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Review

The importance of integrons for development and propagation of resistance in *Shigella*: the case of Latin America

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ABSTRACT

In Latin America, the disease burden of shigellosis is found to coexist with the rapid and rampant spread of resistance to commonly used antibiotics. The molecular basis of antibiotic resistance lies within genetic elements such as plasmids, transposons, integrons, genomic islands, etc., which are found in the bacterial genome. Integrons are known to acquire, exchange, and express genes within gene cassettes and it is hypothesized that they play a significant role in the transmission of multidrug resistance genes in several Gram-negative bacteria including *Shigella*. A few studies have described antibiotic resistance genes and integrons among multidrug resistant *Shigella* isolates found in Latin America. For example, in Brazil, Bolivia, Chile, Costa Rica and Peru, class 1 and class 2 integrons have been detected among multidrug resistant strains of *Shigella;* this phenomenon is more frequently observed in *S. flexneri* isolates that are resistant to trimethoprim, sulfamethoxazole, streptomycin, ampicillin, chloramphenicol, and tetracycline. The gene cassette *sul2*, which is frequently detected in *Shigella* strains resistant to the sulfonamides, suggests that the sulfonamide-resistant phenotype can be explained by the presence of the *sul2* genes independent of the integron class detected. It is to be noted that *sul3* was negative in all isolates analyzed in these studies.

The high frequency of sulfonamide (as encoded by *sul2*) and trimethoprim resistance is likely to be a result of the recurrent use of trimethoprim sulfamethoxazole as a popular regimen for the treatment of shigellosis. The observed resistance profiles of *Shigella* strains confirm that ampicillin and trimethoprim-sulfamethoxazole are ineffective as therapeutic options. In-depth information regarding antibiotic resistance mechanism in this pathogen is needed in order to develop suitable intervention strategies. There is a pressing need for regional and local antimicrobial resistance profiling of *Shigella* to be included as a part of the public health strategy.

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Introduction

Shigellae are global in terms of their prevalence and distribution[1] and shigellosis, which represents a significant cause of morbidity and mortality in developing countries, is still observed to be present in the industrialized nations.[1–3]

The disease process of shigellosis can be accurately summed up as an invasive infection of the human colon that encompasses a wide spectrum of clinical manifestations ranging from short-lasting watery diarrhea to acute inflammatory bowel disease that can cause fever, tenesmus as well as neurologic symptoms.[4,5]

On the basis of biochemical and serological characteristics, shigellae can be classified into four species: *Shigella dysenteriae*,[6] *S. flexneri*,[7] *S. sonnei* [8] and *S. boydii*.[9] The first three species include multiple serotypes. While *S. dysenteriae* and *S. flexneri* are the most commonly found species in developing countries, *S. sonnei* is responsible for most of the cases reported in industrialized nations.[1,4]

Prevalence of *Shigella* is predominantly observed in regions that have poor hygienic and environmental conditions as is common in developing countries. In industrialized nations, prevalence is found to be associated with groups or institutions such as day-care centers.[2,5,9]

The infective dose of *Shigella* is reportedly as low as 10–100 bacterial cells. Transmission occurs from person to person through the fecal and oral pathway, and also by consumption of contaminated food or water.[1,5] After an incubation period of 1–4 days, patients typically present with diarrhea, i.e., liquid stools that contain visible blood either with or without mucus.[1] Severe, acute complications of shigellosis may include acute hypoglycemia, seizures, toxic megacolon, hemolytic uremic syndrome, intestinal perforation, peritonitis, septicemia, etc., and can result in high mortality rates especially among infants and malnourished children.[4,5]

Shigellosis is endemic to most developing countries and is responsible for nearly 165 million cases and more than a million deaths annually. In the developing world, *Shigella* is associated with a high burden of illness particularly among children under five years of age, who present with the majority of cases (70%) and deaths (60%).[1,10,11] This pathogen is also frequently associated with epidemic outbreaks that are associated with high morbidity and mortality.[1]

Methodology

The literature search for this review article included the following databases: SciELO (Scientific Electronic Library on Line), Science Direct, OVID, Clinical Key, EBSCOhost, PubMed (National Library of Medicine); LILACS (Latin American and Caribbean Center on Health Sciences Information) for peer-reviewed literature, and Google. The search terms used in English and Spanish were *Shigella*, antibiotic resistance, resistencia a antibióticos, integron, Latin America and América Latina.

