The genomic signatures of *Shigella* evolution, adaptation and geographical spread

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Abstract | *Shigella* spp. are some of the key pathogens responsible for the global burden of diarrhoeal disease. These facultative intracellular bacteria belong to the family Enterobacteriaceae, together with other intestinal pathogens, such as *Escherichia coli* and *Salmonella* spp. The genus *Shigella* comprises four different species, each consisting of several serogroups, all of which show phenotypic similarity, including invasive pathogenicity. DNA sequencing suggests that this similarity results from the convergent evolution of different *Shigella* spp. founders. Here, we review the evolutionary relationships between *Shigella* spp. and *E. coli*, and we highlight how the genomic plasticity of these bacteria and their acquisition of a distinctive virulence plasmid have enabled the development of such highly specialized pathogens. Furthermore, we discuss the insights that genotyping and whole-genome sequencing have provided into the phylogenetics and intercontinental spread of *Shigella* spp.

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Diarrhoea remains one of the main causes of mortality in young children in low-income countries[1,2]. Although the number of children aged <5 years who die owing to diarrhoea has declined steadily over the past decade, the incidence of diarrhoeal disease has remained comparatively stable over the same period, at ~2.9 episodes per child per year in 2010 (REFS 1,3). In 2012, the WHO estimated that diarrhoeal disease contributes ~3.6% of the global burden of disease in disability adjusted life years (DALY) and results in ~1.5 million deaths annually.

The recent Global Enteric Multicenter Study (GEMS), an expansive case–control study of moderate-to-severe paediatric diarrhoeal disease, identified enterotoxigenic *Escherichia coli* (ETEC) and *Shigella* spp. as the most common bacterial pathogens in sub-Saharan Africa and South Asia[4,5], and found *Shigella* spp. to be the most prevalent pathogens among children 24–59 months old[5]. Historical data suggest that there were ~165 million cases of shigellosis annually mainly in low-income countries and in children <5 years old between 1966 and 1997, resulting in 1.1 million deaths worldwide[6]. More recently, it was estimated that *Shigella* spp. cause ~125 million disease cases annually[7], and that the incidence of shigellosis is 13.2 cases per 1,000 children per year in children aged <5 years in Asia[8]. Importantly, despite the continued high incidence of *Shigella* spp. infections, the mortality rate per case has dropped by 98%; this is probably due to the disappearance of epidemics associated with the highly pathogenic species *Shigella dysenteriae* and improved, more rapid treatment[7].

The Gram-negative bacterial genus *Shigella* belongs to the family Enterobacteriaceae, which also encompasses other enteric pathogens, including ETEC, enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC; also known as Shiga toxin-producing *E. coli* (STEC)), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and enteroinvasive *E. coli* (EIEC)[9,10]. The pathogenesis and epidemiology of each of these *E. coli* pathovars are distinct and complex, and reflect the diverse catalogue of phenotypic traits that *E. coli* has acquired during its evolution from commensal to pathogen in humans and other mammals[11]. However, *Shigella* spp. stand out from other Enterobacteriaceae: their evolutionary history, mechanism of pathogenesis and human-restricted nature make them unique.

Shigella spp. are intracellular pathogens and are transmitted through the faecal–oral route. They can induce a symptomatic infection via an exceptionally low infectious dose (<10 bacteria), as opposed to *Salmonella* spp. and the various diarrhoeagenic *E. coli* pathovars, which have infectious doses of at least four orders of magnitude greater[12]. *Shigella* spp. cause bacillary dysentery, a severe form of diarrhoea in which blood and mucus can be observed in the stool as a consequence of epithelial cell

damage in the lower gut (BOX 1). The highly pathogenic and exotoxin-producing species *S. dysenteriae* was first described in 1897 by Kiyoshi Shiga, who isolated this species from the stool sample of a patient with epidemic dysentery in Japan[13,14]. The genus was expanded soon after: *Shigella flexneri* was identified in 1899, *Shigella sonnei* in 1906 and *Shigella boydii* in 1921 (REFS 15,16). *Shigella* bacteria are non-motile, non-sporulating and non- or late-lactose-fermenting, and classical taxonomy places all *Shigella* spp. into one major group, which is distantly related to *E. coli*[17]. However, even on their initial characterization, the biochemical and morphological proximity of members of the genus *Shigella* with *E. coli* was noted[13]. Biochemical differences exist between the two genera: *S. dysenteriae* is negative in an indole reaction and cannot ferment mannitol[13], and all *Shigella* spp. are negative for lysine decarboxylation (LDC)[18], whereas the opposites are true for *E. coli*. Current serological classification divides the genus *Shigella* into four species (also known as subgroups), which are further subdivided into serotypes according to type-specific antigens: *S. dysenteriae* (subgroup A) has 15 serotypes; *S. flexneri* (subgroup B) has 19 serotypes and subserotypes; *S. boydii* (subgroup C) has 20 serotypes; and *S. sonnei* (subgroup D) consists of a single serotype.

S. flexneri is currently the major cause of bacillary dysentery in low-income settings (in parts of Asia and sub-Saharan Africa, this species accounts for up to 62% of all *Shigella* spp. infections), whereas *S. sonnei* is the most common pathogen in transitional or high-income countries (up to 80% of all *Shigella* spp. infections in Europe and North America are caused by this species)[19]. *S. boydii* and *S. dysenteriae* cause <5% each of all cases of shigellosis globally. Notably, *S. dysenteriae* was the main cause of dysentery when it was first identified more than a century ago, but today it is infrequently isolated from patients with dysenteric diarrhoea[7,19]. It is thought that poor sanitation, malnutrition and unavailability of clean water, and an exceptionally low infectious dose, genomic plasticity and an ability to accept antimicrobial-resistance genes are all potential reasons why *Shigella* spp. are such successful pathogens and why particular human populations are specifically vulnerable to infection with these species.

The genomics revolution has revealed the dynamic genome plasticity of *Shigella* spp. and their close evolutionary history with *E. coli*[20]. The pathogenesis of *Shigella* spp. depends on a large virulence plasmid that, during its enigmatic evolutionary history, has acquired several factors that are essential for invasion and subversion of host defences[21]. Recent advances in high-throughput genomics and phylogenetics have detailed the emergence and spread of different *Shigella* spp. serogroups, and this information can in turn be used to inform control and public health polices for shigellosis[22–24].

In this Review, we discuss the evolution of *Shigella* spp. to highly specialized, human-specific pathogens, taking into account both insights from traditional genotyping methods and current perspectives achieved from phylogenomics. We focus on the most commonly isolated *Shigella* spp., *S. sonnei* and *S. flexneri*, as these are the dominant species responsible for the global burden of shigellosis.

The evolutionary history of *Shigella* spp.

The acquisition of the *Shigella* virulence plasmid was the key event in the formation of the different *Shigella* spp.[25], but the origins of this plasmid and the relationship between the species was contentious for a long time. The advent of DNA sequencing and accompanying phylogenetic analyses have led to a much clearer picture of the evolutionary relationships between the different *Shigella* spp. and their emergence from *E. coli*.

The phylogenetic relationships of* Shigella *spp. Pioneering research in the early genomics era, carried out by aligning and comparing the DNA sequences of eight chromosomal housekeeping genes, found the *Shigella* genus to contain three major clades or clusters (C1, C2 and C3) and a limited number of outliers, all of which are distinct from, but nested within lineages of *E. coli*[26]. A further examination of 23 chromosomal genes reached a similar phylogenetic conclusion, albeit with increased resolution, subdividing C1 into 3 subclusters (SC1, SC2 and SC3)[27] (FIG. 1a). Most *Shigella* spp. serotypes are distributed over the three major clusters, demonstrating an incongruence between evolutionary history and the conventional serology-based nomenclature. Cluster C1 contains a combination of serotypes from *S. dysenteriae* and *S. boydii*, as well as *S. flexneri* serotype 6: SC1 includes only *S. dysenteriae* (serotypes 3, 4, 6, 9, 11, 12 and 13); SC2 contains mostly *S. boydii* (serotypes 1, 3, 6, 8, 10 and 18), as well as *S. dysenteriae* serotype 5; and SC3 is composed of three other *S. boydii* serotypes (2, 4 and 14) and *S. flexneri* serotype 6. Cluster C2 comprises *S. boydii* (serotypes 5, 7, 9, 11, 15, 16 and 17) and *S. dysenteriae* serotype 2. All *S. flexneri* serotypes except 6 (that is, 1, 2, 3, 4, 5, X and Y) fall into cluster C3, as well as *S. boydii* serotype 12. In this analysis, C2 and C3 were found to share a more recent common ancestor than their common ancestor with C1, thus emphasizing the close phylogenetic relationship between these two clusters.

An analysis of short DNA sequences yielded an estimation of the age of the various clusters (50,000–270,000 years for each of C1 and C2; 35,000–170,000 years for C3)[26]; however, whole-genome sequencing and Bayesian phylogenetic tools are expected to provide a more accurate genome-wide dating of these clusters. Notable outliers not belonging to any of the three major clusters include *S. sonnei*, *S. dysenteriae* 1, 8 and 10, and *S. boydii* 13 (FIG. 1a). The position of *S. boydii* 13 on the tree topology indicates that it is also distant from the *E. coli*–*Shigella* clade. This genetic distance is consistent with the finding that *S. boydii* 13 and an *Escherichia albertii* group form a discrete lineage that separated from an *E. coli* ancestor ~28 million years ago[28].

As highlighted above, *S. sonnei* is an outlier from the other *Shigella* spp., and the precise phylogenetic relationship between *S. sonnei* and the other *Shigella* spp. remains ambiguous. It is assumed that *S. sonnei* emerged more recently than the other *Shigella* spp. and serotypes[29].

