The expression of lipopolysaccharide by strains of Shigella dysenteriae, Shigella flexneri and Shigella boydii and their cross-reacting strains of Escherichia coli

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Abstract
Strains of Shigella dysenteriae, Shigella flexneri and Shigella boydii express lipopolysaccharides, that enable the serotyping of strains based on their antigenic structures. Certain strains of S. dysenteriae, S. flexneri and S. boydii are known to share epitopes with strains of Escherichia coli; however, the lipopolysaccharide profiles of the cross-reacting organisms have not been compared by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) lipopolysaccharides profiling. In the present study, type strains of these bacteria were examined using SDS-PAGE/silver staining to compare their respective lipopolysaccharide profiles. Strains of S. dysenteriae, S. boydii and S. flexneri all expressed long-chain lipopolysaccharide, with distinct profile patterns. The majority of strains of Shigella spp., known to cross-react with strains of E. coli, had lipopolysaccharide profiles quite distinct from the respective strain of E. coli. It was concluded that while cross-reacting strains of Shigella spp. and E. coli may express shared lipopolysaccharide epitopes, their lipopolysaccharide structures are not identical.

Introduction
Strains of Shigella dysenteriae, Shigella flexneri, Shigella boydii and Shigella sonnei continue to be a significant cause of human gastroenteritis in the UK (data from the Health Protection Agency: http://www.hpa.org.uk). Strain characterization relies on serotyping strains, and the key antigenic structures of Shigella bacteria are located on the lipopolysaccharide (lipopolysaccharide or somatic) antigens.

Strains of S. dysenteriae can be subdivided into 13 serotypes and S. boydii into 18 serotypes, while a combination of group- and type-specific antigens enabled the subdivision of strains of S. flexneri into six serotypes, each of which can be further subdivided.

All strains of Shigella spp. probably express long-chain lipopolysaccharide; the chemical structures of these somatic antigens have been elucidated (Liu et al., 2008) and they appear to share a common core lipopolysaccharide comprising phosphoryl-ethanolamine, 2-keto-3-deoxy-octanoate, glycerol-D-manno-heptose phosphate, α-D-glucose, α-D-galactose and N-acetyl-D-glucosamine (Simmons & Romanowska, 1987). The long-chain lipopolysaccharide of strains of S. flexneri have a common component comprising α-L-rhamnose, β-D-galactose and N-acetyl galactosamine, with an O-acetyl side group in various arrangements (Simmons & Romanowska, 1987; Liu et al., 2008), while the lipopolysaccharide of S. dysenteriae comprises N-acetyl glucosamine, N-acetyl galactosamine, α-L-rhamnose, α-D-galactose, α-D-glucose, α-D-mannose and/or α-L-fucose (Brahmbhatt et al., 1992). The lipopolysaccharide of S. boydii contains N-acetyl glucosamine, β-L-rhamnose, α-D-glucose, α-D-galactose and α-D-mannose (Kenne & Lindberg, 1983; Brahmbhatt et al., 1992), and all strains of S. sonnei belong to the same serotype and express lipopolysaccharide comprising a disaccharide repeating unit containing two unusual amino sugars: 2-amino-2-deoxy-L-altruronic acid and 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose (Xu et al., 2002).
The genes encoding the lipopolysaccharide expressed by strains of *S. dysenteriae* 1 are located on a plasmid of 6 MDa (Schnaitman & Klene, 1993), while for *S. sonnei* these genes are located on a plasmid of 120 MDa (Sansonetti et al., 1982). On bacterial culture, the plasmid appears to be lost spontaneously at a high frequency, resulting in avirulent variants that lack somatic antigens (Kopecko et al., 1980). In contrast, for strains of *S. flexneri*, most of the genes encoding lipopolysaccharide expression are located chromosomally (Kopecko et al., 1980), but plasmid-encoded elements are required for the assembly of long-chain lipopolysaccharide (Morona et al., 2003).

The technique of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and silver staining has proved very useful for examining the structure of lipopolysaccharide based on the patterns produced during electrophoresis and this has been applied successfully to members of the *Enterobacteriaceae* (Chart et al., 1993; Chart & Cheasty, 2006), but there have been only limited studies using SDS-PAGE profiling with strains of *Shigella* spp. (de Silva et al., 1992). Furthermore, there are known antigenic similarities between strains of *Shigella* and *Escherichia coli* (Rowe et al., 1976; Cheasty & Rowe, 1983; Feng et al., 2004, 2007). Because specific antibodies bind to the cross-reacting lipopolysaccharides, it can be concluded that the two lipopolysaccharides have the same epitopes, but it is not known whether the lipopolysaccharide profiles are the same. In the present study, type-strains of *S. dysenteriae*, *S. flexneri* and *S. boydii* were examined using SDS-PAGE/silver staining to compare the lipopolysaccharide profiles of strains of *E. coli* known to share lipopolysaccharide epitopes.