Emergence of multi drug resistance in *Shigella* isolates of Latin America

In Latin America, as in the rest of the world, the burden of shigellosis has been greatly increased by the emergence of microbial resistance toward antibiotics commonly used for therapy; this usually results in failure of treatment regimen.[12–14] Therefore, early and appropriate antibiotic therapy is crucial for reducing the duration of symptoms as well as for preventing the development of life-threatening complications.[1] Additionally, antibiotics also greatly reduce the excretion of the pathogen in stools which in turn reduces the spread of bacteria into the environment.[13]

Antibiotic resistant *Shigella* was initially noticed in 1940 in Japan when a serious outbreak of dysentery caused by *S. dysenteriae* was found to respond with decreasing effectiveness to sulfonamides, the principal antibacterial treatment available at the time. When antibiotics such as streptomycin, tetracycline, and chloramphenicol became available in that country, it was found that their efficacy was temporary as resistant strains were observed to develop soon after the drugs came into the clinical use.[15,16] Along similar lines, studies in Mexico have described resistance of the species toward tetracycline and chloramphenicol[17] and, in 1977, *Shigellae* strains resistant to ampicillin were isolated from similar epidemics in Mexico and Central America.[18,19] A few years later, an ampicillin resistant phenotype of *Shigella* was detected in Asian and African countries.[20]

Some reports in literature have documented an increased resistance to antibiotics such as gentamicin trimethoprim-sulfamethoxazole and β-lactams in Latin American countries.[5,12,21–24] In 2008, the first isolate of *Shigella* harboring the CMY-2 AmpC-lactamase enzyme was reported in Argentina.[22] This type of class C-lactamase is a clinically relevant cephalosporinase known to be produced by several Enterobacteriaceae strains; it is also known to mediate resistance to antibacterial agents such as cephalothin, cefazolin, cefoxitin, most derivatives of penicillin and β-lactam/β-lactamase inhibitor combinations.[25]

The rapid emergence and propagation of resistance has shifted the recommended treatment modalities to ciprofloxacin and azithromycin.[1] Ciprofloxacin, a fluoroquinolone antibiotic formerly used as a backup drug, is now the drug of choice for all patients that present with bloody diarrhea irrespective of their age. Alarmingly, fluoroquinolone-resistant *Shigella* sp. is an emerging phenotype around the world including Latin America.[12,13,24] Recently, a multistate cluster of *Shigella sonnei* that had an uncommon pulsed-field gel electrophoresis profile and was resistant to ciprofloxacin was detected in the U.S. by the Centers of Disease Control and Prevention (CDC)-National Antimicrobial Resistance Monitoring System (NARMS).[26] According to CDC-NARMS, 109 out of the 126 (87%) *Shigella* isolates that were tested were found to be non susceptible to ciprofloxacin. Ciprofloxacin-resistant *Shigella* isolates were obtained from patients who had traveled to the Dominican Republic (one of five isolates tested) and India (one of one isolate tested), and also among non-travelers (four of seven isolates tested).[26]

ReLAVRA (Latin American Network for the Surveillance of Antimicrobial Resistance), a regional surveillance system supported by PAHO, has reported that *S. flexneri* presents with an elevated ciprofloxacin resistant rates as compared to *S. sonnei* with a general upward trend observed in resistance over time in Argentina, Bolivia, Chile, Dominican Republic and Venezuela.[27] Additionally, the appearance of quinolone-resistance was also reported in 5% of the *Shigella* isolates analyzed in Peru.[24]