Disability adjusted life years (DALY). A measure of overall disease burden, expressed as the cumulative number of years lost owing to ill health, disability or early death.

Pathovars
Groups of bacterial strains that have similar characteristics and are differentiated at the subspecies level on the basis of their distinctive pathogenicity in one or more hosts.

Lysine decarboxylation (LDC). A reaction that is used in a biochemical test to determine the ability of a microorganism to use lysine as a source of carbon for growth. In a positive LDC test, lysine is metabolized into the amine cadaverine through the activity of the enzyme lysine decarboxylase.

Microfold cell
(M cell). A specialized epithelial cell type found in the follicle-associated epithelium of the gastrointestinal tract. Their function is to transport macromolecules and microorganisms across the epithelial barriers to the immune cells, thus inducing mucosal immunity.

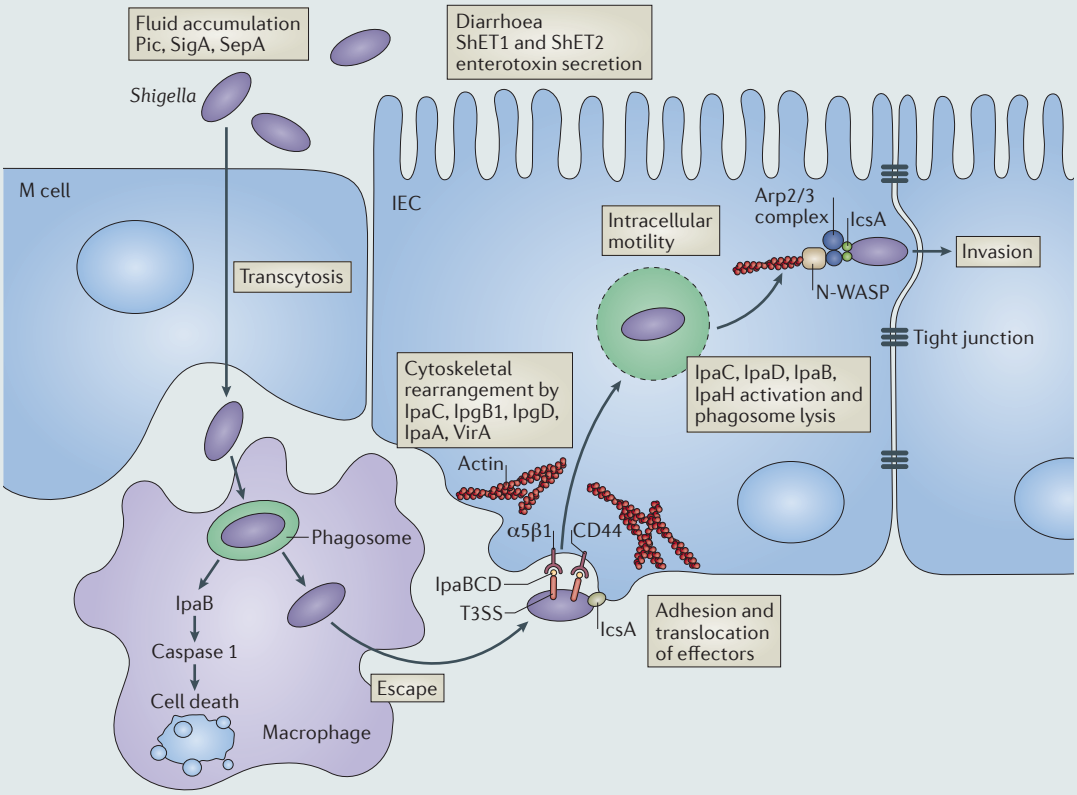
Transcytosis
The selective vesicular transport of macromolecules from one side of the cell to the other while maintaining the unique compositions of these vesicular environments.

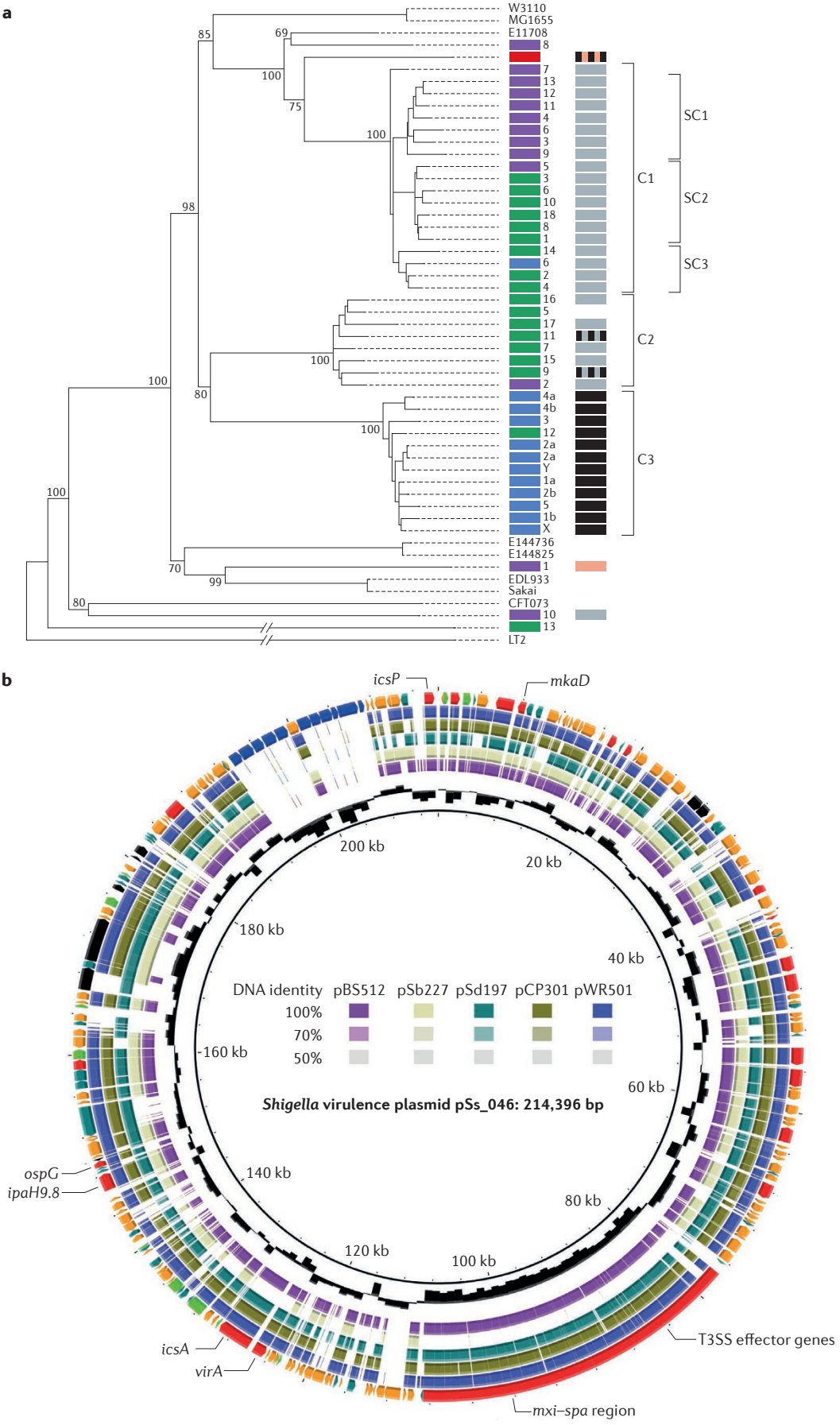
Box 1 | The molecular mechanisms of *Shigella* spp. pathogenesis

Infection with *Shigella* spp. usually results in self-limiting diarrhoea, which is initially watery and then bloody and/or mucus containing. Pathogenesis is tightly orchestrated by the Mxi–Spa type III secretion system (T3SS) and a plethora of effector proteins encoded on the virulence plasmid, including invasion plasmid antigen B (IpaB), IpaC and serine protease SepA, and has been proposed to be a multistep process[37,125]. First, the *Shigella* sp. crosses the intestinal epithelium via a microfold cell (M cell)[126]. This process, termed transcytosis, allows the *Shigella* sp. to cross the physical protective barrier of the host and exposes the bacterium to macrophages in the submucosa of the gastrointestinal tract. The bacterium is phagocytosed, but escapes destruction by inducing rapid macrophage apoptosis through a caspase 1-dependent pathway[127,128]. After its release, the bacterium comes into contact with the basolateral side of an intestinal epithelial cell (IEC) and triggers effector-mediated endocytosis. The Mxi–Spa T3SS needle-like structure is coated by the protein IpaB at the tip, and IpaB has a high affinity for cholesterol-rich areas of eukaryotic cell membranes[129]. The adhesion of the *Shigella* sp. to an IEC is achieved by the binding of IpaB and IpaBCD to the host hyaluronan receptor CD44 and α5β1 integrin, respectively[130,131]. Recently, IcsA was shown to function as an indispensible adhesin to promote contact with host IECs[119]. This close contact facilitates the fusion of the T3SS apparatus and the host cell membrane, leading to the translocation of further effectors. Successful engulfment requires extensive reorganization of the host cell cytoskeleton and modulation of other intracellular pathways, involving the activity of the bacterial effectors IpaC, IpgB1, IpgD, IpaA and VirA in concert[132–136]. *Shigella* spp. are also capable of invading the IECs on the apical side through filopodial capture, which probably requires an interaction between IpaB, IpaD and receptors present on the filopodial extensions but without constitutive binding[137]. After invasion, the *Shigella* sp. quickly lyses its surrounding phagosome by activating IpaC, IpaD, IpaB and IpaH, of which IpaC has been shown to have the pivotal role[138,139]. The cytoplasm serves as a new niche for *Shigella* sp. survival and replication, until diminished resources prompt the invasion of neighbouring cells. The motility of the intracellular *Shigella* sp. is supported by IcsA through the recruitment of the host's neural Wiskott–Aldrich syndrome protein (N-WASP) and actin-related protein 2/3 (ARP2/3) complex, creating a nucleator site for directional actin polymerization to propel the bacterium through the cytoplasm[140]. Intercellular dissemination occurs by this same mechanism when the *Shigella* sp. is endocytosed primarily at tricellular tight junctions, and a new cycle of release–replication–spread takes place.

