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Cholera and Typhoid Vaccines

A Review of Current Status

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Summary

The recent spread of cholera in the Americas and the emergence of *Vibrio cholerae* 0139 in Asia have stimulated researchers to design efficient vaccine formulations to combat increasing cholera morbidity and mortality. Although the conventional heat-killed whole cell cholera vaccine continues to be offered during epidemics and in endemic foci, the novel whole cell killed plus recombinant cholera toxin B subunit vaccine and the live attenuated CVD 103-HgR vaccine are undergoing extensive field trials. Preliminary trials with Bengal-15, a vaccine composed of live *V. cholerae* 0139 without the toxin gene but containing recombinant B subunit, are promising.

Newly emerged clinical features of typhoid, such as increasing antibiotic resistance in *Salmonella typhi*, an aggravated clinical picture during concomitant infection with HIV and an increased risk for cancer among chronic typhoid and paratyphoid carriers, are alarming. Efficacy studies on live typhoid oral vaccine, Ty21a, and the parenteral Vi polysaccharide vaccine have confirmed their superiority over the conventional vaccine. Similar vaccines are indicated for protection against paratyphoid infections.

The development of simple 1- or 2-step laboratory tests for diagnosis of cholera, typhoid and paratyphoid would assist in therapeutic and prophylactic measures, and in monitoring the humoral and cellular immune response of patients, carriers or vaccine recipients.

From the beginning of the twentieth century, active immunisation against cholera and typhoid has been through a parenteral injection of whole-cell, heat-killed, phenol-preserved vaccines. While the past decade has witnessed an almost exponential rise in the number of newer vaccine formulations, including controlled-release delivery systems composed of polymers or microspheres, combination vaccines, novel adjuvants and new vector systems,[1] an improved vaccine against cholera has been a global priority only following the recent outbreak of cholera in the Americas. After a gap of almost a century, and starting from Peru during 1990, many countries in the Americas recorded during a 4-year interval a cholera morbidity of more than 1 million with a mortality approximating 10 000.[2] A simultaneous emergence of *Vibrio cholerae* 0139 (synonym Bengal) in Asia, and its rapid spread among all ages to other areas where 01 cholera was epidemic,[3] has stimulated vaccine researchers.

During the 1980s, about 16.59 million cases of typhoid with 0.58 million deaths were documented globally. In industrialised countries, there were 22 620 cases (0.14% of the total) and 74 deaths (0.01% of the total).[4] The emergence of *Salmonella typhi* and *S. paratyphi* as opportunistic organisms in individuals infected with HIV has been alarming. All those infected with HIV have an increased risk of infection with various typhoid and paratyphoid organisms. These co-infections present atypically with an aggressive clinical picture. In typhoid-endemic countries, fulminant diarrhoea and/or colitis have been the aberrant clinical presentations.[5] Identical cases have been documented in HIV/AIDS cases in non-endemic foci of typhoid fever.[6] Furthermore, *S. typhi* isolates have rapidly acquired resistance to chloramphenicol, ampicillin and cotrimoxazole (trimethoprim-sulfamethoxazole). Multidrug-resistant isolates are very frequent in Africa,[7] Asia[8] and elsewhere.[9] Neonatal typhoid, which used to be uniformly fatal in the pre-antibiotic era, has recently been responsible for sepsis neonatorum in rural areas in Africa.[10] Infection from an exogenous source and vertical intrauterine infection are implicated in the pathogenesis of neonatal typhoid.

Apart from the development of refined products that could be used as active immunogens against cholera or typhoid, simple, specific laboratory tests for their rapid diagnosis have been standardised.[11] Clinicians, specialists, general practitioners and epidemiologists have a crucial role in tackling various prophylactic and therapeutic issues relating to the existing and emerging strains of vibrios and the multidrug-resistant salmonellae. This review documents the current advances in immunisation against cholera and typhoid, including the prospective issues needing attention.

1. Cholera Vaccines

The conventional and 'newer' cholera vaccines have been offered by the parenteral or oral route.