Azithromycin is also considered as an alternative for the treatment of shigellosis. However, its use is currently handicapped by the limited data available with respect to efficacy, high cost and formulation.[1] It is to be noted however that some studies regarding azithromycin have already reported *Shigella* spp. as presenting with a decreased level of susceptibility to the drug.[28,29] Cases of *Shigella* isolates with increased minimum inhibitory concentrations (MICs) for azithromycin have already been documented in India and the Netherlands.[28,29]

The first report of *Shigella* that exhibited an increased MIC to azithromycin was among pediatric patients in the Peruvian Amazon.[30] In the above mentioned study, a total of 403 *Shigella* isolates were analyzed and results were found to reveal an increased level of resistance toward trimethoprim-sulfamethoxazole, ampicillin, erythromycin, and a decreased level of susceptibility to ceftriaxone, azithromycin, nalidixic acid, and ciprofloxacin.[30]

Another population cohort where this phenomenon was initially identified was in the United States among men who had sex with men (MSM).[31] Since 1970 this route of infection has been recognized as an important epidemiological component of Shigellosis transmittance especially in high-income nations. More recently, it has been identified as an important route for intercontinental spread of *Shigella* in MSM and included disease cases from 29 countries collected between 1995 and 2014.[32] In the above mentioned study, more than 300 whole-genome *Shigella* isolates were analyzed and data thus obtained revealed the presence of antibiotic resistance genes in the MSM-outbreak associated lineage which suggested that the ancestral strain of the lineage was multidrug resistant prior to its introduction into the MSM population.[32,33] The results of this study confirmed that shigellosis is an important emerging sexually transmitted infection among MSM and it is already showing potential for global spread as well as for resistance to commonly available antibiotics.

To date, the aforementioned report from Peru is the only observation in all of Latin America for the presence of a resistant phenotype of *Shigella* that exhibits a decreased susceptibility to azithromycin and ciprofloxacin, but such reports can increase in frequency as more intense research into *Shigella* is undertaken and completed in other Latin American countries.

Integrons: molecular mechanisms behind antibiotic resistance

Understanding the changing resistance patterns of *Shigella* spp. is important in terms of standardizing the appropriate treatment. As early as 1959–1960, it was found that multiple antibiotic resistance characteristics present in certain *Shigella* strains could be transferred to other Enterobacteriaceae simply by mixing liquid cultures of resistant and sensitive bacteria and plating on solid medium containing the appropriate antibiotics as selective agents.[16,34] In 1976, the R-plasmid associated with ampicillin resistance in *Shigella dysenteriae* type 1 was characterized from a strain that was isolated in a geographically widespread dysentery epidemic in Mexico, Central America and Bangladesh.[19] Since then, the number of different studies that have described isolates of "transferable drug resistance" has rapidly increased especially when multidrug resistant strains were studied in different countries indicating that the resistance determinants, called R plasmids, often reflected the geography of antibiotic usage.[16,18]

Antibiotic resistance determinants in bacteria are a consequence of antimicrobial use and abuse. In terms of origin and sources of the antibiotic resistance, a broader view must include resistance genes of pathogenic as well as non-pathogenic bacteria so as to encompass the full pan-microbial genome or the resistome.[35]

In case of *Shigella*, similar to several other enteropathogens, the molecular basis of antibiotic resistance resides in a small part of the resistome within mobile genetic elements such as the R plasmids, transposons, integrons, and genomic islands.[36]

Although integrons were first described only in 1989,[30] bioinformatics based-analysis of sequenced bacterial genomes clearly demonstrates that integrons or integrase genes are very common in bacterial populations and are known to sometimes occur with frequencies as high as 10–17%.[37]