In contrast to the rapid killing of macrophages, *Shigella* sp. must prolong the survival of infected IECs to ensure its own replication. Inside IECs, the secreted IpaB targets mitotic arrest deficient 2-like protein 2 (MAD2L2; also known as MAD2B), arresting cell maturation[141]. Inhibition of cell death and detachment are triggered by the activity of IpgD and OspE, respectively[142,143]. Cellular secretory functions, including that of pro-inflammatory cytokines, are blocked by the disruption of the Golgi and ER–Golgi trafficking by the activation of the multifunctional effectors IpaB and VirA, respectively[144,145].

Profuse diarrhoea in a *Shigella* sp. infection is attributed to the production of *Shigella* enterotoxin 1 (ShET1) and ShET2, which are encoded in the genomic pathogenicity island *Shigella* island 1 (SHI-1) and the virulence plasmid pINV, respectively[146,147]. In addition, several members of the serine protease autotransporter of Enterobacteriaceae (SPATE) family enhance bacterial virulence. The chromosomally encoded Pic and SigA, as well as the pINV-encoded SepA, intensify intestinal fluid accumulation in an animal model, a hallmark of the early stages of the infection[42,148].





O antigen
A repeating glycan polymer attached to the outer core in lipopolysaccharide. This structure is on the very outer surface of the bacterial cell and is therefore a target for recognition by the host immune system.

Unlike the other *Shigella* spp., *S. sonnei* expresses an O antigen, encoded by a genetic locus that is also found in the genetically distant Gram-negative organism, *Plesiomonas shigelloides*[29]. A sequence comparison of the O antigen loci from *S. sonnei* and *P. shigelloides* predicts that the O antigen genes diverged approximately 10,000 years ago, placing an upper limit on the age of the formation of *S. sonnei*[29]. However, a more recent study using whole-genome sequencing data from globally distributed isolates estimated that all extant strains of *S. sonnei* descend from a common ancestor that existed <400 years ago, implying that a historical evolutionary bottleneck might have resulted in the extinction of the pre-existing *S. sonnei* strains[22].

The early sequence-based genotyping studies described above largely resolved the phylogenetic relationships of the different *Shigella* spp., but more recent studies have exploited *Shigella* spp. and *E. coli* whole-genome sequences to investigate the evolutionary relationship between these two taxa in more detail. Phylogenetic trees for the entire *E. coli–Shigella* group were constructed using an alignment-free feature frequency profile (FFP), which compares genomes based on the frequencies of oligonucleotide sequences with an optimal length for analysis (so-called features)[30]. These phylogenies, together with those deduced from other studies using core genetic features (present in all genomes and with low variability), have confirmed that the genus *Shigella* is composed of several clusters interspersed in the *E. coli–Shigella* phylogeny, strongly supporting the notion that *Shigella* spp. have emerged from several *E. coli* ancestors on multiple independent occasions[31–33]. The phylogenomic structure of the genus *Shigella* derived from a collection of 336 *E. coli–Shigella* isolates correlates with the grouping from the aforementioned studies based on a limited number of genetic markers[34]. In addition, whole-genome resolution phylogenomics also resolves the context for the origins of these major clades: it places C1 and *S. sonnei* in *E. coli* group B1; C2 and C3 in *E. coli* group A; and *S. dysenteriae* 1 in *E. coli* group E[34]. This supports the theory that the phenotypic similarity observed across the *Shigella* spp. is the result of convergent evolution, in which different *Shigella* founders independently gained genes that facilitate invasive pathogenicity. Only one *E. coli* pathovar, EIEC, has also acquired invasiveness; EIEC comprises several discrete lineages and exhibits pathogenic and biochemical features that are indistinguishable from those of *Shigella* spp. Notably, both EIEC and *Shigella* spp. harbour an analogous virulence plasmid, are non-motile and show a negative LDC test[35]. These similarities have led to the speculation that EIEC represents a distinct non-toxin-producing *Shigella* 'prototype', which could be a precursor for a 'complete' *Shigella* sp. if selective pressure favours further adaptation of this invasive *E. coli* pathovar[36].

The* Shigella *virulence plasmid. The *Shigella* virulence plasmid, which can be as large as ~220 kb, encodes essential virulence factors that facilitate the invasion and spread of *Shigella* spp. into human macrophages and enterocytes[37] (BOX 1). The virulence plasmid contains the conserved 30 kb *mxi–spa* locus, which encodes the Mxi–Spa type III secretion system (T3SS), and genes encoding invasion plasmid antigens (Ipas). The Mxi–Spa T3SS is a molecular syringe that injects effector proteins directly into host cells. This secretion apparatus enables a complex interaction between the bacterium and the host cell, ultimately resulting in a disruption of the intestinal mucosa and the distinctive symptoms of bacillary dysentery. Therefore, the virulence plasmid is the key molecular signature of *Shigella* spp. pathogenesis and is fundamental for initiating infection and manipulating the immune response of the host (BOX 2).

Various DNA sequencing projects have been carried out across several different *Shigella* spp. lineages to elucidate the structure and functions of the virulence plasmid. These projects have uncovered a complex plasmid configuration with a mosaic nature, which is the result of numerous horizontal gene transfer and rearrangement events[21,38,39] (FIG. 1b). The evaluation of three genes in the *mxi–spa* region (*mxiA*, *mxiC* and *ipgD*) revealed two isoforms of the *Shigella* virulence plasmid (pINV A and pINV B) with greater divergence in *ipgD* than in the two *mxi* genes[25]. pINV A and pINV B exhibited incompatibility grouping (plasmids of the same incompatibility group cannot be stably inherited in the same cell)[40]. When plasmid subtype is mapped onto the *Shigella* spp. phylogeny (FIG. 1a), all C1 isolates harbour pINV A, whereas all C3 isolates possess the pINV B isoform. Both forms of the plasmid can be found in C2 isolates. The outlier strains harbour either of the two plasmid forms, which is a sign of lateral gene transfer in their history. For example, *S. dysenteriae* 10 and most EIEC strains harbour pINV A, whereas *S. sonnei* retains pINV B[36,41]. By contrast, *S. dysenteriae* 1 harbours a unique mixed plasmid form (*ipgD* derived from pINV A, and *mxiA* and *mxiC* derived from pINV B). This suggests that several ancestral virulence plasmids, from an unknown source, have entered into a diverse background of *E. coli*

◀ Figure 1 | **The phylogenetic structure of the four *Shigella* spp. and the signature virulence plasmid. a** | A neighbour-joining phylogenetic tree generated by sequencing 23 chromosomal genes[27]. Strains are labelled by serotype and coloured by species: *Shigella sonnei* in red, *Shigella flexneri* in blue, *Shigella boydii* in green and *Shigella dysenteriae* in purple; *Escherichia coli* isolates are uncoloured. Bootstrap values of >50% are indicated at the major nodes, and the three major *Shigella* genus clusters (C) and subclusters (SC) are indicated. The carriage of the two specific isoforms of virulence plasmids is additionally indicated in the second column of coloured blocks: pINV A (grey), pINV B (black), either pINV A or pINV B (hatched black and grey), a unique form of pINV (pink), and either pINV B or a unique isoform (hatched black and pink). **b** | A comparative gene map of the *Shigella* virulence plasmid, using the *S. sonnei* virulence plasmid pSs_046 as a reference; the innermost ring represents pSs_046, with coordinates. The second ring (black) shows the GC content of the reference pSs_046 sequence. The following purple, pale green, teal, khaki, and blue rings show BLASTN comparisons between pSs_046 and the virulence plasmids of *S. boydii* str. BS512, *S. boydii* str. Sb227, *S. dysenteriae* str. Sd197, *S. flexneri* F2a str. 301 (pCP301) and *S. flexneri* F5a (pWR501), respectively. The outer ring represents annotations of genes or genetic clusters based on function: known virulence factor genes (red); plasmid replication, transfer and maintenance genes (black); transposon, phage-borne and insertion sequence elements (orange); genes encoding hypothetical proteins (teal); the *S. sonnei*-specific O antigen biosynthesis cluster (blue); and genes encoding proteins with other known functions (green). *ipa*, invasion plasmid antigen gene; *icsP*, also known as *sopA*; T3SS, type III secretion system. Part **a** is modified with permission from REF. 27, Springer.

Box 2 | **The immune response against *Shigella* spp.**

Immune modulation has a major role in *Shigella* spp. pathogenesis, beginning with the ability of the organisms to manipulate the innate immune response[149]. In the initial stages of infection, rapid killing of infected macrophages by the caspase 1 pathway releases the pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18 (REF. 150). This acute inflammation, heightened by the secretion of CXC-chemokine ligand 8 (CXCL8; also known as IL-8) from infected epithelial cells, triggers the transepithelial migration of neutrophils and an influx of more *Shigella* sp. cells[149,151]. By contrast, inside enterocytes, the *Shigella* sp. releases a cascade of effectors, such as MkaD, which inactivate the mitogen-activated protein kinases (MAPKs) p38 and ERK2; OspG, which targets the ubiquitin-conjugating enzyme E2; OspI, which deamidates the E2 enzyme UBC13 (also known as UBE2N); OspZ, which prevents the nuclear translocation of the transcription factor NF-κB; and invasion plasmid antigen H9.8 (IpaH9.8), which targets the NF-κB essential modulator (NEMO; also known as IKKγ) complex. These pathways inhibit the NF-κB-dependent inflammatory responses, masking the bacteria from detection by the immune system and maintaining their intracellular proliferation[152–154].

