Original Article

Investigation of a food-borne *Salmonella* Oranienburg outbreak in a Mexican prison

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Abstract

Introduction: Gastroenteritis outbreaks in prisons represent a public health risk worldwide. Identifying and characterizing the etiological agents of gastroenteritis outbreaks in prisons is important for implementing effective prevention and infection control measures. We present the first studied case of a gastroenteritis outbreak in a Mexican prison.

Methodology: Rectal swab samples were obtained from affected inmates. Standard microbiological techniques were used for isolating *Salmonella enterica*. Isolates were typed by PCR assays of DNA repetitive elements (ERIC, BOX, REP) and RAPD. Antibiotic resistance profiles were performed by the Kirby-Bauer method.

Results: *S. enterica* serotype Oranienburg was responsible for the outbreak affecting 150 inmates. All patients presented diarrhea, and 70% of them also presented vomiting, with no fatal cases. The origin of the outbreak was undetermined due to the difficulty of gathering epidemiological information, but was likely the result of consumption of shrimp broth or a cantaloupe melon beverage. REP, BOX, and ERIC analyses of 26 serotype Oranienburg strains resulted in Simpson discrimination index (*D*) values of 0, 0.5507, and 0.5661, respectively. The *D* values from DG93-RAPD analyses and from the combined ERIC-BOX-DG93 markers were 0.7753 and 0.6092, respectively. All strains showed multiresistance to antibiotics.

Conclusions: This is the only studied case of a gastroenteritis outbreak in a Mexican prison, and of the first such outbreak caused by serotype Oranienburg. The combined ERIC, BOX, and RAPD markers adequately assessed the genotype diversity of analyzed strains. Penitentiary personnel or inmates involved in outbreaks might spread multiresistant strains outside of the facility.

Key words: *Salmonella* serotype Oranienburg; prison outbreak; ERIC-BOX-RAPD; multiple antibiotic resistance; Mexico

J Infect Dev Ctries 2014; 8(2): 143-153. doi:10.3855/jidc.3367

(Received 31 January 2013 – Accepted 16 June 2013)

Introduction

Acute diarrheic diseases are predominantly caused by food contamination [1] and are widespread throughout the world, killing nearly 1.8 million infants each year, mostly in developing countries [2]. Greig *et al*. [3] recently reviewed the epidemiologic risks associated with gastrointestinal disease outbreaks occurring in prisons in many countries, of which detailed studies are limited to North America (US and Canada) and Europe. Within prisons, overcrowding of prisoners and security personnel boosts pathogen dispersal, mortality, and the costs of disease control and treatment. Asymptomatic prisoners, once released, and prison staff members can carry enteric pathogens, threatening the general population [3]. These epidemiologic characteristics make the analysis and monitoring of outbreaks in prisons relevant for public health. At present, there are no studies of gastroenteritis outbreaks in Mexican prisons.

The main etiologic agent associated with gastroenteritis outbreaks in prisons worldwide is *Salmonella enterica* [3]. The gold standard for differentiating epidemiologically related strains of *S. enterica* is the analysis of polymorphisms through pulsed-field gel electrophoresis (PFGE) [4]. However, analysis of repetitive DNA elements has also been proven to be successful [5,6], and even equally or more effective than PFGE for that purpose [7,8]. Additionally, it has been reported that random amplified polymorphic DNA (RAPD) assays combined with other typing techniques are useful for monitoring outbreaks and clinical cases of

gastroenteritis caused by serotypes of *S. enterica* [9-11]. Repetitive DNA elements and RAPD analyses are advantageous due to their speed, low cost, and easiness of interpretation [12], but the RAPD assay has been criticized for its lack of reproducibility in laboratories [13]. This disadvantage becomes irrelevant when studying outbreaks within limited space and time, in which comparisons with unrelated strains are unnecessary. Consequently, repetitive DNA elements and RAPDs remain valid analysis tools for epidemiologists [4,14], being particularly valuable in countries with limited public health resources.

In this study, we investigated an acute gastroenteritis outbreak in a prison in central Mexico, and determined that *S. enterica* serotype Oranienburg was the etiological agent. We further characterized these isolates through the analysis of repetitive DNA elements and RAPD assays. We also analyzed antibiotic resistance patterns of the pathogen isolates, and discussed the significance of our findings compared with reports from similar outbreaks in prisons from other countries, contrasting the characteristics of our isolates with reports about the serotype.