**Materials and methods**

**Bacteria**

The strains of *S. dysenteriae*, *S. flexneri*, *S. boydii* and *E. coli* are listed in Table 1. All were clinical isolates forming part of the culture collection held by the Laboratory of Gastrointestinal Pathogens (LGP). For the preparation of lipopolysaccharide, strains were cultured on horse blood agar at 37°C overnight.

**Serotyping**

Strains of *E. coli* and *Shigella* spp. were serotyped and antibody–antigen cross-reactions were detected using serotyping methods established in the LGP (Gross & Rowe, 1985).

**Normal rabbit serum**

Complement proteins, present in fresh normal rabbit serum, were used to select strains of *Shigella* spp. expressing long-chain lipopolysaccharide based on studies described previously (Chart et al., 2000). Strains were grown in nutrient broth (37°C, overnight) and diluted in phosphate-buffered saline by a factor of 10⁷. Bacterial suspensions were mixed with normal rabbit serum (50 µL mL⁻¹) before incubation at room temperature for 1 h. The serum suspensions were plated onto blood agar, and following overnight incubation at 37°C, the resultant colonies were confirmed to be a descendant of the original test strain by routine biochemistry and serotyping.

**Lipopolysaccharide**

For SDS-PAGE, lipopolysaccharide was prepared by digesting whole bacteria with proteinase-K (Chart et al., 1989). Bacteria were placed in preweighed Eppendorf tubes, and the cells were suspended in SDS-PAGE sample buffer (Laemmli, 1970) to yield a concentration of 1 mg per 30 µL.
Chemical Co. Ltd; product code P6556) before incubation at 100 °C for 10 min. After cooling, samples were mixed with an equal volume of SDS-PAGE buffer containing 100 μg per 30 μL proteinase-K (Sigma Chemical Co. Ltd; product code P6556) before incubation at 60 °C for 1 h.

**SDS-PAGE and gel staining**

SDS-PAGE was performed using an Atto™ minigel apparatus (Genetic Research Instruments, Braintree, Essex, UK) using the method of Laemmli (1970). Preparations of lipopolysaccharide, representing 80 μg of digested cell mass, were used per lane of gels comprising a 4.5% stacking gel and a 12.5% separation gel. Profiles of lipopolysaccharide were detected with a sensitive silver stain (Tsai & Frasch, 1982). For SDS-PAGE, involving proteins, gels were stained with Coomassie brilliant blue (Chart & Griffiths, 1985). Protein standards (Bio-Rad; product code 161-0304) were used to calculate the molecular size of proteins.

**Results**

**Antibody cross-reactions between Shigella spp. and E. coli**

Certain strains of *Shigella* spp. are known to cross-react with strains of *E. coli* (Table 2), indicating shared epitopes between the respective bacteria, but whether the cross-reacting bacteria express the same lipopolysaccharide structure has been less well understood. Strains of *S. dysenteriae* 1 share epitopes with *E. coli* O148 and both serotypes express long-chain lipopolysaccharide, that give similar, but not identical, lipopolysaccharide profiles (Fig. 1, lanes 1 and 2). Strains of *S. dysenteriae* 2 share epitopes with *E. coli* O112 (Fig. 1, lanes 3 and 4), but their lipopolysaccharide profiles were quite distinct. The lipopolysaccharide profiles of *S. dysenteriae* 3 and *E. coli* O124 had very similar lipopolysaccharide profiles (Fig. 1, lanes 5 and 6). The lipopolysaccharide profiles of *S. dysenteriae* 4 and *E. coli* O159 were very dissimilar, with the type 4 lipopolysaccharide migrating with several ‘rungs’ of the long-chain ladder, while the lipopolysaccharide of *E. coli* O159 comprised some four rungs migrating very closely together (lanes 7 and 8). Strains of *S. dysenteriae* 5 and *E. coli* O58 expressed lipopolysaccharide, that had similar lipopolysaccharide profiles (Fig. 1, lanes 9 and 10). The lipopolysaccharide profiles of *S. dysenteriae* 12 and *E. coli* O152 were also very similar, with the ‘rungs’ of the long-chain lipopolysaccharide matching very closely (Fig. 1, lanes 11 and 12).