1.1 Parenteral Vaccines

The traditional whole-cell parenteral vaccine consists of a mixture of equal parts of the 2 main serological types, Inaba and Ogawa, of the classical biotype with or without the El Tor biotype.[12] The usual liquid or freeze-dried form contains not less than 8×10^9 organisms. Nevertheless, the parenterally administered vaccines, whole-cell, lipopolysaccharide or toxoid,[13] induce a weak and short-term immunity. They fail to induce any intestinal or 'mucosal' immune response, and cause, apart from local erythema or tenderness, anaphylaxis and hypersensitivity reactions. A causal relationship between parenteral cholera vaccine and transverse myelitis was evident recently in a report of a 24-year-old woman who developed paraesthesia of her feet within 2 days of vaccination, which progressed to flaccid paraplegia, retention of urine and sensory loss below the level of the tenth thoracic nerve.[14]

1.2 Oral Vaccines

Oral cholera vaccines, killed or live, have been developed to select the ideal formulations which

would induce local intestinal mucosal immunity and would not be toxigenic (toxin-forming).

1.2.1 Killed Vaccines

The oral use of killed vibrios and inactivated toxoid for immunisation was discontinued during the 1970s,[15] and systematic investigations on oral inactivated cholera vaccines were started during the mid-1980s. They incorporated both the non-toxic component of cholera toxin and killed vibrios.

Inactivated *Vibrio cholerae* 01 Vaccine (WCV)

This vaccine contains 10^{11} inactivated bacteria representing a mixture of both El Tor and classical biotypes of both Inaba and Ogawa serotypes.[15]

Nontoxic B Subunit of Cholera Toxin and Killed Whole Cell Vaccine (BS-WC)

This formulation contains 1.0mg of cholera toxin B subunit, which is the immunogenic and non-toxic subunit of cholera toxin, along with the inactivated organisms contained in WCV.[16]

Whole Cell Inactivated/Recombinant B Subunit (WC/rBS) Vaccine

Each dose of WC/rBS vaccine contains 1mg of recombinant cholera toxin B subunit and 10^{11} *V. cholerae* (2.5×10^{10} each of heated-killed classical Inaba, heat-killed classical Ogawa, formalin-killed El Tor Inaba and formalin-killed classical Ogawa) suspended in 3ml of phosphate-buffered saline. The inclusion of Inaba and Ogawa ensures immune response to both lipopolysaccharide antigens. Use of formalin ensures preservation of protein antigens, while heat killing preserves lipopolysaccharide antigens.[17]

1.2.2 Live Vaccines

The live vaccine formulations lack the toxigenic component of cholera toxin, and are designed to offer protection after a single dose.

CVD 103-HgR

CVD 103-HgR is an attenuated *V. cholerae* 01 strain that has been derived from CVD 103,[15] attenuated from the wild type *V. cholerae* 01 classical Inaba strain 569B. The enzymatically active A subunit of cholera toxin has also been deleted from CVD 103-HgR.[18] By introduction of the gene encoding for resistance to Hg^{++} into the *hylA* locus of the CVD 103 chromosome, it has been possible to confer an indelible marker of mercury resistance in CVD 103-HgR.

Peru-14

An attenuated *V. cholerae* El Tor mutant, Peru-14, has been produced by recombinant technology from the parental strain, C6709 Inaba. The chromosomal deletions, in loci of the *ctx* genetic element *recA* and *lacZ* of *V. cholerae*,[19] appear to offer a safe, effective, single-dose oral vaccine against El Tor cholera.

Bengal-15

An attenuated *V. cholerae* 0139 vaccine, Bengal-15, has been prepared by deleting multiple copies of the cholera toxin genetic element from virulent strains of *V. cholerae* 0139. The deletion mutants were also modified by insertion of a construct encoding for the B subunit of cholera toxin.[20] Bengal-15, a stable non-motile derivative, offered 83% protection among volunteers.

1.2.3 Miscellaneous Preparations

While the prospective strains for use as an oral vaccine have included various genetically engineered strains like the Texas Star-SR, JBK 70[13] and Ty21a-*V. cholerae* Inaba hybrid,[15] immunisation has also been attempted with *V. cholerae* lipopolysaccharides and outer membrane proteins or cell-wall ghosts.