Methodology

Location of the prison and development of outbreak

The studied gastroenteritis outbreak affected prisoners of the Centro de Readaptación Social Mil Cumbres, located in the city of Morelia, Michoacán, in central Mexico. The outbreak was initiated on 3 May 2010, and new cases were manifested on the next day, affecting a total of 150 male prisoners between 18 and 71 years of age. All patients presented diarrhea and 105 (70%) also presented vomiting. In all cases, patients recovered in less than one week after they manifested acute gastroenteritis symptoms.

Sampling and isolation of the etiological agent

Stool samples were taken with rectal swabs from 35 patients randomly chosen from the 150 prisoners displaying acute gastroenteritis symptoms. It was impossible to take samples from healthy prisoners to be used as controls. Guided by the observed symptoms, samples were screened for common enteropathogenic bacteria found in Mexico.

The stool samples were processed according to the Instituto Nacional de Referencia Epidemiológica (InDRE) 1994 NORM for *Salmonella enterica*. Isolates from symptomatic individuals identified as *S. enterica* were serotyped at the InDRE in Mexico City following the White-Kauffman-Le Minor scheme [15].

The foodstuffs and beverages consumed by inmates one day before and on the day of the outbreak onset (May 3) had been discarded prior to sampling, making analysis of them impossible. The prison's kitchenware and food preparation installations also could not be analyzed. Food handlers and prison authorities were interviewed in order to determine the menu consumed by the inmates on the day of the outbreak.

Samples of cream, cheese, lettuce, sauce, shrimp cakes, rice, and chicken meat consumed on May 4, as well as samples of well and kitchen tap water, were analyzed for *S. enterica* according to the corresponding Mexican norm (NOM-114-SSA1-1994). All these samples were also analyzed for fecal coliforms and *Escherichia coli* according to the Mexican norm CCAYAC-M-004. The most probable numbers (MPN)/100 mL were determined for water samples.

Molecular genetics techniques

DNA was extracted from colonies grown in Luria Bertani agar medium by the phenol-chloroform protocol [16]. PCR reactions were made to a final volume of 25 µL of 20 mM Tris-HCl buffer pH 8.4, and contained 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dATP, dGTP, dCTP, and dTTP, and 1.5 U of *Taq* DNA polymerase (Invitrogen, Carlsbad, USA). Primers used for assays are listed in Table 1, each one in the amounts of 10 pmol for enterobacterial repetitive intergenic consensus (ERIC) and BOX elements, 20 pmol for repetitive extragenic

Table 1. Primers used in PCR assays

Primer key	Sequence (5′→3′)	Reference
ERIC1R	ATGTAAGCTCCTGGGGATTAC	[17]
ERIC2	AAGTAAGTGACTGGGGTGAGC	
REP1R-I	IIIICGICGICATCIGGC	[17]
REP2 -I	ICGICTTATCIGGCCTAC	
BOXA1R	CTACGGCAAGGCGCTGACG	[18]
DG93	AGCAGCGCCTCA	[19]
DG102	GGTGCGGGAA	

palindromes (REP), and 15 pmol for RAPD. A total of 50 pmol of template DNA were used for all assays. Standard PCR conditions for repetitive elements ERIC, REP, and BOX were used as previously reported [17,18]. RAPD assays were made in the conditions described by Lim *et al.* [19]. All PCR assays were run in duplicate in a Gene AmpPCR System 2700 (Applied Biosystems, Foster City, USA) thermocycler. Amplification patterns were visualized in 2% (w/v) agarose gels stained with ethidium bromide. Images of gels were obtained in a Chemi-Doc Molecular Viewer (Bio-Rad, Berkeley, USA).

Antibiotic susceptibility assays

Antibiotic susceptibility patterns of isolates were generated by the Kirby-Bauer disk diffusion assay following the standards and interpretation guides of the Clinical and Laboratory Standards Institute [20,21]. The assays were made in sensi-discs (Bio-Rad, Berkeley, USA) containing the following antibiotics: 10 µg ampicillin (AMP), 100 µg carbenicillin (CB), 30 µg cefotaxime (CTX), 30 µg ceftriaxone (CRO), 30 µg cephalothin (CF), 30 µg amikacin (AK), 10 µg gentamicin (GEN), 30 µg netilmicin (NET), 300 µg nitrofurantoin (NF), 30 µg chloramphenicol (CHL), 5 µg pefloxacin (PEF), and 25 µg sulphamethoxazole/trimethoprim (SXT).

