Serotype Distribution and Antimicrobial Resistance of Shigella Species in Bangui, Central African Republic, from 2002 to 2013

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Short article

Title

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Running title

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Abstract

Shigella is a major cause of severe diarrhea in children under five years old in Sub-Saharan Africa. The aim of this study was to describe the (sub-)serotype distribution and antimicrobial susceptibility of *Shigella* serogroups from Centrafrican patients with diarrhea between 2002 and 2013. We collected 443 *Shigella* isolates in total. The most common serogroups were *S. flexneri* (*n*=243, 54.9%), followed by *S. sonnei* (*n*=90, 20.3%) and *S. dysenteriae* (*n*=72, 16.3%). The high diversity of (sub-)serotypes of *S. flexneri* and *S. dysenteriae* may impede the development of an efficient vaccine. Rates of resistance were high for ampicillin, chloramphenicol, tetracycline and cotrimoxazole, but low for many other antimicrobials, confirming recommendations for the use of third-generation cephalosporins (only one organism resistant) and fluoroquinolones (no resistance). However, the detection of one extended-spectrum beta-lactamase-producing *Shigella* organism highlights the need for continued monitoring of antimicrobial drug susceptibility.

Introduction

In 2013, 6.3 million deaths were recorded in children under the age of five years, 578,000 of which were due to diarrheal disease, the second leading cause of death in this age group. Almost half of these diarrhea-related deaths occurred in Sub-Saharan Africa.[1] Recent studies (Torcadia for Central African Republic [CAR]) and GEMS for other sites) in The Gambia and Mali (West Africa), Mozambique and Kenya (East Africa), and CAR (Central Africa) have confirmed the continuing importance of *Shigella* as a major cause of severe diarrhea in children under the age of five years. *Shigella* spp. are the third most important pathogen in these regions.[2, 3]

High rates of resistance to conventional antimicrobials, such as ampicillin, chloramphenicol, tetracycline and cotrimoxazole, have been reported for *Shigella* spp. in many studies.[4] This resistance has led to third-generation cephalosporins (C3G), fluoroquinolones and azithromycin becoming the first-line antimicrobials for treating these infections. However, the clinical severity of shigellosis and the emergence of resistance to first-line treatments highlight the growing need to develop alternative prophylactic and therapeutic strategies. The development of a safe and effective anti-*Shigella* vaccine for controlling shigellosis is enshrined in WHO public health policy. However, the presence of four different serogroups (formerly known as species), *S. flexneri, S. dysenteriae*, *S. boydii*, and *S. sonnei,* made up of at least 50 antigenically different (sub-)serotypes may impede vaccine development.[5] Indeed, the larger the number of serotypes to be included, the more complex and expensive the vaccine becomes. The lack of data from Central Africa and from very low-income countries, such as CAR,[6] which suffers from long-term instability, highlights the need to improve our understanding of the spatial and temporal distribution of (sub-)serotypes in Sub-Saharan Africa.[7, 8] CAR is a resource-limited country in equatorial Africa (ranked 180/187 according to the Human Development Index in 2013). Here, we

describe the (sub-)serotype distribution and antimicrobial susceptibility of *Shigella* species isolated from patients in CAR during the 2002–2013 period.

Clinical isolates of *Shigella* were obtained between January 2002 and December 2013, from Centrafrican outpatients with diarrhea attending the Institut Pasteur in Bangui. If more than one isolate of the same (sub-)serotype and serogroup, and with the same antimicrobial drug resistance phenotype was recovered from a given patient, only the first isolate was included. *Shigella* was identified by conventional methods and (sub-)serotyping was performed by slide agglutination assays with a complete set of antisera recognizing all the described *Shigella* serotypes.[9] Antimicrobial drug susceptibility was assessed by the disk diffusion method, and extended-spectrum beta-lactamase (ESBL) production was evaluated in the double-disc synergy test, as previously described.[10]

Date, site of isolation, patient age and sex were recorded for each isolate. We used Chi-squared test, Student's *t* test, and ANOVA (analysis of variance – with Lilliefors' test for normality and Levene's test for homoscedasticity) to compare categorical and continuous variables in univariate analysis. Multivariate logistic regression was performed to explore high rates of multidrug resistance (MDR, defined by resistance to more than five of the 13 antimicrobials tested). Factors with p values <0.2 in univariate analysis were retained for the multivariate analysis. We considered p-values <0.05 to be statistically significant.

In total, 443 clinical isolates of *Shigella* were collected between January 2002 and December 2013, from 443 Centrafrican outpatients with diarrhea (238 male and 205 female patients; mean age: 27.2 years; median age: 29.5 years; 25th percentile: 8 years; 75th percentile: 40 years). The small number of organisms isolated during the study period reflects the poor access to healthcare services in CAR, particularly for the laboratory diagnosis of diarrhea. Significant differences in the number of isolates obtained were also observed between years, due to the economic and political crisis that occurred during the study period,

further restricting patient access to healthcare facilities. However, the distribution of serogroups recovered in our study for 2004-2005 was consistent with that reported for the same period in a study conducted at four healthcare centers in Bangui.[6] The data reported here may therefore be considered representative of the global situation in Bangui.