These molecular platforms, first discovered in clinical contexts, can be classified as class 1, class 2, and class 3 integrons. Integrons have been prolifically studied and analyzed as a model system in order to arrive at a formal definition for the structure of these genetic elements.[38] Integrons are well known as naturally occurring genetic elements that are capable of acquiring, exchanging and expressing genes within gene cassettes.[37] Structurally, they are composed of three key elements: a tyrosine recombinase gene or integrase (*intI*), a recombination site (*attI*) and a promoter site (Pc).[36,30] All integrons analyzed till today are composed of an *intI* gene that encodes for an integrase enzyme. This enzyme which belongs to the tyrosine recombinase family can recombine discrete units of circularized DNA that are also known as genetic cassettes.[36,39] The other crucial integron component is the primary recombination site (*attI*). A wide variety of genetic cassettes that confer resistance to antibiotics can be found in this specific region.[36] Lastly, the third important component of an integron is a strong promoter (Pc, Pant) that is capable of directing transcription of the captured genes or genetic cassettes.[40] Genetic cassettes are small mobile units composed of a coding sequence and a recombination site, *attC*. Integrons exchange gene cassettes through integrase catalyzed site specific recombination either between an *attI* and *attC* sites or between two *attC* sites both of which lead to excision of the gene cassette.[36,39]

Each class of integron is uniquely different in terms of the sequence of the integrase gene it encodes.[37] While both

class 1 and class 2 integrons are known to contain antibiotic resistant gene cassettes, class 1 is the best example that demonstrates evolution as driven by natural selection; this is so because class1 integrons have been observed and characterized from a variety of different locations, from different plasmids and transposons, and their appearance is coincident with the widespread use of antibiotics.[36,39]

Integrons and gene cassettes are hypothesized to play a crucial role in the mechanism that is responsible for transmission of multidrug resistant genes in several Gram-negative bacteria.[36,41,42] In *Shigella*, the dissemination of resistance-inducing genes is mostly facilitated by the ability of the bacteria to acquire transposons or plasmids that may contain integrons, especially class 1 and class 2 integrons, that are known to have a high density of resistance gene cassettes.[36,42]

The distribution of integrons varies according to the species and the resistant phenotype. *S. sonnei* and *S. boydii* strains contain a single class 2 integron whereas *S. flexneri* and *S. dysenteriae* strains are known to carry a class 1 integron, either alone or associated with a class 2 integron.[36,43,44]

Detection of integrons and genetic cassettes in *Shigella* isolates from Latin America

In Latin America, the public information available regarding *Shigella* is sufficient for the emergence of multidrug resistant varieties to be classified as a major public health problem. Published literature regarding phenotypic methodology based antibiotic resistance profiling of this pathogen clearly establishes a high prevalence of multidrug resistant *Shigella* among Latin American countries; however, the information regarding molecular mechanisms responsible for antibiotic resistance in this pathogen is very limited.

The INTEGRALL database (www.integrall.bio.ua.pt) has a substantial amount of information regarding classes or families of integrons isolated from other South American pathogens. This web-based platform, which has been designed for the purpose of organizing all data available for these genetic structures, is very useful tool for compiling information on integrons.[45] Till date, the INTEGRALL database contains more than 7664 entries, including sequences of 1509 integrase genes and 8559 gene cassettes, isolated from 144 genera and 323 species of bacteria. Of these available sequences, only 53 belong to *Shigella* and, unfortunately, there are no studies regarding integrases and gene cassette arrays from isolates belonging to Latin America. Other South American pathogens that have been described are: *Acinetobacter*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Salmonella* spp.; these include several novel gene cassette arrays located in unusual class 1 integrons.