Data analyses

The size of amplification bands was established with the software Quantity One 4.4.1 (Bio-Rad, Berkeley, USA) using a 1 Kb molecular ladder (Invitrogen, Carlsbad, USA) as reference. Binary matrixes were built from the obtained bands from each marker and strain, in which the digit 1 designated presence of band in a strain and the digit 0, its absence. The resulting binary matrixes were used for the calculation of genetic distances by the Dice coefficient, from which a dendrogram was built by the UPGMA (unweighted pair group method with arithmetic mean) algorithm in the free software PAST version 1.77 developed by Hammer *et al.* [22].

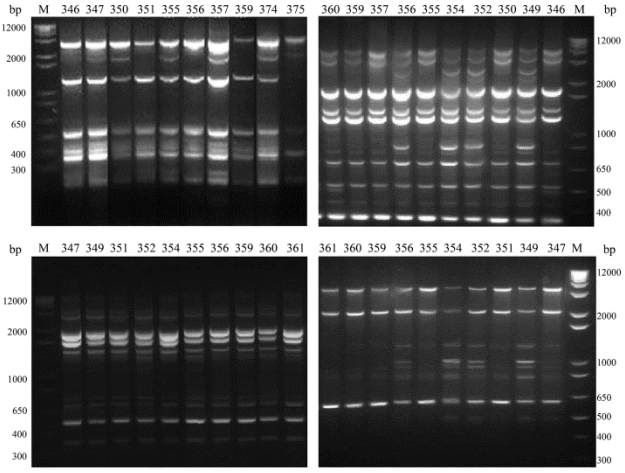
The discrimination index of Simpson (*D*) was calculated for each marker according to Hunter and Gaston [23].

Results

Identification of the etiological agent

Salmonella enterica serotype Oranienburg was isolated from all 35 patients with symptoms of a gastrointestinal infection that could be sampled, which indicated that it was the etiological agent of the outbreak. Cream, cheese, lettuce, and sauce samples were positive for fecal coliforms and *E. coli*. Shrimp cakes, rice, and chicken meat were negative for bacteria. Kitchen tap water and well water were negative for *E. coli*, having 76 MPN/100 mL and 1 MPN/100 mL of total coliforms, respectively. All analyzed samples of food and water were negative for *S. enterica*. The conducted survey of the food consumed the day of the outbreak onset (May 3) showed that all affected prisoners consumed shrimp broth and the fresh melon beverage, and although these food items were impossible to be analyzed, they were considered to be the most probable causes of the outbreak.

Figure 1. 2% agarose gels stained with ethidium bromide showing examples of amplification patterns obtained for different SOM strains from *Salmonella* serotype Oranienburg with the markers ERIC (top left), BOX (top right), REP (bottom left), and DG93-RAPD (bottom right). M, molecular weight marker, 1 Kb DNA ladder (Invitrogen, USA). SOM strain numbers are indicated on each lane.



Molecular genetic characterization

Only 26 of the 35 initial *S. enterica* isolates were characterized by molecular genetic analysis; the remaining were lost before serotyping.

ERIC amplification patterns revealed five to eight bands with molecular weights ranging from 220 to 3300 bp (Figure 1). The four polymorphic loci detected by this marker grouped isolates in five genotype clusters at the 95% similitude level (Figure 2), and generated the highest discrimination value among the analyzed repetitive DNA elements (*D* = 0.5661).

Figure 2. UPGMA dendrograms of studied *Salmonella* serotype Oranienburg strains derived from amplification patterns of the markers ERIC (left) and BOX (right). The vertical dashed line indicates the 95% similitude level. Genotypes clustered at a 95% similitude level are indicated with Roman numerals