The death of B cells and T cells has been observed during infection with *Shigella flexneri*[155]. The type III secretion system (T3SS) effector IpgD has been shown to impair the migration of activated T cells *in vitro* probably through phosphatidylinositol hydrolysis, which impedes the reorganization of the cytoskeleton[156]. Inhibition of T cell migration compromises T cell contact with antigen-presenting cells and thus dampens the adaptive immune response. An *in vivo* study of *Shigella flexneri* infection in lymph nodes has confirmed the capacity of the bacterium to invade T cells and arrest their movements by the T3SS[157]. In addition, there is evidence that the T3SS-coating protein IpaD targets Toll-like receptor 2 (TLR2) on B cells and induces apoptosis, irrespective of invasion[158].

founder strains. Such introductions include pINV A and pINV B into major *Shigella* clusters C1 and C3, respectively, thus giving rise to these two lineages. Independent acquisitions of either plasmid isoform by *Shigella* spp. isolates not belonging to the main clades, as well as lateral gene transfer in the C2 isolates, complicate the evolutionary history of *Shigella* spp.

Plasmid sequences have also been compared for five virulence genes (*mkaD* (also known as *ospF*), CP0014, *parA*, *parB* and *repA*) located outside the core entry region (defined as the ~30 kb cluster encoding the T3SS and associated effector proteins that facilitate the mechanistic invasion of the bacteria into enterocytes). The constructed phylogeny was consistent with the one based on chromosomal genes[27], except for a close relationship between C1 and C2 isolates in the plasmid phylogeny; by contrast, the C2 and C3 clusters showed close proximity in the chromosomal phylogeny. The authors of the plasmid phylogeny report argued that the virulence plasmid acquired by C1 and C2 isolates differed from the one obtained by C3 isolates. Interestingly, the virulence plasmids from the outliers *S. dysenteriae* 1 and *S. sonnei* share considerable homogeneity and can be grouped together outside of the three major clusters. These data suggest that *Shigella* spp. have arisen on several independent occasions owing to the transmission of multiple virulence plasmid forms to many *E. coli* ancestors. The authors suggested that the subsequent loss of the *tra* locus, which aids the exchange of plasmids between bacteria by conjugation, on the virulence plasmid restricted its transmissibility and enabled parallel evolution of the virulence plasmid and the bacterial chromosomes, thus creating the several discrete *Shigella* lineages observed today.

Gain and loss of gene function. The ability to invade host cells and escape the competitive environment of the gastrointestinal tract was pivotal in the emergence of *Shigella* spp. Although acquisition of the virulence plasmid is a 'foothold moment' in the evolution of this pathogen, it is not the only long-term evolutionary change. Numerous other plasmids with different functions have been crucial during the evolution of *Shigella* spp. (BOX 3). In addition to the genes encoded on the virulence plasmid, several clusters of horizontally acquired genetic material, carrying genes that facilitate interactions with the host and contribute to pathogenesis, have been incorporated into the chromosome of *Shigella* spp.

These pathogenesis-associated genomic regions are pathogenicity islands (PAIs) and have various functions; the largest PAI encodes an enterotoxin (*Shigella* island 1 (SHI-1)) and it enables the sequestration of iron (SHI-2, SHI-3 and *sitABCD*), the ability to modify the O antigen (SHI-O) and resistance to antimicrobials (*Shigella* resistance locus (SRL))[37,42–46]. PAIs have enhanced the virulence and adaptability of *Shigella* spp. and are commonly associated with bacteriophage integrases, which highlights the fact that bacteriophages had a major role in the evolution of *Shigella* spp. One such bacteriophage-associated element is the Shiga toxin (Stx) prophage in *S. dysenteriae* 1; Stx expression can have severe complications, including haemolytic uraemic syndrome (HUS). Recently, an alternative prophage (φPOC-J13) encoding Stx1a was identified in several clinical isolates of *S. flexneri* and *S. dysenteriae* 4 from patients returning from or residing in Hispaniola[47–50]. Unlike the cryptic prophage in *S. dysenteriae* 1, φPOC-J13 seems to be capable of disseminating the stx_{1a} gene into other *Shigella* spp. isolates by transduction[50]. Insertion sequence elements — small transposable DNA sequences that can 'jump' within bacterial genomes — are also highly abundant in *Shigella* spp. chromosomes and virulence plasmids. These elements have shaped the genome architecture of *Shigella* spp., causing gene inactivation and genome rearrangement[20,21,51]. An analysis of >400 genomes from a range of bacterial species found that, in relation to genome size, *E. coli* and *Shigella* spp. possess the highest number of insertion sequence elements[52].

Linked to this insertion sequence expansion, *Shigella* spp. genomes have also undergone substantial functional gene loss[53]. Similar phenomena have been observed in other human-restricted pathogens, such as *Yersinia pestis*, *Mycobacterium leprae* and *Salmonella enterica* subsp. *enterica* serovar Typhi[54–56]. The modes of gene inactivation are variable in different *Shigella* spp. strains and range from the complete deletion of a locus, to missense point mutations, to insertions. However, gene inactivation has occurred preferentially in specific genetic regions and operons rather than being randomly distributed throughout the genome[20,57]. Independent inactivation of the same or functionally similar genes in different *Shigella* spp. represents a major pathway of convergent evolution, resulting in similar phenotypic changes that are associated with adaptation to new niches. For example, different mutations have resulted

Conjugation
The transfer of genetic material between bacterial cells through cell-to-cell contact or by a bridge-like connection between two cells.

Transduction
A mode of horizontal gene transfer whereby genetic material is transferred from one bacterium to another by a virus.

Flagellum
A multiprotein thread-like structure protruding from prokaryotic or eukaryotic cells that is used for motility and for the sensory perception of extracellular chemicals and temperature.

Fimbriae
Appendages composed of the protein curlin and found on many Gram-negative and some Gram-positive bacteria. Fimbriae are used mainly for adherence to bacterial cells, host cells and abiotic surfaces.

Box 3 | ***Shigella* spp. plasmids**

The main plasmid that enables the intracellular lifestyle of *Shigella* spp. is the large virulence plasmid pINV, which encodes a type III secretion system (T3SS) and an arsenal of virulence factors[21,159]. In *Shigella sonnei*, pINV also harbours an O antigen biosynthesis cluster homologous to that of *Plesiomonas shigelloides*[29]. Under laboratory conditions, this plasmid is usually lost from *S. sonnei* after subsequent culturing, a factor that has undergone limited detailed investigation[22,160]. An additional small plasmid, pHS-2, harbours a single gene, chain length determinant (*cld*; also known as wzz_{pHS-2}), which is instrumental in controlling O antigen chain length and, thus, determining resistance to serum killing[116,161]. Although pHS-2 was originally thought to be associated with reactive arthritis induced by shigellosis as a result of a hypothesized molecular mimicry of human HLA-B27 by a pHS-2-encoded protein, this suggestion has been challenged by recent findings, including the occurrence of reactive arthritis in patients infected with pHS-2-negative *S. sonnei*[162–164]. Colicins are bactericidal proteins that act on those bacterial species which are closely related to the colicin-producing bacteria, and it has been proposed that colicins provide *Shigella* spp. with a competitive advantage against other susceptible *E. coli* or *Shigella* spp. populations[165]. It has been speculated that the introduction of colicin-encoding plasmids initiated the recent clonal expansions of specific *S. sonnei* populations, such as the acquisition of pDPT1 by *S. sonnei* in Vietnam and of pSSE3 by *S. sonnei* in South Asia[75,165,166].

Shigella spp. have also acquired several antimicrobial-resistance plasmids. The small 8 kb spA plasmid, encoding resistance to sulfonamide, streptomycin and tetracycline, was introduced into multiple *S. sonnei* populations during the latter part of the twentieth century, rendering treatments with these drugs ineffective[22]. More recently, stably inherited extended-spectrum β-lactamase (ESBL)-producing plasmids are increasingly being recovered from both *S. sonnei* and *S. flexneri* in developing countries, which raises concerns about restricted therapeutic options, in particular as fluoroquinolone resistance is increasing worldwide[19,24,75]. Other modern antimicrobials are also at risk of becoming inactive, as plasmid pKSR100, carrying resistance against azithromycin, erythromycin, β-lactams and aminoglycosides, has been commonly found in the *S. flexneri* 3a lineage circulating in the men who have sex with men (MSM) community[101].

in the loss of flagellum biosynthesis and specific structures of the fimbriae[20,58]. Importantly, flagellum loss results in reduced immunogenicity and in evasion of the human immune system, as flagellin is a pathogen-associated molecular pattern (PAMP)[59]. In addition, computational reconstruction of metabolic functions (or their loss) based on genomic data groups the different *Shigella* spp. together and away from *E. coli* pathovars and commensals, purely by their catabolic function[60]. For example, the *E. coli* genes *cadA* and *nadAB* (responsible for the synthesis of cadaverine and the NAD precursor quinolinate, respectively) hinder intercellular spread, phagosomal escape and antigen secretion[61–63]. Likewise, *ompT* and *argT* inhibit intracellular motility and invasive capacity, respectively[64,65]. Therefore, the loss of these genes in *Shigella* spp. ensures patho-adaptation for an intracellular lifestyle. Alternatively, the loss of gene function can increase the survival rate in a new niche. Disruption of *speG*, which encodes spermidine acetyltransferase, leads to the accumulation of the polyamine spermidine, which acts as scavenger of free radicals and thereby provides resistance to oxidative stress in macrophages[66].