Pasteur Oral Cholera Vaccine

Thai volunteers were offered, during the late 1980s, lyophilised lipopolysaccharides and outer membrane proteins of *V. cholerae* El Tor Ogawa and Inaba in enteric coated capsules.[21]

V. cholerae Ghosts

Ghosts, produced by expression of the cloned lysis gene from the phage ϕX174 in intact *V. cholerae*, were employed for intraperitoneal immunisation of mice. Seroconversions to reactivity with whole-cell vibrio antigens with such ghosts or the heat-killed whole cells were identical.[22]

1.3 Efficacy Trials with 'New' Cholera Vaccines

While preliminary phase I trials have been conducted with various candidate vaccine strains or formulations right through the 1980s, phase III trials have been in progress in the Americas and Asia for the killed whole cell B subunit vaccine and the live attenuated CVD 103-HgR.

1.3.1 Whole Cell B Subunit Vaccine

The 1980s phase III trial in Bangladesh with BS-WC vaccine involved 63 498 individuals.[16] After 3 doses, protection was 85% during the 6-month period and 50% for 3 years. Protection against the El Tor infection among recipients with blood group O was poor. The vaccine failed to block the intestinal carriage of vibrios.[23]

1.3.2 Whole Cell Inactivated/Recombinant B Subunit Vaccine

The safety of the recombinant B subunit vaccine, WC/rBS,[17] was established during the early 1990s in volunteers in Sweden[24] and the US.[25] A phase II trial was initiated in Peru in young men, with 2 doses given 1 to 2 weeks apart. There was 86% protection lasting for 4 to 6 months.[26] Another trial involved 1426 army personnel in Peru, who received 2 doses at 7- to 14-day intervals. The vaccine offered 86% protection against cholera but not against symptomless vibrio excretion. Cholera cases were confined to those in blood group O. The 2-week interval between the doses was optimal for adequate protection.[27]

In mid-1994, an efficacy trial involving 90 000 individuals aged 2 to 60 years was initiated in Peru to determine the duration and efficacy of protection attributable to WC/rBS in the cholera endemic population.[28]

1.3.3 CVD 103-HgR

The safety of CVD 103-HgR was evident in phase I trials in the Americas and Asia.[29,30] Studies in volunteers have established that 1 dose offers high-level protection against experimental cholera within 8 days, lasting for 6 months. There was no shedding of *V. cholerae* 01 in stools.

During 1993, a field trial was initiated in Indonesia involving administration of 1 vaccine dose to 66 000 people. Efficacy will be known at the end of the trial in 1996.[28]

1.4 Unaddressed Issues

Results of phase III trials with 2 cholera vaccine preparations will be known during 1996 (sections 1.3.2 and 1.3.3) In the interim period, a number of issues need attention. These include:

- vaccine interference in laboratory diagnosis of cholera
- monitoring the immune response of vaccine recipients
- multiplication of vibrios at unusual anatomical and environmental locations
- multivalent vaccines
- emergence of yet another new epidemic strain of *V. cholerae*.

1.4.1 Rapid Laboratory Diagnosis of Cholera

Direct detection of *V. cholerae* in stools has been performed via a polymerase chain reaction (PCR) procedure employing oligonucleotide primers for the genes of the toxin subunits A (*ctxA*) and B (*ctxB*). With 2 consecutive PCRs, including one with a nested primer, a positive result was obtained even when *V. cholerae* were not culturable or dead.[31]

The sensitivity of the *ctxA* and *ctxB* PCR means that the results could be confounded both by the whole cell/recombinant B subunit vaccine and by CVD 103-HgR, as both vaccines contain the genetic sequences of the B subunit of cholera toxin.[17,18] Furthermore, both the vaccines would also interfere in the sensitivity of the colloidal gold–based colorimetric assay for direct detection of *V. cholerae* 01 in stools,[32] or the rapid monoclonal antibody–based coagglutination test for *V. cholerae* 0139.[33]

During the limited phase I and II trials, CVD 103-HgR excretion from children was negligible.[15] That might not be true among vaccine recipients with HIV-induced immunosuppression. The ever-increasing burden of paediatric AIDS in cholera endemic foci could be associated with increasing

excretion of CVD 103-HgR and an interference with the laboratory diagnosis of cholera by molecular or immunological techniques.[31-33]