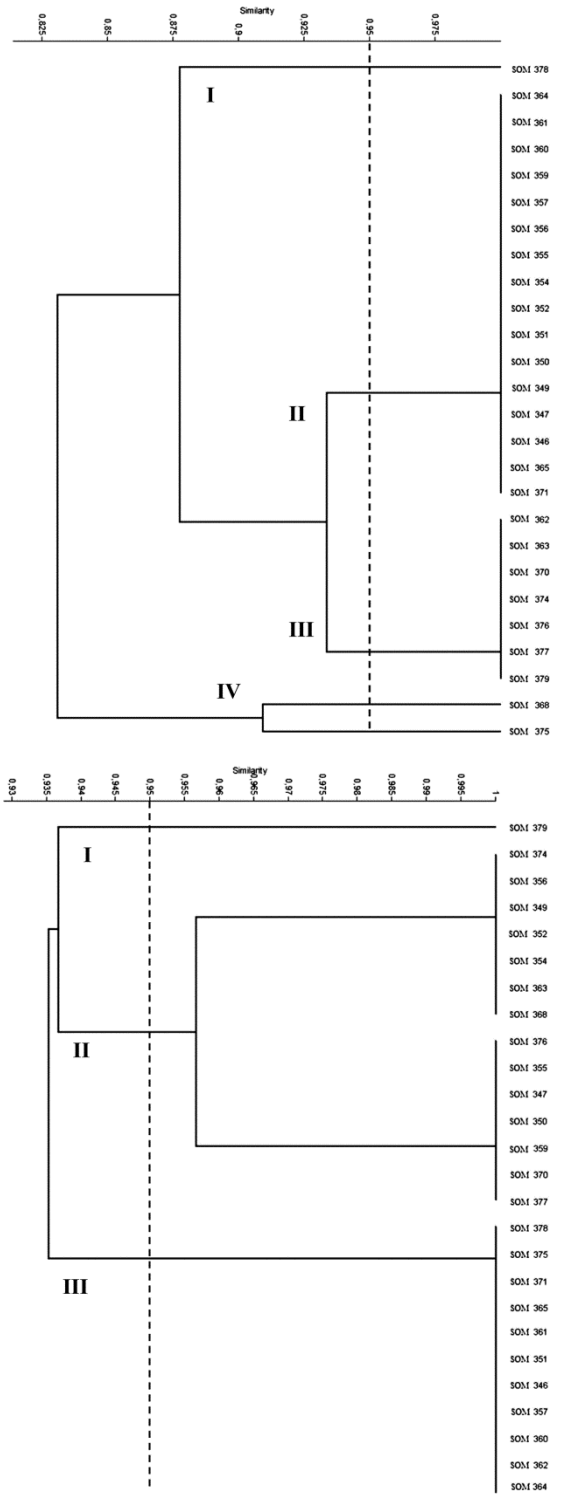


Figure 3. UPGMA dendrograms of studied *Salmonella* serotype Oranienburg strains derived from amplification patterns of the markers DG93-RAPD (left), and the combination of ERIC, BOX, and RAPD (right). The vertical dashed line indicates the 95% similitude level. Genotypes clustered at a 95% similitude level are indicated with Roman numerals.