Strains of *S. boydii* 1, 2, 4, 5, 8, 11, 14 and 15 cross-react with strains of *E. coli* O149, O87, O53, O79, O143, O105, O32 and O112, respectively (Table 2); however, the profiles in Fig. 2 show that the patterns of the cross-reacting lipopolysaccharides were dissimilar despite sharing epitopes. These dissimilarities ranged from very distinct differences in the lipopolysaccharide profile, for example, *S. boydii* 11 (Fig. 2, lane 11) and *E. coli* O105 (Fig. 2, lane 12), to only minor differences such as in *S. boydii* 8 (Fig. 2, lane 9) and *E. coli* O143 (Fig. 2, lane 10).

Strains of *S. flexneri* 2b, 3a, 4b and 5a cross-react with lipopolysaccharide expressed by *E. coli* O147, O16, O135 and O129, respectively (Table 2). With reference to Fig. 3, *S. flexneri* 2b expressed lipopolysaccharide with a profile quite distinct from that of *E. coli* O147, with the even rung

![Fig. 1. Strains of Shigella dysenteriae 1, 2 and 4 (lanes 1, 3 and 7) share epitopes with Escherichia coli O148, O112 and O159 (lanes 2, 4 and 8), but their lipopolysaccharide profiles were dissimilar. In contrast, S. dysenteriae 3, 5 and 12 (lanes 5, 9 and 11) and E. coli O124, O58 and O152 (lanes 6, 10 and 12) shared lipopolysaccharide epitopes, but had similar lipopolysaccharide profiles. Certain profiles had a nonstaining band (arrows, lane 5).](https://academic.oup.com/femsle/article-abstract/292/1/21/489982/200121-489982)

Table 2. Strains of *Shigella* spp. and the respective serogroup of cross-reacting *Escherichia coli*

<table>
<thead>
<tr>
<th>Serogroup of <em>Shigella</em> spp.</th>
<th>Serogroup of <em>E. coli</em></th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. dysenteriae 1</td>
<td>O148</td>
<td>Feng et al. (2007)</td>
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<tr>
<td>S. dysenteriae 2</td>
<td>O112</td>
<td>Cheasty &amp; Rowe (1983)</td>
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<tr>
<td>S. dysenteriae 3</td>
<td>O124</td>
<td>Cheasty &amp; Rowe (1983)</td>
</tr>
<tr>
<td>S. dysenteriae 4</td>
<td>O159</td>
<td>Rowe et al. (1976)</td>
</tr>
<tr>
<td>S. dysenteriae 5</td>
<td>O58</td>
<td>Edwards &amp; Ewing (1972)</td>
</tr>
<tr>
<td>S. dysenteriae 12</td>
<td>O152</td>
<td>Edwards &amp; Ewing (1972)</td>
</tr>
<tr>
<td>S. flexneri 2b</td>
<td>O147</td>
<td>Edwards &amp; Ewing (1972)</td>
</tr>
<tr>
<td>S. flexneri 3a</td>
<td>O16</td>
<td>Edwards &amp; Ewing (1972)</td>
</tr>
<tr>
<td>S. flexneri 4b</td>
<td>O135</td>
<td>Edwards &amp; Ewing (1972)</td>
</tr>
<tr>
<td>S. flexneri 5a</td>
<td>O129</td>
<td>Edwards &amp; Ewing (1972)</td>
</tr>
<tr>
<td>S. boydii 1</td>
<td>O149</td>
<td>Rowe et al. (1976)</td>
</tr>
<tr>
<td>S. boydii 2</td>
<td>O87</td>
<td>Edwards &amp; Ewing (1972)</td>
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<td>O53</td>
<td>Edwards &amp; Ewing (1972)</td>
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<td>O79</td>
<td>Edwards &amp; Ewing (1972)</td>
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<td>Edwards &amp; Ewing (1972)</td>
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<td>S. boydii 14</td>
<td>O32</td>
<td>Edwards &amp; Ewing (1972)</td>
</tr>
<tr>
<td>S. boydii 15</td>
<td>O112</td>
<td>Cheasty &amp; Rowe (1983)</td>
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</tbody>
</table>
lipopolysaccharide using rabbit serum

The lipopolysaccharide profiles of cross-react with lipopolysaccharide expressed by strains of bacteria without lipopolysaccharide and successfully selected a source of active complement proteins, that killed bac-

In the present study, fresh rabbit serum was used as a source of active complement proteins, that killed bacteria without lipopolysaccharide and successfully selected strains of S. sonnei and S. dysenteriae 1 with long-chain lipopolysaccharide.