1.4.2 Immune Response of Vaccine Recipients

While vaccine efficacy in the community would be evident in terms of protection offered against the disease profile attributable to cholera, serum vibriocidal antibody response has been monitored to assess the immunogenicity of CVD 103 HgR.[30] A ≥4-fold increase is considered significant and indicative of seroconversion. That has also been apparent with Bengal-15, the *V. cholerae* 0139 vaccine prototype.[20] Assays for anti–cholera toxin IgG have been made by enzyme-linked immunosorbent assay and the antitoxin titre equated with a susceptibility or otherwise to cholera.[34]

The complex assay procedures to determine susceptibility against *V. cholerae* involve many steps and should be simplified to a dipstick format for field use. For uniformity of results, the threshold protective level of anti-vibriocidal antibody and antitoxin should be defined in international units.

1.4.3 V. cholerae at Unusual Sites

Anatomical

V. cholerae El Tor serotype Ogawa was responsible during the early 1990s for acute acalculous cholecystitis in a 57-year-old woman in Ecuador. There was possible penetration of vibrios through the papilla of Vater in a retrograde fashion from the duodenum.[35]

Environmental

V. cholerae is speculated to have entered South America via shipping. Organisms were isolated from ballast, bilge and sewage of 3 of 14 cargo ships docked in the Gulf of Mexico.[36] *V. cholerae* 01 Inaba, biotype El Tor, was found in oysters and oyster-eating fish from closed oyster beds in Mobile Bay, Alabama, USA.[37]

Molecular biological studies, including *in situ* PCR, would enable a search of aberrant multiplication sites for *V. cholerae* both *in vivo* and in the aquatic environment.

1.4.4 Multivalent Vaccines

The simultaneous occurrence of both the classical *V. cholerae* and 0139 in endemic areas[38] could be effectively prevented by a multivalent vaccine incorporating various strains. By entrapping B subunit toxin in microparticles,[39] it might be feasible to offer continued protection against various strains even with a single dose. Parenteral microspheres or oral microparticles would be ideal for mass immunisation in explosive outbreaks, or for regular use in endemic areas.

1.4.5 Emergence of New Strains

The recent emergence of *V. cholerae* 0139 as an additional agent of cholera epidemics is speculated to have occurred through a genetic recombination between the El Tor 01 and an as yet to be identified non-01 strain of *V. cholerae*.[40] The aquatic environment that provides an ecological shelter for vibrios[41] might be responsible for the future emergence of yet another epidemic vibrio.

The recombinational events between various *V. cholerae* strains have the potential to allow attenuated strains to acquire cholera toxin and virulence genes. This process is exemplified by the acquisition by CVD-103-HgR of the toxin A subunit gene from a *V. cholerae* strain possessing a conjugate factor.[42] Laboratory-based surveillance is essential to characterise and differentiate isolates from virgin-soil non-endemic foci[43] by ribotyping with the restriction enzyme Bg/I.[44]

2. Typhoid Vaccines

The whole cell inactivated typhoid vaccines known since the 1890s[12,45] were modified during the 1960s. Through the 1980s large clinical trials have been in progress with a live *S. typhi* strain vaccine[46] and the purified Vi polysaccharide preparation.[47] A large number of genetically engineered salmonellae preparations have been available for preliminary safety studies (table I).

2.1 Vaccine Types

2.1.1 Vaccines Originating from the Last Century

Whole cell or heat-, acetone-, phenol- or alcohol-killed vaccines were offered parenterally in liquid

Table I. Development of typhoid vaccines

Originating from the 1890s	From the 1960s onwards[44]	From the 1980s with extensive field trials	Experimental, with rather limited safety trials
Whole cell, heat-, phenol- or alcohol-inactivated[12,44]	**Liquid** 1. Heat-inactivated, phenol-preserved 2. Alcohol-inactivated and preserved	**Oral, attenuated** Ty21a[45]	**Conjugated Vi polysaccharide**[47]
	Dried 1. Acetone-inactivated 2. Heat- and phenol-inactivated	**Parenteral** Purified Vi capsular polysaccharide[46]	**Genetically engineered mutants** *Affecting regulatory pathways*[44] X3927, X4073, PhoP, PhoQ, PagC, PagD *Affecting biosynthetic pathways* Chi 3297,[48] 541Ty, 543Ty, CVD 906, CVD 908[48] **Others** 1. *Salmonella typhi* O-polysaccharide tetanus toxoid conjugate[49] 2. Temperature-sensitive strain Ts 51-1[50] 3. *S. typhimurium* Vi4072[51]