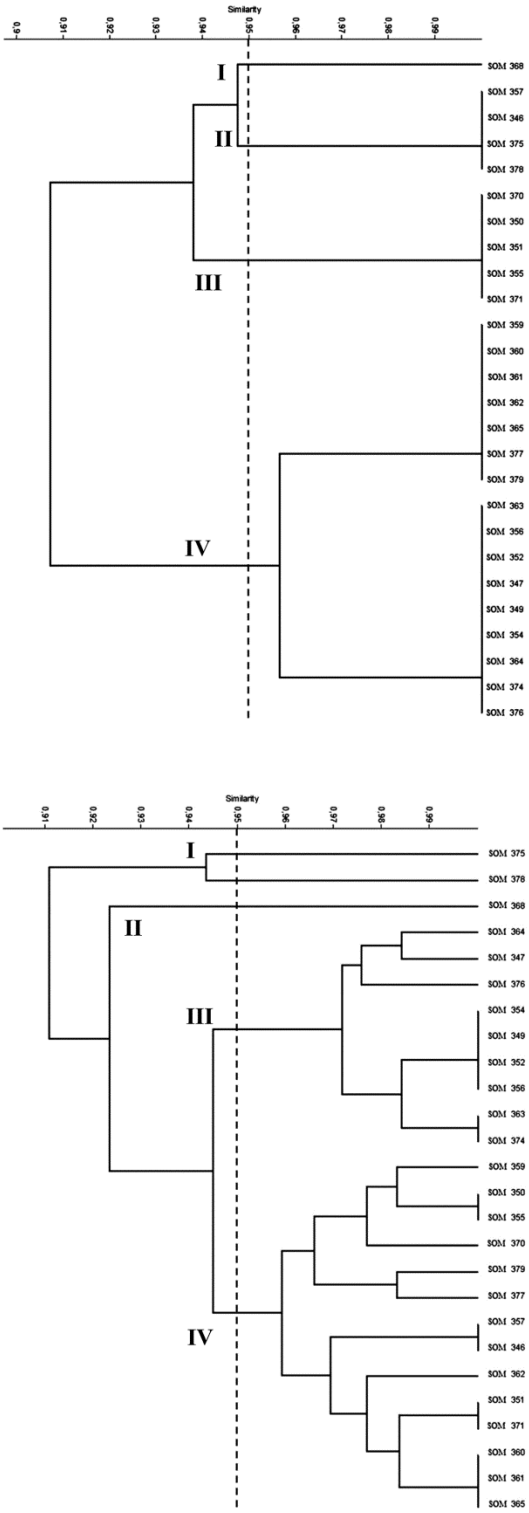


Table 2. Grey tone heat-map of the antibiotic resistance profile of the studied strains of *Salmonella* serotype Oranienburg. For each antibiotic (columns), a dark area represents resistance (R), a half-tone area represents intermediate resistance (I), and a light area represents sensitivity (S). Number of resistance profiles is indicated for each isolate. Strain genotypes, according to dendrograms of Figures 2 and 3, are indicated for each marker (ERIC, BOX and RAPD) and for their combination (E+B+R).

	Antibiotic												
Strain	**CF**	**AMP**	**CB**	**NF**	**AK**	**CTX**	**GEN**	**NET**	**CHL**	**CRO**	**PEF**	**SXT**	**Resistance profile**
SOM 368	R	R	R	R	R	R	R	R	S	I	R	S	1
SOM 376	R	R	R	R	R	R	R	S	R	R	S	S	2
SOM 352	R	R	R	R	R	I	R	R	R	I	I	S	3
SOM 364	R	R	R	R	R	I	R	R	I	I	S	S	4
SOM 350	R	R	R	R	R	R	R	I	I	I	S	S	5
SOM 375	R	R	R	R	R	R	R	I	S	I	S	S	5
SOM 371	R	R	R	R	R	R	I	R	S	S	S	S	6
SOM 362	R	R	R	R	R	R	R	I	S	S	S	S	7
SOM 377	R	R	R	R	R	R	R	I	S	S	S	S	7
SOM 378	R	R	R	R	R	R	R	S	S	S	S	S	7
SOM 363	R	R	R	R	R	I	R	I	I	I	S	S	8
SOM 370	R	R	R	R	R	R	I	I	I	I	S	S	9
SOM 360	R	R	R	R	R	R	I	I	I	I	S	S	9
SOM 374	R	R	R	R	R	R	I	I	S	I	S	S	9
SOM 355	R	R	R	R	R	I	R	S	S	I	S	S	10
SOM 356	R	R	R	R	R	R	I	I	S	S	S	S	11
SOM 359	R	R	R	R	I	I	R	S	S	I	S	S	12
SOM 365	R	R	R	R	I	I	R	I	S	S	S	S	12
SOM 357	R	R	R	R	R	I	I	I	S	S	S	S	13
SOM 349	R	R	R	R	I	R	S	I	S	S	S	S	14
SOM 347	R	R	R	R	R	I	S	S	I	S	S	S	15
SOM 351	R	R	R	R	R	I	S	S	I	S	S	S	15
SOM 354	R	R	R	R	R	I	I	S	S	S	S	S	15
SOM 379	R	R	R	S	R	I	I	S	S	S	S	S	16
SOM 346	R	R	R	R	S	I	S	S	S	S	S	S	17

Antibiotic abbreviations: ampicillin (AMP), carbenicillin (CB), cephalothin (CF), nitrofurantoin (NF), amikacin (AK), cefotaxime (CTX), gentamicin (GEN), netilmicin (NET), chloramphenicol (CHL), ceftriaxone (CRO), pefloxacin (PEF), sulphamethoxazole/trimethoprim (SXT)

BOX showed between 11 and 12 bands weighing from 440 to 4570 bp, to be the most complex banding patterns compared with the repetitive elements that were analyzed (Figure 1). BOX displayed three polymorphic loci, allowing for distinguishing three genotypes at the 95% similitude level (Figure 2) with an intermediate discrimination value ($D = 0.5507$).

Amplification patterns for REP displayed 10 bands per isolate with molecular weights of between 550 and 3900 bp (Figure 1), representing 10 monomorphic loci, which, at the 95% similitude level, were interpreted as all strains belonging to the same genotype ($D = 0$).