Reports regarding the identification and characterization of these molecular structures in *Shigella* are scarce. In Brazil, Bolivia, Chile, Costa Rica and Peru class 1 and class 2 integrons have been detected among multidrug resistant *Shigella* isolates; such integrons are frequently associated with resistance of *Shigella* to trimethoprim, sulfamethoxazole, streptomycin, ampicillin, chloramphenicol, and tetracycline.[24,46–49] Studies from Chile, Costa Rica and Peru showed that class 1 and class 2 integrases are commonly found among *S. flexneri* isolates.[24,46–48]

In a study conducted in Brazil, Peirano et al.[42] reported the presence of class 1 integrons in a single isolate of *S. flexneri* and *S. sonnei*, whereas class 2 integrons were reportedly found in 56 of the 62 (90.3%) strains that were analyzed [*S. flexneri* ($n = 43$), *S. sonnei* ($n = 13$)]. Curiously, an isolate carrying a class 1 integron was also found to carry an additional class 2 integron. Another study, also conducted in Brazil, reported a higher frequency of *intI-1* (92%, 23 out of 25 isolates) and *intI-2* (100% of 25 isolates) in *S. sonnei* isolates.[43] In this study, 92% of the strains were found to be positive for two integrons and all the strains that exhibited resistance toward two or more tested antibiotics revealed the presence of at least one of the integrons. The detection rate of *intI-2* in *Shigella* (100%) was substantially higher than that reported for Costa Rica [47] whereas; class 1 integrase (*intI-1*) was detected in all of the 30 *Shigella* isolates analyzed [*S. flexneri* ($n = 25$), *S. sonnei* ($n = 5$)], whereas class 2 integrase was detected in 27 of the 30 isolates (90%). In the case of Bolivia, a study conducted on *Shigella* spp. by Rodas et al.[49] reported that the class 1 integrase was present in 20 out of 45 isolates (44%) analyzed, whereas class 2 integrase was found in 31 out of the 45 (69%) strains.

In Peru, Lluque et al.,[24] reported the presence of class 1 and class 2 integrases in similar frequencies in case of *S. flexneri* ($n = 42$, 50–55%) and *S. dysenteriae* ($n = 4$, 25%). In *S. sonnei* ($n = 10$) *intI-1* was found to occur more frequently (90%) than *intI-2* (10%) whereas in *S. boydii* ($n = 11$) the opposite was found to be true as *intI-2* was more frequently observed (45%) than *intI-1* (9%).

In Brazil, class 2 integrons were more prevalent in *S. flexneri* and *S. sonnei* than class 1 integrons.[42,43] It is of importance that class 3 integrons were not detected in Brazil, Chile, Costa Rica and Peru.

In Brazil, the sulfonamide resistant gene (*sul1*) was detected in 2 of the 62 (3%) multidrug resistant-*Shigella* that were also positive for presence of class 1 integrons [41] and results along similar lines were also reported from Peru. Curiously, it was found that *sul1*, in bacterial strains harboring typical class 1 integrons, is associated with resistance to trimethoprim sulfamethoxazole.[50,51] A study conducted by Reyes et al.[52] reported that in case of isolates identified from Chilean hospitals, Class 1 integrons were found to be most prevalent in enterobacterial isolates (62 out of 191 strains, 62.4%) including the *Shigella* spp. They further specified that among *Shigella* spp., class 2 integrons were found only in five strains. Interestingly, One of these isolates was also found to contain a class 1 integron. The authors went on to characterize the variable regions of 13 class 1 integrons and identified four distinct types: type 1 carrying *ant(3″)I*, type 2 carrying *ant(2″)I* and *ant(3″)I*, type 3 carrying *aac(6′)Ib* and *ant(3″)I*, and type 4 was an empty integron.

The class 1 integrons differ from the class 2 in their capacity to integrate and excise gene cassettes, and also in terms of the presence of *sul1* gene in the 3′ conserved segment (3′CS).[42] In Brazil, Costa Rica and Peru the gene cassette *sul2* is more frequently detected in *Shigella* isolates that are resistant to sulfonamides, which suggests that sulfonamide-resistant

phenotype could be explained by the detection of *sul2* genes independent of the integron class detected; *sul3* was negative in all isolates.[42,47]