The incidence of *S. dysenteriae* infection has been reported to be higher in men than in women in China.[11] By contrast, we found a significant association between *S. dysenteriae* infection and being female (OR=1.86 95% CI [1,11-3,12]; p=0.018). No significant association was found between sex and the other serogroups, or between *Shigella* serogroup and age. Contrary to several other reports,[11, 12] we observed no significant seasonality in the distribution of *Shigella* isolates or in serogroup distribution (i.e. erratic variation between months, but no difference between the wet and dry seasons).

The most common serogroup was *S. flexneri* (n=243, 54.9%), consistent with several reports from developing countries in Africa and Asia,[5, 13] followed by *S. sonnei* (n=90, 20.3%) and *S. dysenteriae* (n=72, 16.3%). S. *boydii* (n=34, 7.7%), which is generally restricted to North and East Africa (Ethiopia and Egypt) and South Asia (Bangladesh and Nepal),[13] was rarely encountered in our study (Table 1). Unsurprisingly, no significant difference in the prevalence of *Shigella* serogroups was observed during the study period, except for *S. sonnei* (p<0.001). However, the prevalence of the *S. sonnei* serogroup fluctuated over time, with no significant trend (Table 1). This finding is consistent with the known distribution of *Shigella* serogroups in countries with a low socioeconomic level,[4] CAR being one of the poorest countries of Sub-Saharan Africa.

An increase in the frequency of *S. sonnei* isolates relative to *S. flexneri* has been reported worldwide, in regions in which sanitation and clean water provision have been improved.[14] Such interactions between these two serogroups were detected here, by analyzing the negative correlation between proportions and incidence, which was strong year after year

(r=-0.8164; *p*=0.001), whereas an erratic pattern was observed between years (Table 1). Six serotypes/subserotypes of *S. flexneri* accounted for 50.9% of all isolates: 6 (14.4% of the total), 1b (13.3%), 2a (9.0%), 3a (8.6%) and 4 (5.6%). Only minimal changes in serotype distribution were observed from year to year, for most of the (sub-)serotypes, and any significant variation detected was inconsistent (Table 1). *S. dysenteriae* serotype 1, which is a source of great concern as it has caused devastating epidemics of shigellosis in various developing countries, including CAR,[15] was recovered only rarely in our laboratory (one organism in 2006).

No significant cross-reactions were observed between serotypes in *S. dysenteriae* (15 serotypes) and *S. boydii* (20 serotypes), but major cross-reactions were observed for 14 of the 19 serotypes of *S. flexneri*, due to a degree of antigenic relatedness attributable to a common repeating tetrasaccharide unit.[16] Thus, a multivalent vaccine including O antigens from *S. flexneri* 2a and 3a, in addition to direct protection against *S. flexneri* 2b and 3b, would provide cross protection against *S. flexneri* 1a, 1b, 4a, 4b, 5a, 5b, 7b, X and Y.[17, 18] Extrapolating these data to humans, a multivalent vaccine including *S. sonnei* (only one serotype described), *S. flexneri* 2a, 3a, and 6 would have provided direct protection for 52.3% and cross protection for 72.9% of these infections. This level is lower than that estimated for two multicenter studies at four sites in Africa and nine sites at Asia,[5, 19] in which a quadrivalent vaccine including the serotypes listed above would have provided protection against at least 85% of the serotyped organisms. However, these cross-reactions remain theoretical and discrepancies exist between data for humans and animals, as highlighted by the appearance of cross-reactive type 6 antibodies in humans, but not in mice, after vaccination with *S. flexneri* 2a conjugate.[20] Together with the considerable diversity of (sub-)serotypes in two of the three major serogroups (*S. flexneri* and *S. dysenteriae*) recovered in our study, this constitutes a real barrier to the development of a cheap, safe vaccine providing broad coverage against *Shigella.*

The ability of *Shigella* to acquire antimicrobial drug resistance rapidly is a major challenge in the control of infections with this bacterium. Overall resistance rates to antimicrobials were low during the study period, for all classes other than conventional antimicrobials (chloramphenicol (62%), amoxicillin (64%), cotrimoxazole (92%) and tetracycline (98%)), confirming recommendations for first-line treatment based on C3G and fluoroquinolones (Table 2). However, although no resistance to ciprofloxacin was detected, we report the first case of ESBL-producing *Shigella* (*S. flexneri*) in Sub-Saharan Africa, which is of major concern. Unlike *S. sonnei*, the *S. flexneri*, *S. dysenteriae* and *S. boydii* serotypes were all strongly associated with high rates of MDR (Table 2). After adjustment for sex and serogroup, multivariate analysis highlighted a significant contribution of the antimicrobial drug resistance pattern of *S. sonnei* to the rates of MDR of *Shigella* isolates (OR=0.02 95% CI [0.007-0.65]; $p<0.001$), despite the lower prevalence of *S. sonnei* than of *S. flexneri* (Table 3).

The data reported here are particularly important given the difficulty of carrying out such studies in countries with inadequate healthcare systems. The high diversity of *S. flexneri* and *S. dysenteriae* (sub-)serotypes observed here may act as a major obstacle to the development of a vaccine. Resistance to front-line antimicrobials is low, but it will be important to continue monitoring antimicrobial drug susceptibility in *Shigella* isolates.

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Disclosures regarding conflicts of interest

The authors have no conflict of interest to declare.

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