The banding pattern that emerged from the RAPD assays using the DG102 primer showed five invariable bands in all isolates ($D = 0$) weighting from 610 to 2800 bp (data not shown), because of which the DG102 amplification products were excluded from the UPGMA analysis. In the RAPD assay using the DG93 primer, the banding patterns displayed five to eight bands with molecular weights of 514 to 4000 bp (Figure 1), three of which were considered to be polymorphic loci, allowing for differentiating four genotypes at 95% similitude level (Figure 3). The *D* value calculated for this marker was 0.7753, the highest discrimination index found among all used markers.

The discrimination index value (*D*) for the combined markers with variable banding patterns (ERIC, BOX, and DG93-RAPD) was 0.6092, corresponding to 30 loci, of which 10 were polymorphic. The UPGMA dendrogram for the combined markers revealed five genotypes at a similitude level of 95% (Figure 3).

Susceptibility to antibiotics

The assays of susceptibility to 12 antibiotics made on the 25 isolates resulted in 17 resistance profiles, all displaying multiple resistances (Table 2). It was impossible to test antibiotic susceptibility for isolate SOM 361 because it was lost. All isolates tested were sensitive to sulphamethoxazole/trimethoprim and resistant to ampicillin, carbenicillin, and cephalothin, while most tolerated nitrofurantoin (96%) and amikacin (84%). The remaining antibiotics tested elicited variable levels of resistance and susceptibility, the most resilient strains being SOM368 and SOM376, with resistance to nine of the tested antibiotics. On the

Table 3. Distribution of antibiotic resistance profiles among genotypes defined for each molecular marker

	Genotypes[a]			
Resistance profile[b]	**ERIC**	**BOX**	**RAPD**	**E+B+R**
1 (1)	IV	II	I	II
2 (1)	III	II	IV	III
3 (1)	II	II	IV	III
4 (1)	II	III	IV	III
5 (2)	II	II	III	IV
	IV	III	II	I
6 (1)	II	III	III	IV
7 (3)	III	III	IV	IV
	III	II	IV	IV
	I	III	II	I
8 (1)	III	II	IV	III
9 (3)	III	II	III	IV
	II	III	IV	IV
	III	II	IV	III
10 (1)	II	II	III	IV
11 (1)	II	II	IV	III
12 (2)	II	II	IV	IV
	II	III	IV	IV
13 (1)	II	III	II	IV
14 (1)	II	II	IV	III
15 (3)	II	II	IV	III
	II	III	III	IV
	II	II	IV	III
16 (1)	III	I	IV	IV
17 (1)	II	III	II	IV

[a] Roman numerals correspond to genotypes identified for each marker and their combination (E+B+R) (Figures 2 and 3)
[b] The number of strains having the same antibiotic resistance profile is shown between parenthesis

opposite extreme, strains SOM379 and SOM346 were the most susceptible, only resisting four antibiotics (Table 2).

There was no correlation between genotype and resistance profile for either of the used molecular markers and their combination (Table 3).

Discussion

Salmonella enterica was identified as the etiological agent responsible for the gastroenteritis outbreak among prisoners. This pathogen has been found to be the cause of 27% of 72 similar cases reported in the literature from prisons throughout the world, in which the serotypes Typhimurium, Newport, Muenster, Heidelberg, Hadar, Montevideo, and Enteritidis were identified [3,24,25].

The present is the first report of a gastroenteritis outbreak in a prison that involved the serotype Oranienburg, which was reported in North and Latin America as the 10th and 15th most common serotype, respectively [26]. This serotype was the 12th most frequently found in human stool samples in Mexico among 199 serotypes reported by the country's health services between 1972 and 1999 [27]. A more recent study conducted between 2002 and 2005 covering four Mexican states including Michoacán, where the present study was conducted found that this serotype was fifth in frequency of isolation from patients with gastrointestinal symptoms, and sixth among asymptomatic children [28]. These data, together with our results, suggest that Oranienburg may become a relevant pathogen among *S. enterica* serotypes causing gastrointestinal diseases in Mexico, a finding that should draw the attention of public health authorities in the country. Increases in the incidence of serotypes causing gastrointestinal diseases have been reported from several countries. The reasons associated with this include an increase in multidrug-resistant strains, a change in reservoir availability, and a change in travel and food trade patterns [26,29,30,31]. It is important to know which of these factors are causing the rise in incidence of the serotype Oranienburg in Mexico.