**Selection of Shigella spp. expressing long-chain lipopolysaccharide using rabbit serum**

Profiles of lipopolysaccharide prepared from certain strains of S. dysenteriae, S. boydii and S. sonnei were found to contain a ‘ghost’ band in their lipopolysaccharide profiles (e.g. see the arrows in Fig. 1, lane 5). To investigate this, lipopolysaccharide preparations were electrophoresed alongside outer membranes prepared from a strain of S. dysenteriae, and the gel stained for protein with Coomassie blue. The proteinase-K-resistant protein was found to have a mass of 40 000 and comigrated with the major outer membrane protein OmpC (data not shown).

**Proteinase-K-resistant membrane protein**

Profiles of lipopolysaccharide prepared from certain strains of S. dysenteriae, S. boydii and S. sonnei were found to contain a ‘ghost’ band in their lipopolysaccharide profiles (e.g. see the arrows in Fig. 1, lane 5). To investigate this, lipopolysaccharide preparations were electrophoresed alongside outer membranes prepared from a strain of S. dysenteriae, and the gel stained for protein with Coomassie blue. The proteinase-K-resistant protein was found to have a mass of 40 000 and comigrated with the major outer membrane protein OmpC (data not shown).

**Discussion**

Antigenic cross-reactions between strains of Shigella spp. and E. coli are well known, but whether the cross-reacting bacteria express the same lipopolysaccharide structures appears not to have been investigated. Strains of S. dysenteriae 1 share epitopes with E. coli O148 and both serotypes express long-chain lipopolysaccharide, that give similar, but not identical, lipopolysaccharide profiles. The subunits of lipopolysaccharide expressed by S. dysenteriae 1 have two molecules of α-L-rhamnose and one of N-acetyl-D-glucosamine (Brahmbhatt et al., 1992), and the lipopolysaccharide of E. coli O148 has two molecules of α-D-glucose and one of N-acetyl-D-glucosamine (Feng et al., 2007), suggesting that the shared epitopes involve the two molecules of α-L-rhamnose and the N-acetyl-D-glucosamine. Strains of S. dysenteriae 2 share epitopes with E. coli O112, but their lipopolysaccharide profiles were quite distinct. The lipopolysaccharide of both organisms contains N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, α-D-glucose and two molecules of β-D-galactose (Liu et al., 2006), suggesting that these sugars form part of the cross-reacting epitopes. The lipopolysaccharide profiles of S. dysenteriae serotype 3 and E. coli O124 had very similar lipopolysaccharide profiles and both species share the lipopolysaccharide components N-acetyl-D-galactosamine, β-D-galactose and α-D-glucose (Liu et al., 2006). In contrast,
the lipopolysaccharide profiles of \textit{S. dysenteriae} 4 and \textit{E. coli} O159 were very dissimilar, with the serotype 4 lipopolysaccharide migrating with several 'rungs' of the long-chain ladder, while the lipopolysaccharide of \textit{E. coli} O159 comprised some four rungs migrating very closely together. The structure of these two lipopolysaccharides has been studied in detail and the backbones of both contain N-acetyl-D-glucosamine and \(\alpha-L\)-fucose, but differ in \textit{S. dysenteriae} 4 having a molecule of \(\alpha-D\)-glucose, whereas \textit{E. coli} O159 has a molecule of \(\alpha-D\)-galactose (Rosen et al., 2004). Also, \textit{E. coli} O159 has a side-chain molecule of \(\alpha-L\)-fucose, whereas \textit{S. dysenteriae} 4 has side-chain \(O\)-acetyl \(\alpha-L\)-fucose and it was within these side-chain moieties that the shared epitope was thought to be located (Rosen et al., 2004).

Strains of \textit{S. dysenteriae} 5 and \textit{E. coli} O58 expressed lipopolysaccharide, that had similar lipopolysaccharide profiles. The lipopolysaccharide structure of \textit{S. dysenteriae} 5 has been deduced and shown to comprise N-acetyl-D-glucosamine and \(\alpha-D\)-mannose with a side chain of \(\alpha\)-l-rhamnose (Brahmbhatt et al., 1992), while the lipopolysaccharide of \textit{E. coli} O58 contains N-acetyl-D-glucosamine and \(\alpha\)-D-mannose (Ørskov et al., 1977), suggesting that these sugars might form the cross-reacting epitopes between these bacterial species. The lipopolysaccharide profiles of \textit{S. dysenteriae} 12 and \textit{E. coli} O152 were also very similar, with the 'rungs' of the long-chain lipopolysaccharide matching very closely. The lipopolysaccharide structures of both organisms contain N-acetyl-D-glucosamine, \(\beta-D\)-glucose and \(\beta-L\)-rhamnose (Liu et al., 2006), and these most likely comprise the cross-reacting epitopes, but the lipopolysaccharide structures are not identical.