In comparison to *E. coli*, *Shigella* spp. have lost more genes, which is attributed to a reduction in genome-wide purifying selection and the fixation of inactivating mutations without greatly compromising fitness. It has been suggested that this resulted from a decrease in effective population size when *Shigella* spp. became human-restricted pathogens, compared with their *E. coli* ancestor. In addition, the intracellular niche in which *Shigella* spp. began to thrive imposed a more relaxed selective pressure owing to abundant resources and a relative lack of competitors[67,68].

Pathogen-associated molecular pattern
(PAMP). A set of specific molecules that are present on groups of pathogens and are recognized by the innate immune system. These conserved molecular motifs in bacteria, such as lipopolysaccharide and flagellin, are usually recognized by Toll-like receptors and other pattern-recognition receptors.

Cadaverine
A diamine with a putrid odour. It is the product of lysine decarboxylation.

Quinolinate
A dicarboxylic acid generated as the downstream product of tryptophan catabolism. It acts as a substrate for the biosynthesis of nicotinic acid mononucleotide and, ultimately, the formation of the coenzyme NAD.

Genomic insights into *Shigella* spp.

The advent of high-throughput whole-genome sequencing has permitted the detection of genomic variation in the form of single-nucleotide polymorphisms (SNPs) and accessory genome content. These techniques give us an unprecedented view of how *Shigella* spp. have emerged and been transmitted globally, and how antimicrobial resistance has swept through the population throughout the later part of the twentieth century.

Shigella sonnei. Although *S. sonnei* is the most common *Shigella* species in middle-income and high-income countries, the recent emergence of this species in transitional lower-income countries has highlighted the need for more effective surveillance systems and has opened new avenues of vaccine research[69–73] (BOX 4). In a key study, the genomes of 132 globally representative *S. sonnei* isolates were sequenced and analysed to investigate the recent evolution of the species[22]. Three main lineages of *S. sonnei* were identified (I, II and III), which share a most recent common ancestor <400 years ago. All lineages probably originated in Europe, as the oldest lineages, and the majority of all genetic diversity, were detected in European isolates (FIG. 2a). Although all three lineages were distributed in Europe, not all of them have spread globally. Isolates from Asia, Africa and South America were predominantly representatives of the more recently expanded lineage III. Lineage III (particularly clade Global III) emerged in the 1970s and spread internationally in the 1980s and 1990s, establishing more distant endemic populations in other regions of the world (FIG. 2a). Importantly, there was a correlation between global dissemination and the acquisition of resistance to multiple antimicrobials. Resistance was mediated through the gain of class II integrons and mutations in DNA gyrase subunit A (*gyrA*), which encodes the target protein for fluoroquinolones (a family of broad-spectrum antimicrobials). These modifications probably resulted from strong selective pressures induced by antimicrobial exposure; indeed, antimicrobial resistance may be advantageous in promoting post-symptomatic shedding of bacteria and sustained short-term transmission in the host population[74].

A study that investigated more than 250 *S. sonnei* samples in Vietnam expanded the observations of the global study[75]. Genomic and phylogeographical analyses showed that the Global III lineage became established in Ho Chi Minh City following the reunification of the country in 1975. The founder clone later spread north to other provinces, where it established, albeit after multiple introductions, further discrete endemic populations (FIG. 2b). Clonal expansion in these regions contributed to the increase in *S. sonnei*-associated dysentery in Vietnam. In larger human populations, such as in Ho Chi Minh City, a series of bottlenecks in the bacterial population and the stepwise accumulation of antimicrobial resistance were observed, probably as a consequence of the

Box 4 | **Vaccines against *Shigella* spp.**

The adaptive immune response to *Shigella* spp. infection largely targets the bacterial O antigen[167], rendering this structure a sound candidate for vaccine development. However, this approach is hindered by the wide geographical distribution of numerous serotypes, highlighting the requirement for large-scale surveillances. It was calculated that ~64% of the shigellosis episodes in the Global Enteric Multicenter Study (GEMS) project were caused by only four serotypes, *Shigella sonnei*, *Shigella flexneri* 2a, *S. flexneri* 3a and *S. flexneri* 6 (REF. 113). Owing to the extensive cross-protection provided by *S. flexneri* 2a and *S. flexneri* 3a O antigens against other serotypes of this species[168], a quadrivalent vaccine composed of the O antigens of the four serotypes could in theory provide ~88% coverage (in the case of a 100% efficacious vaccine) against shigellosis[113].

The rise of *S. sonnei* in economically transitioning nations poses questions about the management and control of shigellosis worldwide and highlights the feasibility of a vaccine against a single-serotype enteric pathogen. It has been suggested that because *S. sonnei* and *Plesiomonas shigelloides* have an almost identical O antigen structure, exposure to the latter in contaminated water provides immunity to the former by passive 'environmental' immunization[29]. An improvement in access to clean water facilities in transitional economies reduces the occurrence of environmental *P. shigelloides* and any potential cross-protective passive immunization. This may explain why *S. sonnei* is able to thrive in transitional countries. However, despite being a fascinating hypothesis, the exact correlation between these two phenomena remains unclear[169].

Shigella vaccine development involves consortia of experts, and it has been reviewed elsewhere[170–172]. Currently available candidate vaccines can be classified into three major approaches: those targeting specific O antigens, those targeting common conserved proteins and those targeting a combination of both. Live-attenuated variants of *S. sonnei*, *S. flexneri* 2a and *Shigella dysenteriae* 1 are entering different phases in clinical studies; these variants have been engineered to harbour mutations in essential virulence genes, such as *guaA*, *guaB*, *icsA*, or enterotoxin genes *senA* (*Shigella* enterotoxin 2), *senB*, *stxA* (Shiga toxin subunit A) or *stxB*. Furthermore, serotype-specific lipopolysaccharides conjugated with carrier proteins (*Pseudomonas* exoprotein A or tetanus toxoid) are also potential candidates. Purified virulence plasmid-encoded proteins invasion plasmid antigen B (IpaB) and IpaD were shown to confer protection in animal models, as well as the IcsP and SigA proteins[173]. Invaplex, a combination of highly conserved *Shigella* spp. IpaBCD and lipopolysaccharide, induces a serotype-specific immune response after intranasal delivery[174].

strong selective pressure exerted by the high use of antimicrobials in the country. Furthermore, plasmid pDPT1, encoding an E5 type colicin (a bactericidal toxin with RNA degradation potency) and an associated immunity protein (protecting the producer from the activity of the corresponding colicin), became fixed in the Ho Chi Minh City population following the first selective sweep in 1994, providing a crucial selective advantage over other non-immune *Shigella* spp. and *E. coli* strains. In the 2006 selective sweep, the population acquired plasmid pKHSB1, which harbours an extended-spectrum β-lactamase (ESBL) gene. This explains the sudden increase in the isolation rate of cephalosporin-resistant *S. sonnei* in the following years in the region[76]. The acquisition of a plasmid conferring resistance to third-generation cephalosporins (FIG. 2b) reoccurred in satellite populations in the central region of Vietnam, namely in the Khanh Hoa province. Similarly, other signs of convergent evolution included the independent emergences of *gyrA* mutations in Ho Chi Minh City and other provinces, reducing the susceptibility to fluoroquinolones. With such a detailed understanding of the *S. sonnei* population in Vietnam, the authors suggested that *S. sonnei* could act as a sentinel organism for the surveillance of human enteric bacterial pathogens by providing a tractable window onto the circulating antimicrobial-resistance elements in other Gram-negative enteric bacteria in a specific region. Indeed, the transfer of third-generation cephalosporin resistance plasmids between *S. sonnei* and commensal *E. coli* in the human gut might occur, as the expansion of the *S. sonnei* population during an episode of infection greatly increases the chance of contact between these two organisms[77].

Shigella flexneri. Alongside *S. sonnei*, *S. flexneri* remains a major aetiological agent of bacillary dysentery, particularly in low-income and middle-income countries. Much of our epidemiological knowledge about *S. flexneri* comes from serotyping data. *S. flexneri* serotypes differ in their O antigens, and there is experimental evidence that the O antigen conformation is important for invasion and the evasion of innate immunity[78]. However, serotype conversion (that is, the modification of the serotype in a clonal population) is well documented in *S. flexneri* and mediated by bacteriophages and plasmids carrying genes that contribute to variation of the O antigen structure. The bacteriophages often integrate as prophages into the chromosomal *thrW* tRNA site, for prophages carrying the glycosylation (*gtr*) operon, or into the *argW* tRNA site, for those carrying the *O*-acetylation (*oac*) gene, and lead to changes in the O antigen structure[79,80]. Many O antigen-modifying bacteriophages have been identified to date, including SfI, SfII, Sf6, SfIV, SfV and SfX, which convert *S. flexneri* Y into serotypes 1a, 2a, 3b, 4a, 5a and X, respectively[81–86]. Furthermore, several novel *S. flexneri* serotypes have been discovered in the past decade, which complicates the epidemiology and potential protective efficacy of any potential O antigen-based vaccines (BOX 4).