Outbreaks associated with serotype Oranienburg have been documented elsewhere in the world. Its causes include the consumption of black pepper [32], cheese [33], fruit salad (including cantaloupe and honeydew melons) [34], semi-dry cuttlefish [35], and chocolate [36]. In Mexico, this serotype has been isolated from farm [37] and zoo [38] animal feces, from meat for human consumption [28], and from fields where cantaloupe melon was grown [39].

As mentioned above, foodstuffs consumed by prisoners during the day before the outbreak and at the onset of the outbreak were unavailable for analysis, and food and water samples later analyzed were negative for *S. enterica*, but we were informed that some inmates were served a cantaloupe melon beverage and shrimp broth on the day of the outbreak. Given that the serotype Oranienburg has been isolated worldwide from cantaloupe melon and seafood, we considered that the consumption by patients of any of these foodstuffs was the most likely source of infection. Other researchers have reported similar difficulties in studying infectious disease outbreaks in prisons [3];these difficulties also obstruct attempts to establish prevention and control strategies in penitentiary institutions around the world. This important issue must be analyzed by competent authorities and organizations in order to mitigate the negative effects of the illness.

The general causes associated with food contamination by *S. enterica* in prisons are personnel or asymptomatic carriers' lack of hygiene, inadequate food storage and handling, and deficient cleaning of materials and installations used for food preparation [3]. Any of these causes could have contributed to the outbreak we analyzed. Prevention and control measures adopted in cases of diarrheagenic outbreaks in prisons aim at correcting any of the above-mentioned circumstances, including, among others, washing and sanitation of kitchen utensils and counters, adequate storage and preparation of foodstuffs, proper hand washing by kitchen personnel, and promoting health education campaigns for prisoners and prison staff members [3].

The Simpson discrimination index (*D*) values we determined for repetitive elements varied from a minimum of 0 for REP to a maximum of 0.5661 for ERIC. The discrimination capability of ERIC agrees with the report of Kumao *et al.* [5], who, in a study of Oranienburg strains involved in a gastroenteritis outbreak, found that the intraserotype discriminatory power of ERIC2 was higher than that of PFGE and ribotyping; however, the authors did not calculate *D* values. In fact, there are no available reports of *D* values for strains involved in outbreaks caused by the serotype Oranienburg to be contrasted with our results. However, other serotypes that were analyzed by techniques similar to the ones we used allow for such comparison. De Oliveira *et al.* [6] assessed the diversity of 111 isolates of serotype Enteriditis associated with a diarrheagenic outbreak using the repetitive elements ERIC, REP, and BOX, for which

they calculated the *D* values of 0.04, 0.05, and 0.04, respectively; these figures are one order of magnitude below our *D* estimates for ERIC and BOX. In contrast, an analysis of serotype Typhi strains from clinical samples obtained along a period of 20 years estimated a *D* value of 0.9821 for ERIC [40].

The RAPD primer DG93 displayed a higher *D* value (0.7753) than that for repetitive elements. The values of *D* resulting from RAPD assays showed considerable variation when analyzing strains of the same serotype isolated from outbreaks or clinical cases. Eriksson *et al.* [41] found a *D* value of 0.236 in strains of the serotype Livingstone from an outbreak. In analyses of isolates of the serotype Ohio from clinical cases, the highest reported *D* value was 0.22 [42]. For clinical isolates of serotypes Typhimurium, Virchow, Hadar, and Panama, Soto *et al.* [43] reported values of 0.72, 0.66, 0.78, and 0.60, respectively. Nath *et al.* [40] obtained a *D* value of 0.8978 for the serotype Typhi. In the case of the serotype Enteritidis, Landeras and Mendoza [44] reported a *D* value of 0.31, Soto *et al.* [43] of 0.52, and Fernandez *et al.* [45] of 0.68.

Several studies documented that combining ERIC and RAPD analyses increases the discriminatory power between isolates of *S. enterica* [19,40,43]. In this study, the combination of BOX, ERIC, and RAPD-DG93 increased the *D* value between analyzed isolates relative to BOX and ERIC, but not so for the RAPD marker alone. Our results show that the REP marker was inadequate as a typing tool in outbreaks caused by the serotype Oranienburg.