On the basis of the model of a culture-based methodology without antibiotic selection, it was suggested that the *sul1* gene cassette is a genetic determinant associated with urbanization.[53] In a study developed at Tierra de Fuego, the Patagonian Island of Argentina, antibiotic resistant genes such as *sul 1* and *qacE1/qacED1*, both of which are embedded in class 1 integrons, exhibited varying ecological and molecular behavior in environmental samples. While the *sul1* gene frequency was clearly related to urbanization, the *qacE1/qacED1* gene demonstrated an adaptive role to several habitats indicating that its presence has been widespread for a considerable time and emphasizing its role in terms of a large variety of genomes, habitats, and possibly different types of stressors. Gene cassette *sul1* exhibited a high frequency in urban sites, which could possibly be the consequence of the genetic flow of clinical *sul1* alleles from the hospital toward the open environment, where they are absorbed by non clinical strains and, in all likelihood, maintained by the presence of contaminants that are co-selected for sulfonamide resistance.[53]

The high frequency of sulfonamide (encoded by *sul2*) and trimethoprim resistance is possibly a direct result of the recurrent use of trimethoprim sulfamethoxazole as a treatment modality for shigellosis.[42] The generalized resistance profile of *Shigella* spp. toward ampicillin (40–100% of isolates), and trimethoprim–sulfamethoxazole (27–100% of isolates) confirms that these antibiotics are ineffective for empirical therapy.[42,47,54,55] Most of the ampicillin-resistant strains are associated with the presence of an OXA-type β-lactamase. This gene cassette was found to occur with a higher frequency as compared to the CARB-like and TEM-1 β-lactamases, which were also detected in some of the isolates.[24,42,46,48]

The chloramphenicol resistance gene *catA1*, which encodes for chloramphenicol o-acetyl-transferase, was detected in 100% of the chloramphenicol-resistant strains in Brazil and Chile and in about 86% of similarly resistant strains in Peru. In addition, in all the three countries, *tet(B)* was the dominant tetracycline-resistant gene detected, which explains the high level of chloramphenicol and tetracycline resistance profile that had been observed.[24,42,46]

Some studies have hypothesized that insertion of the class 1 integrons within the *Shigella* resistance locus (SRL) may be able to explain the chloramphenicol and tetracycline resistance observed in strains containing class 1 integron. It is also a distinct possibility that the insertion-sequence regions may be deleted or inserted independently at hot spots, such as *orfB* of *IS600*, as is the case for integration of the class 1 integrons within the transposons of the *Tn3* family. Conserved organization of the class 1 integron in unrelated *Shigella* strains might be linked to the insertion and stabilization of these elements within mobile genetic superstructures such as SRL and PAI; such phenomenon may be dependent upon mechanisms such as the expression of the distal gene cassettes and hence merits an in-depth exploration.[36,44] This observation could aid in explaining the phenotypes of chloramphenicol and tetracycline-resistance in *Shigella* harboring class 1 integrons in the specific case of Latin America.

Conclusion

Increased prevalence of antibiotic resistance is a major threat against controlling shigellosis. In Latin America, a few studies have proposed that antibiotic resistance genes together with integrons make up the molecular mechanism that has the potential to be involved in transmission of multidrug resistance genes in this pathogen. Studies conducted in Brazil, Bolivia, Chile, Costa Rica and Peru have shown that class 1 and class 2 integrons are present among multidrug resistant *Shigella* isolates harboring the OXA-type β-lactamase, and less frequently, the CARB-like and TEM-1 β-lactamases. In one of these studies class 1 integrons were characterized and certain types of integrons were described comprehensively.

The observed antibiotic resistance profile confirmed that ampicillin and trimethoprim–sulfamethoxazole are ineffective for therapeutic purposes against *Shigella*. More comprehensive and in-depth information about antibiotic resistance mechanisms in this pathogen is needed in order to develop suitably effective interventional strategies. To this purpose, acquiring regional and local antimicrobial resistance profiles for all *Shigella* patients should be considered for inclusion as a part of the public health strategy.

Conflicts of interest

The authors declare no conflicts of interest.

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