Strains of \textit{S. boydii} 1, 2, 4, 5, 8, 11, 14 and 15 cross-react with strains of \textit{E. coli} O149, O87, O53, O79, O143, O105, O32 and O112, respectively; however, the profiles in Fig. 2 show that the patterns of the cross-reacting lipopolysaccharides were dissimilar despite sharing epitopes. The structures of lipopolysaccharide expressed by \textit{S. boydii} 1 and \textit{E. coli} O149 share the sugars containing N-acetyl-D-glucosamine and \(\beta-L\)-rhamnose (Liu et al., 2006), and \textit{S. boydii} 4 and \textit{E. coli} O53 have been shown to have a carbohydrate backbone comprising N-acetyl-D-glucosamine and \(\alpha-D\)-glucose and \(\alpha-L\)-rhamnose (Wang et al., 2004). The structure of lipopolysaccharide expressed by \textit{S. boydii} 8 and \textit{E. coli} O143 shares the sugars containing N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, \(\beta-D\)-glucose and \(\beta-D\)-galactose (Liu et al., 2008). \textit{Shigella boydii} 15 and \textit{E. coli} O112 have also been determined and both have a carbohydrate backbone comprising both \(\alpha\)-D- and \(\beta\)-D-N-acetyl-D-galactosamine and \(\alpha\)-D-glucose, with a side group of N-acetyl-D-glucosamine (Liu et al., 2006).

Strains of \textit{S. flexneri} 2b, 3a, 4b and 5a cross-react with lipopolysaccharide expressed by \textit{E. coli} O147, O16, O135 and O129, respectively. The somatic antigens of these organisms share an lipopolysaccharide backbone comprising N-acetyl-D-glucosamine and \(\alpha-L\)-rhamnose, and differ in structure by \textit{S. flexneri} 2b having an additional \(O\)-acetyl group (Hygge-Blakeman et al., 1998). Furthermore, the shared epitopes on \textit{S. flexneri} 2b and \textit{E. coli} O147 were confirmed with a monoclonal antibody prepared to \textit{S. flexneri} 2b (Hygge-Blakeman et al., 1998). The lipopolysaccharide profiles of \textit{S. flexneri} 3a and \textit{E. coli} O16 differed both in the ladder pattern and in the intensity of staining. The chemical structures of these lipopolysaccharides have been determined and both have a sugar backbone containing N-acetyl-D-glucosamine and \(\alpha-L\)-rhamnose (Batley et al., 1997), but the location of the shared epitope(s) appears not to have been identified. The lipopolysaccharide profiles of \textit{S. flexneri} 4b and \textit{E. coli} O135, and \textit{S. flexneri} 5a and \textit{E. coli} O129 are shown in Fig. 3, and although the cross-reacting lipopolysaccharides did not have identical lipopolysaccharide ladder patterns, all four strains had the evenly spaced rung pattern generally associated with strains of \textit{S. flexneri}. The structure of these lipopolysaccharides appears not to have been determined and the sugars comprising shared epitopes remain unknown.

The genes encoding the expression of lipopolysaccharide by strains of \textit{S. sonnei} are located on a 120 MDa plasmid (Sansonetti et al., 1982) and on subculture \textit{in vitro}, the plasmid was shed, resulting in a population predominated by bacteria unable to synthesize lipopolysaccharide. Fresh rabbit serum complement was used successfully to enrich for bacteria with long-chain lipopolysaccharide and has become a routine procedure in the Laboratory of Gastrointestinal Infections. The 'ghost' band observed in certain lipopolysaccharide profiles was thought to be OmpC, known to have a mass of 40 000 (Roy et al., 1994), suggesting that certain moieties of the OmpC protein were resistant to the action of protease-K.

Serotyping has proved vital for characterizing strains of \textit{S. dysenteriae}, \textit{S. flexneri} and \textit{S. boydii}. Chemical and nuclear magnetic resonance studies have elucidated the sugar composition of many of the known serotypes of these serotypes of \textit{Shigella}. This study is the first to describe the SDS-PAGE profiles of lipopolysaccharide from the recognized type-strains of \textit{S. dysenteriae}, \textit{S. flexneri} and \textit{S. boydii}, and relate lipopolysaccharide profiles to their respective antigenically cross-reacting strains of \textit{E. coli}. It has also demonstrated that lipopolysaccharide profiling can provide useful information when comparing strains, in particular when examining strains of \textit{Shigella} spp. and \textit{E. coli}, which share epitopes.

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