When considering isolates of the same serotype using repetitive DNA elements and RAPD, differences in reported *D* values could be due to methodological issues, such as the space and time extension of the sampling, and the number of analyzed isolates. The number and type of primers tested is another factor for discordances in the case of RAPD. Reported variations of *D* values among different serotypes may in part be explained by known dissimilarities in the genetic variation mechanisms of the different serotypes of *S. enterica* [46,47]. Taken together, the high *D* values calculated in this study, despite the relative low number of Oranienburg outbreak related isolates analyzed, suggest this serotype has a high genetic variation potential; the reasons for this potential remain unknown. Using the genomic analysis approach, it has been shown that prophage-related genes are the main source of genetic variation among outbreak related strains of the serotypes Enteritidis [11] and Typhimurium [48]. It will be desirable to make a genomic analysis of Oranienburg strains in order to shed light on the genetic variation displayed by this serotype.Interestingly, despite deriving from the same outbreak, the studied isolates differed in antibiotic resistance profiles; also remarkable was that all isolates had multiple resistance profiles. No data are available about the antibiotic resistance of serotype Oranienburg from clinical cases of diarrhea in Mexico, but our results contrast with a single report about strains from animal excreta, which found that 21.05% of isolates were resistant to ampicillin, and that all were sensitive to amikacin and gentamicin; however, this study found a similar susceptibility of all isolates to chloramphenicol and sulphamethoxazole / trimethropin to that which we observed [37].

The susceptibility patterns herein reported show both similarities and differences relative to those reported for the serotype Oranienburg from outbreaks in other countries. In Argentina, gastrointestinal and extra-intestinal isolates from pediatric patients showed co-resistance to gentamicin, netilmicin, and cefotaxime, but susceptibility to amikacin, which agrees with our results regarding the sensitivity of both types of isolates to sulphamethoxazole/trimethoprim and chloramphenicol [49]. Also in Argentina, Orman *et al.* [50] found that the serotype associated with outbreaks was resistant to cefotaxime, gentamicin, and amikacin. A clinical isolate from Turkey was resistant to ampicillin and clavulanic acid, but susceptible to chloramphenicol, tetracycline, and sulphamethoxazole / trimethoprim [51].

Among the known genetic factors associated with multiresistance to antibiotics in clinical isolates of the serotype Oranienburg are the multiple antibiotic resistance operon (marRAB) [52] and class I transposons and integrons [50]. However, it is important not to discard the possibility that the strains we studied could carry other chromosomal and plasmid resistance genes previously reported for serotypes of *S. enterica* [53]. A genetic analysis is needed to determine if these or other genetic factors are involved in the multiresistance to antibiotics of the isolates we studied.

An interesting finding of this work is that 52% of isolates were resistant to cefatoxime, an antibiotic used in combination with ceftazidime and others to reveal strains producing extended spectrum beta lactamases (ESBL), particularly of the CTX-M type [54]. Given the reports of the capacity of serotype Oranienburg from clinical isolates to produce ESBL [49,50,55] – a characteristic with clinical and public health relevance – it is important to determine if some of the isolates

we studied display the production of ESBL as a resistance factor.

The lack of correlation between genotype and the antibiotic resistance profile pattern we detected has previously been documented for different *S. enterica* serotypes isolated from humans, animals, and food using the same molecular markers we used [6,56,57,58]. This lack of correlation might be partially explained by the fact that both repetitive and RAPD markers are dispersed throughout the bacterial chromosome, while genetic resistance determinants are allocated in restricted genetic regions, or even outside chromosomes, as in the case of plasmids [53]. As stated above, the determinants of the genetic resistance of the strains we studied remain to be disclosed.

Conclusion

We present the only known study of a diarrheic outbreak in a Mexican prison, and the first report of serotype Oranienburg as a causal agent. The results herein presented reinforce previous observations showing that the combination of molecular genetic markers can more effectively discriminate *S. enterica* isolates associated with diarrheagenic outbreaks, and demonstrate that the use of repetitive DNA elements and RAPDs is a first approximation to the analysis of strains of the serotype Oranienburg. The profiles of multiresistance to antibiotics we found in isolates have a profound relevance for public health in terms of optimization of treatments. Our study notes the limitations encountered in the documentation of gastroenteritis outbreaks in Mexican prisons, a limitation that hinders the design of strategies for their prevention and control.

Acknowledgements

We are grateful for the support provided to the project SALUD-2009-01-115172 by the program S0008- FONSEC CONACYT/SSA/IMSS/ISSSTE.

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Conflict of interests: No conflict of interests is declared.