospital of icddrb from January 2012 to December 2012. In total, 143 typhoid patients of all age groups were admitted during this period. The age distribution were: 0-5 y (41%); 6-12y (20%); 13-20y (25%); 21-45y (13%). Of these patients 60% were male and 40% female. The mean ±SD of duration (days) of fever and duration of diarrhea on admission were 6.5±3.9 and 4.6±3.2 respectively. About 8% patients presented with altered consciousness (encephalopathy) and this feature was common in children and young adults. Half of the patients’ stool microscopy have shown invasive picture (stool WBC & RBC > 20cells/HPF. The time of defervescence of fever varied from one to 9 days. All the patients were treated with Injection Ceftriaxone 3- 4 g per day for adults and 75- 100 mg/Kg once daily for children. The isolates were susceptible (100%) to Ceftriaxone during this period. Comparatively more typhoid patients were admitted during the winter (January and December). Most (93%) patients were recovered and discharged as usual, 6 patients were referred for complication (renal failure) and one died. To conclude that young children are common sufferers of this illness and older patients with typhoid fever is rare among the admitted patients in icddrb Dhaka hospital. So, vaccination of children in young age might prevent them from this illness.
Abstract #: 114

Multidrug-Resistant Salmonella enterica in the Democratic Republic of the Congo (DR Congo)

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Background:
Salmonella Typhi and non-typhi Salmonella (NTS) are leading causes of invasive blood stream infections in central Africa, however very little data on the pathogen from the area is available.

Methods:
In the course of a microbiological survey from 2007 to 2011 a total of 9.634 patients were enrolled at several sites in the Democratic Republic of the Congo (DR Congo), blood was collected to assess for the presence of Salmonella enterica spp. Antibiotic susceptibility patterns for ampicillin, cefotaxime and trimethoprim-sulphamethoxazole (TMP-SMX) were assessed using the Vitek II system. Minimal inhibitory concentrations for nalidixic acid, ciprofloxacin, chloramphenicol and azithromycin were determined using the E-test. On a subset of isolates PCR, PFGE and MLVA were performed.

Results:
A total of 201 Salmonella Typhi isolates were detected. Resistance to ampicillin, TMP-SMX and chloramphenicol were observed in 64.7%, 57.7% and 41.3% of all isolates respectively, multi drug resistant strains were found in 30.3% of all cases and 15.4% had a decreased ciprofloxacin susceptibility (DCS). DCS was associated with point mutations in the gyrA gene at codons 83 and 87.

In total 233 NTS cases were isolated, leading serotypes were Salmonella Typhimurium and Enteritidis with 79% and 18% respectively. The proportions of MDR, DCS and resistance to azithromycin were 81.0%, 4.3% and 3.0% respectively. Resistance to ampicillin, TMP-SMX and chloramphenicol and MDR strains were significantly higher in Salmonella Typhimurium than in Salmonella Enteritidis (94.0%, 94.0%, 90.2% and 86.9% versus 64.3%, 64.3%, 61.9 and 59.5% respectively, p<0.001). DCS was associated with a single mutation at codon 87 in the gyrA gene.

Conclusion:
This study is the first to describe widespread antibiotic resistance among invasive Salmonella enterica spp. in the DR of Congo, highlighting the need for increased efforts in antibiotic stewardship and continuous monitoring.
Abstract #: 119

Typhoid control in Fiji through sero-epidemiology, social mixing surveys, economics and modeling

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Background:

Confirmed typhoid case numbers in Fiji have been rising since 2005, particularly affecting 15-29 year old men of the indigenous iTaukei community, both as sporadic cases and in outbreaks. This presents a significant ongoing public health concern, particularly for village communities with difficulty accessing medical care. Typhoid also has adverse trade and economic impacts for affected families and villages. While the Fijian population is small in global terms at 840,000, confirmed incidence rates are amongst the highest in the world.

Proposed Methods:

The proposed research will use serology – measuring immune antibodies in blood serum samples – to understand age-based typhoid exposure. Cost and burden of disease will be estimated. Data from serology and a social mixing survey will be developed into a transmission model.

