Molecular diagnosis of non-serotypeable Shigella spp.: problems and prospects

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Abstract

It is not always possible to identify Shigella serogroups/serotypes by biochemical properties alone. Specific identification requires serotyping. Occasionally, isolates that resemble Shigella spp. biochemically, but are non-agglutinable with available antisera, have been observed. Several mechanisms have been reported to limit the efficiency of the serotyping assay. Serotype conversion is a major mechanism in Shigella spp. to escape protective host immune responses. This easy conversion through significant modification of the O-antigen backbone results in different serotypes, which makes laboratory identification difficult. Furthermore, members of the family Enterobacteriaceae are closely related and there is antigenic cross-over (intra- and inter-specific cross-reaction) which affects the agglutination reaction. The performance of the available methods for identification of non-serotypeable Shigella is discussed here, and reveals them to be non-reliable. This shows a need for an alternative method for identification and typing of Shigella spp.

Sir,

Shigellae are the primary causative agents of bacterial diarrhoea, representing a significant public health problem, especially in developing countries like India. Shigella spp. belong to the extremely diverse species Escherichia coli. Shigella spp. are still very significant pathogens, particularly where the standard of hygiene is poor. In the 1940s, Shigella strains were put in a different genus from E. coli to distinguish pathogenic forms from the non-pathogenic strains of E. coli and based on their medical significance [1]. Although Shigella was grouped as a subgenus of E. coli in the later 1990s, it is still difficult to distinguish atypical E. coli from non-serotypeable Shigella, where timely diagnosis is essential.

Serological identification is the crucial step in diagnosing Shigella infection. Occasionally an isolate will be biochemically indistinguishable from Shigella spp. and non-agglutinable with the standard antisera. Several intra- and inter-specific cross-reactions, morphological transitions from smooth to rough forms and mutations in the O-antigen synthesis/modification genes of Shigella spp. have been reported to limit the efficiency of serotyping assays [2]. The commercially available antisera could not discriminate all possible epitopes of the Shigella O-antigen. In addition, among Shigella, new serotypes/sub-serotypes are not unusual and are reported from different parts of the world [3].

Molecular identification of Shigella using 16S rRNA sequencing could not distinguish atypical E. coli from Shigella spp. The sequence similarities of Shigella flexneri, Shigella sonnei and Shigella boydii with E. coli were reported to be 99.8, 99.9 and 99.7 %, respectively. Although studies have been reported that a matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) MS with specialized automated data analysis approach could replace the traditional phenotypic and biochemical methods for microbial identification [4, 5], MALDI-TOF MS has several limitations, such as the database diversity and resolution of closely related species, which results in its inability to discriminate atypical E. coli from Shigella spp. Further automated analysis is difficult to implement in less sophisticated laboratories. This implies a major diagnostic challenge in identification of Shigella spp. in clinical microbiology laboratories.

The rfb region of the O-antigen gene cluster in Shigella spp. was known to harbour the enzyme coding genes responsible for O-antigen synthesis. The number of genes in the rfb cluster varies from 6 to 19, and strains of different serotypes can show completely different gene sets [2]. This study aims...
to characterize the non-serotypeable *Shigella* by molecular typing.

A total of 3647 faecal specimens were processed between January and December 2014. Among these, 27.5% (n=176) were identified as *Shigellae*, of which eight were non-serotypeable. Antimicrobial susceptibility testing showed that 50% (n=4) were resistant to ampicillin, 87% to cotrimoxazole (n=7), 75% to nalidixic acid (n=6), 25% to cefixime (n=2) and all were susceptible to norfloxacin and cefotaxime. Multi-drug resistance is rarely reported in non-serotypeable *Shigella* [3]. These isolates (n=8) were also screened for the antimicrobial resistance (AMR) genes and integrons (*dhfr1A*, *sulII*, *blaOXA*, *blaTEM*, *blaCTX-M*, AmpC, *qnrA*, B, S, *intI1* and *intI2*) [6-13]. This revealed the presence of *dhfr1A* (n=2), *sulII* (n=5), *blaTEM* (n=4) and *qnrS* (n=4). All eight isolates were negative for *blaOXA*, *blaCTX-M*, AmpC, *intI1* and *intI2* genes. Although studies on antimicrobial-resistant non-serotypeable *Shigella* are scarce, there is one study from India where 10 such isolates were characterized. The results showed that seven isolates were positive for *dhfr1A* and *tetB*, five for *sulII*, *catA*, *intI1* and *intI2*, six for *aadA1*, four for both *strA* and *strB*, three for *blaOXA* and two for *tetA* genes with IncFIIA incompatibility plasmids present in all strains [3]. Available data on antimicrobial susceptibility and genes responsible for resistance in non-serotypeable *Shigella* reveal that they harbour genes for almost all classes of antibiotics, which may vary from place to place, thereby limiting therapeutic options.

The *rfb* region (longer than 10 kb) of *Shigella* was amplified and characterized by *rfb*-PCR and endonuclease restriction was done using the *MboII* enzyme as described earlier [2]. Studies have reported that identification of *Shigella* serotypes using *rfb*-RFLP has closer resolution of full serotyping compared with other methods. The restriction results showed six clearly identifiable and reproducible O-patterns (Fig. 1). Coimbra et al. [2] have created a database of O-patterns for known *Shigella* serotypes. However, open data or a database on the O-patterns of non-agglutinable *Shigella* are still lacking. Further analysis of O-antigen gene cassette arrangements using whole-genome sequencing (WGS) could identify the serotype [14].

In recent years, the conventional bacterial typing methods such as AMR patterns, bacteriophage typing or serotyping have been replaced by molecular techniques such as RFLP targeting the *rfb* and *fbC* gene, ribotyping, PFGE, MALDI-TOF MS and PCR-based methods including ERIC (Enterobacterial Repetitive Intergenic Consensus)-PCR. However these methods have some limitations, such as that they do not provide clonal information, are time-consuming, expensive, could not differentiate between closely related species and can only give information on a limited number of species. Indeed, WGS was found to have improved identification of species from clinical samples which can provide more clinically relevant information than other methods yet no studies were done on identification of *Shigella* spp. [15, 16].

![Fig. 1. (a) Undigested rfb-PCR product of eight non-serotypeable Shigella. Lanes: 1, FC2045; 2, FC1661; 3, FC2928; 4, FC1882; 5, FC1544; 6, FC1764; 7, FC2383; 8, FC2710. Marker, 100 bp. (b) rfb-RFLP using MboII enzyme showing six different O-patterns from eight non-serotypeable Shigella (lanes 1–8). M, Marker 100 bp; lanes 1 and 4 and lanes 6 and 8 show identical O-patterns.](image-url)

Choi et al. [17] studied *S. flexneri* isolates collected mainly in Korea and Asian countries using a multilocus sequence typing (MLST) scheme and have reported that several sequence types have been recognized within a serotype. Hence, categorizing each serotype according to sequence types is not possible.

Therefore, it is imperative to characterize the non-serotypeable *Shigella* using WGS and it is also essential to have an open access database for future identification of such strains. This would help to understand and track the disease burden caused by the new *Shigella* serotypes and could possibly help in studying the phylogenicity of non-serotypeable *Shigellae*. Notably, the Global Antimicrobial Resistance Surveillance System (GLASS) developed by the WHO in 2015 reported that *Shigella* spp. are one of the priority pathogens among enteric organisms for AMR surveillance. Also, the resistance pattern changes with the change in the serogroup. This highlights the importance
of molecular typing of *Shigella* spp. for proper antimicrobial therapy of shigellosis.

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**Conflicts of interest**
The authors declare that there are no conflicts of interest.

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