The emergence of novel *S. flexneri* serotypes has been widely observed. For example, *S. flexneri* 1c was first identified in Bangladesh in the 1980s, and an unrelated clone of this serotype was then also found to be prevalent in rural northern Vietnam and several other Asian countries[8,87,88]. Furthermore, the emergence of *S. flexneri* 1d, X variant (Xv) and 4s has been reported in China[89–91]. Many of these novel serotypes harbour more than one O antigen-modifying operon, resulting in additional modifications in the already highly modified tetrasaccharide. For example, the introduction of *gtr1C* into *S. flexneri* 1a leads to the addition of a glucosyl group on the glucosyl-linked *N*-acetylglucosamine, effectively converting this serotype into the novel serotype 1c[92]. Unpredictably, the *gtr1C* cluster shares similarities with genes from *Citrobacter koseri* rather than with previously characterized orthologues in other *S. flexneri* serotypes. This suggests that *S. flexneri* can sample from a large pool of O antigen-modifying genes. Plasmid-mediated serotype conversion has also been reported in *S. flexneri* Xv, 4s and Yv. The plasmid-borne O antigen phosphoethanolamine transferase (*opt*) gene was found to be essential for the transfer of phosphoethanolamine (PEtN) to the second rhamnose (RhaII) and RhaIII of the O antigen in *S. flexneri* Xv and *S. flexneri* Yv, respectively[93,94].

Class II integrons
Mobile genetic elements that are capable of carrying genes, including antimicrobial-resistance genes, and integrating into bacterial chromosomes by site-specific recombination. An integron contains at least an integrase, an attachment site and a promoter. Classification is based on the type of integrase.

Molecular typing of *S. flexneri* has, to date, largely relied on pulsed-field gel electrophoresis (PFGE) and/or multilocus sequence typing (MLST), using the sequences of the seven housekeeping genes: *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*[95,96]. MLST of more than 100 Asian *S. flexneri* isolates revealed that serotypes 1–5, X and Y belong to a discrete clonal complex (ST245 of the ST245 complex), whereas serotype 6 forms a distinct clonal complex (ST145 of the ST243 complex)[97,98]. Although the resolution of MLST for *S. flexneri* is limited because of an inadequate number of differentiating mutations in the selected housekeeping genes, especially for investigating local clonal expansion or fine-scaled phylogenetic relationships, this method has provided insights into the genetic relationship between major *S. flexneri* serotypes. For example, studies examining the spread of the epidemic *S. flexneri* clone ST91 in China have low resolution, but have aided the tracking of this pathogen across the region[90]. *S. flexneri* clone ST91, which was typed using another *E. coli* genotyping scheme[90], is actually typed ST245 using the *Shigella* spp. MLST approach described above[95]. The alternative *E. coli* typing scheme relies on 15 housekeeping genes — *aspC*, *clpX*, *fadD*, *icdA*, *lysP*, *mdh*, *uidA*, *arcA*, *aroE*, *cyaA*, *dnaG*, *grpE*, *mtlD*, *mutS* and *rpoS* — and provides better resolution for MLST, especially for clonal populations, such as the *S. flexneri* ST245 complex. To obtain even higher resolution, this expanded MLST scheme was combined with PFGE to investigate the expansion of *S. flexneri* clone ST91. Somewhat atypically for members of the genus *Shigella*, *S. flexneri* clone ST91 underwent at least 57 independent serotype switching events during its clonal expansion in China[90], illustrating the potential problem with using serotyping as a proxy for genetic relatedness. A major serotype conversion in the *S. flexneri* ST245 complex led to the rise of a novel variant, *S. flexneri* Xv, which then rapidly spread and became one of the most prevalent serotypes in China since 2000 (REF. 90). The spread of *S. flexneri* Xv is concerning, as this serotype is resistant to several antimicrobials (see below).

Extensive serotype switching and the success of specific clones highlight the need for higher-resolution tracking and monitoring of *S. flexneri*. Whole-genome sequencing provides such higher-resolution data; for example, this method showed that *S. flexneri* ST91 serotype Xv had acquired a plasmid carrying *opt*, leading to O antigen modification, on three independent occasions[94]. Before the *opt*-harbouring plasmid was introduced, clone ST91 had already carried antimicrobial-resistance genes, including the SRL locus (a multidrug-resistance (MDR) genomic island harbouring resistance genes against tetracycline (*tetACDR*), streptomycin (*aadA2*), ampicillin (*oxa1*) and chloramphenicol (*cat*)), Tn*7* (an MDR transposon carrying resistance genes against trimethoprim (*dfrA1*), streptothricin (*sat1*) and streptomycin (*aadA1*)) and two mutations in *gyrA* facilitating resistance against nalidixic acid. The rapid expansion of the ST91 clone in different geographical locations can be explained by O antigen switching and the evasion of pre-existing immunity in host populations, and by the ineffectiveness of antimicrobials owing to the MDR background, which promotes prolonged faecal shedding and sustained circulation[74].

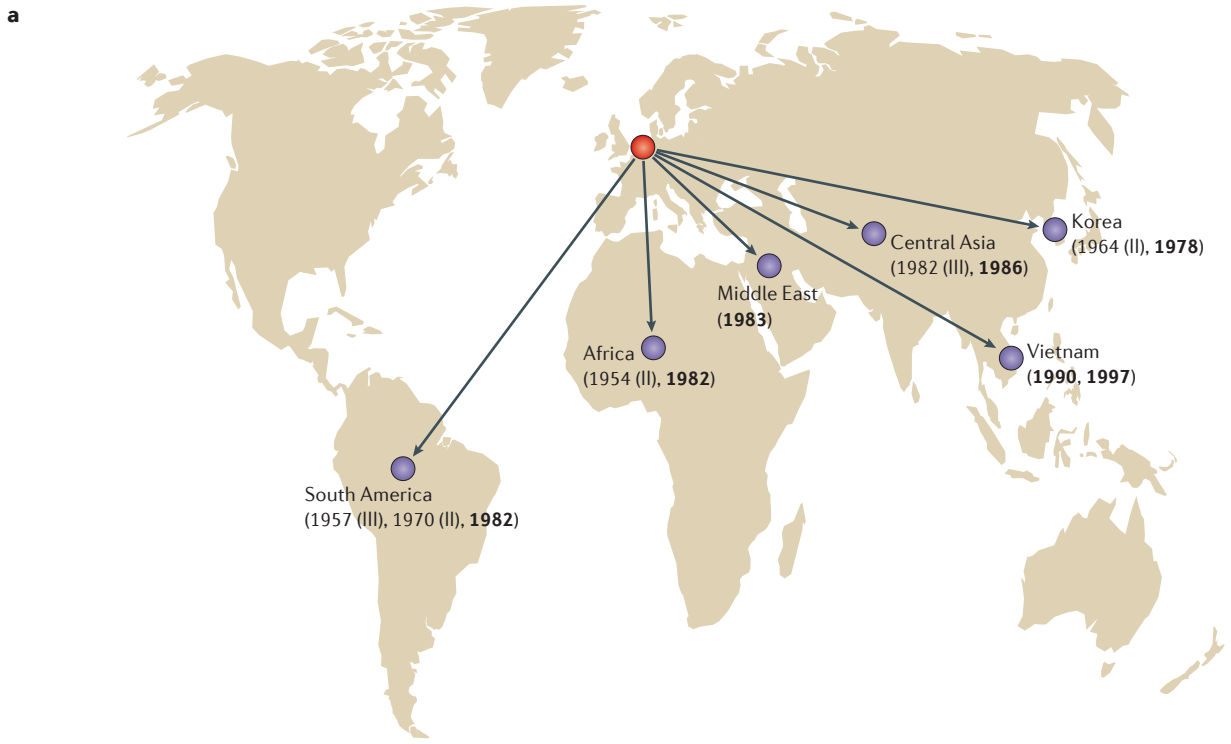
In addition to the substantial species shift observed in developing countries, *S. flexneri* epidemiology has also changed in certain populations in developed countries. The isolation rate of *S. flexneri* 3a has increased steadily in men who have sex with men (MSM) communities in Canada, England and Wales[99,100]. This increased isolation rate is not attributable to an introduction (or introductions) from the low-income countries, suggesting that the ecology of this particular variant may now be better adapted to transmission within MSM populations[99]. A recent genomic analysis of a global collection of this serotype indicated the emergence of an *S. flexneri* 3a lineage attributed to infections in MSM populations in higher-income countries[101]. This lineage has spread globally since its emergence in 1998, and as is common for current populations of *Shigella* spp., has acquired resistance to multiple antimicrobials, most notably azithromycin, a frequent antimicrobial treatment for sexually transmitted diseases, including gonorrhoea, syphilis and chlamydia. This change in antimicrobial susceptibility, seen as the response to selective pressure exerted by azithromycin treatment for comorbid infections, has contributed to the dominance of this organism in MSM populations[101].