Expected Outputs:

Serology will directly inform control measures in Fiji, such as vaccination programmes and water and sanitation improvements. The model will build on this to assess the potential impact of different control strategies. For example, school-based vaccination may also indirectly protect unimmunised adults, so placing less of a demand on the health service than delivering a whole-population vaccination programme. It is anticipated that this approach can also be used to inform control measures in other typhoid-endemic settings.
Abstract #: 130

Estimating Effectiveness of Typhoid Vaccination Using National Surveillance Data

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Typhoid vaccination is recommended for travelers to countries where typhoid fever (TF) is common. Two vaccines are available in the United States; little information is available about their effectiveness in travelers. We estimated vaccine effectiveness (VE) by comparing vaccination rates in travelers with TF and travelers with paratyphoid fever (PF), a closely-related infection against which typhoid vaccines offer no protection.

State and local health officials report cases of TF and PF to the Centers for Disease Control and Prevention. Reports include demographic information, travel history, and history of typhoid vaccination in the 5 years before illness onset. We analyzed data from 2008-2010 on cases of TF and PF (defined as Salmonella Paratyphi A infection) in travelers, persons who spent time outside of the United States in the 30 days before illness onset. We included patients ≥ 2 years old whose reports indicated that they were 1) not vaccinated or 2) had received either licensed vaccine. We calculated the odds ratio with 95% confidence intervals (95% CI) for vaccination among persons with TF, as compared to those with PF, and calculated VE as 1/OR x 100%.

In all, 996 travelers were reported with TF and 247 with PF; vaccination information was available in 648 (65%) TF and 123 (50%) PF reports. For travelers with vaccination information, the most common countries reported were India (359 TF, 81 PF), Bangladesh (86 TF, 20 PF), and Pakistan (58 TF, 8 PF); other countries were reported for 145 patients with TF and 14 with PF. Among those with TF, 40 (6.2%) had been vaccinated, whereas among those with PF, 35 (28.5%) had been vaccinated. Estimated VE was 83.5% (95% CI 72.6%, 90.0%). Restricting the analysis to US citizens did not substantially change the VE estimate. Information on the vaccine administered was available for 39 of the 75 (52%) vaccinated patients; 16 received the oral vaccine and 23 the single-dose parenteral vaccine.

We estimate moderate VE, 83.5%, for typhoid vaccination based on US surveillance data, supporting the recommendation that vaccination be offered to travelers to areas where TF is common. Increased vaccine use among travelers to these destinations and development of an effective paratyphoid fever vaccine could reduce the burden of illness.
Abstract #: 131

The Coalition against Typhoid: History of the Conference on Typhoid Fever and Other Invasive Salmonelloses

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Background
In 1984 a small gathering was held in Washington, DC to discuss the lack of disease control policies for typhoid fever. In 2013, 29 years later, there is now a third generation of typhoid vaccines in clinical development and a fourth generation of bivalent typhoid-paratyphoid A vaccines on the horizon.

Results
In the eight meetings, the two overarching themes have been the reduction of mortality through case identification and the management of antibiotic resistance, as well as the prevention of morbidity — through preventative vaccination and other strategies.

The challenge of diagnosing typhoid fever in resource poor settings was a central focus of the early meetings. However, as a result of advances in the description of clinical presentation and treatment strategies, later conferences reported reductions in the prevalence of severe disease and case fatality.

The first meeting barely touched on the issue of antimicrobial resistant typhoid fever. In stark contrast, less than 20 years later in 2004, researchers began reporting high rates of multi-drug resistance strains throughout Asia and Africa.

In 1984, the Vi-polysaccharide (ViPS) vaccine candidate was reported as showing promising results, especially in relation to mass vaccinations. Later research sparked discussion on a second generation of vaccines, particularly ones that could provide lasting protect, herd immunity and were safe in children. Recently the discussion shifted toward conjugate vaccines candidates. Similarly, later conferences reported difficulties in implementing vaccination programs in developing countries. As a result, the WHO South East Asian Regional Office prioritized typhoid vaccines for immediate introduction in 2009.