or freeze-dried form in a combined formulation against *S. typhi* and *S. paratyphi* A and B. They were associated with local and systemic reactions.[12] Field trials during the 1960s and 1970s pointed to the vast superiority of the acetone-inactivated vaccines over the heat/phenol-inactivated vaccines, which in turn performed better than the alcohol-inactivated and preserved vaccines.[45] During the 1960s, in the absence of a clear evidence of efficacy against *S. paratyphi* A and B, incorporation of paratyphoid components was discontinued.

2.1.2 Vaccines of the 1980s with Meritorious Trials

Two vaccines that were offered during the late 1970s and 1980s have had extensive field trials. Both have far fewer adverse effects than the whole cell inactivated vaccines.

Ty21a

Ty21a is an oral vaccine containing attenuated *S. typhi* strain Ty21a, which induces mucosal immunity without a systemic dissemination of *S. typhi*.[45] Ty21a, a mutant of *S. typhi* Ty, carries a mutation of the *galE* gene that results in the absence of the enzyme uridine diphosphate galactose 4-epimerase.[46] Ty21a grows in the intestine, but owing to a deficiency of *galE* it is destroyed with hardly any faecal excretion. Oral doses containing 10^{11} organisms are dispensed in enteric-coated capsules or sachets containing powdered buffer and lyophilised vaccine. Ty21a is well tolerated, safe and immunogenic in vaccine recipients.

Vi Polysaccharide Vaccine

The vaccine contains purified Vi polysaccharide, a linear homopolymer of 1→4-linked *N*-acetyl-α-D-galactosaminuronic acid, acetylated at O-3.[47] Each dose of 25μg (0.5ml) of Vi polysaccharide vaccine is given by a deep subcutaneous or intramuscular injection. In adults and children over 5 years, immunity persists for at least 3 years. Local adverse reactions in about 20% of recipients include minor pain, swelling or erythema. Systemic reactions such as headache, fever, malaise or nausea are transient and only occur in about 3% of recipients.

2.1.3 Experimental Vaccines with Limited Trials

Efforts have been made to increase the immunogenicity of purified Vi polysaccharides by conjugation to tetanus toxoid, diphtheria toxoid and cholera toxin.[48] Genetically engineered mutants have been prepared by affecting their regulatory or biosynthetic pathways (table I).[49] The somatic antigen polysaccharide of *S. typhi* has also been conjugated to tetanus toxoid.[50] Initial trials with a new temperature-sensitive (ts) 51-1 strain of *S. typhi* have been encouraging.[51] *S. typhimurium* Vi4072 induces humoral and cellular immunity in

mice and could be useful for protection against typhoid and salmonellosis caused by *S. typhimurium*.[52]

2.2 Efficacy Trials with Current Vaccines

2.2.1 Ty21a

Efficacy trials have been conducted since 1978 in Egypt, Chile and Indonesia. During 1978, about 32 000 children aged 6 to 7 years were randomised at Alexandria in Egypt to receive 3 doses of 10^9 organisms on every other day. The lyophilised vaccine was reconstituted in the field and the liquid suspension ingested by the child 1 minute after chewing a 1.0g tablet of $NaHCO_3$.[45] During the 3-year follow-up of passive surveillance at Alexandria, with an annual incidence of 40 to 50 typhoid cases/100 000 population, the protective efficacy was 96%.

In Chile, efficacy trials have been in progress through the early 1980s. The earlier trials during 1982 to 1984 demonstrated the superiority of enteric-coated capsules over gelatin capsules. Ty21a protected for at least 7 years. While the incidence of typhoid was lowest after 4 doses on alternate days, 2 doses were insufficient for protection. During 1986 at Santiago, Chile, 81 621 schoolchildren aged 5 to 19 years received 3 doses within 1 week of Ty21a in enteric-coated capsules or in a new liquid suspension. During the 36-month surveillance, with the Santiago typhoid incidence exceeding 150/100 000, vaccine efficacy was 76.9% with the liquid formulation and 33.2% with the enteric-coated capsules.[53] Moreover, the liquid formulation protected younger (aged 5 to 9 years) children, with 82.3% efficacy, better than older children, with 69.3% efficacy.