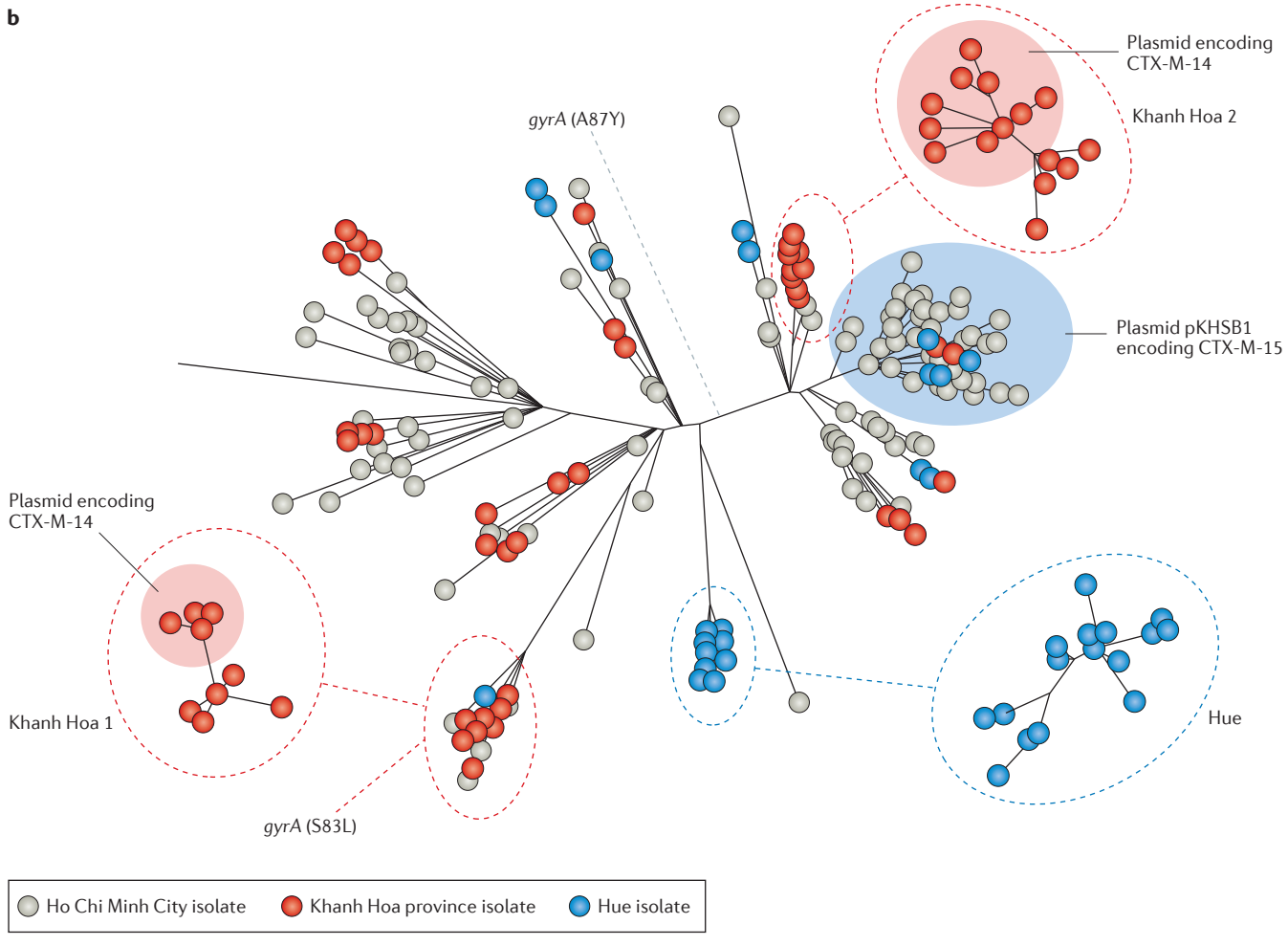
Studying the evolution and epidemiology of *S. flexneri* has proved complicated owing to serotype diversity, until a recent study of 351 whole-genome sequences from different serotypes of this species[24]. This study concluded that *S. flexneri*, with the exclusion of the diverging serotype 6, consists of seven phylogenetic groups (FIG. 3). Notably, these phylogenetic groups are inconsistent with serotype groupings and have arisen on several occasions between the 1300s and the 1800s[24]. The presence of numerous serotypes in all phylogenetic groups suggests that serotype switching is common, consistent with previous research[90].

This study also revealed substantial variability in the composition of *S. flexneri* virulence factors (for example, the genomic island SHI-1, and genes encoding iron uptake systems, such as the enterobactin genes and the ferric dicitrate transport (*fec*) locus) and antimicrobial-resistance genes (for example, the SRL island). SHI-1, SRL and enterobactin genes exclusively co-occur in phylogenetic group 3 (PG3), which is composed predominantly of *S. flexneri* serotype 2a, and this may account for the enhanced virulence and international dominance of this serotype[24]. The accumulation of antimicrobial-resistance genes in *S. flexneri* over the past three decades is considered to be essential for maintaining successful lineages. However, unlike for *S. sonnei*, this has neither led to the displacement of pre-existing antimicrobial-susceptible lineages nor resulted in substantial international transmission, with the exception of the global spread of the MSM-associated serotype *S. flexneri* 3a[22,101]. This finding supports the concept of longer-term colonization, in which diverse populations of both antimicrobial-resistant and antimicrobial-susceptible lineages co-circulate in endemic locations. These data also imply that *S. flexneri* is persisting in the environment, where selection for antimicrobial resistance may be less favourable.

Pulsed-field gel electrophoresis
(PFGE). A molecular typing technique based on the migration pattern of DNA fragments of variable lengths, generated by restriction enzyme treatment, in an electrical field.

Multilocus sequence typing
(MLST). A DNA sequence-based molecular typing scheme in which each isolate is distinguished by a combination of unique alleles of housekeeping genes (by comparing their genetic variations).





Homoplasies
Phenotypic or genotypic characteristics that are shared by a set of organisms but not inherited from a common ancestor.

Shigella dysenteriae *and* Shigella boydii. As *S. dysenteriae* and *S. boydii* account for <10% of the cases of shigellosis, research into these organisms is less of a priority for global health research[19]. Furthermore, research is complicated by the sheer diversity of serotypes in these species (15 for *S. dysenteriae*, and 20 for *S. boydii*) and by a lack of large, well-characterized, geographically diverse collections of isolates.

One of the best studied *S. dysenteriae* serotypes is *S. dysenteriae* serotype 1, which induces a more severe disease phenotype than other *Shigella* spp. and serotypes. The hypervirulence of *S. dysenteriae* 1 can be explained by the release of Stx, which inhibits protein synthesis[102], and could also be partly attributed to the presence of the *shu* cluster, which is upregulated in response to the host body temperature and uses haem as an iron source, leading to better adaptation in the human host[103,104]. Notably, EHEC O157:H7 also carries the *stx*-encoding prophage and the *shu* cluster and can also cause severe complications, demonstrating that *S. dysenteriae* 1 and EHEC have inherited and maintained these virulence factors from a common ancestor[103]. A detailed study of the proteomic profile of *S. dysenteriae* 1 revealed several proteins that are expressed preferentially in the host environment, including the Mxi–Spa T3SS, and proteins that are involved in anaerobic energy metabolism, acid resistance, modulation of the outer membrane and modification of peptidoglycan structure[105]. The last reported dysentery outbreak caused by *S. dysenteriae* 1 occurred in Sierra Leone in 1999 (REF. 106); since then, the prevalence of disease caused by this serotype has become negligible[7]. The most recent pandemic clone of *S. dysenteriae* 1 emerged from a common ancestor at the beginning of the twentieth century, which is much more recent than the common ancestor of the major current clones of both *S. sonnei* and *S. flexneri*[23]. Two lineages of *S. dysenteriae* 1 rapidly disseminated intercontinentally, facilitated by poor sanitation and excessive human migration during the two world wars[23]. Furthermore, these lineages independently acquired antimicrobial-resistance genes, seemingly through selection during outbreaks. However, directional selection in the chromosome is unlikely to occur, as inactivating mutations equally affected all metabolic functions[23]. Such unbiased mutations and a generally high mutation rate suggest that *S. dysenteriae* 1 could be maintained and transmitted through long-term human carriage, similarly to *S.* Typhi[107]. This theory may explain the infrequent isolation rate of *S. dysenteriae* 1 but its ability to cause devastating outbreaks in vulnerable populations.

S. boydii was first isolated in the Indian subcontinent and seems to be restricted to this region, as it is rarely isolated elsewhere[108]. However, a new serotype, *S. boydii* serotype 20, was discovered in travellers to Central America, which demonstrates that the epidemiology of *S. boydii* is more complicated than previously described or predicted[109,110]. In developing countries, *S. boydii* serotype 2 is the most prevalent and clinically relevant serotype, with an isolation rate of ~50% of all *S. boydii* isolates[111–113]. Other *S. boydii* serotypes are rare, but several O antigen clusters from *S. boydii* have been transferred to different members of the genus *Escherichia*; for example, *S. boydii* O antigens 10 and 15 can be found in EHEC and *Escherichia fergusonii*, respectively[114,115].

Linking genomics and pathogenesis. As members of the genus *Shigella* do not form a single monophyletic group, distinct *Shigella* spp. can differ in both physiology and pathogenesis. *Shigella* pathogenesis mainly relies on the Mxi–Spa T3SS and its effector proteins, so the subtle phenotypic variation seen in host–pathogen interactions could be caused by the gain and/or loss of other genetic material. Alternatively, convergent evolution has enabled several *Shigella* spp. to adopt an intracellular lifestyle, exemplified by the independent loss of flagella, fimbriae, and metabolic pathways, such as LDC, carbon utilization and transporters (of carbohydrates, amino acids and amines)[20,57,58].