Conclusion
The routine meeting of experts in the field of typhoid, paratyphoid, and other invasive Salmonelloses has played an important role in the dissemination of new and important information. For example, when the GAVI Alliance announced in 2008 that it will only support the typhoid conjugate vaccine (ViCV), CaT likewise shifted its focus to the development and implementation of these vaccines and bivalent typhoid paratyphoid conjugate vaccines. As such, gathering the typhoid community at conferences serves to coordinate and catalyze the global control and prevention of enteric fever.
Abstract #: 132

Typhoid Fever: A Review of Case Definitions and Surveillance Standards

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Background
Public health surveillance is defined as the ongoing, systematic collection, analysis, interpretation, and dissemination of data regarding a health-related event. Surveillance data is used to inform public health actions focused on reducing morbidity and mortality and improving health. Strong national surveillance systems form the basis of an effective regional and global network for the control of communicable diseases. Health managers need timely and scientifically sound evidence in order to make informed decisions on interventions to control both endemic and epidemic disease.

Results
A proper surveillance system should utilize systematic observation and measurement in data collection, precise analysis, and effective data dissemination. Public health surveillance requires clear definitions (particularly case definitions) to identify and track cases (e.g. suspected and confirmed), and often benefits from parameterized definitions that are specific to a particular outbreak investigation, season, or location.

The primary goal of disease surveillance is the identification of populations with endemic disease as well as the early identification of outbreak and epidemic disease. Routine surveillance data can be collected through active or passive methods and case incidence above the average can trigger alert and action/epidemic actions at predetermined thresholds.

As with other epidemic-prone diseases, typhoid outbreaks/epidemics are detected through reports from the field. Suspected cases of typhoid are typically reported within 24 hours. Specific countries may define their own locally adapted alert and action/epidemic thresholds, as they see fit.

Conclusions
Ongoing public health surveillance with local alert and action thresholds are an integral part of infectious disease control programs. Clear and consistent case definitions must be utilized within a robust reporting mechanism. The 2010 WHO AFRO IDSR is an excellent reference and reflects current best practices in enteric fever epidemiology and surveillance and other regions would benefit from similar guidance and the establishment of similar standards and case definitions.

ACKNOWLEDGEMENT: The Coalition against Typhoid (CaT) Secretariat would like to thank several colleagues from the United States Centers for Disease Control and Prevention (CDC) for their comments on a draft version of this document: Dr. Eric Mintz, Ms. Helen Perry, Dr. Terry Hyde, and Dr. Kashmira Date.
Abstract # 133:

The Coalition against Typhoid: Addressing Barriers to Typhoid Vaccine Implementation

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Background
Typhoid fever, caused by the bacterium Salmonella enterica serovar Typhi (S. Typhi) and associated with unsafe water and food and poor sanitation, continues to be a major public health problem in middle and low income countries of Asia and Africa, particularly among school-aged children.

Results
The programmatic use of typhoid vaccines is a global health priority supported by WHO and prioritized by the GAVI Alliance. Despite these recommendations, the implementation of typhoid vaccines as part of national or municipal immunization programs has been slow.

In response, the Coalition against Typhoid (CaT), a global forum of health and immunization experts working to expedite and sustain evidence-based decisions at the global, regional and national levels regarding the use of typhoid vaccination to prevent childhood enteric fever, was established in 2009. The Sabin Vaccine Institute serves as the secretariat of the CaT and is supported by a three-year grant from the Bill & Melinda Gates Foundation. The goals of CaT are to:

· Advance a comprehensive and integrated typhoid control strategy in endemic countries through sustained advocacy, communications, consultation, collaboration, and coordination;
· Facilitate rational, evidence-based, and sustainable decisions regarding the use of typhoid vaccines;
· Assist countries with decision-making and the implementation and monitoring of typhoid vaccination programs and their impact; and
· Promote research and development of next generation’s vaccines and improved diagnostics.

Barriers to widespread use of typhoid vaccines include limited surveillance data and diagnostic tools; low awareness of the prevalence of MDR and NAR typhoid; limited understanding of vaccine options, vaccination strategies and the successful use of vaccines to rapidly control disease; and the absence of public sector financial support, even though the cost of typhoid vaccine is relatively low, vaccination strategies are very cost-effective, and there is high demand for vaccine in targeted populations.

Conclusion
The control of enteric fever caused by S. Typhi is a global health priority. Safe, effective and affordable vaccines can be used as part of a comprehensive and integrated strategy to control typhoid fever. By prioritizing typhoid on the global health agenda and developing a comprehensive work plan to combat this disease, the Coalition against Typhoid eagerly anticipates expanding access to these life-saving vaccines.
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