The relative protective efficacy of Ty21a given in solution with $NaHCO_3$ and in enteric-coated capsules has been determined in Indonesia under conditions of intensive typhoid transmission. A total of 20 543 Indonesians, aged 3 to 44 years, were randomised to receive 3 doses of Ty21a either in enteric-coated capsules or reconstituted with phosphate buffer.[54] During the 30-month follow-up of recipients between 3 and 19 years old, who accounted for 91% of local typhoid cases, the liquid formulation was more efficient (53% protection) rather than the enteric-coated capsules (42%).

Ty21a at a dose of 10^9 live *S. typhi* did not induce immune responses in children under 2 years in an endemic region.[55] In Thailand, preliminary studies showed the liquid Ty21a formulation to be effective in children aged 2 to 6 years, with 69% seroconversion compared with 14% among placebo recipients.[56]

Ty21a has been licensed for use in many countries, and is marketed in sachets containing powdered buffer and lyophilised vaccine.[57] A total of 4 doses should be taken, on alternate days before meals.

2.2.2 Vi Polysaccharide Vaccine

Efficacy studies on *S. typhi* Vi polysaccharide vaccine were carried out in Nepal and South Africa. In Nepal, with an annual typhoid incidence of 655/100 000 population, vaccine was given in 5 villages outside Kathmandu as a single 25μg intramuscular dose. The trial involved 6438 individuals; controls received 25μg of pneumococcal 23-valent polysaccharide. The results of active surveillance during 17 months indicated a 72% efficacy in culture-positive cases, 80% in clinically suspected cases and 78% when the two groups were combined.[58]

The second randomised and controlled trial was conducted in eastern Transvaal, South Africa, with 11 384 participants aged from 5 to 16 years. The control vaccine was the meningococcal groups A and C polysaccharide. A single 25μg dose conferred 64% protection against culture-confirmed typhoid for at least 21 months.[59] Following injections, local reactions were transient and there were no systemic reactions such as fever.[60]

2.3 Unaddressed Issues

A comprehensive approach for typhoid containment would include considerations of vaccines, vaccine recipients and the auxiliary problems linked with salmonellae isolated from endemic and non-endemic areas.

2.3.1 Vaccines

Additional protection from Ty21a vaccine, which lacks Vi capsular polysaccharide, may be expected by an appropriately engineered expression of Vi polysaccharide[61] and the subsequent elevated serum antilipopolysaccharide response. Freeze-dried Vi polysaccharide vaccine formulation, an equally efficient immunogen as the liquid formulation,[62] should resist environmental degradation. Any of the Vi vaccine formulations may be useful for immunisation of infants and children below 2 years of age after antigen binding to the B subunit of the heat-labile toxin (LT-B) of *Escherichia coli* or the recombinant exoprotein A of *Pseudomonas aeruginosa*.[48] The Vi lipopolysaccharide from *S. paratyphi* C, which resembles *S. typhi* Vi in its antigenic or immunogenic properties,[63] could be easily incorporated into the existing *S. typhi* Vi vaccine.

An alternative strategy for a multivalent typhoid/paratyphoid vaccine might involve conjugation of O antigens. The experimental linkage of *S. typhi* O polysaccharides to tetanus toxoid protected Balb/c mice against an *S. typhi* Ty2 challenge.[50]

The performance of Ty21a and liquid or lyophilised Vi polysaccharide vaccines could be suboptimal in adverse environments involving spikes in humidity or temperature, or extremes of temperature.[64] In travellers from non-endemic areas to hyperendemic foci of malaria and typhoid, coprophylaxis of typhoid by Ty21a and malaria by mefloquine and chloroquine could have a deleterious effect on the immunogenicity of Ty21a.[65] That could be overcome by entrapment of *S. typhi* Ty21a or Vi, or somatic antigens of *Salmonella*, in microparticles for oral administration.[39]