Information related to pathogenic differences between and in the various *Shigella* spp. is scarce, because most experimental studies have used *S. flexneri*. The other species are used less frequently for experiments owing to the instability of their virulence plasmid (*S. sonnei*), their unavailability or simply the fact that they are less of a global health priority (*S. dysenteriae* and *S. boydii*). Nevertheless, recent findings have shed more light on variation between the different species. For example, it has been shown that bacteriophage-borne glycosylation of the O antigen in *S. flexneri* 5 optimizes its length, enhancing the exposure of the T3SS apparatus without making it more of a target for host antibodies[78]. There is a fine balance between virulence and immune protection: in *S. flexneri* serotype 2a, the plasmid pHS-2 carries a gene that results in very long O antigen chains which mask the cell from serum killing, whereas the chromosomally determined chains are short and unmask the T3SS structure to enhance functionality[116,117]. *S. sonnei* uses a different mechanism: this species expresses a group 4 capsule composed of pINV-borne O antigen sugars[118]. Removal of the capsule

◀ Figure 2 | **The intercontinental and regional dissemination of *Shigella sonnei*.** **a** | A global map showing the spread of *Shigella sonnei* out of Europe, using data from REF. 22. *S. sonnei* diverged into three main lineages (I, II and III) that have been circulating in Europe since the early nineteenth century (red). Years represent the estimated dates of introduction of these strains from Europe into new human populations. The most successful of these global lineages is lineage III, which harbours a combination of antimicrobial-resistance genes (bold dates indicate the introduction of the Global III clade). **b** | An unrooted phylogenetic tree showing the relationship between sequenced *S. sonnei* strains isolated in three different locations across Vietnam: Ho Chi Minh City in the south, Khanh Hoa province on the south-central coast and Hue in the central region; based on data from REF. 75. The tree shows that strains from Ho Chi Minh City are frequently transferred to other Vietnamese cities and rarely form new populations. However, as highlighted by two clonal expansions in Khanh Hoa (Khanh Hoa 1 and Khanh Hoa 2) and one in Hue, pioneering *S. sonnei* strains can form new location-specific subpopulations. The ongoing selection of these organisms seems to be driven by antimicrobials, as there is evidence of homoplasies by the acquisition and maintenance of differing DNA gyrase subunit A (*gyrA*) mutations and of differing plasmids encoding extended-spectrum β-lactamases (ESBLs), which confer resistance to fluoroquinolones and third-generation cephalosporins, respectively. Strains harbouring ESBL-encoding plasmids are highlighted by background shading (blue for the incompatibility group I1 (IncI1) plasmid pKHSB1 encoding CTX-M-15, and red for the IncA/C plasmid encoding CTX-M-14). Part **a** is reproduced from REF. 169, Nature Publishing Group.

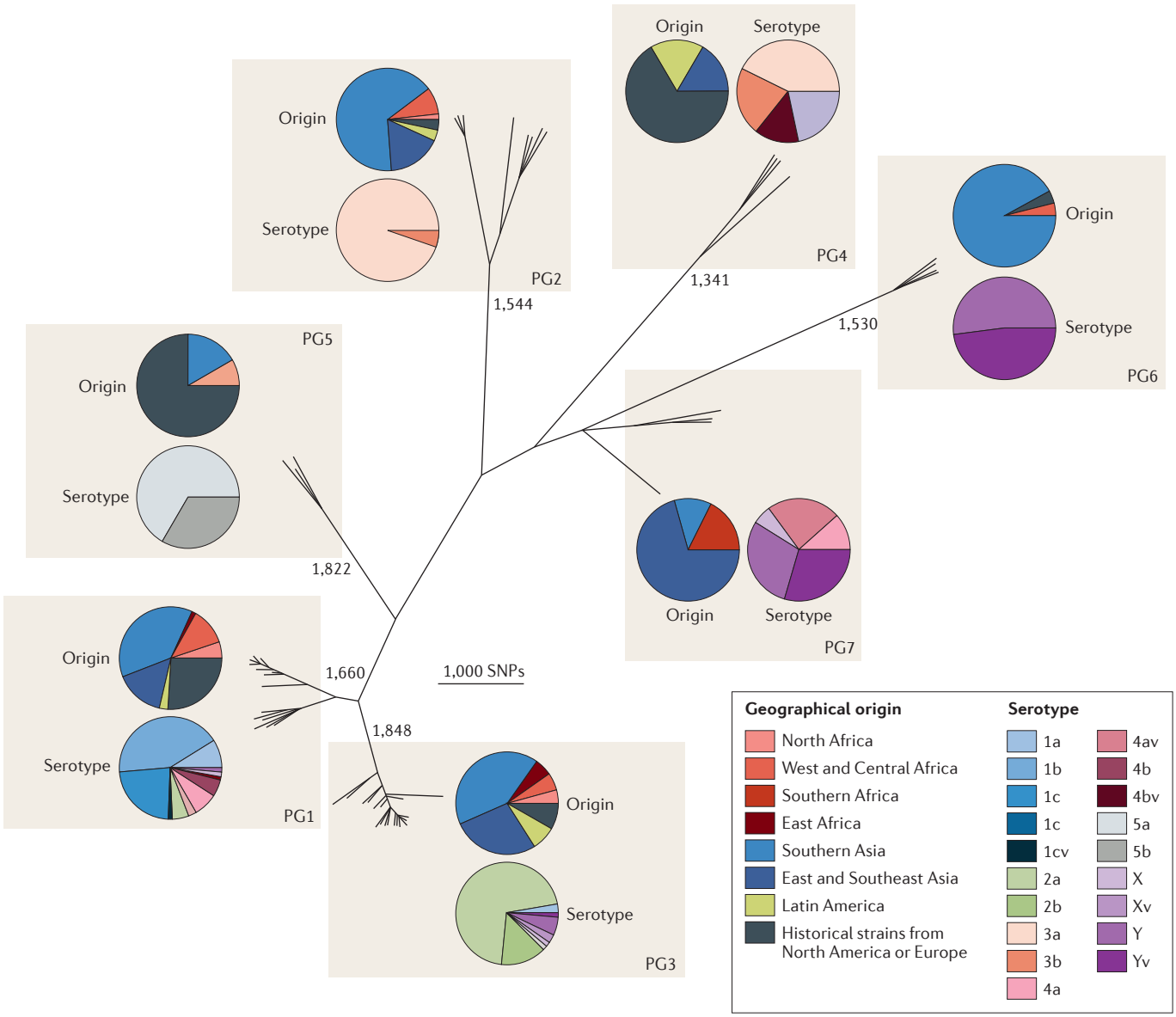


Figure 3 | **The phylogenetic structure and global distribution of the 19 *Shigella flexneri* serotypes.** The figure shows a maximum likelihood phylogeny of *Shigella flexneri* serotypes, created from genome sequences of a representative global collection of 351 isolates of S. *flexneri*, spanning serotypes F1–F5, FX, FXv, FY and FYv. The isolates were collected from the main foci of endemic disease today (Africa, Asia, and South and Central America), and historical isolates from reference collections dating back to 1914 were also used. Phylogenetic groups (PGs) determined by Bayesian analysis of population structure clustering are boxed, and the geographical and serotypic composition of isolates in each PG are inlaid as pie charts. This figure is reproduced from REF. 24.

increases invasiveness and inflammation, but decreases the capacity to spread from cell to cell and increases susceptibility to immune killing, thus showing that the capsule is crucial for the balance between virulence and immune evasion. The *g4c* operon, which encodes this capsule, is intact in *S. sonnei* but is lacking in *S. flexneri* serotype 2a owing to a frame-shift deletion[118]. This variance may explain, in part, the differential virulence and immunogenicity of *Shigella* spp. Differences also exist in the use of adhesins for attachment to host cells. In *S. flexneri*, the T3SS-dependent protein IcsA (also known as VirG) mediates both adhesion and actin-based motility, facilitating invasion into the host cell[119]. In *S. sonnei*, an additional multivalent adhesion molecule (MAM), SSO1327, has been shown to function as a non-redundant adhesin to IcsA[120]. Deletion of either of these proteins in *S. sonnei* reduces attachment and invasion *in vivo*. The gene encoding SSO1327 is intact in isolates of *S. sonnei*, *S. dysenteriae* and *S. boydii*, but is a pseudogene in *S. flexneri*[120]. This difference in adhesin composition may explain the differential interaction of *S. flexneri* and *S. sonnei* with the host; for example, bile salts stimulate the attachment of *S. flexneri* but impede the attachment of *S. sonnei*[119,120].

Conclusions and outlook

The evolutionary history of the bacterial genus *Shigella* is shaped by three key processes. First, *Shigella* spp. have arisen from different ancestral *E. coli* strains on several independent occasions. Second, the acquisition of plasmids that encode virulence genes into numerous ancestral *Shigella* strains were 'foothold moments' in their evolution; similar observations have been made for other enteric human pathogens, such as *Y. pestis* and, more recently, *Yersinia enterocolitica*[121]. The acquisition and adaptation of these plasmids has shaped all existing *Shigella* spp. Third, convergent evolution, by the independent acquisition of mobile elements and loss of gene function, has further transformed these organisms to become restricted to humans and exquisitely customized to interact with the human intestinal mucosa.

The shift in dominance from *S. flexneri* to *S. sonnei* in economically transitioning nations should prompt more in-depth studies of the evolution and epidemiology of these two species. Although whole-genome analyses of *S. sonnei* and *S. flexneri* provided insights into their evolution and spread, comparatively little is currently understood about *S. dysenteriae* and *S. boydii*. As genome sequencing becomes more accessible and affordable, it will be essential to apply this tool to investigate the evolution of other *Shigella* spp. locally and globally. Greater insights into the epidemiology of these species should aid their control in disease-burdened regions as well as facilitate vaccine development and distribution. Conserved proteins across all *Shigella* spp., such as the T3SS proteins IpaB and IpaD, have been identified as promising candidates for a serotype-independent pan-*Shigella* vaccine. Preclinical testing in mice indicates that IpaB and IpaD are safe and provide substantial protection against challenges with *S. flexneri* and *S. sonnei*[122–124]. However, the utility of these antigens needs to be further validated in human studies. Owing to the multiple serotypes of *S. flexneri*, their complex evolutionary history and the extent of horizontal gene transfer, studying this species is more challenging. Further, *S. boydii* and *S. dysenteriae* research has been neglected owing to their lower disease burdens. *S. dysenteriae* serotype 1, in particular, warrants more attention because it can cause severe disease and has the potential to cause major epidemics. Future laboratory research should be integrated with genomics to address the survival, transmission and evolution of *Shigella* spp., focusing on how their lifestyle in the environment can affect disease epidemiology and global public health.

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Competing interests statement
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