Simultaneous administration of typhoid vaccine Vi with other vaccines, including Vero cell rabies,[66] yellow fever 17D[67] and meningococcal polysaccharide group A and C,[68] has been satisfactory with good immunogenicity, tolerance and general reactions. Interference of Ty21a with the live poliovirus or measles vaccines, if administered simultaneously during national immunisation days, could lead to disappointing results.[45]

2.3.2 Vaccine Recipients

The Vi vaccine produces a marked anti-Vi IgG response,[62] but with Ty21a there is a specific antilipopolysaccharide faecal IgA rise that lasts for up to 8 months.[69] An *in vitro* assessment of the specific IgA has been possible using circulating peripheral blood lymphocytes.[70] Typhoid vaccination of Indonesian neonates and infants with Vi polysaccharide vaccine appeared safe with a specific antibody response,[71] although Ty21a was less immunogenic in children in Kurdish refugees camps.[72]

The modern typhoid vaccines might be useful as a specific therapeutic agent for chronic typhoid carriers, in the same way as a specific hepatitis B vaccine appears to have stopped or reduced virus replication in 14 of 32 patients with chronic viral replication.[73]

Ty21a and other live genetically engineered hybrid vaccines (table I) could interfere with PCR-based investigations using 323 base pair fragments of the flagellin gene of *S. typhi*.[74] The sensitivity of the *S. typhi* 50kD outer membrane protein enzyme immunoassay,[75] which has emerged to be as sensitive as the Widal agglutination diagnostic test in endemic areas, may be poor in visitors to endemic areas if they had received Ty21a prior to departure from the native countries. Ty21a-induced antibody might well exceed the respective antibody threshold level of 1/100 in such febrile patients.

Aberrant sites for multiplication of *S. typhi* have recently included hydronephrosis from a typhoid abscess,[76] central nervous system,[77] adult respiratory system,[78] cholestatic jaundice[79] and common iliac artery occlusion with HIV co-infection.[80] Chronic carriers of typhoid and paratyphoid run an excessive risk for cancer of the gall bladder, pancreas and colorectum.[81]

2.3.3 Salmonella Isolates

The 2 efficient typhoid vaccines are expected to lower the high incidence of typhoid and the dramatic rise in multidrug resistance of *S. typhi* isolates. Nevertheless, *S. typhi* and *paratyphi* A, B and C isolates should be characterised through im-

proved culture facilities, measurement of antibiotic resistance and bacteriophage typing.

2.3.4 Other Approaches

The prospective search for still more efficient antityphoid chemotherapeutics[82] should engage clinicians, industry and academics. In industrialised countries, general practitioners are interested in surveillance of uncommon and serious illnesses including preventable diseases.[83] In developing countries, the majority of the population prefer the private services of non-government clinicians to fully established governmental facilities in the locality.[84] Involvement of such primary healthcare clinicians is critical for better compliance with improved cholera, typhoid or other vaccines and a prompt notification of emerging multidrug-resistant salmonellae.

In Argentina, preliminary screening for *in vitro* anti-*S. typhi* activity in extracts from the medicinal plants *Cassia occidentalis*, *Heimia salicifolia*, *Punica granatum* and *Rosa borboniana* has been encouraging.[85] Potentially useful formulations from plants elsewhere could be valuable in treatment of multidrug-resistant salmonellae and could supplement typhoid control plans involving Ty21a or Vi polysaccharide typhoid vaccine.

3. Conclusions

The relative efficacy of 2 modern cholera or typhoid vaccines would be evident after a concurrent trial in an endemic area. Any extra cost for a successful oral formulation would be partly offset by the requirement for a large supply of needles and syringes for a parenteral vaccine.

The intestinal rather than humoral immune response to oral cholera vaccination in HIV-1 infected individuals, with very low CD4+ T cells in circulation, is intact.[86] Oral immunisation with attenuated *S. typhi* elicits a strong systemic cell-mediated immunity, including cytokine production attributable to a type 1 T cell response.[87] An innovative oral single-dose formulation incorporating cholera, typhoid and paratyphoid antigens might be ideal to immunise individuals with HIV infection or AIDS and to tackle explosive epidemics or endemic disease foci.

Last but not least, it may be possible to predict outbreaks of cholera with a better understanding of the role of crustacean copepods, zooplankton, that favour environmental survival of *V. cholerae* 01 and 0139 in non-culturable but viable form.[88]

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